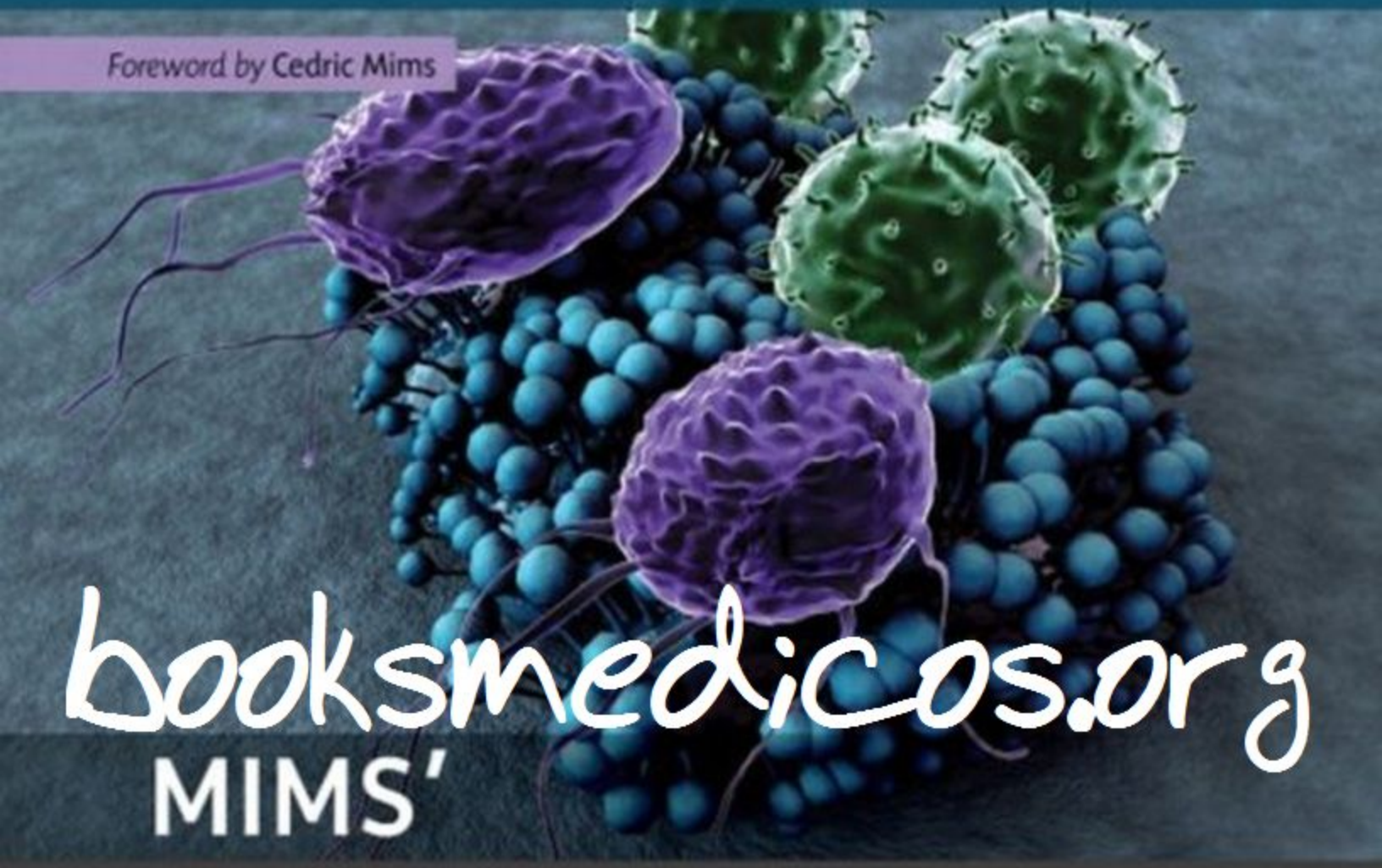
Richard V. Goering ■ Hazel M. Dockrell
Mark Zuckerman ■ Peter L. Chiodini

Foreword by Cedric Mims



MIMS' Medical Microbiology AND Immunology

SIXTH EDITION

ELSEVIER

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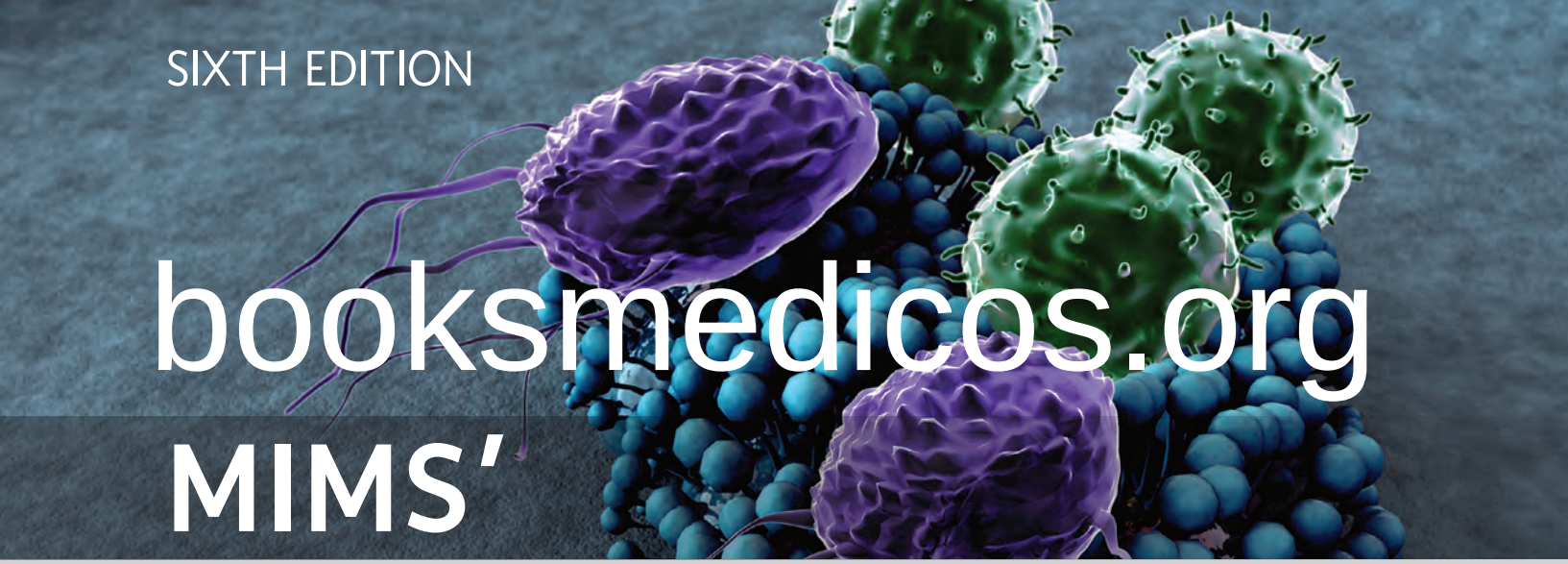

MIMS' Medical Microbiology AND Immunology

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ELSEVIER
EDINBURGH LONDON NEW YORK OXFORD PHILADELPHIA ST LOUIS SYDNEY TORONTO 2019

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ELSEVIER

First edition 1993
Second edition 1998
Third edition 2004
Fourth edition 2008
Fifth edition 2013
Sixth edition 2019

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ISBN: 978-0-7020-7154-6
978-0-7020-7156-0

Printed in China
Last digit is the print number: 9 8 7 6 5 4 3 2 1

Foreword by Cedric Mims

When I sat down with immunologist Ivan Roitt to think about writing this book, we agreed that it was to be more than a mere listing of microbial diseases with their diagnosis and treatment. All these infections result from the interplay between microbial cunning in relation to the immunological and inflammatory defences of the host, and Ivan's contribution meant that the immunology would be relevant and up-to-date.

During my 60 years as a physician and zoologist in England, America, Africa, and Australia, I have been able to study in some detail the mechanism by which microbial parasites enter the body, spread, and cause disease. It was always useful to think of those invaders as parasites, to look at it from their point of view, with the same forces governing the outcome in all cases, whether worms, bacteria, or viruses. It turns out that of all the different living species on earth, nearly half have opted for the parasitic way of life.

While the life of a parasite may sound attractive, with free board and lodging in or on the host, only a few invaders manage to survive those powerful defences. Over millions of years of evolution, their ability to avoid or evade the defences has been perfected and should never be underestimated.

Since the first edition of this book we have incorporated several improvements to make learning easier, including case studies, chapter key facts and chapter questions. My hope is that although what you learn from it will undoubtedly help you with final and board examinations, and although over the years many of the details may slip from your memory, you will have retained a useful way of looking at infectious diseases. To put it in military terms, every infection sets in train an armed conflict, with possible disease or death awaiting the loser.

This way of looking at infectious diseases will I hope stay with you and prepare you for the astonishing advances and the new treatments that await you during your career – in particular, new diseases from animals or birds, perhaps transmitted by biting insects or bats, as well as possible super-strains of influenza virus from birds that spread effectively in our species and make us ill, and also of course new antimicrobial drugs to which resistance is impossible. And we expect an unravelling of the influence on human health of that vast and mysterious collection of resident microbes living in our intestines.

I have always felt a personal as well as a scientific interest in these invaders. They killed both my parents when I was a child long before the development of antibiotics, and were responsible for my attacks of measles, mumps, diphtheria, whooping cough, tuberculosis, and much later Rift Valley Fever in Africa.

Cedric Mims
Canberra, Australia
October 2016

Preface to the sixth edition

Previous editions of *Mims' Medical Microbiology* have adopted the approach that the interaction between infectious disease and host response is best understood as a give-and-take conflict. The sixth edition continues this tradition, revising the title to *Mims' Medical Microbiology and Immunology* to better reflect the subject. Continued recognition of Cedric Mims' founding contribution to this work is seen not only in the title but also in the foreword to this sixth edition. Ivan Roitt, who played a major role in earlier editions, has relinquished his role as a main author and we gratefully acknowledge his contribution.

Overall, this edition benefits from significant revision in multiple areas. The introductory chapters continue to present fundamental principles of infectious agents and host defences but now include the newly recognized importance of the human microbiota. Subsequent chapters present an updated overview of the general principles behind the infectious agent – immune response conflict, followed by a chapter-specific consideration of system-oriented conflict scenarios. Final chapters provide a revised consideration of issues affecting diagnosis and control of the conflict especially centring on newer molecular (especially DNA-sequence-based) approaches.

Bibliographic references continue to include current Internet resources. Online access to interactive extras is provided via Elsevier's STUDENT CONSULT website (www.studentconsult.com) including questions and answers, mostly in USMLE format, the Pathogen Parade (infectious agent) index, and a new Vaccine Parade index.

Molecular approaches continue to inform and enlarge our understanding of pathogen–host interaction at a record pace. In this new edition of *Mims' Medical Microbiology and Immunology*, we believe the student will find a logical and unified approach to the subject that is readable, exciting, and informative.

Richard V Goering, Hazel M Dockrell,
Mark Zuckerman, Peter L Chiodini
2017

Acknowledgements

We would like to acknowledge the following colleagues for their helpful suggestions during the preparation of the new edition: Paul Fine, Kate Gallagher, Punam Mangtani, John Raynes, Eleanor Riley, Anthony Scott, Mel Smith, Steven Smith and Sara Thomas. We also acknowledge significant current and past content provided by Katharina Kranzer and Ivan Roitt.

A contemporary approach to microbiology

INTRODUCTION

Microbes and parasites

The conventional distinction between 'microbes' and 'parasites' is essentially arbitrary

Microbiology is sometimes defined as the biology of microscopic organisms, its subject being the 'microbes'. Traditionally, clinical microbiology has been concerned with those organisms responsible for the major infectious diseases of humans and whose size makes them invisible to the naked eye. Thus, it is not surprising that the organisms included have reflected those causing diseases that have been (or continue to be) of greatest importance in those countries where the scientific and clinical discipline of microbiology developed, notably Europe and the USA. The term 'microbes' has usually been applied in a restricted fashion, primarily to viruses and bacteria. Fungi and protozoan parasites have historically been included as more minor contributors, but in general they have been treated as the subjects of other disciplines (mycology and parasitology).

Although there can be no argument that viruses and bacteria are, globally, the most important pathogens, the conventional distinction between these as 'microbes' and the other infectious agents (fungi, protozoan, worm and arthropod parasites) is essentially arbitrary, not least because the criterion of microscopic visibility cannot be applied rigidly (Fig. Intro.1). Perhaps we should remember that the first 'microbe' to be associated with a specific clinical condition was a parasitic worm – the nematode *Trichinella spiralis* – whose larval stages are just visible to the naked eye (though microscopy is needed for certain identification). *T. spiralis* was first identified in 1835 and causally related to the disease trichinellosis in the 1860s. Viruses and bacteria comprise just over half of all human pathogen species (Table Intro.1).

THE CONTEXT FOR CONTEMPORARY MEDICAL MICROBIOLOGY

Many microbiology texts deal with infectious organisms as agents of disease in isolation, both from other infectious organisms and from the biological context in which they live and cause disease. It is certainly convenient to consider organisms group by group, to summarize the diseases they cause, and to review the forms of available control, but this approach produces a static picture of what is a dynamic relationship between the organism and its host.

Host response is the outcome of the complex interplay between host and parasite. Host response can be discussed in terms of pathological signs and symptoms and in terms of immune control, but it is better treated as the outcome of the complex interplay between two organisms – host and parasite; without this dimension a distorted view of infectious disease results. It simply is not true that 'microbe+host=disease', and clinicians are well aware of this. Understanding why it is that most host–microbe contacts do not result in disease, and what changes so that disease does arise, is as important as the identification of infectious organisms and a knowledge of the ways in which they can be controlled.

We therefore continue to believe that our approach to microbiology, both in terms of the organisms that might usefully be considered within a textbook and also in terms of the contexts in which they and the diseases they cause are discussed, provides a more informative and more interesting picture of these dynamic interrelationships. There are many reasons for having reached this conclusion, the most important being the following:

- A comprehensive understanding now exists at the molecular level of the biology of infectious agents and of the host–parasite interactions that lead to infection and disease. It is important for students to be aware of this understanding so that they can grasp the connections between infection and disease within both individuals and communities and to be able to use this knowledge in novel and changing clinical situations.
- It is now realized that the host's response to infection is a coordinated and subtle interplay involving the mechanisms of both innate and acquired resistance, and that these mechanisms are expressed regardless of the nature and identity of the pathogen involved. Our present understanding of the ways in which these mechanisms are stimulated and the ways in which they act is very sophisticated. We can now see that infection is a conflict between two organisms, with the outcome (resistance or disease) being critically dependent upon molecular interactions. Again, it is essential to understand the basis of this host–pathogen interplay if the processes of disease and disease control are to be interpreted correctly.

Emerging or re-emerging diseases continue to pose new microbiological problems

Three other factors have helped to mould our opinion that a broader view of microbiology is needed to provide a firm basis for clinical and scientific practice:

- There is an increasing prevalence of a wide variety of opportunistic infections in patients who are hospitalized or immunosuppressed. Immunosuppressive therapies are now common, as are diseases in which the immune system is compromised – notably, of course, acquired immunodeficiency disease (AIDS).
- Newly emerging disease agents continue to be identified, and old diseases previously thought to be under control, re-emerge as causes of concern. Of the 1407 species

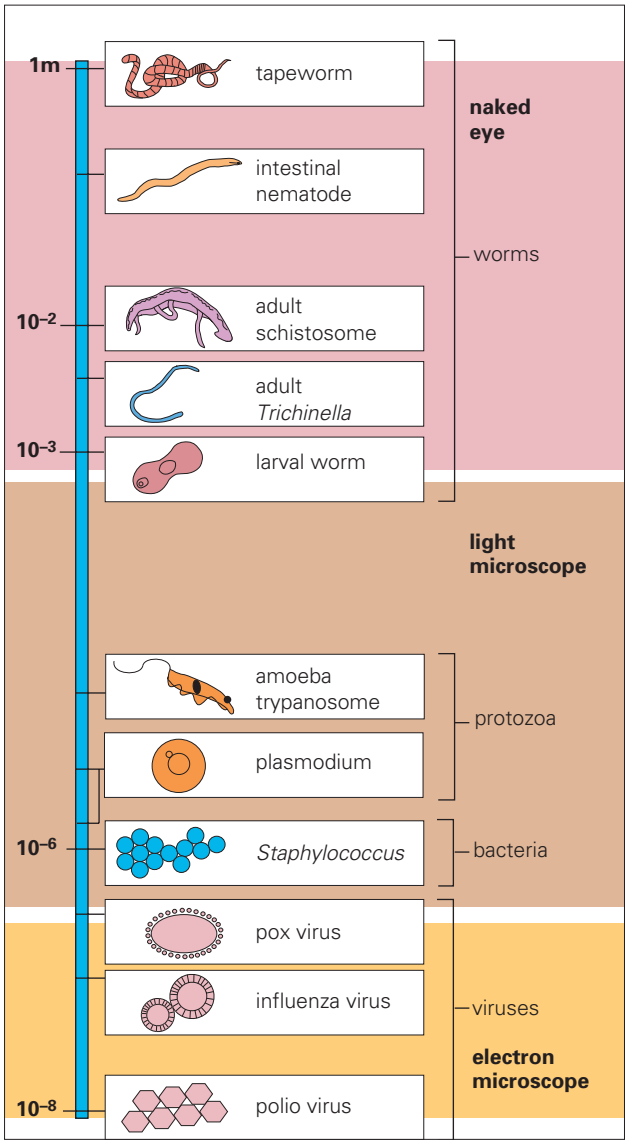


Figure Intro.1 Relative sizes of the organisms covered in this book.

Table Intro.1 Distribution of 1407 human pathogen species among the major groups of organisms (excluding arthropods)

Group	% of total
Viruses and prions	14–15
Bacteria	38–41
Fungi	22–23
Protozoa	4–5
Helminths	20

(Data from average of multiple studies summarized by Smith K.F., Guegan J.-F. Changing geographic distributions of human pathogens. *Annu Rev Ecol Evol* 2010; 41:231–250.)

identified as pathogenic for humans, 183 are regarded as emerging or re-emerging pathogens, almost half being viruses, some of animal origin (see Table Intro.1).

- Tropical infections are now of much greater interest. Clinicians see many tourists who have been exposed to the quite different spectrum of infectious agents found in tropical countries (at least 80 million people travel from resource-rich to resource-poor countries each year), and practising microbiologists may be called upon to identify and advise on these organisms. There is also greater awareness of the health problems of the resource-poor world.

Thus, a broader view of microbiology is necessary: one that builds on the approaches of the past, but addresses the problems of the present and of the future.

MICROBIOLOGY PAST, PRESENT AND FUTURE

The demonstration in the nineteenth century that diseases were caused by infectious agents founded the discipline of microbiology. Although these early discoveries involved tropical parasitic infections as well as the bacterial infections common in Europe and the USA, microbiologists increasingly focused on the latter, later extending their interests to the newly discovered viral infections. The development of antimicrobial agents and vaccines revolutionized treatment of these diseases and raised hopes for the eventual elimination of many of the diseases that had plagued the human race for centuries. Those in the resource-rich world learned not to fear infectious disease and believed such infections would disappear in their lifetime. To an extent, this was realized; through vaccination, many familiar childhood diseases became uncommon, and those of bacterial origin were more easily controlled by antibiotics. Encouraged by the eradication of smallpox during the 1970s, and the success of polio vaccines, the United Nations in 1978 announced programmes to obtain 'health for all' by 2000. However, this and other optimistic targets have required re-evaluation.

Infectious diseases are killers in both resource-rich and resource-poor countries

Globally, infectious diseases (especially lower respiratory infections) are second only to heart disease as most frequent cause of death. The World Health Organization (WHO) has now listed 12 antibiotic-resistant bacterial pathogens as priorities for the development of new antibiotics – 75% of which are categorized as critical or of high importance. However, infectious diseases are not evenly distributed worldwide (Fig. Intro.2)

The burden of infectious disease in the resource-poor world is especially concerning. Although sub-Saharan Africa has only about 10% of the world's population, it has the clear majority of AIDS infections and AIDS-related deaths, the highest HIV-TB co-infection rates and most of the global malaria burden. Tuberculosis (TB) and HIV-AIDS are of increasing importance in South-East Asia and the Pacific, where drug-resistant malaria is also common. Children younger than 5 years are most at risk from infectious diseases. It is obvious that the prevalence and importance of infectious diseases in the resource-poor world are directly linked to poverty.

Infections continue to emerge or re-emerge

On a worldwide basis, infectious diseases continue to emerge in the human population for the first time. Recent examples include the MERS coronavirus, the H7N9 avian influenza virus, and the Zika virus. Concern regarding spread of the Ebola virus and the lack of effective antibiotics for treating

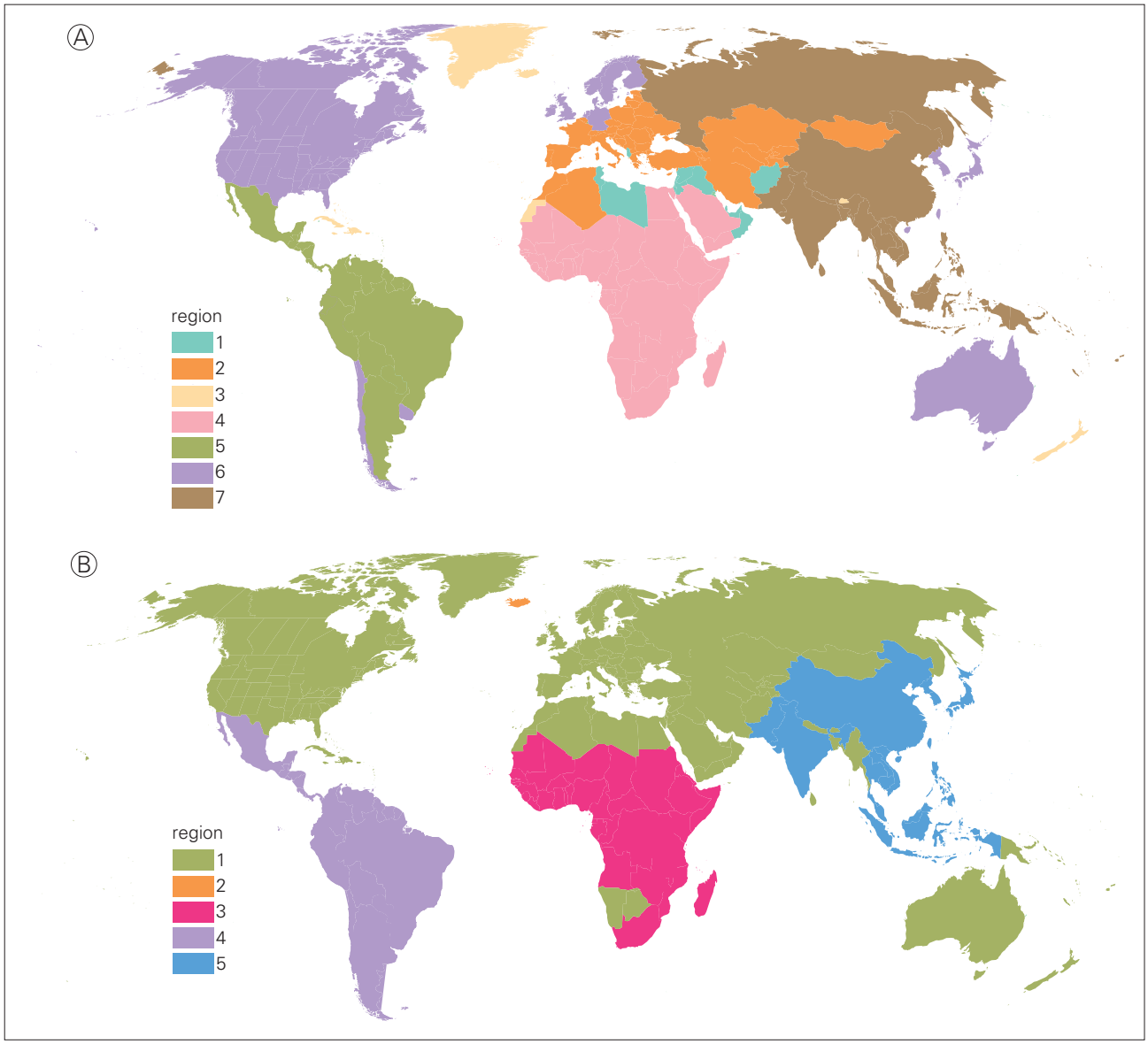


Figure Intro.2 Geographic distribution of 301 diseases in 229 countries. (A) 93 vector-associated diseases predominant in seven geographic regions and (B) 208 non-vector predominant in five geographic regions. Colours indicate groups of similar diseases (vector or non-vector) tending to predominate in specific geographic regions. (Redrawn from Just M.G., Norton J.F., Traud A.L. et al. [2014] Global biogeographic regions in a human-dominated world: the case of human diseases. *Ecosphere*. Chichester: John Wiley & Sons, Fig 1.)

bacterial infections (see above) further underscore the negative global impact of infectious diseases.

Modern lifestyles and technical developments facilitate transmission of disease

The reasons for the resurgence of infectious diseases are multiple. They include:

- New patterns of travel and trade (especially food commodities), new agricultural practices, altered sexual behaviour, medical interventions and overuse of antibiotics.
- The movement of multidrug-resistant bacteria, such as multiply resistant *Staphylococcus aureus* (MRSA), and virulent pathogens such as *Clostridium difficile* from the healthcare setting into the community. The issue of antimicrobial resistance is compounded in resource-poor countries by inability or unwillingness to complete programmes of treatment and by the use of counterfeit drugs with, at best, partial action. The World Health Organization (WHO) has now catalogued the existence of over 900 counterfeit medical products representing the full spectrum of medical therapies.
- Breakdown of economic, social and political systems especially in the resource-poor world has weakened medical services and increased the effects of poverty and malnutrition.
- The dramatic increase in air travel over the last few decades has facilitated the spread of infection and increased the threat of new pandemics. The Spanish influenza pandemic in 1918 spread along railway and sea links. Modern air

travel moves larger numbers of people more rapidly and more extensively and makes it possible for microbes to cross geographical barriers.

What of the future?

Predictions based on data from the United Nations and WHO give a choice of scenarios. Optimistically, the aging population, coupled with socioeconomic and medical advances, could be expected to see a fall in the problems posed by infectious disease, and a decrease in deaths from these causes. The pessimistic view is that population growth in resource-poor countries, especially in urban populations, the increasing gap between rich and poor countries and continuing changes in lifestyle will result in surges of infectious disease. Even in resource-rich countries, increasing drug resistance and a slowing of developments in new antimicrobials and vaccines will create additional problems in control. Added to these are three additional factors. These are:

- the emergence of new human infections such as a novel strain of influenza virus, or a new infection of wildlife origin
- climate change, with increased temperatures and altered rainfall adding to the incidence of vector-borne infection
- the threat of bioterrorism, with the possible deliberate spread of viral and bacterial infections to human populations with no acquired immunity or no history of vaccination.

One thing is certain: whether optimistic or pessimistic scenarios prove true, microbiology will remain a critical medical discipline for the foreseeable future.

THE APPROACH ADOPTED IN THIS BOOK

The factors outlined above indicate the need for a text with a dual function:

1. It should provide an inclusive treatment of the organisms responsible for infectious disease.
2. The purely clinical / laboratory approach to microbiology should be replaced with an approach that will stress the biological context in which clinical / laboratory studies are to be undertaken.

The approach we have adopted in this book is to look at microbiology from the viewpoint of the conflicts inherent in all host–pathogen relationships. We first describe the adversaries: the infectious organisms on the one hand, and the innate and adaptive defence mechanisms of the host on the other. The outcome of the conflicts between the two is then amplified and discussed system by system. Rather than taking each organism or each disease manifestation in turn, we look at the major environments available for infectious organisms in the human body, such as the respiratory system, the gut, the urinary tract, the blood and the central nervous system. The organisms that invade and establish in each of these are examined in terms of the pathological responses they provoke. Finally, we look at how the conflicts we have described can be controlled or eliminated, both at the level of the individual patient and at the level of the community. We hope that such an approach will provide readers with a dynamic view of host–pathogen interactions and allow them to develop a more creative understanding of infection and disease.

KEY FACTS

- Our approach is to provide a comprehensive account of the organisms that cause infectious disease in humans, from the viruses to the worms, and to cover the biological bases of infection, disease, host–pathogen interactions, disease control and epidemiology.
- The diseases caused by microbial pathogens will be placed in the context of the conflict that exists between them and the innate and adaptive defences of their hosts.
- Infections will be described and discussed in terms of the major body systems, treating these as environments in which microbes can establish themselves, flourish and give rise to pathological changes.

1 Pathogens as parasites

Introduction

The interaction between pathogen and host can be viewed as a parasitic relationship. The pathogenic process involves the establishment, persistence, and reproduction of the infecting agent at the expense of the host. How this is accomplished depends on multiple factors including microbial anatomy, size (macro vs micro parasites), and whether the organisms live inside or outside of host cells. Understanding these issues in the context of a classification system that provides a view of microbe interrelationships provides an important foundation for the study of pathogen-host interaction

THE VARIETIES OF PATHOGENS

Prokaryotes and eukaryotes

A number of important and distinctive biological characteristics must be taken into account when considering any microorganism in relation to infectious disease. In general, these can be considered in terms of comparative microbial anatomy – the way in which organisms are constructed, and particularly the way in which genetic material and other cellular components are organized.

All organisms other than viruses and prions are made up of cells

Although viruses have genetic material (DNA or RNA) they are not cellular, lacking cell membranes, cytoplasm and the machinery for synthesizing macromolecules, depending instead upon host cells for this process. Conventional viruses have their genetic material packed in capsids. The agents (prions) which cause diseases such as Creutzfeldt–Jakob disease (CJD), variant CJD and kuru in humans, and scrapie and bovine spongiform encephalopathy (BSE) in animals, lack nucleic acid and consist only of infectious proteinaceous particles.

All other organisms have a cellular organization, being made up of single cells (most 'microbes') or of many cells. Each cell has genetic material (DNA) and cytoplasm with synthetic machinery, and is bounded by a cell membrane.

Bacteria are prokaryotes; all other organisms are eukaryotes

There are many differences between the two major divisions – prokaryotes and eukaryotes – of cellular organisms (Fig. 1.1). These include the following.

In prokaryotes:

- a distinct nucleus is absent
- DNA is in the form of a single circular chromosome; additional 'extrachromosomal' DNA is carried in plasmids
- transcription and translation can be carried out simultaneously.

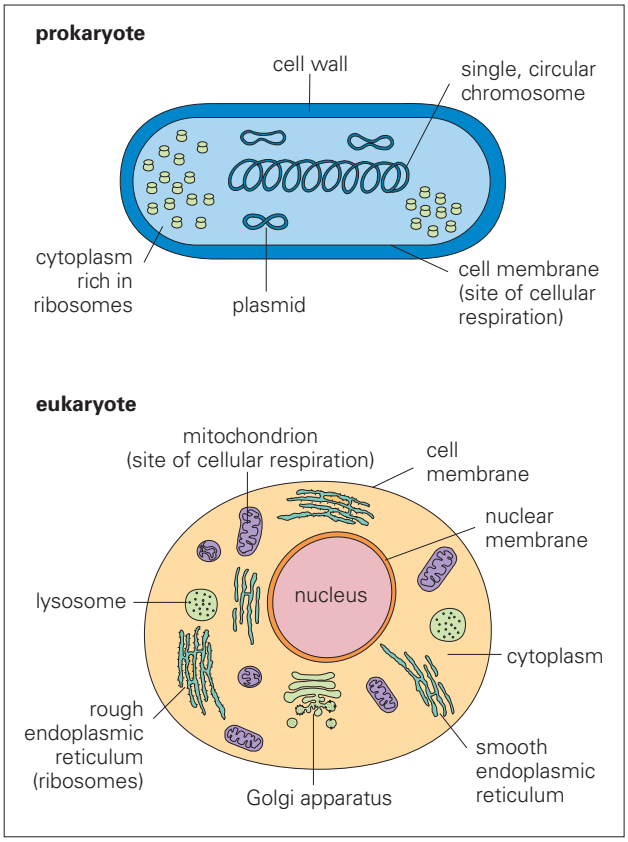


Figure 1.1 Prokaryote and eukaryote cells. The major features of cellular organization are shown diagrammatically.

In eukaryotes:

- DNA is carried on several chromosomes within a nucleus
- the nucleus is bounded by a nuclear membrane
- transcription and translation are carried out separately with transcribed messenger RNA (mRNA) moving out of the nucleus into the cytoplasm for ribosomal translation

- the cytoplasm is rich in membrane-bound organelles (mitochondria, endoplasmic reticulum, Golgi apparatus, lysosomes), which are absent in prokaryotes.

Gram-negative bacteria have an outer lipopolysaccharide-rich layer

Another important difference between prokaryotes and the majority of eukaryotes is that the cell membrane (plasma membrane) of prokaryotes is covered by a thick protective cell wall. In Gram-positive bacteria this wall, made of peptidoglycan, forms the external surface of the cell, whereas in Gram-negative bacteria there is an additional outer layer rich in lipopolysaccharides. These layers play an important role in protecting the cell against the host immune system and chemotherapeutic agents, and in stimulating certain pathological responses. They also confer antigenicity.

Microparasites and macroparasites

Microparasites replicate within the host

There is an important distinction between microparasites and macroparasites that overrides their differences in size. *Micro*parasites (viruses, bacteria, protozoa, fungi) replicate within the host and can, theoretically, multiply to produce a very large number of progeny, thereby causing an overwhelming infection. In contrast, *macro*parasites (worms, arthropods), even those that are microscopic, do not have this ability: one infectious stage matures into one reproducing stage and, in most cases, the resulting progeny leave the host to continue the cycle. The level of infection is therefore determined by the numbers of organisms that enter the body. This distinction between microparasites and macroparasites has important clinical and epidemiological implications.

The boundary between microparasites and macroparasites is not always clear. The progeny of some macroparasites do remain within the host, and infections can lead to the build-up of overwhelming numbers, particularly in immune-suppressed patients. The roundworms *Trichinella*, *Strongyloides stercoralis* and some filarial nematodes, and *Sarcoptes scabiei* (the itch mite), are examples of this type of parasite.

Organisms that are small enough can live inside cells

Absolute size has other biologically significant implications for the host–pathogen relationship, which cut across the divisions between micro- and macroparasites. Perhaps the most important of these is the relative size of a pathogen and its host's cells. Organisms that are small enough can live inside cells and, by doing so, establish a biological relationship with the host that is quite different from that of an extracellular organism – one that influences both disease and control.

LIVING INSIDE OR OUTSIDE CELLS

The basis of all host–pathogen relationships is the exploitation by one organism (the pathogen) of the environment provided by another (the host). The nature and degree of exploitation varies from relationship to relationship, but the pathogen's primary requirement is a supply of metabolic materials from the host, whether provided in the form of nutrients or (as in the case of viruses) in the form of nuclear synthetic machinery. The reliance of viruses upon host synthetic machinery requires an obligatory intracellular habit: viruses must live within host cells. Some other groups of pathogens (e.g. *Chlamydia*, *Rickettsia*) also live only within cells. In the remaining groups of pathogens, different species have adopted either the intracellular or the extracellular habit or, in a few cases, both. Intracellular microparasites other than viruses take their metabolic requirements directly from the pool of nutrients available in the cell itself, whereas extracellular organisms take theirs from the nutrients present in tissue fluids or, occasionally, by feeding directly on host cells (e.g. *Entamoeba histolytica*, the organism associated with amoebic dysentery). Macroparasites are almost always extracellular (though *Trichinella* is intracellular), and many feed by ingesting and digesting host cells; others can take up nutrients directly from tissue fluids or intestinal contents.

Pathogens within cells are protected from many of the host's defence mechanisms

As will be discussed in greater detail in Chapter 15, the intracellular pathogens pose problems for the host that are quite different from those posed by extracellular organisms. Pathogens that live within cells are largely protected against many of the host's defence mechanisms while they remain there, particularly against the action of specific antibodies. Control of these infections depends therefore on the activities of intracellular killing mechanisms, short-range mediators or cytotoxic agents, although the latter may destroy both the pathogen and the host cell, leading to tissue damage. This problem, of targeting activity against the pathogen when it lives within a vulnerable cell, also arises when using drugs or antibiotics, as it is difficult to achieve selective action against the pathogen while leaving the host cell intact. Even more problematic is the fact that many intracellular pathogens live inside the very cells responsible for the host's immune and inflammatory mechanisms and therefore depress the host's defensive abilities. For example, a variety of viral, bacterial and protozoal pathogens live inside macrophages, and several viruses (including human immunodeficiency virus, HIV) are specific for lymphocytes.

Intracellular life has many advantages for the pathogen. It provides access to the host's nutrient supply and its genetic machinery and allows escape from host surveillance and antimicrobial defences. However, no organism can be wholly intracellular at all times: if it is to replicate successfully, transmission must occur between the host's cells, and this inevitably involves some exposure to the extracellular environment. As far as the host is concerned, this extracellular phase in the development of the pathogen provides an opportunity to control infection through defence mechanisms such as phagocytosis, antibody and complement. However, transmission between cells can involve destruction of the initially infected cell and so contribute to tissue damage and general host pathology.

Living outside cells provides opportunities for growth, reproduction and dissemination

Extracellular pathogens can grow and reproduce freely, and may move extensively within the tissues of the body. However, they also face constraints on their survival and development. The most important is continuous exposure to components of the host's defence mechanisms, particularly antibody, complement and phagocytic cells.

The characteristics of extracellular organisms lead to pathological consequences that are quite different from those associated with intracellular species. These are seen most dramatically with the macroparasites, whose sheer physical size, reproductive capacity and mobility can result in extensive destruction of host tissues. Many extracellular pathogens have the ability to spread rapidly through extracellular fluids or to move rapidly over surfaces, resulting in a widespread infection within a relatively short time. The rapid colonization of the entire mucosal surface of the small bowel by *Vibrio cholerae* is a good example. Successful host defence against extracellular parasites requires mechanisms that differ from those used in defence against intracellular parasites. The variety of locations and tissues occupied by extracellular parasites also poses problems for the host in ensuring effective deployment of defence mechanisms. Defence against intestinal parasites requires components of the innate and adaptive immune systems that are quite distinct from those effective against parasites in other sites, and those living in the lumen may be unaffected by responses operating in the mucosa. These problems in mounting effective defence are most acute where large macroparasites are concerned, because their size often renders them insusceptible to defence mechanisms that can be used against smaller organisms. For example, worms cannot be phagocytosed; they often have protective external layers, and can actively move away from areas where the host response is activated.

SYSTEMS OF CLASSIFICATION

Infectious diseases are caused by organisms belonging to a very wide range of different groups – prions, viruses, bacteria, fungi, protozoa, helminths (worms) and arthropods. Each has its own system of classification, making it possible to identify and categorize the organisms concerned. Correct identification is an essential requirement for accurate diagnosis and effective treatment. Identification is achieved by a variety of means, from simple observation to molecular analysis. Classification is being revolutionized by the application of genome sequencing. Many of the major pathogens in all categories have now been sequenced and this is allowing not only more precise identification but also a greater understanding of the interrelationships of members within each taxonomic group.

The approaches used vary between the major groups. For the protozoa, fungi, worms and arthropods, the basic unit of classification is the species, essentially defined as a group of organisms capable of reproducing sexually with one another. Species provide the basis for the binomial system of classification, used for eukaryote and some prokaryote organisms. Species are in turn grouped into a 'genus' (closely related but non-interbreeding species). Each organism is identified by two names, indicating the 'genus' and the 'species' respectively, for example, *Homo sapiens* and *Escherichia coli*. Related genera are grouped into progressively broader and more inclusive categories.

Classification of bacteria and viruses

The concept of 'species' is a basic difficulty in classifying prokaryotes and viruses, although the categories of genus and species are routinely used for bacteria. Classification of bacteria uses a mixture of easily determined microscopic, macroscopic and biochemical characteristics, based on size, shape, colour, staining properties, respiration and reproduction, and a more sophisticated analysis of immunological and molecular criteria. The former characteristics can be used to divide the organisms into conventional taxonomic groupings, as shown for the Gram-positive bacteria in Fig. 1.2 (see also Ch. 2).

Correct identification of bacteria below the species level is often vital to differentiate pathogenic and non-pathogenic forms

Correct treatment requires correct identification. For some bacteria, the important subspecies groups are identified on the basis of their immunological properties. Cell wall, flagellar and capsule antigens are used in tests with specific antisera to define serogroups and serotypes (e.g. in salmonellae, streptococci, shigellae and *E. coli*). Biochemical characteristics can be used to define other subspecies groupings (biotypes, strains, groups). For example, *Staphylococcus aureus* strains typically release a beta-haemolysin (causing red blood cells to lyse). Production of other toxins is also important in differentiating between

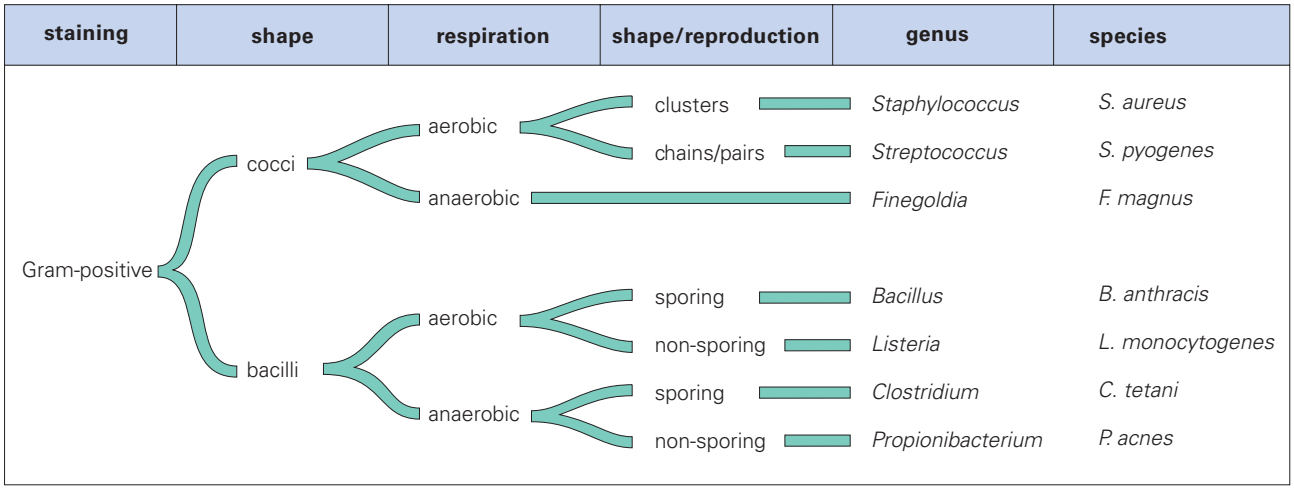


Figure 1.2 How the structural and biological characteristics of bacteria can be used in classification, taking Gram-positive bacteria as an example.

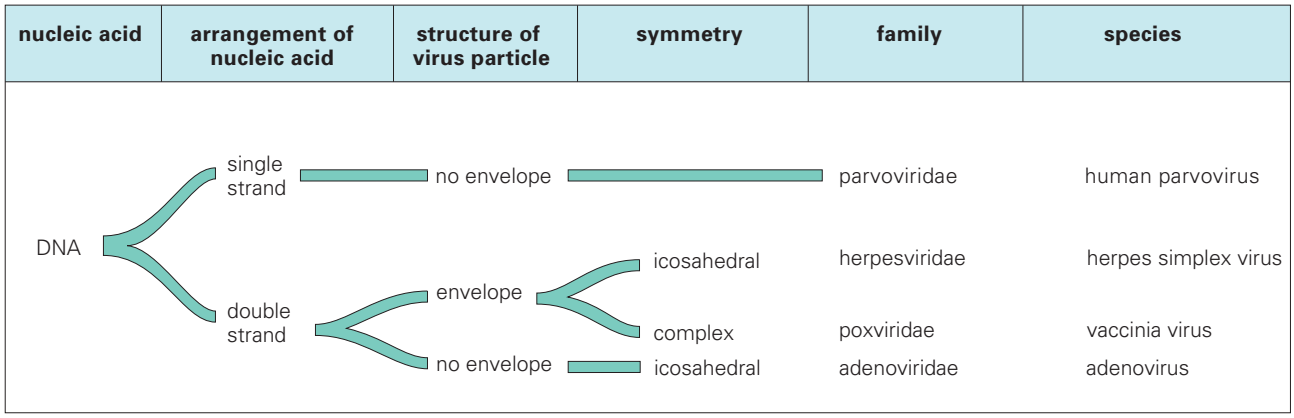


Figure 1.3 How the characteristics of viruses can be used in classification, taking DNA viruses as an example.

groups, as in *E. coli*. Antibiotic susceptibility can also be helpful in identification. Matrix-assisted laser desorption ionization time of flight (MALDI TOF) mass spectrometry is being increasingly used as a rapid and cost-effective means of identification. Direct genetic approaches are also used in identification and classification such as the use of the polymerase chain reaction (PCR) and probes to detect organism-specific sentinel DNA sequences. These tests are particularly useful for those organisms which grow poorly or not at all in vitro.

Classification of viruses departs even further from the binomial system

Virus names draw on a wide variety of characteristics (e.g. size, structure, pathology, tissue location or distribution). Groupings are based on characteristics such as the type of nucleic acid present (DNA or RNA), the symmetry of the virus particle (e.g. icosahedral, helical, complex) and the presence or absence of an external envelope, as shown for the DNA viruses in Fig. 1.3 (see also Ch. 3). The equivalents of subspecies categories are also used including serotypes, strains, variants and isolates and are determined primarily by serological reactivity of virus material. The influenza virus, for example, can be considered as the equivalent of a genus containing three types (A, B, C). Identification can be carried out using the stable nucleoprotein antigen, which differs between the three types. The neuraminidase and haemagglutinin antigens are not stable and show variation within types. Characterization of these antigens in an isolate enables the particular variant to be identified, haemagglutinin (H) and neuraminidase (N) variants being designated by numbers (e.g. H5N1, the variant associated with fatal avian influenza; see Ch. 20). A further example is seen in adenoviruses, for which the various antigens associated with a component of the capsid can be used to define groups, types and finer subdivisions. The rapid rate of mutation shown by some viruses (e.g. HIV) creates particular problems for classification. The population present in a virus-infected individual may be genetically quite diverse and may best be described as a quasispecies – representing the average of the broad spectrum of variants present.

Classification assists diagnosis and the understanding of pathogenicity

Prompt identification of organisms is necessary clinically so that diagnoses can be made and appropriate treatments advised. To understand host–parasite interactions, however, not only should the identity of an organism be known, but also as much as possible of its general biology; useful predictions can then be made about the consequences of infection. For these reasons, in subsequent chapters, we have included outline classifications of the important pathogens, accompanied by brief accounts of their structure (gross and microscopic), modes of life, molecular biology, biochemistry, replication and reproduction.

KEY FACTS

- Organisms that cause infectious diseases can be grouped into seven major categories: prions, viruses, bacteria, fungi, protozoa, helminths and arthropods.
- Identification and classification of these organisms are important parts of microbiology and are essential for correct diagnosis, treatment and control.
- Each group has distinctive characteristics (structural and molecular make-up, biochemical and metabolic strategies, reproductive processes) which determine how the organisms interact with their hosts and how they cause disease.
- Many pathogens live within cells, where they are protected from many components of the host's protective responses.

2 The bacteria

Introduction

Although free-living bacteria exist in huge numbers, relatively few species cause disease. The majority of these are well known and well studied; however, new pathogens continue to emerge and the significance of previously unrecognized infections becomes apparent. Good examples of the latter include Ebola virus disease and Zika fever, while infection with *Legionella*, the cause of Legionnaires' disease and gastric ulcers associated with *Helicobacter pylori* infection, are good historical bacterial examples.

Bacteria are single-celled prokaryotes, their DNA forming a long circular molecule, but not contained within a defined nucleus. Many are motile, using a unique pattern of flagella. The bacterial cell is surrounded by a complex cell wall and often a thick capsule. They reproduce by binary fission, often at very high rates, and show a wide range of metabolic patterns, both aerobic and anaerobic. Classification of bacteria uses both phenotypic and genotypic data. For clinical purposes, the phenotypic data are of most practical value, and rest on an understanding of bacterial structure and biology (see Fig. 32.2). Detailed summaries of members of the major bacterial groups are given in the Pathogen Parade (see online appendix).

STRUCTURE

Bacteria are 'prokaryotes' and have a characteristic cellular organization

The genetic information of bacteria is carried in a long, double-stranded (ds), circular molecule of deoxyribonucleic acid (DNA) (Fig. 2.1). By analogy with eukaryotes (see Ch. 1), this can be termed a 'chromosome', but there are no introns; instead, the DNA comprises a continuous coding sequence of genes. The chromosome is not localized within a distinct nucleus; no nuclear membrane is present and the DNA is tightly coiled into a region known as the 'nucleoid'. Genetic information in the cell may also be extrachromosomal, present as small circular self-replicating DNA molecules termed plasmids. The cytoplasm contains no organelles other than ribosomes for protein synthesis. Although ribosomal function is the same in both pro- and eukaryotic cells, organelle structure is different. Ribosomes are characterized as 70 S in prokaryotes and 80 S in eukaryotes (the 'S' unit relates to how a particle behaves when studied under extreme centrifugal force in an ultracentrifuge). The bacterial 70 S ribosome is specifically targeted by antimicrobials such as the aminoglycosides (see Ch. 34). Many of the metabolic functions performed in eukaryote cells by membrane-bound organelles such as mitochondria are carried out by the prokaryotic cell membrane. In all bacteria except mycoplasmas, the cell is surrounded by a complex cell wall. External to this wall may be capsules, flagella and pili. Knowledge of the cell wall and these external structures is important in diagnosis and pathogenicity and for understanding bacterial biology.

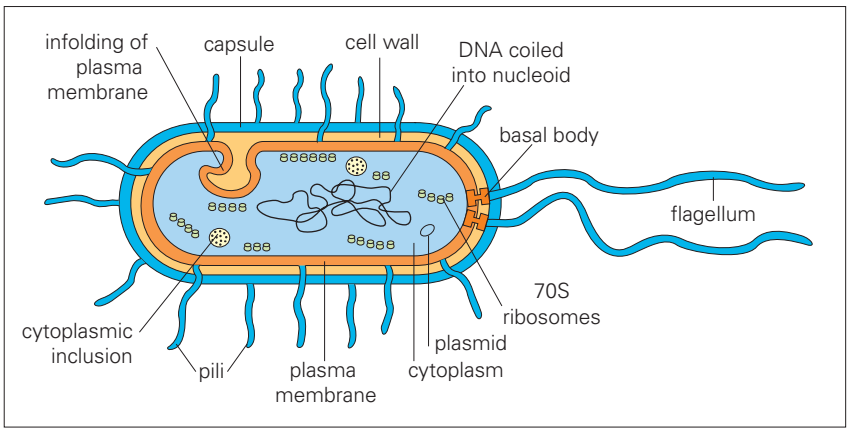


Figure 2.1 Diagrammatic structure of a generalized bacterium.

Bacteria are classified according to their cell wall as Gram-positive or Gram-negative

Gram staining is a basic microbiological procedure for identification of bacteria (see Ch. 32). The main structural component of the cell wall is 'peptidoglycan' (mucopeptide or murein), a mixed polymer of hexose sugars (*N*-acetylglucosamine and *N*-acetylmuramic acid) and amino acids (Fig. 2.2):

- In Gram-positive bacteria, the peptidoglycan forms a thick (20–80 nm) layer external to the cell membrane, and may contain other macromolecules.
- In Gram-negative species, the peptidoglycan layer is thin (5–10 nm) and is overlaid by an outer membrane, anchored to lipoprotein molecules in the peptidoglycan layer. The principal molecules of the outer membrane are lipopolysaccharides and lipoprotein.

The polysaccharides and charged amino acids in the peptidoglycan layer make it highly polar, providing the bacterium with a thick hydrophilic surface. This property allows Gram-positive organisms to resist the activity of bile in the intestine. Conversely, the layer is digested by lysozyme, an enzyme present in body secretions, which therefore has bactericidal properties. Synthesis of peptidoglycan is disrupted by beta-lactam and glycopeptide antibiotics (see Ch. 34).

In Gram-negative bacteria, the outer membrane is also hydrophilic, but the lipid components of the constituent molecules give hydrophobic properties as well. Entry of hydrophilic molecules such as sugars and amino acids is necessary for nutrition and is achieved through special channels or pores formed by proteins called 'porins'. The lipopolysaccharide (LPS) in the membrane confers both antigenic properties (the 'O antigens' from the carbohydrate chains) and toxic properties (the 'endotoxin' from the lipid A component; see Ch. 18).

While staining weakly Gram-positive, mycobacteria also possess an outer membrane, which contains a variety of complex lipids (mycolic acids). These create a waxy layer, which both alters the staining properties of these organisms (the so-called acid-fast bacteria) and gives considerable resistance to drying and other environmental factors. Mycobacterial cell wall components also have a pronounced adjuvant activity (i.e. they promote immunological responsiveness).

External to the cell wall may be an additional capsule of high molecular weight polysaccharides (or amino acids in anthrax bacilli) that gives a slimy surface. This provides protection against phagocytosis by host cells and is important in determining virulence. With *Streptococcus pneumoniae* infection, only a few capsulated organisms can cause a fatal infection, but unencapsulated mutants cause no disease.

The cell wall is a major contributor to the ultimate shape of the organism, an important characteristic for bacterial identification. In general, bacterial shapes are categorized as spherical (cocci), rods (bacilli) or helical (spirilla) (Fig. 2.3), although there are variations on these themes.

Many bacteria possess flagella

Flagella are long helical filaments extending from the cell surface, which enable bacteria to move in their environment. These may be restricted to the poles of the cell, singly (polar) or in tufts (lophotrichous), or distributed over the general surface of the cell (peritrichous). Bacterial flagella are structurally quite different from eukaryote flagella. In addition, the forces that result in movement are generated quite differently, being proton dependent (i.e. driven by movement of hydrogens across the cell membrane) in prokaryotes but adenosine triphosphate (ATP) dependent in eukaryotes. Motility allows positive and negative responses to environmental stimuli such as chemicals (chemotaxis). Flagella are built of protein components (flagellins), which are strongly antigenic. These antigens, the H antigens, are important targets of protective antibody responses.

Pili are another form of bacterial surface projection

Pili (fimbriae) are more rigid than flagella and function in attachment, either to other bacteria (the 'sex' pili) or to host cells (the 'common' pili). Adherence to host cells involves

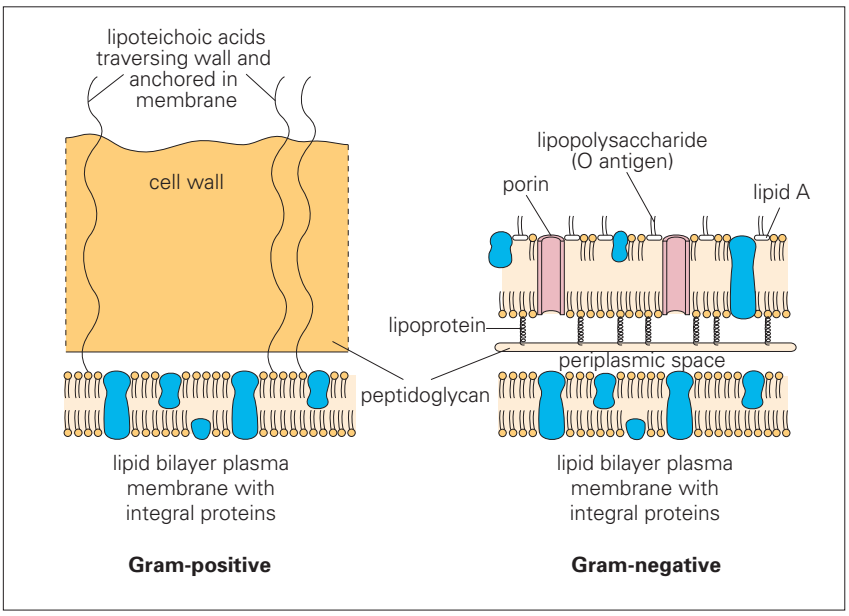


Figure 2.2 Construction of the cell walls of Gram-positive and Gram-negative bacteria.

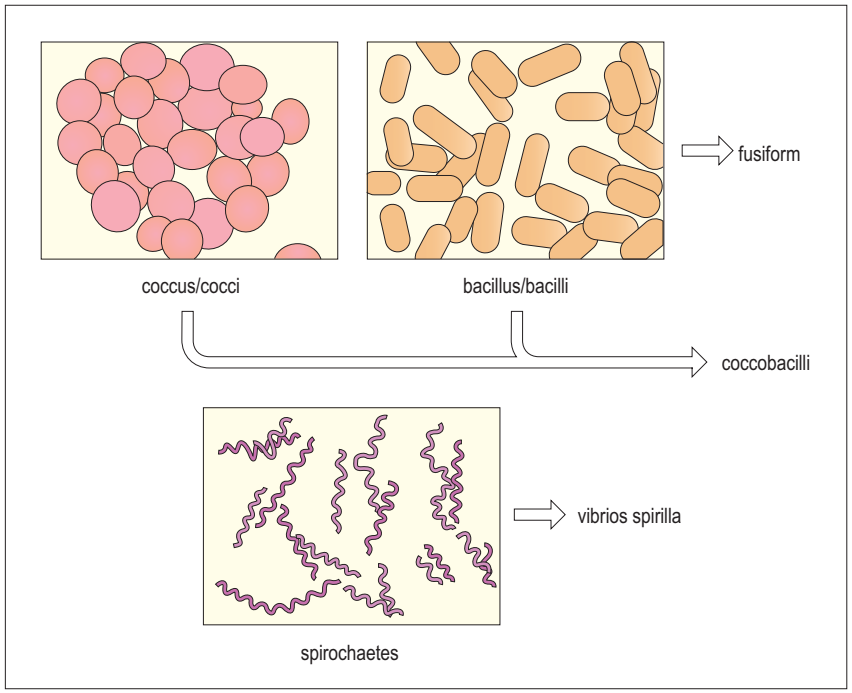


Figure 2.3 The three basic shapes of bacterial cells.

specific interactions between component molecules of the pili (adhesins) and molecules in host cell membranes. For example, the adhesins of *Escherichia coli* interact with fucose / mannose molecules on the surface of intestinal epithelial cells (see Ch. 23). The presence of many pili may help to prevent phagocytosis, reducing host resistance to bacterial infection. Although immunogenic, their antigens can be changed, allowing the bacteria to avoid immune recognition. The mechanism of 'antigenic variation' has been elucidated in organisms such as the gonococci and is known to involve recombination of genes coding for 'constant' and 'variable' regions of pili molecules.

NUTRITION

Bacteria obtain nutrients mainly by taking up small molecules across the cell wall

Bacteria take up small molecules such as amino acids, oligosaccharides and small peptides across the cell wall. Gram-negative species can also take up and use larger molecules after preliminary digestion in the periplasmic space. Uptake and transport of nutrients into the cytoplasm is achieved by the cell membrane using a variety of transport mechanisms including facilitated diffusion, which utilizes a carrier to move compounds to equalize their intra- and extracellular concentrations, and active transport, where energy is expended to deliberately increase intracellular concentrations of a substrate. Oxidative metabolism (see below) also takes place at the membrane–cytoplasm interface.

Some species require only minimal nutrients in their environment, having considerable synthetic powers, whereas others have complex nutritional requirements. *E. coli*, for example, can be grown in media providing only glucose and inorganic salts; streptococci, on the other hand, will grow only in complex media providing them with many organic compounds. Nevertheless, all bacteria have similar general nutritional requirements for growth, which are summarized in Table 2.1.

All pathogenic bacteria are heterotrophic

All bacteria obtain energy by oxidizing preformed organic molecules (carbohydrates, lipids and proteins) from their environment. Metabolism of these molecules yields ATP as an energy source. Metabolism may be aerobic, where the final electron acceptor is oxygen, or anaerobic, where the final acceptor may be an organic or inorganic molecule other than oxygen.

- In aerobic metabolism (i.e. aerobic respiration), complete utilization of an energy source such as glucose produces 38 molecules of ATP.
- Anaerobic metabolism utilizing an inorganic molecule other than oxygen as the final hydrogen acceptor (anaerobic respiration) is incomplete and produces fewer ATP molecules than aerobic respiration.
- Anaerobic metabolism utilizing an organic final hydrogen acceptor (fermentation) is much less efficient and produces only two molecules of ATP.

Anaerobic metabolism, while less efficient, can thus be used in the absence of oxygen when appropriate substrates are available, as they usually are in the host's body. The requirement for oxygen in respiration may be 'obligate' or it may be 'facultative', some organisms being able to switch between aerobic and anaerobic metabolism. Those that use fermentation pathways often use the major product pyruvate in secondary fermentations by which additional energy can be generated. The interrelationship between these different metabolic pathways is illustrated in Fig. 2.4.

The ability of bacteria to grow in the presence of atmospheric oxygen relates to their ability to deal enzymatically with

Table 2.1 Major nutritional requirements for bacterial growth

Element	Cell dry weight (%)	Major cellular role
Carbon	50	Molecular 'building block' obtained from organic compounds or CO_2
Oxygen	20	Molecular 'building block' obtained from organic compounds, O_2 or H_2O; O_2 is an electron acceptor in aerobic respiration
Nitrogen	14	Component of amino acids, nucleotides, nucleic acids and coenzymes obtained from organic compounds and inorganic sources such as $NH4^+$
Hydrogen	8	Molecular 'building block' obtained from organic compounds, H_2O, or H_2; involved in respiration to produce energy
Phosphorus	3	Found in a variety of cellular components including nucleotides, nucleic acids, lipopolysaccharide (lps) and phospholipids; obtained from inorganic phosphates (PO_4^{3-})
Sulphur	1–2	Component of several amino acids and coenzymes; obtained from organic compounds and inorganic sources such as sulphates (SO_4^{2-})
Potassium	1–2	Important inorganic cation, enzyme cofactor, etc., obtained from inorganic sources

Table 2.2 Bacterial classification in response to environmental oxygen

Environmental oxygen			
Category	**Present**	**Absent**	**Oxygen-detoxifying enzymes (e.g. superoxide dismutase, catalase, peroxidase)**
Obligate aerobe	Growth	No growth	Present
Microaerophile	Growth in low oxygen levels	No growth	Some enzymes absent; reduced enzyme concentration
Obligate anaerobe	No growth	Growth	Absent
Facultative (anaerobe/aerobe)	Growth	Growth	Present

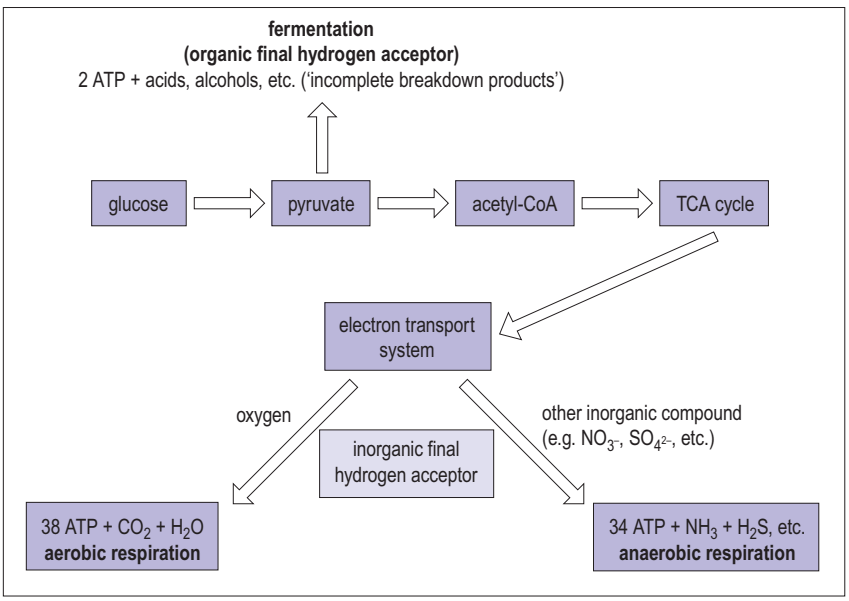


Figure 2.4 Catabolic breakdown of glucose in relationship to final hydrogen acceptor.

potentially destructive intracellular reactive oxygen species (e.g. free radicals, anions containing oxygen, etc.) (Table 2.2). The interaction between these harmful compounds and detoxifying enzymes such as superoxide dismutase, peroxidase and catalase is illustrated in Fig. 2.5 (also see Ch. 10 and Box 10.2).

GROWTH AND DIVISION

The rate at which bacteria grow and divide depends in large part on the nutritional status of the environment. The growth and division of a single *E. coli* cell into identical 'daughter cells' may occur in as little as 20–30 min in rich laboratory

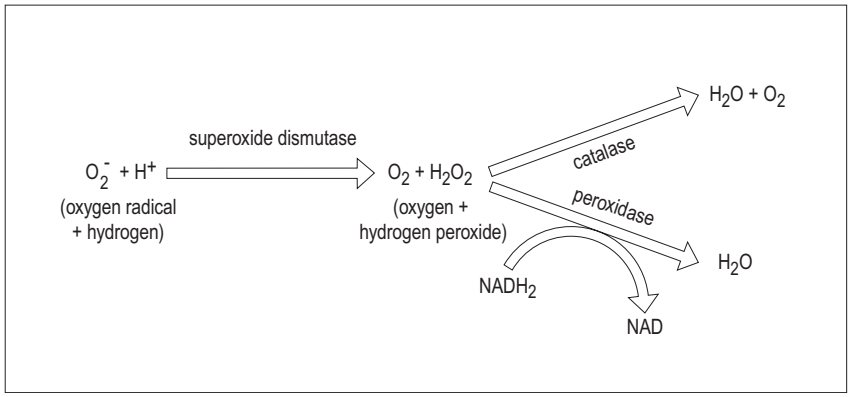


Figure 2.5 Interaction between oxygen detoxifying enzymes.

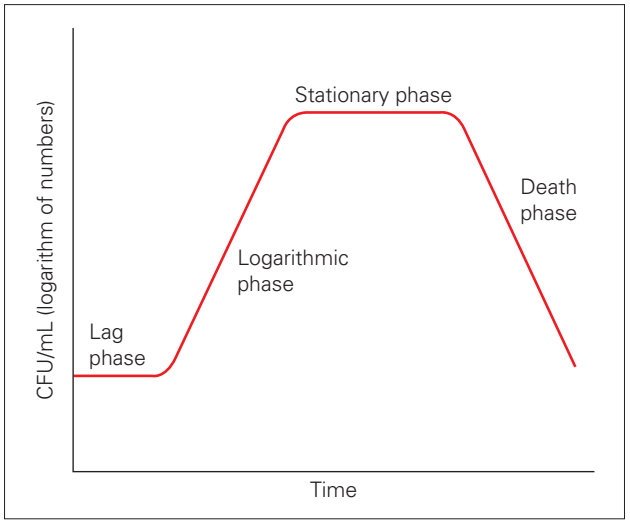


Figure 2.6 The bacterial growth curve. CFU, colony-forming units.

media, whereas the same process is much slower (1–2 h) in a nutritionally depleted environment. Conversely, even in the best environment, other bacteria such as *Mycobacterium tuberculosis* may grow much more slowly, dividing every 24 h. When introduced into a new environment, bacterial growth follows a characteristic pattern depicted in Fig. 2.6. After an initial period of adjustment (lag phase), cell division rapidly occurs, with the population doubling at a constant rate (generation time), for a period termed log or exponential phase. As nutrients are depleted and toxic products accumulate, cell growth slows to a stop (stationary phase) and eventually enters a phase of decline (death).

A bacterial cell must duplicate its genomic DNA before it can divide

All bacterial genomes are circular, and their replication begins at a single site known as the origin of replication (termed OriC). A multienzyme replication complex binds to the origin and initiates unwinding and separation of the two DNA strands, using enzymes called helicases and topoisomerases (e.g. DNA gyrase). Each of the separated DNA strands serves as a template for DNA polymerase. The polymerization reaction involves incorporation of deoxyribonucleotides, which correctly base pair with the template DNA. Two characteristic replication forks are formed, which proceed in opposite directions around the chromosome. Each of the two copies of the total genetic information (genome) produced during replication comprises one parental strand and one newly synthesized strand of DNA.

Replication of the genome takes approximately 40 min in *E. coli*, so when these bacteria grow and divide every 20–30 min they need to initiate new rounds of DNA replication before an existing round of replication has finished to provide complete chromosomal copies at an accelerated rate. In such instances, daughter cells inherit DNA that has already initiated its own replication.

Replication must be accurate

Accurate replication is essential because DNA carries the information that defines the properties and processes of a cell. It is achieved because DNA polymerase is capable of proofreading newly incorporated deoxyribonucleotides and excising those that are incorrect. This reduces the frequency of errors to approximately one mistake (an incorrect base pair) per 10^{10} nucleotides copied.

Cell division is preceded by genome segregation and septum formation

The process of cell division (or septation) involves:

- segregation of the replicated genomes
- formation of a septum in the middle of the cell
- division of the cell to give separate daughter cells.

The septum is formed by an invagination of the cytoplasmic membrane and ingrowth of the peptidoglycan cell wall (and outer membrane in Gram-negative bacteria). Septation and DNA replication and genome segregation are not tightly coupled, but are sufficiently well coordinated to ensure that the overwhelming majority of daughter cells have the correct complement of genomic DNA.

The mechanics of cell division result in reproducible cellular arrangements, when viewed by microscopic examination. For example, cocci dividing in one plane may appear chained (streptococci) or paired (diplococci), while division in multiple planes results in clusters (staphylococci). As with cell shape, these arrangements have served as an important characteristic for bacterial identification.

Bacterial growth and division are important targets for antimicrobial agents

Antimicrobials that target the processes involved in bacterial growth and division include:

- quinolones (ciprofloxacin and levofloxacin), which inhibit the unwinding of DNA by DNA gyrase during DNA replication
- the many inhibitors of peptidoglycan cell wall synthesis (e.g. beta-lactams such as the penicillins, cephalosporins and carbapenems, and glycopeptides such as vancomycin).

These are considered in more detail in Chapter 34.

GENE EXPRESSION

Gene expression describes the processes involved in decoding the 'genetic information' contained within a gene to produce a functional protein or ribonucleic acid (RNA) molecule.

Most genes are transcribed into messenger RNA (mRNA)

The overwhelming majority of genes (e.g. up to 98% in *E. coli*) are transcribed into mRNA, which is then translated into proteins. Certain genes, however, are transcribed to produce ribosomal RNA species (5 S, 16 S, 23 S), which provide a scaffold for assembling ribosomal subunits; others are transcribed into transfer RNA (tRNA) molecules, which together with the ribosome participate in decoding mRNA into functional proteins.

Transcription

The DNA is copied by a DNA-dependent RNA polymerase to yield an RNA transcript. The polymerization reaction involves incorporation of ribonucleotides, which correctly base pair with the template DNA.

Transcription is initiated at promoters

Promoters are nucleotide sequences in DNA that can bind the RNA polymerase. The frequency of transcription initiation can be influenced by many factors, for example:

- the exact DNA sequence of the promoter site
- the overall topology (supercoiling) of the DNA
- the presence or absence of regulatory proteins that bind adjacent to and may overlap the promoter site.

Consequently, different promoters have widely different rates of transcriptional initiation (of up to 3000-fold). Their activities can be altered by regulatory proteins. Sigma factor (a protein specifically needed to begin RNA synthesis) plays an important role in promoter recognition. The presence of several different sigma factors in bacteria enables sets of genes to be switched on simply by altering the level of expression of a particular sigma factor (e.g. spore formation in Gram-positive bacteria).

Transcription usually terminates at specific termination sites

These termination sites are characterized by a series of uracil residues in the mRNA following an inverted repeat sequence, which can adopt a stem-loop structure (which forms as a result of the base-pairing of ribonucleotides) and interfere with RNA polymerase activity. In addition, certain transcripts terminate following interaction of RNA polymerase with the transcription termination protein, rho.

mRNA transcripts often encode more than one protein in bacteria

The bacterial arrangement seen for single genes (promoter–structural-gene–transcriptional-terminator) is described as monocistronic. However, a single promoter and terminator may flank multiple structural genes, a polycistronic arrangement known as an operon. Operon transcription thus results in polycistronic mRNA encoding more than one protein (Fig. 2.7). Operons provide a way of ensuring that protein subunits that make up particular enzyme complexes or are required for a specific biological process are synthesized simultaneously and in the correct stoichiometry. For example, the proteins required for the uptake and metabolism of lactose are encoded by the lac operon. Many of the proteins responsible for the pathogenic properties of medically important microorganisms are likewise encoded by operons, for example:

- cholera toxin from *Vibrio cholerae*
- fimbriae (pili) of uropathogenic *E. coli*, which mediate colonization.

Translation

The exact sequence of amino acids in a protein (polypeptide) is specified by the sequence of nucleotides found in the mRNA transcripts. Decoding this information to produce a protein is achieved by ribosomes and tRNA molecules in a process known as translation. Each set of three bases (triplet) in the mRNA sequence corresponds to a codon for a specific amino acid. However, there is redundancy in the triplet code resulting in instances of more than one triplet encoding the same amino acid (i.e. also referred to as code degeneracy). Thus, a total of 64 codons encode all 20 amino acids as well as start and stop signal codons.

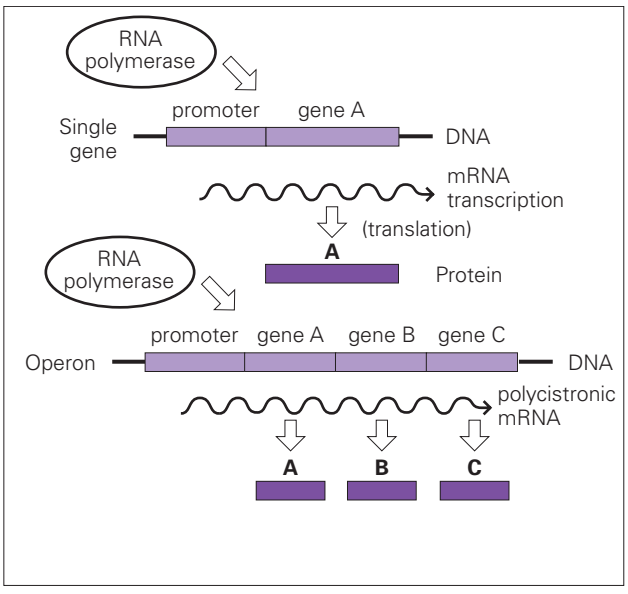


Figure 2.7 Bacterial genes are present on DNA as separate discrete units (single genes) or as operons (multigenes), which are transcribed from promoters to give, respectively, monocistronic or polycistronic messenger RNA (mRNA) molecules; mRNA is then translated into protein.

Translation begins with formation of an initiation complex and terminates at a STOP codon

The initiation complex comprises mRNA, ribosome and an initiator tRNA molecule carrying formylmethionine. Ribosomes bind to specific sequences in mRNA (Shine–Dalgarno sequences) and begin translation at an initiation (START) codon, AUG (i.e. the bases adenosine, uracil, guanine), which hybridizes with a specific complementary sequence (the anti-codon loop) of the initiator tRNA molecule. The polypeptide chain elongates as a result of movement of the ribosome along the mRNA molecule and the recruitment of further tRNA molecules (carrying different amino acids), which recognize the subsequent codon triplets. Ribosomes carry out a condensation reaction, which couples the incoming amino acid (carried on the tRNA) to the growing polypeptide chain.

Translation is terminated when the ribosome encounters one of three termination (STOP) codons: UGA, UAA or UAG.

Transcription and translation are important targets for antimicrobial agents

Such antimicrobial agents include:

- inhibitors of RNA polymerase, such as rifampicin
- a wide array of bacterial protein synthesis inhibitors including macrolides (e.g. erythromycin, aminoglycosides, tetracyclines, chloramphenicol, lincosamides, streptogramins, and oxazolidinones) (see Ch. 34).

Regulation of gene expression

Bacteria adapt to their environment by controlling gene expression

Bacteria show a remarkable ability to adapt to changes in their environment. This is predominantly achieved by controlling gene expression, thereby ensuring that proteins are produced only when and if they are required. For example:

- Bacteria may encounter a new source of carbon or nitrogen and as a consequence switch on new metabolic pathways that enable them to transport and use such compounds.
- When compounds such as amino acids are depleted from a bacterium's environment, the bacterium may be able to switch on the production of enzymes that enable it to synthesize de novo the particular molecule it requires.

Expression of many virulence determinants by pathogenic bacteria is highly regulated

This makes sense as it conserves metabolic energy and ensures that virulence determinants are produced only when their particular property is needed. For example, enterobacterial pathogens are often transmitted in contaminated water supplies. The temperature of such water will probably be lower than 25°C and low in nutrients. However, upon entering the human gut there will be a striking change in the bacterium's environment – the temperature will rise to 37°C, there will be an abundant supply of carbon and nitrogen and a low availability of both oxygen and free iron (an essential nutrient). Bacteria adapt to such changes by switching on or off a range of metabolic and virulence-associated genes.

The analysis of virulence gene expression is one of the fastest-growing aspects of the study of microbial pathogenesis. It provides an important insight into how bacteria adapt to the many changes they encounter as they initiate infection and spread into different host tissues.

The most common way of altering gene expression is to change the amount of mRNA transcription

The level of mRNA transcription can be altered by altering the efficiency of binding of RNA polymerase to promoter sites. Environmental changes such as shifts in growth temperature (from 25°C to 37°C) or the availability of oxygen can change the extent of supercoiling in DNA, thereby altering the overall topology of promoters and the efficiency of transcription initiation. However, most instances of transcriptional regulation are mediated by regulatory proteins, which bind specifically to the DNA adjacent to or overlapping the promoter site and alter RNA polymerase binding and transcription. The regions of DNA to which regulatory proteins bind are known as operators or operator sites. Regulatory proteins fall into two distinct classes:

- those that increase the rate of transcription initiation (activators)
- those that inhibit transcription (repressors) (Fig. 2.8).

Genes subject to positive regulation need to bind an activated regulatory protein (apoinducer) to promote transcription initiation. Gene transcription subject to negative regulation is inhibited by the binding of repressor proteins.

The principles of gene regulation in bacteria can be illustrated by the regulation of genes involved in sugar metabolism

Bacteria use sugars as a carbon source for growth and prefer to use glucose rather than other less well-metabolized sugars. When growing in an environment containing both glucose and lactose, bacteria such as *E. coli* preferentially metabolize glucose and at the same time prevent the expression of the lac operon, the products of which transport and metabolize lactose (Fig. 2.9). This is known as catabolite repression. It occurs because the transcriptional initiation of the lac operon is dependent upon a positive regulator: the cyclic adenosine monophosphate (cAMP)-dependent catabolite activator protein (CAP), which is activated only when cAMP is bound. When bacteria grow on glucose the cytoplasmic levels of cAMP are low and so CAP is not activated. CAP is therefore unable to bind to its DNA binding site adjacent to the lac promoter and facilitate transcription initiation by RNA polymerase. When the glucose is depleted, the cAMP concentration rises resulting in the formation of activated cAMP–CAP complexes, which bind the appropriate site on the DNA, increasing RNA polymerase binding and lac operon transcription.

CAP is an example of a global regulatory protein that controls the expression of multiple genes; it controls the expression of over 100 genes in *E. coli.* All genes controlled by the same regulator are considered to constitute a regulon (see Fig. 2.8). In addition to the influence of CAP on the lac operon, the operon is also subject to negative regulation by the lactose repressor protein (LacI, see Fig. 2.9). LacI is encoded by the *lacI* gene, which is located immediately upstream of the lactose operon and transcribed by a separate promoter. In the absence of lactose, LacI binds specifically to the operator region of the lac promoter and blocks transcription. An inducer molecule, allolactose (or its non-metabolizable homologue,

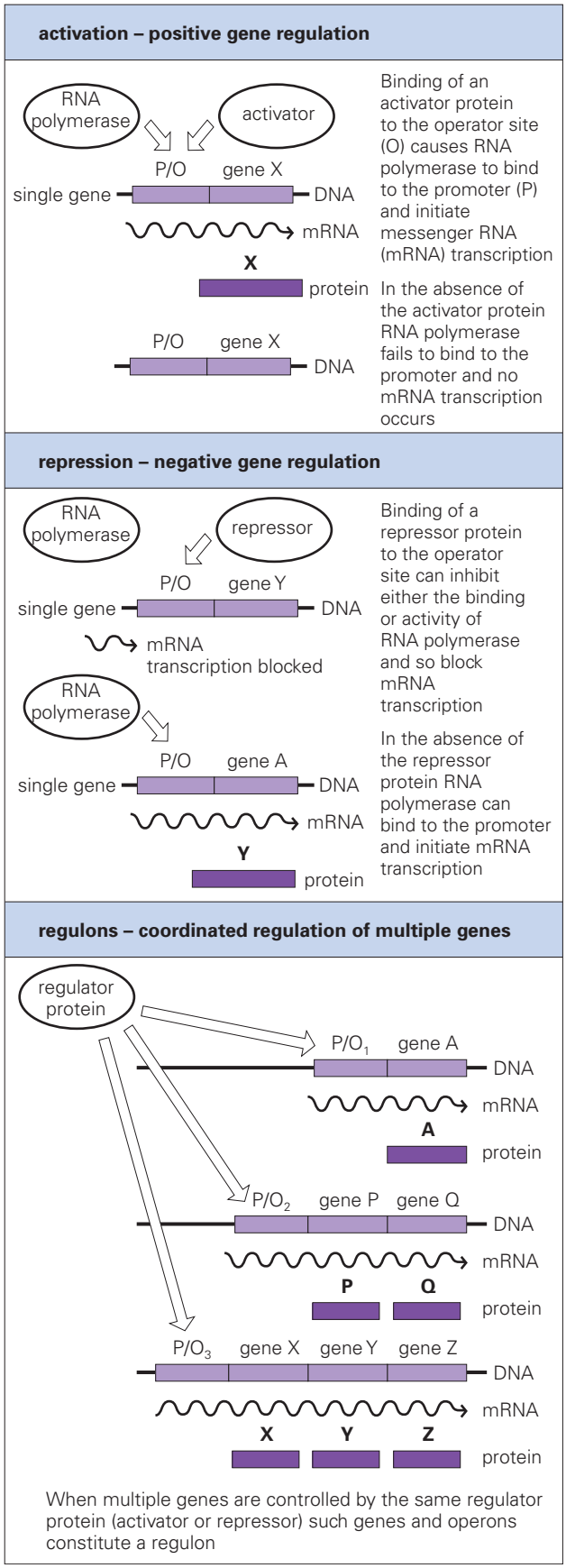


Figure 2.8 Expression of genes in bacteria is highly regulated, enabling them to switch genes on or off in response to changes in available nutrients or other changes in their environment. Genes and operons controlled by the same regulator constitute a regulon.

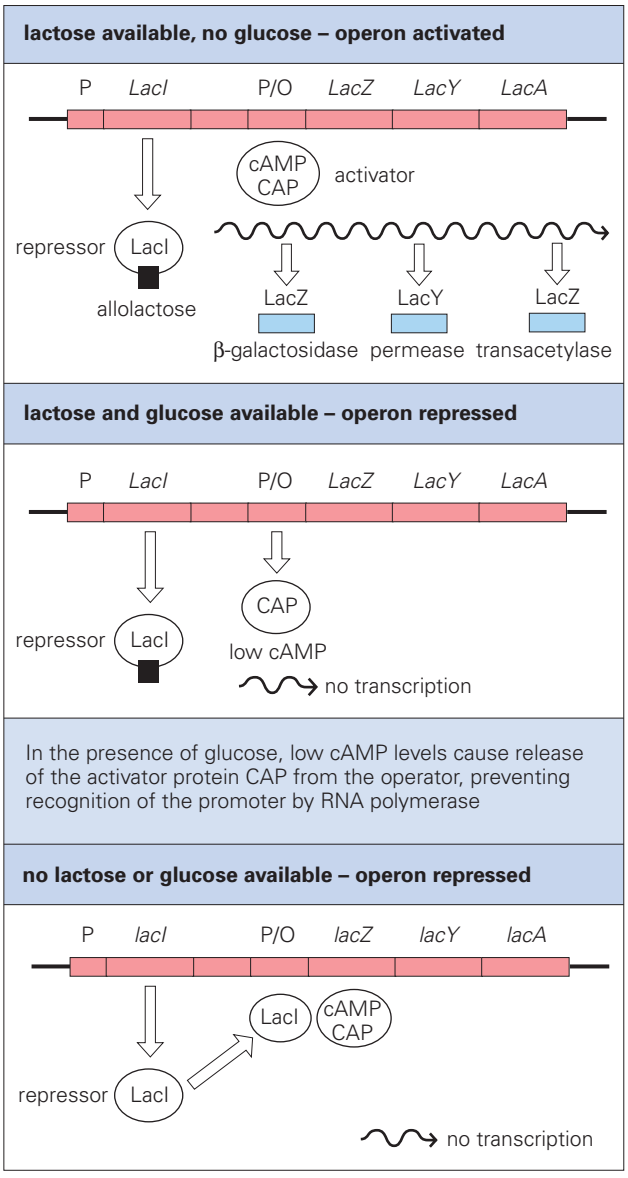


Figure 2.9 Control of the lac operon. Transcription is controlled by the lactose repressor protein (LacI, negative regulation) and by the catabolite activator protein (CAP, positive regulation). In the presence of lactose as the sole carbon source for growth, the lac operon is switched on. Bacteria prefer to use glucose rather than lactose, so if glucose is also present the lac operon is switched off until the glucose has been used.

isopropyl-thiogalactoside – IPTG) is able to bind to LacI, causing an allosteric change in its structure. This releases it from the DNA, thereby alleviating the repression. The lac operon therefore illustrates the fine tuning of gene regulation in bacteria – the operon is switched on only if lactose is available as a carbon source for cell growth, but remains unexpressed if glucose, the cell's preferred carbon source, is also present.

Expression of bacterial virulence genes is often controlled by regulatory proteins

An example of such regulation is the production of diphtheria toxin by *Corynebacterium diphtheriae* (see Ch. 19), which is subject to negative regulation if there is free iron in the growth environment. A repressor protein, DtxR, binds iron and undergoes a conformational change that allows it to bind with high affinity to the operator site of the toxin gene and inhibit transcription. When *C. diphtheriae* grow in an environment with a very low concentration of iron (i.e. similar to that of human secretions), DtxR is unable to bind iron, and toxin production occurs.

Many bacterial virulence genes are subject to positive regulation by 'two-component regulators'

These two-component regulators typically comprise two separate proteins (Fig. 2.10):

- one acting as a sensor to detect environmental changes (such as alterations in temperature)
- the other acting as a DNA-binding protein capable of activating (or repressing in some cases) transcription.

Bacteria may possess multiple two-component regulators recognizing different environmental stimuli. Thus, bacteria residing in more complex environments tend to carry increased numbers of two-component regulators.

In *Bordetella pertussis*, the causative agent of whooping cough (see Ch. 20), a two-component regulator (encoded by the bvg locus) controls expression of a large number of virulence genes. The sensor protein, BvgS, is a cytoplasmic membrane-located histidine kinase, which senses environmental signals (temperature, Mg^{2+}, nicotinic acid), leading to an alteration in its autophosphorylating activity. In response to positive regulatory signals such as an elevation in temperature, BvgS undergoes autophosphorylation and then phosphorylates, so activating the DNA-binding protein BvgA. BvgA then binds to the operators of the pertussis toxin operon and other virulence-associated genes and activates their transcription.

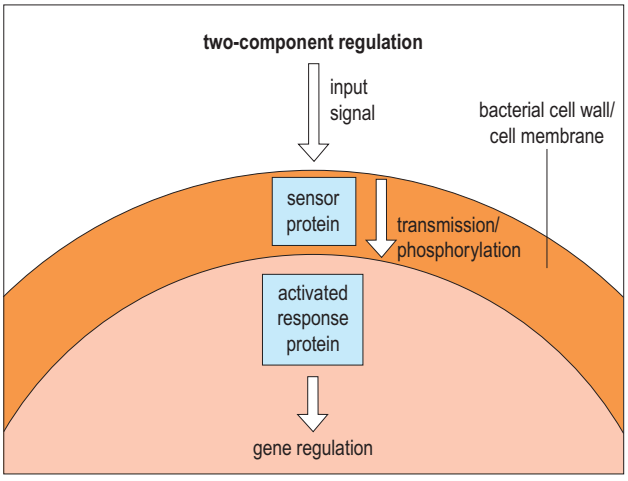


Figure 2.10 Two-component regulation is a signal transduction process that allows cellular functions to react in response to a changing environment. An appropriate environmental stimulus results in autophosphorylation of the sensor protein, which, by a phosphotransfer reaction, activates the response protein that affects gene regulation.

In *Staphylococcus aureus*, a variety of virulence genes are influenced by global regulatory systems, the best studied and most important of which is a two-component regulator termed accessory gene regulator (*agr*). Agr control is complex in that it serves as a positive regulator for exotoxins secreted late in the bacterial life cycle (post-exponential phase) but behaves as a negative regulator for virulence factors associated with the cell surface.

The control of virulence gene expression in *V. cholerae* is under the control of ToxR, a cytoplasmic membrane-located protein, which senses environmental changes. ToxR activates both the transcription of the cholera toxin operon and another regulatory protein, ToxT, which in turn activates the transcription of other virulence genes such as toxin-co-regulated pili, an essential virulence factor required for colonization of the human small intestine.

In some instances the pathogenic activity of bacteria specifically begins when cell numbers reach a certain threshold

Quorum sensing is the mechanism by which specific gene transcription is activated in response to bacterial concentration. While quorum sensing is known to occur in a wide variety of microorganisms, a classic example is the production of biofilms by *Pseudomonas aeruginosa* in the lungs of cystic fibrosis (CF) patients. The production of these tenacious substances allows *P. aeruginosa* to establish serious long-term infection in CF patients, which is difficult to treat (see Ch. 20; Fig. 20.23). As illustrated in Fig. 2.11, when quorum-sensing bacteria reach appropriate numbers, the signalling compounds they produce are at sufficient concentration to activate transcription of specific response genes such as those related to biofilm production. Current research is aimed at better understanding the quorum-sensing process in different bacterial pathogens and exploring potential therapeutic approaches (e.g. inhibitory compounds) to interfere with this coordinated mechanism of bacterial virulence.

SURVIVAL UNDER ADVERSE CONDITIONS

Some bacteria form endospores

Certain bacteria can form highly resistant spores – endospores – within their cells, and these enable them to survive adverse conditions. They are formed when the cells are unable to grow (e.g. when environmental conditions change or when nutrients are exhausted), but never by actively growing cells. The spore has a complex multilayered coat surrounding a new bacterial cell. There are many differences in composition between endospores and normal cells, notably the presence of dipicolinic acid and a high calcium content, both of which are thought to confer the endospore's extreme resistance to heat and chemicals.

Because of their resistance, spores can remain viable in a dormant state for many years, re-converting rapidly to normal existence when conditions improve. When this occurs,

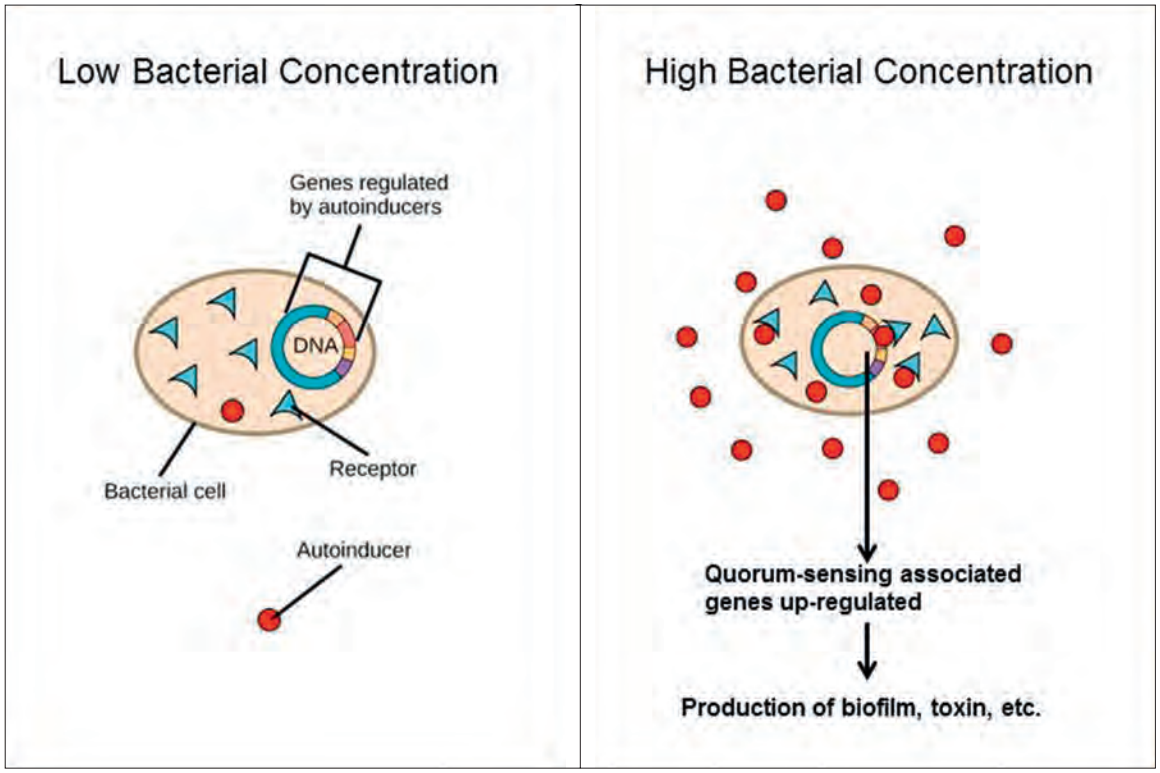


Figure 2.11 Quorum sensing bacteria produce autoinducer signaling compounds which, in sufficient concentration, bind to receptors that activate transcription of specific response genes (e.g. for biofilm production, etc.). (Adapted from https://www.boundless.com/biology/textbooks/boundless-biology-textbook/cell-communication-9/signaling-in-single-celled-organisms-86/signaling-in-bacteria-391-11617/images/fig-ch09_04_02/.)

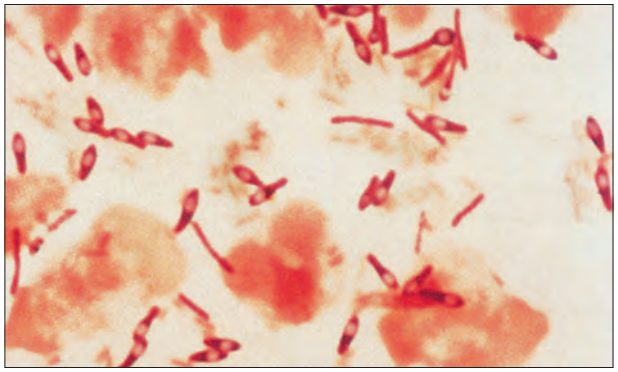


Figure 2.12 *Clostridium tetani* with terminal spores.

a new bacterial cell grows out from the spore and resumes vegetative life. Endospores are abundant in soils, and those of the *Clostridium* and *Bacillus* are a particular hazard (Fig. 2.12). Tetanus and anthrax caused by these bacteria are both associated with endospore infection of wounds, the bacteria developing from the spores once they are in appropriate conditions.

MOBILE GENETIC ELEMENTS

The bacterial chromosome represents the primary reservoir of genetic information within the cell. However, a variety of additional genetic elements may also be present which are capable of independently moving to different locations within a cell or between cells (also termed horizontal gene transfer).

Many bacteria possess small, independently replicating (extrachromosomal) nucleic acid molecules termed plasmids and bacteriophages

Plasmids are independent, self-replicating, circular units of dsDNA, some of which are relatively large (e.g. 60–120 kb) whereas others are quite small (1.5–15 kb). Plasmid replication is similar to the replication of genomic DNA, though there are differences. Not all plasmids are replicated bidirectionally – some have a single replication fork, others are replicated like a 'rolling circle'. The number of plasmids per bacterial cell (copy number) varies for different plasmids, ranging from 1 to 1000s of copies per cell. The rate of initiation of plasmid replication determines the plasmid copy number; however, larger plasmids generally tend to have lower copy numbers than smaller plasmids. Some plasmids (broad-host-range plasmids) are able to replicate in many different bacterial species; others have a more restricted host range.

Plasmids contain genes for replication, and in some cases for mediating their own transfer between bacteria (*tra* genes). Plasmids may additionally carry a wide variety of additional genes (related to the overall size of the plasmid) which can confer a variety of advantages to the host bacterial cell (e.g. antibiotic resistance, toxin production).

Widespread use of antimicrobials has applied a strong selection pressure in favour of bacteria able to resist them

In the majority of cases, resistance to antimicrobials is due to the presence of resistance genes on self-transferrable

(conjugative) plasmids (R plasmids; see Ch. 34). These are known to have existed before the era of mass antibiotic treatments, but they have become widespread in many species as a result of selection. R plasmids may carry genes for resistance to multiple antimicrobials. For example, one of the earliest-studied R plasmids, R100, confers resistance to sulphonamides, aminoglycosides, chloramphenicol, and tetracycline, and there are many others carrying genes for resistance to an even greater spectrum of antimicrobials. R plasmids can recombine, resulting in individual replicons encoding new combinations of multiple drug resistance.

Plasmids can carry virulence genes

Plasmids may encode toxins and other proteins that increase the virulence of microorganisms. For example:

- The virulent enterotoxinogenic strains of *E. coli* that cause diarrhoea produce different types of plasmid-encoded enterotoxins that alter the secretion of fluid and electrolytes by the intestinal epithelium (see Ch. 23).
- In *Staph. aureus*, both an enterotoxin and a number of enzymes involved in bacterial virulence (haemolysin, fibrinolysin) are encoded by plasmid genes.

The production of toxins by bacteria, and their pathological effects, is discussed in detail in Chapter 18.

Plasmids are valuable tools for cloning and manipulating genes

Molecular biologists have generated a wealth of recombinant plasmids to use as vectors for genetic engineering (Fig. 2.13). Plasmids can be used to transfer genes across species barriers so that defined gene products can be studied or synthesized in large quantities in different recipient organisms.

Bacteriophages are bacterial viruses that can survive outside as well as inside the bacterial cell

Bacteriophages differ from plasmids in that their reproduction usually leads to destruction of the bacterial cell. In general, bacteriophages consist of a protein coat or head (capsid), which surrounds nucleic acid which may be either DNA or RNA but not both. Some bacteriophages may also possess a tail-like structure which aids them in attaching to and infecting their bacterial host. As illustrated in Fig. 2.14 for DNA-containing bacteriophages, the virus attaches and injects its DNA into the bacterium, leaving the protective protein coat behind. Virulent bacteriophages instigate a form of molecular mutiny to commandeer cellular nucleic acid and protein to produce new virus DNA and protein. Many new virus particles (virions) are then assembled and released into the environment as the bacterial cell ruptures (lyses), thus allowing the cycle to begin again.

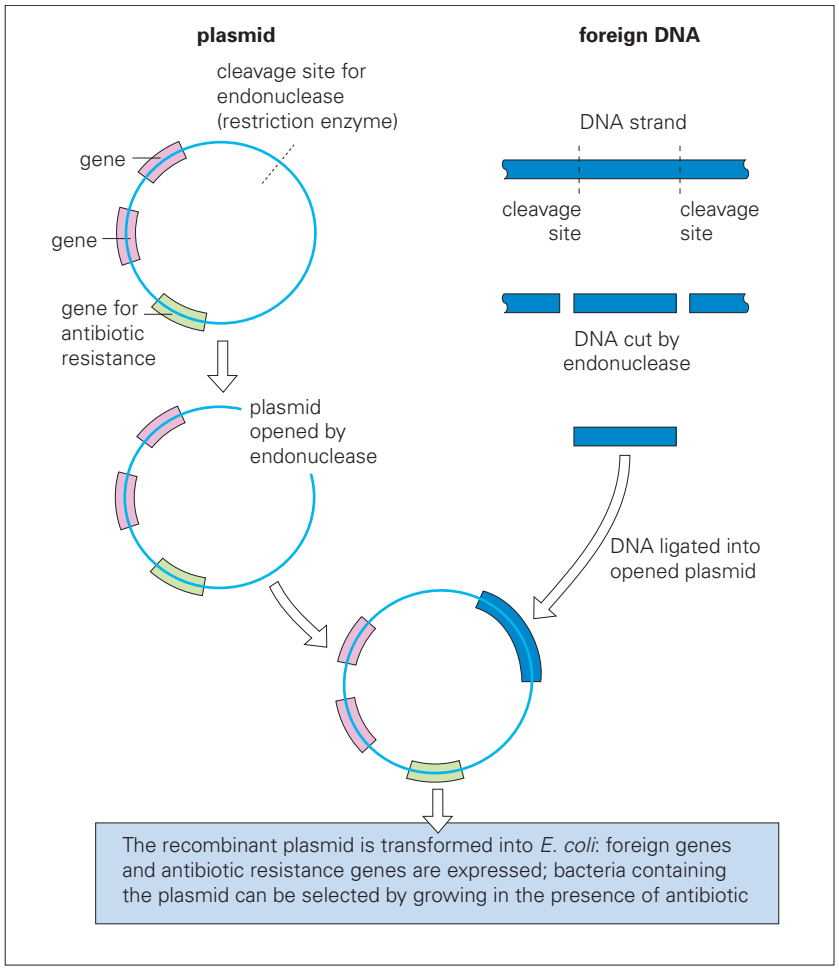


Figure 2.13 The use of plasmid vectors to introduce foreign DNA in *E. coli* – a basic step in gene cloning.

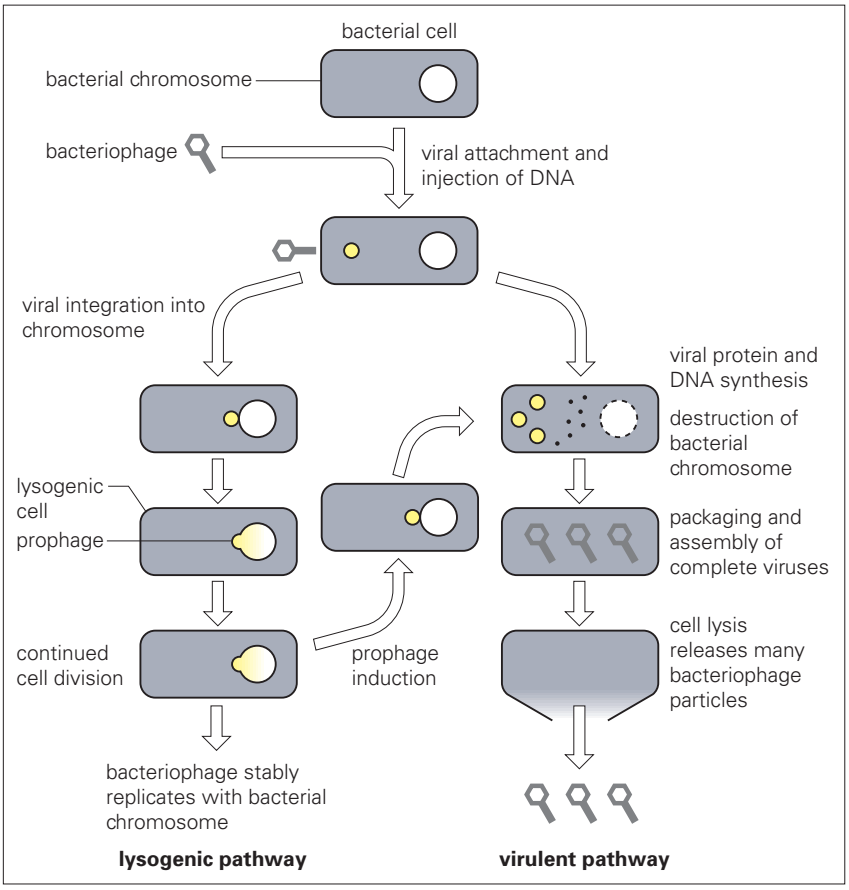


Figure 2.14 The life cycle of bacteriophages.

While destruction of the host is always the direct consequence of virulent bacteriophage infection, temperate bacteriophages may exercise a 'choice'. Following infection, they may immediately reproduce in a manner similar to their virulent counterparts. However, in some instances they may insert into the bacterial chromosome. This process, termed lysogeny, does not kill the cell as the integrated viral DNA (now called a prophage) is quiescently carried and replicated within the bacterial chromosome. New characteristics may be expressed by the cell as a result of prophage presence (prophage conversion), which, in some instances, may increase bacterial virulence (e.g. the gene for diphtheria toxin resides on a prophage). Nevertheless, this latent state is eventually destined to end, often in response to some environmental stimulus inactivating the bacteriophage repressor which normally maintains the lysogenic condition. During this induction process, the viral DNA is excised from the chromosome and proceeds to active replication and assembly, resulting in cell lysis and viral release.

Whether virulent or temperate, bacteriophage infection ultimately results in death of the host cell which, given current problems with multiple resistance, has sparked a renewed interest in their use as 'natural' antimicrobial agents. However, a variety of issues related to dosing, delivery, quality control, etc. have impeded the use of 'bacteriophage therapy' in routine clinical practice.

Transposition

Transposable elements are DNA sequences that can jump (transpose) from a site in one DNA molecule to another in a cell. This movement does not rely on host-cell (homologous) recombination pathways which require extensive similarity between the resident and incoming DNA. Instead, movement involves short target sequences in the recipient DNA molecule where recombination / insertion is directed by the mobile element (site-specific recombination).

While plasmid transfer involves the movement of genetic information between bacterial cells, transposition is the movement of such information between DNA molecules. The most extensively studied transposable elements are those found in *E. coli* and other Gram-negative bacteria, although examples are also found in Gram-positive bacteria, yeast, plants and other organisms.

Insertion sequences are the smallest and simplest 'jumping genes'

Insertion sequence elements (ISs) are generally <2 kb in length and only encode functions such as the transposase enzyme, which is required for transposition from one DNA site to another. At the ends of ISs, there are usually short inverted repeat sequences (36 nucleotides long in IS*911*), which are also important in the process of locating and inserting into a DNA target (Fig. 2.15A). During the transposition process, a portion of the target sequence is duplicated, resulting in short direct repeat sequences (the same sequence in the same orientation) on each side of the newly inserted IS element. Many aspects of the target selection process remain unclear. While adenine / thymine (A / T)-rich regions of DNA appear to be preferred, some ISs are highly selective, whereas others

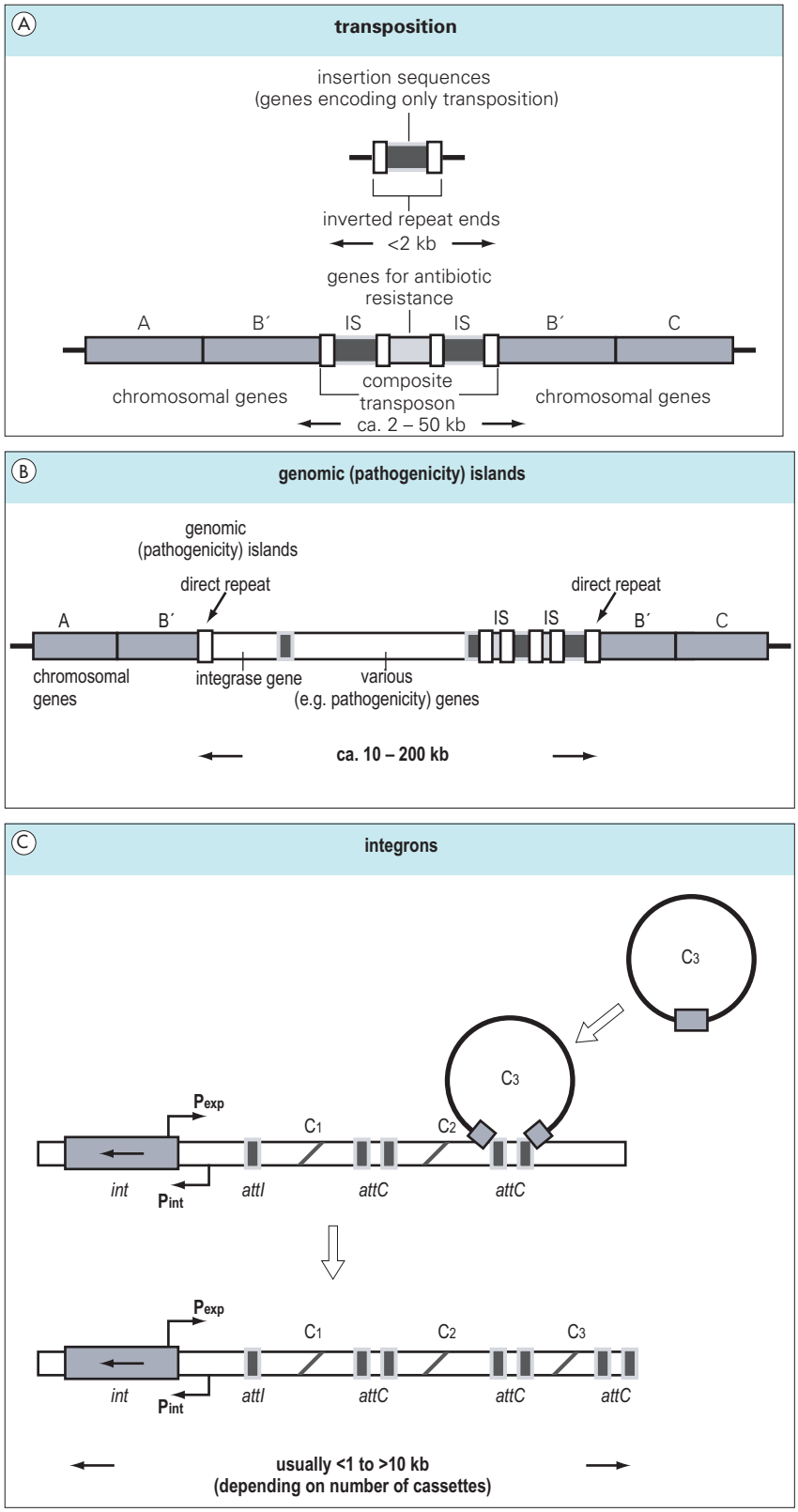


Figure 2.15 (A) Transposons (jumping genes) can move from one DNA site to another; they inactivate the recipient gene into which they insert. Transposons often contain genes which confer resistance to antibiotics. (B) Genomic islands are regions of DNA with 'signature sequences' (e.g. direct repeats) indicative of mobility. Their encoded functions increase bacterial fitness (e.g. pathogenicity). (C) Integrons are genetic regions into which independent open reading frames, also termed gene cassettes, can integrate and become functional (e.g. under control of the promoter P_{exp}). The integration process occurs by site-specific recombination between circular cassettes and their recipient integron, which is directed by an integrase gene (*intI*) with promoter P_{int} and an associated attachment site (*attI*).

are generally indiscriminate. Transposition does not rely on enzymatic processes typically used by the cell for homologous recombination (recombination between highly related DNA molecules) and is thus termed 'illegitimate recombination'. The result is a number of ISs in bacterial genomes. For example, some *E. coli* strains carry 19 copies of IS*629*, and three copies of IS*677*. Multiple IS copies serve an important function as 'portable regions of homology' throughout a bacterial genome where homologous recombination may occur between different DNA regions or molecules (e.g. chromosome and plasmid) carrying the same IS sequence. Two IS elements inserting relatively near to each other would allow the entire region to become transposable, further promoting the potential for genetic movement and exchange in bacterial populations.

Transposons are larger, more complex elements, which encode multiple genes

Transposons are >2 kb in size and contain genes in addition to those required for transposition (often encoding resistance to one or more antibiotics) (Fig. 2.15A). Furthermore, virulence genes, such as those encoding heat-stable enterotoxin from *E. coli*, have been found on transposons.

Transposons can be divided into two classes:

1. composite transposons, where two copies of an identical IS element flank antibiotic-resistance genes (kanamycin resistance in Tn5)
2. simple transposons, such as Tn3 (encoding resistance to beta-lactams).

ISs at the ends of composite transposons may be either in the same or in an inverted orientation (i.e. direct or indirect repeats). Although part of the composite transposon structure, the terminal IS elements are fully intact and capable of independent transposition.

Simple transposons move only as a single unit, containing genes for transposition and other functions (e.g. antibiotic resistance) with short, inversely oriented sequences (indirect repeats) at each end.

Mobile genetic elements promote a variety of DNA rearrangements which may have important clinical consequences

The ease with which transposons move into or out of DNA sequences means that transposition can occur:

- from host genomic DNA harbouring a transposon to a plasmid
- from one plasmid to another plasmid
- from a plasmid to genomic DNA.

Transposition onto a broad-host-range self-transferrable (conjugative) plasmid can lead to the rapid dissemination of resistance among different bacteria. The transposition process (whether by ISs or transposons) can be deleterious if insertion occurs within, and disrupts, a functional gene. However, transpositional mutagenesis has been effectively utilized in the molecular biology laboratory to produce extremely specific mutations without the harmful secondary effects often seen with more generally acting chemical mutagens.

Other mobile elements also behave as portable cassettes of genetic information

Pathogenicity islands (Fig. 2.15B) are a special class of mobile genetic elements containing groups of coordinately controlled virulence genes, often with ISs, direct repeat sequences, etc. at their ends. Though originally observed in uropathogenic *E. coli* (encoding haemolysins and pili), pathogenicity islands have now been found in a number of additional bacterial species including *H. pylori*, *V. cholerae*, *Salmonella* spp., *Staph. aureus* and *Yersinia* spp. Such regions are not found in non-pathogenic bacteria, may be quite large (up to hundreds of kilobases), and may be unstable (spontaneously lost). Differences in DNA sequence (guanine+cytosine [G+C] content) between such elements and their host genomes and the presence of transposon-like genes support speculation regarding their origin and movement from unrelated bacterial species. The term 'genomic island' has been given to DNA sequences similar to pathogenicity islands but not contributing directly to virulence or pathogenicity.

Integrons are mobile genetic elements that are able to use site-specific recombination to acquire new genes in 'cassette-like' fashion and express them in a coordinated manner (Fig. 2.15C). Integrons lack terminal repeat sequences and certain genes characteristic of transposons but, similar to transposable elements, often carry genes associated with antibiotic resistance (see Fig. 34.5).

Another important type of mobile element includes staphylococcal cassette chromosomes (SCCs) such as SCC*mec*, which not only encodes methicillin resistance but also serves as a recombinational hot spot for the acquisition of other mobile sequences. SCCs influencing virulence and antimicrobial resistance include SCC*capI* encoding capsular polysaccharide I and SCC_{476} and SCC*mercury* conferring resistance to fusidic acid and mercury, respectively. The arginine catabolic mobile element (ACME) is a cassette-like element potentially contributing to the virulence of the important USA300 community-associated methicillin-resistant *Staph. aureus* (MRSA) strain originally reported in the United States but now globally disseminated. An example of the interrelationship between the bacterial core genome and additional mobile genetic elements is depicted in Fig. 2.16.

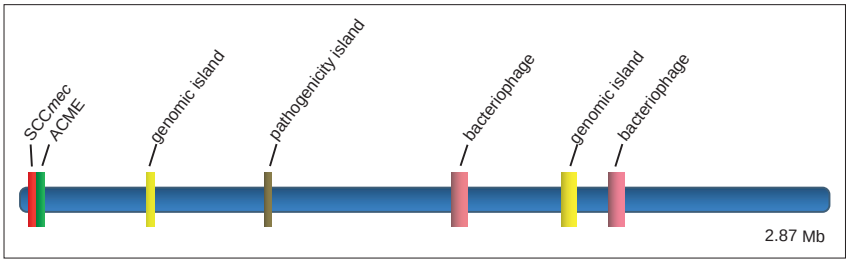


Figure 2.16 Linear depiction of the interrelationship between the USA300 MRSA core genome and key mobile genetic elements SCC*mec*, ACME, two different bacteriophages, two different genomic islands, and a pathogenicity island encoding antibiotic resistance and a variety of virulence factors.

MUTATION AND GENE TRANSFER

Bacteria are haploid organisms, their chromosomes containing one copy of each gene. Replication of the DNA is a precise process resulting in each daughter cell acquiring an exact copy of the parental genome. Changes in the genome can occur by two processes:

- mutation
- recombination.

These processes result in progeny with phenotypic characteristics that may differ from those of the parent. This is of considerable significance in terms of virulence and drug resistance.

Mutation

Changes in the nucleotide sequence of DNA can occur spontaneously or under the influence of external agents

While mutations may occur spontaneously as a result of errors in the DNA replication process, a variety of chemicals (mutagens) brings about direct changes in the DNA molecule. A classic example of such an interaction involves compounds known as nucleotide-base analogues. These agents mimic normal nucleotides during DNA synthesis but are capable of multiple pairing with a counterpart on the opposite strand. While 5-bromouracil is considered a thymine analogue, for example, it may also behave as a cytosine, thus allowing the potential for a change from T–A to C–G in a replicating DNA duplex. Other agents may cause changes by inserting (intercalating) and distorting the DNA helix or by interacting directly with nucleotide bases to alter them chemically.

Regardless of their cause, changes in DNA may generally be characterized as follows:

- Point mutations – changes in single nucleotides, which alter the triplet code. Such mutations may result in:
  - no change in the amino acid sequence of the protein encoded by the gene, because the different codons specify the same amino acid and are therefore silent mutations
  - an amino acid substitution in the translated protein (missense mutation), which may or may not alter its stability or functional properties
  - the formation of a STOP codon, causing premature termination and production of a truncated protein (nonsense mutation).
- More comprehensive changes in the DNA, which involve deletion, replacement, insertion or inversion of several or many bases. The majority of these changes are likely to harm the organism, but some may be beneficial and confer a selective advantage through the production of different proteins.

Bacterial cells are not defenceless against genetic damage

As the bacterial genome is the most fundamental molecule of identity in the cell, enzymatic machinery is in place to protect it against both spontaneously occurring and induced mutational damage. As illustrated in Fig. 2.17, these DNA repair processes include the following:

- Direct repair, which either reverses or simply removes the damage. This may be regarded as 'first-line' defence. For example, abnormally linked pyrimidine bases in DNA (pyrimidine dimers) resulting from ultraviolet radiation are directly reversed by a light-dependent enzyme through a repair process known as photoreactivation.
- Excision repair, where damage in a DNA strand is recognized by an enzymatic 'housekeeping' process and excised, followed by repair polymerization to fill the gap using the intact complementary DNA strand as a template. This is also a primary form of defence, as the goal is to correct damage before it encounters and potentially interferes with the moving DNA replication fork. Some of these housekeeping genes are also part of an inducible system (SOS repair), which is activated by the presence of DNA damage to quickly respond and effect repair.
- 'Second line' repair, which operates when DNA damage has reached a point where it is more difficult to correct. When normal DNA replication processes are blocked, permissive systems may allow the interfering damage to be inaccurately corrected, allowing errors to occur but improving the probability of cell survival. In other instances, where damage has passed the DNA replication fork, post-replication or recombinational repair processes may 'cut and paste' to construct error-free DNA from multiple copies of the sequence found in parental and daughter strands.

Bacterial DNA repair has provided a model for understanding similar, more complex processes in humans

DNA repair mechanisms appear to be present in all living organisms as a defence against environmental damage. The study of these processes in bacteria has led to an important understanding of general principles that apply to higher organisms, including issues of cancer and aging in humans. For example, several human disorders are known to be DNA-repair related, including:

- xeroderma pigmentosum, characterized by extreme sensitivity to the sun, with great risk for development of a variety of skin cancers such as basal cell carcinoma, squamous cell carcinoma and melanoma
- Cockayne syndrome, characterized by progressive neurological degeneration, growth retardation, and sun sensitivity not associated with cancer
- trichothiodystrophy, characterized by mental and growth retardation, fragile hair deficient in sulphur, and sun sensitivity not associated with cancer.

Gene transfer and recombination

New genotypes can arise when genetic material is transferred from one bacterium to another. In such instances, the newly transferred DNA is expressed when it:

- inserts into or recombines with the genome of the recipient cell
- or is on a plasmid capable of replication in the recipient without recombination.

Recombination can bring about large changes in the genetic material and, as these events usually involve functional genes, they are likely to be expressed phenotypically. DNA can be transferred from a donor cell to a recipient cell by:

- transformation
- transduction
- conjugation.

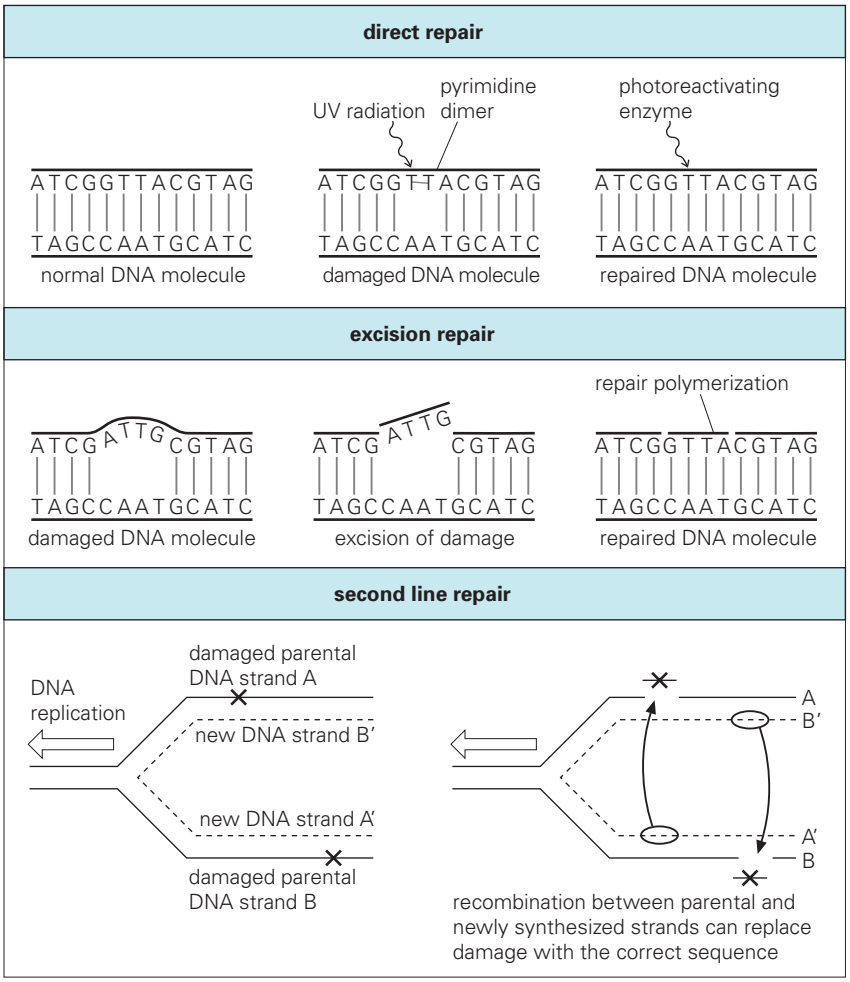


Figure 2.17 Mechanisms of DNA repair.

Transformation

Some bacteria can be transformed by DNA present in their environment

Certain bacteria such as *S. pneumoniae*, *Bacillus subtilis*, *Haemophilus influenzae* and *Neisseria gonorrhoeae* are naturally 'competent' to take up DNA fragments from related species across their cell walls. Such DNA fragments may result from lysis of organisms, the release of their DNA and its cleavage into smaller fragments, which are then available for uptake by available (competent) recipient cells. Once taken into the cell, chromosomal DNA must recombine with a homologous segment of the recipient's chromosome to be stably maintained and inherited. If the DNA is completely unrelated, the absence of homology prevents recombination and the DNA is degraded. However, plasmid DNA may be transformed into a cell and expressed without recombination. Thus, transformation has served as a powerful tool for molecular genetic analysis of bacteria (Fig. 2.18).

Most bacteria are not naturally competent to be transformed by DNA, but competence can be induced artificially by treating cells with certain bivalent cations and then subjecting them to a heat shock at 42°C or by electric shock treatment (electroporation).

Prior to uptake by competent cells, DNA is extracellular, unprotected and thus vulnerable to destruction by environmental extremes (e.g. DNA-degrading enzymes – DNases). Thus, it is the least important mechanism of gene transfer from the standpoint of clinical relevance (e.g. probability of transfer within a patient).

Transduction

Transduction involves the transfer of genetic material by infection with a bacteriophage

During the process of virulent bacteriophage replication (or temperate bacteriophages direct replication upon infection, rather than lysogeny), other DNA in the cell (genomic or plasmid) is occasionally erroneously packaged into the virus head, resulting in a 'transducing particle', which can attach to and transfer the DNA into a recipient cell. If chromosomal, the DNA must be incorporated into the recipient genome by homologous recombination to be stably inherited and expressed. As with transformation, plasmid DNA may be transduced and expressed in a recipient without recombination. In either case, this type of gene transfer is known as generalized transduction (see Fig. 2.18).

Another form of transduction occurs with 'temperate' bacteriophages, since they may integrate at specialized attachment sites in the bacterial genome. As the resulting prophages prepare to enter the lytic cycle, they occasionally incorrectly excise from the site of attachment. This can result in

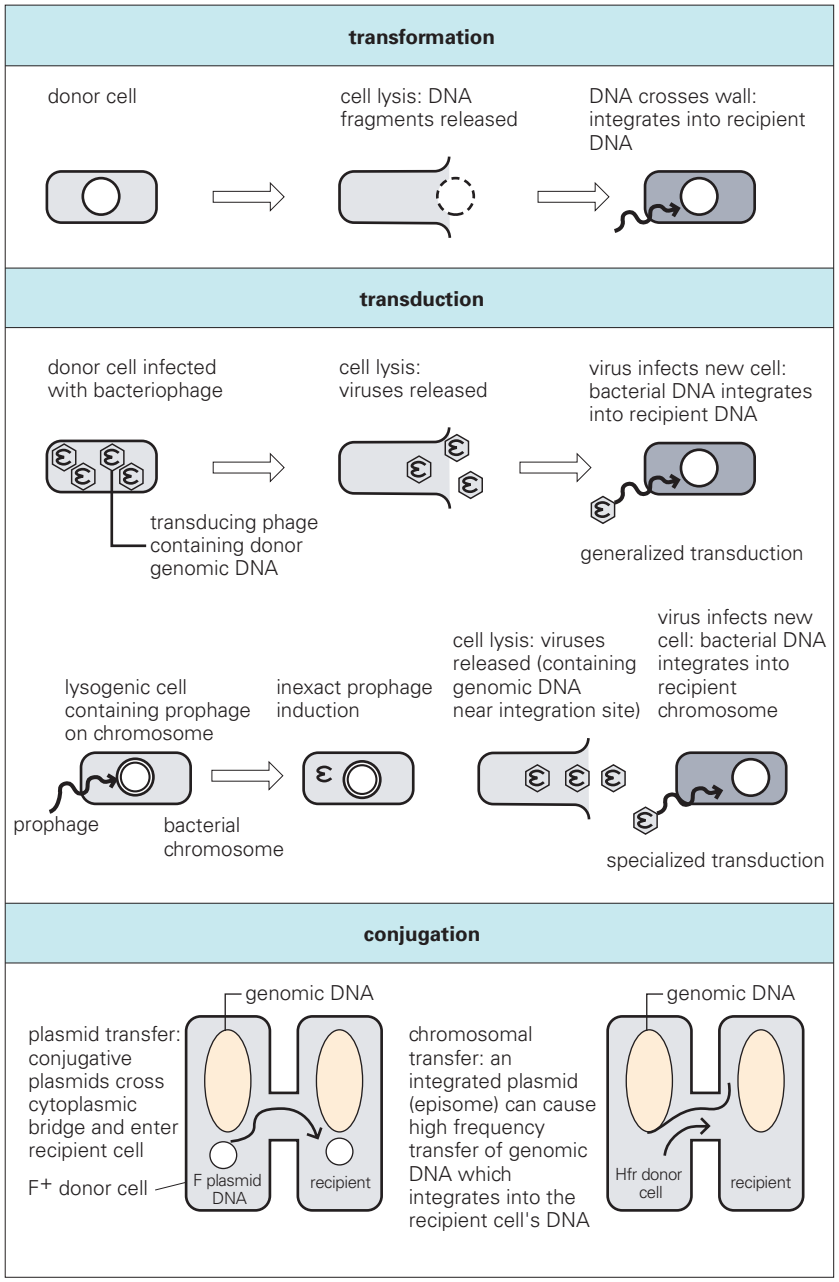


Figure 2.18 Different ways in which genes can be transferred between bacteria. With the exception of plasmid transfer, donor DNA integrates into the recipient's genome by a process of either homologous or illegitimate (in the case of transposons) recombination.

phages containing a piece of bacterial genomic DNA adjacent to the attachment site. Infection of a recipient cell then results in a high frequency of recombinants where donor DNA has recombined with the recipient genome in the vicinity of the attachment site. As this 'specialized transduction' is based on specific chromosome–prophage interaction, only genomic DNA, and not plasmids, is transferred by this process.

In contrast to transformation, transduced DNA is always protected, thus increasing its probability of successful transfer and potential clinical relevance. However, bacteriophages are extremely host-specific 'parasites' and therefore unable to move any DNA between bacteria of different species.

Conjugation

Conjugation is a type of bacterial 'mating' in which DNA is transferred from one bacterium to another

Conjugation is dependent upon the *tra* genes found in 'conjugative' plasmids, which, among other things, encode instructions for the bacterial cell to produce a sex pilus – a tube-like appendage which allows cell-to-cell contact to ensure the protected transfer of a plasmid DNA copy from a donor cell to a recipient (see Fig. 2.18). Since the *tra* genes take up genetic space, 'conjugative' plasmids are generally larger than non-conjugative ones.

Occasionally, conjugative plasmids such as the fertility plasmid (F plasmid or F factor) of *E. coli* integrate into the

bacterial genome (e.g. facilitated by identical IS elements on both molecules as noted earlier), and such integrated plasmids are called episomes. When an integrated F episome attempts conjugative transfer, the duplication-transfer process eventually moves into regions of adjacent genomic DNA, which are carried along from the donor cell into the recipient. Such strains, in contrast to cells containing the unintegrated F plasmid, mediate high-frequency transfer and recombination of genomic DNA (Hfr strains). However, conjugation with Hfr donor cells does not result in complete transfer of the integrated plasmid. Thus, the recipient cell does not become Hfr and is incapable of serving as a conjugation donor. The circular nature of the bacterial genome and the relative 'map' positions of different genes were established using interrupted mating of Hfr strains.

When a non-conjugative plasmid is present in the same cell as a conjugative plasmid, they are sometimes transferred together into the recipient cell by a process known as mobilization. Conjugative transfer of plasmids with resistance genes has been an important cause of the spread of resistance to commonly used antibiotics within and between many bacterial species, since no recombination is required for expression in the recipient. Of all the mechanisms for gene transfer, this rapid and highly efficient movement of genetic information through bacterial populations is clearly of the highest clinical relevance.

THE GENOMICS OF MEDICALLY IMPORTANT BACTERIA

Bacteria have been historically identified and characterized by phenotypic methods. However, advances in molecular biology have increasingly focused attention on analysis of the bacterial genome as it represents the ultimate source of information regarding bacterial identity, potential for pathogenicity, etc.

Various targeted approaches to the detection and utilization of genomic sequence information exist

Methods such as the polymerase chain reaction (PCR) and nucleic acid probes have clearly had a pivotal role in providing sequence-based answers to clinical microbiology questions (see Ch. 37).

- *Identification and classification.* The genes encoding ribosomal RNA (16 S, 23 S and 5 S) are typically found together in an operon where their transcription is coordinated (Fig. 2.19). This rDNA operon is found at least once and often in multiple copies distributed around the chromosome, depending on the bacterial species (*Borrelia burgdorferi* has one copy; *Clostridium difficile* may have up to 12 copies). While the rDNA operon contains many conserved sequences (identical in different bacterial species), a portion of the 16 S- and 23 S-encoding regions have been found to be species specific. In between them, an 'internally transcribed spacer' (ITS) region exhibits sequence variability that may be analysed in PCR products providing utility in differentiating closely related bacterial isolates. Such information may also allow the rapid identification, classification and epidemiology of clinically important microorganisms (see Chs. 32 and 37).
- *Resistance to antimicrobial agents.* Genes specifically mediating antimicrobial resistance are well known (see Ch. 34) and may be detected by a variety of targeted genomic approaches including PCR and probes.
- *Molecular epidemiology.* While a variety of phenotypic and genotypic methods have been employed to assess interrelationships in clinical isolates (see Ch. 37), epidemiological analysis has now moved toward sequence-based approaches. In contrast to earlier methods, sequence data are highly portable (internet transfer, etc.), less ambiguous (encoded entirely in the characters A, T, G and C, corresponding to the four bases adenine, thymine, guanine and cytosine, respectively), and easily stored in databases.

Microarrays provide a more global targeted genomic analysis

DNA microarrays are a means for the 'parallel processing' of genomic information. Traditionally, molecular biology has operated by analysing one gene in one experiment. Although yielding important information, this approach is time consuming and does not afford ready access to the information (chromosomal organization and multiple-gene interaction) contained within genomic-sequence databases. Microarrays acquire information from multiple queries simultaneously posed to a genomic-sequence database (parallel processing). DNA microarrays are based on the principles of nucleic hybridization (A pairs with T; G pairs with C). While there are a number of variations on the theme, the general format is the arrangement of samples (e.g. gene sequences) in a known matrix on a solid support (nylon, glass, etc.). Using specialized robotics, individual 'spots' may be less than 200 mm in diameter, allowing a single array (often called a DNA chip) to contain thousands of spots. Different fluorescently labelled probes of known sequence may then be simultaneously applied followed by monitoring to detect whether complementary binding has occurred.

DNA microarrays have been especially useful in the identification of mutations and studies on bacterial gene expression

In a number of instances, specific point mutations are clinically important in pathogenic bacteria. Since these changes involve only one nucleotide base they are often referred to as single nucleotide polymorphisms (SNPs). Resistance to the quinolone class of antibiotics, for example, may result from a single base change within the bacterial *gyrA* gene (see Ch. 34). In the past, such mutations have been detected by PCR amplification of

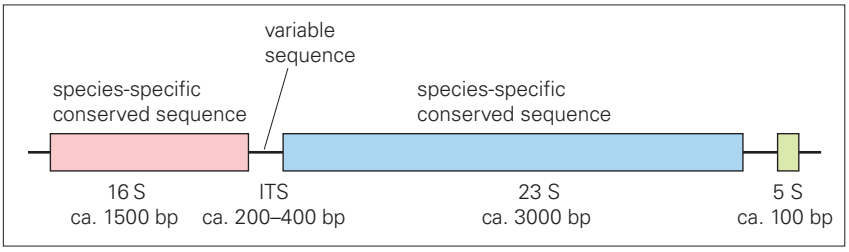


Figure 2.19 Typical arrangement of the bacterial operon encoding ribosomal RNA. Sizes of the genes for 16 S, 23 S, 5 S rRNA and the internally transcribed spacer (ITS) region are indicated in nucleotide base pairs (bp). Regions encoding sequences helpful for species identification or epidemiology are indicated.

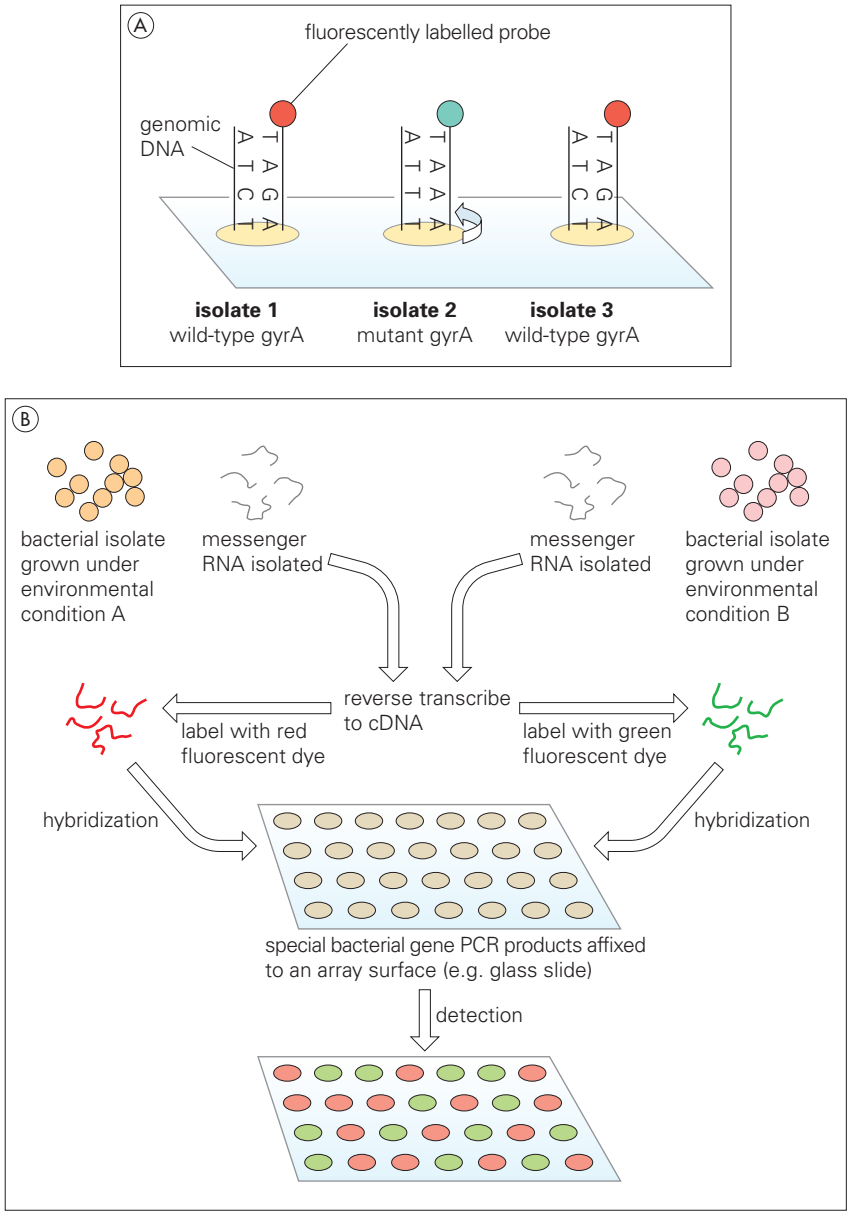


Figure 2.20 (A) Microarray detection of mutations and (B) analysis of gene expression.

the desired *gyrA* region followed by DNA sequencing and analysis. As illustrated in Fig. 2.20A, DNA microarrays allow *gyrA* amplicons from different bacterial isolates to be applied to the same chip. Two *gyrA* probes (wild type, fluorescently labelled red; mutant, fluorescently labelled green) are applied to the array under conditions so stringent that only 100% homology will result in hybridization. In this way, the presence or absence of the specific mutation may be quickly and accurately assessed in a large number of isolates simultaneously.

Studies of gene expression are extremely important to the understanding of numerous bacterial processes, including virulence. For example, analysis might involve a comparison of gene expression (transcription) in an organism under different environmental conditions (Fig. 2.20B). In such an experiment, genomics can provide data allowing sequences from every known chromosomal gene of the organism to be applied to a unique position on the chip. Messenger RNA (the result of gene expression) may be isolated from the same bacteria grown under either environmental condition A or B. Using the enzyme reverse transcriptase in a process similar to that naturally employed by retroviruses (see Ch. 3), the mRNA is copied into complementary DNA (termed cDNA). Different fluorescent dyes (red or green) are bound to the A or B cDNA, respectively, which is then allowed to hybridize to complementary sequences on the chip. Array spots with red fluorescence will indicate genes expressed in environment A. Those appearing green will correspond to genes active in environment B, while yellow spots (red+green) will indicate genes active under both conditions.

Sequence of the entire bacterial chromosome (whole genome sequencing; WGS) represents the most global approach to genomic analysis

Targeted sequence-based genomic analysis continues to be of great value in providing results rapidly and comparatively inexpensively. However, the specific nature of these assays

is also a limitation since only previously identified genomic regions can be analysed, whereas uncharacterized potentially novel genomic sequences are not detected or investigated. Conversely, WGS data encompasses both characterized and uncharacterized regions of an organism's genome, which may be re-analysed in light of new data to provide information on novel, previously untargeted, gene presence and function. Since the first complete bacterial genome sequence was published in 1995, advances in DNA-sequencing techniques have led to an ever-increasing number of bacterial pathogens for which the total genomic sequence is known (see Fig. 2.21). This evolving database represents a powerful resource with enormous application for the understanding and treatment of infectious disease.

WGS methods continue to evolve in what has been described as generational increments

However, the scientific literature is somewhat confusing on this issue. The most historically used method for sequencing individual PCR products a few hundred bases in length was developed by Frederick Sanger in 1977. Sanger sequencing is generally considered a first-generation approach. Subsequent next-generation (i.e. next-gen) approaches (more applicable to WGS) have sometimes also been described as second generation (parallel sequencing of a group of DNA molecules) or third generation (sequencing of longer single DNA molecules).

Current WGS methods have some common challenges

Current WGS approaches have three basic steps in common (although the specific details differ):

- DNA library preparation
- sequencing
- sequence analysis.

It is important to note that these steps are not fully automated or 'push button' in nature. Proper preparation of high-quality genomic DNA (i.e. the library) from the organism to be sequenced is critical for a meaningful outcome. The way in which this is accomplished depends on the requirements of the specific DNA-sequencing method. However, regardless of the approach, the ultimate end product is a computer file containing the sequence data. Thus, a computerized approach to sequence analysis is necessary, which can be visualized as having two goals:

- to construct the whole genome as accurately as possible by connecting generated sequences together (assembly)
- genetic analysis of the WGS (i.e. identification of specific genes or gene changes and other genetic 'signatures' of interest).

Depending on the instrumentation, generated sequence lengths (read lengths) may range from several hundred to tens or even hundreds of thousands of base pairs. Since this is less than the total genome size the sequence reads must be connected to produce the WGS. This is accomplished by computer programs that either identify common overlapping regions in sequence reads (de novo assembly) or connect reads together using a closely related reference genome as a template (reference mapping) (Fig. 2.22A and B, respectively). Ensuring proper quality control (e.g. sequence error rates) is very important but beyond discussion here.

The identification of specific genes, gene changes and other important genomic information from WGS data is termed bioinformatics and also involves extensive computer analysis. At the moment, this is the most challenging aspect of WGS data interpretation. However, there is an intense effort to develop user-friendly software for this purpose, which has currently resulted in an expanding list of free 'stand alone' as well as commercially available software packages dedicated to this purpose. As will be discussed more thoroughly in subsequent chapters (e.g. Ch. 37), bioinformatic analysis has demonstrated that bacterial genomes can be subdivided into core and accessory regions. The core genome represents conserved genes that are found in all members of a bacterial species, while the presence or absence of accessory genomic regions is variable. Taken together, all the core and variable sequences found in members of a bacterial species are termed the pan-genome, which is finding increasing use in identifying microorganisms present in specific (e.g. human, environmental) settings (i.e. metagenomics).

Major groups of bacteria

Detailed summaries of members of the major bacterial groups are given in the Pathogen Parade appendix available online.

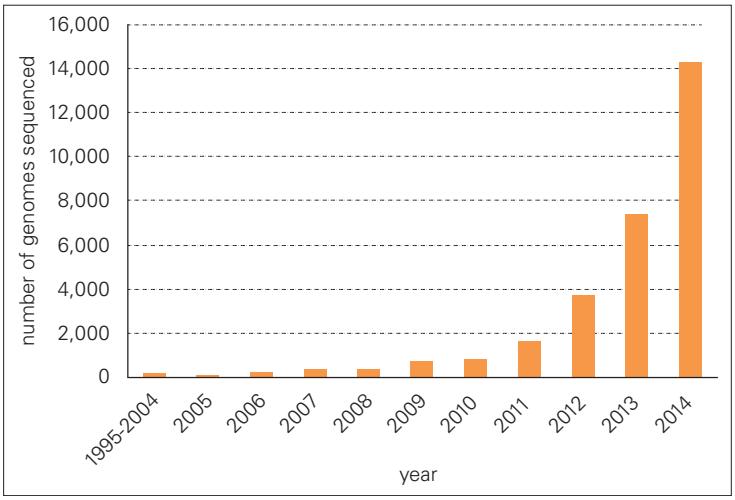


Figure 2.21 Number of sequenced bacterial and archaeal genomes submitted to NCBI. (Source: GenBank prokaryotes.txt file taken from https://www.ncbi.nlm.nih.gov/pubmed/25722247.)

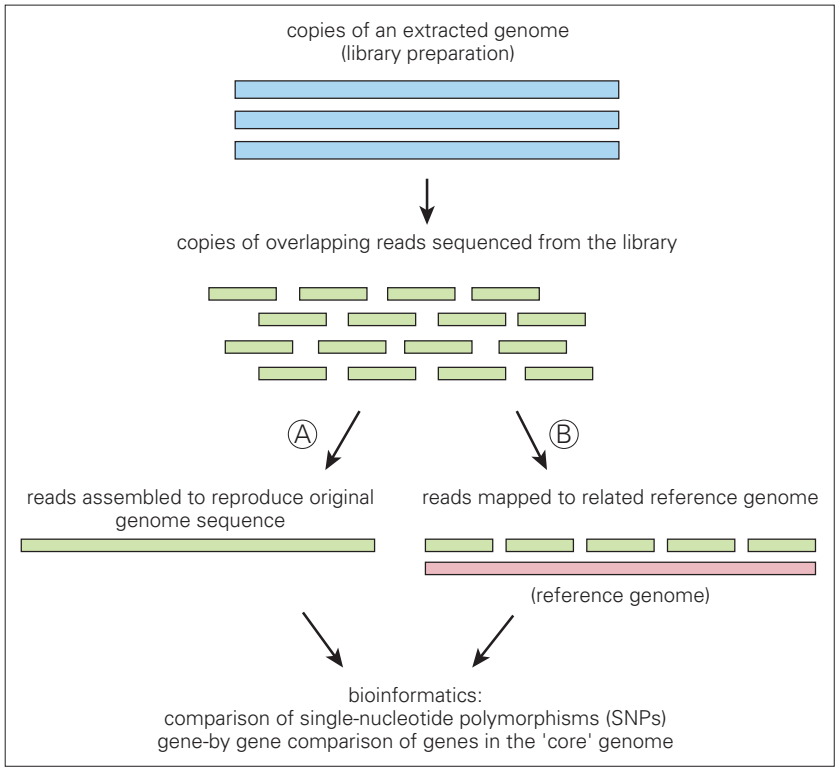


Figure 2.22 Illustration of the principal steps involved in whole genome sequence analysis. Overlapping genomic sequences (reads) are ordered and analysed either following (A) de novo sequence assembly or (B) reference mapping to a related genomic template.

KEY FACTS

- Bacteria are prokaryotes. Their DNA is not contained within a nucleus and there are relatively few cytoplasmic organelles.
- The cell wall is a key structure in metabolism, virulence and immunity. Its staining characteristics define the two major divisions: the Gram-positive and Gram-negative bacteria. Flagella may be present and confer motility.
- Bacteria metabolize aerobically and anaerobically and can utilize a range of substrates.
- The bacterial cell walls and their reproductive processes are targets for antimicrobial agents.
- Transcription of bacterial DNA may involve single or multiple genes. The arrangement of promoter and terminal sequences flanking multiple genes forms an operon.
- Bacteria can regulate gene expression to optimize exploitation of their environment.
- Plasmids and bacteriophages are independently replicating extrachromosomal agents. Plasmids may carry genes that affect resistance to antimicrobials or virulence.
- Genetic material can be carried from one bacterium to another in several ways; this can result in the rapid spread of resistance to antimicrobials.
- Genomics is revolutionizing the study of bacterial pathogenicity and the control of associated infections.

CONFLICTS

Bacteria have many ways of coming out on top in the conflict with the host. A number produce highly resistant spores that can survive for long periods in the external world, increasing the chances of infection. Once in the host there are many ways of evading host responses. For example, some hide within cells, some have external surfaces that prevent host cells binding to them, while others suppress host immunity. Perhaps the most significant advantage bacteria have in their conflict with the host is their ability to sidestep the antibiotics designed to inhibit or eliminate them. Either by mutation, facilitated by their rapid generation/duplication time, or by externally acquired genetic information they are able to engage in a game of 'cat and mouse', where repeated introduction of new and improved antimicrobial compounds is met with equally innovative mechanisms of resistance. A classic example of this interaction is seen with the Gram-positive bacterium *Staphylococcus aureus*. Although initially susceptible to penicillin, introduced in the 1950s, subsequent development and spread of resistant organisms rendered the antibiotic ineffective. This was countered with the introduction of methicillin in the 1980s leading to the development of methicillin-resistant *Staph. aureus* (MRSA), which has now been followed by isolates with resistance to the historically effective antibiotic, vancomycin. Unfortunately, a survival-of-the-fittest environment ensures the perpetuation of this conflict, underscoring the importance of the continued development of novel antimicrobial agents.

3 The viruses

Introduction

Viruses differ from all other infectious organisms in their structure and biology, particularly in their reproduction. Although viruses carry conventional genetic information in their DNA or RNA, they lack the synthetic machinery necessary for this information to be processed into new virus material. Viruses are metabolically inert and can replicate only after infecting a host cell and parasitizing the host's ability to transcribe and / or translate genetic information. Viruses infect every form of life. They cause some of the most common and many of the most serious diseases of humans, including cancer. Some insert their genetic material into the human genome and others can remain latent in different cell types and then reactivate at any time, but especially if the body is stressed or the immune system is compromised. Viruses are difficult targets for antiviral agents as it is difficult to target only those cells infected by the virus. However, many can be controlled by vaccines.

MAJOR GROUPS OF VIRUSES

The classification of viruses into major groups (families) is based on a few simple criteria (see Pathogen Parade available online). These include:

- the type of nucleic acid in the genome
- the number of nucleic acid strands and their polarity
- the mode of replication
- the size, structure and symmetry of the virus particle.

Viruses share some common structural features

Viruses range from very small, (parvovirus, from the Latin *parvo* meaning small, at 18–26 nm in diameter) to quite large (vaccinia virus, at 400 nm, is as big as small bacteria). Their organization varies considerably between the different groups, but there are some general characteristics common to all:

- The genetic material, in the form of single-stranded (ss) or double-stranded (ds), linear or circular RNA or DNA, is contained within a coat or capsid, made up of a number of individual protein molecules (capsomeres).
- The complete unit of nucleic acid and capsid is called the 'nucleocapsid', and often has a distinctive symmetry depending upon the ways in which the individual capsomeres are assembled (Fig. 3.1). Symmetry can be icosahedral, helical or complex.
- In many cases, the entire virus particle or 'virion' consists only of a nucleocapsid. In others, the virion consists of the nucleocapsid surrounded by an outer envelope or membrane (Fig. 3.2). This is generally a lipid bilayer of host cell origin, into which virus proteins and glycoproteins are inserted.

The outer surface of the virus particle is the part that first makes contact with the membrane of the host cell

The structure and properties of the outer surface of the virus particle are therefore of vital importance in understanding the process of infection. In general, naked (envelope-free)

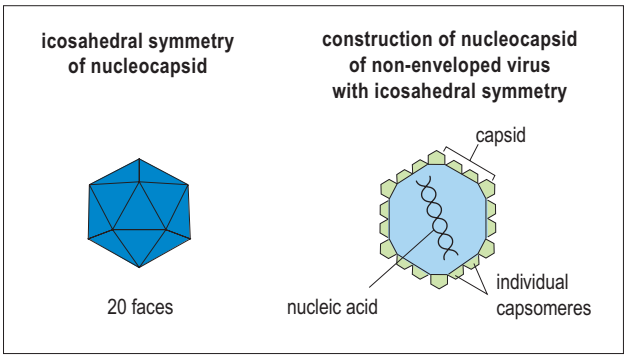


Figure 3.1 Symmetry and construction of the viral nucleocapsid.

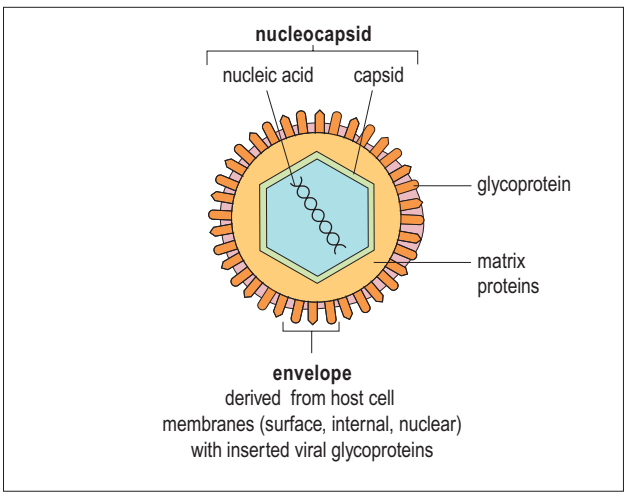


Figure 3.2 Construction of an enveloped virus.

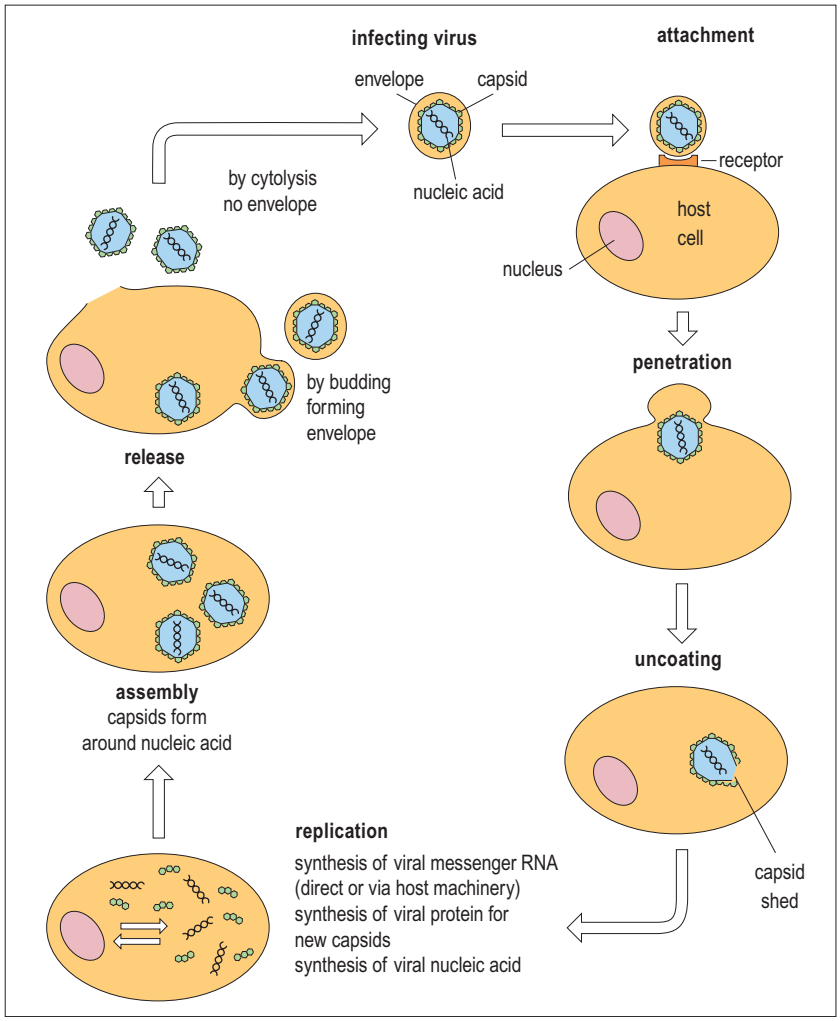


Figure 3.3 Stages in the infection of a host's cell and replication of a virus. Several thousand virus particles may be formed from each cell.

viruses are resistant and survive well in the outside world; they may also be acid and bile resistant, allowing infection through the gastrointestinal tract. Enveloped viruses are more susceptible to environmental factors such as drying, gastric acidity and bile. These differences in susceptibility influence the ways in which these viruses can be transmitted.

INFECTION OF HOST CELLS

The stages involved in infection of host cells are summarized in Fig. 3.3 (see also Fig. 2.6).

Virus particles enter the body of the host in many ways

The most common forms of virus transmission (Fig. 3.4; see also Ch. 13) are:

- via inhaled droplets (e.g. rhinovirus, influenza viruses, MERS coronavirus)
- in food or water (e.g. hepatitis A virus, hepatitis E virus, noroviruses)
- by direct transfer from other infected hosts such as infected body fluids by sexual transmission or blood-borne routes (e.g. HIV, hepatitis B virus, Ebola virus)
- from bites of vector arthropods (e.g. yellow fever virus, West Nile virus, Zika virus).

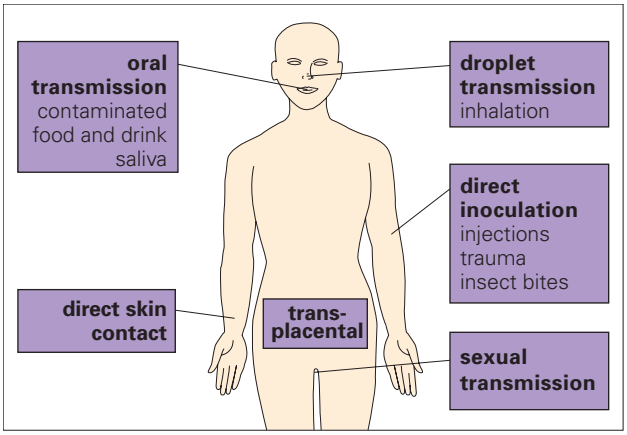


Figure 3.4 Routes by which viruses enter the body.

Viruses show host specificity and usually infect only one or a restricted range of host species. The initial basis of specificity is the ability of the virus particle to attach to the host cell

The process of attachment to, or adsorption by, a host cell depends on general intermolecular forces, then on more

Table 3.1 Viruses may use more than one receptor to gain entry into the host cell

Cell membrane receptors for virus attachment	
Virus	**Receptor molecule**
Influenza	Sialic acid receptor on lung epithelial cells and upper respiratory tract
Rabies	Acetylcholine receptor Neuronal cell adhesion molecule
HIV	CD4: Primary receptor CCR5 or CXCR4: chemokine receptors
Epstein–Barr virus	CD21 (also called CR2) receptor on B cells
Human parvovirus B19	P antigen on erythoid progenitor cells Ku80 antoantigen and $\alpha 5\beta 1$ integrin proposed co-receptors
Hepatitis C virus	Scavenger receptor class B, CD81, claudin-1, occluding and very-low-density lipoprotein receptors are host co-factors for viral entry
Human rhinoviruses A and B	Intercellular adhesion molecule 1 (ICAM-1) Low-density lipoprotein receptor (LDL-R)
Human rhinovirus C	Cadherin-related family member 3 (CDHR3) – a cell surface protein involved in cell communication

specific interactions between the molecules of the nucleocapsid in unenveloped viruses, or the virus membrane in enveloped viruses, and the molecules of the host cell membrane. In many cases, there is a specific interaction with a particular host molecule, which therefore acts as a receptor. Influenza virus, for example, attaches by its haemagglutinin to a glycoprotein (sialic acid) found on cells of mucous membranes and on red blood cells; other examples are given in Table 3.1. Attachment to the receptor is followed by entry into the host cell.

Once in the host's cytoplasm the virus is no longer infective

After fusion of viral and host membranes, or uptake into a phagosome, the virus particle is carried into the cytoplasm across the plasma membrane. At this stage, the envelope and / or the capsid are shed and the viral nucleic acid released. The virus is now no longer infective: this 'eclipse phase' persists until new complete virus particles reform after replication. The way in which replication occurs is determined by the nature of the nucleic acid concerned.

REPLICATION

Viruses must first synthesize messenger RNA (mRNA)

Viruses contain either DNA or RNA, never both. The nucleic acids are present as single or double strands in a linear (DNA or RNA) or circular (DNA) form. The viral genome may be carried on a single molecule of nucleic acid or on several molecules. With these options, it is not surprising that the process of replication in the host cell is also diverse. In viruses containing DNA, mRNA can be formed using the host's own RNA polymerase to transcribe directly from the viral DNA. The RNA of viruses cannot be transcribed in this way, as host polymerases do not work from RNA. If transcription is necessary, the virus must provide its own polymerases. These may be carried in the nucleocapsid or may be synthesized after infection.

RNA viruses produce mRNA by several different routes

In dsRNA viruses, one strand is first transcribed by viral polymerase into mRNA (Fig. 3.5). In ssRNA viruses, there are three distinct routes to the formation of mRNA:

1. Where the single strand has the positive (+) sense configuration, meaning it has the same base sequence as that required for translation, it can be used directly as mRNA.
2. Where the strand has the negative (–) sense configuration, it must first be transcribed using viral polymerase into a positive sense strand, which can then act as mRNA.
3. Retroviruses follow a completely different route. Their positive sense ssRNA is first made into a negative sense ssDNA, using the viral reverse transcriptase enzyme carried in the nucleocapsid, and dsDNA is then formed, which enters the nucleus and becomes integrated into the host genome. This integrated viral DNA is then transcribed by host polymerase into mRNA.

Viral mRNA is then translated in the host cytoplasm to produce viral proteins

Once viral mRNA has been formed, it is translated using host ribosomes to synthesize viral proteins (Fig. 3.6). Viral mRNA, which is usually 'monocistronic' (i.e. has a single coding region) can displace host mRNA from ribosomes so that viral products are synthesized preferentially. In the early phase, the proteins produced are enzymes (regulatory molecules) that will allow subsequent replication of viral nucleic acids; in the later phase, the proteins necessary for capsid formation are produced.

In viruses where the genome is a single nucleic acid molecule, translation produces a large multifunctional protein, a polyprotein, which is then cleaved enzymatically to produce a number of distinct proteins. In viruses where the genome is distributed over a number of molecules, several mRNAs are produced, each being translated into separate proteins. After translation, the proteins may be glycosylated, again using host enzymes.

Viruses must also replicate their nucleic acid

In addition to producing molecules for the formation of new capsids, the virus must replicate its nucleic acid to provide genetic material for packaging into these capsids. In positive sense ssRNA viruses such as poliovirus, a polymerase translated from viral mRNA produces negative sense RNA from the positive sense template, which is then transcribed repeatedly into more positive strands. Further cycles of transcription then occur, resulting in the production of very

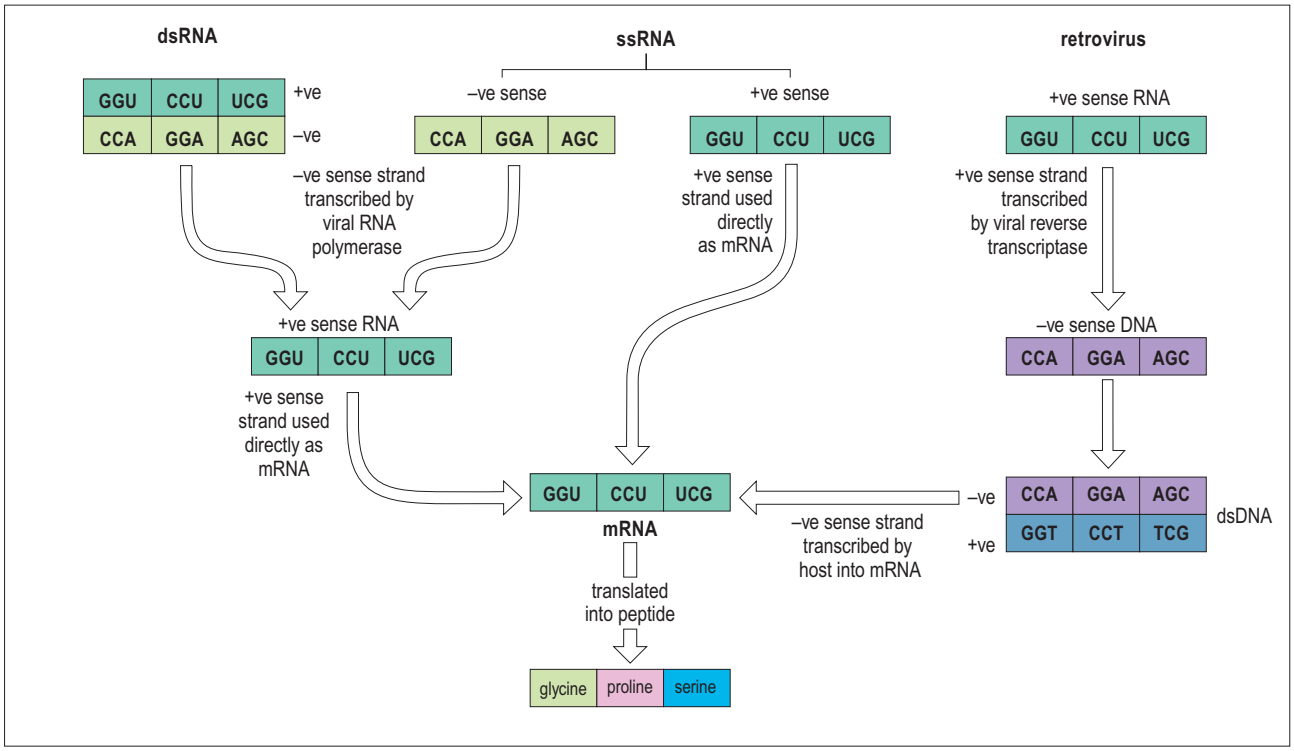


Figure 3.5 Ways in which genomic RNA of RNA viruses can be transcribed into messenger RNA (mRNA) before translation into proteins. +ve, positive sense; –ve, negative sense; ds, double stranded; ss, single stranded.

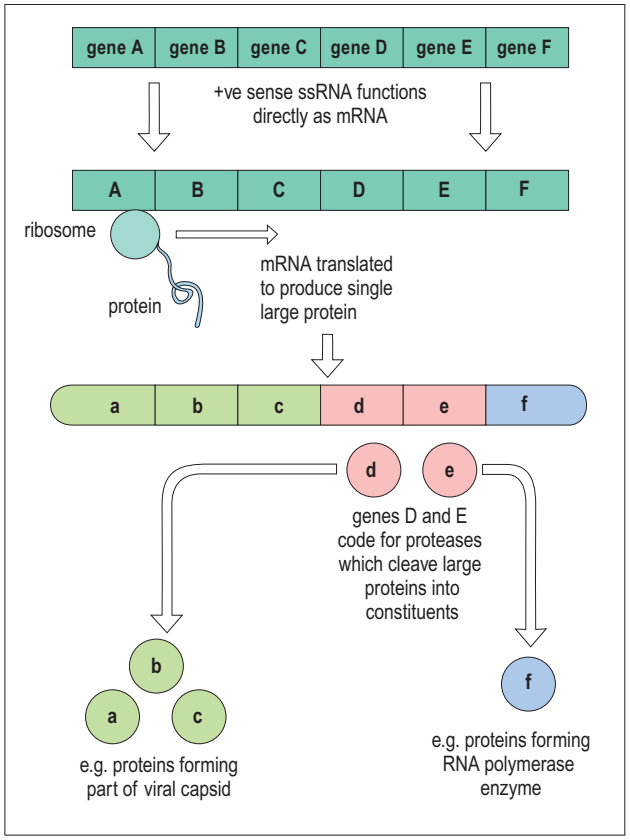


Figure 3.6 Translation and cleavage of viral proteins from messenger RNA (mRNA). +ve, positive sense; ss, single stranded.

large numbers of positive strands, which are packaged into new particles using structural proteins translated earlier from mRNA (Fig. 3.7).

In negative sense ssRNA viruses, such as rabies virus, transcription by viral polymerase produces positive sense RNA strands from which new negative sense RNA is produced (Fig. 3.7). In the rabies virus this replication occurs in the host cell cytoplasm, but in others, such as measles and influenza virus, replication takes place within the nucleus – large numbers of negative sense RNA molecules being transcribed for new particles.

Nucleic acid replication follows a similar pattern in dsRNA viruses such as rotavirus, in that positive sense RNA strands are produced. These then act as templates in a subviral particle for the synthesis of new negative sense strands to restore the double-stranded condition.

Replication of viral DNA occurs in the host nucleus – except for poxviruses, where it takes place in the cytoplasm

Viral DNA may become complexed with host histones to produce stable structures. With herpesviruses, mRNA translated in the cytoplasm produces a DNA polymerase that is necessary for the synthesis of new viral DNA; adenoviruses use both viral and host enzymes for this purpose. With retroviruses (e.g. HIV), synthesis of new viral RNA occurs in the nucleus, host RNA polymerase transcribing from the viral DNA that has become integrated into the host genome (see Fig. 3.5). Hepatitis B virus, a partially dsDNA virus, is unique in using an ssRNA intermediate transcribed from its DNA in order to synthesize new DNA. Retroviruses and

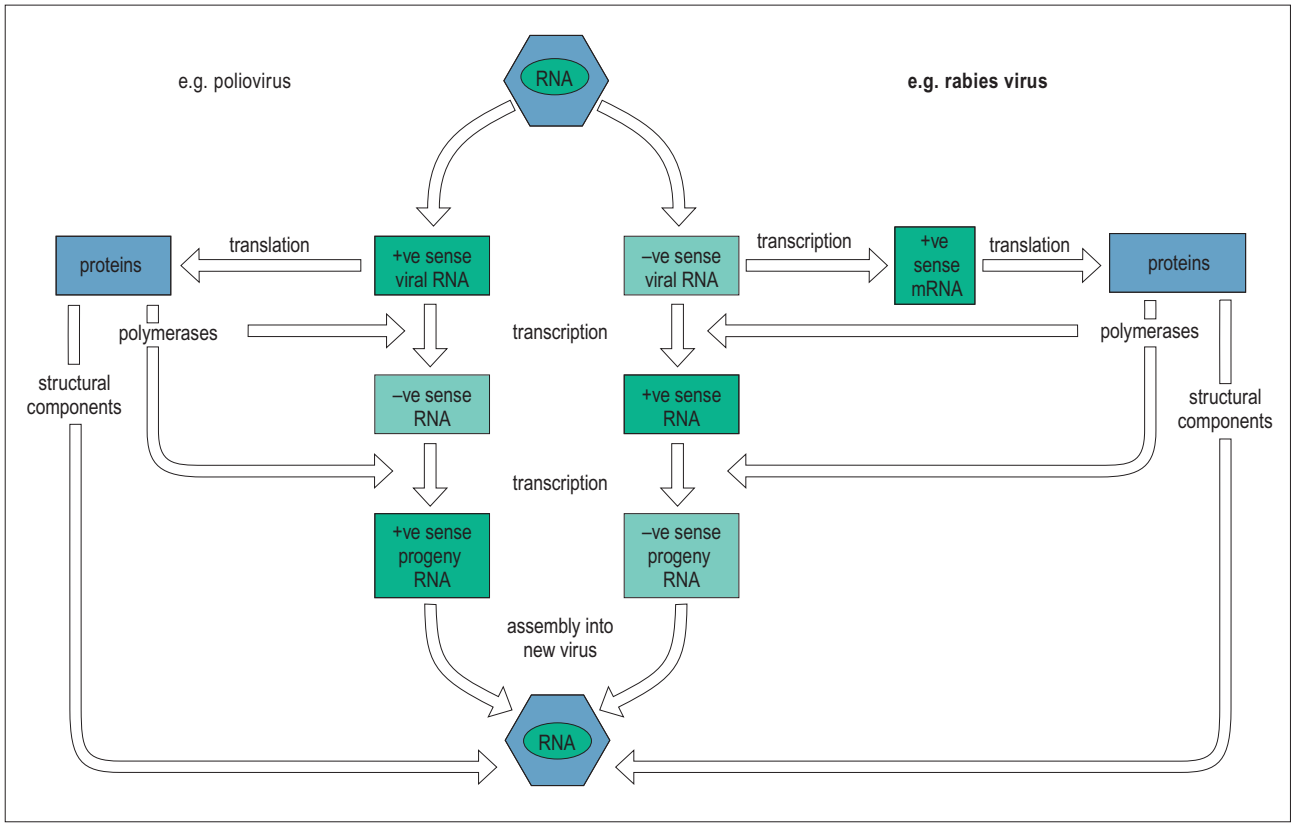


Figure 3.7 The ways in which genomic RNA of RNA viruses is replicated. +ve, positive sense; –ve, negative sense; mRNA, messenger RNA.

hepatitis B are the only viruses affecting humans that have reverse transcriptase activity.

The final stage of replication is assembly and release of new virus particles

Assembly of virus particles involves the association of replicated nucleic acid with newly synthesized capsomeres to form a new nucleocapsid. This may take place in the cytoplasm or in the nucleus of the host cell. Enveloped viruses go through a further stage before release. Envelope proteins and glycoproteins, translated from viral mRNA, are inserted into areas of the host cell membrane (usually the plasma membrane). The progeny nucleocapsids associate specifically with the membrane in these areas, via the glycoproteins, and bud through it (Fig. 3.8). The new virus acquires the host cell membrane plus viral molecules as an outer envelope, and viral enzymes, such as the neuraminidase of influenza virus, may assist in this process (see details for influenza virus in Ch. 20). Host enzymes (e.g. cellular proteases) may cleave the initial large envelope proteins, a process that is necessary if the progeny viruses are to be fully infectious. In herpesviruses, acquisition of a membrane occurs as the nucleocapsids bud from the inner nuclear membrane. Release of enveloped viruses can occur without causing cell death so that infected cells continue to shed virus particles for long periods.

Insertion of viral molecules into the host cell membrane results in the host cell becoming antigenically different. Expression of viral antigens in this way is a major factor in the development of antiviral immune responses.

OUTCOME OF VIRAL INFECTION

Viral infections may cause cell lysis or be persistent or latent

In lytic infections, the virus goes through a cycle of replication, producing many new virus particles. These are released by cell lysis. This host cell destruction is the typical consequence of infection with polio or influenza viruses. With other infections, such as hepatitis B, the cell may remain alive and continue to release virus particles at a slow rate. These 'persistent' infections are of great epidemiological importance, as the infected person may act as a symptomless carrier of the virus, providing a continuing source of infection (see Ch. 17). In both lytic and persistent infections, the virus undergoes replication. However, in latent infections, the virus remains quiescent, and the genetic material of the virus may:

- exist in the host cell cytoplasm (e.g. herpesvirus)
- be incorporated into the genome (retroviruses, hepatitis B virus)

Replication does not take place until some signal triggers a release from latency. The stimuli that result in release are not fully understood in all cases. In herpes simplex infection, stress can activate the virus, resulting in an active infection seen as cold sores.

Some viruses can 'transform' the host cell into a tumour or cancer cell

Lytic, persistent and latent infections involve essentially normal host cells, although cellular metabolic and regulatory

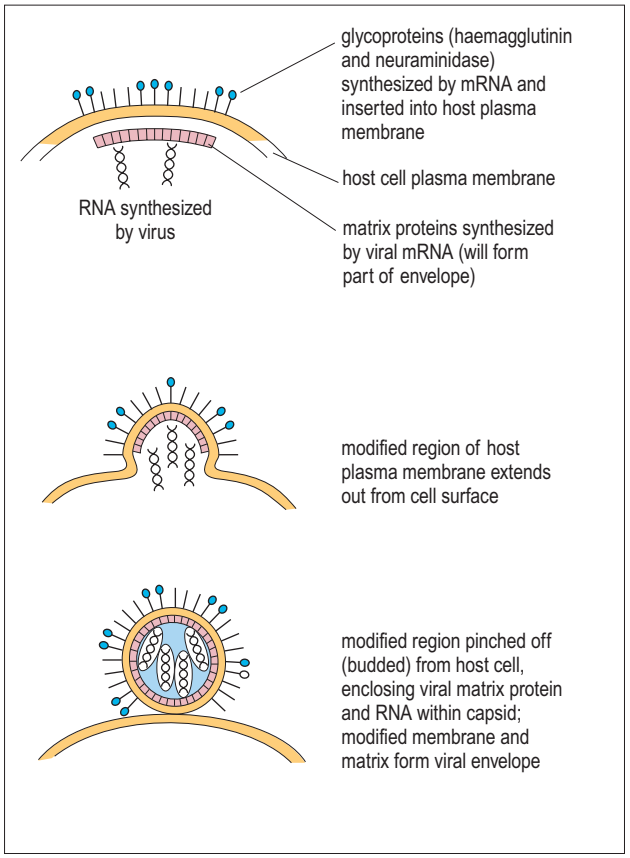


Figure 3.8 Release of enveloped RNA virus by budding through host cell membrane. Influenza A virus is shown in this example.

processes can be severely disrupted. Some viruses, however, can 'transform' the host cell, malignant transformation being the change of a differentiated host cell into a tumour or cancer cell (see Ch. 18). Transformed cells show changes in morphology, behaviour and biochemistry. Controlled growth patterns and contact inhibition are lost, so that cells continue to divide and form random aggregations. They become invasive and can form tumours if injected into animals. However, not all transformed cells give rise to harmful tumours in vivo. Warts, for example, may be benign growths on the skin of the hands or feet caused by one group of papillomaviruses, or genital warts caused by a different group of specific papillomaviruses may lead to cervical cancer.

Cancer-inducing (oncogenic) viruses are found in several different groups including both DNA and RNA viruses. Of the seven oncogenic viruses affecting humans, which cause 15% of cancers, hepatitis B virus, human papillomaviruses, Merkel cell virus and human T-cell lymphotropic virus type 1 (HTLV-1) integrate and are therefore part of the host genome. Those that do not integrate are Epstein–Barr virus (EBV), hepatitis C virus (HCV) and human herpesvirus 8 (HHV-8).

Cell proliferation is helped by genes called proto-oncogenes. If these change, for example a viral integration event occurs, the cell can become activated continuously. The changed gene, referred to as an oncogene, causes cell overproliferation and can lead to cancer.

Although the end results of transformation may be similar, the mechanisms involved vary between different viruses. It is a multiple step model and the end results of cell transformation are similar, but the mechanisms used by these different viruses are diverse and include inflammation, induced expression of viral and cellular oncogenes and epigenetic changes. High throughput, whole genome sequencing has allowed host–pathogen sequence analysis identifying links between viral integration and changes in gene expression. These techniques demonstrated viral sequences in tumour genomes that, in 2008, resulted in detecting the Merkel cell virus causing Merkel cell carcinoma.

The mechanisms all involve interference with the normal regulation of division and response to external growth-promoting and growth-inhibiting factors. These epigenetic and genetic changes come about after viral nucleic acid is incorporated into the host genome. Finally, cancer is not always the result of some of these infections. Papillomaviruses are present in cervical cancer but additional cellular events are needed for most of the other viral infections to result in tumours.

A classic example is the Rous sarcoma virus, a retrovirus that causes cancer in chickens. 2011 was the hundredth anniversary of Francis Rous demonstrating that this chest tumour could be transmitted by giving tumour extracts that were cell free to chickens related to the same brood. Transformation arises from the introduction into the host genome of a viral oncogene, v-*src*. This codes for an activated and overexpressed protein – tyrosine kinase, an enzyme involved in the phosphorylation of tyrosine residues in target proteins. This leads to some molecular events and changes in phenotype in transformed host cells and subsequent tumorigenesis as a result of the viral infection. A urokinase-type plasminogen activator (PLAU) gene is induced by v-*src* and highly up-regulated. PLAU is a protease enzyme that lyses fibrin and breaks down the extracellular matrix, promoting cancer cell stickiness and spread.

The first human tumour virus was discovered in 1964 when Epstein–Barr virus (EBV) was found by electron microscopic analysis of cells of a tumour called Burkitt's lymphoma seen in African patients.

More than 20 retroviral oncogenes are now known (Table 3.2). Of the retrovirus family, the human T-cell lymphotropic virus (HTLV type 1) is a cancer-causing virus in humans despite neither possessing a viral oncogene nor directly activating a cellular oncogene (see below). In contrast, HIV type 1 and 2 virus infections compromise the host's immune system, resulting in tumours associated with other viruses including EBV and Kaposi's sarcoma herpesvirus (KSHV) also known as human herpesvirus type 8 (HHV-8). A larger number of retroviruses cause cancers in animals.

Tumour formation as a result of viral infection: direct and indirect mechanisms

Viruses associated with cancer may do so by direct means, by expressing viral oncogenes that transform the cell as mentioned above. They may also do so indirectly by chronically infecting the cells resulting in inflammation and mutations that result in tumour formation. An example is hepatitis B that activates cell signalling pathways via the HBx oncoprotein.

Viral oncogenes have probably arisen from incorporation of host oncogenes into the viral genome during viral replication

Oncogenes are designated by short acronyms, preceded by 'v' if a viral oncogene is described (e.g. v-*myc*) or by 'c' for

Table 3.2 Oncogenes, gene products, viruses known to carry them and associated human and animal diseases

Examples of retroviral oncogenes			
Class of gene product	**Oncogene**	**Virus**	**Disease**
Tyrosine kinases	*fms* *ros* *src* *yes*	FeLV ALV ALV ALV	Sarcoma
Serine / kinase threonine	*mos*	MuLV	Sarcoma
Examples of human oncogenic viruses			
	HBx	Hepatitis B virus	Hepatocellular carcinoma
	LMP-1, BARF-1	Epstein–Barr virus	Burkitt's lymphoma, B-cell lymphoma, nasopharyngeal carcinoma
	vGPCR	Human herpesvirus 8	Kaposi's sarcoma, primary effusion lymphoma
	E6, E7	Human papillomavirus	Cervical, anal and oral cancer
	T antigens	Merkel cell polyomavirus	Merkel cell carcinoma
	Tax	Human T-cell leukaemia lymphoma virus	Adult T-cell leukaemia / lymphoma

ALV / FeLV / MuLV, avian, feline and murine leukaemia viruses; GTP, guanosine triphosphate; HBx, hepatitis B x gene; LMP-1, latent membrane protein-1; vGPCR, virus G protein-coupled receptor.

a cellular (host) oncogene (e.g. c-*myc*). In HPV infection, recurrent integration of the HPV DNA in upstream regions of c-*myc* causes c-*myc* up-regulation, resulting in proliferation and immortalization of cells. This recurrent integration occurs in other oncogenes which have similar functions, such as *NOTCH1* and *ERBB2* and tumour suppressor genes too. The integration events may cause instability of the genome and resultant changes in gene expression.

Retroviral oncogene sequences can make up as much as 0.03–0.3% of the mammalian genome. Oncogene sequences have been identified in a wide variety of animals, from humans to fruit flies, implying that they are conserved because of some valuable function. Which came first, host or viral oncogenes? The fact that host oncogenes contain introns, whereas viral oncogenes do not, and that their chromosomal positions are fixed, implies that they, and not the viral forms, are the original genes.

From what we now know about the gene products of viral oncogenes, we can guess that cellular oncogenes (or 'proto-oncogenes') probably play an important role in host cell growth regulation. They may code for growth factors themselves, for cell surface receptor molecules that bind specific growth factors, for components of intracellular signalling systems, or for DNA-binding proteins that act as transcription factors.

The Rous sarcoma virus *src* oncogene is incorporated within the viral genome adjacent to the gene coding for viral envelope proteins (Fig. 3.9). Unlike other strongly transforming viruses, the Rous virus has all three genes (*gag*, *pol* and *env*) necessary for replication. In the others, termed 'defective' transforming viruses, incorporation of an oncogene results in deletion of genetic material in the regions coding for the *pol* and / or *env* genes, so preventing replication. This becomes possible only with help from genetically complete helper viruses.

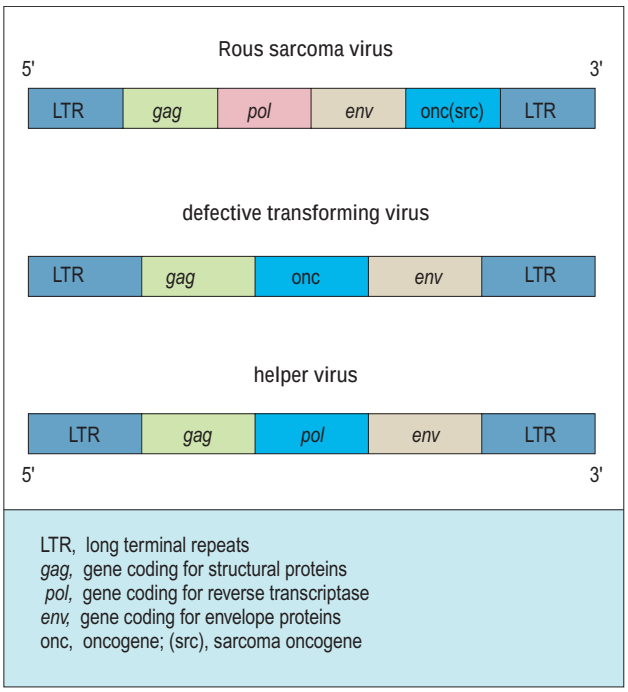


Figure 3.9 Rous sarcoma virus can transform the host cell and replicate because it has both the oncogene *src* and a complete genome. Some transforming viruses are defective – they carry the oncogene, but lack genes for full replication. Helper viruses can supply these genes.

Oncogenes can be carried from one cell to another within the same host or from one host to another. This can occur through 'vertical' transmission, from mother to offspring, through passage of viruses in gametes, across the placenta or in milk. It can also occur by 'horizontal' transmission, the virus passing in, for example, saliva or urine (see Ch. 14).

Transformation of a cell occurs:

- when viral oncogenes are incorporated into the host genome (as in Rous sarcoma virus)
- when viral DNA is inserted near to a cellular oncogene

The former may be due to mutations in the oncogene sequence while in the viral genome; single base changes in cellular oncogenes are known to confer the ability to transform normal cells. The latter may reflect altered expression of the host oncogene through disturbance of normal regulatory influences. Altered expression can occur whether the insertion is of a retroviral oncogene or of non-oncogenic viral DNA; it can also occur as a result of exposure to a variety of carcinogens. The products of cellular oncogenes are normally used in series to regulate cellular proliferation in a carefully controlled manner. Viral oncogene products or overexpressed cellular oncogene products short circuit and overload this complex control system, resulting in unregulated cell division.

KEY FACTS

- Viruses have RNA or DNA but absolutely depend on the host to process their genetic information into new virus particles.
- The outer surface of a virus (capsid or envelope) is essential for host cell contact and entry, and determines the capacity to survive in the outside world.
- Viruses can be transmitted in droplets, in food and water or by intimate contact.
- Replication of viral RNA or DNA is a complex process, making use of host and/or viral enzymes.
- RNA of retroviruses becomes integrated into the host genome.
- New virus particles are released by cell lysis or by budding through the host cell membrane.
- Some viruses, such as herpesviruses, may become latent and require a trigger to resume replication; others replicate at a slow rate, persisting as a source of infection in symptomless carriers.
- A number of viruses transform the host cell, by interfering with normal cellular regulation, resulting in the development of a cancer cell. This may be the result of the activity of viral or cellular oncogenes.

CONFLICTS

Viruses have developed a cunning strategy as hardy infectious agents as, once they have infected the host cell, they may lie latent or integrate within the host cell chromosome and reactivate, potentially transmitting the infection to others. The host may not be too incapacitated, ensuring they can infect those susceptible. In addition, the host has to have a full immunosurveillance repertoire to suppress all these viruses waiting to step up to the plate. Once the defences are lowered by stress, immunosuppression or trauma, for example, active viral replication can occur.

Viruses may have a number of options with respect to receptors they can attach to and subsequently infect the host. They may be able to cross species barriers as well and not affect the reservoir host. With respect to transmissibility, their job description includes the ability to exist in blood and other body fluids, to be aerosolized and to be carried by insect vectors. The route of transmission is crucial in order to maximize their potential for infection. If you, the reader, thought of how you would be a successful virus, you would want to infect as many people as possible, either integrate or lie latent in the host cell and kill neither the cell nor the host. You might also be musing about preferable routes of transmission, so some may say that EBV infection, sealed with a kiss, might be a more desirable option.

To keep the host's immune system on its toes, most of the RNA viruses can subtly change their genetic make-up and drift away from the circulating strain, thus evading the immune response. Alternatively, they may have a number of genotypes with a different susceptibility to antiviral agents, are not cross-protective therefore ensuring a multivalent vaccine is required as a preventative measure, and are associated with a different clinical illness spectrum.

Viruses make full use of the cellular replicative machinery and therefore an antiviral agent has difficulty targeting the virus without affecting the host cell. As a result, most antiviral agents can adversely affect the host. This means that individuals taking certain antiviral agents have to be monitored carefully, as treatment can potentially lead to side effects including bone marrow suppression, renal toxicity and mitochondrial disorders.

What can the host do to offset all these advantages? Antiviral vaccines have been a major success, behavioural changes can limit the chances of infection and, increasingly, more precise chemotherapeutic targets are being identified.

4 The fungi

Introduction

The study of fungi is known as mycology and fungal infections are known as mycoses

Fungi are eukaryotes, but are quite distinct from plants and animals and occupy their own kingdom. Characteristically, they have a thick carbohydrate cell wall containing chitin, glucans, mannans and glycoproteins. They are found either as filamentous fungi (moulds) or as yeasts. Filamentous fungi exist as multinucleate thread-like filaments (hyphae), which may show septation and which grow longitudinally and by branching. Yeasts are unicellular, round or oval in appearance and reproduce by budding. Other growth forms such as mushrooms also occur. Fungi are ubiquitous as free-living organisms and are of enormous importance commercially in baking, brewing and pharmaceuticals, producing antibiotics for example. Some form part of the body's normal flora, and others are common causes of local infections on skin and hair. A number of fungi are associated with significant disease and many of these are acquired from the external environment. Pathogenic species invade tissues and digest material externally by releasing enzymes; they also take up nutrients directly from host tissues. In recent years, invasive fungal disease has assumed much greater prominence in clinical practice as a result of the rise in number of severely immunocompromised patients.

MAJOR GROUPS OF DISEASE-CAUSING FUNGI

Importance of fungi in causing disease

There are more than 70 000 species of fungi but only about 300 are identified as pathogens in humans and animals. Some of these are cosmopolitan; others are found mainly in tropical regions. Some – those that infect superficially – cause only minor health problems but those that invade deeper tissues can be life threatening. These systemic forms have become much more serious problems as medical advances have taken place (e.g. immunosuppressive and antibiotic therapies, transplantation, invasive procedures and AIDS) such that opportunistic infections are now significant components of hospital-acquired infection. Only *Candida* and the dermatophytes are transmitted between humans; the remainder of disease-causing fungal infections are acquired from the environment, including the hospital environment.

Fungal pathogens can be classified on the basis of their growth forms or the type of infection they cause

Fungi were reclassified down to the level of order in 2007 following advances in fungal molecular taxonomy. Whilst this has no immediate effect on the practice of clinical microbiology, it will lead to greater understanding of the biology of the Kingdom Fungi and the diseases its members may cause.

Examples of branched filamentous forms or yeasts are shown in Fig. 4.1. Some show both growth forms in their cycle, with hyphae in the environment and yeasts in humans and are known as dimorphic fungi. In filamentous forms (e.g. *Trichophyton*), a mass of hyphae forms and is termed a mycelium. Asexual reproduction results in the formation of sporangia, which are sacs that contain and then liberate spores by which the fungus is dispersed; spores are a common cause of infection after inhalation. In yeast-like forms (e.g. *Cryptococcus*) the characteristic form is the single cell, which reproduces by budding. The bud may remain attached, with further budding leading to the formation of chains known as pseudohyphae. Dimorphic forms (e.g. *Histoplasma*) form hyphae at environmental temperatures, but occur as yeast cells in the body, the switch being temperature induced. *Candida* is an important exception in the dimorphic group, showing the reverse and forming hyphae within the body.

Three types of infection (mycoses) are recognized:

- Superficial mycoses where the fungus grows on body surfaces (skin, hair, nails, mouth, vagina). Examples are tinea pedis (athlete's foot) and vaginal candidiasis (thrush).
- Subcutaneous mycoses where nails and deeper layers of the skin are involved. Examples are mycetoma (Madura foot) and sporotrichosis.
- Systemic or deep mycoses with involvement of internal organs. This category includes fungi capable of infecting individuals with normal immunity and the opportunistic fungi that cause disease in patients with compromised immune systems. Examples are histoplasmosis and systemic candidiasis.

The superficial mycoses are spread by person-to-person contact or from animal-to-human contact (e.g. from cats and dogs); the subcutaneous mycoses infect humans via the skin (e.g. following skin penetration in the case of mycetoma); the deep mycoses often result from the opportunistic growth of fungi in individuals with impaired immune competence and are primarily acquired via the respiratory tract (see Ch. 31), with intravenous lines an important portal of entry for *Candida*. Free-living fungi can also cause disease. This occurs indirectly when toxins produced by fungi are present in items used

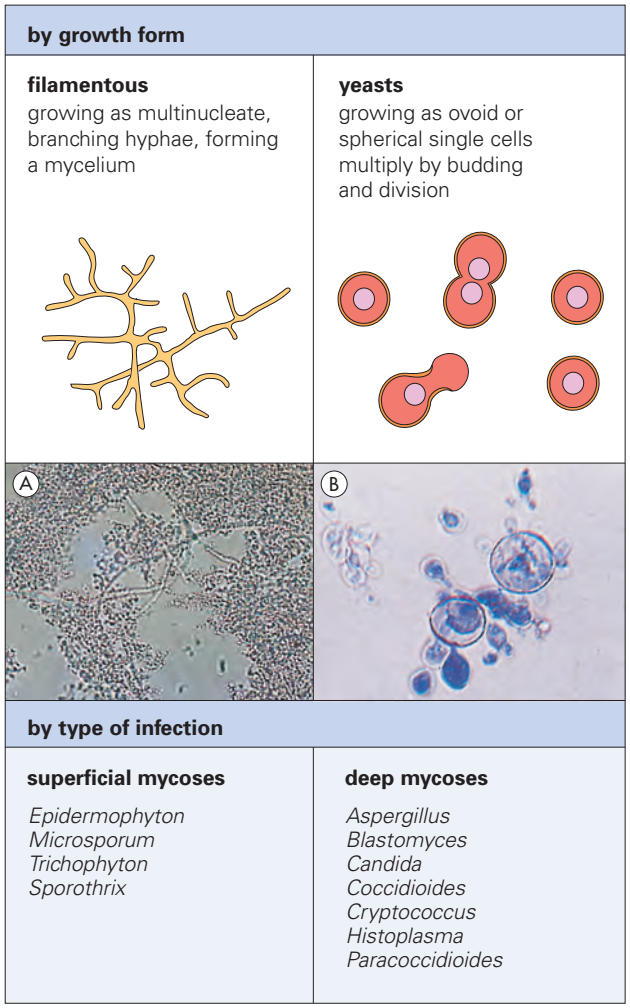


Figure 4.1 Two ways to classify fungi that cause disease: by growth form and by type of infection. (A) Hyphae in skin scraping from ringworm lesion. (B) Spherical yeasts of *Histoplasma*. ([A] Courtesy of D.K. Banerjee. [B] Courtesy of Y. Clayton and G. Midgley.)

as food (e.g. aflatoxin, a carcinogen produced by *Aspergillus flavus*) or when their spores are inhaled, an immune response occurs and a hypersensitivity pneumonitis develops (allergic bronchopulmonary aspergillosis).

Many of the fungi that cause disease are normally free-living in the environment, but can survive in the body if acquired by inhalation or by entry through wounds. Some fungi are part of the normal flora (e.g. *Candida*) and are innocuous unless the body's defences are compromised (e.g. by underlying malignancy, diabetes mellitus or intravenous drug use). The filamentous forms grow extracellularly, but yeasts can survive and multiply within macrophages and neutrophils. Neutrophils can play a major role in controlling the establishment of invading fungi. Species that are too large for phagocytosis can be killed by extracellular factors released from phagocytes as well as by other components of the immune response. Some species, notably *Cryptococcus neoformans*, prevent phagocytic uptake because they are surrounded by a polysaccharide capsule (see Chs 25 and 31). Until recently, *Pneumocystis jiroveci*, an important opportunistic infection in AIDS patients, was classified as a protozoan, but it is now regarded as an atypical fungus. It attaches to lung cells (pneumocytes) and can give rise to a fatal pneumonia. The microsporidia, previously thought to be protozoa, now turn out to be closely related to the fungi. The major groups of fungi causing human disease are shown in Table 4.1.

Control of fungal infection

The echinocandins inhibit glucan synthesis in the fungal cell wall. Below the fungal cell wall lies the plasma membrane or plasmalemma. Unlike human plasma membranes, where the dominant sterol is cholesterol, the fungal membrane is rich in ergosterol. Compounds that selectively bind to ergosterol can therefore be used as effective antifungal agents. These include the polyenes nystatin and amphotericin B. The azoles (e.g. miconazole) and the allylamines (e.g. terbinafine) inhibit ergosterol synthesis. The pyrimidines (e.g. flucytosine) inhibit nucleic acid synthesis.

Table 4.1 Summary of fungi that cause important human diseases

Important fungal diseases				
Type	**Anatomic location**	**Representative disease**	**Causative organisms**	**Growth form**
Superficial	Hair shaft, dead layer of skin	Pityriasis versicolor, tinea nigra, piedra	*Trichosporon*, *Malassezia*, *Exophiala*	Y/F
Cutaneous	Epidermis, hair, nails	Tinea (ringworm)	*Microsporum*, *Trichophyton*, *Epidermophyton*	F
Subcutaneous	Dermis, subcutis	Sporotrichosis Mycetoma	*Sporothrix* several genera	Y[a] F
Systemic	Internal organs	Coccidioidomycosis Histoplasmosis Blastomycosis Paracoccioidomycosis	*Coccidioides* *Histoplasma* *Blastomyces* *Paracoccidioides*	Form[b] Y Y Y
Opportunistic	Internal organs	Cryptococcosis Candidiasis Aspergillosis Pneumocystis pneumonia	*Cryptococcus* *Candida* *Aspergillus* *Pneumocystis*	Y Y[c] F[a] N/A

[a]Growth from the body.
[b]*Coccidioides* has an unusual growth form with yeast-like endospores within a spherule.
[c]Also forms pseudohyphae.
Y, yeast; F, filamentous; N/A, Y/F forms are not applicable.

KEY FACTS

- Fungi are distinct from plants and animals, have a thick chitin-containing cell wall, and grow as filaments (hyphae) or single-celled yeasts.
- Species causing disease may be acquired from the environment or occur as part of the normal flora.
- Infections may be located superficially, in cutaneous and subcutaneous sites, or in deep tissues.
- Infections are most serious in immunocompromised individuals.

CONFLICTS

Fungi are versatile; the same species can be both free-living in the external environment and cause disease. Thus, there is always a plentiful reservoir of infection. Fungi are physiologically versatile too and can grow at a wide range of temperatures. Their reproductive stages (spores) are small, can be air-borne and are easily inhaled. As they have a resistant chitinous cell wall and may produce antiphagocytic factors, they can be difficult for innate defence systems to deal with. Once past the defences of the respiratory system, many fungi change growth form and invade deeper tissues, often forming a network of elongate hyphae (e.g. in aspergillosis), which are even more difficult to defend against; indeed, immunological responses may aggravate systemic pathology. The prevalence of infective stages in the environment and the ability of fungi to grow rapidly in the absence of effective defences makes fungal infection a major hazard for immunocompromised patients. The balance is further tipped in their favour by the difficulty in diagnosing deep-seated mycoses and by the toxicity to the host of some of the drugs used to treat them. Fortunately, immunologically competent individuals appear to deal well with what must be frequent exposure. However, the potential for disease is always present and a new combatant has joined the conflict.

In 2009, *Candida auris* was isolated in Japan from a patient's external ear canal. Since then it has been responsible for bloodstream, wound and ear infections in at least nine countries. Prolonged hospital outbreaks have occurred. *C. auris* is a formidable opponent because it can be misidentified as a different yeast; it is usually resistant to fluconazole and is often multidrug resistant; environmental contamination can take place in healthcare facilities and result in secondary infections.

5 The protozoa

Introduction

Protozoa are single-celled animals, ranging in size from 2 to 100 nm. Like human cells, they are eukaryotic. Many protozoal species are free-living, but others are important parasites of humans. Some free-living species can infect humans opportunistically. Protozoa continue to multiply in their host until controlled by its immune response or by treatment and thus may cause particularly severe disease in immunocompromised individuals. Protozoal infections are most prevalent in tropical and subtropical regions, but also occur in temperate regions. Protozoa may cause disease directly (e.g. the rupture of red cells in malaria), but more often the pathology is caused by the host's response. Of all parasites, malaria presents the most severe global problem and kills approximately 500 000 people each year, mostly young children.

Protozoa can infect all the major tissues and organs of the body

Protozoa infect body tissues and organs as:

- intracellular parasites in a wide variety of cells (red cells, macrophages, epithelial cells, brain, muscle)
- extracellular parasites in the blood, intestine or genitourinary system.

The locations of the species of greatest importance are shown in Fig. 5.1. Intracellular species obtain nutrients from the host cell by direct uptake or by ingestion of cytoplasm. Extracellular species feed by direct nutrient uptake or by ingestion of host cells. Reproduction of protozoa in humans is usually asexual, by binary or multiple division of growing stages (trophozoites). Sexual reproduction is normally absent or occurs in the insect vector phase of the life cycle, where present. *Cryptosporidium* is exceptional in undergoing both asexual and sexual reproduction in humans. Asexual reproduction gives the potential for a rapid increase in number, particularly where host defence mechanisms are impaired. For this reason some protozoa are most pathogenic in the very young (e.g. *Toxoplasma* in the fetus and in neonates). The AIDS epidemic focused attention on a number of protozoa which give rise to opportunistic infections in immunocompromised individuals. These include *Cryptosporidium*, *Cystoisospora* and members of the Microsporidia. New parasites continue to emerge, e.g. *Cyclospora cayetanensis*, a food-borne and water-borne cause of diarrhoea, which became recognized as a clinical problem in the early 1990s (Fig. 5.2).

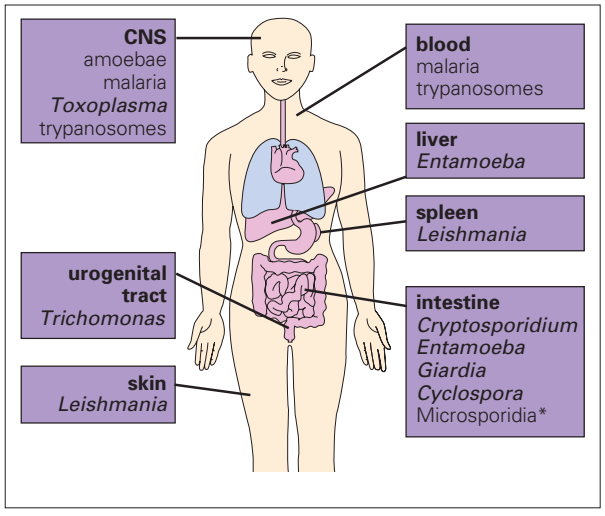


Figure 5.1 The occurrence of protozoan parasites in the body. * Can also occur in other sites. CNS, central nervous system.

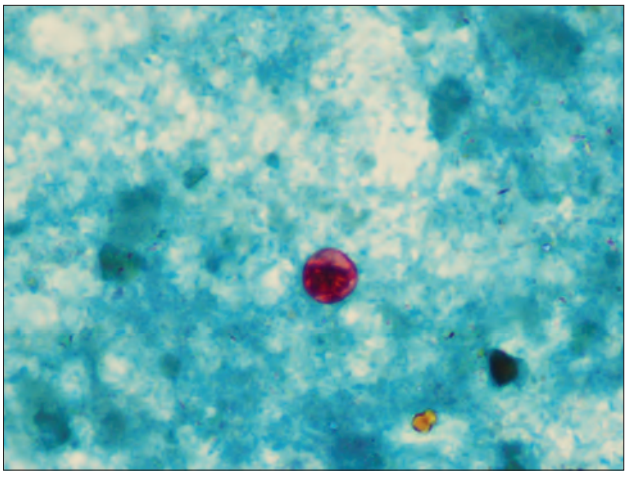


Figure 5.2 Oocyst of *Cyclospora cayetanensis*. Modified Ziehl–Neelsen stain. (Courtesy of Peter Chiodini.)

Table 5.1 Summary of the location, transmission and diseases caused by protozoan parasites

Features of medically important protozoa			
Location	**Species**	**Mode of transmission**	**Disease**
Intestinal tract	*Entamoeba histolytica* *Giardia intestinalis* *Cryptosporidium* spp. *Cystoisospora belli* *Cyclospora cayetanensis* Microsporidia	Ingestion of cysts in food or water	Amoebiasis Giardiasis Cryptosporidiosis Cystoisosporiasis Cyclosporiasis Microsporidiosis
Urogenital tract	*Trichomonas vaginalis*	Sexual	Trichomoniasis
Blood and tissue	*Trypanosoma* spp.: *T. cruzi* *T. b. gambiense,* *T. b. rhodesiense*	Reduviid bug Tsetse fly	Trypanosomiasis Chagas disease Sleeping sickness
	Leishmania spp.: *L. donovani* complex *L. tropica, L. major,* *L. mexicana, L. (Viannia) braziliensis*	Sandfly	Visceral leishmaniasis (kala-azar) Cutaneous leishmaniasis Mucosal leishmaniasis
	Plasmodium spp.: *P. vivax, P. ovale, P. malariae,* *P. falciparum, P. knowlesi*	*Anopheles* mosquito	Malaria
	Toxoplasma gondii	Ingestion of cysts in raw meat; ingestion of oocysts from cat faeces via environmental contamination	Toxoplasmosis

Protozoa have evolved many sophisticated strategies to avoid host responses

Extracellular species evade immune recognition of their plasma membrane. The interface between host and extracellular protozoa is the parasite's plasma membrane, and examples of strategies to avoid immune recognition of this surface include the following:

- Trypanosomes undergo repeated antigenic variation of surface antigens.
- Malaria parasites show polymorphisms in dominant surface antigens.
- Amoebae can consume complement at the cell surface.

Intracellular species evade host defence mechanisms. Although intracellular stages are removed from direct contact with antibody, complement and phagocytes, their antigens may be expressed at the surface of the host cell, which can then be a target for cytotoxic effectors. Survival within cells, particularly within macrophages (*Leishmania*, *Toxoplasma*), involves a variety of devices to evade or inactivate the harmful effects of intracellular enzymes or reactive oxygen and nitrogen metabolites.

Protozoa use a variety of routes to infect humans

Many extracellular protozoa are transmitted by ingestion of food or water contaminated with transmission stages such as cysts, but *Trichomonas vaginalis* is transmitted through sexual activity, and the trypanosomes by insect vectors. The most important intracellular species – *Plasmodium* and *Leishmania* – are also insect transmitted. *Trypanosoma cruzi*, another insect-transmitted protozoan which has both intracellular and extracellular stages in humans, can additionally be transmitted to the fetus in utero. *Toxoplasma*, a common and important intracellular protozoan, can be acquired by ingestion or from the mother in utero (Table 5.1).

KEY FACTS

- Protozoa are single-celled animals, occurring both as free-living organisms and as parasites. Both can cause disease in humans.
- The single most important protozoal disease is malaria, which causes some 500 000 deaths each year.
- Protozoa live both outside and within cells, and have complex ways of avoiding the responses of their hosts.
- Most infections are acquired through ingestion of contaminated water or food, or via insect vectors. A few are transmitted from mother to fetus.

CONFLICTS

Malaria provides a good example of human–protozoan conflict. After a period in the liver, the malaria parasite spends all of its time inside the red cell. It grows, divides and releases new parasites by rupturing the red cell. At this stage, the parasite wins the conflict by hiding away inside a cell, a non-nucleated cell that cannot respond defensively. How can the host protect itself immunologically? It has a number of difficult choices. It can try to destroy the parasite inside the cell by producing toxic mediators, or it can try to destroy the parasite and the cell together by targeting antibodies against antigens from the parasite that appear on the red cell surface, though the parasite presents a moving target as *Plasmodium falciparum* is adept at antigenic variation. Both of these are risky strategies. Toxic mediators can affect the host as well as the parasites, particularly if, as in *P. falciparum* malaria, the parasite-infected cells are lodged inside capillaries in vital organs. Destroying red cells can contribute to anaemia, and the by-products of destruction can also be toxic. A significant part of the pathology associated with malaria is therefore a cost of the host defending itself – game, set and match to the parasite, although a dead host is of no further use to the parasite. Though treatment with antimalarials can be highly effective, if they are given late the patient may still succumb as a result of complications despite clearance of parasites from the blood. Furthermore, the malaria parasite is adept at developing drug resistance, another example of the moving target.

6 The helminths

Introduction

The term 'helminth' is used for all groups of parasitic worms. Three main groups are important in humans: the tapeworms (Cestoda), the flukes (Trematoda) and the roundworms (Nematoda). The first two belong to the phylum Platyhelminthes or flatworms; the roundworms are in a separate phylum: Nematoda. Platyhelminths have flattened bodies with muscular suckers and/or hooks for attachment to the host. Nematodes (roundworms) have long cylindrical bodies and generally lack specialized attachment organs. Helminths are often large organisms with a complex body organization. Although invading larval stages may measure only 100–200 µm, adult worms may be centimetres or even metres long. Infections are commonest in warmer countries, but intestinal species also occur in temperate regions.

Transmission of helminths occurs in four distinct ways

Transmission routes are summarized in Fig. 6.1. Infection can occur after:

- swallowing infective eggs or larvae via the faecal–oral route
- swallowing infective larvae in the tissues of another host
- active penetration of the skin by larval stages
- the bite of an infected blood-sucking insect vector.

The greater frequency of helminths in tropical and subtropical regions reflects the climatic conditions that favour survival of infective stages, the socioeconomic conditions that facilitate faecal–oral contact, the practices involved in food preparation and consumption, and the availability of suitable vectors. Elsewhere, infections are commonest in children, in individuals closely associated with domestic animals and in individuals with particular food preferences.

Many helminths live in the intestine, whereas others live in the deeper tissues. Almost all organs of the body can be parasitized. Flukes and nematodes actively feed on host tissues or on the intestinal contents; tapeworms have no digestive system and absorb predigested nutrients.

The majority of helminths do not replicate within the host, although certain tapeworm larval stages can reproduce asexually in humans. In most, sexual reproduction results in the production of eggs, which are released from the host in faecal material. In others, reproductive stages may accumulate within the host, but do not mature. The nematode *Strongyloides* is exceptional in that eggs produced in the intestine can hatch there, releasing larvae which can mature to the infective stage and re-invade the body – the process of 'autoinfection' (Fig. 6.2).

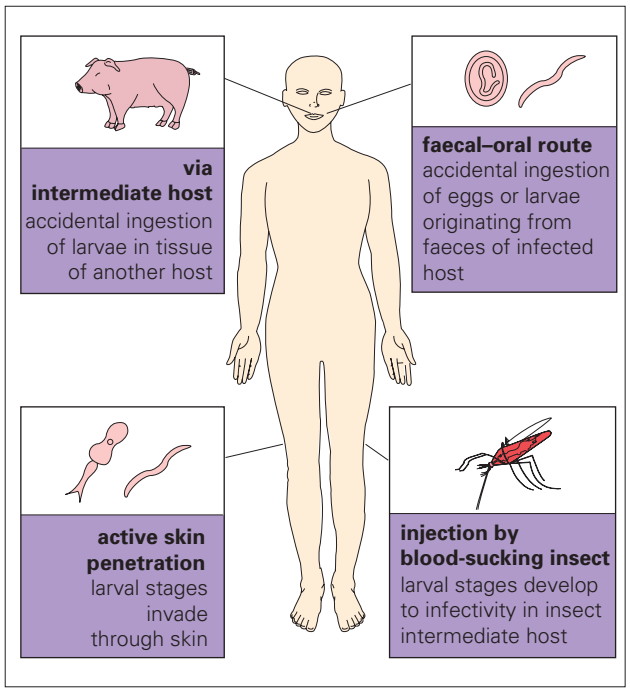


Figure 6.1 How helminth parasites enter the body.

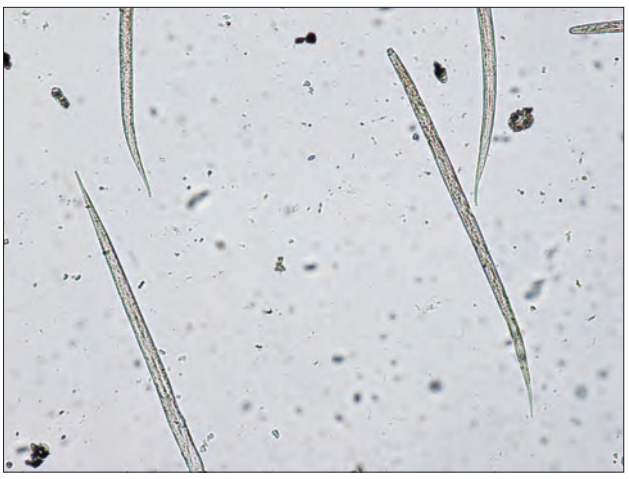


Figure 6.2 Filariform larvae, the infective stage of *Strongyloides stercoralis*. (Courtesy of Peter Chiodini.)

The outer surfaces of helminths provide the primary host–parasite interface

In tapeworms and flukes, the surface is a complex plasma membrane and in both there are protective mechanisms to prevent the host damaging the outer surface. The nematode outer surface is a tough collagenous cuticle, which, although antigenic, is largely resistant to immune attack. However, smaller larval stages may be damaged by host granulocytes and macrophages. Worms release large amounts of soluble antigenic material in their excretions and secretions, and this plays an important role both in immunity and in pathology.

LIFE CYCLES

Many helminths have complex life cycles

In direct life cycles, reproductive stages produced by sexually mature adults in one host are released from the body and can develop directly to adult stages after infection of another host via the faecal–oral route (*Ascaris*) or by direct penetration (hookworm). Indirect cycles are those where reproductive stages must undergo further development in an intermediate host (tapeworms) or vector (filarial worms) before sexual maturity can be achieved in the final host.

The larvae of flukes and tapeworms must pass through one or more intermediate hosts, but those of nematodes can develop to maturity within a single host

Most flukes are hermaphrodites, except the schistosomes, which have separate sexes. The reproductive organs of tapeworms are replicated along the body (the strobila) in a series of identical segments or 'proglottids'. The terminal gravid proglottids become filled with mature eggs, detach and pass out in the faeces. The eggs of both flukes and tapeworms develop into larvae that must pass through one or more intermediate hosts and develop into other larval stages before the parasite is again infective to humans. The dwarf tapeworm *Hymenolepis nana*, which is occasionally found in humans, is exceptional as it can go through a complete cycle from egg to adult in the same host.

In nematodes, the sexes are separate. Most species liberate fertilized eggs, but some release early-stage larvae directly into the host's body. Development from egg or larva to adult can be direct and occur in a single host, or may be indirect, requiring development in the body of an intermediate host. Classification of nematodes is complex, and for practical purposes only two categories of human-specific nematodes are considered here:

- those that mature within the gastrointestinal tract, some of which may migrate through the body during development (e.g. *Ascaris*, hookworms, *Trichinella*, *Strongyloides*, *Trichuris*)
- those that mature in deeper tissues (e.g. the filarial nematodes).

In addition, humans can be infected with the larvae of species that mature in other hosts (e.g. the dog parasites *Toxocara canis* and *Ancylostoma brasiliense*).

HELMINTHS AND DISEASE

Adult tapeworms are acquired by eating undercooked or raw meat containing larval stages

Tapeworms frequently infect humans, but the adult tapeworms are relatively harmless despite their potential for reaching a large size. Humans can also act as the intermediate hosts for certain species, and the development of larval stages in the body can cause severe disease (Table 6.1).

Table 6.1 Summary of the location, transmission and other hosts used by tapeworms that infect humans

Human tapeworm infections			
Species	**Acquired from**	**Other hosts**	**Site in humans**
Adult worms			
Taenia saginata	Larvae in beef	None	Intestine
Taenia solium	Larvae in pork	None	Intestine
Diphyllobothrium latum	Larvae in fish	Fish-eating mammals	Intestine
Hymenolepis nana	Eggs; or larvae in beetles	Rodents	Intestine
*Hymenolepis diminuta**	Larvae in insects	Rats, mice	Intestine
*Dipylidium caninum**	Larvae in fleas	Dogs, cats	Intestine
Larval worms			
Taenia solium (cysticercosis)	Eggs in food or water contaminated with human feces	Pigs	Brain, eyes
Echinococcus granulosus (cystic echinococcosis; cystic hydatid disease)	Eggs passed by dogs	Sheep	Liver, lung, brain
*Echinococcus multilocularis** (alveolar echinococcosis; alveolar hydatid disease)	Eggs passed by carnivores	Rodents	Liver
Pseudophyllidean tapeworms* (sparganosis)	Larvae in other hosts	Many vertebrates	Subcutaneous tissues, eyes
*Taenia multiceps**	Eggs passed by dogs	Sheep	Brain, eye, subcutaneous tissue

*Rare infections.

The most important flukes are those causing schistosomiasis

Several species of fluke can mature in humans, developing in the intestine, lungs, liver and blood vessels. The most important, both in terms of prevalence and pathology, are the blood flukes or schistosomes, the cause of schistosomiasis, also known as bilharzia. Three main species – *Schistosoma haematobium, S. japonicum* and *S. mansoni* – infect many millions and are responsible for severe disease (Table 6.2). Like all flukes, schistosomes have an indirect life cycle involving stages of larval development in the body of a snail, in this case freshwater aquatic snails. Humans become infected when they come into contact with water containing infective larvae released from the snails, the larvae penetrating the skin. Other important species are *Clonorchis sinensis*, the oriental liver fluke, and *Paragonimus westermani*, the lung fluke, transmitted by eating infected freshwater fish or freshwater crabs, respectively.

Certain nematodes are highly specific to humans; others are zoonoses

Several of the many species of nematode that infect humans are highly specific and can mature in no other host. Others have much lower host specificity, being acquired accidentally as zoonoses, with humans acting as either the intermediate or the final host after picking up infection from domestic animals or in food (Table 6.3).

Table 6.2 Summary of the location and transmission of flukes that infect humans

Human fluke infections		
Species	**Acquired from**	**Site in humans**
Schistosoma haematobium	Penetration of skin by larval stages released from snails	Blood vessels of bladder
S. japonicum		Blood vessels of intestine
S. mansoni		Blood vessels of intestine
Clonorchis sinensis	Ingesting fish infected with larval stages	Liver
Fasciola hepatica	Ingesting vegetation (e.g. watercress) infected with larval stages	Liver
Paragonimus westermani	Ingesting freshwater crabs infected with larval stages	Lungs

Table 6.3 Summary of the location and transmission of nematodes that infect humans

Human nematode infections		
Species	**Acquired by**	**Site in humans**
Transmitted person to person		
Ascaris lumbricoides	Ingestion of eggs	Small intestine
Enterobius vermicularis	Ingestion of eggs	Large intestine
Hookworms:		
Ancylostoma duodenale	Skin penetration by infective larvae	Small intestine
Necator americanus	Skin penetration by infective larvae	Small intestine
Strongyloides stercoralis	Skin penetration by infective larvae; autoinfection	Small intestine (adults), general tissues (larvae)
Trichuris trichiura	Ingestion of eggs	Large intestine
Transmitted person to person via arthropod vector		
Brugia malayi	Bite of mosquito carrying infective larvae	Lymphatics (adults), blood (larvae)
Onchocerca volvulus	Bite of *Simulium* fly carrying infective larvae	Skin (larvae, adults), eye (larvae)
Wuchereria bancrofti	Bite of mosquito carrying infective larvae	Lymphatics (adults), blood (larvae)
Loa loa	Bite of deer fly-carrying infective larvae	Subcutaneous tissues (adults), blood (larvae)
Zoonoses transmitted from animals		
Angiostrongylus cantonensis	Ingestion of larvae in snails, crustaceans	CNS (larvae)
Anisakis simplex	Ingestion of larvae in fish	Stomach, small intestine (larvae)
Capillaria philippinensis	Ingestion of larvae in fish	Small intestine (adults, larvae)
*Toxocara canis**	Ingestion of eggs passed by dogs	Tissues, eye, CNS (larvae)
*Trichinella spiralis**	Ingestion of larvae in pork, meat of wild mammals	Small intestine (adults), muscles (larvae)

*These species are the commonest in this group.

Survival of helminths in their hosts

Many helminth infections are long lived, the worms surviving in their hosts for many years, despite living in parts of the body where there are effective immune defences. How this is achieved has been worked out in several species. Some, such as the schistosomes, disguise themselves from the immune system by acquiring host molecules on their outer surface, so they are less easily recognized as foreign invaders. Others actively suppress the host's immune responses by releasing factors that interfere with, or divert, protective responses. For example, the human host shows a degree of immune tolerance to hookworm infection. The ability of worms to do this is being actively investigated as a potential therapeutic approach to the control of immunologically mediated conditions such as coeliac disease and inflammatory bowel disease. It may one day be possible to protect patients at risk from these conditions by giving them a parasite infection!

KEY FACTS

- Helminths are multicellular worms that parasitize many organs of the body, most commonly the gastrointestinal tract.
- Transmission may be direct, through swallowing infective stages or by larvae penetrating the skin, or indirect via intermediate hosts or insect vectors.
- The most serious helminth infection is schistosomiasis, caused by infection with blood flukes. The pathology is primarily due to hypersensitivity reactions to eggs as they pass through tissues.

CONFLICTS

Helminths are typically large parasites, often covered by a protective outer layer, so they are difficult for the immune system to deal with – too big for phagocytosis or cytotoxic T cells and unaffected by direct antibody activity. They are often active and mobile and can move away from host defences, damaging host tissues as they do so. Many disguise their outer surfaces or produce immunosuppressive factors. Because they are long lived and able to survive despite immune responses, they can produce chronic disease, either as a consequence of their activity or because of misdirected and pathological host immune responses. Reliance on direct infection through faecal–oral contact, or transmission by vectors, makes it difficult to avoid infection when climate and low standards of hygiene combine to tilt the balance in favour of the parasite. Treatment with anthelminthics works against many intestinal worms, but re-infection is almost routine in areas of poor sanitation, necessitating regular re-treatment programmes. Those living in the tissues are much more difficult to deal with; for example, hydatid cysts may require major surgery as well as antiparasitic drugs and there are still no effective drugs for the treatment of Guinea worm.

7 The arthropods

Introduction

The phylum Arthropoda is the largest in the animal kingdom. It is remarkably diverse and arguably the most successful single group of animals. Arthropods are characterized by having an exoskeleton composed of a rigid cuticle which contains chitin, a segmented body, and jointed appendages. Examples with which most people will be familiar are crustaceans, centipedes, insects, ticks and mites. The latter three are the most relevant to human disease.

Members of the class Insecta have segmented bodies with a head, thorax and abdomen and three pairs of legs. They usually have wings, but some insects are wingless.

Ticks are members of the class Arachnida, which includes spiders. Adult ticks have four pairs of legs; larvae have three pairs.

Mites are also in the class Arachnida. Adults have one to four pairs of legs, usually four; larvae have a maximum of three pairs.

Many of these arthropods have adapted to live on humans or use humans as sources of food (blood and tissue fluid). Linked with these feeding habits is the ability of many arthropods to transmit a very wide variety of microbial pathogens. Others, acting as intermediate hosts, may transmit helminth parasites when eaten, and yet other species can inflict dangerous bites and stings.

Many arthropods feed on human blood and tissue fluids

Blood feeders include mosquitoes, midges, biting flies, bugs, fleas and ticks. Some mites also feed in this way – chiggers, the larvae of trombiculid mites, being a familiar example. Contact may be temporary or permanent. Mosquitoes are temporary ectoparasites, feeding for only a few minutes; ticks feed for much longer. The louse *Pediculus humanus* and the crab louse *Phthirus pubis* spend almost all of their lives on humans, feeding on blood and reproducing on the body or in clothing. The scabies mite *Sarcoptes scabiei* (Fig. 7.1) lives permanently on humans, burrowing into the superficial layers of skin to feed and lay eggs. Heavy infections can build up, particularly on individuals with reduced immune responsiveness, causing a severe inflammatory condition (see Ch. 27). In tropical and subtropical regions the larvae (or maggots) of certain flies enter the skin and develop into boil-like lesions under the skin, a condition known as myiasis. One remarkable way in which this occurs is exemplified by *Dermatobia hominis*, the human botfly from Central and South America, the adult female of which attaches her eggs to mosquitoes. When the mosquito bites, larvae leave the mosquito and enter human skin at the sites of the mosquito bites.

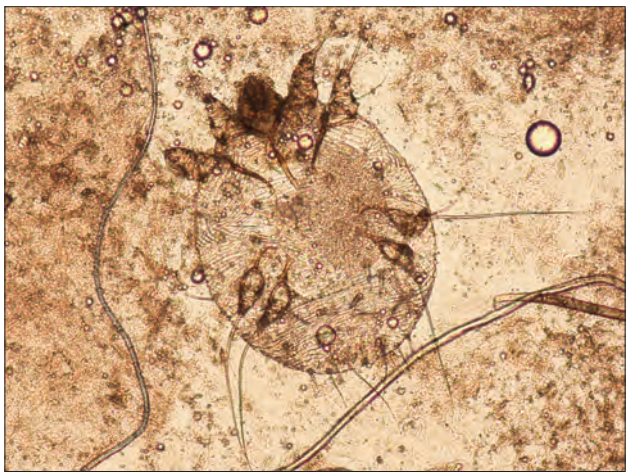


Figure 7.1 *Sarcoptes scabiei*, the scabies mite. (Courtesy of Peter Chiodini.)

Arthropod infestation carries the additional hazard of disease transmission

Arthropods transmit pathogens of all major groups, from viruses to worms and some (e.g. mosquitoes and ticks) transmit a wide variety of organisms (Table 7.1). The ability to transmit infections acquired from animals to humans poses a constant threat of acquiring zoonoses. Some vector-borne infections, such as yellow fever, have been known for centuries, whereas others, such as the viral encephalitides and Lyme disease, have been recognized more recently (1920s and 1975, respectively). Mosquito-transmitted West Nile virus has become a significant threat in North America, with sporadic cases and outbreaks reported from Europe (see Ch. 28).

KEY FACTS

- Arthropods of importance in human disease are those that feed on blood or body tissues (insects, ticks, mites) and those that transmit other infections, particularly viruses, bacteria and protozoa.
- Insecticide resistance is a major threat to the success of malaria control and eradication programmes.

Table 7.1 Summary of infectious diseases transmitted by arthropods

Infectious diseases transmitted by arthropods		
	Disease	**Arthropod vector**
Viruses		
Arboviruses	Zika virus	Mosquitoes
	Dengue fever	Mosquitoes
	Yellow fever	Mosquitoes
	Encephalitides	Mosquitoes, ticks
	Hemorrhagic fevers	Ticks, mosquitoes
Bacteria		
Yersinia pestis	Plague	Fleas
Borrelia recurrentis	Relapsing fever	Soft ticks
Borrelia burgdorferi	Lyme disease	Hard ticks
Rickettsias:		
Orientia tsutsugamushi	Scrub typhus	Larval mites
R. prowazekii	Epidemic typhus	Lice, (ticks)
R. mooseri	Endemic (murine) typhus	Fleas
R. rickettsii	Spotted fever	Ticks
R. akari	Rickettsial pox	Mites
Protozoa		
Trypanosoma cruzi	American trypanosomiasis (Chagas disease)	Reduviid bugs
T. b. rhodesiense	African trypanosomiasis (sleeping sickness)	Tsetse flies
T. b. gambiense		
Plasmodium spp.	Malaria	Mosquitoes
Leishmania spp.	Leishmaniasis	Sandflies
Worms		
Wuchereria and *Brugia*	Lymphatic filariasis	Mosquitoes
Onchocerca	Onchocerciasis	*Simulium* flies
Loa loa	Loiasis (eye worm)	*Chrysops* flies

CONFLICTS

Prevention of human infection with insect-transmitted organisms depends very heavily on bite avoidance, as only yellow fever is readily prevented by vaccination. Insect bite avoidance relies on barrier methods (e.g. mosquito nets), insect repellents and insecticides.

The insects have fought back and are capable of becoming resistant to insecticides in a variety of ways:

- by changing their metabolism so that their enzyme systems detoxify, destroy or excrete the insecticide more rapidly
- by modifying the site at which the insecticide acts, to prevent its binding or interacting there
- by developing barriers to penetration in their outer cuticle, thus slowing absorption of the insecticide into their tissues
- by recognizing the presence of the insecticide and moving away from it where possible.

Since the year 2000, substantial gains have been made in combating malaria using long-lasting insecticidal nets and indoor residual spraying. However, these advances are threatened by the emergence of resistance among *Anopheles* mosquitoes. World Health Organization information for the years 2010 to 2015 shows 60 countries having reported anopheline resistance to at least one insecticide class, and 49 of them with resistance to two or more classes. Given the presence of multidrug resistance in the malaria parasite *Plasmodium falciparum*, we can be sure that the conflict with this insect-transmitted infection will be long and arduous; and there are many other battles between humans and insects where insecticide resistance threatens the outcome.

8 Prions

Introduction

Prions are infectious proteins that acquire alternative conformations and are associated with a number of human, animal and fungal diseases. In humans they can cause degenerative changes in the brain: the transmissible spongiform encephalopathies. Kuru is the classic example of such a condition, epidemiological studies confirming human–human transmission due to cannibalistic rituals among the Fore people of Papua New Guinea. Prions lack a nucleic acid genome and are highly resistant to all conventional forms of disinfection processes. They are small proteinaceous particles that are modified forms of a normal cellular protein, and cause disease by converting normal protein into further abnormal forms. Prion-related conditions can arise endogenously by mutation (and be inherited), or be acquired exogenously during medical procedures or by ingestion of contaminated material. The prion diseases are part of a spectrum of neurodegenerative disorders in which soluble proteins are modified and accumulate as insoluble beta-sheet rich amyloid fibrils. The other neurogenerative disorders that include different types of dementia are not infectious but are sporadic or inherited, sharing a common pathogenesis. Endogenous sporadic Creutzfeldt–Jakob disease (CJD) has been known for some time, as have Gerstmann–Sträussler–Scheinker disease, fatal familial insomnia and kuru. However, in the 1990s another form of this disease (variant CJD, vCJD) was associated with eating beef from cattle infected with the prion that causes bovine spongiform encephalopathy (BSE).

'ROGUE PROTEIN' PATHOGENESIS

Prions are unique infectious agents

There are a number of human and animal diseases – the spongiform encephalopathies – whose pathology is characterized by the development of large vacuoles in the central nervous system (CNS). These include kuru and Creutzfeldt–Jakob diseases (CJD) in humans, bovine spongiform encephalopathy (BSE) in cattle and scrapie in sheep. Sporadic CJD is the most common prion disease in humans worldwide and the incidence is approximately 1.5 per million people. For a long time, these diseases were thought to be caused by so-called unconventional slow viruses, but it is now known that the agents concerned are prions: small, proteinaceous infectious particles. Their characteristics include:

- small size (<100 nm, therefore filterable)
- lack of a nucleic acid genome
- extreme resistance to heat, disinfectants and irradiation (but susceptible to high concentrations of phenol, periodate, sodium hydroxide and sodium hypochlorite)
- slow replication – typically diseases have a long incubation period and usually appear late in life; incubation periods of up to 35 years have been recorded in humans, but variant CJD can produce symptoms much more rapidly
- cannot be cultured in vitro
- do not elicit immune or inflammatory responses.

Prions are host-derived molecules

Studies on scrapie, a fatal transmissible spongiform encephalopathy of sheep and goats, gave some insight into the nature of prions and their role in disease. In the 1960s, it had been proposed that proteins could be infectious pathogens and were thought to be involved in scrapie. It was not until 20 years later that Stanley Prusiner demonstrated that the infectious particles purified from hamster brains, having been infected with scrapie, were proteinaceous infectious particles that were called prions. The infectious agent is a host-derived 30–35 kDa glycoprotein (termed PrP^{Sc}, prion protein scrapie) that is associated with the characteristic intracellular fibrils seen in diseased tissue. PrP^{Sc} is derived from a naturally occurring cellular prion protein (PrP^{C}), a membrane glycoprotein expressed predominantly on the surface of nerve cells in the CNS, but is also found in non-neurological tissues and organs. PrP^{C} is encoded by the prion protein gene *PRNP* found on chromosome 20 and may have a role in oxidative stress reduction, signal transduction apoptosis and forming and maintaining synapses. This means that it is involved in key physiological processes in the nervous and immune systems.

Mice with the *PrP*^{C} gene disrupted are resistant to scrapie, and they show no gross abnormalities. The two proteins have a similar sequence, but differ in structure and protease resistance; PrP^{Sc} is globular and enzyme resistant; PrP^{C} is linear and enzyme susceptible. The association of PrP^{Sc} with PrP^{C} results in conversion of the latter into the abnormal form, the change being largely conformational, from alpha helices to beta-pleated sheets. This conformational change explains why PrP^{Sc} forms compact protein aggregates that accumulate in the brain. Affected cells produce more PrP^{C} and the process is then repeated, the accumulating PrP^{Sc} aggregating into amyloid fibrils and plaques (Fig. 8.1). PrP^{Sc} continues to accumulate

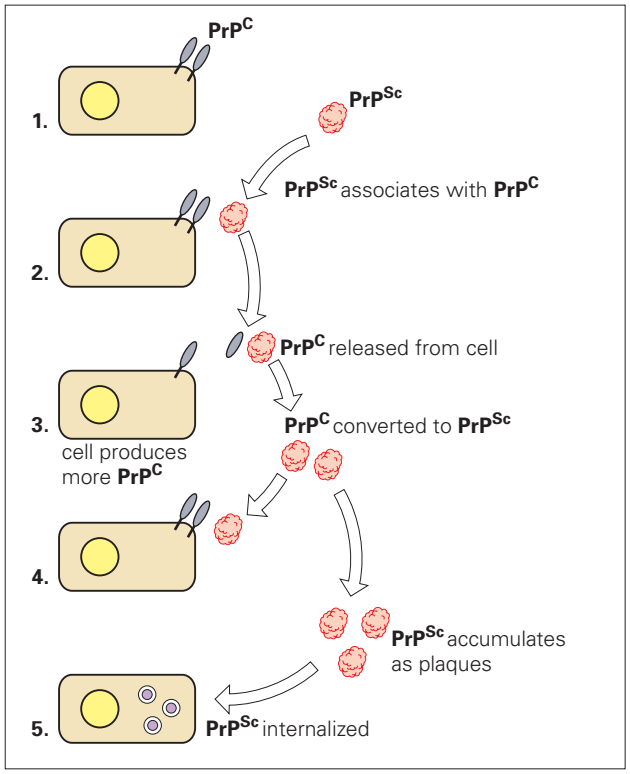


Figure 8.1 How prions may damage cells. (1) Normal cells express PrP^{C} at the cell membrane as linear proteins. (2) PrP^{Sc} exists as a free globular glycoprotein, which can interact with PrP^{C}. (3) PrP^{C} is released from the cell membrane and is converted into PrP^{Sc}. (4) Cells produce more PrP^{C} and the cycle is repeated. (5) PrP^{Sc} accumulates as plaques, and is internalized by cells.

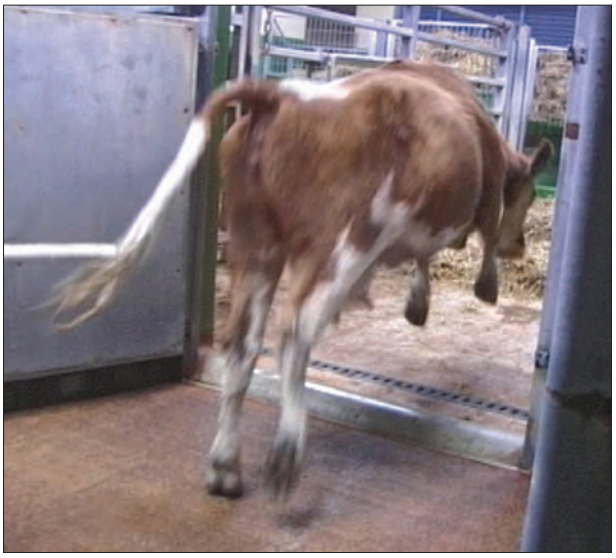


Figure 8.2 A cow with bovine spongiform encephalopathy jumping over a minor step. (Crown copyright 2003. Courtesy of Dr Timm Konold. Reproduced with permission of the Animal and Plant Health Agency.)

Box 8.1 Animal and human prion diseases

ANIMAL (SPORADIC OR ACQUIRED)	HUMAN
Scrapie	Kuru (sporadic and acquired)
Chronic wasting disease	CJD (sporadic, inherited or acquired)
Bovine spongiform encephalopathy	GSS syndrome (inherited or sporadic)
Transmissible mink encephalopathy	Fatal familial insomnia (inherited or sporadic)
Feline spongiform encephalopathy	VPSPr (sporadic or familial)
Exotic ungulate encephalopathy	

CJD=Creutzfeldt–Jakob disease; GSS=Gerstmann–Sträussler–Scheinker syndrome; VPSPr=variably protease-sensitive prionopathy.

replacing the normal PrP^{C}, resulting in neurodegeneration. Replication can lead to very high titres of infectious particles and up to 10^{8}–10^{9} / g of brain tissue have been recorded.

Evidence that the interaction of PrP^{Sc} with PrP^{C} causes these events is based on extensive experiments in sheep and mice, the main conclusions being:

- Scrapie infectivity in material co-purifies with PrP^{Sc}.
- Purified PrP^{Sc} confers greater scrapie activity.
- Mice lacking the *PrP*^{C} gene do not develop disease when injected with prions.
- Introduction of a *PrP* transgene from a prion donor species (e.g. hamster) into a recipient species (e.g. mouse) facilitates cross-species transmission, suggesting that homology between the *PrP* genes of donor and recipient is the main molecular determinant of such transmission.
- In vitro, PrP^{Sc} can convert PrP^{C} into PrP^{Sc}, with the transfer of biochemical characteristics.

The development of scrapie in sheep shows strong genetic influences, some breeds being much more resistant than others, and similar genetic effects have been shown in mice. In humans, homozygosity for methionine at codon 129 of the prion protein gene is a major determinant of susceptibility to sporadic, iatrogenic and variant CJD. There is also variation in prions, different strains being described. These combinations of host and prion variation result in a spectrum of disease onset and severity. Animal prion diseases (Box 8.1) include scrapie, chronic wasting disease (CWD) and bovine spongiform encephalopathy (BSE) (Fig. 8.2), all of which are sporadic or acquired. CWD affects some North American deer (Fig. 8.3) and Rocky Mountain elk and moose populations. These animals are all hunted and eaten, so the concern is that although CWD has not been transmitted to humans, there is the possibility that this could happen after eating infected meat.

DEVELOPMENT, TRANSMISSION AND DIAGNOSIS OF PRION DISEASES

PrP is a modified host protein and the gene is located on chromosome 20. The normal form of the prion protein is referred to as PrP^{C}. PrP^{Sc} is an abnormal isoform of PrP and accumulates in brain tissue. It differs from PrP^{C} only by having an increased beta-sheet content, which makes it more stable and is responsible for the ability of PrP^{Sc} to form aggregates, which then form amyloid fibrils. In addition, it

is quite resistant to proteolysis. The normally folded protein PrP^{C} is converted to an abnormal conformation by direct contact with the misfolded form PrP^{Sc}. If the load of the latter increases it can lead to a rapid neurodegenerative phenotype. PrP^{Sc} can be built into different structures and so these PrP^{Sc} species can result in a variety of human prion diseases (Box 8.1) that include sporadic and acquired kuru, sporadic, inherited or acquired CJD and sporadic or inherited Gerstmann–Sträussler–Scheinker (GSS) syndrome. There is some evidence that people have a genetic predisposition for sporadic CJD. There is a naturally occurring polymorphism at codon 129 of the PrP^{C} gene on chromosome 20 and this codes for the amino acid methionine or valine. Compared with the unaffected population, people with sporadic CJD are many times more likely to be methionine homozygous at this locus.

With the exception of those cases where prions arise by mutation, transmission and spread of prion disease require exposure to the infective agent. Ways in which this could occur include eating contaminated food material, use of contaminated medical products (blood, hormone extracts, transplants), the introduction of prions from contaminated instruments during surgical procedures, as prions bind strongly to metal surfaces, and possibly mother–fetus transmission during pregnancy (although none of the hundreds of infants born to mothers with kuru developed the disease). The disease kuru was transmitted by eating the brains of dead humans in funeral rites, and vCJD is associated with eating contaminated beef products. In these cases, prions survive digestion and are taken up across the intestinal mucosa. They are then carried in lymphoid cells, eventually being transferred into neural tissues and entering the CNS.

Figure 8.3 A deer with chronic wasting disease. (Courtesy of Professor Jason Bartz, Department of Medical Microbiology and Immunology, Creighton University School of Medicine, Omaha, Nebraska, USA.)

Prions can cross species boundaries

Although prions from one species are more effective in transmitting disease to the same species, transmission can occur between different species (Fig. 8.4). An example of this is the transfer of prions from cattle infected with BSE to humans through consumption of infected meat, which was associated with outbreaks of vCJD. BSE itself arose as a result of transfer to cattle of prions from sheep infected with scrapie, and in 1996 it became clear that human vCJD and BSE were caused by the same prion strain. Unlike CJD itself, vCJD caused disease in younger individuals (14 years and upwards) with a much shorter incubation period. The number of human infections likely to arise from the UK epidemic of BSE in cattle (thought to have affected more than 2 million animals) is still controversial, though some believe the potential to be quite small. CJD surveillance was started in the United Kingdom in 1990 in order to identify the number of human infections arising from the UK epidemic of BSE in cattle that was thought to have affected more than 3 million animals. This estimate was based on the likely number of asymptomatic animals and the clinical diagnosis of BSE made in over 180 000 cattle. vCJD was first reported in the UK in 1996 by the National CJD Surveillance Unit. Those affected had a clinical and pathological phenotype distinct from sporadic CJD and were homozygous for methionine at codon 129. Again, this demonstrated a genetic predisposition for vCJD. vCJD is the only prion disease affecting humans that can be acquired from another species and is caused by BSE. This has also been shown by animal transmission studies in which the infectious agent associated with vCJD was shown to have the same biological properties as that causing BSE.

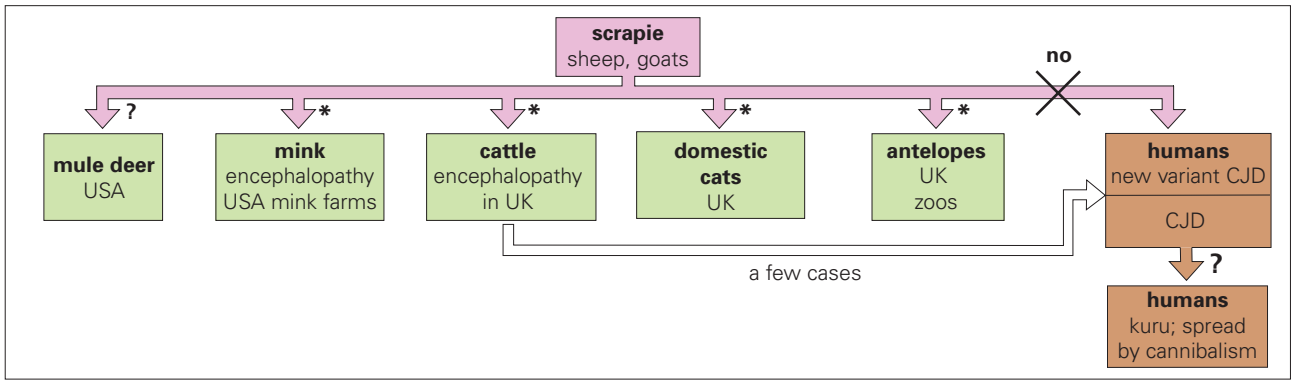


Figure 8.4 The spread of scrapie agents between species. Nearly all have been transmitted to laboratory rodents and primates. (* Infections transferred by scrapie-infected sheep materials present in foodstuff. Most of these infectious agents have mutations at amino acid residue 129 of the prion protein, which are thought to cause conversion of the protein into the pathogenic form.)

Epidemiological studies suggest that the most likely route of transmission is the oral route, the affected individual having eaten beef contaminated with the BSE agent. PrP^{Sc} has been found in the lymphoreticular system including the tonsils and spleen as well as neurological tissues, and the prion may be carried in the blood by lymphocytes.

Overall, by July 2010, 220 people had developed vCJD in 11 countries around the world, 171 of whom were diagnosed in the UK. By 2016, 178 people in the UK in total had died of vCJD. That number was much lower than had been predicted by mathematical modellers in the 1990s. As the incubation period can be very long, it is unclear how many people could be at risk and asymptomatic. Issues surrounding diagnostic tests include assay sensitivity and specificity, resulting in difficulty in comparing studies. A large study was carried out in the UK investigating more than 32 000 anonymized tonsil tissues for disease-related prion protein referred to as PrP^{CJD} from people who underwent an elective tonsillectomy. Of these, 12 753 were from the 1961–1985 birth cohort that included the time most vCJD cases had arisen and 19 908 were from the 1986–1995 cohort that would potentially have been exposed to BSE-contaminated meat products. PrP^{CJD} was not detected in any samples.

Prion diseases are difficult to diagnose

Because prions cannot be cultured, and as there is no immune response, prion disease in its early stages cannot be diagnosed easily. Clinical appearances usually indicate the probable occurrence of prion disease and this can be confirmed histologically post mortem. Tonsillar tissue is a good source of PrP^{Sc} in clinical cases and these prions can be identified by immunoblotting or immunohistochemistry. Tonsillar and other tissue homogenates can also be tested for the presence of the abnormal prion protein by enzyme immunoassays. These have been used in a number of studies and the development of diagnostic tests is important not only to make a diagnosis but also from a public health standpoint to prevent infection, as transmission by blood and blood products has been reported. Clinical diagnostic criteria include brain imaging and specific biomarkers in the cerebrospinal fluid. Assays such as protein misfolding cyclic amplification (PMCA) based on PrP^{Sc} polymerization were developed, but there were false positive and negative results. The amyloid seeding assay (ASA), a marker of amyloid formation, was more sensitive but strain dependent. However, the test demonstrating most promise in making the preclinical diagnosis of prion diseases was the brilliantly named real-time quaking-induced conversion (RT-QuIC) assay. The sample was added to recombinant PrP, an amyloid-sensitive fluorescent dye and a chaotropic agent and the incubated plate vigorously shaken (quaked) and fluorescence measured as fibrils formed. Femtogram amounts of PrP^{Sc} could be detected.

Lessons from kuru

Kuru is a condition that was identified with cannibalistic behaviour in Papua New Guinea. There were more than 2700 infections between 1957 and 2004, the incubation period of the disease being estimated at more than 50 years. The fatality rate fell from over 200 per year in the late 1950s to 6 per year in the early 1990s. This reduction followed the prohibition of cannibalistic behaviour in the 1950s. A study investigating suspected kuru cases between 1996 and 2004 identified 11 infected individuals. The minimum estimated incubation periods in this group ranged from 34 to 41 years, the range in males being from 39 to at least 56 years. Analysis of the prion protein gene (*PRNP*) showed that most patients with kuru were heterozygous at codon 129.

PREVENTION AND TREATMENT OF PRION DISEASES

Prion diseases are incurable

Although, as of 2017, there is neither an effective treatment nor vaccine, chemotherapeutic strategies involve stopping the conversion of the normal form of prion protein to the abnormal form PrP^{Sc}. However, little success has been reported using a number of different agents including antimalarials and antibiotics that had been shown to reduce the growth of abnormal prion protein deposits in vitro.

Humanized versions of antibodies that bind to PrP that had activity on prion-infected nerve cells growing in vitro have been prepared for a clinical trial. The hypothesis was that the normal form of PrP required for prions to grow could be removed. Extended survival time was reported when prion-infected mice were treated.

Understanding the nature of the interactions between PrP^{Sc} and PrP^{C} may eventually offer some hope of regulating the development of disease by reducing or destabilizing the formation of PrP^{Sc}. Immunomodulation and mucosal immunization may be potential therapeutic and preventative approaches, especially as the alimentary tract is likely to be the main route of transmission.

KEY FACTS

- Prions are unusual infectious agents, causing diseases characterized by changes in the brain (spongiform encephalopathies) and motor disturbances.
- Prions are host-derived glycoproteins and lack a nucleic acid genome. They are extremely resistant to disinfection procedures.
- Transmission of prions is usually by ingestion of contaminated tissues, but can occur via medical procedures.
- Diseases in humans caused by prions include kuru, Creutzfeldt–Jakob disease (CJD), variant CJD (vCJD) and bovine spongiform encephalopathy (BSE).
- The prion diseases are difficult to diagnose, but assays are being developed that may help make a preclinical diagnosis.

CONFLICTS

Of all the pathogens covered in this book, prions win the human–pathogen conflict. However, one could argue that they may win the battle but lose the war, as they kill the host and cannot be transmitted further. They are resistant to almost all disinfectant procedures and elicit minimal immune responses. They are never exposed to the outside world and cannot therefore be intercepted. They have no nucleic acids and no metabolic systems, so cannot be targeted by antimicrobial drugs. Prions can arise by mutation and hijack normal protein-folding control, producing abnormal molecules that are resistant to enzymes. Prions can cross from one species to another, and have crossed from animals to humans. Infection is therefore possible from meat-based food products. The presence of prions in meat is hard to detect; once ingested, prions can travel from the intestine to lymphoid and then to nervous tissues, ultimately causing profound and usually fatal changes in the CNS. Genetic characteristics of potential hosts seem to play an important role in determining the course of disease after exposure. Examples of prion-induced diseases are Creutzfeldt–Jakob disease, variant CJD (linked to 'mad cow disease') and kuru. These diseases can be diagnosed but there is currently no effective treatment.

9 The host–parasite relationship

Introduction

Historically, the study of the host–pathogen (parasite) interrelationship has relied on information gained from the study of specific organisms examined under laboratory conditions. However, advances in molecular biology and DNA sequencing have revealed the existence of microorganisms in the host which cannot be cultured or directly observed. This has led to a quest to understand more completely the full range of microorganisms present in the host, collectively referred to as the microbiota and its genetic content – the microbiome. Analysis of the microbiome is an aspect of what is generally referred to as metagenomics: the study of genomic content and diversity in a given environment. In 2007 a concerted large-scale effort in this regard began as The Human Microbiome Project, a 5-year initiative of the United States National Institutes of Health (NIH). As a result, the terms microbiota and microbiome are largely replacing the phrase 'normal flora' although the latter will still be used on occasion in this book. Preceding book chapters have focused primarily on organisms that are disease agents. Small numbers may be found in healthy individuals, but their presence in large numbers is usually associated with pathological changes. The first section of this chapter considers members of the microbiota found in the normal healthy individual, in some cases necessary for normal functioning of the human body but able to cause disease under certain circumstances (e.g. in the newborn or in stressed, traumatized or immunocompromised individuals). Their relationship with the host makes an interesting comparison with that of species that are considered as true parasites or pathogens discussed later in this chapter in the broader context of symbiotic relationships and the evolution of host–parasite relationships.

THE MICROBIOTA AND MICROBIOME

Identifying and understanding the microbiota and microbiome

It has been estimated that humans have approximately 10^{13} cells in the body and something like 10^{14} bacteria (and 100× more genes) associated with them, the majority in the large bowel. Studies of the microbiota and microbiome rely on advances in high-throughput DNA sequencing and extensive DNA sequence databases. Thus, microbial DNA samples can be analysed for (1) the presence of known or new species by comparison with species-specific sequences in a 16S ribosomal gene (see Fig. 2.19) database and (2) potential gene function by comparison of all gene sequences with a database of known genes. Although bacteria are the major contributors to the microbiome, viruses, fungi and protozoa are also regularly found in healthy individuals, but are far less frequent. The microbiome content is a consequence of different body areas (e.g. the skin, nose and mouth and intestinal and urogenital tracts) exposed to, or communicating with, the external environment.

The microbiome is acquired rapidly during and shortly after birth and changes continuously throughout life

The organisms present at any given time are influenced by the age, nutrition and environment of the individual. For example, the bowel microbiome of children in developing countries is quite different from that of children in developed countries. In addition, breast-fed infants have lactic acid streptococci and lactobacilli in their gastrointestinal tracts, whereas bottle-fed children show a much greater variety of organisms. Thus, the human body can be thought of as a complex of microenvironments with characteristic differences in microbial composition. In this context, organisms present at a given body site of least 95% of individuals are considered to represent a core microbiome whereas more minor fluctuating organisms represent the variable microbiome.

The skin is an example of a complex microbiome due to multiple microenvironments

Exposed dry areas are not a good environment for bacteria and consequently have relatively few resident organisms on the surface, whereas moister areas (axillae, perineum, between the toes, scalp) support much larger populations. *Staphylococcus epidermidis* is one of the commonest species, making up some 90% of the aerobes and occurring in densities of 10^3–10^4 / cm^2; *Staph. aureus* may be present in the moister regions.

Anaerobic diphtheroids occur below the skin surface in hair follicles, sweat and sebaceous glands, *Propionibacterium acnes* being a familiar example. Changes in the skin occurring during puberty often lead to increased numbers of this species, which can be associated with acne.

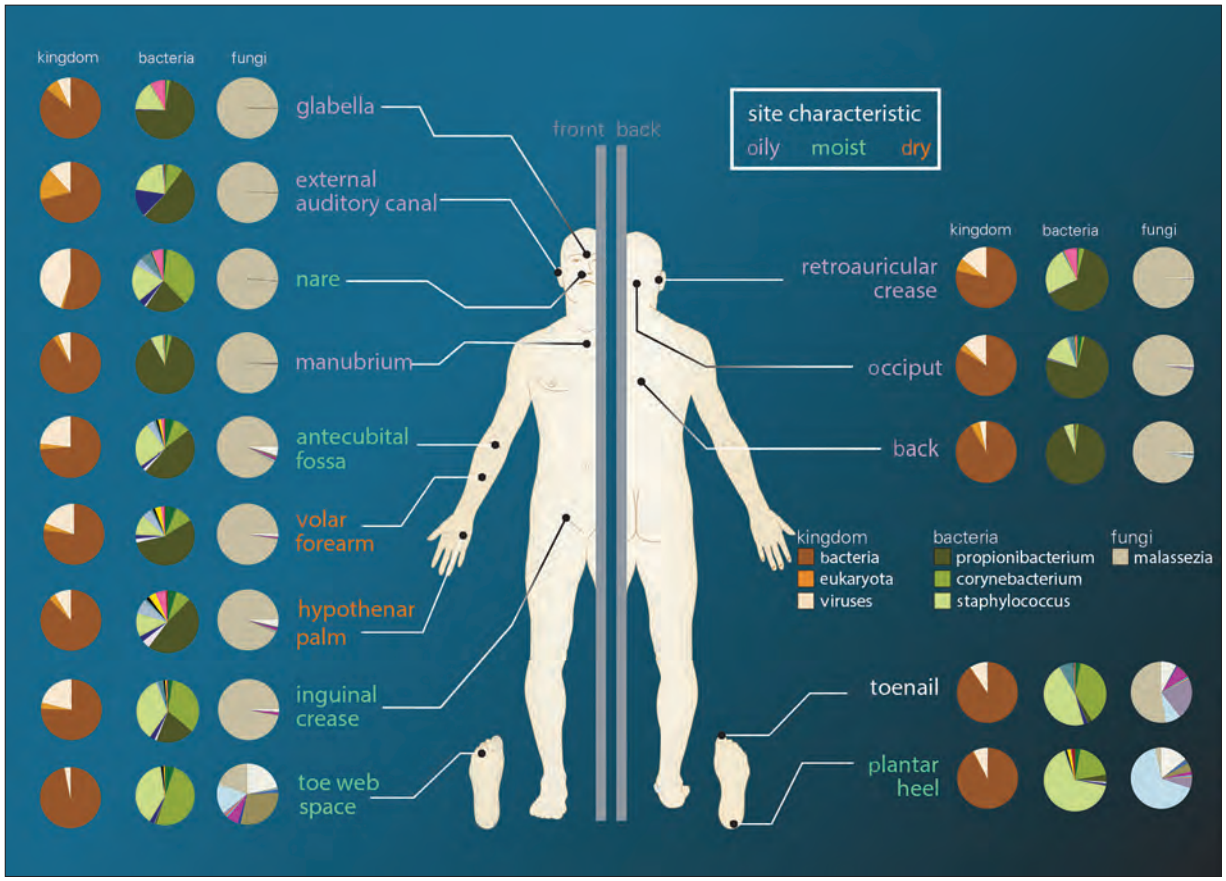


Figure 9.1 Examples of organisms that occur as members of the skin microbiota and their location. (Reproduced with permission from Belkaid Y, Segre JA: Dialogue between skin microbiota and immunity. *Science* 346(6212), 954–959 (Nov 2014), doi: 10.1126/science.1260144.)

A number of fungi, including *Candida*, occur on the scalp and around the nails. They are infrequent on dry skin, but can cause infection in moist skinfolds (intertrigo). An overview of the diversity found in the skin microbiome is shown in Fig. 9.1.

Both the nose and mouth can be heavily colonized by bacteria

The majority of bacteria here are anaerobes. Common species colonizing these areas include streptococci, staphylococci, diphtheroids and Gram-negative cocci. Some of the aerobic bacteria found in healthy individuals are potentially pathogenic (e.g. *Staph. aureus*, *Streptococcus pneumoniae*, *Strep. pyogenes*, *Neisseria meningitidis*); *Candida* is also a potential pathogen.

The mucous membranes of the mouth can have the same microbial density as the large intestine, numbers approaching 10^{11} / g wet weight of tissue.

Dental caries is one of the most common infectious diseases in developed countries

The surfaces of the teeth and the gingival crevices carry large numbers of anaerobic bacteria. Plaque is a film of bacterial cells anchored in a polysaccharide matrix, which the organisms secrete. When teeth are not cleaned regularly, plaque can accumulate rapidly and the activities of certain bacteria, notably *Streptococcus mutans*, can lead to dental decay (caries), as acid fermented from carbohydrates can attack dental enamel. The prevalence of dental decay is linked to diet.

The pharynx and trachea carry their own microbiota

Microorganisms in the pharynx and trachea may include both α- and β-haemolytic streptococci as well as a number of anaerobes, staphylococci (including *Staph. aureus*), *Neisseria* and diphtheroids. The respiratory tract is normally quite sterile, despite the regular intake of organisms by breathing. However, substantial numbers of clinically normal people may carry the fungus *Pneumocystis jirovecii* (previously known as *P. carinii*) in their lungs.

In the gut the density of microorganisms increases from the stomach to the large intestine

The stomach normally harbours only transient organisms, its acidic pH providing an effective barrier. However, the gastric mucosa may be colonized by acid-tolerant lactobacilli and streptococci. *Helicobacter pylori*, which can cause gastric ulcers (see Ch. 23), is carried without symptoms by large numbers of people, the bacterium being in mucus and neutralizing the local acidic environment. The upper intestine is only lightly colonized (10^4 organisms / g), but populations increase markedly in the ileum, where streptococci, lactobacilli, Enterobacteriaceae and *Bacteroides* may all be present. Bacterial numbers are very high (estimated at 10^{12} / g) in the large bowel, and many species can be found (Fig. 9.2). The vast majority (95–99%) is anaerobes, *Bacteroides* being especially common and a major component of faecal material; *E. coli* is also carried by most individuals. *Bacteroides* and *E. coli* are

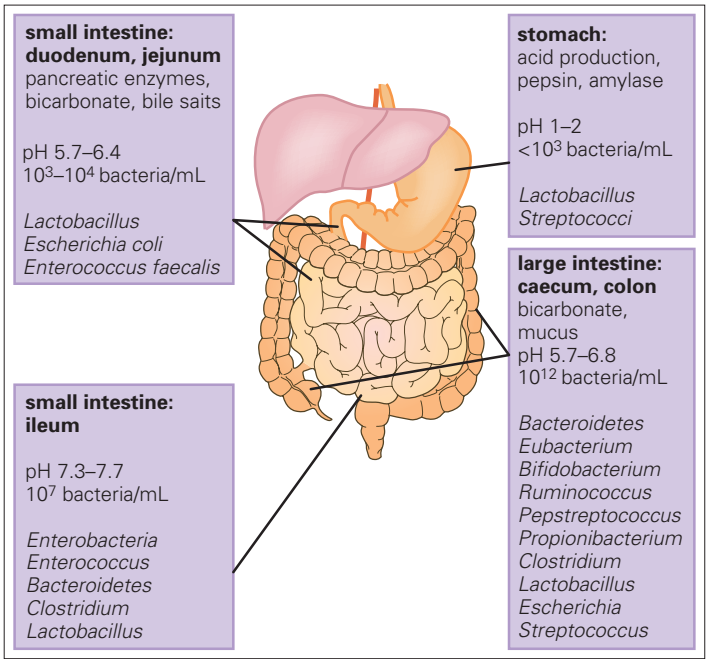


Figure 9.2 Examples of organisms that occur as members of the microbiota of the human gastrointestinal tract and their location. (Redrawn after J.A.Wisnewsky, J. Doré and K. Clement. The importance of the gut microbiota after bariatric surgery. *Nature Reviews Gastroenterology & Hepatology* 9, 590–598 (October 2012), doi:10.1038/nrgastro.2012.161.)

among the species capable of causing severe disease when transferred into other sites in the body. Harmless protozoans can also occur in the intestine (e.g. *Entamoeba coli*) and these can be considered as part of the variable microbiota, despite being animals.

The urethra is lightly colonized in both sexes, but the vagina supports an extensive presence of bacteria and fungi

The urethra in both sexes is relatively lightly colonized, although *Staph. epidermidis*, *Strep. faecalis* and diphtheroids may be present. In the vagina, the composition of bacterial and fungal microbiota undergoes age-related changes:

- Before puberty, the predominant organisms are staphylococci, streptococci, diphtheroids and *E. coli*.
- Subsequently, lactobacilli predominate, fermenting glycogen for the maintenance of an acid pH, which prevents overgrowth by other vaginal organisms.

A number of fungi occur, including *Candida*, which can overgrow to cause the pathogenic condition 'thrush' if the vaginal pH rises and competing bacteria diminish. The protozoan *Trichomonas vaginalis* may also be present in healthy individuals.

Advantages and disadvantages of the microbiota

Studies of the microbiome have confirmed the benefit of various species to the host

The contribution of such species to host health is directly shown in instances of dysbiosis, the disruption or disturbance of the microbiota. Broad-spectrum antibiotic therapy can drastically reduce the presence of beneficial microbiota, and the host may then be over-run by introduced pathogens or by overgrowth of organisms normally present in small numbers. After treatment with clindamycin, overgrowth by *Clostridium difficile*, which survives treatment, can give rise to antibiotic-associated diarrhoea or, more seriously, pseudomembranous colitis.

Ways in which the microbiota inhibits potential pathogens include the following:

- Skin bacteria produce fatty acids, which discourage other species from invading.
- Gut bacteria release a number of factors with antibacterial activity (bacteriocins, colicins) as well as metabolic waste products that help prevent the establishment of other species.
- Vaginal lactobacilli maintain an acid environment, which suppresses growth of other organisms.
- The sheer number of bacteria present in the microbiota of the intestine means that almost all of the available ecological niches become occupied; these species therefore out-compete others for living space.

Gut bacteria also release organic acids, which may have some metabolic value to the host; they also produce B vitamins and vitamin K in amounts that are large enough to be valuable if the diet is deficient. The antigenic stimulation provided by the intestinal flora helps to ensure the normal development of the immune system.

Studies of germ-free animals underscore the importance of the microbiota

Germ-free animals tend to live longer, presumably because of the complete absence of pathogens, and develop no caries (see Ch. 19). However, humans acquire microbiota during and immediately after birth, with the accompaniment of intense immunological activity. Thus, the immune system of germ-free animals is less well developed and they are vulnerable to introduced microbial pathogens, underscoring the important interaction between the microbiota and immune response (see Ch. 13).

Problems arise if members of the microbiota spread into previously sterile parts of the body

Examples of this include:

- when the intestine is perforated or the skin is broken
- during extraction of teeth (when *Streptococcus viridans* may enter the bloodstream)
- when organisms from the perianal skin ascend the urethra and cause urinary tract infection.

Members of the microbiota may cause hospital-acquired infection when patients are exposed to treatments that are invasive or that reduce the host's capacity for immune response. Patients suffering burns are also at risk.

As noted earlier, overgrowth by potential pathogens can occur when the composition of the microflora changes (e.g. after antibiotics) or when:

- the local environment changes (e.g. increases in stomach or vaginal pH)
- the immune system becomes ineffective (e.g. AIDS, clinical immunosuppression).

Under these conditions, the potential pathogens take advantage of the opportunity to increase their population size or invade tissues, so becoming harmful to the host. An account of diseases associated with such opportunistic infections is given in Chapter 31.

SYMBIOTIC ASSOCIATIONS

All living animals are used as habitats by other organisms; none is exempt from such invasion – bacteria are invaded by viruses (bacteriophages) and protozoans have their own microbiota – for example, amoebas are natural hosts for *Legionella pneumophila* infection. As evolution has produced larger, more complex and better-regulated bodies, it has increased the number and variety of habitats for other organisms to colonize. The most complex bodies, those of birds and mammals (including humans), provide the most diverse environments and are the most heavily colonized. Relationships between two species – interspecies associations or symbiosis – are therefore a constant feature of all life.

As the microbiota demonstrates, disease is not the inevitable consequence of interspecies associations between humans and microbes. Many factors influence the outcome of a particular association, and organisms may be pathogenic in one situation but harmless in another. To understand the microbiological basis of infectious disease, host–microbe associations that can be pathogenic need to be placed firmly in the context of other symbiotic relationships, such as commensalism or mutualism, where the outcome for the host does not normally involve any damage or disadvantage.

Commensalism, mutualism and parasitism are categories of symbiotic association

All associations in which one species lives in or on the body of another can be grouped under the general term 'symbiosis' (literally 'living together'). Symbiosis has no overtones of benefit or harm and includes a wide diversity of relationships. Attempts have been made to categorize types of association very specifically, but these have failed because all associations form part of a continuum (Fig. 9.3). Three broad categories of symbiosis – commensalism, mutualism and parasitism – can be identified on the basis of the relative benefit obtained by each partner. None of these categories of association is restricted to any particular taxonomic group. Indeed, some organisms can be commensal, mutualist or parasitic depending upon the circumstances in which they live (Fig. 9.4).

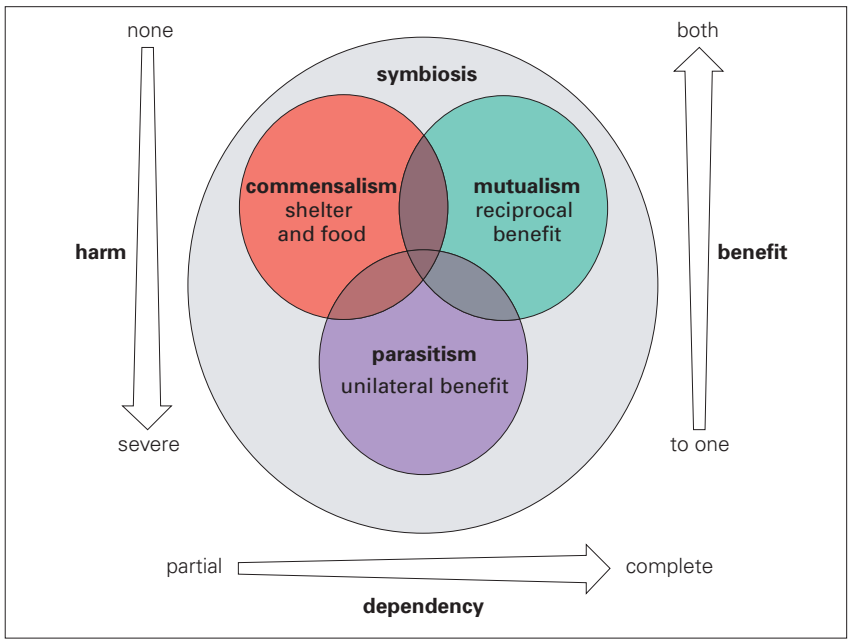
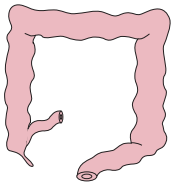


Figure 9.3 The relationships among symbiotic associations. Most species are independent of other species or rely on them only temporarily for food (e.g. predators and their prey). Some species form closer associations termed 'symbioses' and there are three major categories – commensalism, mutualism and parasitism – though each merges with the other and no definition separates one absolutely from the others.

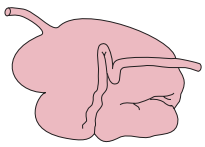
commensalism – large intestine of humans



Bacteroides spp.

Host provides environment. Bacteria ferment digested food. Present in large numbers (10^{10}/g) but usually harmless. May be harmful if tissues damaged (surgery), gut flora changes (antibiotics), or immunity reduced.

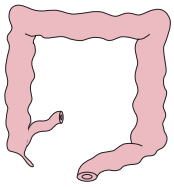
mutualism –rumen of cattle



Bacteroides spp.

Host provides environment. Bacteria metabolize host food to fatty acids and gases. Host uses fatty acids as energy source.

parasitism – large intestine of humans



Entamoeba histolytica

Host provides environment. Protozoa feed on mucosa causing ulcers and dysentery.

Figure 9.4 Examples of commensalism, mutualism and parasitism. These examples show how difficult it is to categorize any organism as entirely harmless, entirely beneficial or entirely harmful.

Commensalism

In commensalism, one species of organism lives harmlessly in or on the body of a larger species

At its simplest, a commensal association is one in which one species of organism uses the body of a larger species as its physical environment and may make use of that environment to acquire nutrients.

Like all animals, humans support an extensive commensal microbiota on the skin, in the mouth and in the alimentary tract. The majority of these microbes are bacteria, and their relationship with the host may be highly specialized, with specific attachment mechanisms and precise environmental requirements. Normally, such microbes are harmless, but they can become harmful if their environmental conditions change in some way (e.g. *Bacteroides*, *E. coli*, *Staph. aureus*). Conversely, the ability of the intestinal microbiota to prevent colonization by more pathogenic species could also be considered mutualistic. Thus, the normal definition of commensalism is not very exact, as the association can merge into mutualism or parasitism.

Mutualism

Mutualistic relationships provide reciprocal benefits for the two organisms involved

Frequently, the relationship is obligatory for at least one member, and may be for both. Good examples are the bacteria and protozoa living in the stomachs of domestic ruminants, which play an essential role in the digestion and utilization of cellulose, receiving in return both the environment and the nutrition essential for their survival. The dividing line between commensalism and mutualism can be hard to draw. In humans, good health and resistance to colonization by pathogens can depend upon the integrity of the normal commensal enteric bacteria, many of which are highly specialized for life in the human intestine, but there is certainly no strict mutual dependence in this relationship.

Parasitism

In parasitism, the symbiotic relationship benefits only the parasite

The terms 'parasites' and 'parasitism' are sometimes thought to apply only to protozoans and worms, but all pathogens are parasites. Parasitism is a one-sided relationship in which the benefits go only to the parasite, the host providing parasites with their physicochemical environment, their food, respiratory and other metabolic needs, and even the signals that regulate their development. Although parasites are thought of as necessarily harmful, this is a view coloured by human and veterinary clinical medicine, and by the results of laboratory experimentation. In fact, many 'parasites' establish quite innocuous associations with their natural hosts but may become pathogenic if there are changes in the host's health or they infect an unnatural host; the rabies virus, for example, coexists harmlessly with many wild mammals but can cause fatal disease in humans. This state of 'balanced pathogenicity' is sometimes explained as the outcome of selective pressures acting upon a relationship over a long period of evolutionary time. It may reflect selection of an increased level of genetically determined resistance in the host population and decreased pathogenicity in the parasite (as has happened with myxomatosis in rabbits). Alternatively, it may be the evolutionary norm, and 'unbalanced pathogenicity' may simply be the consequence of organisms becoming established in 'unnatural' (i.e. new) hosts. Thus, like the other categories of symbiosis, parasitism is impossible to define exclusively except in the context of clear-cut and highly pathogenic organisms. The belief that the ability to cause harm is a necessary characteristic of a parasite is difficult to sustain in any broader view (though it is a convenient assumption for those working with infectious diseases) and the reasons for this are discussed in more detail below.

THE CHARACTERISTICS OF PARASITISM

Many different groups of organisms are parasitic and all animals are parasitized

Parasitism as a way of life has been adopted by many different groups of organisms. Some groups, such as viruses, are exclusively parasitic (see below), but the majority include both parasitic and free-living representatives. Parasites occur in all animals, from the simplest to the most complex, and are an almost inevitable accompaniment of organized

animal existence. We can see, then, that parasitism has been an evolutionary success; as a way of life, it must confer very considerable advantages.

Parasitism has metabolic, nutritional and reproductive advantages

The most obvious advantage of parasitism is metabolic. The parasite is provided with a variety of metabolic requirements by the host, often at no energy cost to itself, so it can devote a large proportion of its own resources to replication or reproduction. This one-sided metabolic relationship shows a broad spectrum of dependence, both within and between the various groups of parasites. Some parasites are totally dependent upon the host, whereas others are only partly dependent.

Viruses are completely dependent upon the host for all their metabolic needs

Viruses are at one extreme of the 'parasite dependency' spectrum. They are obligate parasites, possessing the genetic information required for production of new viruses, but none of the cellular machinery necessary to transcribe or translate this information, to assemble new virus particles or to produce the energy for these processes. The host provides not only the basic building blocks for the production of new viruses, but also the synthetic machinery and the energy required. Retroviruses go one stage further in dependence, inserting their own genetic information into the host's DNA in order to parasitize the transcription process. Viruses therefore represent the ultimate parasitic condition and are qualitatively different from all other parasites in the nature of their relationship with the host (see Ch. 3).

The basis for the fundamental difference between viruses and other parasites is the difference between virus organization and the cellular organization of prokaryotic and eukaryotic parasites. Non-viral parasites have their own genetic and cellular machinery, and multienzyme systems for independent metabolic activity and macromolecular synthesis. The degree of reliance on the host for nutritional requirements varies considerably and follows no consistent pattern between the various groups, nor does it follow that smaller parasites tend to be more dependent (e.g. some of the largest parasites, the tapeworms, are wholly reliant upon the host's digestive machinery to provide their nutritional needs). All, of course, receive nutrition from the host but, whereas some use macromolecular material (proteins, polysaccharides) of host origin and digest it using their own enzyme systems, others rely on the host for the process of digestion as well, being able to take up only low-molecular-weight materials (amino acids, monosaccharides). Nutritional dependence may also include host provision of growth factors that the parasite is unable to synthesize itself. All internal parasites rely upon the host's respiratory and transport systems to provide oxygen, although some respire anaerobically in either a facultative or obligate manner.

Parasite development can be controlled by the host

The advantage that parasitism confers in reproductive terms makes it vital to coordinate parasite development with the availability of suitable hosts. Indeed, one of the characteristic features of parasites is that their development may be controlled partly or completely by the host, the parasite having lost the ability to initiate or to regulate its own development. At its simplest, host control is limited to providing the cell surface molecules necessary for parasite attachment and internalization. Many parasites, from viruses to protozoa, rely on the recognition of such molecular signals for their entry into host cells, and this process provides the trigger for their replicative or reproductive cycles.

Other parasites, primarily the eukaryotes, require more comprehensive and sophisticated signals, often a complex of signals, to initiate and regulate their entire developmental cycle. The complexity of the signal required for development is one of the factors determining the specificity of the host–parasite relationship. Where the availability of one of the signals entails that parasite development can occur in only one species, host specificity is high. Where many host species are capable of providing the necessary signals for a parasite, specificity is low.

Disadvantages of parasitism

The most obvious disadvantage of parasitism arises from the fact that the host controls the development of the parasite. No development is possible without a suitable host, and many parasites will die if no host becomes available. For this reason, several adaptations have evolved to promote prolonged survival in the outside world and so maximize the chances of successful host contact (e.g. virus particles, bacterial spores, protozoan cysts and worm eggs). The prolific replication of parasites is another device to achieve the same end. Nevertheless, where parasites fail to make contact with a host, their powers of survival are ultimately limited. Adaptation to host signals can therefore have a reproductive cost (i.e. the loss of many potential parasites).

THE EVOLUTION OF PARASITISM

As so many organisms are parasitic and every group of animals is subject to invasion by parasites, the development of parasitism as a way of life must have occurred at an early stage in evolution and at frequent intervals thereafter. How this occurred is not fully understood, and it may well have been different in different groups of organisms. In many, parasitism most probably arose as a consequence of accidental contacts between organism and host. Of many such contacts, some would have resulted in prolonged survival and, under favourable nutritional circumstances, prolonged survival would have been associated with enhanced replication, giving the organism a selective advantage within the environment. Many parasites of humans and other mammals may have originated via the route of accidental contact, but it is clear that others have become adapted to these hosts after initially becoming parasitic in other species. For example, parasites of blood-feeding arthropods have ready access to the tissues of the animals on which the arthropods feed. Where the parasite becomes specialized for the non-arthropod host it may lose the ability to be transmitted by blood feeding. Where the arthropod host is retained in the life cycle the parasite is faced by competing demands for survival in each host, which probably explains why, for example, arboviruses are restricted to only a few families of RNA viruses and a single DNA virus, African swine fever virus.

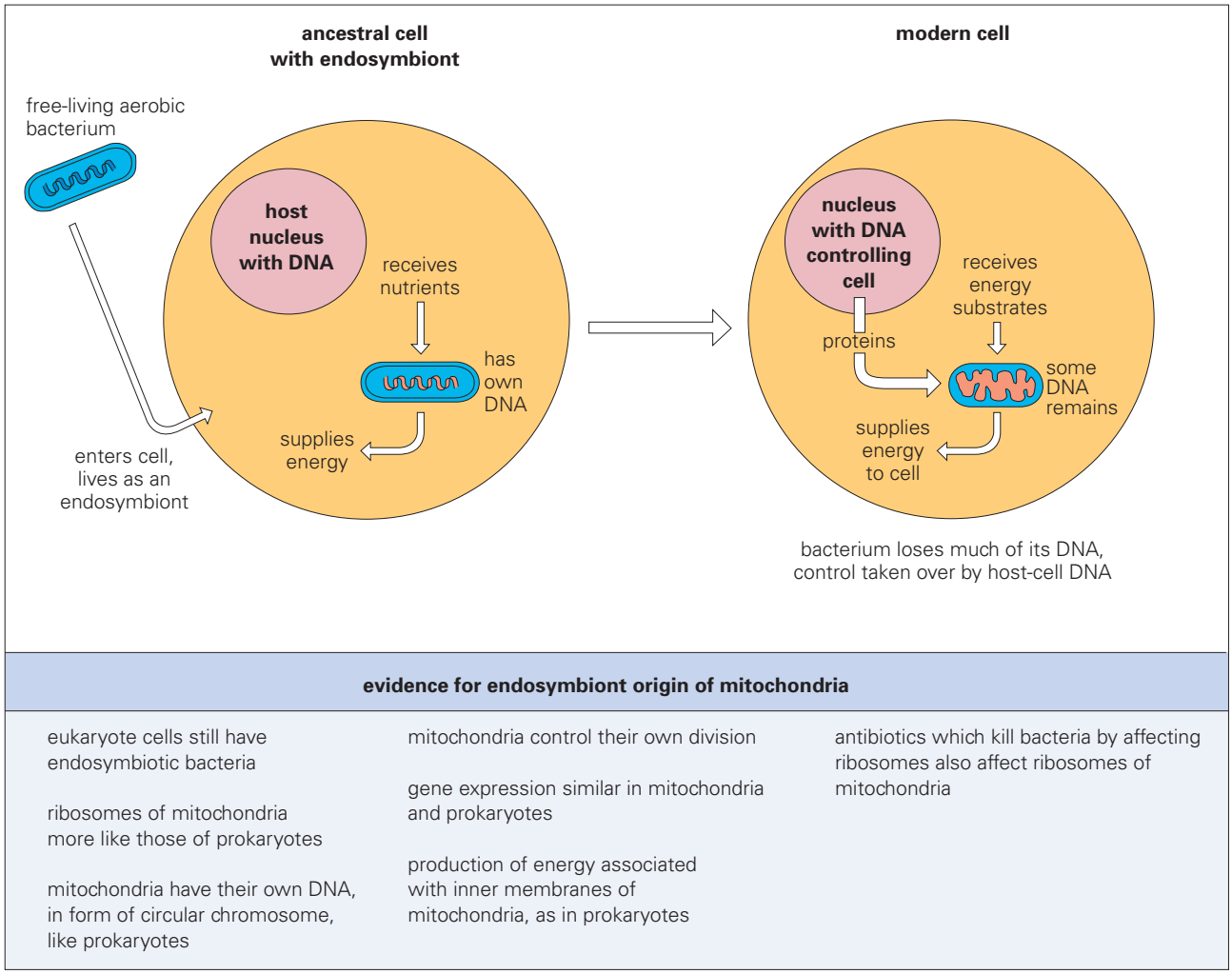


Figure 9.5 The evolution of mitochondria. Many lines of evidence suggest that mitochondria of modern eukaryote cells evolved from bacteria that established symbiotic (mutualistic) relationships with ancestral cells.

Bacterial parasites evolved through accidental contact

In the case of bacteria, it is easy to see how accidental contact in environments rich in free-living bacteria could lead to successful invasion of external openings such as the mouth and eventual colonization of the gastrointestinal tract. Initially, the organisms concerned would have had to be facultative parasites, capable of life both within or outside host organisms (many pathogenic bacteria still have this property, e.g. *Legionella*, *Vibrio*), but selective pressures would have forced others into obligatory parasitism. Such events are of course speculative, but are supported by the close relationship of enteric bacteria such as *E. coli* with free-living photosynthetic purple bacteria.

Many bacterial parasites have evolved to live inside host cells

Bacteria that became parasitic by accidental contact would have lived outside host cells at first and would not have had the advantages of being intracellular. The evolution of the intracellular habit required further modifications to allow survival within host cells, but could easily have been initiated by passive phagocytic uptake. Subsequent survival of the pathogen would depend upon the possession of surface or metabolic properties that prevented digestion and destruction by the host cell. The success of intracellular life can be measured not only by the large number of bacteria that have adopted this habit, but also by the extent to which some organisms have integrated their biology with that of the host cell. The end point of such integration is perhaps to be seen in the evolution of the eukaryote mitochondrion, which may have evolved from symbiotically associated heterotrophic purple bacteria (Fig. 9.5).

The pathway of virus evolution is uncertain

Clearly, parasitism by bacteria, which are undoubtedly ancient organisms (they can be traced back 3–5 billion years in the fossil record), depended upon the evolution of higher organisms to act as hosts. Whether the same is true of viruses is open to question, and depends upon whether viruses are considered primarily or secondarily simple. If viruses evolved from cellular ancestors by a process of secondary simplification, then parasitism must have evolved

long after the evolution of prokaryotes and eukaryotes. If viruses are primitively non-cellular then it is possible that they became parasitic at a very early stage in the evolution of cellular life, at some point when, because of environmental change, independent existence became impossible. A third alternative is that viruses were never anything other than fragments of the nuclear material of other organisms and have in effect always been parasitic. Modern viruses may, in fact, have arisen by all three pathways.

Eukaryote parasites have evolved through accidental contact

The evolution of parasitism by eukaryotes is likely to have arisen much as it may have done in prokaryotes (i.e. through accidental contact and via blood-feeding arthropods). Examples can be found among protozoan and worm parasites to support this view:

- There are protozoa such as the free-living amoeba *Naegleria* which can opportunistically invade the human body and cause severe and sometimes fatal disease.
- There are several species of nematode worms that can live either as parasites or as free-living organisms, *Strongyloides stercoralis* being the most important in humans.
- It is likely that trypanosomes (the protozoans responsible for sleeping sickness) were primarily adapted as parasites of blood-feeding flies and only secondarily became established as parasites of mammals, though most retain the arthropod in their life cycle.

Parasite adaptations to overcome host inflammatory and immune responses

We can view the evolution of parasitism and the adaptations necessary for life within another animal as being exactly analogous to the adaptations necessary for life within any other specialized habitat: the environment in which parasites live is merely one of the many to which organisms have become adapted in evolution (comparable with life in soil, fresh water, salt water, decaying material and so on). However, it is always necessary to remember that in one major respect parasitism is quite different from any other specialist mode of life. This difference is that the environment in which a parasite lives, the body of the host, is not passive; on the contrary, it is capable of an active response to the presence of the parasite.

The attractiveness of animal bodies as environments for parasites means that hosts are under continual pressures from infection, and these pressures are increased when hosts live:

- close together
- in insanitary conditions
- in climates that favour the survival of parasite stages in the external world.

Pressure of infection has been a major influence in host evolution

Pressure of infection has been a major selective influence in evolution, and there is little doubt that it has been largely responsible for the development of the sophisticated inflammatory and immune responses we see in humans and other mammals. In evolutionary terms, all infection has its costs to the host because it diverts valuable resources from the activities of survival and reproduction; there has therefore been pressure to develop means of overcoming infection whether or not it causes disease. Of course, this is not the focus of clinical microbiology, which legitimately places emphasis on the costs of infection in terms of frank disease, but it should be remembered because it explains more fully the nature of the continuing battle between host and parasite – the former attempting to contain or destroy, the latter attempting to evade or suppress – and why the emergence of new, and the return of old, infectious diseases are a constant threat.

Parasites are faced with the problems not only of surviving within the environment they experience initially, but also of surviving in that environment as it changes in ways that are likely to be harmful to them. The inflammatory and immune responses that follow the establishment of infection are the most important means by which the host can control infections by those organisms able to penetrate its natural barriers and survive within its body. These responses represent formidable obstacles to the continued survival of parasites, forcing them to evolve strategies to cope with harmful changes in their environment. The successful parasite is therefore one that can cope with, or evade, the host's response in one of the ways shown in Table 9.1.

All of these adaptations are known to exist within different groups of parasites and they are well documented in the case of some of the major human pathogens. Indeed, they are often the very reason why such organisms are major pathogens.

Table 9.1 Evasion strategies of parasites

Evasion strategies	
Strategy	**Example**
Elicit minimal response	Herpes simplex virus – survives in host cells for long periods in a latent stage – no pathology
Evade effects of response	Mycobacteria – survive unharmed in granulomas designed to localize and destroy infection
Depress host's response	HIV – destroys T cells; malaria – depresses immune responsiveness
Antigenic change	Viruses, spirochaetes, trypanosomes – all change target antigens so host response is ineffective
Rapid replication	Viruses, bacteria, protozoa – producing acute infections before recovery and immunity
Survival in weakly responsive individuals	Genetic heterogeneity in host population means some individuals respond weakly or not at all, allowing organism to reproduce freely; examples in all groups

Table 9.2 Lifestyle changes and infectious diseases

Social and behavioural changes and infectious diseases	
The causes	**The results**
Altered environments (e.g. air conditioning)	Water in cooling systems provides growth conditions for *Legionella*
Changes in food production and food-handling practices	Intensive husbandry under antibiotic protection leads to drug-resistant bacteria; deep-freeze, fast-food and inadequate cooking allow bacteria and toxins to enter body (e.g. *Listeria*, *Salmonella*)
Routine use of antibiotics in medicine	Emergence of antibiotic-resistant bacteria as hazards to hospitalized patients (e.g. MRSA – methicillin-resistant *Staphylococcus aureus*)
Routine use of immunosuppressive therapy	Development of opportunistic infections in patients with reduced resistance (e.g. *Pseudomonas*, *Candida*, *Pneumocystis*)
Altered sexual habits	Promiscuity increases sexually transmitted diseases (e.g. gonorrhoea, genital herpes, AIDS)
Breakdown of filtration systems, overuse of limited water supplies	Transmission of animal infections leading to diarrhoeal and other infections (e.g. cryptosporidiosis, giardiasis, leptospirosis)
Increase in ownership of pets, particularly exotic species	Transmission of animal infections (e.g. *Chlamydia*, *Salmonella*, *Toxoplasma, Toxocara)*
Increased frequency of journeys to tropical and subtropical countries	Exposure to exotic organisms and vectors (e.g. malaria, viral encephalitis)

Nevertheless, transmission and survival of many parasites depends upon the existence of particularly susceptible host individuals (e.g. children) to provide a continuing reservoir of infective stages.

Changes in parasites create new problems for hosts

From what has been said above, it can be appreciated that there is no such thing as a static host–parasite relationship, and that concepts of unchanging 'pathogenicity' or 'lack of pathogenicity' cannot be justified. Each relationship is an 'arms race'; changes in one member being countered by changes in the other. Quite subtle changes in either can completely change the balance of the relationship, towards greater or lesser pathogenicity, for example.

One of the most important and dramatic illustrations of this situation has been the explosive appearance of HIV infections. This group of viruses was originally restricted to non-human primates, but changes in the virus permitted extensive infections in humans. Similarly, changes in the SARS coronavirus allowed human infection from bats and there is concern about the avian virus H5N1, which is able to spread to humans from infected poultry. Of a different nature, but relevant to the general theme, is the acquisition of drug resistance in bacteria, viruses and protozoa (see Ch. 34). Although the underlying genetic and metabolic changes do not by themselves influence pathogenicity, the expression of such changes in the face of intense and selective chemotherapy certainly does, allowing overwhelming infection to occur with major concerns regarding diminished therapeutic options.

Host adaptations to overcome changes in parasites

Changes in the host can also alter the balance of a host–parasite relationship. A particularly dramatic example is the intense selection for resistant genotypes in rabbit populations exposed to the myxomatosis virus, which took place concurrently with selection for reduced pathogenicity in the virus itself (see Ch. 13). There are no exactly equivalent examples in humans, but in evolutionary time there have been major selective influences on populations prompting changes to permit survival in the face of life-threatening infections. A good example is the selective pressure exerted by falciparum malaria, which has been responsible for the persistence in human populations of many alleles associated with haemoglobinopathies (e.g. sickle cell haemoglobin). Although these abnormalities are detrimental to varying degrees, they persist because they are (or were) associated with resistance to malarial infection. Studies suggest that malaria may change the frequency of certain HLA antigens in areas where infection is severe.

Social and behavioural changes can be as important as genetic changes in altering host–parasite relations

Social and behavioural changes can alter host–parasite relations both positively and negatively (Table 9.2). Although many bacterial infections of the intestine have declined in importance with changes in human lifestyle, there are other contemporary microbiological problems in the resource-rich world whose onset can be traced directly to sociological, environmental and even medical change. A particularly good example is disease arising from domestication of pets (e.g. toxoplasmosis) because it illustrates that human freedom from some infections arises primarily because of lack of contact with the organisms and not from any innate resistance to the establishment of the infection itself. Diseases arising from contact with infected animals or animal products (zoonotic infections) constitute a constant threat that can be realized by behavioural or environmental changes that alter established patterns of human–animal contact.

KEY FACTS

- The body is colonized by many organisms (the microbiota), which can be positively beneficial. They live on or within the body without causing disease, and play an important role in protecting the host from pathogenic microbes.
- The microbiota is predominantly made up of bacteria, but includes fungi and protozoa.
- Members of the microbiota can be harmful if they enter previously sterile parts of the body. They can also be causes of hospital-acquired infections.
- The usual relationship between the microbiota and the body is an example of beneficial symbiosis; parasitism (in the broad sense, covering all pathogenic microbes) is a harmful symbiosis.
- The biological context of host–parasite relationships, and the dynamics of the conflict between two species in this relationship, provide a basis for understanding the causes and control of infectious diseases.
- Changes in medical practice, in human behaviour and, not least, in infectious organisms, are broadening the spectrum of organisms responsible for disease.

10 The innate defences of the body

Introduction

The immune system has a challenge. It needs to defend us against pathogens that range in size from the smallest viruses to the large helminth worms. These pathogens may also infect us by different routes, including by aerosol, by ingestion, through the skin, or through sexual contact. In the preceding chapters, we have outlined some of the fundamental characteristics of the many types of microparasites and macroparasites (here collectively called pathogens) that may infect the body. We now turn to consider the ways in which the body seeks to defend itself against infection by these organisms, starting with the innate immune responses that are the first line of defence.

The body has both 'innate' and 'adaptive' immune defences

When an organism infects the body for the first time, the defence systems already in place may well be adequate to prevent replication and spread of the infectious agent, thereby preventing development of disease. These established mechanisms are referred to as constituting the 'innate' immune system. However, should innate immunity be insufficient to deal with the invasion by the infectious agent, the 'adaptive' immune system then comes into action, although it takes time to reach its maximum efficiency (Fig. 10.1). When it does take effect, it generally eliminates the infective organism, allowing recovery from disease.

The main feature distinguishing the adaptive response from the innate mechanism is that specific memory of infection is imprinted on the adaptive immune system, so that should there be a subsequent infection by the same agent a particularly effective response comes into play with remarkable speed. It is worth emphasizing, however, that there is close synergy between the two systems, with the adaptive mechanism greatly improving the efficiency of the innate response and vice versa, and that innate immunity does show some evidence of 'memory'.

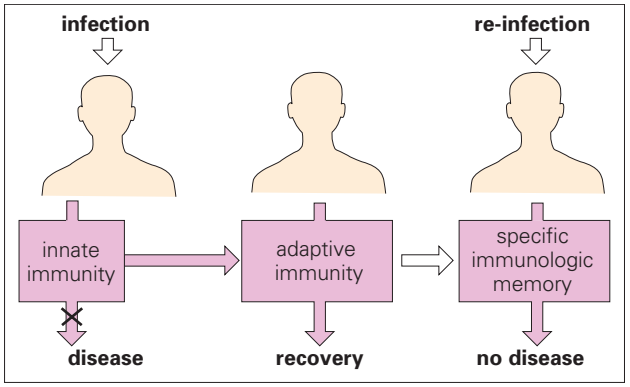


Figure 10.1 Innate and adaptive immunity. An infectious organism first encounters the cells and molecules of the innate immune system. If these do not prevent infection, the adaptive immune system is needed with its specific and specialized cells and mediators. Following recovery, specific immunological memory will prevent re-infection.

The contrasts between these two systems are set out in Table 10.1. On the one hand, the soluble factors such as lysozyme and complement, together with the phagocytic cells, contribute to the innate system, while on the other the T- and B-lymphocyte-based mechanisms that produce cytokines and toxicity or antibodies are the main elements of the adaptive immune system. Not only do these lymphocytes provide improved resistance by repeated contact with a given infectious agent, but also the memory with which they become endowed shows very considerable specificity to that infection. For instance, infection with measles virus will induce a memory to that microorganism alone and not to another virus such as rubella.

DEFENCES AGAINST ENTRY INTO THE BODY

A variety of biochemical and physical barriers operate at the body surfaces

Before an infectious agent can penetrate the body, it must overcome biochemical and physical barriers that operate at the body surfaces. One of the most important of these is the skin, which is normally impermeable to the majority of infectious agents. Many bacteria fail to survive for long on the skin because of the direct inhibitory effects of lactic acid and fatty acids present in sweat and sebaceous secretions and the lower pH to which they give rise (Fig. 10.2). However, should there be skin loss, as can occur in burns, for example, infection becomes a major problem.

The membranes lining the inner surfaces of the body secrete mucus, which acts as a protective layer outside the epithelium,

inhibiting the adherence of bacteria to the epithelial cells, thereby preventing them from gaining access to the body. Microbial and other foreign particles trapped within this adhesive mucus may be removed by mechanical means such as ciliary action, coughing and sneezing. The flushing actions of tears, saliva and urine are other mechanical strategies that help to protect the epithelial surfaces. In addition, many of the secreted body fluids contain microbicidal factors (e.g. the acid in gastric juice, spermine and zinc in semen, lactoperoxidase in milk, and lysozyme in tears, nasal secretions and saliva).

Harmless commensal organisms that are part of our microbiome (see Ch. 9) also protect us through microbial antagonism. These commensal organisms suppress the growth of many potentially pathogenic bacteria and fungi at superficial sites, first by virtue of their physical advantage of previous occupancy, especially on epithelial surfaces, second by competing for essential nutrients, and third by producing inhibitory substances such as acid or colicins. The latter are a class of bactericidins that have a number of modes of action including binding to the negatively charged surface of susceptible bacteria and forming a voltage-dependent channel in the membrane, which kills by destroying the cell's energy potential.

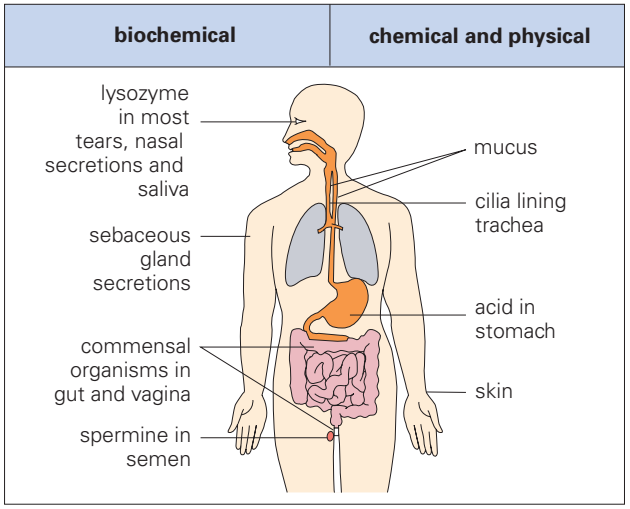


Figure 10.2 Exterior defences. Most of the infectious agents encountered by an individual are prevented from entering the body by a variety of biochemical and physical barriers. The body tolerates a huge number of commensal, harmless, organisms, now collectively called the microbiome; these organisms may prevent pathogens from invading through competition, and also have a major impact on immune function.

DEFENCES ONCE THE MICROORGANISM PENETRATES THE BODY

Despite the general effectiveness of the various barriers, microorganisms can often successfully penetrate the body. When this occurs, two main defensive strategies come into play, based on:

- the destructive effect of soluble antimicrobial factors, such as defensins and cathelicidin
- the mechanism of phagocytosis, involving engulfment and killing of microorganisms by specialized cells, the 'professional phagocytes'.

There are two types of antimicrobial molecules secreted by epithelial cells as well as by phagocytic cells. The **defensins**, which are small cationic peptides, are directly toxic to not only bacteria but also fungi and encapsulated viruses; some are made by epithelial cells in mucosa and Paneth cells in the gut, others by neutrophils and cytotoxic T cells. **Cathelicidin** is another useful molecule with antibacterial effects (see Ch. 15).

Phagocytosis

The innate immune system has an efficient way of removing and killing pathogens – phagocytosis. Phagocytes engulf the pathogen, and if we are lucky, kill them. The phagocytes consist of two major cell families, as originally defined by Elie Metchnikoff, the Russian zoologist (Box 10.1; Fig. 10.3):

- the larger macrophages, which are resident in tissues that develop from monocytes circulating in the blood

Table 10.1 Comparison of innate and adaptive effector immune systems

	Innate immune system	Adaptive immune system
Major elements		
Soluble factors	Lysozyme, complement, acute phase proteins, e.g. C-reactive protein, interferon, other cytokines	Antibody Cytokines
Cells	Phagocytes Innate lymphoid cells including natural killer cells	T lymphocytes B lymphocytes
Response to microbial infection		
First contact	+	+
Second contact	+	+ + +
	Broad specificity; no specific memory Resistance not improved by repeated contact	Antigen specificity; specific memory Resistance improved by repeated contact

Innate immunity is sometimes referred to as 'natural', and adaptive as 'acquired'. The two systems are bridged by innate lymphoid cells, and innate immunity is needed for an effective adaptive immune response. Humoral immunity due to soluble factors uses antibody (made by B cells) to provide specific protection whereas cellular immunity relies on antigen-specific T cells. If the same organism persists or is encountered a second time, a more effective specific adaptive response to its antigens is induced. Although this immunological memory largely relies on memory B cells and memory T cells, some cells of the innate immune system can show a form of memory although this lacks the antigen specificity shown by T and B cells.

Box 10.1 Lessons in Microbiology

Elie Metchnikoff – the father of phagocytosis

Metchnikoff (1845–1916) was a Russian zoologist who became fascinated by how cells deal with bacteria. He observed that if he introduced a rose thorn into a transparent starfish larva the thorn became surrounded by motile cells. He then went on to investigate mammalian leukocytes, showing that they could engulf microorganisms, which he termed phagocytosis. He defined two types of circulating phagocytes: the smaller microphage (now called a polymorphonuclear leukocyte) and the larger macrophage. We now know that phagocytosis is further enhanced when the humoral components antibody and complement are present.

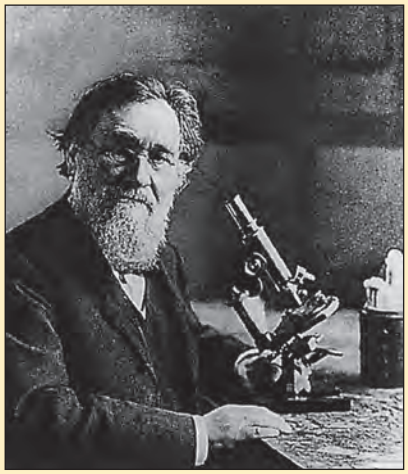


Figure 10.3 Elie Metchnikoff, the father of phagocytosis. (Courtesy of the Wellcome Institute Library, London.)

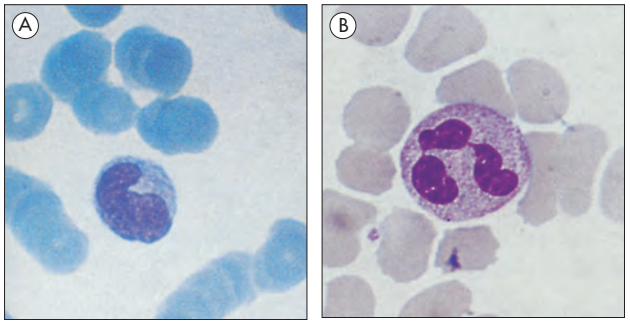


Figure 10.4 Phagocytic cells. (A) Blood monocyte and (B) polymorphonuclear neutrophil, both derived from bone marrow stem cells. (Courtesy of P.M. Lydyard.)

- the smaller neutrophils, which are generally referred to as polymorphs or neutrophils (polymorphonuclear leukocytes, PMNs) because their cytoplasmic granules do not stain with haematoxylin and eosin (Fig. 10.4).

As a very crude generalization, the PMNs provide the major defence against pyogenic (pus-forming) bacteria, whereas the macrophages are best at combating organisms capable of living within the cells of the host. Neutrophils are closely related to eosinophils and basophils but are more phagocytic.

Most monocytes in the blood are termed 'classical' monocytes; a small subset that express both the CD14 and CD16 markers patrol the endothelial surfaces. Dendritic cells, particularly immature dendritic cells, can also phagocytose microorganisms, but less efficiently.

Pathogens can be opsonized or coated by the binding of plasma proteins from the complement system. Opsonization increases the efficiency of uptake of the particle or pathogen. This process becomes even more efficient if specific antibodies are around and can bind to Fc receptors on the macrophage surface. The phagocytes have receptors for the C3a and C5a complement components. These complement components are examples of molecules that recognize pathogen-associated molecular patterns or PAMPs. Other phagocytic receptors include dectin-1, the mannose receptor and a group of receptors called scavenger receptors. As well as internalizing the pathogen, other G-protein coupled receptors on phagocytes sense bacteria and trigger killing mechanisms such as production of reactive oxygen species. Once the pathogen is contained within a membrane vesicle called a phagosome that contains the external phagocyte membrane on the inside, the phagosome can fuse with lysosomes that contain an unpleasant mix of digestive enzymes that need an acidic pH to function effectively. So, rather cleverly, some pathogens such as *Mycobacterium tuberculosis* have developed ways of blocking phagosome–lysosome fusion and acidification of the phagolysosome.

Macrophages are widespread throughout the tissues

The majority of tissue-resident macrophages originate during embryogenesis and enter the tissue, where they differentiate into a macrophage that has properties dependent on the site they have entered (Fig. 10.5). They are particularly concentrated in the lung (alveolar macrophages), liver (Kupffer cells) and the lining of lymph node medullary sinuses and splenic sinusoids (Fig. 10.6), where they are well placed to filter out foreign material. Other examples are the brain microglia, kidney mesangial cells, synovial A cells and osteoclasts in bone. These tissue macrophages are long-lived cells that depend upon mitochondria for their metabolic energy and show elements of rough-surfaced endoplasmic reticulum (Fig. 10.7) related to the formidable array of different secretory proteins that these cells generate.

It is also possible for bone marrow promonocytes to develop into circulating blood monocytes (Fig. 10.4), which finally become mature macrophages, which are enriched in tissues in states of disease and inflammation. Collectively these cells are termed the 'mononuclear phagocyte system' (Fig. 10.5).

Macrophages live much longer than neutrophils or monocytes. But they have other interesting properties. If the cytokine interferon gamma (IFNγ) is around, they can become activated, becoming more efficient at killing intracellular pathogens. This is another example of where innate cell function is enhanced by adaptive immunity, as a subset of T cells as well as the innate natural killer (NK) cells make IFNγ. These IFNγ-activated macrophages are called classically activated or M1 macrophages. Other cytokines such as interleukin (IL)-4 and IL-13 drive the development of alternatively activated or M2 macrophages (Fig. 10.8). These

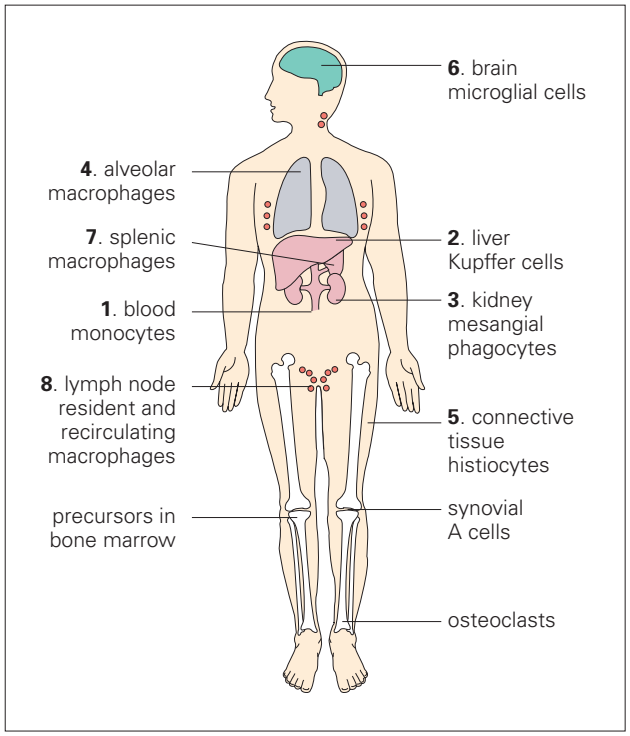


Figure 10.5 The mononuclear phagocyte system. Most tissue macrophages are derived very early in life and differentiate in the organs to which they have homed. (The numbers relate to those in Fig. 10.6.)

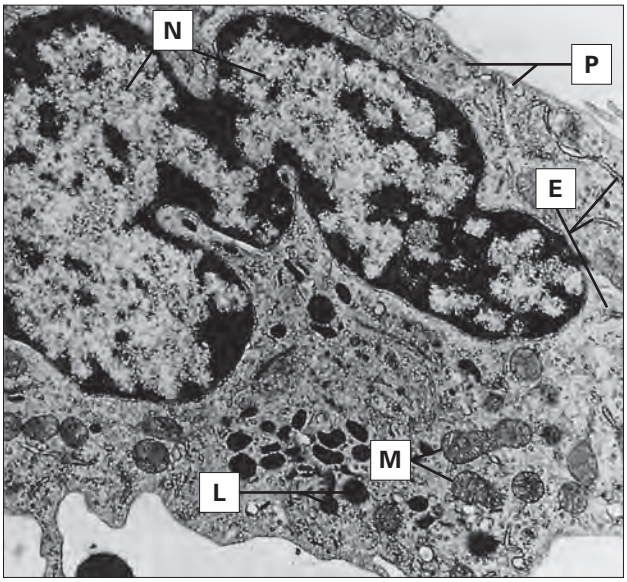


Figure 10.7 Monocyte (×8000), with 'horseshoe' nucleus (N). Phagocytic and pinocytic vesicles (P), lysosomal granules (L), mitochondria (M) and isolated profiles of rough-surfaced endoplasmic reticulum (E) are evident. (Courtesy of B. Nichols; ©Rockefeller University Press.)

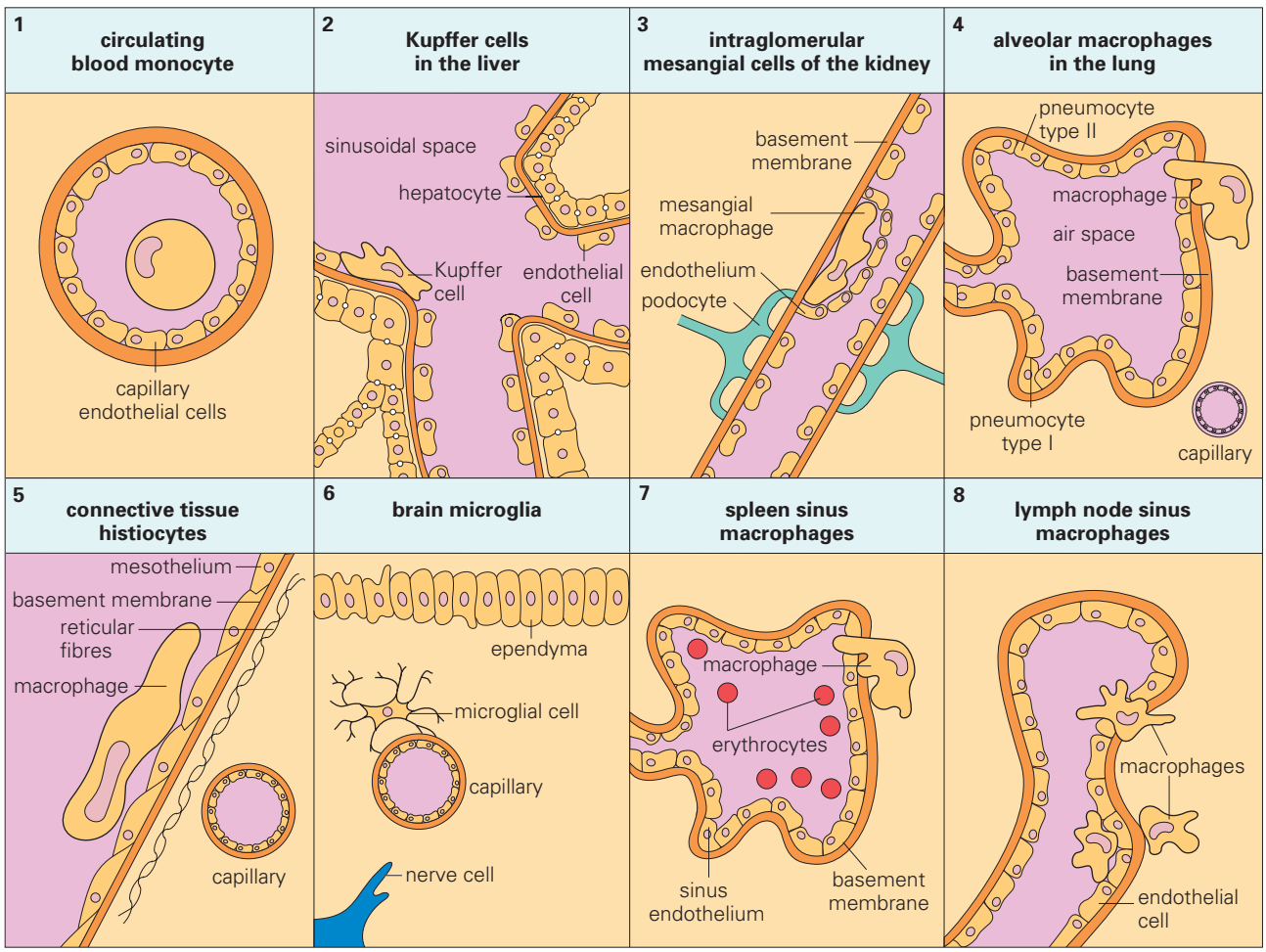


Figure 10.6 Tissue location of mononuclear phagocytes.

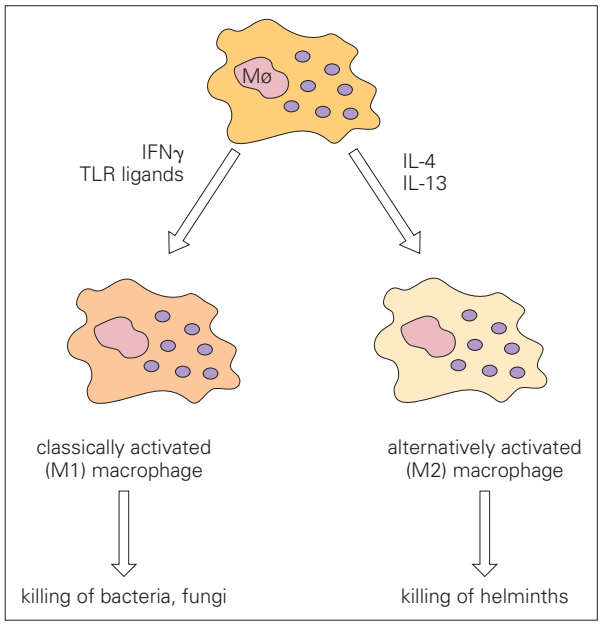


Figure 10.8 Classically and alternatively activated macrophages. Macrophages can be activated in two ways to form activated macrophages that can kill different types of pathogens. IFNγ derived from NK cells, ILC1 cells or Th1 T cells and Toll-like receptor (TLR) ligands will induce classically activated (M1) macrophages; IL-4 and IL-13 from Th2 T cells will induce alternatively activated (M2) macrophages. Alternatively activated macrophages also play a role in tissue repair.

two macrophage subsets play particular roles in our defence against intracellular infections and against helminth infections, as discussed in Chapter 15.

Polymorphs possess a variety of enzyme-containing granules

The polymorph is the dominant white cell in the bloodstream and, like the macrophage, shares a common haemopoietic stem cell precursor with the other formed elements of the blood. It has no mitochondria, but rather uses its abundant cytoplasmic glycogen stores for its energy requirements; therefore, glycolysis enables these cells to function under anaerobic conditions, such as those in an inflammatory focus. The polymorph is a non-dividing, short-lived cell, with a segmented nucleus; the cytoplasm is characterized by an array of granules, which are illustrated in Fig. 10.9 and Table 10.2. Polymorphs can also produce IL-8 as well as other chemokines and cytokines. Polymorphs provide a major defence against extracellular and acute bacterial infections such as with staphylococci or streptococci, but also play a role in chronic infections – in tuberculosis, a very chronic intracellular bacterial infection, there are huge numbers of polymorphs in the lungs that have phagocytosed mycobacteria.

Phagocytosis and killing

How do phagocytes sense infection?

Before the professional phagocyte can phagocytose a microorganism, it must first attach to the phagocyte surface. Pattern recognition receptors (PRRs) on the phagocyte surface bind repeating pathogen-associated molecular patterns (PAMPs) (Fig. 10.10).

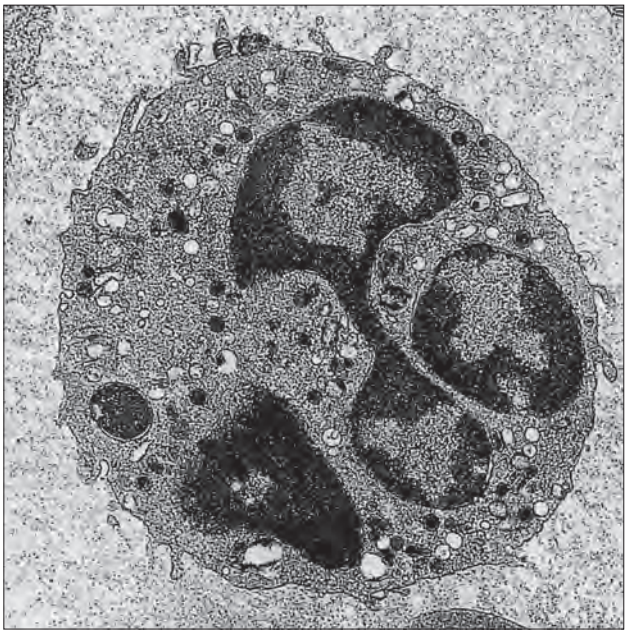


Figure 10.9 The neutrophil. The multi-lobed nucleus and primary azurophilic, secondary specific and tertiary lysosomal granules are well displayed. There is an overlap in the contents between some of the granules. Typical conventional lysosomes with acid hydrolase are also seen. (Courtesy of D. McLaren.)

Table 10.2 Cytoplasmic granules

Primary azurophilic granules	Secondary specific granules	Tertiary granules
Lysozyme	Lysozyme	Lysozyme
Myeloperoxidase	Cytochrome b_{558}	Cytochrome b_{558}
Elastase	OH phosphatase	Gelatinase (MMP9)
Cathepsins	Lactoferrin	
Acid hydrolases	Collagenase matrix metalloproteinase (MMP8)	
Defensins		
Bactericidal permeability increasing protein (BPI)		

The 'Toll-like receptors' (TLRs) are a major family of PPRs. The TLRs are so called because of their similarity to the Toll receptor in the fruit fly, *Drosophila*, which, in the adult fly, triggers an intracellular cascade generating the expression of antimicrobial peptides in response to microbial infection. A series of cell surface TLRs acting as sensors for extracellular infections have been identified (Fig. 10.11) which are activated by microbial elements such as peptidoglycan, lipoproteins, mycobacterial lipoarabinomannan, yeast zymosan and flagellin.

Other PRRs displayed by phagocyte on the cell surface include the cell-bound 'C-type (calcium-dependent) lectins', of which the macrophage mannose receptor is an example, and 'scavenger receptors', which recognize a variety of anionic polymers and acetylated low-density proteins. Some TLRs

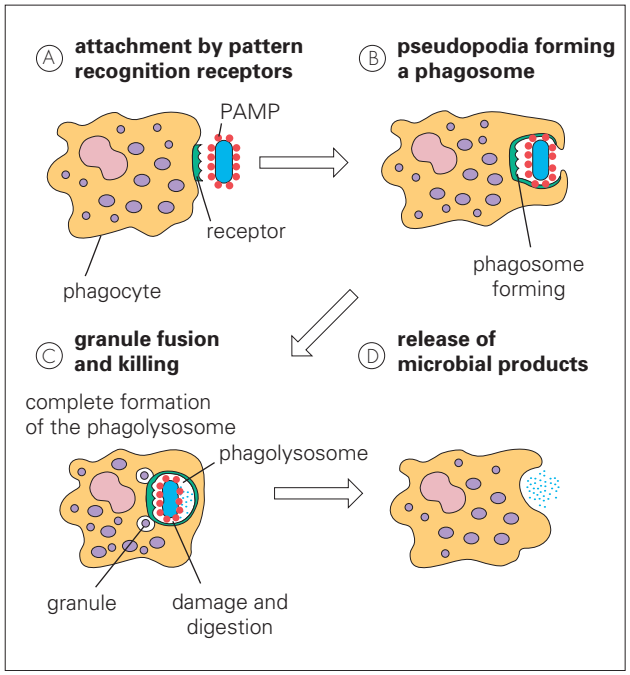


Figure 10.10 Phagocytosis. (A) Phagocytes attach to microorganisms (blue icon) via their cell surface receptors, which recognize pathogen-associated molecular patterns (PAMPs) such as lipopolysaccharide. (B) If the membrane now becomes activated by the attached infectious agent, the pathogen is taken into a phagosome by pseudopodia, which extend around it. (C) Once inside the cell, the various granules fuse with the phagosome to form a phagolysosome. (D) The infectious agent is then killed by a battery of microbicidal degradation mechanisms, and the microbial products are released.

(TLR 3,7 and 9) are also found in the endosomal environment and here they recognize PAMPs such as the unmethylated guanosine–cytosine (CpG) sequences of bacterial DNA and double-stranded RNA from RNA viruses. There are also cytoplasmic PRRs that can recognize pathogens (Fig. 10.11).

The phagocyte is activated through PAMP recognition

Signals are sent through the phagocyte's receptors to initiate the ingestion phase by activating an actin–myosin contractile system, which sends arms of cytoplasm around the particle until it is completely enclosed within a vacuole (phagosome; Fig. 10.12; see also Fig. 10.10). Shortly afterwards, the cytoplasmic granules fuse with a phagosome and discharge their contents around the captive microorganism.

The internalized pathogen is the target for a fearsome array of killing mechanisms

As phagocytosis is initiated, the attached microbes also signal through one of the PRRs to engineer an appropriate defensive response to the different types of infection through a number of nuclear factor (NF)-κB-mediated responses. This activation of a unique plasma membrane reduced nicotinamide adenine dinucleotide phosphate (NADPH) oxidase reduces oxygen to a series of powerful microbicidal agents, namely superoxide anion, hydrogen peroxide, singlet oxygen and hydroxyl radicals (Box 10.2; see also Ch. 15). Subsequently, the peroxide, in association with myeloperoxidase, generates a potent halogenating system from halide ions, which is capable of killing both bacteria and viruses.

As superoxide anion is formed, the enzyme superoxide dismutase acts to convert it to molecular oxygen and hydrogen peroxide, but in the process consumes hydrogen ions. Therefore initially there is a small increase in pH, which facilitates the antibacterial function of the families of cationic proteins derived from the phagocytic granules. These molecules damage microbial membranes by the proteolytic action of cathepsin G and by direct adherence to the microbial surface. The defensins have an amphipathic structure, which allows them to interact with and disrupt the structure and function of microbial membranes. These antibiotic peptides reach extraordinarily high concentrations within the phagosome and act as disinfectants against a wide spectrum of bacteria, fungi and enveloped viruses. Other important factors are:

- lactoferrin, which complexes iron to deprive bacteria of essential growth elements
- lysozyme, which splits the proteoglycan cell wall of bacteria
- nitric oxide, which together with its derivative, the peroxynitrite radical, can also be directly microbicidal.

The pH now falls so that the dead or dying microorganisms are extensively degraded by acid hydrolytic enzymes, and the degradation products released to the exterior.

NF-κB activation can also lead to the release of proinflammatory mediators. These include the antiviral interferons, the small protein *cytokines* IL-1β, IL-6, IL-12 and tumour necrosis factor alpha (TNFα, a proinflammatory cytokine produced by macrophages and other cell types), which activate other cells through binding to specific receptors, and *chemokines* such as IL-8, which represent a subset of chemoattractant cytokines.

Phagocytes are mobilized and targeted onto the microorganism by chemotaxis

Phagocytosis cannot occur unless the bacterium first attaches to the surface of the phagocyte, and clearly this cannot happen unless both have become physically close to each other. There is therefore a need for a mechanism that mobilizes phagocytes from afar and targets them onto the bacterium. Many bacteria produce chemical substances, such as formyl methionyl peptides, which directionally attract leukocytes – a process known as 'chemotaxis'. However, this is a relatively weak signalling system, and evolution has provided the body with a far more effective 'magnet' that uses a complex series of proteins collectively termed 'complement'.

Activation of the complement system

Complement resembles blood clotting, fibrinolysis and kinin formation in being a major triggered enzyme cascade system. Such systems are characterized by their ability to produce a rapid, highly amplified response to a trigger stimulus mediated by a cascade phenomenon in which the product of one reaction is the enzymic catalyst of the next. The most abundant and most central component is C3 (complement components are designated by the letter 'C' followed by a number), and the cleavage of this molecule is at the heart of all complement-mediated phenomena.

In normal plasma, C3 undergoes spontaneous activation at a very slow rate to generate the split product C3b. This

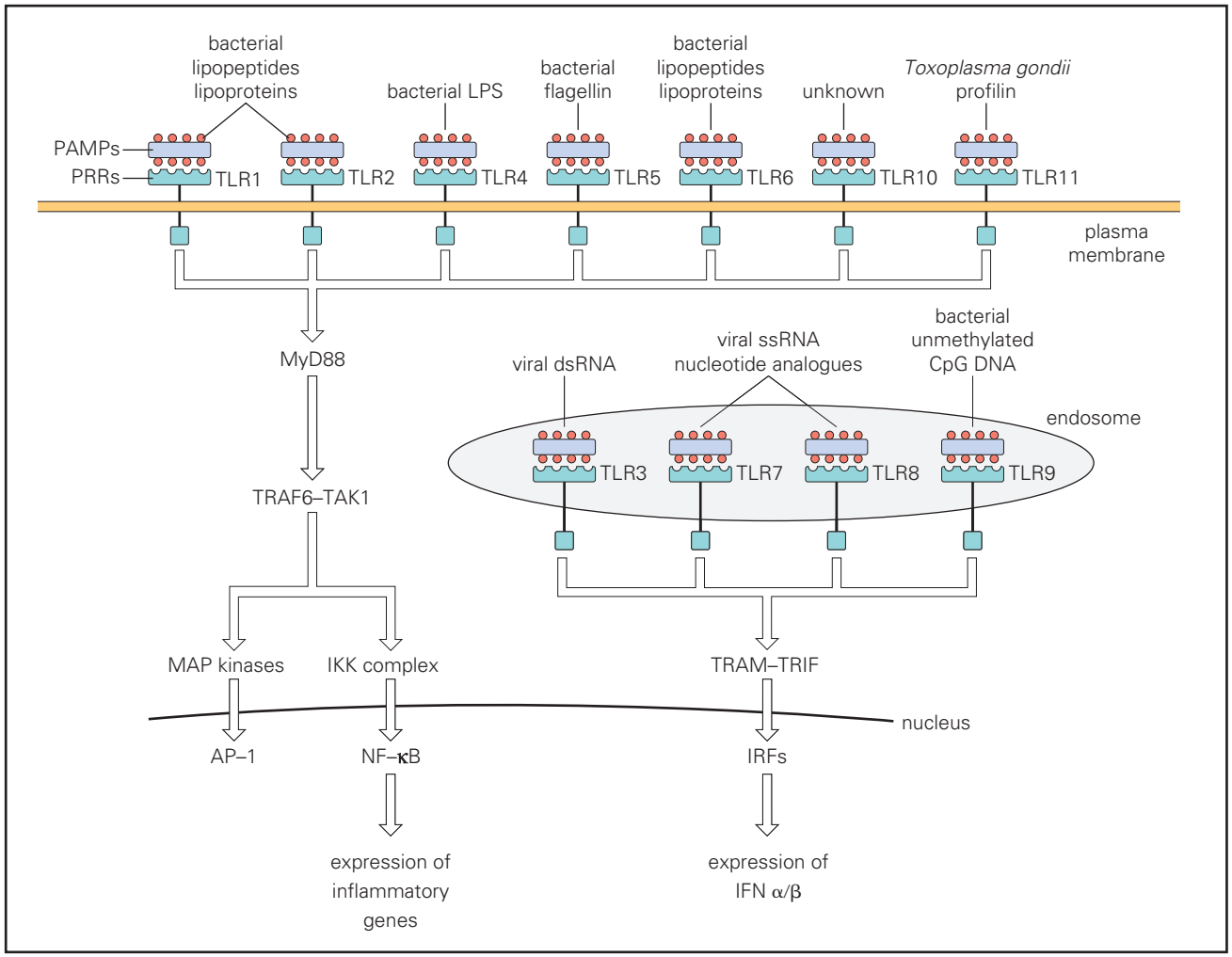


Figure 10.11 Recognition of PAMPs by a subset of pattern recognition receptors (PRRs) termed Toll-like receptors (TLRs). TLRs reside within plasma membrane or endosomal membrane compartments, as shown. All TLRs have multiple *N*-terminal leucine-rich repeats forming a horseshoe-shaped structure which acts as the PAMP-binding domain. Upon engagement of the TLR ectodomain with an appropriate PAMP (some examples are shown), signals are sent that ultimately activate the activation protein 1 (AP-1), nuclear factor-κB (NF-κB) and/or interferon-regulated factor (IRF) transcription factors, as shown. NF-κB and IRF transcription factors then direct the expression of numerous antimicrobial gene products such as cytokines and chemokines, as well as proteins that are involved in altering the activation state of the cell. There are also cytosolic PPRs that can sense bacterial peptidoglycan (NOD-like receptors), viral RNA (RIG-like receptors) and cytosolic DNA sensors. MyD88 is a signalling adaptor that is part of the supramolecular organizing centre; TRAF6 is a ubiquitin ligase; TAK1 phosphorylates the mitogen-activated protein (MAP) kinases; the IKK complex contains I kappa kinases; TRAM is a signalling adaptor molecule; TRIF is TIR domain containing adaptor inducing IFNβ.

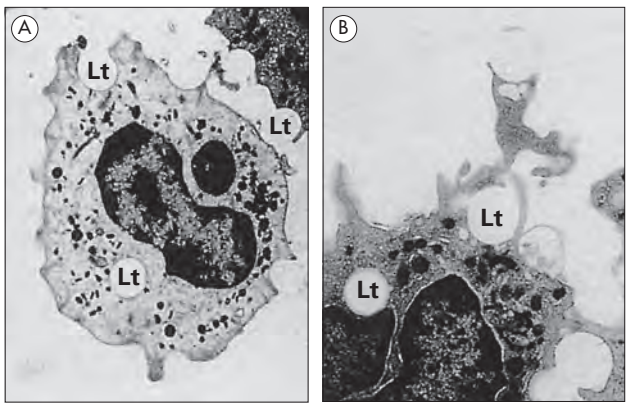


Figure 10.12 Electron micrographic study of phagocytosis. These two micrographs show human phagocytes engulfing latex particles (Lt). (A) ×3000; (B) ×4500. (Courtesy of C.H.W. Horne.)

can complex with another complement component, factor B, which is then acted upon by a normal plasma enzyme, factor D, to produce the C3-splitting enzyme C3bBb. This C3 convertase can then split new molecules of C3 to give C3a (a small fragment) and further C3b. This represents a positive feedback circuit with potential for runaway amplification; however, the overall process is restricted to a slow turn-over rate by powerful regulatory mechanisms, which break the unstable soluble-phase C3 convertase into inactive cleavage products (Fig. 10.13).

In the presence of certain molecules, such as the carbohydrates on the surface of many bacteria, the C3 convertase can become attached and stabilized against breakdown. Under these circumstances, there is active generation of new C3 convertase molecules, and what is known as the 'alternative' complement pathway is activated

Box 10.2 Antimicrobial Mechanisms in Phagocytic Vacuoles

Oxygen-independent antimicrobial mechanisms

Cathepsin G and elastase Low-molecular-weight ***defensins*** High-molecular-weight ***cationic proteins*** Bactericidal ***permeability-increasing protein***	Damage to microbial membranes
Lactoferrin	Complex with iron
Lysozyme	Splits peptidooglycan
Acid hydrolases	Degrade dead pathogens

Oxygen-dependent antimicrobial mechanisms

Generation of reactive oxygen intermediates

O_2+NADPH	NADPH oxidase →	**O_2^-**+NADP
$2O_2+2H^+$	Superoxide dismutase →	**H_2O_2**+$^1O_2^-$
$H_2O_2+O_2^-$, Cl^-, Br^-	Myeloperoxidase →	**·OH, OCl^-, OBr^-**+H_2O

Nitric oxide reaction sequence

O_2+L–arginine	→	**NO·**
NO·+·O^-_2	→	**·$ONOO^-$**
NO· + Fe/RSH	→	$Fe(RS)_2$ $(NO)_2$

Microbicidal species in bold letters. O_2^-, superoxide anion; H_2O_2, hydrogen peroxide; $^1O_2$ singlet oxygen; ·OH, hydroxyl radical; OCl^-, OBr^-, hypohalous anions; NO· nitric oxide; ·$ONOO^-$, peroxynitrite radical.

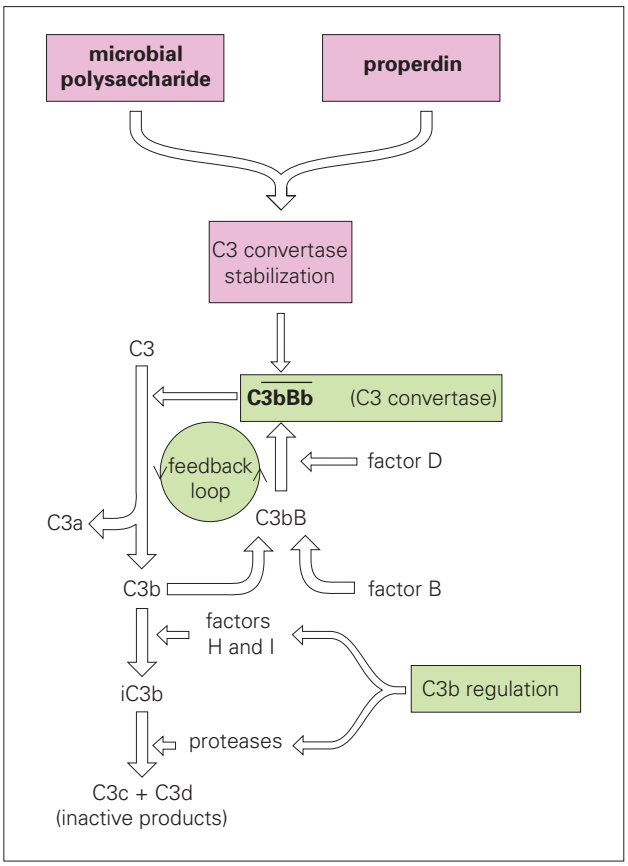


Figure 10.13 Activation of complement by microorganisms. C3b is formed by the spontaneous breakdown of C3 complexes with factor B to form C3bB, which is split by factor D to produce a C3 convertase $\overline{C3bBb}$ capable of further cleaving C3. The convertase is heavily regulated by factors H and I but can be stabilized on the surface of microbes and properdin. The horizontal bar indicates an enzymically active complex. iC3b, inactive C3b.

(see Ch. 15). Complement can also be activated by another innate pathway (the lectin pathway), which involves binding to carbohydrates on lectins like mannan-binding lectin and ficolins. A third pathway, the classical pathway, is mainly known as the pathway for acquired immunity but even here there are innate initiators, such as C-reactive protein (CRP) or natural antibody.

Complement synergizes with phagocytic cells to produce an acute inflammatory response

Activation of the alternative complement pathway with the consequent splitting of very large numbers of C3 molecules has important consequences for the orchestration of an integrated antimicrobial defense strategy (Fig. 10.14). Large numbers of C3b produced in the immediate vicinity of the microbial membrane bind covalently to that surface and act as opsonins (molecules that make the particle they coat more susceptible to engulfment by phagocytic cells; see below). This C3b, together with the C3 convertase, acts on the next component in the sequence, C5, to produce a small fragment, C5a, which, together with C3a, has a direct effect on mast cells to cause their degranulation (Fig. 10.15). This results in the release not only of mediators of vascular permeability, but also of factors chemotactic for polymorphs (Table 10.3). The circulating equivalent of the tissue mast cell, the basophil, is also shown in Fig. 10.15.

The vascular permeability mediators increase the permeability of capillaries by modifying the intercellular forces between the endothelial cells of the vessel wall. This allows the leakage or exudation of fluid and plasma components, including more complement, to the site of the infection. These mediators (Table 10.3) also up-regulate molecules such as intercellular adhesion molecule-1 (ICAM-1) and E-selectin, which bind to specific complementary molecules on the polymorphs and encourage them to stick in stages to the walls of the capillaries, a process termed 'margination'.

The chemotactic factors, on the other hand, provide a chemical gradient that attracts marginated polymorphs from their intravascular location, through the walls of the blood vessels, to the site of the C3b-coated bacteria that initiated the whole activation process. Polymorphs have a receptor for C3b on their surface, and as a result, the opsonized bacteria adhere very firmly to the surface of these newly arrived cells.

The processes of capillary dilation (erythema), exudation of plasma proteins and of fluid (oedema) due to hydrostatic and osmotic pressure changes, and the accumulation of neutrophils are features of the 'acute inflammatory response', and result in a highly effective way of focusing phagocytic cells onto complement-coated microbial targets.

The macrophage can also be stimulated by certain bacterial toxins such as the lipopolysaccharides (LPS), by the action of C5a, and by the phagocytosis of C3b-coated bacteria, to secrete other potent mediators of acute inflammation, independently of the mast-cell-directed pathway (Fig. 10.16).

C9 molecules form the 'membrane attack complex', which is involved in cell lysis

We have already introduced the idea that following the activation of C3 the next component to be cleaved is C5; the larger C5b fragment that results becomes membrane

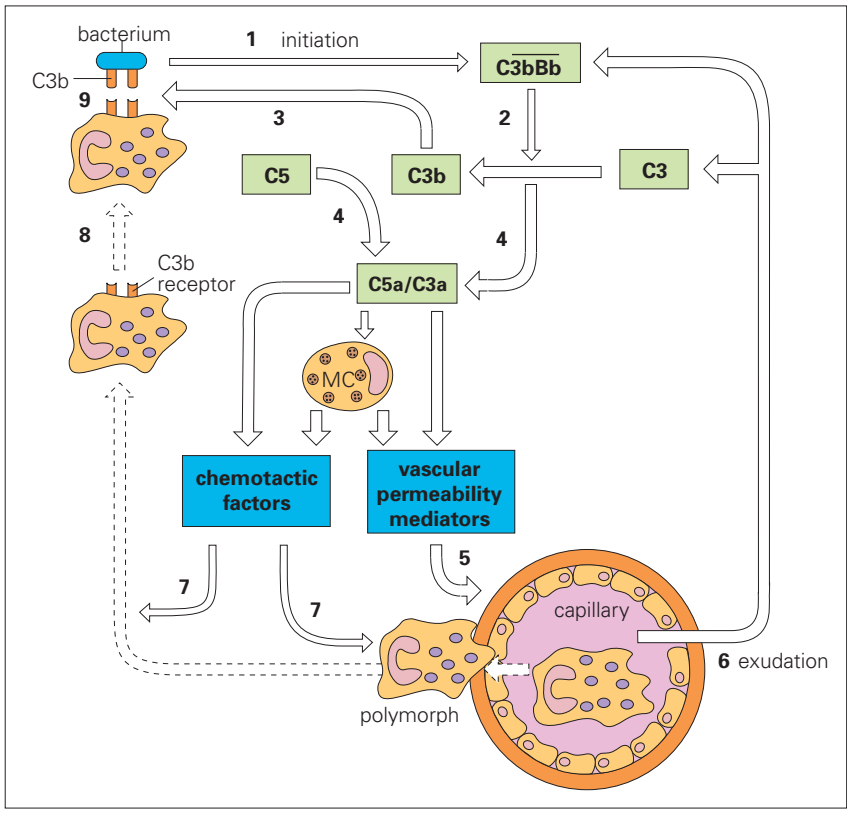


Figure 10.14 The defensive strategy of the acute inflammatory reaction initiated by bacterial activation of the alternative complement pathway. Activation of the $\overline{C3bBb}$ C3 convertase by the bacterium (1) leads to the generation of C3b (2) (which binds to the bacterium [3]), C3a and C5a (4), which recruit mast cell (MC) mediators. These in turn cause capillary dilation (5), exudation of plasma proteins (6), and chemotactic attraction (7) and adherence of polymorphs to the C3b-coated bacterium (8). Note that C5a itself is also chemotactic. The polymorphs are then activated for phagocytosis and the final kill (9).

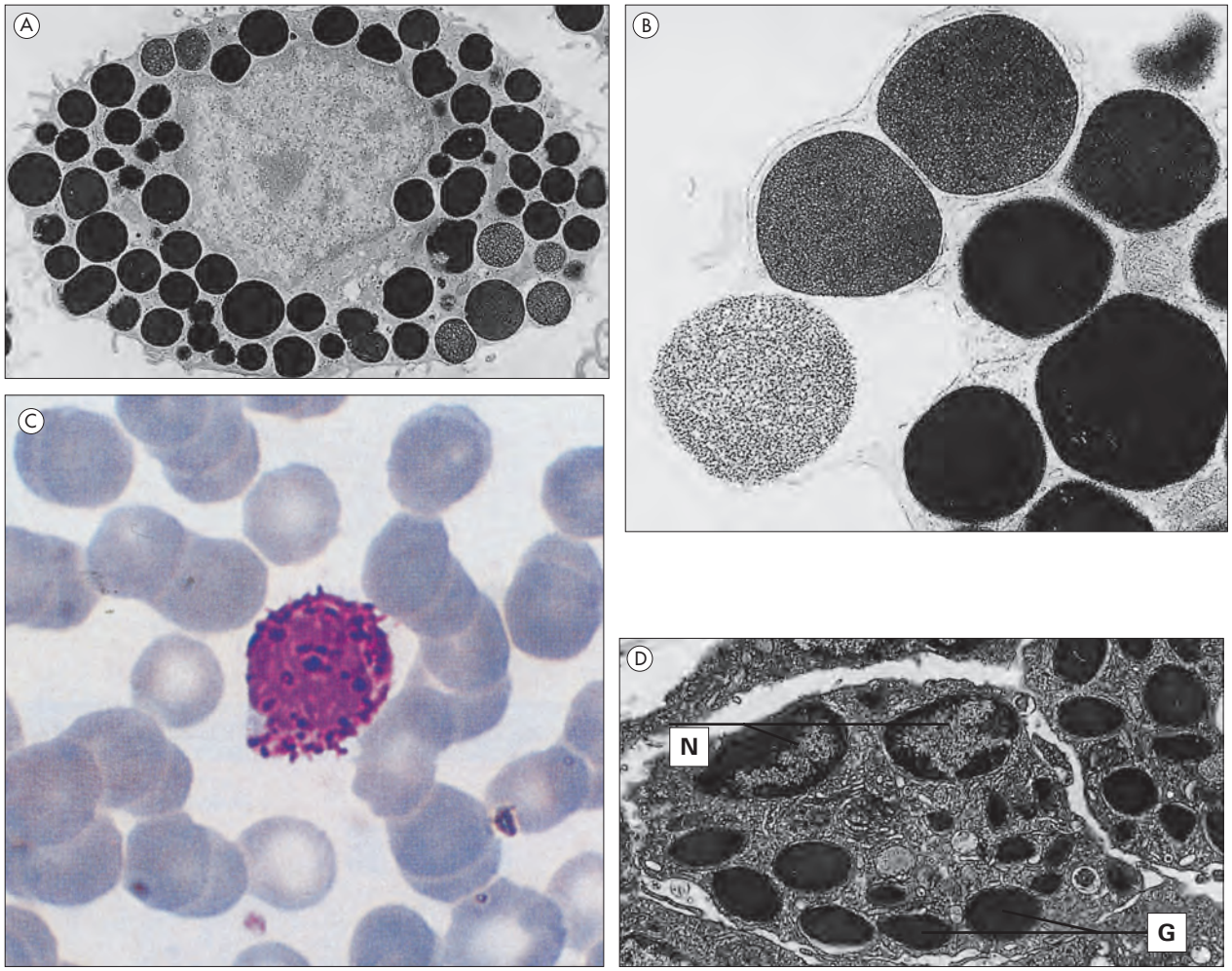


Figure 10.15 Electron micrographs of mast cells and basophils. These show (A) the resting rat peritoneal mast cell with its electron-dense granules (×6000) and (B) a granule in the process of exocytosis (×30 000). The morphology of a circulating human basophil is shown in (C) which shows a typical basophil with its deep violet-blue granules in a blood film stained with Wright's stain (×1500) and in (D) an electron micrograph shows the ultrastructure of a basophil in guinea pig skin showing the nuclei (N) and characteristic randomly distributed granules (G) (×6000). ([A,B] Courtesy of T.S.C. Orr; [C,D] Courtesy of D. McLaren.)

bound. This subsequently binds components C6, C7 and C8, which form a complex capable of inducing a critical conformational change in the terminal component C9. The unfolded C9 molecules become inserted into the lipid bilayer and polymerize to form an annular 'membrane attack complex' (MAC) (Figs 10.17, 10.18). This behaves as a transmembrane channel that is fully permeable to electrolytes and water; because of the high internal colloid osmotic pressure of cells, there is a net influx of sodium (Na^+) and this frequently leads to cell lysis.

Inflammasomes

Within the cytoplasm of phagocytes, special complexes of cytoplasmic proteins called inflammasomes recruit and activate critical enzymes such as caspases. The enzyme caspase 1 cleaves a precursor molecule to produce the cytokines IL-1α and Il-1β, which act synergistically with TNFα. Different types of inflammasomes are activated by different bacterial components, for example the NLRP3 inflammasome recognizes the PAMPs discussed above, bacterial flagellin activates the NLRP4 inflammasome and the AIM2 inflammasome senses cytoplasmic viral DNA. Once activated, a form of proinflammatory cell death called pyropoptosis occurs in which the cell swells up in size, lyses and releases its cytoplasmic contents. Special proteins from the gasdermin family are needed to induce this form of cell death, which can release bacteria from macrophages that can then be phagocytosed and killed by neutrophils. Of course the inflammasomes themselves then have to be regulated, through a series of regulator proteins.

Acute phase proteins

Certain proteins in the plasma, collectively termed 'acute phase proteins', increase in concentration in response to early 'alarm' mediators such as the cytokines IL-1, IL-6 and TNF, released as a result of infection or tissue injury. Many acute phase reactants such as mannose-binding lectin and CRP increase dramatically during inflammation (Fig. 10.19). Like

Table 10.3 The major inflammatory mediators that control blood supply and vascular permeability or modulate cell movement

Inflammatory mediators		
Mediator	**Main source**	**Actions**
Histamine	Mast cells, basophils	Increased vascular permeability, smooth muscle contraction, chemokinesis
5-hydroxytryptamine (5HT – serotonin)	Platelets, mast cells (rodent)	Increased vascular permeability, smooth muscle contraction
Platelet activating factor (PAF)	Basophils, neutrophils, macrophages	Mediator release from platelets, increased vascular permeability, smooth muscle contraction, neutrophil activation
Interleukin-8 (IL-8, CXCL8)	Mast cells, endothelium, monocytes and lymphocytes	Polymorph and monocyte localization
C3a	Complement C3	Mast cell degranulation, smooth muscle contraction
C5a	Complement C5	Mast cell degranulation, neutrophil and macrophage chemotaxis, neutrophil activation, smooth muscle contraction, increased capillary permeability
Bradykinin	Kinin system (kininogen)	Vasodilation, smooth muscle contraction, increased capillary permeability, pain
Fibrinopeptides and fibrin breakdown products	Clotting system	Increased vascular permeability, neutrophil and macrophage chemotaxis
Prostaglandin E_2 (PGE_2)	Cyclo-oxygenase pathway, mast cells	Vasodilation, potentiates increased vascular permeability produced by histamine and bradykinin
Leukotriene B_4 (LTB_4)	Lipoxygenase pathway, mast cells	Neutrophil chemotaxis, synergizes with PGE_2 in increasing vascular permeability
Leukotriene D_4 (LTD_4)	Lipoxygenase pathway	Smooth muscle contraction, increasing vascular permeability

Other mediators are generated from the coagulation process. Chemotaxis refers to directed migration of granulocytes up the concentration gradient of the mediator, whereas chemokinesis describes randomly increased motility of these cells.
(Reproduced from Male D., Brostoff J., Roth D.B., Roitt I. *Immunology*, 7th edition, 2006. Mosby Elsevier, with permission.)

the professional phagocytes, both use pattern recognition receptors to bind to molecular patterns on the pathogen (PAMPs) to generate defensive effector functions. Other acute phase reactants show more moderate rises, usually less than fivefold (Table 10.4). This response involves a considerable energy and resource cost for the host and these proteins have a wide range of roles that include homeostatic roles as well as pathogen defence. Acute phase proteins like CRP can be used clinically as a marker of inflammation.

Other extracellular antimicrobial factors

There are many microbicidal agents that operate at short range within phagocytic cells, but also appear in various body fluids in sufficient concentration to have direct inhibitory effects on infectious agents. For example, lysozyme is present in fluids such as tears and saliva in amounts capable of acting against the proteoglycan wall of susceptible bacteria. Other proteins such as collectins bind to carbohydrates on microbial surfaces (see Ch. 15). Whether agents that normally act over a short range, such as reactive oxygen metabolites or TNFα, can reach concentrations in the body fluids that are adequate to allow them to act at a distance from the cell producing them will be discussed in Chapter 15.

Interferons are a family of broad-spectrum antiviral molecules

Interferons (IFNs) are widespread throughout the animal kingdom and are again discussed further in Chapter 15. They were first recognized by the phenomenon of viral interference, in which a cell infected with one virus is found to be resistant to superinfection by a second, unrelated virus. Leukocytes produce many different alpha interferons (IFNα), whereas fibroblasts and probably all cell types synthesize IFNβ. A third type (IFNγ) is made by natural killer (NK) cells and other innate lymphoid cells (see below) as well as by the Th1 subset of T cells (see Ch. 15). When cells are infected by a virus, they synthesize and secrete IFNs α and β, which bind to specific receptors on nearby uninfected cells. The bound IFN exerts its antiviral effect by facilitating the synthesis of two new enzymes, which interfere with the machinery used by the virus for its own replication. The mechanism of action of IFN is discussed more fully in Chapter 15; the net result is to set up a cordon of infection-resistant cells around the site of virus infection, so restraining its spread (Fig. 10.20). IFN is highly effective in vivo, as supported by experiments in which mice injected with an antiserum to murine IFN were found to be killed by several hundred times less virus than

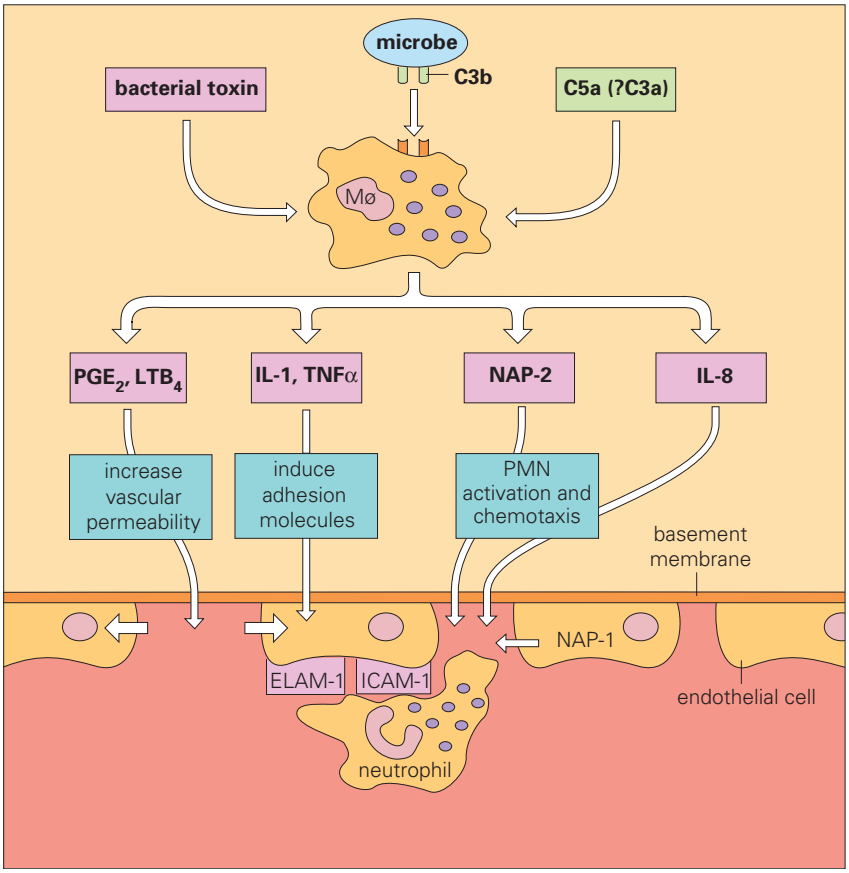


Figure 10.16 A role for the macrophage (Mø) in the initiation of acute inflammation. Stimulation induces macrophage secretion of mediators. Blood neutrophils stick to the adhesion molecules on the endothelial cell and use them to provide traction as they force their way between the cells, through the basement membrane (with the help of secreted elastase) and up the chemotactic gradient. During this process they become progressively activated by neutrophil-activating peptide 2 (NAP-2). PGE_2, prostaglandin E_2; LTB_4, leukotriene B_4; IL-1, interleukin-1; IL-8, interleukin-8; NAP-1, neutrophil-activating peptide 1; PMN, polymorphonuclear neutrophil; TNFα, tumour necrosis factor alpha; ELAM-1, endothelial cell leukocyte adhesion molecule 1; ICAM-1, intercellular adhesion molecule 1.

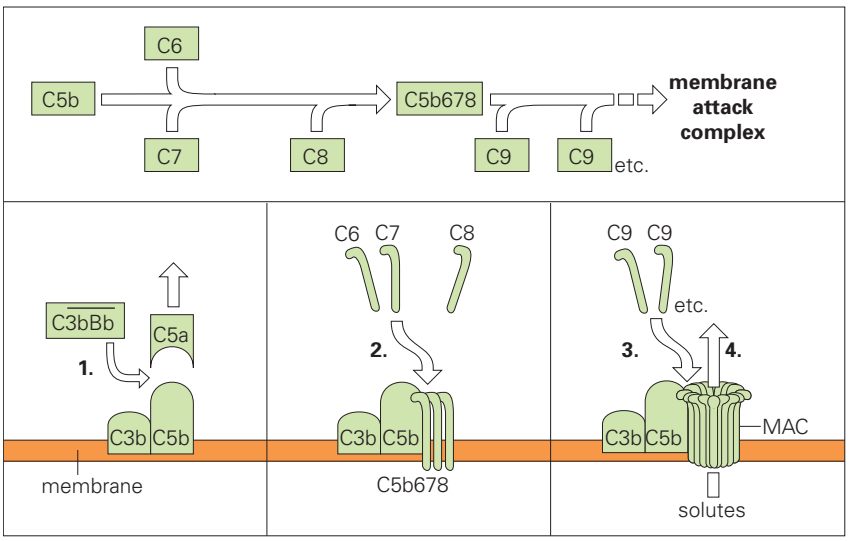


Figure 10.17 Assembly of the C5b-9 membrane attack complex (MAC). (1) Recruitment of a further C3b into the $\overline{C3bBb}$ enzymic complex generates a C5 convertase, which cleaves C5a from C5 and leaves the remaining C5b attached to the membrane. (2) Once C5b is membrane bound, C6 and C7 attach themselves to form the stable complex C5b67, which interacts with C8 to yield C5b678. (3) This unit has some effect in disrupting the membrane, but primarily causes the polymerization of C9 to form tubules traversing the membrane. The resulting tubule is referred to as a MAC. (4) Disruption of the membrane by this structure permits the free exchange of solutes, which are primarily responsible for cell lysis.

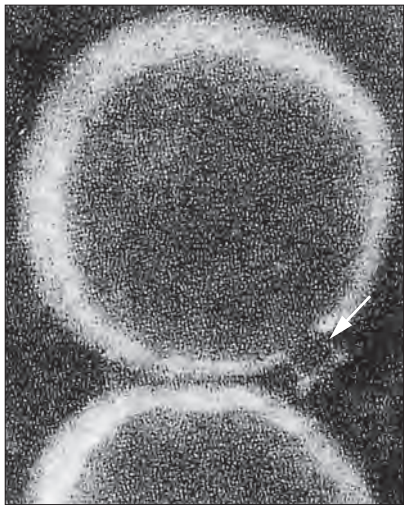


Figure 10.18 Electron micrograph of the membrane attack complex. The funnel-shaped lesion (*arrowed*) is due to a human C5b–9 complex that has been re-incorporated into lecithin liposomal membranes (×234 000). (Courtesy of J. Tranum-Jensen and S. Bhakdi.)

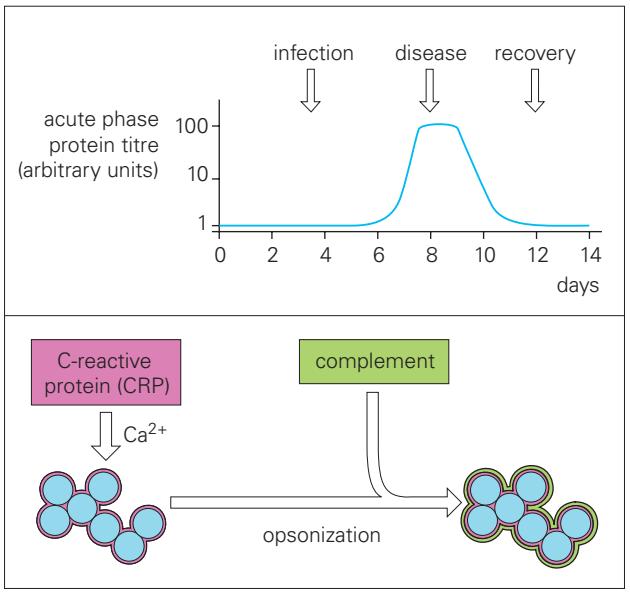


Figure 10.19 Acute phase proteins, here exemplified by C-reactive protein (CRP), are serum proteins that increase rapidly in concentration (sometimes up to 100-fold) following inflammation induced by infection (graph). They are important in innate immunity to infection. CRP recognizes and binds in a calcium (Ca^{2+})-dependent fashion to molecular groups found on a wide variety of bacteria and fungi. In particular, it uses its pattern recognition to bind the phosphocholine moiety of pneumococci. The CRP acts as an opsonin and activates complement with all the associated sequelae. Mannose-binding protein reacts with not only mannose but also several other sugars, enabling it to bind to a wide variety of Gram-negative and -positive bacteria, yeasts, viruses and parasites, subsequently activating the complement system and phagocytic cells. The structurally related ficolins typically recognize PAMPs containing *N*-acetylglucosamine and can also activate the lectin complement pathway.

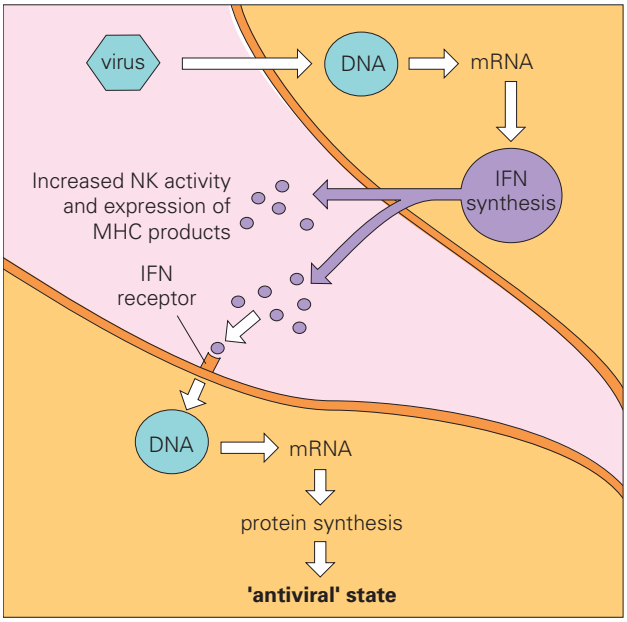


Figure 10.20 The action of interferon (IFN). A virus infecting a cell induces the production of IFNα/β. This is released and binds to IFN receptors on other cells. The IFN induces the production of antiviral proteins, which are activated if virus enters the second cell, and increased synthesis of surface MHC molecules, which enhance susceptibility to cytotoxic T cells (see Ch. 11). NK, natural killer cell; MHC, major histocompatibility complex.

Table 10.4 Acute phase proteins produced in response to infection in the human

Acute phase reactant	Function
Dramatic increases in concentration	
C-reactive protein	Fixes complement, opsonizes
Mannose-binding lectin	Fixes complement, opsonizes
spLA2	Kills Gram-positive bacteria
Serum amyloid A protein	Unknown
Moderate increases in concentration	
α_1 proteinase inhibitors	Inhibit bacterial proteases
α_1 anti-chymotrypsin	Inhibits bacterial proteases
α_1 acid glycoprotein	Unknown but binds many drugs/lipophilic compounds
C3, C9, factor B	Increase complement function
Ceruloplasmin	O_2 scavenger
Fibrinogen	Coagulation
Angiotensin	Blood pressure
Haptoglobin	Binds haemoglobin
Fibronectin	Cell attachment

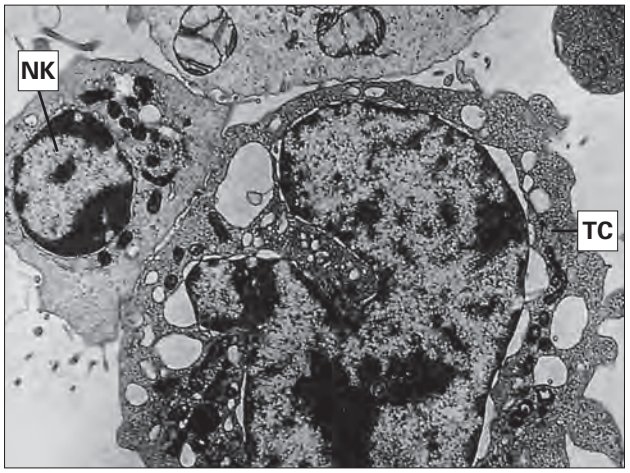


Figure 10.21 Electron micrograph of an natural killer (NK) cell killing a tumour cell (TC). NK cells bind to and kill IgG antibody-coated (see Fig. 10.13) and non-coated tumour cells. It is essential for the membranes of the two cells to be in contact in order for the NK cell to deliver the 'kiss of death' (×4500). (Courtesy of P. Lydyard.)

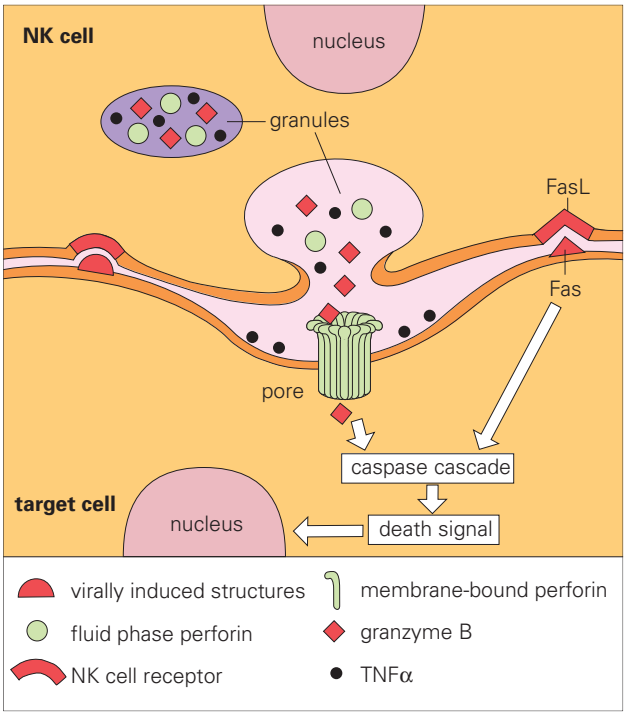


Figure 10.22 Schematic model of lysis of virally infected target cell by a natural killer (NK) cell. As the NK cell receptors bind to the surface of the virally infected cell, and if signals from activation receptors exceed those from the inhibitory receptors that recognize normal MHC class I molecules, there is exocytosis of granules and release of cytolytic mediators into the intercellular cleft. A calcium (Ca^{2+})-dependent conformational change in the perforin enables it to insert and polymerize within the membrane of the target cell to form a transmembrane pore, which allows entry of granzyme B into the target cell, where it causes programmed cell death (apoptosis). A back-up cytolytic system using engagement of the Fas receptor with its ligand (FasL) can also trigger apoptosis, as can binding of granule-derived tumour necrosis factor alpha (TNFα) to its receptor. Unlike the PRR-mediated activation of phagocytes by intracellular components – so-called danger-associated molecular patterns (DAMPs) – released on *necrotic* cell death typically caused by tissue trauma, burns and other non-physiological stimuli, cells undergoing *apoptotic* death do not activate the immune system because they express surface molecules such as phosphatidyl serine, which mark them out for phagocytic removal before they release their intracellular DAMPs.

was needed to kill the controls. It should be emphasized, however, that IFN seems to play a significant role in recovery from, rather than prevention of, viral infections.

Natural killer cells attach to virally infected cells, allowing them to be differentiated from normal cells

Viruses need to infect host cells to utilize the host cell's machinery in order to replicate. Clearly, it is in the interests of the host to try to kill such infected cells before the virus has had a chance to reproduce. NK cells are cytotoxic cells that appear to have evolved to carry out just such a task. These are large granular lymphocytes (LGLs) (Fig. 10.21) that recognize virus-infected or stressed cells and allow them to be differentiated from normal cells; this clever discrimination is mediated by activating receptors on the NK cells such as NKG2D, which recognize ligands on the infected cell that are related to MHC class I molecules, and inhibitory receptors which bind to MHC class I molecules on normal cells, generating signals that counteract those from the activating receptors. Activation of the NK cell results in the extracellular release of its granule contents into the space between the target and effector cells. These contents include perforin molecules, which resemble C9 in many respects, especially in their ability to insert into the membrane of the target cell and polymerize to form annular transmembrane pores, like the MAC. This permits the entry of another granule protein, granzyme B, which leads to death of the target cell by apoptosis (programmed cell death), a process mediated by a cascade of proteolytic enzymes termed caspases, which terminates with effector caspases that process the cell for clearance including the ultimate fragmentation of DNA by a Ca-dependent endonuclease (Fig. 10.22).

Subsidiary mechanisms that can activate the caspase pathway include engagement of Fas on the target cell by Fas ligand, and binding of tumour necrosis factor released from the NK granules to surface receptors. TNF was first recognized as a product of activated macrophages known to be capable of killing certain other cells, particularly some tumour cells.

Innate lymphoid cells (ILCs)

The NK cells discussed above are one of a group of ILCs (Fig. 10.23). As we will see in Chapter 11, in many ways ILCs duplicate the functions of the subsets of T cells; however, they lack the specific antigen receptors expressed by T cells and do not have pattern recognition receptors. Instead they respond to tissue damage in terms of cytokines, alarmins and inflammatory mediators secreted by myeloid or epithelial cells. ILCs are derived from bone marrow precursors and, apart from the circulating NK cells, are resident in the tissues including skin, lung and intestine. There are three main groups

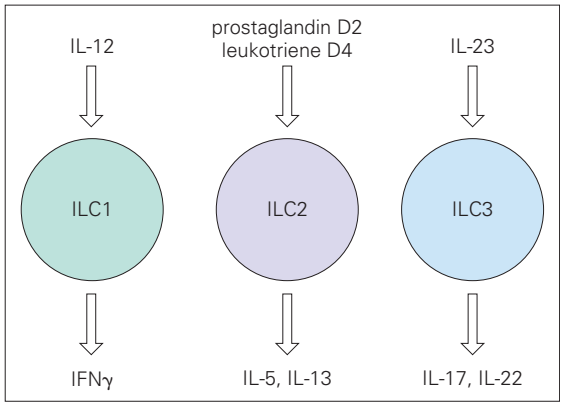


Figure 10.23 Innate lymphoid cells. There are three main groups of innate lymphoid cells (ILC1, 2 and 3) that respond to different signals of inflammation or tissue damage and that produce particular cytokines including those illustrated. Natural killer cells are part of the group 1 ILCs. IFNγ, interferon gamma; IL, interleukin.

of ILCs: Group 1 ILCs including NK cells make IFNγ, Group 2 ILCs make IL-5 and IL-13, and Group 3 ILCs make IL-17 and IL-22, although, as we will see later for T cells, there can be some 'plasticity' where exposure to particular cytokines will alter the cytokines produced by ILCs.

Eosinophils act against large parasites

It takes little imagination to realize that professional phagocytes are far too small to be capable of physically engulfing larger parasites such as helminths. An alternative strategy, such as killing by an extracellular surface attack of the type discussed above would seem to be a more appropriate form of defence. Eosinophils appear to have evolved to fulfil this role. These polymorphonuclear relatives of the neutrophil have distinctive cytoplasmic granules, which stain strongly with acidic dyes (Fig. 10.24) and have a characteristic ultrastructural appearance. The core of the granule contains a major basic protein (MBP), while the matrix contains an eosinophilic cationic protein, a peroxidase and a perforin-like molecule. Eosinophils have surface receptors for C3b and when activated generate copious amounts of active oxygen metabolites.

Many helminths can activate the alternative complement pathway but, although resistant to C9 attack, their coating with C3b allows adherence to the eosinophils through their C3b surface receptors. Once activated, the eosinophil launches its extracellular ammunition, which includes the release of major basic proteins and the cationic protein to damage the parasite membrane, with a possibility of a further 'chemical burn' from the oxygen metabolites and 'leaky pore' formation by the perforins.

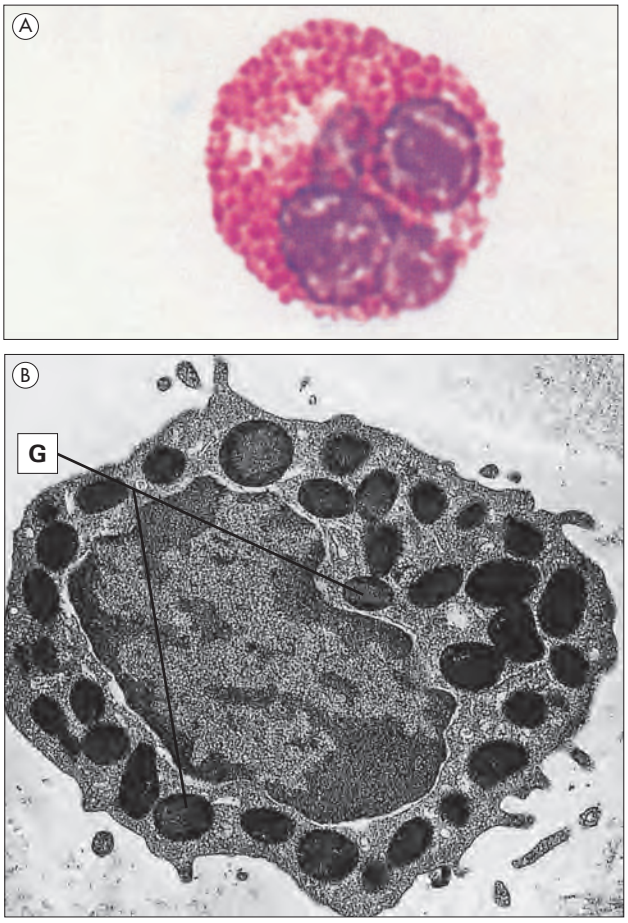


Figure 10.24 The eosinophil granulocyte is capable of extracellular killing of parasites (e.g. worms) by releasing its granule contents. (A) Morphology of the eosinophil. This blood smear enriched for granulocytes shows an eosinophil with its multilobed nucleus and heavily stained cytoplasmic granules. Leishman's stain (×1800). (B) Electron micrograph showing the ultrastructure of a guinea pig eosinophil. The mature eosinophil contains granules (G) with central crystalloids (×8000). ([A] Courtesy of P. Lydyard; [B] Courtesy of D. McLaren.)

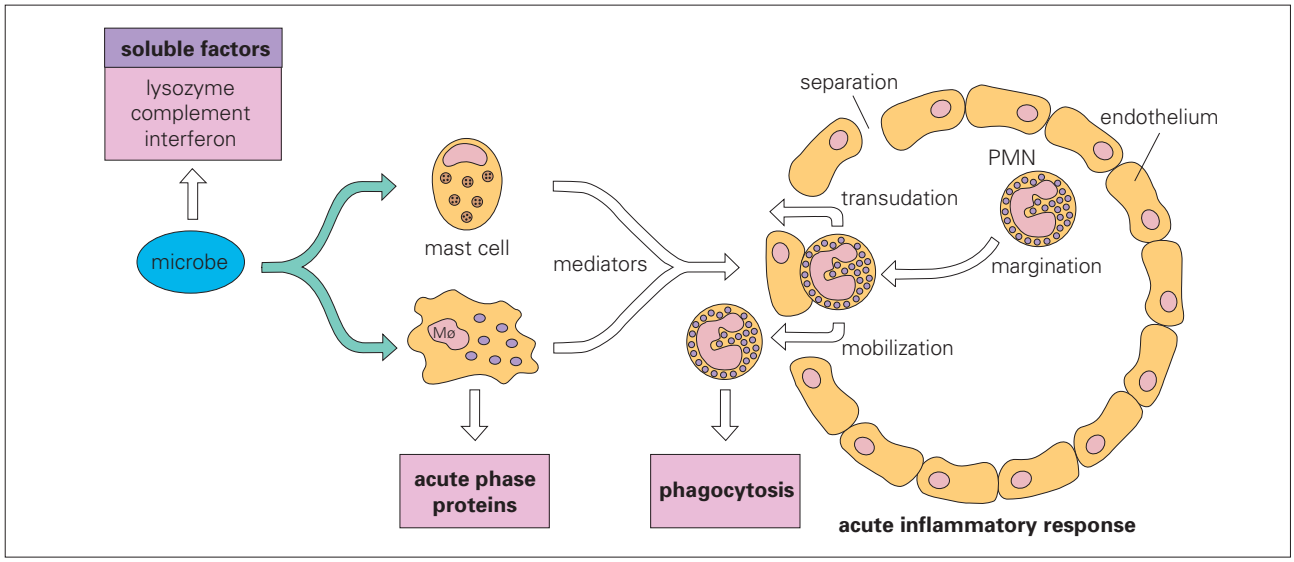


Figure 10.25 Mobilization of defensive components of innate immunity. Microbes, either through complement activation or through direct effects on macrophages (Mø), release mediators that increase capillary permeability to allow transudation of plasma bactericidal molecules, and chemotactically attract plasma polymorphs from the bloodstream to the infection site. PMN, polymorphonuclear neutrophil.

KEY FACTS

- The innate system of immune defence consists of a formidable barrier to entry, followed by a second line of defence by phagocytes and circulating soluble factors. Colonization of the body by normally non-pathogenic ('opportunistic') microorganisms occurs whenever there is a hereditary or acquired deficiency in any of these functions.
- There are mechanisms to recognize and respond to pathogens in extracellular, cell surface and intracellular compartments.
- The main phagocytic cells are polymorphonuclear neutrophils and macrophages. Receptor-mediated phagocytosis and vacuole fusion lead to oxygen-dependent and oxygen-independent microbicidal mechanisms.
- 'Complement' consists of a series of components that are cleaved by enzymes in an amplifying cascade. The C3a and C5a components bind to receptors on neutrophils and monocytes as well as on basement membranes, but also induce further cytokine release and cell and fluid extravasation. As well as inducing inflammation, complement components improve phagocytosis through opsonization and can cause direct lysis of bacteria or cells through punching holes in their membranes.
- Inflammation can also be initiated by tissue macrophages as signalling by bacterial toxins, C5a or by C3b-coated bacteria causes the release of TNFα, LTB_4, PGE_2, the neutrophil chemotactic factor, IL-8, and a neutrophil-activating peptide.
- Other humoral defences include the acute phase proteins such as CRP, and the type 1 IFNs, which can block viral replication.
- Virally infected cells can be killed by NK cells, following increased recognition by activation receptors that overcomes inhibitory signals from normal MHC class I recognition. NK cells are one of the group 1 innate lymphoid cells, which are activated by IL-12. Group 2 ILCs secrete IL-5 and IL-13, and group 3 ILCs secrete IL-17. ILCs lack the ability to sense pathogens directly, but respond to tissue damage and inflammation.
- Extracellular killing can also be effected by C3b-bound eosinophils, which may be responsible for the failure of many large parasites to establish a foothold in potential hosts.
- Engulfment and killing by phagocytic cells is the mechanism used to dispose of the majority of microbes, and the mobilization and activation of these cells by orchestrated responses such as the acute inflammatory response (Fig. 10.25) is a key feature of innate immunity. However, not every organism is readily susceptible to phagocytosis or even to killing by complement or lysozyme, which explains why the additional specificity of the adaptive immune response is needed, which is explored in Chapter 11.

11 Adaptive immune responses bring specificity

Introduction

How to recognize an extensive repertoire of foreign antigens in an efficient way

To protect us against pathogens, those that we have never been exposed to as well as those we have met before, the immune system needs to be permanently on stand-by but in an effective and efficient way. There are many instances where the cells and molecules of innate immunity are insufficient to cope effectively with these infections, and a greater degree of specificity is needed. Cells that defend us against different types of infections and that have specialized functions are needed. This is where the antigen-specific T and B lymphocytes come to our defence.

However, the immune system faces the same challenge as many armies. How to defend the body when it doesn't know what the next threat will be or where an attack will be launched? Will it need specialized marines, pilots or ground troops and where should they be based? It has solved this dilemma by having cells recirculate through the body and by sending out signals to attract its troops to the site of attack.

LYMPHOID TISSUES PRIMARY AND SECONDARY

The cells that provide the immune system with its exquisite antigen specificity are lymphocytes (Fig. 11.1). When not activated, T and B cells have a small rim of cytoplasm, and are often referred to as 'resting'. Once activated they become larger and more granular, and are called lymphoblasts. Producing the T and B cells that are needed is a more complex process than the maturation of innate immune cells in the bone marrow.

Although resting T and B cells look similar, luckily, they (and many other cell types) can be distinguished by the surface antigens they express, often referred to as markers. Most of these have been given CD numbers – CD stands for 'cluster of differentiation', a designation to allow antibodies that recognize different epitopes on the same molecule to be grouped together (Table 11.1).

The tissues of the immune system divide into those where the lymphocytes develop – the primary lymphoid organs – and the secondary lymphoid organs where immune responses are initiated and where the mature cells wait while on stand-by (Fig. 11.2).

Like the cells of the innate immune system, B lymphocytes or B cells develop in the bone marrow (although chickens have a specialized organ for this called the bursa of Fabricius). Each mature B cell expresses antibody of one specificity on its surface, formed by splicing different variable and constant region genes together, and the first stages of these gene rearrangements take place in the bone marrow. B cells that recognize self-antigens are deleted at this stage as these cells would only cause autoimmunity. The bone marrow also contains the precursors of T lymphocytes or T cells. The presence of these and many other precursor stem cells in the bone marrow explains why bone marrow transplants work so well to replace damaged cells. The bone marrow is critical for both production and replacement of the cells of the immune system.

Immature T cells have a specialized organ in which they develop, called the thymus. The thymus carries out two important roles, giving positive survival signals to immature cells to keep them alive but also carrying out negative selection to remove cells that might cause damage by recognizing our own cells or 'self'. T-cell maturation is therefore a more complicated process than the production of a useful repertoire of antigen recognition receptors in B cells.

Once the cells needed for innate and acquired immunity are mature they circulate via the blood and lymph, which also allows them to enter the specialized secondary lymphoid organs such as spleen, lymph nodes and the lymphoid tissues associated with the gut (gut-associated lymphoid tissue, GALT) and the mucosa (mucosa-associated lymphoid tissue, MALT). When needed, the cells of the immune system can reach anywhere in the body particularly if there is inflammation.

The thymus is a highly specialized organ producing mature T cells

In the thymus, T cells develop from immature pre-T cells or thymocytes into mature T cells (Fig. 11.3). In the outer cortex the immature thymocytes first receive maturation signals including interleukin 7 (IL-7) produced by cortical epithelial cells. They already express the main T-cell marker antigen CD3 but now also start to express both CD4 and CD8, two surface markers that will later identify the CD4 and CD8 T-cell subsets. If the thymus is absent, as in the congenital DiGeorge syndrome in humans, or in nude (athymic) mice, no mature T cells develop. The thymus decreases in size as

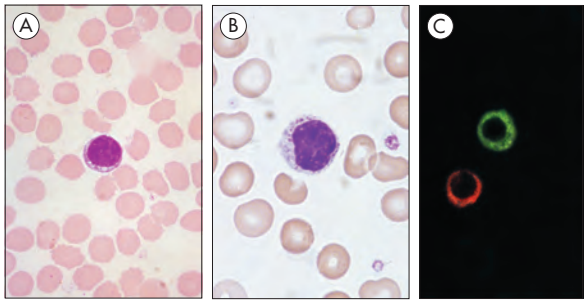


Figure 11.1 Lymphocytes and plasma cells. (A) Small B and T lymphocytes have a round nucleus and a high nuclear : cytoplasmic ratio. (B) A large granular lymphocyte with a lower nuclear : cytoplasmic ratio, an indented nucleus and azurophilic cytoplasmic granules. Fewer than 5% of T helper cells, and 30–50% of cytotoxic T cells, γδ T cells and natural killer (NK) cells have this morphology. (C) Antibody formed when B cells differentiate into plasma cells, here stained with fluoresceinated anti-human IgM (green) and rhodaminated anti-human IgG (red) showing extensive intracytoplasmic staining. Note that plasma cells produce only one class of antibody as the distinct staining reveals. (A and B, stained with Giemsa, courtesy of A. Stevens and J. Lowe; C, adapted from: Zucker-Franklin A. et al. [1988] *Atlas of Blood Cells: Function and Pathology*, 2nd edn, Vol. 11, Milan: EE Ermes; Philadelphia: Lea and Febiger.)

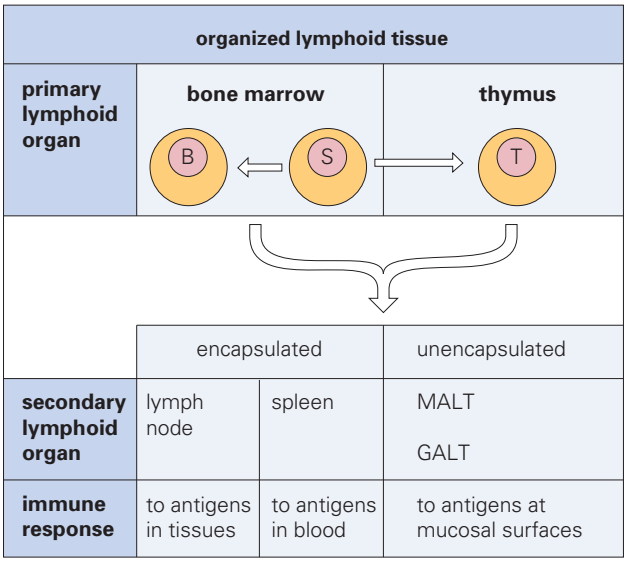


Figure 11.2 Organized lymphoid tissue. Stem cells (S) arising in the bone marrow differentiate into immunocompetent B and T cells in the primary lymphoid organs. These cells then colonize the secondary lymphoid tissues where immune responses are organized. MALT, mucosa-associated lymphoid tissue; GALT, gut-associated lymphoid tissue.

Table 11.1 Useful CD markers that can be used to identify different cell types

CD molecule	Cell expression	Function
CD3 (δ, γ, ε-chains)	All T cells	Signal transduction from T-cell antigen receptor (TCR)
CD4	CD4 T cells	Binds to MHC II
CD8 (α, β-chains)	CD8 T cells	Binds to MHC I
CD14	Monocytes / macrophages, dendritic cells, polymorphs	Binds LPS–LPS binding protein complex
CD16α (FcRIIIA)	NK cells, macrophages	Fc receptor for IgG, phagocytosis, ADCC
CD19	B cells	B-cell activation
CD20	B cells	B-cell activation?
CD25 (α-chain)	Activated T and B cells; Tregs	Binds IL-2
CD45R	RA on naive T cells, RO on antigen-experienced / memory T cells (also on B cells)	Splice variant of common leukocyte antigen
CD69	Activated T cells, B cells, NK cells	Early activation marker
CD158 (killer Ig-like receptor, KIR)	NK cells, T cell subset	Activation / inhibition of NK cells (interaction with MHC I)
CD159a (NKG2D)	NK cells	Activation / inhibition of NK cells (interaction with MHC I)
CD206 (mannose receptor)	Monocytes / macrophages	Phagocytosis of microorganisms

CD, cluster of differentiation (antibodies that recognize the same chain or molecule); LPS, lipopolysaccharide; MHC, major histocompatibility class; NK, natural killer; Treg, regulatory T cell.

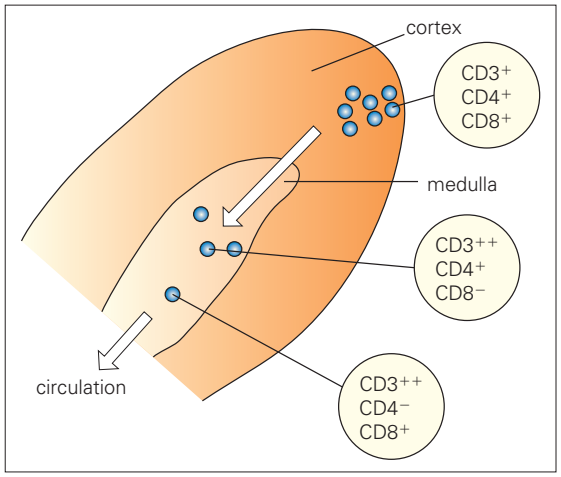


Figure 11.3 Structure and function of the thymus. The bi-lobed thymus is subdivided into lobules by fibrous trabeculae. The most immature thymocytes are found in the outer cortex, where gene rearrangement to form the T-cell receptor (TCR) occurs. Following positive and negative selection once in the medulla the T cells start to express only CD4 or CD8, before entering the circulation via blood vessels. CD, cluster of differentiation.

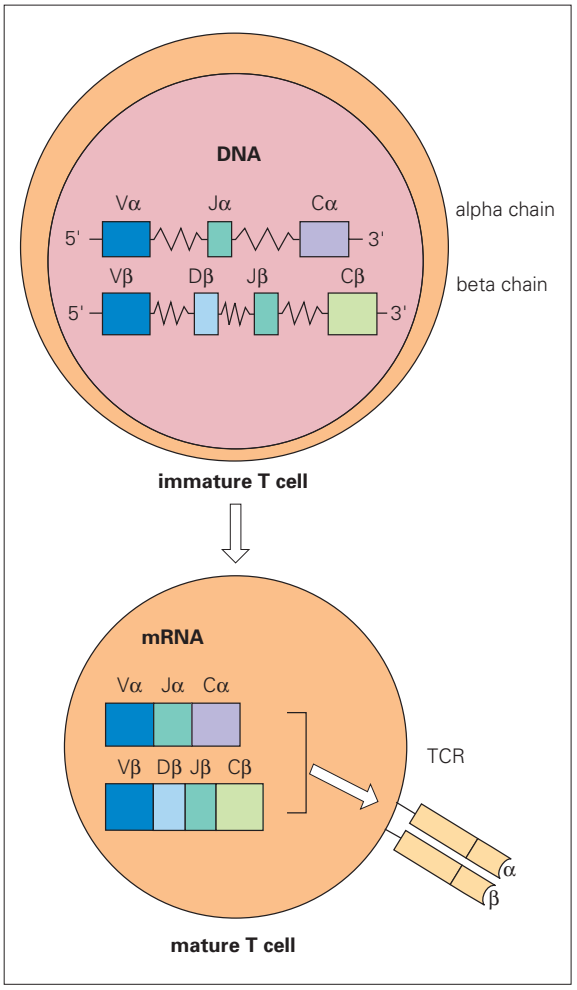


Figure 11.4 T-cell receptor (TCR) rearrangement. Rearrangements of the gene segments for the α– and β-chains (or γ– and δ-chains) of the TCR is similar to that for the immunoglobulin heavy and light chains. Spliced transcribed RNA codes for the individual α– and β-chains that are expressed on the T-cell surface with specificity for a peptide presented in the peptide-binding groove of MHC. The combination of V, J and C gene segments for the α-chain and of V, D, J and C gene segments for the β-chain provides even greater total diversity in antigen recognition than for the immunoglobulin molecule.

we age, but is still capable of producing some new T cells in adults.

The next step involves complicated genetic rearrangements, similar to those that occur in B cells as they differentiate (see below). The repertoire or number of potential shapes or antigens that need to be recognized by T cells is very large but the T-cell receptor (TCR) repertoire is generated in a very efficient way, by combining or splicing different gene segments together, just as for the antibody molecule described below. The alpha (α)-chain contains variable (V), and joining (J) gene segments that are combined with a constant or C region (Fig. 11.4). The second beta (β)-chain combines V, diversity (D) and J gene segments as well as its own C regions. Further diversity results from reading the D segments of the β-chain in all three reading frames, and from adding N and P nucleotides to the V–D and D–J junctions of the α-chain and the V–J junctions of the β-chain. A subset of T cells expresses a γδ–TCR rather than an αβ–TCR, but if the α-chain is expressed this results in the deletion of the Vδ region, committing the cell to express an αβ-TCR. Again similar to the antibody molecule, the most variable regions are in the complementarity-determining region hypervariable loops on the α– and β-chains. But the TCR does have important differences from immunoglobulin – it is not secreted, it does not change its C regions, and there is no somatic mutation to further increase the repertoire of antigens that can be recognized.

Two further selection processes take place in the thymus. First, only those T cells whose TCR can recognize self-major histocompatibility complex (MHC) molecules presenting self-peptides are given a survival signal in a process that is called positive selection. Any T cells unable to recognize antigens presented by the individual's MHC molecules would be useless and only occupy valuable space, so are better deleted. A final step of maturation in the thymus involves negative selection, through which any T cell whose TCR binds too strongly to the self-MHC molecules are deleted by apoptosis (programmed cell death) as these cells could be dangerous, inducing autoimmunity (i.e. an immune response against the body or self). From the combinations of gene segments and chains there is a T-cell repertoire even larger than that of the starting B-cell repertoire; however, this repertoire does not expand further in the periphery owing to mutation, as is the case for antibodies.

During these selection processes the T cells express the signature T-cell antigen CD3, and are also positive for both the CD4 and CD8 antigens. In the medulla the T cells then become either CD4 or CD8 positive and start expressing more of the CD3 antigen. The T cells that enter the circulation are mature $CD3^+$ T cells expressing a functional TCR and CD4 or CD8. However, in immunology speak they are still 'naive' T cells as they have not yet been activated by the signals they receive when they recognize antigen presented by self-MHC.

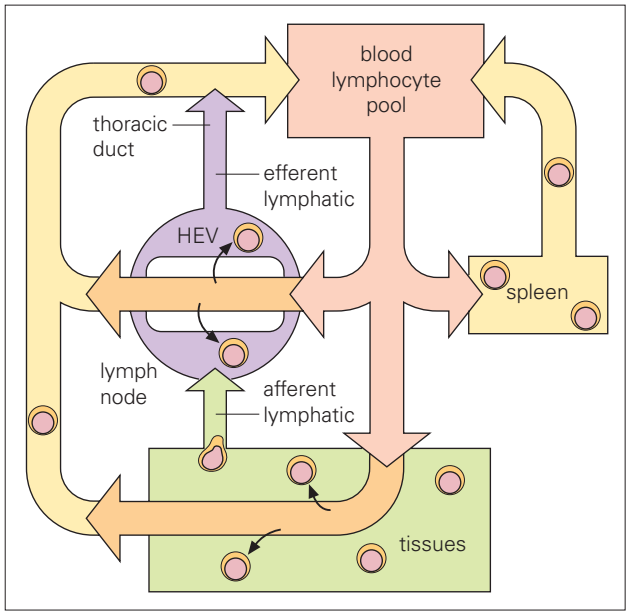


Figure 11.5 Recirculation of T and B cells. The lymphocytes move through the circulation and enter the lymph nodes via the specialized endothelial cells of the postcapillary venules (HEVs). They leave through the efferent lymphatic vessels and pass through other lymph nodes, finally entering the thoracic duct, which empties into the circulation at the left subclavian vein (in humans). Lymphocytes enter the white pulp areas of the spleen in the marginal zones; they pass into the sinusoids of the red pulp and leave via the splenic vein. (Adapted from: Roitt, I.M., Brostoff, J., Male, D. [2002] *Immunology*, 6th edn. London: Elsevier Science.)

The thymus medulla also contains structures called Hassall's corpuscles, formed of epithelial cells whose function is still not understood.

Once they are ready the naive T cells join the naive B cells (and innate immune cells) in the blood. They can then enter the secondary lymphoid organs (spleen, lymph nodes and the mucosa and gut-associated lymphoid tissue) and recirculate around the body through the lymphatic vessels and the blood (Fig. 11.5). They can also enter other tissues. And most importantly they can go to sites of infection and inflammation when they are needed.

SECONDARY LYMPHOID ORGANS

Lymphoid organs like the lymph nodes and spleen are compartmentalized into T-cell and B-cell areas. In the lymph node B cells are found in B-cell follicles, surrounded by T-cell zones where T cells respond to antigens brought there by dendritic cells (Fig. 11.6). B and T cells both enter through the high endothelial venules but are then directed to their respective B- and T-cell zones by particular chemokines. T cells express CCR7, which binds to CXCL19 and CXCL21 expressed by stromal cells in the T-cell regions. B cells are attracted to the B-cell follicles where the CXCR5 on their surface binds to CXCL13 on the surface of follicular dendritic cells. The B-cell primary follicles develop into germinal centres where B cells are activated and proliferate following antigen stimulation; once they become mature plasma cells that secrete large quantities of antibody they move into the medulla. In the spleen B cells are again found in follicles, in this case in the white pulp, surrounded by T cells in an area called the periarteriolar lymphoid sheath.

The mucosal-associated lymphoid tissue also contains foci of lymphocytes in Peyer's patches, where lymphocytes can respond to antigens from the environment, and particularly to the heavy bacterial load in the intestine, by producing IgA antibodies for mucosal secretions. The lymphocytes that form the MALT recirculate between these mucosal tissues using specialized homing receptors (Fig. 11.7). There are further fat-associated lymphoid clusters in the pleural, pericardial and peritoneal cavities.

SUBSETS OF T CELLS

Just as there are subsets of innate lymphoid cells, subsets of T cells also develop specialized functions.

T cells can be subdivided by function (for example as helper, cytotoxic and regulatory T cells), and by production of particular cytokines or cytotoxic mediators. CD4 T cells are generally referred to as T-helper (Th) cells and CD8 T cells as T-cytotoxic cells, although both CD4 and CD8 T cells can have a variety of functions. The initial division of CD4 helper T cells into Th1 and Th2 T cells, making the defining cytokines interferon gamma (IFNγ) and IL-4 respectively, has been expanded to include Th17 T cells making IL-17, Th9 T cells making IL-9, and regulatory T cells (Treg) that make the immunosuppressive cytokines transforming growth factor-β (TGFβ) and IL-10 (Fig. 11.8). Another T-cell subset, follicular helper T cells (Tfh) provides specialized help to B cells. Further markers such as CD69 and CD25 identify activated T cells, and finally more markers can distinguish naive T cells (CD45RA+) from antigen-stimulated effector cells or memory cells (CD45RO+) that will provide better and faster protection if we meet the same infection again. Within the cell there are signalling cascades and transcription factors associated with these functions. As more sophisticated ways of analysing individual T cells are developed, it has become clear that the immune system does not always keep T cells in these clearly defined subsets, and that T cells can show the ability to change from one subset to another, a phenomenon known as plasticity. This is presumably another way in which the immune system can generate more T cells of the type it needs to defend the body against a particular infection, as and when they are needed.

Like CD4 T cells, CD8 T cells can make cytokines such as IFNγ and IL-4, but are also good at killing virus-infected 'target' cells. The CD8 T cell recognizes viral peptides derived from cytoplasmic viruses that presented by MHC class I molecules on the host cell surface. Once activated, the T cell can punch holes in the infected target cell membrane using molecules such as perforin with structural similarities to the C9 terminal complement component. Natural killer (NK) cells can also kill target cells using perforin. Other ways cytotoxic T cells can kill include using granzyme, a molecule that is delivered into the target cells but that can kill some pathogens directly, and by inducing apoptosis (programmed cell death) through Fas–FasL interactions (Fig. 11.9).

Why do we need so many types of T cell?

Firstly the immune system has to defend us from a range of pathogens: bacteria, viruses and parasites. This requires specialized immune cells, but within the complexity of all

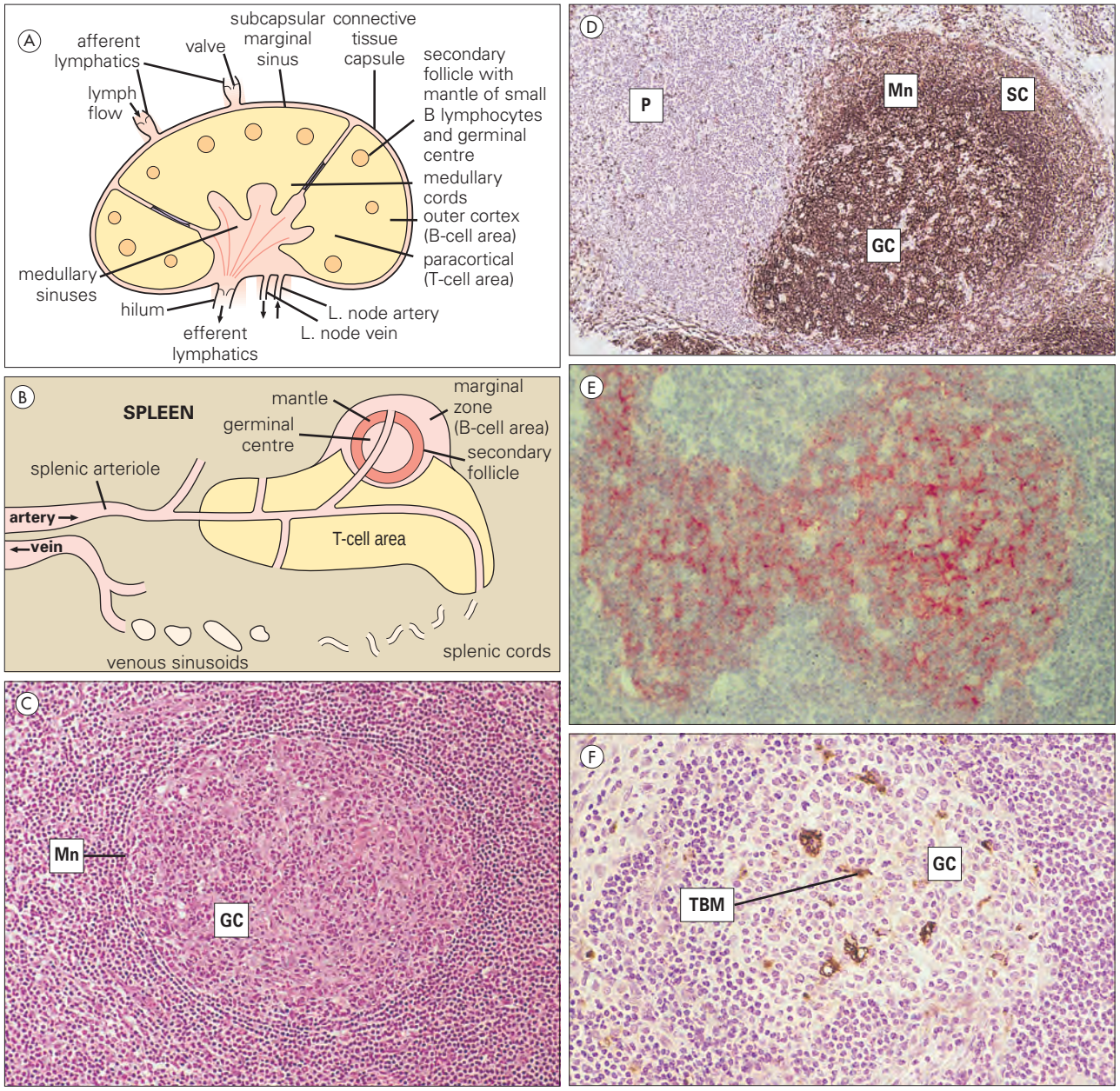


Figure 11.6 Structure of a lymph node and spleen. (A) Diagrammatic representation of section through a whole lymph node. The cortex is essentially a B-cell region where differentiation within the germinal centres of secondary follicles to antibody-forming plasma cells and memory cells occurs. (B) Diagrammatic representation of spleen showing B- and T-cell areas. (C) Structure of a secondary follicle. A large germinal centre (GC) is surrounded by the mantle zone (Mn). (D) Distribution of B cells in lymph node cortex. Immunochemical staining of B cells for surface immunoglobulin shows that they are concentrated largely in the secondary follicle, germinal centre (GC), mantle zone (Mn), and between the capsule and the follicle – the subcapsular zone (SC). A few B cells are seen in the paracortex (P), which contains mainly T cells. (E) Follicular dendritic cells in a secondary lymphoid follicle. This lymph node follicle is stained with enzyme-labelled monoclonal antibody to demonstrate follicular dendritic cells. (F) Germinal centre macrophages. Immunostaining for cathepsin D shows several macrophages localized in the germinal centre (GC) of a secondary follicle. These macrophages, which phagocytose apoptotic B cells, are called tingible body macrophages (TBM). (Courtesy of A. Stevens and J. Lowe; C–F reproduced from Male D., Brostoff J., Roth D.B., Roitt I. *Immunology*, 7th edition, 2006. Mosby Elsevier, with permission.)

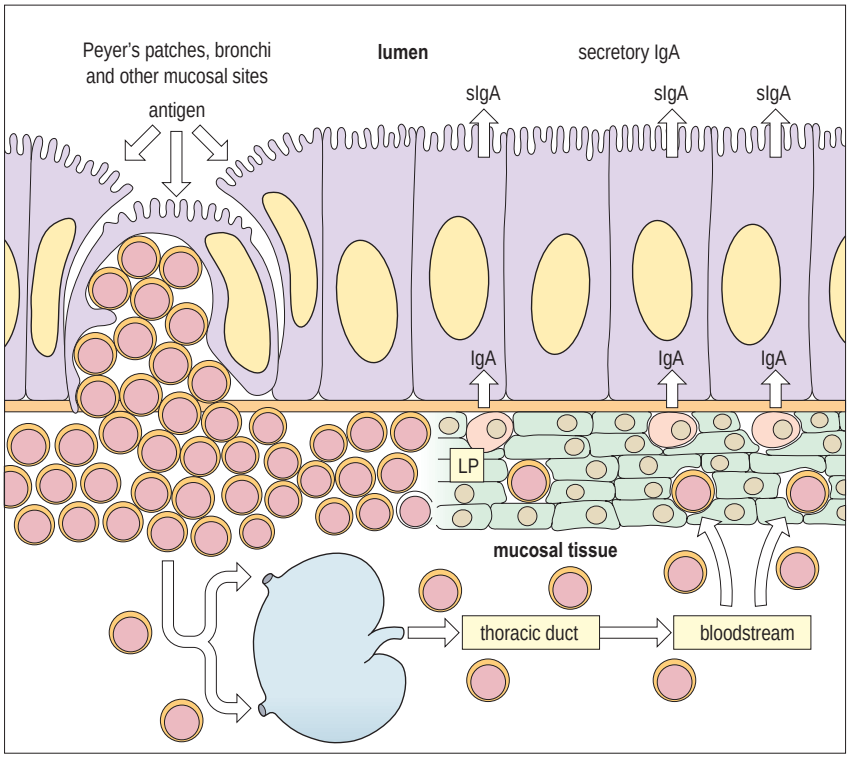


Figure 11.7 Mucosa-associated lymphoid tissue (MALT). Lymphoid cells which are stimulated by antigen in Peyer's patches (or the bronchi or another mucosal site) migrate via the regional lymph nodes and thoracic duct into the bloodstream and thence to the lamina propria (LP) of the gut or other mucosal surfaces which might be close to or distant from the site of priming. Thus lymphocytes stimulated at one mucosal surface may become distributed selectively throughout the MALT system. This is mediated through specific adhesion molecules on the lymphocytes and the mucosal high-walled endothelium of the postcapillary venules. (Adapted from: Roitt I.M., Brostoff J., Male D. [2002] *Immunology*, 6th edn. London: Elsevier Science.)

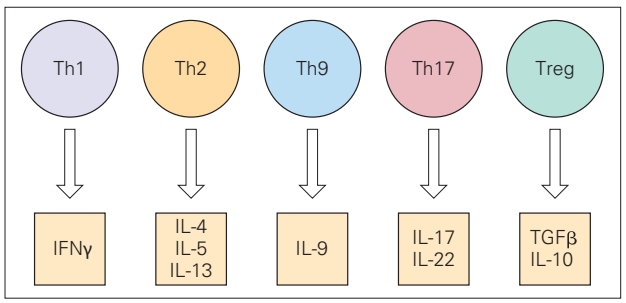


Figure 11.8 CD4 T-cell subsets. CD4 helper T cells can develop into a number of CD4 T-cell subsets in response to stimulation with particular cytokines; using particular transcription factors and gene regulators these T cells then produce particular cytokines that carry out distinct functions. IFN, interferon; IL, interleukin; TGF, transforming growth factor; Th, T-helper cell; Treg, regulatory T cell.

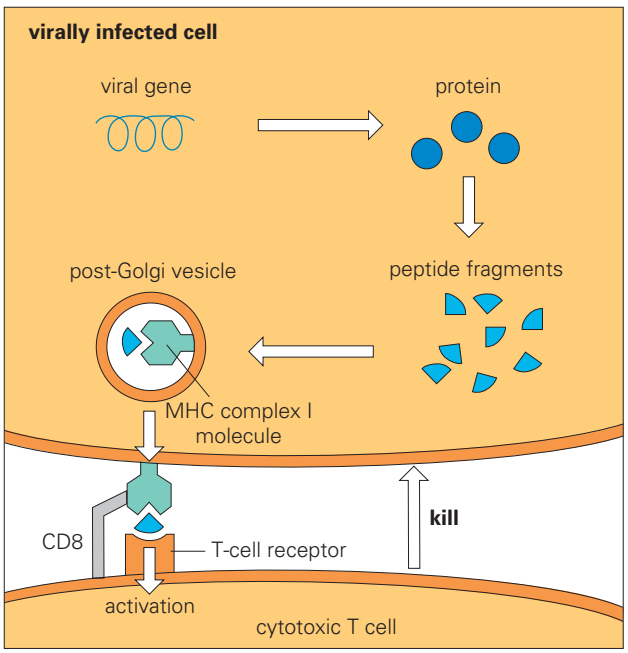


Figure 11.9 Cytotoxic T lymphocytes are activated when their specific cell surface receptors recognize an infected cell by binding to a surface MHC class I molecule that is associated with a peptide fragment derived from a degraded intracellular viral protein. CD, cluster of differentiation; MHC, major histocompatibility complex.

these subsets there is also redundancy where evolution has resulted in not only Th2 CD4 T cells but also type 2 CD8 T cells, but there are parallels in cytokine production between the Th1 and Th2 subsets and innate lymphoid cell types ILC1 and ILC2 (Fig. 11.10).

ANTIBODY STRUCTURE AND FUNCTION

The antibody molecule is the B cell's antigen recognition molecule. The term 'antigen' was given to the parts of foreign microorganisms that were *anti*body *gen*erating. Antibodies are expressed on the surface of the B cells where they can bind to parts of an antigen directly, but they can also be secreted by the B cell as soluble antibodies.

All antibodies have the same four-chain structure, with two longer 'heavy' chains and two shorter 'light' chains (Fig. 11.11). Their specificity is determined by the sequences of three hypervariable regions on both the heavy chain and the light chain that together form the Fab (or fragment antigen binding) region. These hypervariable regions are also called complementarity-determining regions, as their sequence is

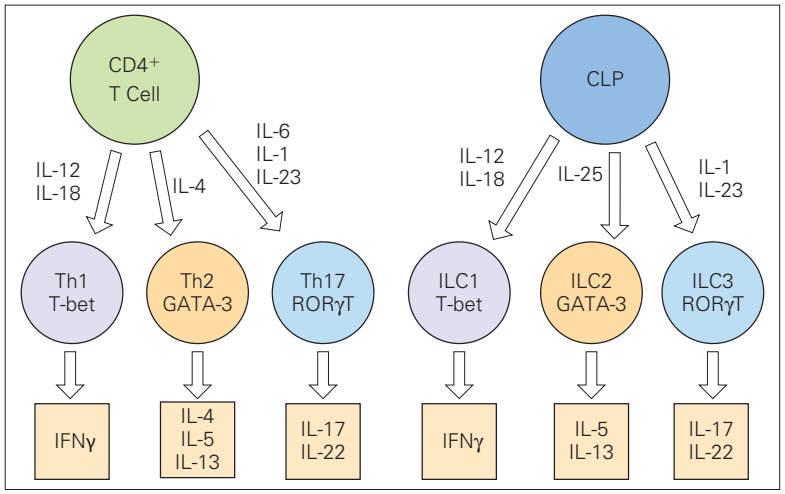


Figure 11.10 Parallels between the main CD4 T-cell subsets and innate lymphoid cell (ILC) subsets. ILC1, ILC2 and ILC3 cells use the same transcription factors and produce similar cytokines to the CD4 Th1, Th2 and Th17 subsets. Thus both ILC1 and Th1 cells provide protection against intracellular pathogens, ILC2 and Th2 help with defence against helminths and ILC3 and Th17 help with protection against fungi. Natural killer (NK) cells can be grouped with ILC1; they also make IFNγ but do not depend on the transcription factor T-bet. The ILC subsets develop from a precursor in the bone marrow; the CD4 subsets develop from naive CD4 T cells. CD, cluster of differentiation; GATA, GATA transcription factor; IFN, interferon; IL, interleukin; ROR, RAR-related orphan receptor; Th, T helper.

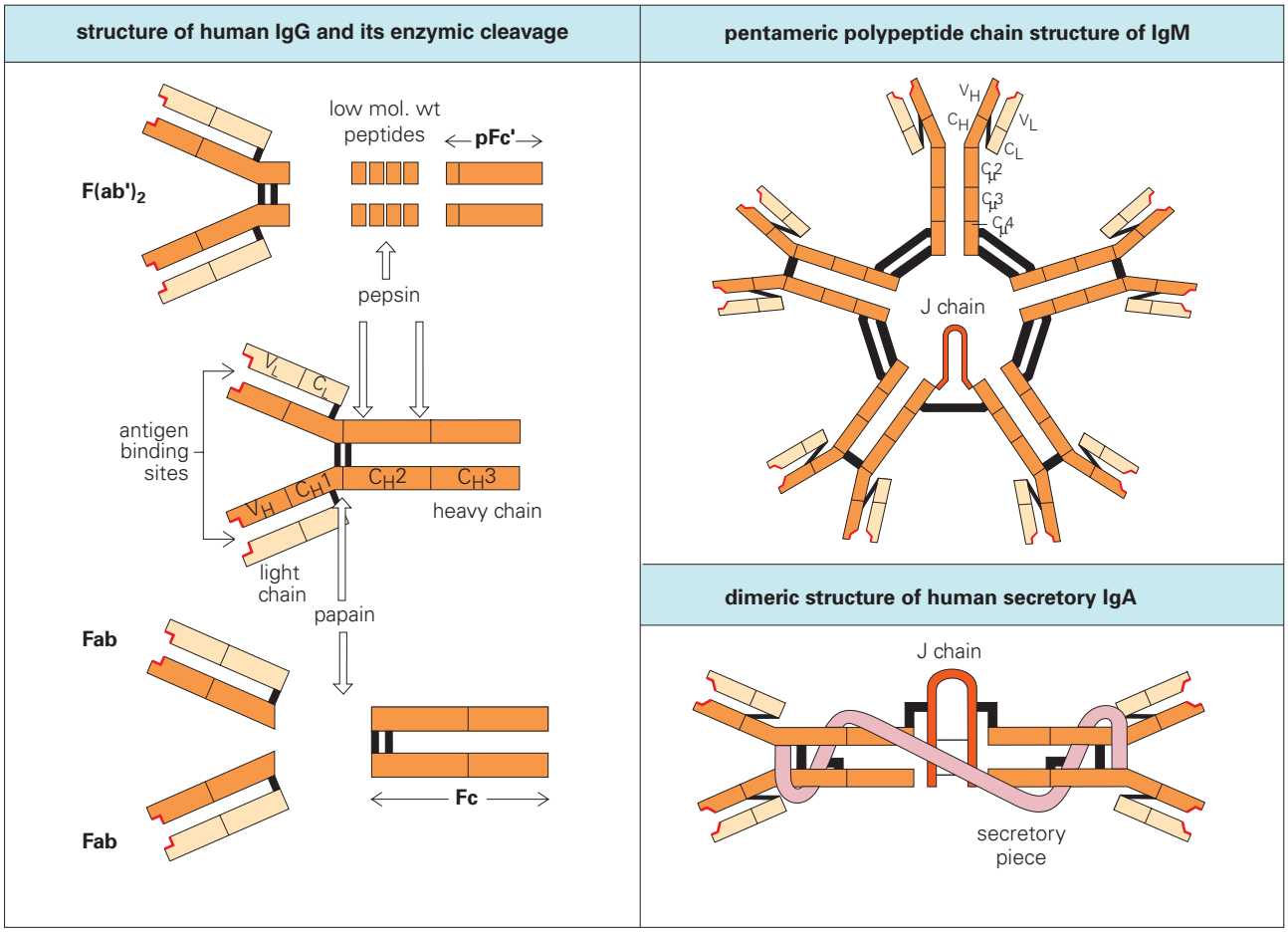


Figure 11.11 The structure of immunoglobulins. The basic structure of immunoglobulins is a unit consisting of two identical light polypeptide chains and two identical heavy polypeptide chains linked together by disulphide bonds (*black bars*). Each chain is made up of individual globular domains. Different antibodies have different V_L and V_H domains, the highly variable regions of the light and heavy chains, respectively. This hypervariability is confined to three loops on the V_L and three on the V_H domains. These make up the antigen-binding site (*highlighted in red*). In contrast, the remaining domains (C_L, C_H1, etc.) are relatively constant in amino acid structure. Cleavage of human immunoglobulin G (IgG) by pepsin induces a divalent antigen-binding fragment, F(ab')$_2$ and a pFc′ fragment composed of two terminal C_H3 domains. Papain produces two univalent antigen-binding fragments, Fab, and an Fc portion containing the C_H2 and C_H3 heavy chain domains. Polymerization of the basic immunoglobulin units to form IgM and IgA is catalysed by the J (joining) chain. The portion of the transporter (which transfers IgA across the mucosal cell to the lumen) which remains attached to the IgA is termed the 'secretory piece'.

complementary to the antigen. The molecule has a flexible hinge joint where the Fab region joins the constant or Fc region. How strongly the single antigen-binding site binds to the antibody defines the affinity.

Just as for the T-cell receptor, the chains of the antibody molecule are put together by splicing variable, diversity, joining and constant region gene segments together (Fig. 11.12). The heavy chains are formed from splicing V, D, J and C region genes together, while the light chains have V, J and C genes. All of this requires recombination signal sequences, a number of enzymes and epigenetic changes to the chromatin. This generates a huge repertoire of antibodies, enabling the immune system to have antibodies able to recognize the many pathogenic threats we may face.

The antigen-binding sites at the end of the two arms of the antibody molecule recognize three-dimensional shapes or antigens. These can be formed by the three-dimensional shape of a molecule or a linear sequence of amino acids. Antibodies can also recognize sugars, lipids and nucleic acids as antigens – it is the shape that determines how well they bind to, or recognize, an antigen. The strength of the binding of the bivalent basic IgG antibody molecule (or the four-valent secretory IgA or the ten binding sites of the pentavalent IgM antibody molecule) defines the overall strength of binding or avidity. High affinity and avidity binding is important to prevent the antibody from dissociating from its antigen, as this binding is not irreversible. During an immune response the affinity of the antibodies made can increase owing to somatic mutation in the Fab (variable) region and selection of higher affinity antibodies.

Antibodies come in different classes and subclasses, with different structures and functions

B cells initially make IgD, with two heavy and two light chains, which is expressed on the surface of naive B cells. The clever thing is that this basic antibody molecule can retain its antigen specificity, but change its other properties encoded in the sequence of the Fc region. This **class switching** involves switching the constant or C regions of the heavy and light chains, but fusing them to the same antigen-binding sequences that give the antibody its antigen specificity. Alternative splicing then combines the same Fab regions with the Fc region for IgM, which forms a pentameric IgM antibody that is particularly good at agglutinating bacteria and binding complement, and then immunoglobulin G (IgG). The IgG molecule itself comes in different subclasses, with different functions (IgG1, IgG2, IgG3 and IgG4 in humans). The bivalent secretory IgA molecules protect mucosal surfaces and IgE antibodies help defend us against helminth parasites as well as causing unwanted allergies (Table 11.2).

The process of stimulating B cells to proliferate, so that useful B-cell clones expand, with further increases in the affinity of the antibodies they make, due to somatic hypermutation, and class switching and differentiation of B cells into plasma cells, takes place in the germinal centres of the lymph nodes and spleen. Some memory B cells are also exported to provide better protection against future attacks by the same pathogen, as discussed in Chapter 12.

The antibody molecules themselves are part of a 'superfamily' of molecules that include not only the antibodies,

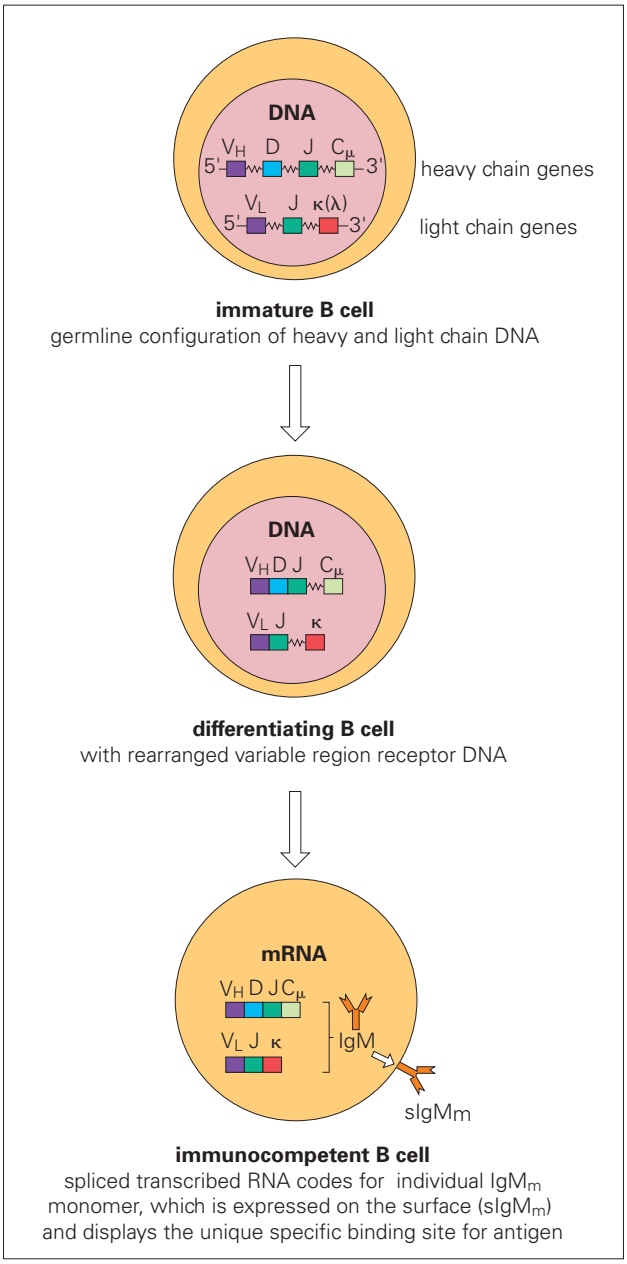


Figure 11.12 Differentiation events leading to the expression of unique sIgM monomers on the surface of an immunocompetent B lymphocyte. There are 45 germline V_H genes encoding the major portion of the variable region, with 23 minigene segments encoding the D segment and 6 for the J region. As the cell differentiates, V_H, D and J gene segments on one chromosome randomly fuse to generate lymphocytes with a very wide range of individual heavy chain variable domains. There are separate loci for the genes encoding the kappa and lambda light chains. Variable region light chain domains are formed by random V_L to J recombination – there are 35 and 30 kappa and lambda V genes with 5 and 4 J regions for each. Finally, the variable and constant region genes, respectively, recombine to encode a single antibody molecule which is expressed on the mature B-cell surface as an sIgM antigen receptor. When activated for antibody production, the transmembrane segment of IgM, which normally holds the molecule on the surface is spliced out at the RNA stage and the soluble form of the IgM is secreted. Subsequently, heavy chain constant region gene switching can occur to generate the various immunoglobulin classes, IgG, IgA, etc. Leader sequences have been omitted for simplicity.

Table 11.2 Biological properties of major immunoglobulin (Ig) classes in the human

Designation	IgG	IgA[a]	IgM	IgD	IgE
Major characteristics	Most abundant internal Ig	Protects external surfaces	Very efficient against bacteria	Mainly lymphocyte receptor	Initiates inflammation raised in parasitic infections; causes allergy symptoms
Valency[b]	1	1/2	5	1	1
Antigen binding	++	++	++	++	++
Complement fixation (classical)	++	–	+++	+	–
Cross placenta	++	–	–	–	–
Fix to homologous mast cells and basophils	–	–	–	–	++
Binding to macrophages and polymorphs	+++	+	–	–	+

[a]Dimer in external secretion carries secretory piece; IgA dimer and IgM contain J-chains.
[b]Valency or number of four-chain molecules, each with two antigen-binding sites.

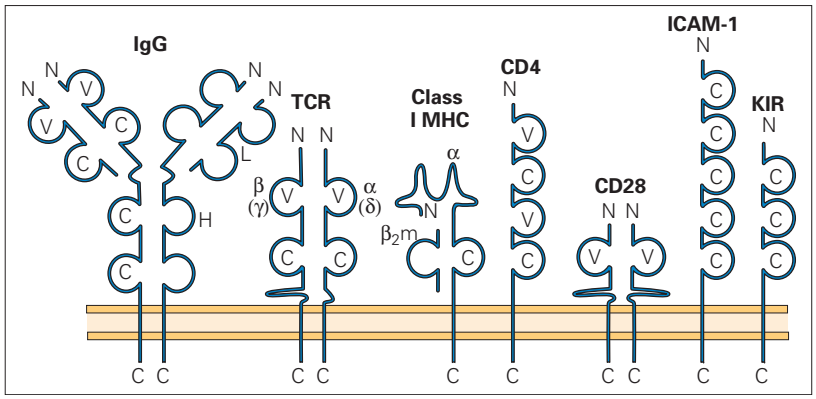


Figure 11.13 Members of the immunoglobulin superfamily. A number of important molecules including the immunoglobulins and the T-cell receptor have a similar overall structure containing a number of domains with β–pleated sheets held together by disulphide bonds. All these V and C domains originally evolved from a single gene precursor. CD, cluster of differentiation; ICAM, intercellular adhesion molecule-1; KIR, killer Ig-like receptor; MHC, major histocompatibility complex; TCR, T-cell receptor.

the T-cell receptor and MHC molecules but also a number of other molecules found in the plasma membranes of the cell, that also have similar domain structures and transmembrane portions (Fig. 11.13).

Subsets of B cells

There are also subsets of B cells. The main B-cell subset responsible for the most effective antibodies, that undergoes both class switching and somatic mutation of the antibodies they make, are the follicular B cells, but these require 'help' from T cells, which will be discussed further in Chapter 12. These B cells can develop into plasma cells, and into memory B cells (see Fig. 12.9). Marginal zone B cells can respond to antigens without help from T cells, but produce only short-lived plasma cells; similar B-1 cells are found in mucosal tissues. These B cells derive from precursors in the liver rather than the bone marrow.

RECIRCULATION OF T AND B CELLS

Naive T and B cells can move from the thymus and the bone marrow into the secondary lymphoid organs where they are activated, while activated effector T and B cells can migrate into tissues and to sites of inflammation and tissue injury. Migration requires adhesion to endothelial cells in the post-capillary venules. Adhesion needs selectins, adhesion molecules that bind carbohydrates, and integrins, a larger family of 30 adhesion molecules. Naive T and B cells use L-selectin and the integrin leukocyte function associated antigen 1 (LFA-1) to reach the secondary lymphoid organs (naive T cells also use LFA-4). To enter sites of inflammation and infection, effector and memory T cells use LFA-1, very late antigen 4 (VLA-4) and the integrin $\alpha_4\beta_7$, while the tissue-homing central memory T cells also use L-selectin to enter tissues. The ligand for LFA-1 is intercellular adhesion molecule 1 (ICAM-1), which is expressed on cytokine-activated endothelial cells. VLA-4 binds to vascular cell adhesion molecule 1 (VCAM-1) (Table 11.3). Some T cells remain in tissues once there, as tissue-resident T cells; for example memory T cells for Epstein–Barr virus (EBV) are retained in the tonsils by local production of the cytokine IL-15 that down-regulates the sphingosine-1-phosphate molecule they need to exit the tonsil.

Chemokines also play a key role in attracting T and B cells to the right place. Naive T cells are directed to the T-cell areas of lymphoid organs by the chemokines CCL19 and CCL21, which bind to CCR7 on the naive T-cell surface. B cells are attracted into the white pulp in the spleen and into germinal

Table 11.3 Important adhesion molecules

Family	Molecule	Distribution	Ligand	Cell types bound
Selectins	L-selectin	Neutrophils, monocytes, naive and central memory T cells, naive B cells	Sialyl Lewis X	Endothelium
	E-selectin	Activated endothelium	Sialyl Lewis X	Neutrophils, monocytes, effector and memory T cells
	P-selectin	Activated endothelium	Sialyl Lewis X	Neutrophils, monocytes, effector and memory T cells
Integrins	LFA-1	Neutrophils, monocytes, naive and central memory T cells, naive B cells	ICAM-1, ICAM-2	Endothelium*
	Mac-1	Neutrophils, monocytes, dendritic cells	ICAM-1, ICAM-2	Endothelium*
	VLA-4	Monocytes, T cells	VCAM-1	Endothelium*
	$\alpha_4\beta_7$	Monocytes, gut-homing T and B cells	VCAM-1, MadCAM-1	Gut endothelium

*Up-regulated when activated by cytokines.
ICAM, intercellular adhesion molecule; LFA, leukocyte function-associated antigen; VCAM, vascular cell adhesion molecule; VLA, very late antigen.

centres by the chemokine CXCL13, which binds to CXCR5 on the B-cell surface. Mature plasma cells move out of the lymphoid organs and enter particular tissues based on the antibody they produce, for example, IgA-producing plasma cells move to mucosal sites as they express the integrin $\alpha_4\beta_7$, and the chemokine receptors CCR9, and CCR10, which bind to MadCAM-1, CCL25, and CCL28 on mucosal epithelial cells.

As well as this increased adhesion and attraction to lymphoid tissues, T and B cells are also attracted to sites of inflammation and infection by chemotaxis in response to signals alerting the body that there are invaders about, or that tissue damage is occurring, as for the cells of the innate immune system.

KEY FACTS

- The lymphoid system has primary lymphoid organs, the thymus and bone marrow, where the T and B cells develop, and secondary lymphoid organs such as the lymph nodes and spleen, where the mature T and B cells are activated to carry out their functions.
- The cells of the immune system recirculate through the body in the blood and lymph, and are attracted to sites of infection by mechanisms that sense pathogens and markers of inflammation.
- The cells of the adaptive immune system, the T cells and B cells, are antigen specific – each T cell or B cell has a single receptor on its surface that recognizes antigen. These antigen receptors are made up of splicing a number of gene segments together, allowing a large repertoire of antigens to be recognized.
- T cells recognize only antigens presented in the groove of an MHC molecule by an antigen-presenting cell, whereas the antibody that forms the antigen recognition molecule on a B cell can recognize and bind to free antigen or to whole pathogens.
- There are subsets of both T cells and B cells that carry out specialized functions; some of these functions are similar to those seen in the cells of the innate immune system.
- Once the body has its armies of antigen-specific T cells and B cells as well as the cells of the innate system, it is ready to defend us against a range of pathogens in an efficient and effective way.

12 Cooperation leads to effective immune responses

Introduction

Once the immune system has a full arsenal of innate and antigen-specific adaptive immune responses at its disposal, it needs to exploit these effectively in defending the body against pathogens. To deliver a protective immune response the various players have to work together. Antigens are 'presented' to T cells by professional antigen-presenting cells (APCs), more than one signal is needed to activate T cells, and although B-cell activation is simpler, it also requires cascades of intracellular events. Activated effector cells can act directly through cell-to-cell contact to kill an infected cell, but also produce soluble cytokines as messengers that act on other cells. And once the immune response has successfully dealt with an invader, it needs to be shut down. This chapter will discuss the many ways in which the cells of the innate and adaptive immune system, and their products, interact to provide effective immunity against infections.

COOPERATION MEANS GREATER EFFICIENCY

Antibodies on their own can serve useful functions, such as blocking the activity of toxins, but combining antibodies with the phagocytes of the innate immune system delivers more effective phagocytosis through opsonization. Additional activation of the complement system will further enhance removal of pathogens, and result in beneficial inflammation and lysis of infected cells.

T cells recognize processed antigen presented by antigen-presenting cells, unlike the B cells that can recognize free antigen or antigens on the surface of a pathogen. Nevertheless to deliver effective antibody responses, T cells need to provide 'help' to B cells, through a specialized subset of T follicular helper cells. Subsets of CD4 T helper cells also provide help to cytotoxic CD8 T cells, and can activate macrophages, making them more effective at killing intracellular organisms. Many of these interactions involve cell-to-cell contact and signalling but others are mediated by cytokines delivered into the cell–cell contact zone or immunological synapse.

As both T cells and B cells have antigen-specific receptors, they can be increased in number as required through clonal expansion. Although the rapid expansion of large numbers of antigen-specific T and B cells is beneficial, once the infection has been dealt with an excess of these cells would only occupy valuable space, and so the numbers are reduced by apoptosis. In case we are threatened by the same pathogen again, the immune system maintains highly specialized elite troops on standby – these antigen-specific memory cells are ready to be deployed but are already trained to kill or to secrete antibodies or cytokines. Finally, all these troops must be kept from getting out of control, so regulatory T cells and mechanisms of immune suppression are needed.

OPSONIZATION BY ANTIBODY ENHANCES PHAGOCYTOSIS AND LEADS TO COMPLEMENT ACTIVATION

Although antibodies can by themselves carry out useful functions such as blocking toxins from binding to their receptors, they work best when cooperating with phagocytes. Opsonization of a microbe when antigen-specific antibodies bind to its surface antigens will make it easier for the phagocyte to phagocytose the microbe. The Fc portion of the antibody molecule can bind to Fc receptors expressed on the phagocyte (Fig. 12.1). As noted in Chapter 11, some antibody classes and subclasses are good at activating the complement cascade, and additional opsonization with C3b, which then binds to the C3b receptor, further enhances phagocytosis (Fig. 12.2). Antibody-coated bacteria can also bind complement leading to lysis. The combined presence of complement and antibodies has a dramatic effect on survival of extracellular bacteria (Fig. 12.3). This illustrates how the innate and adaptive immune systems work together to deal with the removal of microbes.

BENEFICIAL INFLAMMATORY REACTIONS CAN ALSO BE ENHANCED BY ANTIBODIES

As well as increasing the rate of removal of pathogens, antibodies and complement enhance inflammation, with release of cytokines from macrophages. Activation of complement, as well as certain proinflammatory cytokines and mediators such as chemokines, increases vascular permeability, thus enabling greater numbers of circulating monocytes as well as other leukocytes to access the site of an infection. Other cytokines will attract and activate neutrophils. Adhesion molecules will then enhance binding to the vascular endothelium.

Mast cells express receptors for IgE antibodies. Cross-linking of these receptors will result in signalling and mast cell

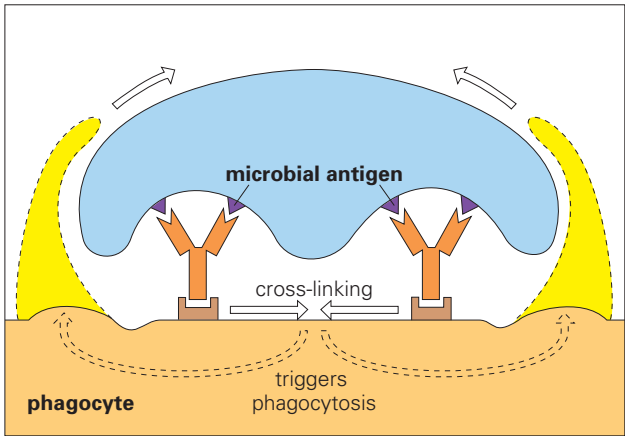


Figure 12.1 The binding of a microbe to a phagocyte by more than one antibody cross-links the antibody (Fc) receptors on the phagocyte surface and triggers phagocytosis of the microorganism, which is engulfed by the extending cytoplasmic projections.

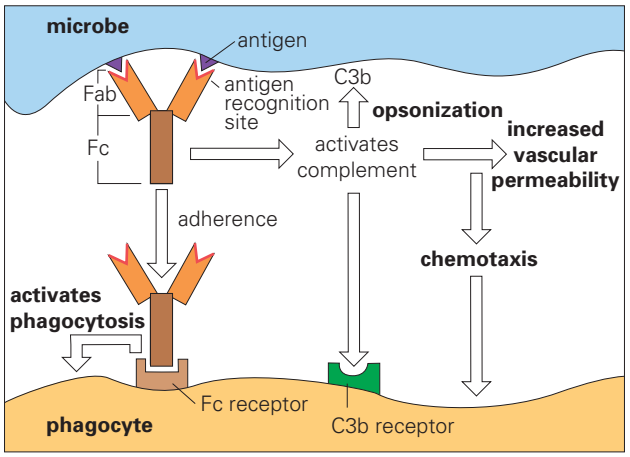


Figure 12.2 The antibody adaptor molecule. Antibodies (anti-foreign bodies) are produced by host lymphocytes on contact with invading microbes, which act as antigens (i.e. generate antibodies). Each antibody (see Fig. 11.11) has a recognition site (Fab) enabling it to bind antigen, and a backbone structure (Fc) capable of some secondary biological action such as activating complement and phagocytosis. Thus, in the present case, antibody bound to the microbe activates complement and initiates an acute inflammatory reaction. The C3b generated fixes to the microbe and, together with the antibody molecules, facilitates adherence to Fc and C3b receptors on the phagocyte and thence microbial ingestion.

degranulation, also leading to increased polymorph chemotaxis and vascular permeability (Fig. 12.4).

ACTIVATION OF T CELLS INVOLVES ANTIGEN-PRESENTING CELLS AND ADDITIONAL CO-STIMULATORY SIGNALS

Once a T cell is mature, it enters the circulation, expressing a T-cell receptor (TCR) on its surface. This receptor is designed to recognize antigen, or rather short linear peptides, presented in the groove of a major histocompatibility complex (MHC) molecule. The peptides are 'presented' to the T cells by highly efficient professional antigen-presenting cells called dendritic

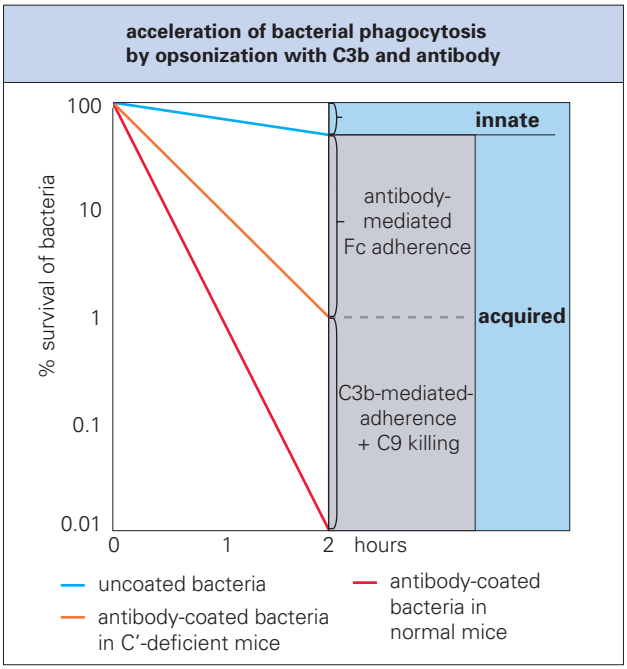


Figure 12.3 The slow rate of phagocytosis of uncoated bacteria (innate immunity) is increased many times by acquired immunity through coating with antibody and then C3b (opsonization). Killing may also take place through the C5–9 terminal complement components. This is a hypothetical but realistic situation; the natural proliferation of the bacteria has been ignored.

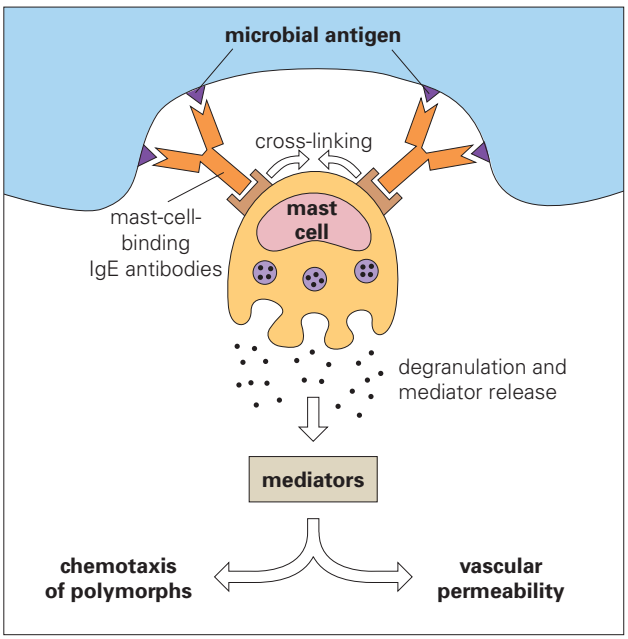


Figure 12.4 Degranulation of mast cells by interaction of microbial antigen with specific antibodies of the IgE class, which bind to special receptors on the mast cell surface. The cross-linking of receptors caused by this interaction leads to the release of mediators, which induce an increase in vascular permeability and attract polymorphs (i.e. they provoke an acute inflammatory reaction at the site of the microbial antigen).

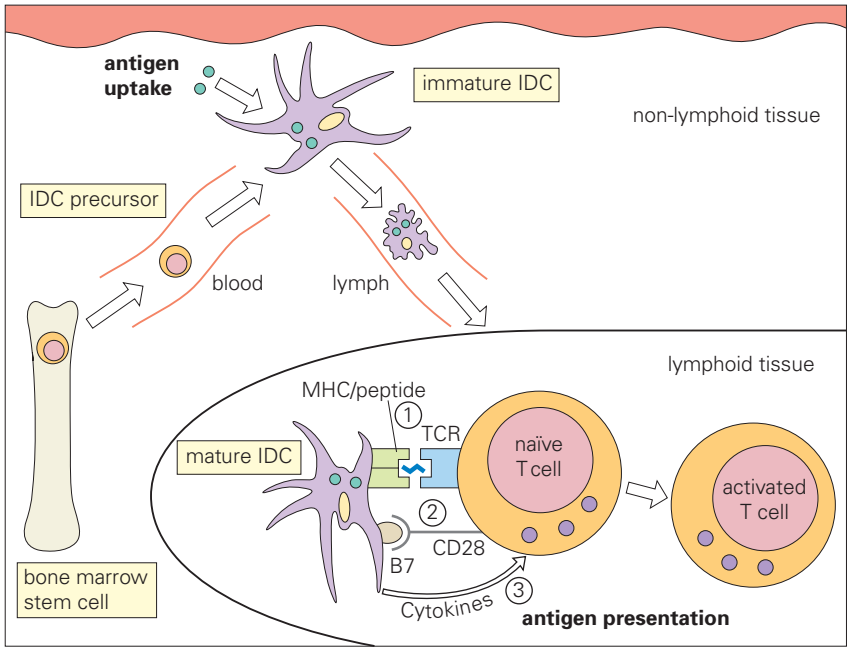


Figure 12.5 Migration and maturation of interdigitating dendritic cells (IDC). The precursors of the IDCs are derived from bone marrow stem cells. They travel via the blood to non-lymphoid tissues. These immature IDCs (e.g. Langerhans cells in skin) are specialized for antigen uptake. Subsequently, they travel via the afferent lymphatics to take up residence within secondary lymphoid tissues, where they express high levels of major histocompatibility complex (MHC) class II and co-stimulatory molecules such as B7. These cells are highly specialized for the activation and differentiation of naive T cells which are effected through three signals: (1) T-cell receptor (TCR) binding to MHC/peptide complex, (2) B7–CD28 co-stimulation and (3) cytokine release. (Reproduced with minor additions with permission from: Roitt I.M., Delves P.J. [2001] *Roitt's Essential Immunology*, 10th edn. Oxford: Blackwell Science.)

cells (Fig. 12.5) within the T-cell areas of the secondary lymphoid organs. The dendritic cells found in lymphoid organs express greater numbers of the MHC molecules on their surface compared with those found resident in tissues. The dendritic cells also need to be good at providing co-stimulatory signals (see below). Macrophages and even B cells can also present antigens to T cells, but although macrophages are better at phagocytosis than dendritic cells, both macrophages and B cells have lower expression of MHC and co-stimulatory molecules than do the dendritic cells in lymphoid tissues, although following activation this can be increased.

CD4 T cells recognize and respond to peptides presented by MHC class II molecules, that are derived from the degradation of proteins from phagocytosed organisms; the CD8 T cells recognize peptides derived from antigens in the cytoplasm that are presented by MHC class I molecules. The peptide-binding cleft or groove of the MHC class I molecule is closed at the ends and so binds only short peptides of 8–9 amino acids in length (Fig. 12.6), whereas the MHC class II molecules have clefts that are open at the ends so the peptides can be up to 30 amino acids long. These MHC molecules are highly heterogeneous and so some people will respond well to some peptides and others weakly or not at all (leaving what is called a 'hole' in their antigen-recognition repertoire). But before there can be an immune response, the peptides have to be loaded into the grooves of the MHC molecules.

Organisms that are phagocytosed, or antigens that are endocytosed (taken up into membrane bound endosomes) are degraded following fusion with lysosomes. The MHC class II molecules are found in a special type of endosome that also contain other key molecules needed to help transfer foreign peptides into the MHC class II groove. First, the invariant chain that has been occupying the groove must be removed, through initial degradation to form a shorter CLIP peptide and then exchanged for the foreign peptide by a peptide exchanger molecule called HLA-DM. Fusion of the two types of endosome, those containing the foreign peptides and those with the MHC molecules, results in suitable foreign peptides occupying the MHC class II groove. The ends of the MHC class II groove are open so any peptide that hangs out from the ends can be trimmed to a final length of 13–30 amino acids. The MHC class II molecule is then cycled out to be expressed on the surface of the cell. Simple really!

To be presented in the groove of the MHC class I molecule, antigens must first get into the cytoplasm of the cell. There they are degraded by a special organelle called a proteasome. Next a transporter – imaginatively called 'transporter associated with antigen processing' or TAP – is needed to get the peptides to where the MHC class I molecules are located within the lumen of the endoplasmic reticulum. MHC I molecules with empty grooves are selected by a molecule called tapasin, and once the peptide has bound into the MHC I groove, the molecule is ready to begin its journey to the cell surface through the Golgi region and via exocytic vesicles. As the MHC class I molecule has a groove with closed ends, only peptides of eight to nine amino acids will fit comfortably.

The MHC antigens or human leukocyte antigens (HLA) are highly variable, so different individuals can respond to or recognize different peptide antigens. So although MHC tetramer reagents can be made containing four copies of particular labelled MHC class I molecule binding a specific antigen, and used to stain antigen-specific CD8 T cells, only T cells from those with that MHC type will bind the tetramer. Tetramers with MHC class II antigens are more complex to make as there are two variable MHC chains.

T cells need additional signals for activation

If a CD4 or CD8 T cell recognizes the peptide MHC complex on the surface of an antigen-presenting cell, often referred

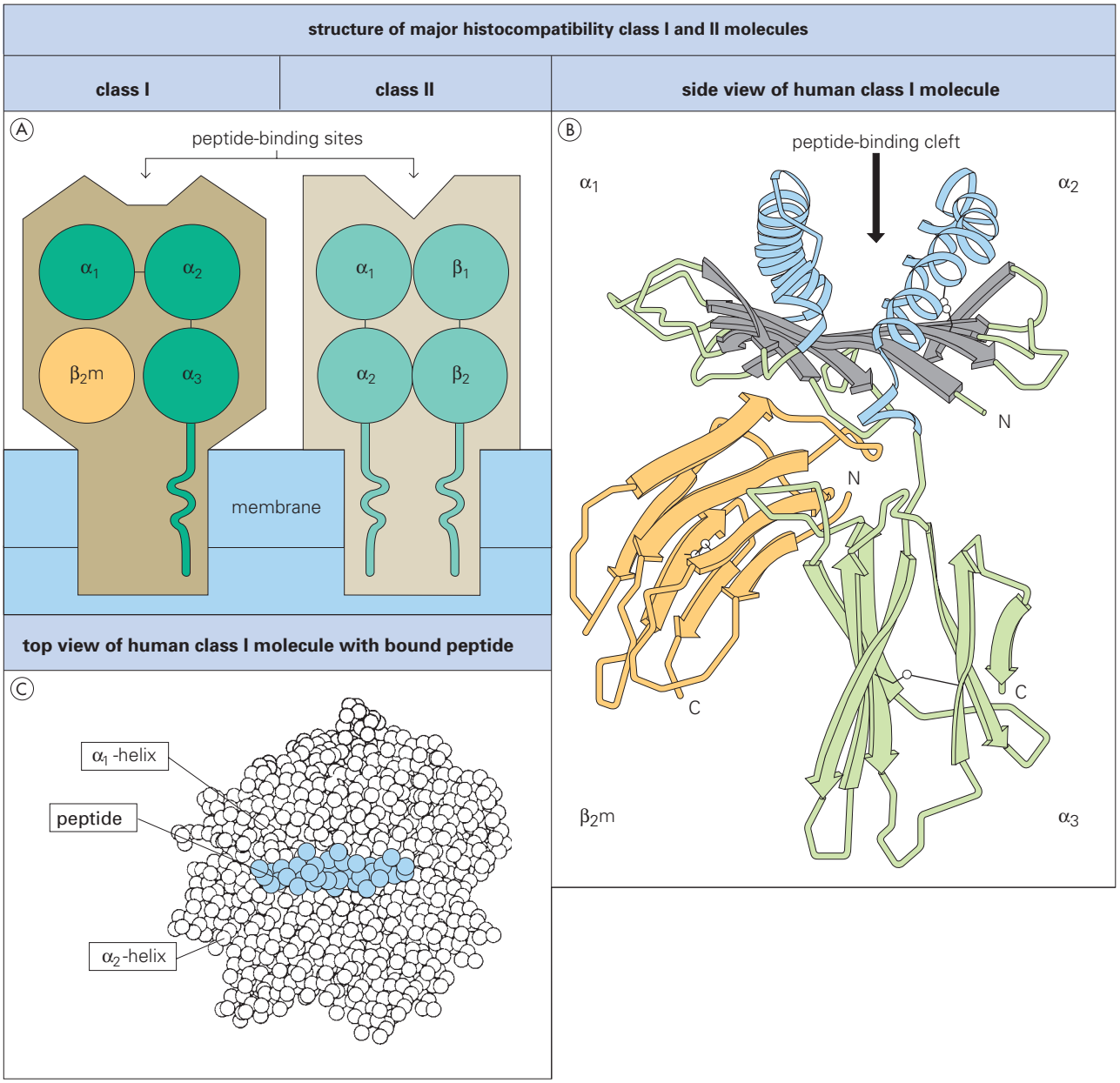


Figure 12.6 Class I and class II major histocompatibility complex (MHC) molecules. (A) Diagram showing domains and transmembrane segments; the α-helices and β-pleated sheets are viewed end-on. (B) Side view of human class I molecule (HLA-A2) based on X-ray crystallographic structure showing the cleft and the typical immunoglobulin folding of the α_3 and β_2-microglobulin (β_2m) domains (four antiparallel β-strands on one face and three on the other). The strands making the β-pleated sheet are shown as thick grey arrows in the amino to carboxyl direction, α-helices are represented as helical ribbons. The inside facing surfaces of the two helices and the upper surface of the β-pleated sheet form a cleft which binds the peptide. (C) Top view of a peptide bound tightly within the MHC class I cleft, in this case peptide 309–317 from HIV-1 reverse transcriptase bound to HLA-A2. This is the 'view' seen by the combining site of the T-cell receptor described below. ([B] Adapted from: Bjorkman, P.I. et al. [1987] *Nature*; 329:512, with permission. [C] Based on Vignali, D.A.A., Strominger, J.L. [1994] *The Immunologist*; 2:112, with permission.)

to as its cognate antigen, this provides the first signal for T-cell activation. However a second signal is also required, delivered by the binding of a molecule called CD28 on the T cell to a molecule called B7 on the antigen-presenting cell. If both signals are sent, then T-cell activation results, helped by T-cell expression of CD40 interacting with CD40L on the antigen-presenting cells and leading to additional cytokine release from the DC. T-cell activation involves a cascade of intracellular enzymes. The T-cell receptor itself does not have a cytoplasmic tail capable of delivering such signals – instead signalling occurs through the associated gamma (γ), delta (δ), epsilon (ε) and zeta (ζ) chains of the CD3 molecule. Such signalling involves the phosphorylation of protein kinases, and for TCR signalling through the CD3-chains there are immunoreceptor tyrosine-based activation motifs (ITAMs) available for tyrosine phosphorylation. ITAMs consist of two copies of the sequence [tyrosine—any amino acid—any amino acid—leucine]. Sometimes the kinase enzymes associate with

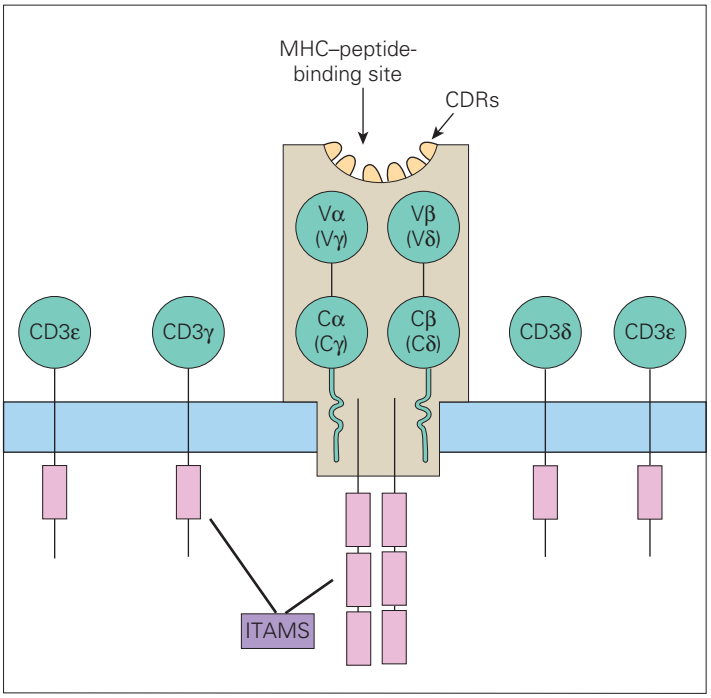


Figure 12.7 The T-cell receptor on αβ-T cells consists of an α- and a β-chain each composed of a variable (V) and a constant (C) domain resembling the immunoglobulin Fab antigen-binding fragment in structure. The highly variable (complementarity-determining) regions (CDRs) on the variable domains contact the major histocompatibility complex (MHC)–peptide antigen complex. This produces a signal which is transduced by the invariant CD3 complex composed of γ-, δ-, ε- and ζ- or η-chains, through their cytoplasmic immune receptor tyrosine-based activation motifs (ITAMs) which contact protein tyrosine kinases. γδ-T cells (see below) have receptors composed of γ and δ-chains as indicated in the figure. CD, cluster of differentiation.

the intracellular portion of a co-receptor such as the CD4 α-chain, or the CD8 α– and β-chains (Fig. 12.7).

The molecular interactions between the T cell and the antigen-presenting cell take place within a contact zone known as the **immunological synapse**, where lipid rafts help bring the molecules on the two cells together. The central zone of the synapse that contains the TCR and associated co-receptors is called the central supramolecular activation cluster, surrounded by a peripheral area that contains adhesins. Cytokines are also secreted directly into this synapse.

The immunoglobulin molecule on the surface of a B cell is also unable to signal directly and is also associated with two invariant chains called immunoglobulin alpha (Igα) and immunoglobulin beta (Igβ) that contain ITAMs. For natural killer (NK) cells that lack both CD3 and immunoglobulin on their surface, signalling occurs through their own ITAM-containing DAP12 protein.

Complex intracellular signalling cascades follow the phosphorylation of ITAMs

T cells use a particular kinase Lck to phosphorylate the ITAMs of the T-cell receptor complex. Next the tyrosine kinase ZAP-70 binds to the phosphorylated ITAMs leading to activation of phosphatidinol inositol. The following steps are even more complex – involving enzyme scaffold proteins, adaptor molecules such as LAT and a host of enzymes – but the end result is the production of transcription factors such as nuclear factor of activated T-cells (NFAT) and nuclear factor kappa B (NFκB), and changes in metabolism, Ca^{2+}, adhesion properties and cytoskeletal reorganization – leading to cell activation and ultimately cell division.

T cells with a γδ TCR and other invariant T cells

A smaller family of T cells have a TCR with γ– and δ-chains rather than the usual αβ-TCR. These γδ-T cells recognize non-protein antigens including phosphorylated molecules and lipids, presented by CD1 molecules that lack the diversity seen in the classical MHC I molecules. Other invariant T cells are found in the mucosa, called mucosal-associated invariant T cells, (Tmait), that recognize vitamin D metabolites from bacteria and fungi presented by MR1, another invariant MHC-like molecule.

Superantigens stimulate too many T cells

Some 'superantigens' can stimulate any TCR expressing particular families of Vβ genes directly, independently of their antigen specificity, activating up to 20% of all T cells. Staphylococcal enterotoxin B can do this, leading to extensive T-cell activation and a 'cytokine storm' due to the excessive cytokine release that results. Both T cells and B cells can also be stimulated non-specifically by mitogens, for example the red-kidney-bean-derived phytohaemagglutinin or concanavalin A for T cells and pokeweed mitogen for B cells. These mitogens can be useful tools for immunologists but are best avoided in real life, which is why it is important to boil uncooked red kidney beans well!

CLONAL EXPANSION

Each T cell and B cell expresses its own antigen receptor, either a TCR or an Ig molecule. Clonal expansion enables a large increase in the numbers of antigen-specific T or B cells. A lymphocyte expressing a receptor for a particular antigen or part of that antigen (the epitope) are activated as described above, leading to clones of cells with the same receptor (and the same function) (Fig. 12.8). Although some bystander proliferation of non-antigen-specific cells occurs through the release of cytokines such as IL-2 that act as growth factors, clonal expansion is a very effective way of producing enough of the right sort of cells on demand.

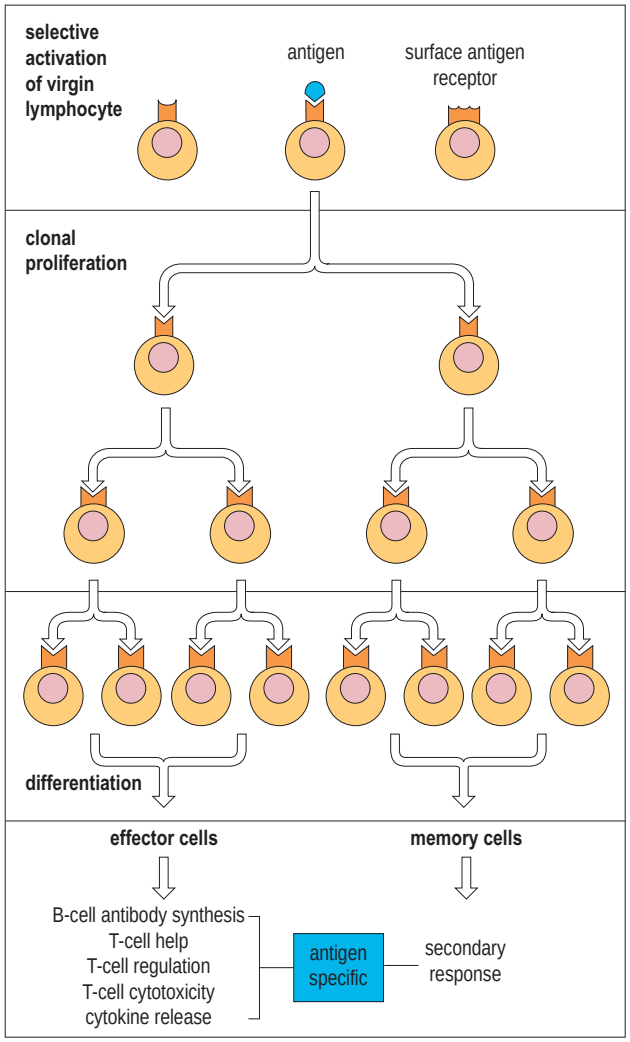


Figure 12.8 Generation of a large population of effector and memory cells by clonal proliferation after primary contact of B or T cell with antigen. A fraction of the progeny of the original antigen-reactive lymphocytes becomes non-dividing long-lived memory cells, whereas the others become the effector cells of humoral or cell-mediated immunity. Memory cells provide a large pool of antigen-specific cells that are activated more easily than naive T or B cells.

The principle of clonal expansion can be used to generate a clone of T cells expressing the same TCR. The T-cell clone must be stimulated by antigen presentation of the epitope it recognizes, as well as growth factors such as IL-2 and IL-7, but can be maintained in tissue culture without being immortalized by fusion with a tumour cell or transformed by infection with a tumour virus.

ANTIBODY PRODUCTION INVOLVES A SERIES OF STEPS WITHIN THE GERMINAL CENTRE

To make an effective antibody response is a complicated process! First, the antigen-specific B cells needs to be activated and to proliferate. However, whereas all the daughter clones of a particular T cell will express exactly the same TCR, somatic mutation in the immunoglobulin genes leads to antibodies of greater affinity, as well as switching of the class and subclass of antibody made by the B cell (Fig. 12.9). An enzyme called activation-induced cytidine deaminase (AID) drives the development of somatic mutations in the immunoglobulin V region genes of germinal centre B cells, and also initiates isotype switching.

T-cell help for antibody production

Within germinal centres in the secondary lymphoid organs, T-cell help is needed for effective B-cell development and to help the affinity maturation of the antibodies produced (Fig. 12.10). The specialized T cells providing this help are called follicular helper T cells (Tfh). The Tfh respond to IL-6 and chemokines such as CXCL13 and move into the germinal centre; IL-6 activates the STAT 3 transcription factor and then the transcription factor Bcl-6. Contact between the Tfh and the B cell involves co-stimulatory molecules such as CD28 as well as through the TCR/MHC but B-cell help is also mediated by IL-4 and IL-21 secreted by the Tfh. IL-21 is particularly important in promoting the proliferation of B cells and their differentiation into plasma calls that produce large quantities of antibody.

Tfh cells seem to be very permissive for viral production in early HIV infection; they are reduced in number as infection progresses, but are maintained in those individuals (called elite controllers) who control their HIV infection without progression.

Sometimes B cells can make antibodies without T-cell help

Some antigens, called T-independent antigens, can stimulate B cells to make antibodies directly, without help from T cells. Just as T cells expressing TCRs with different specificities can be activated by superantigens, B cells expressing different immunoglobulins can also be activated by polyclonal activators called thymus-independent or TI-1 antigens such as lipopolysaccharide (LPS) or bacterial DNA, that activate the B cell via Toll-like receptors – in effect acting as B-cell mitogens (Fig. 12.11). These thymus-independent antigens do not induce affinity maturation or B-cell memory responses.

A second type of T-cell independent antigen has repeating determinants such as those found on the polysaccharide capsules of some bacteria. These are presented to marginal zone B cells by marginal zone macrophages in the spleen or to B cells by macrophages in the subcapsular sinus of lymph nodes. The repeating determinants cross-link the immunoglobulins on the B cell, leading to B-cell activation. But again, the antibody response is not optimal as mainly IgM antibody is produced.

Monoclonal antibody technology exploits clonal expansion and transformation to produce large quantities of monoclonal antibodies

B cells are harder to maintain in culture than T cells, but fusing an individual B cell to a myeloma cell will result in a clone of transformed B cells, producing antibody of one specificity, called monoclonal antibodies (Fig. 12.12, Box 12.1). Monoclonal antibodies are now widely used in medicine, for example to block cytokines such as tumour necrosis factor alpha (TNFα) (see Fig. 15.8). The monoclonal antibody can also be 'humanized' through inserting the critical antigen recognition CDR regions into the basic structure of a human

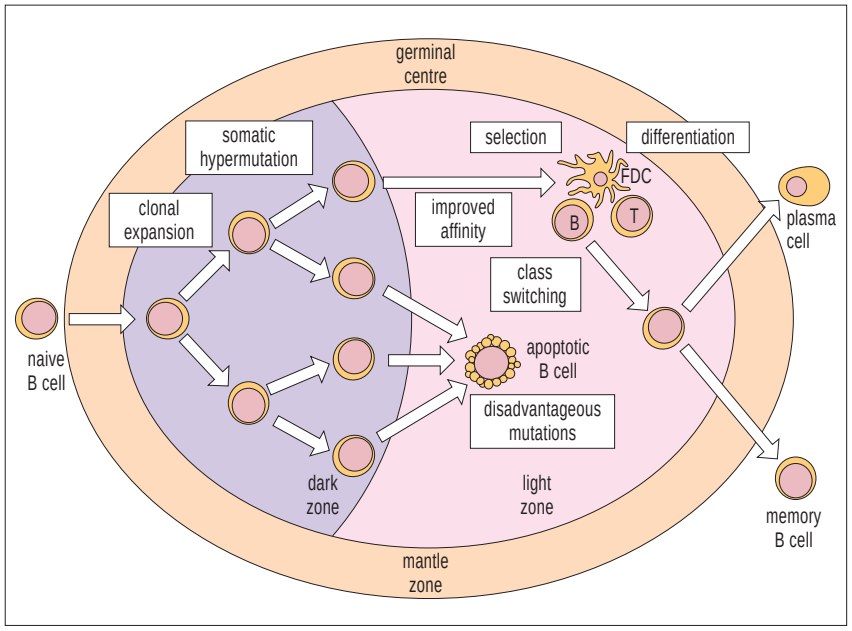


Figure 12.9 Structure and function of the germinal centre. One or a few B cells in the dark zone proliferate actively. This proliferation leads to clonal expansion and is accompanied by somatic hypermutation of the immunoglobulin V region genes. B cells with the same specificity, but various affinities, are therefore generated. In the light zone, B cells with disadvantageous mutations or with low affinity undergo apoptosis (see Fig. 11.6F) and are phagocytosed by macrophages. Cells with appropriate affinity encounter the antigen on the surface of the follicular dendritic cells (FDCs) and, with the help of $CD4^+$ T cells, undergo class switching, leaving the follicle as memory B-cell or plasma cell precursors. (Reproduced from Male D., Brostoff J., Roth D.B., Roitt I. *Immunology*, 7th edition, 2006. Mosby Elsevier, with permission.)

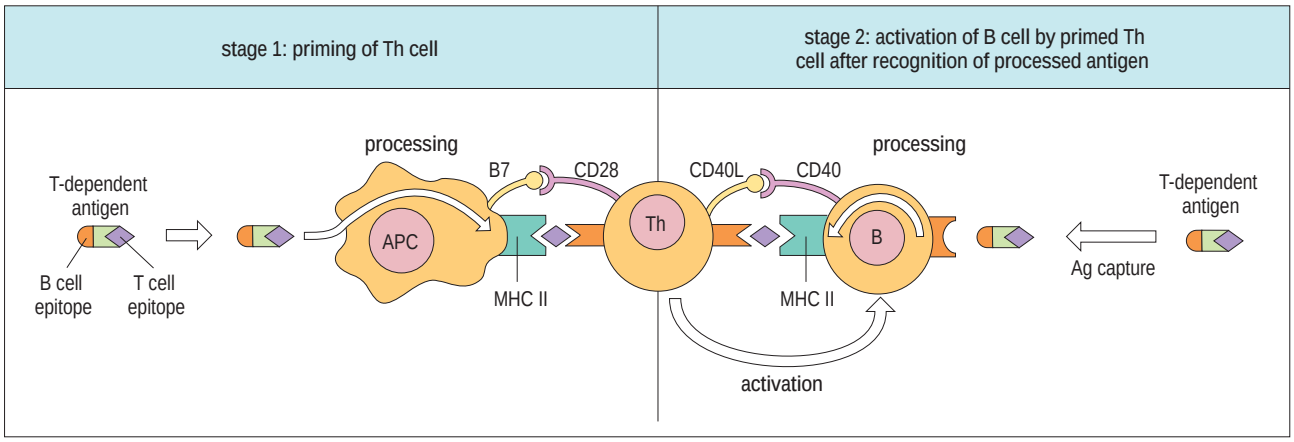


Figure 12.10 The mechanism by which T-helper (Th) cells are primed and then stimulate B cells to synthesize antibody to T-dependent antigens with the help of the cognate co-stimulatory pairs B7/CD28 and CD40L/CD40. See text for a detailed description of the sequence of events. Ag, antigen; APC, antigen-presenting cell; CD40L, CD40 ligand; MHC, major histocompatibility complex.

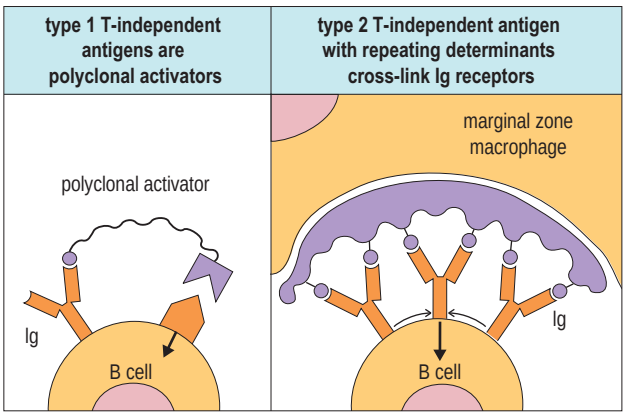


Figure 12.11 B-cell activation by T-independent antigens. Some antigens can directly activate the B cells, other with repeating structures cross-link specific antibodies on the B-cell surface. Ig, immunoglobulin.

antibody. Some of the uses of monoclonal antibodies in diagnosis and in immunotherapy are described in Chapters 32 and 36 respectively.

CYTOKINES PLAY AN IMPORTANT PART IN THESE CELL–CELL INTERACTIONS

As seen above, to make an effective T-cell or antibody response requires cooperation between cells of different types. In addition to direct cell–cell contact that triggers signalling cascades, cytokines can be secreted into the contact zone or immunological synapse between the cells. Cytokines that are secreted by one cell can act as a molecular messenger on the cell itself in an autocrine manner, but most often act on another cell in a paracrine reaction. The antigen-presenting cell delivers cytokines such as IL-12 to the CD4 T cell; the T cell itself secretes growth factors such as IL-2, and cytokines that

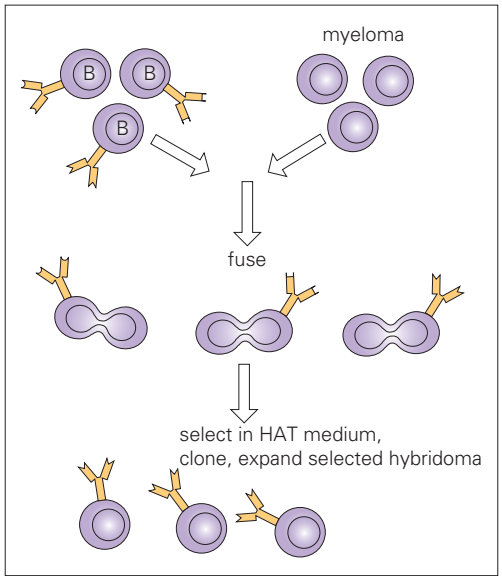


Figure 12.12 Production of monoclonal antibodies. Mouse spleen cells from immunized mice are fused to myeloma cells using polyethylene glycol. As the myeloma cells lack the enzyme hypoxanthine–guanine phosphoribosyltransferase (HGPRT), culture in medium containing hypoxanthine aminopterin thymidine (HAT) allows only fused hybridoma cells to survive and these cells can then be cloned by limiting dilution. Those hybridomas making the antibody you want can then be selected. Newer techniques use genetic engineering to clone the DNA from selected antibody VL and VH regions.

Box 12.1 Monoclonal Antibodies

Georges Kohler and Cesar Milstein first published how to make monoclonal antibodies in 1975. A single B cell (from an animal immunized with the antigen of interest) is fused to a myeloma cell, giving an immortalized cell making antibody of a single specificity. This technology has revolutionized both immunology and medicine, and Kohler and Milstein shared the Nobel Prize for Physiology or Medicine in 1984, along with Niels Jerne 'for theories concerning the specificity in development and control of the immune system and the discovery of the principle for production of monoclonal antibodies'. Monoclonal antibodies are used to identify cell surface and intracellular molecules using flow cytometry. They are the basis of many diagnostic assays for infection and are also used therapeutically.

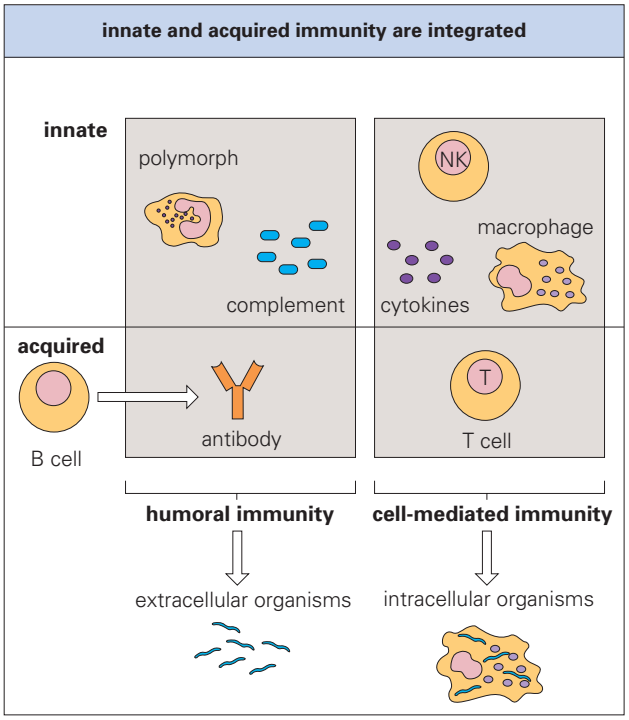


Figure 12.13 The mechanisms of innate and acquired immunity are integrated to provide the basis for humoral and cell-mediated immunity. Deficiencies of humoral immunity predispose to infection with extracellular organisms, and deficiencies of T-cell-mediated responses are associated primarily with intracellular infections.

will help B-cell production of antibodies as well as drive the development of M1 or M2 macrophages. (Some of these many interactions are illustrated in Fig. 12.13, Table 12.1.) In general, cytokines act between adjacent cells; when they are found in large quantities in the circulation it is usually bad news. Some infections can trigger a massive release of cytokines in a 'cytokine storm', which causes a lot of pathology; this was thought to be responsible for a lot of the deaths caused by the Spanish H1N1 influenza outbreak in 1918.

IMMUNOLOGICAL MEMORY ENABLES A SECOND INFECTION WITH THE SAME MICROBE TO BE DEALT WITH MORE EFFECTIVELY

Once the body has dealt with an infection, the adaptive immune system keeps some of the antigen-specific cells it has generated on stand-by, as memory T cells and memory B cells. When a naive T cell has recognized and been activated by its specific antigen presented by the right MHC molecule, it changes both its ability to secrete cytokines (or make cytotoxic mediators) and some of its surface antigens or markers. There are different subsets of memory T cells that express particular markers and that are found in particular locations (Table 12.2). Central memory T cells can recirculate through the peripheral lymphoid tissues as they express the chemokine receptor CCR7. Effector memory T cells are better at migrating to sites of inflammation. Other tissue-resident T memory cells are mainly located in epithelia. CD4 T cells need to provide help to CD8 T cells in order to generate a good CD8 memory T-cell response. Meanwhile memory B cells are mainly located in the spleen and lymph nodes. Human memory B cells express CD27, one member of the TNF receptor family of receptors. As noted above, T-independent antigens do not induce B-cell memory.

Memory cells are easier to activate than naive cells and are present at higher frequencies than antigen-specific naive cells, so will generate a faster and more effective antigen-specific

Table 12.1 Cytokines that play roles in cell–cell cooperation and induction of adaptive immune responses

Factor	Source	Actions
IL-1 α/β	Macrophages	Induce inflammation
IL-2	T cells	T-cell proliferation
IL-3	T cells	Pluripotent growth
IL-4	T cells	B-cell proliferation and IgE selection, Th1 suppression
IL-5	T cells	B-cell growth, IgA and eosinophil differentiation
IL-6	Macrophages, T cells	B-cell differentiation, induce acute phase proteins
IL-7	T cells	B- and T-cell proliferation
IL-10	T cells	Inhibition of Th1 cytokine production
IL-12	Monocytes, Mφ	Induction of Th1 cells
IL-13	T cells	Inhibits mononuclear phagocyte inflammation: proliferation and differentiation of B cells
IL-14	T cells	Proliferation of activated B cells, inhibits Ig secretion
IL-15	Dendritic cells	Maintenance of CD8 T-memory cells
IL-16	$CD8^+$ T cells and eosinophils	Chemotaxis of CD4 T cells
IL-17	$CD4^+$ T cells	Proinflammatory; stimulates production of cytokines including TNFα, IL-1β, IL-6, IL-8, G-CSF
IL-18	Macrophages	Induces IFNγ production by T cells; enhances NK cytotoxicity
IL-21	Th cells	NK differentiation; B activation; T-cell co-stimulation induces acute phase reactants
IL-22	T cells	Inhibits IL-4 production by Th 2; induces production of antimicrobial proteins by epithelial cells
IL-23	Dendritic cells	Induces proliferation and IFNγ production by Th 1; induces proliferation of memory cells
IL-26	Th17 T cells	Lysis of membranes of gram-negative bacteria
IFNγ	T cells, NK cells	Antiviral, activation of macrophages, inhibition of Th 2 cells, MHC class I and II induction
TGFβ	T cells / macrophages	Inhibits activation of NK and T cells, macrophages; inhibits proliferation of B and T cells, promotes wound healing

BM, bone marrow; G-CSF, granulocyte colony-stimulating factor; GM-CSF, granulocyte-macrophage colony-stimulating factor; IFN, interferon; IL, interleukin; M-CSF, macrophage colony-stimulating factor; NK, natural killer cell; PMN, polymorphonuclear lymphocyte; TGF, transforming growth factor; TNFβ, tumour necrosis factor beta.

Table 12.2 Human memory CD4 T-cell subsets

	Naive T cells	Effector T cells	Effector memory T cells	Central memory T cells	Tissue-resident memory T cells
Tissue location	Blood, lymphoid tissues	Blood, lymphoid tissues	Peripheral tissues, mucosal tissues	Lymphoid tissues	Peripheral tissues
CD45 isotype	RA	RO	RO	RO	RO
CCR7	++	+	–	+	±
CD62L (L-selectin)	++	+	±	+	±
Proliferative ability	±	+++	±	++	+
Cytokines produced	IL-2	IFNγ, IL-4 / 5 / 13, IL-17	IFNγ, IL-4 / 5 / 13, IL-17	IL-2	IFNγ
BCL-2 (anti-apoptotic)	–	–	+	+	+

Tissue resident memory T cells also express CD69 and CD103 (αE integrin). There are also similar types of memory CD8 T cells. All memory cells depend on IL-7 for survival, and CD8 memory cells also require IL-15. BCL-2, B-cell lymphoma 2; CCR7, C-C chemokine receptor 7; CD, cluster of differentiation; IFN, interferon; IL, interleukin.

immune response when they are restimulated. For antibody responses, secondary or memory responses will consist mainly of IgG and IgA antibodies that will be of higher affinities than those produced in the primary response. Immunological memory can last for long periods – when the isolated Faroe Islands had a measles epidemic in 1846, those who had measles in the previous epidemic in 1781 were still immune! Although in some cases re-exposure to the same antigens may boost memory responses, memory cells can be maintained without antigen, presumably through cytokine stimulation. Memory T cells express a molecule called Bcl-2 that promotes cell survival, and a receptor for IL-7 which seems important for the maintenance of memory T cells. Memory CD8 T cells also depend on IL-15 for their survival and seem to have larger clones but of fewer different antigen specificities than memory CD4 T cells; they also need CD4 T-cell help for their development and long-term maintenance.

As for the T-cell subsets, T cells exist in a spectrum, as they develop from naive to memory cells. New techniques like cytometry by time-of-flight that use larger panels of surface and intracellular markers than in ordinary flow cytometry are revealing a continuum of cells, and that the type of memory T cells found in different infections differs (Fig. 12.14).

Although memory T cells, or their descendants may be long-lived, too much antigen stimulation can result in the memory T cells losing function and becoming old or senescent, at which point they start to re-express the naive T-cell marker CD45RA.

ARMIES MUST BE KEPT UNDER CONTROL

An immune response will naturally wane once an infection has been dealt with and its antigens removed, so stopping the stimulation of antigen-specific cells. The now-unwanted antigen-specific cells that are not retained as memory cells die due to a lack of cytokines such as IL-2 and IL-7 that promote cell division, but these cytokines also increase the expression of molecules like Bcl-2 that are anti-apoptotic and so without them the cell is more likely to undergo apoptosis. Apoptosis, or programmed cell death can be induced by two pathways: an intrinsic pathway associated with the expression of Bim, but also an extrinsic pathway of apoptosis that is activated through Fas, a molecule that has an intracellular death domain.

There are occasions when excessive immune responses could damage the body – so specialized cells and cytokines are needed to regulate and reduce excessive cytokine damage (Fig. 12.15). Regulatory T cells (Treg) secrete cytokines that inhibit cytokine secretion and T-cell function, such as IL-10 (originally called cytokine synthesis inhibitory factor) and transforming growth factor beta (TGFβ). Regulatory T cells are sometimes subdivided into those that are pre-existing or natural, and those that are induced by antigen or infection. Natural Tregs are associated with tolerance to self-antigens, while induced Tregs are responsible for the down-regulation of immune responses induced by infection. There are also regulatory B cells. In some instances a state of anergy or tolerance can be induced (Fig. 12.16). For example, T cells

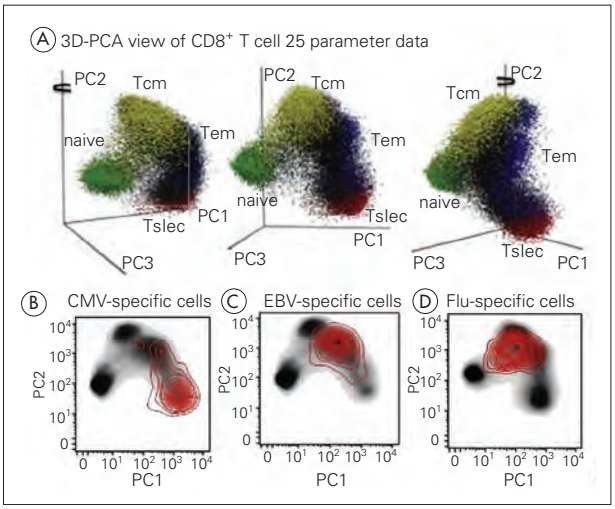


Figure 12.14 (A) Cytometry by time-of-flight (CyTOF) uses specific heavy-metal-labelled antibody to label cells, followed by mass spectrometry to identify the binding of specific antibodies by individual T cells. Human peripheral blood T cells were analysed for expression of 25 parameters that included T-cell markers, memory T-cell markers, activation and functional markers, and in B–D, virus-specific T cells identified using tetramers with CMV, EBV and influenza peptides. The 25 parameters are then grouped by principle component analysis. The 3-D visualization of the principle component analysis in (A) shows that human naive CD8 T cells develop into Tcm and Tem cells; Tslec, a smaller group of short-lived effector cells, is shown in red. The plots in B–D show tetramer-positive cells specific for CMV, EBV or influenza respectively in red. The phenotype of the memory CD8 T cells differs in the three viral infections: CMV infection shows Tem with more short-lived effector cells, EBV infection shows most Tem, and the more acute influenza infection has more Tcm. (Redrawn from Newell E.W., Sigal N., Bendall S.C. et al. Cytometry by time-of-flight shows combinatorial cytokine expression and virus-specific cell niches within a continuum of CD8+ T cell phenotypes. *Immunity* 2012, 36:142–152, with permission.)

can become unresponsive or anergic if they receive the first TCR-MHC activating signal without co-stimulation. This may be a useful way of ensuring that T cells recognizing tissue antigens not found in the thymus (and so not able to direct the removal of such T cells before their export from the thymus) do not induce autoimmunity. T-cell tolerance can also be induced by inhibitory molecules such as CTLA-4 binding to B7-1 on the antigen-presenting cell, or through PD-1, an inhibitory receptor molecule similar to CD28 that is expressed on activated T cells and that binds to PD-L1 and PD-L2. Expression of PD-1 is up-regulated on T cells during chronic infections. B cells are down-regulated if they are activated through the FcRγRIIA receptor and their antigen receptor without co-stimulation through CD19 or CD20 (Fig. 12.17). B cells can also be made anergic if they have weak binding to a self-antigen, or via other inhibitory receptors. Finally, there is an interesting phenomenon called oral tolerance that must have developed to prevent immune reactions to food antigens.

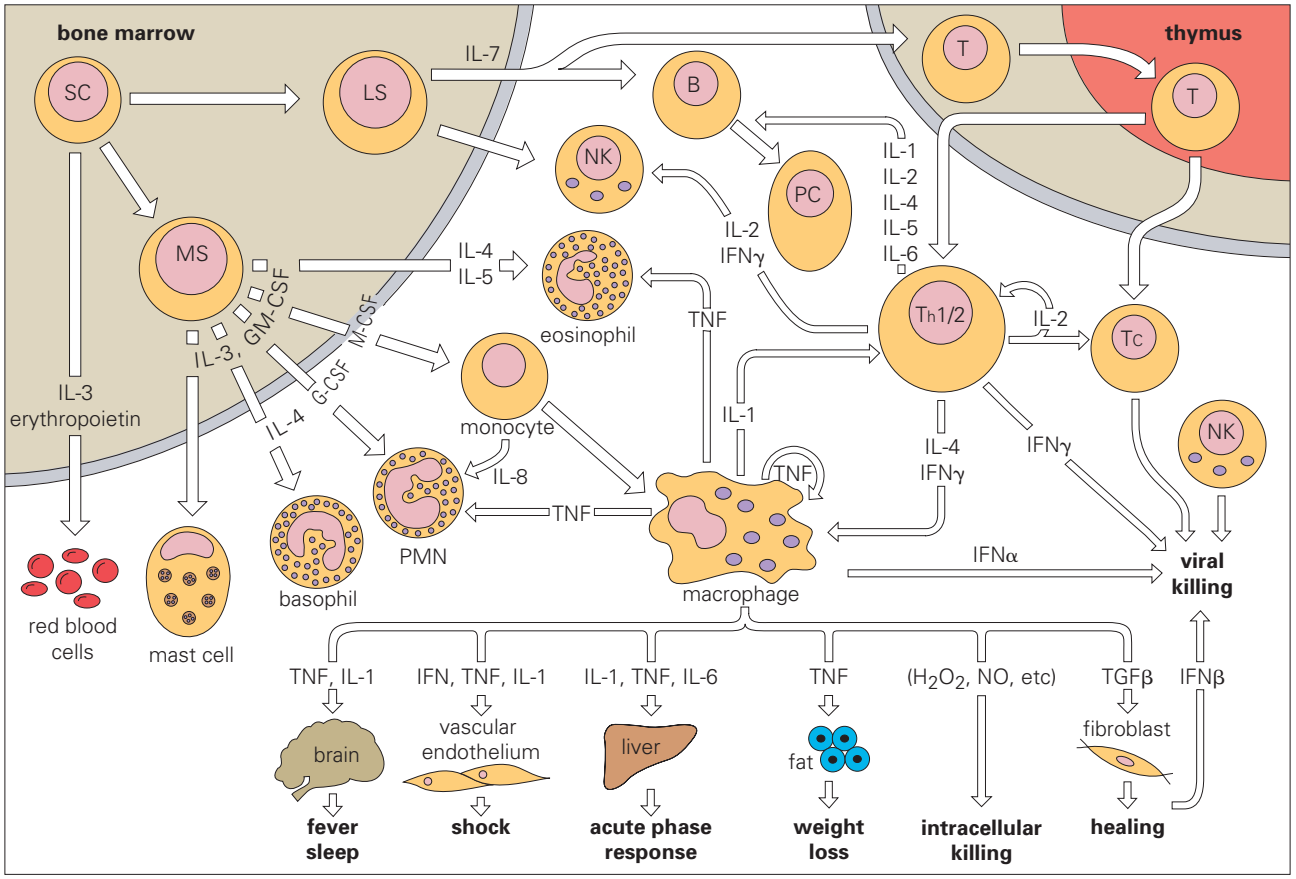


Figure 12.15 Regulation of the immune response. T help for cell-mediated immunity is subject to similar regulation. G-CSF, granulocyte colony-stimulating factor; GM-CSF, granulocyte-macrophage colony-stimulating factor; H_2O_2, hydrogen peroxide; LS, lymphoid stem cell; M-CSF, macrophage colony-stimulating factor; MS, myeloid stem cell; NK, natural killer cell; NO, nitric oxide; PC, plasma cell; PMN, polymorphonuclear lymphocyte; SC, stem cell; Tc, cytotoxic T cell; TGFβ, transforming growth factor beta; Th, T-helper cell; TNF, tumour necrosis factor. (Adapted from Playfair J.H.L. [2001] *Immunology at a Glance*. Oxford: Blackwell Science.)

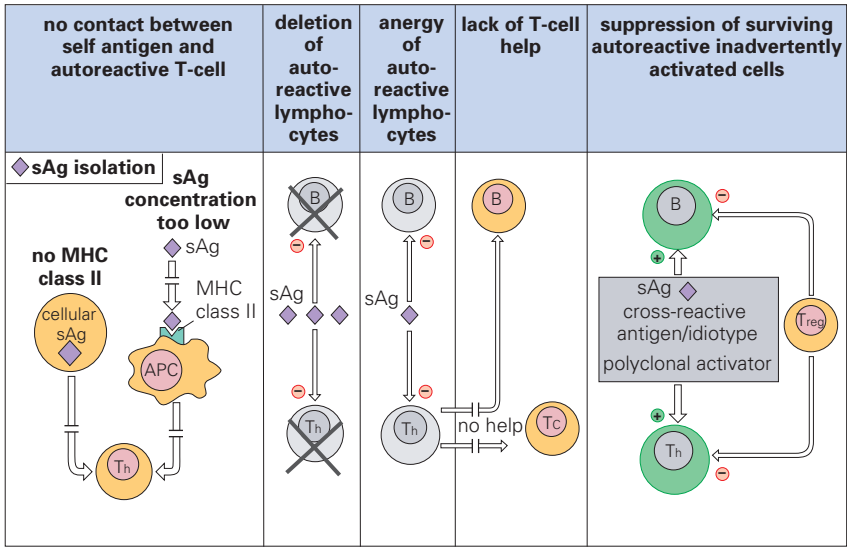


Figure 12.16 Mechanisms of self-tolerance. Self-antigens (sAg) will not stimulate autoreactive Th cells if they are anatomically isolated, if there is too low a concentration of processed peptide–major histocompatibility complex class II (MHC II) molecules, or if there is no MHC II on the cell. Both B and T cells can be silenced by clonal deletion or made anergic (still living, but unresponsive) by contact with self-antigen. Too low a concentration of presented sAg will fail to silence differentiating immature lymphocytes bearing the cognate receptors, leading to the survival of populations of autoreactive T and B cells. Th cells are the most readily tolerized population, and surviving autoreactive B cells and cytotoxic T (Tc) cells cannot function without T-cell help. Furthermore, inadvertent stimulation of surviving autoreactive cells may be checked by regulatory T cells (Treg). Cells that are dead, unreactive or suppressed are shown in grey. APC, antigen-presenting cell. (Modified from: Delves P. J. et al. [2006] *Roitt's Essential Immunology*, 11th edn. Oxford: Blackwell Science.)

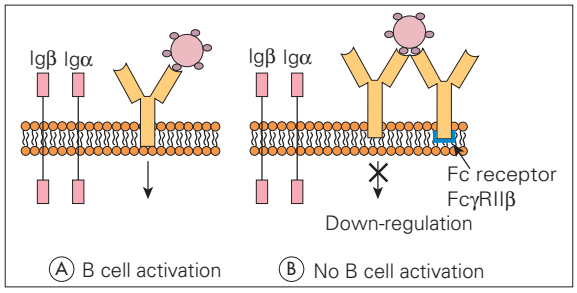


Figure 12.17 B-cell down-regulation. Normally B cells are activated when antigen is recognized by antibody expressed on the surface of the B cell leading to signalling through the B-cell receptor immunoglobulin (Ig)α– and Igβ-chains, with the subsequent involvement of Src kinases, scaffold proteins and tyrosine phosphorylation (A). If antigen binds to both the B-cell surface receptor as well as to antibody bound to the FcγRIIβ receptor, this inhibitory Fc receptor blocks signalling and B-cell activation by activating the Fc receptor-associated SHIP phosphatase that converts phosphatidyl inositol triphosphate (PIP3) to phosphatidyl biphosphate (PIP2) (B).

KEY POINTS

- To make an effective immune response, cooperation between the cells of the innate and adaptive immune systems is needed, including multiple steps such as antigen presentation to T cells, T- and B-cell activation and cytokine secretion.
- Activation of T and B cells involves more than just antigen recognition, including specialized co-stimulatory as well as cytokine signals, resulting in activation of intracellular signalling cascades, metabolic changes and ultimately cell division.
- Cell division will produce clones of daughter T cells of the same antigen specificity; B cells further fine-tune their antigen specificity during an immune response due to somatic mutation, leading to antibodies of greater affinity.
- After the infection has been controlled, the number of antigen-specific T and B cells declines, due to cell death, but some remain as memory cells, enabling a faster and more efficient response to re-infection with the same organism.
- The effector cells are also kept from getting out of control and causing tissue damage by regulatory cells such as Tregs and suppressive cytokines such as IL-10 and TGFβ.

13 Background to the infectious diseases

Introduction

Vertebrates have been continuously exposed to microbial infections throughout their hundreds of millions of years of evolution. Disease or death was the penalty for inadequate defences. Therefore they have developed:

- highly efficient methods for recognizing foreign invaders
- effective inflammatory and immune responses to restrain the growth and spread of foreign invaders and to eliminate them from the body.

The fundamental bases of these defences have been described in Chapters 10–12. If these defences were completely effective, microbial infections would be scarce and terminated rapidly, as microorganisms would not be allowed to persist in the body for long periods.

Microbes rapidly evolve characteristics that enable them to overcome the host's defences

Microorganisms faced with the antimicrobial defences of the host species have evolved and developed a variety of characteristics that enable them to bypass or overcome these defences and carry out their obligatory steps for survival (Table 13.1). Unfortunately, microorganisms evolve with extraordinary speed in comparison with their hosts. This is partly because they multiply much more rapidly, the generation time of an average bacterium being 1 h or less, compared with about 20 years for the human host. Rapid evolutionary change is also favoured in bacteria that can hand over genes (carried on plasmids) directly to other bacteria, including unrelated bacteria. Antibiotic resistance genes, for instance, can then be transferred rapidly between species. This rapid rate of evolution ensures that pathogens are always many steps ahead of the host's antimicrobial defences. Indeed, if there are possible ways around the established defences, microorganisms are likely to have discovered and taken advantage of them. Infectious microorganisms therefore owe their success to this ability to adapt and evolve, exploiting weak points in the host's defences, as outlined in Table 13.2 and Figs 13.1 and 13.2. The host, in turn, has had to respond to such strategies by slowly improving defences, adding extra features, and having multiple defence mechanisms with overlap and a good deal of duplication.

HOST–PARASITE RELATIONSHIPS

The speed with which host adaptive responses can be mobilized is crucial

Every infection is a race between the capacity of the microorganism to multiply, spread and cause disease and the ability of the host to control and finally terminate the infection (Fig. 13.1). For instance, a 24 h delay before an important host

Table 13.1 Successful infectious microorganisms must take certain obligatory steps

Obligatory steps for infectious microorganisms		
Step	**Requirement**	**Outcome**
Attachment±entry into body	Evade natural protective and cleansing mechanisms	Entry (infection)
Local or general spread in the body	Evade immediate local defences	Spread
Multiplication	Increase numbers (many will die in the host, or en route to new hosts)	Multiplication
Evasion of host defences	Evade immune and other defences long enough for the full cycle in the host to be completed	Avoid killing by host defences
Shedding from body (exit)	Leave body at a site and on a scale that ensures spread to fresh hosts	Transmission
Cause damage in host	Not strictly necessary but often occurs[a]	Pathology, disease

[a] The last step, causing damage in the host, is not strictly necessary, but a certain amount of damage may be essential for shedding. The outpouring of infectious fluids in the common cold or diarrhoea, for instance, or the trickle from vesicular or pustular lesions, is required for transmission to fresh hosts.

Table 13.2 Some examples of host defences and microbial evasion strategies

Host's defences and the microbe's answer				
	Defence	**Microbial answer**	**Mechanism**	**Example**
Mechanical and other barriers	Microbe rinsed away from epithelial surface by host secretions (plus ciliary activity in respiratory tract)	Bind firmly to epithelial surface	Surface molecule on microbe attaches to 'receptor' molecule on host epithelial cell	Influenza, rhinovirus, *Chlamydia*, gonococci
		Interfere with ciliary activity	Produce ciliotoxic / ciliostatic molecule	*Bordetella pertussis*, pneumococci, *Pseudomonas*
	Host cell membranes as barrier to pathogen	Traverse host cell membrane	Fusion protein in viral envelope	Influenza, HIV
		Enter cell by active penetration	Microbial enzymes mediate cell penetration	Trypanosomes, *Toxoplasma gondii*
Phagocytic and immediate host defences	Microbe ingested and killed by phagocyte	Inhibit phagocytosis	Microbial outer wall or capsule impedes phagocytosis	Pneumococci, *Treponema pallidum*, *H. influenza*
		Inhibit phagosome-lysosome fusion	Sulphatides of *Mycobacterium tuberculosis* inhibit fusion	*M. tuberculosis*
		Interfere with signal transduction in macrophage	Induction of SOCS[a] proteins	*Toxoplasma gondii*
		Resist killing and multiply in phagocyte	Exit from phagosome into cytoplasm (*Listeria*)	*Brucella* spp., *Listeria monocytogenes*, measles, dengue viruses
	Host molecules (lactoferrin, transferrin etc.) restrict availability of free iron needed by microbe	Microbe competes with host for iron	Microbe possesses avidly iron-binding siderophores	Pathogenic *Neisseria*, *E. coli*, *Pseudomonas*
	Complement activated with antimicrobial effects	Inactivate complement components	Production of an elastase	*Pseudomonas aeruginosa*
		Interfere with complement-mediated phagocytosis	C3b receptor on microbe competes with that on phagocyte and complement access blocked	*Candida albicans*, *Toxoplasma gondii*, M protein of *Strep. pyogenes*
	Infected host produces interferons to inhibit virus replication	Induce a poor interferon response	Core antigen of hepatitis B suppresses IFNβ production	Hepatitis B, rotaviruses
		Insensitive to interferons	Prevent activation of interferon-induced enzymes	Adenovirus

Continued

Table 13.2 Some examples of host defences and microbial evasion strategies—cont'd

Host's defences and the microbe's answer				
	Defence	**Microbial answer**	**Mechanism**	**Example**
Immune defences	Infected host produces antibody	Destroy antibody	Bacterium liberates IgA protease	Gonococci, *H. influenzae*, streptococci
		Display Fc receptor on microbial surface	Antibody bound to microbe in upside-down position	Staphylococci (Protein A), trypanosomes, certain streptococci, herpes simplex virus, cytomegalovirus
	Infected host produces antimicrobial cell-mediated immune response	Invade T cells, and interfere with their function or kill them	Virus envelope molecule binds to CD4 on helper T-cell surface	HIV
		Induce regulatory T cells	Suppress beneficial immunity	*Bordetella pertussis*, *M. tuberculosis*, *Helicobacter pylori*, HIV
	Antimicrobial immune response recognizes infected cells and destroys them	Microbe in cells fails to display microbial antigens on cell surface	Viral antigens not synthesized	Herpes simplex virus latent in sensory neurons
			Virus inhibits transport of MHC class I molecules to cell surface thus avoiding recognition by CD8 T cell	Cytomegalovirus, adenovirus
	Effective immune response produced	Vary microbial antigens in individual host, or during spread in host community	Switch on different surface antigens	*Trypanosoma* spp., *Borrelia recurrentis*
			Mutation, genetic recombination	Influenza virus, streptococci, gonococci

Although inflammation is not listed as a host defence in its own right, many of these defences depend on local inflammation. Inflammation (see Ch. 10) means an increased blood supply and the delivery of antibodies, complement, immune cells and phagocytes to the site of infection. In the days before antibiotics, people applied hot poultices to staphylococcal boils and abscesses so as to increase the amount of inflammation and hasten recovery. Microbes that interfere with the action of complement or with chemotaxis (staphylococci, streptococci, *Pseudomonas aeruginosa*, herpes simplex viruses) will thereby tend to reduce inflammation.

[a] SOCS, Suppressor of cytokine signalling.

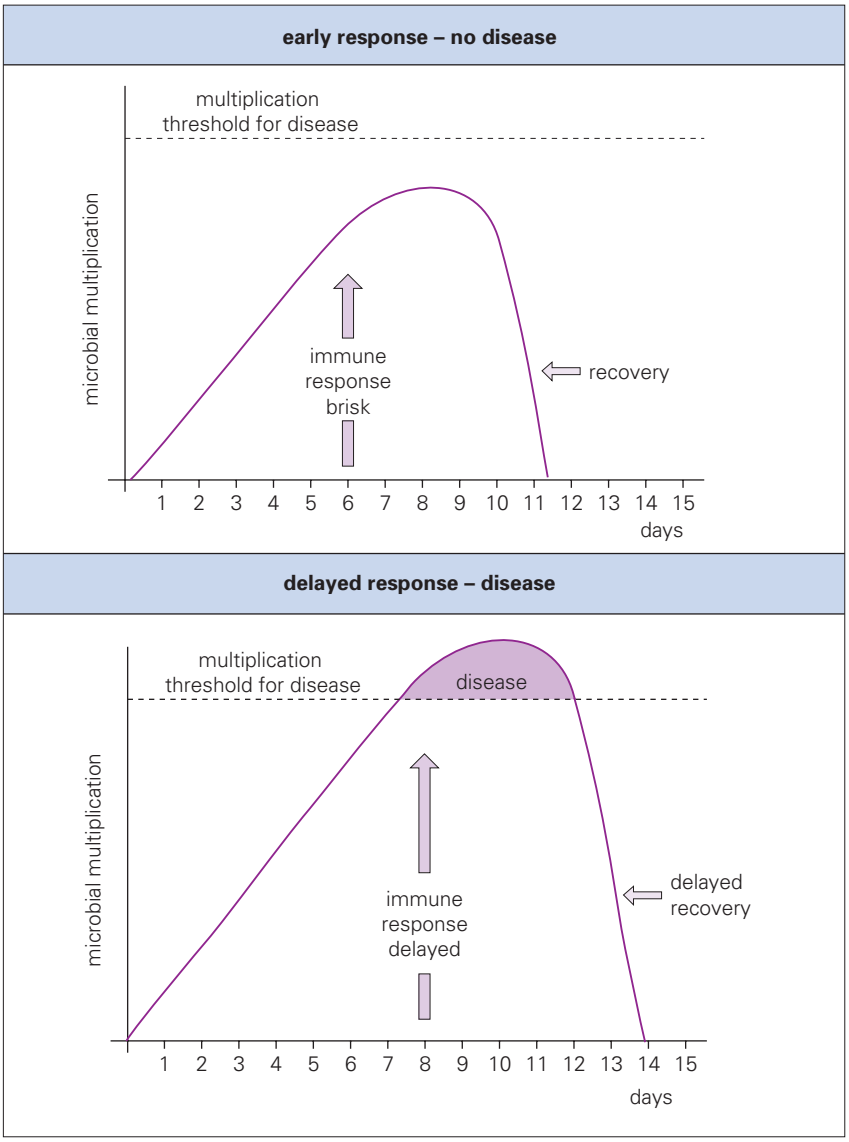


Figure 13.1 Every infection is a race. Delays in mobilizing host adaptive defences can lead to disease or death.

response comes into operation can give a decisive advantage to a rapidly growing microorganism. From the host's point of view, it may allow enough damage to cause disease. More importantly, from the pathogen's point of view, it may give the microbe the opportunity to be shed from the body in larger amounts or for an extra day or two. A pathogen that achieves this will be rapidly selected for in evolution.

Adaptation by both host and parasite leads to a more stable balanced relationship

The picture of conflict between host and parasite, usually and appropriately described in military terms, is central to an understanding of the biology of infectious disease. As with military conflicts, adaptation on both sides (Box 13.1) tends to lessen the damage and incidence of death in the host population, leading to a more stable and balanced relationship. The successful parasite gets what it can from the host without causing too much damage and, in general, the more ancient the relationship, the less the damage. Many microbial parasites, not only the normal flora (see Ch. 9) but also polioviruses, meningococci and pneumococci and others, live for the most part in peaceful coexistence with their human host.

Some microorganisms remain at body surfaces, perhaps spreading locally but failing to invade deeper tissues. These include the common cold viruses, wart viruses, mycoplasmas and skin fungi. Often the disease is mild, but severe illness can occur when powerful toxins are produced and act either locally (cholera) or at distant sites (diphtheria).

Infecting microorganisms can gain entry to a healthy host and cause disease in three ways (Fig. 13.3). There are:

- microorganisms with specific mechanisms for attaching to, or penetrating, the body surfaces (most viruses and certain bacteria)
- microorganisms introduced by biting arthropods (e.g. malaria, plague, typhus, yellow fever)
- microorganisms introduced into otherwise normal healthy hosts via skin wounds or animal bites (clostridia, rabies, *Pasteurella multocida*)

Microorganisms are also able to infect a normal healthy host when surface or systemic defences are impaired (see

Box 13.1 Lessons in Microbiology

Myxomatosis

Myxomatosis provides a well-studied classic example of the evolution of an infectious disease unleashed on a highly susceptible population. This viral disease, which is spread mechanically by mosquitoes, normally infects South American rabbits (*Sylvilagus brasiliensis*), but they remain perfectly well, developing only a virus-rich skin swelling at the site of the mosquito bite. The same virus in the European rabbit (*Oryctolagus cuniculus*) causes a rapidly fatal disease.

Myxomavirus was successfully introduced into Australia in 1950 as an attempt to control the rapidly increasing rabbit population. Initially, more than 99% of infected rabbits died (Fig. 13.2), but then two fundamental changes occurred:

1. New, less lethal strains of virus appeared and replaced the original strain. This occurred because rabbits infected with these strains survived for longer and their virus was therefore more likely to be transmitted.
2. The rabbit population changed its character, as those that were genetically more susceptible to the infection were eliminated. In other words, the virus selected out the more resistant host, and the less lethal virus strain proved to be a more successful parasite. If the rabbit population had been eliminated, the virus would also have died out, but the host–parasite relationship quite rapidly settled down to reach a state of better balanced pathogenicity, and by the 1970s only about half the rabbits died from infection. Australian rabbits have since faced a new threat – a calicivirus introduced from Europe, which spreads by contact and causes a lethal haemorrhagic disease.

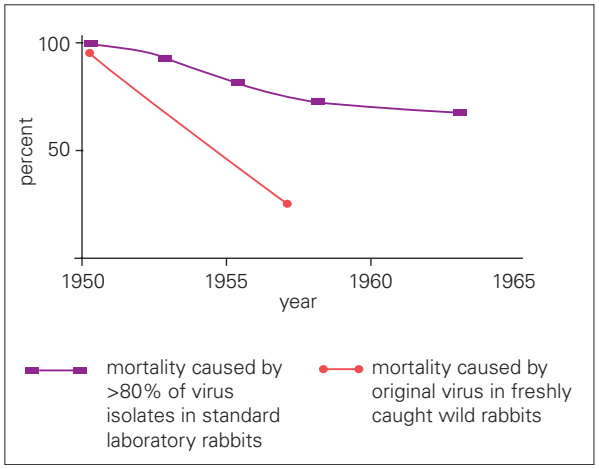


Figure 13.2 Evolution of a pathogen within a host species. Myxomatosis is the best-studied example of the appearance of a highly lethal pathogen in a host population that gradually settles down to a state of more balanced pathogenicity. *Vibrio cholerae* has progressed in this direction, and perhaps HIV is destined to tread the same path.

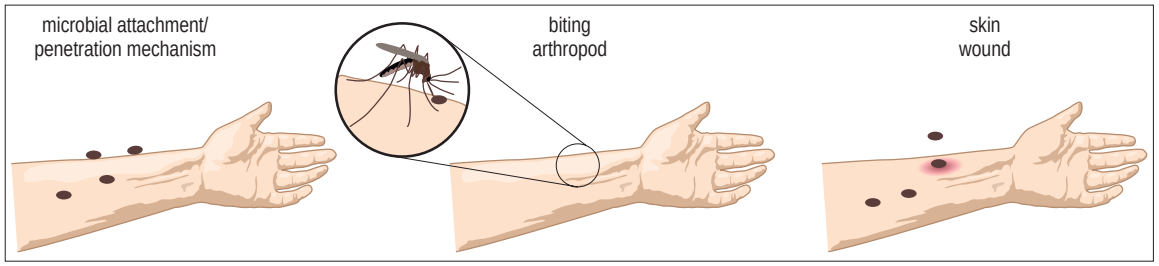


Figure 13.3 Pathogens can invade the healthy host in three main ways. Invasion may also occur if the host is immunosuppressed.

Ch. 31) – as occurs with burns, insertion of foreign bodies (cannulas and catheters), urinary tract infections in men (stones, enlarged prostate, see Ch. 21), bacterial pneumonia following initial viral damage (post-influenza) or depressed immune responses (immunosuppressive drugs or diseases such as AIDS).

CAUSES OF INFECTIOUS DISEASES

More than 100 microbes commonly cause infection

Humans are host to many different microorganisms. In addition to the scores of microbes that form the normal flora or microbiome, there are more than 100 that quite

commonly cause infection, some of them remaining in the body for many years afterwards, and several hundred others that are responsible for less common infections. Against this rich background of parasitic activity, how do we prove that a certain microorganism is the culprit in any given disease? In some instances (anthrax, cholera, tetanus), the causative microorganism is identified and incriminated at an early stage, but in the case of glandular fever and viral hepatitis it is not so easy.

Koch's postulates to identify the microbial causes of specific diseases

In 1890, Robert Koch (Box 13.2) set out as 'postulates' the following criteria he felt to be necessary for a microorganism to be accepted as the cause of a given disease:

- The microbe must be present in every case of the disease.
- The microbe must be isolated from the diseased host and grown in pure culture.
- The disease must be reproduced when a pure culture is introduced into a non-diseased-susceptible host.
- The microbe must be recoverable from an experimentally infected host.

In the early days of microbiology, Koch's postulates brought a welcome clarity. The germ theory of disease causation had only recently been set out following Koch's classic studies on anthrax (1876) and tuberculosis (1882), and methods for isolating microbes in pure culture and identifying them were only just being developed. However, modifications were needed in order to include certain bacterial diseases and the new world of viral diseases. The microbe could not always be grown in the laboratory (*Treponema pallidum*, wart viruses, *Mycobacterium leprae*), and for certain microbes: hepatitis B, Epstein–Barr virus (EBV), there were (initially) no susceptible animal species. The criteria were modified, therefore, on several occasions to accommodate these problems and finally reformulated by A.S. Evans in 1976.

Conclusions about causation are now reached using enlightened common sense

Nowadays, with our vastly increased technology and understanding of infection, those attempts to make lists and apply rigid criteria may seem old fashioned. Perhaps we can now reach conclusions about causation using common sense. For instance, we recognize that diseases sometimes do not appear until many years after a specific infection (subacute sclerosing panencephalitis, Creutzfeldt–Jakob disease; see Ch. 25). Molecular genetic techniques may now identify previously uncultivable causative organisms. The polymerase chain reaction was used to amplify and sequence small amounts of mRNA from the bowel of patients with Whipple's disease, a rare multisystem disorder. A unique 16S mRNA was identified, belonging to a previously uncharacterized, uncultivable bacterium, *Tropheryma whippelii*. Nevertheless, grey areas remain, especially in diseases of possible or probable microbial aetiology where the pathogen does not act alone. Co-factors or genetic and immunological factors in the host may play a vital part. Examples include:

Box 13.2 ■ Lessons in Microbiology

Robert Koch (1843–1910)

In 1876, while in general practice in Berlin, Robert Koch (Fig. 13.4) isolated the anthrax bacillus, and became the first to show a specific organism as the cause of a disease. In 1882, he discovered *Mycobacterium tuberculosis* as the cause of tuberculosis. He then went on to lead the 1883 expedition to Egypt and India, and discovered the cause of cholera: *Vibrio cholerae*.

Koch was the founder of the 'germ theory' of disease, which maintained that certain diseases were caused by a single species of microbe. In 1890, he set out his 'postulates' as ground rules. New techniques were necessary to meet the exacting requirements of the postulates, and Koch became the first to grow bacteria in 'colonies', initially on potato slices and later, with his pupil Petri, on solid gelatin media.

Koch himself could not reproduce cholera in animals, however, and not all microbes could be cultivated. His neat rules therefore had to be modified. Nevertheless, he brought order and clarity to medicine – until then diseases were attributed to miasmas or mists, to punishments from the Gods or devils, or to unfortunate conjunctions of the stars and planets. However, there was resistance to his ideas. A distinguished Munich physician, Max Von Petternkofer, believed that he had put paid to the new theory when he drank a pure culture of *V. cholerae* and suffered no more than mild diarrhoea!

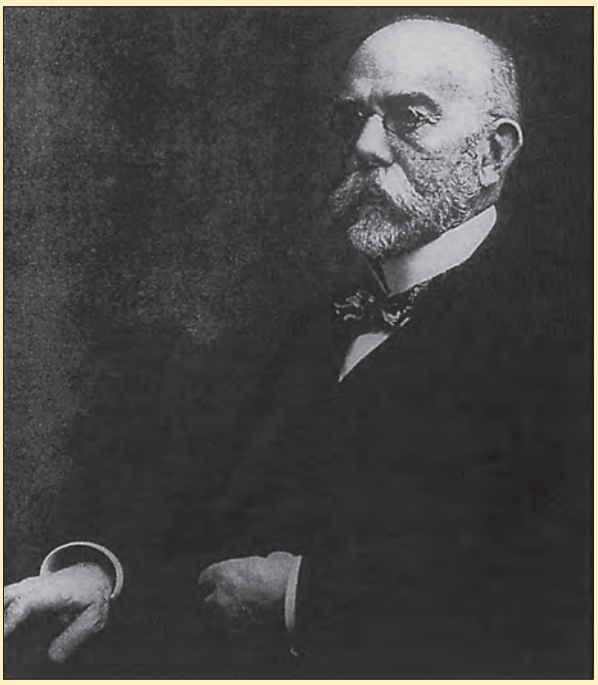


Figure 13.4 Robert Koch (1843–1910).

- the cancers associated with viruses (hepatitis B, genital wart viruses, EBV)
- diseases of possible microbial origin where a number of different pathogens may be involved (post-viral fatigue syndrome, exacerbations of multiple sclerosis)
- diseases that might be infectious, but occur in only a very small proportion of genetically predisposed individuals (rheumatoid arthritis, juvenile diabetes mellitus).

THE BIOLOGICAL RESPONSE GRADIENT

It is uncommon for a pathogen to cause exactly the same disease in all infected individuals

Hence, a physician must be able to make a diagnosis when only some of the possible signs and symptoms are present. The exact clinical picture depends upon many variables such as infecting dose and route, age, sex, presence of other pathogens, nutritional status and genetic background. Infections such as measles or cholera give a fairly consistent disease picture, but others such as syphilis cause such a wide spectrum of pathology that Sir William Osler (1849–1919) stated that 'He who knows syphilis, knows medicine'.

There is great variation not only in the nature, but also in the severity of clinical disease. Many infections are asymptomatic in >90% individuals, the clinically characterized illness applying to only an occasional unfortunate host (Table 13.3). This illness can be mild or severe. Asymptomatically infected individuals are important because, although they develop immunity and resistance to reinfection, they are not identified, move normally in the community and can infect others. Clearly, there is little point in isolating a clinically infected patient when there is a high frequency of asymptomatically infected individuals in the community. This phenomenon can be represented as an iceberg (Fig. 13.5).

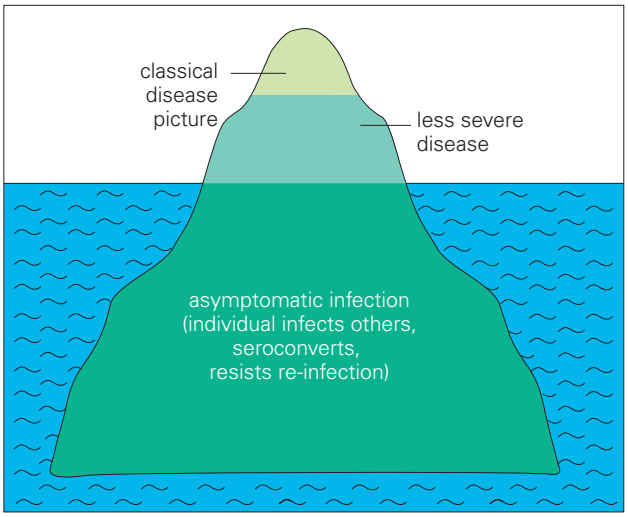


Figure 13.5 The 'iceberg' concept of infectious disease.

Table 13.3 The likelihood of developing clinical disease varies with the infection and often depends upon age

Frequency of clinically apparent disease	
Infection	**Approximate % with clinically apparent disease[a]**
Pneumocystis jirovecii[b]	0
Epstein–Barr virus (1–5 year old child)	1.0 (30–75% in young adults)
Poliomyelitis (child)	24[c]
Malaria (1–5 year old child)	25 (2% in adults)
Rubella	50
Influenza (young adult)	60
Whooping cough Typhoid Anthrax	>90
Gonorrhoea (adult male) Measles	99
HIV[d] Rabies	100

When there is a lengthy incubation period, the proportion with clinical disease may increase with time, from a few percent to (nearer) 100% in the case of HIV.
[a]On primary infection.
[b]Formerly *P. carinii*.
[c]1% develop paralytic poliomyelitis.
[d]Some individuals infected with HIV can maintain high CD4 counts and very low viral loads for >5 years, and are called 'long-term non-progressors' or 'controllers', with a few individuals called 'elite controllers' controlling progression to disease for >20 years.

KEY FACTS

- Faced with host defences (see Chs. 10–12), the pathogens (see Chs. 1–7) have developed mechanisms to bypass them, and in turn the host defences have had to be modified, although slowly, in response.
- There is a conflict between the pathogen and host, and every infectious disease is the result of this ancient battle. Details of the host–pathogen conflict are given in Chapters 13–18, an outline of diagnostic methods in Chapter 32, and a central account of infectious diseases according to the body systems involved in Chapters 19–31.
- Speed matters. Every infection is a race between microbial replication and spread and the mobilization of host responses.
- Some organisms can invade a healthy host but others are injected via insect bite, or gain entry through wounds.
- Molecular techniques have helped identify the cause of a disease.
- Pathogens do not necessarily produce the same disease in all infected individuals. A biological response gradient causes a spectrum that can range from an asymptomatic to a lethal infection.

14 Entry, exit and transmission

Introduction

Microorganisms must attach to, or penetrate, the host's body surfaces

The mammalian host can be considered as a series of body surfaces (Fig. 14.1). To establish themselves on or in the host, microorganisms must either attach to, or penetrate, one of these body surfaces. The outer surface, covered by skin or fur, protects and isolates the body from the outside world, forming a dry, horny, relatively impermeable outer layer. Elsewhere, however, there has to be more intimate contact and exchange with the outside world. Therefore in the alimentary, respiratory and urogenital tracts, where food is absorbed, gases exchanged and urine and sexual products released respectively, the lining consists of one or more layers of living cells. In the eye, the skin is replaced by a transparent layer of living cells: the conjunctiva. Well-developed cleansing and defence mechanisms are present at all these body surfaces, and entry of microorganisms always has to occur in the face of these natural mechanisms. Successful microorganisms therefore possess efficient mechanisms for attaching to, and often traversing, these body surfaces.

Receptor molecules

There are often specific molecules on pathogens that bind to receptor molecules on host cells, either at the body surface (viruses, bacteria) or in tissues (viruses). These receptor molecules, of which there may be more than one, are not present for the benefit of the virus or other infectious agent; they have specific functions in the life of the cell. Very occasionally, the receptor molecule is present only in certain cells, which are then uniquely susceptible to infection. Examples include the CD4 molecule and the CCR5 beta-chemokine receptor for HIV, the C3d receptor (CR_2) for Epstein–Barr virus, and alpha-dystroglycan seems to act as receptor for *M. leprae* in Schwann cells (the same receptor can be used by arenaviruses). In these cases, the presence of the receptor molecule determines microbial tropism and accounts for the distinctive pattern of infection. Receptors are therefore critical determinants of cell susceptibility, not only at the body surface, but in all tissues. After binding to the susceptible cell, the microorganism can multiply at the surface (mycoplasma, *Bordetella pertussis*) or enter the cell and infect it (viruses, chlamydia; see Ch. 16).

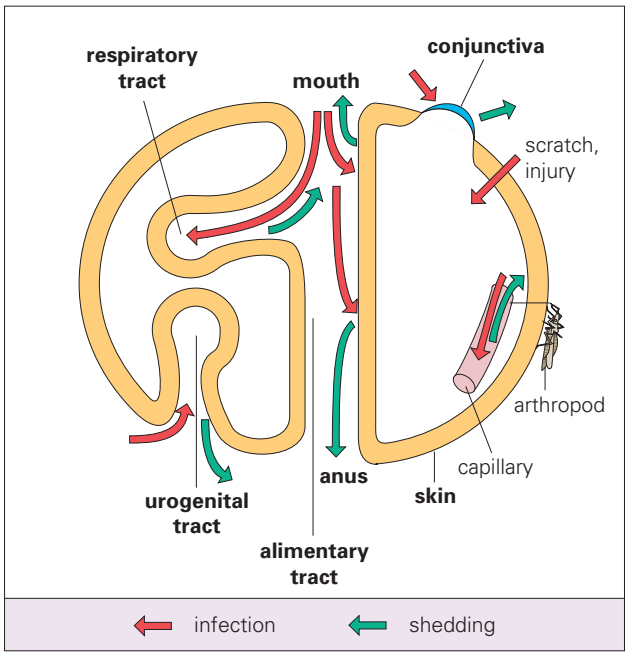


Figure 14.1 Body surfaces as sites of microbial infection and shedding.

Exit from the body

Microorganisms must also exit from the body if they are to be transmitted to a fresh host. They are either shed in large numbers in secretions and excretions or are available in the blood for uptake, for example by blood-sucking arthropods or needles.

SITES OF ENTRY

Skin

Microorganisms gaining entry via the skin may cause a skin infection or infection elsewhere

Microorganisms which infect or enter the body via the skin are listed in Table 14.1. On the skin, microorganisms other than residents of the normal flora (see Ch. 9) are soon inactivated, especially by fatty acids (skin pH is about 5.5), and probably by substances secreted by sebaceous and other glands, and certain peptides formed locally by keratinocytes protect against invasion by group A streptococci. Materials produced by the normal flora of the skin also protect against infection. Skin bacteria may enter hair follicles or sebaceous glands to cause styes and boils, or teat canals to cause staphylococcal mastitis.

Table 14.1 Microorganisms that infect via the skin

Microorganism	Disease	Comments
Arthropod-borne viruses	Fever and various organ systems can be affected such as: West Nile encephalitis Japanese encephalitis Yellow fever Zika virus-related microcephaly Congo–Crimean haemorrhagic fever	150 distinct viruses, transmitted by bite of infected arthropod
Rabies virus	Rabies	Bite from infected animals
Human papillomaviruses	Warts	Infection restricted to epidermis
Staphylococci	Boils	Commonest skin invaders
Rickettsia	Typhus, spotted fevers	Infestation with infected arthropod
Leptospira	Leptospirosis	Contact with water containing infected animals' urine
Streptococci	Impetigo, erysipelas	Concurrent pharyngeal infection in one-third of cases
Bacillus anthracis	Cutaneous anthrax	Systemic disease following local lesion at inoculation site
Treponema pallidum and *T. pertenue*	Syphilis, yaws	Warm, moist skin susceptible
Yersinia pestis, *Plasmodia*	Plague, malaria	Bite from infected rodent flea or mosquito
Trichophyton spp. and other fungi	Ringworm, athlete's foot	Infection restricted to skin, nails, hair
Ancylostoma duodenale (or *Necator americanus*)	Hookworm	Silent entry of larvae through skin of, e.g. foot
Filarial nematodes	Filariasis	Bite from infected mosquito, midge, blood-sucking fly
Schistosoma spp.	Schistosomiasis	Larvae (cercariae) from infected snail penetrate skin during wading or bathing

Some remain restricted to the skin (papillomaviruses, ringworm), whereas others enter the body after growth in the skin (syphilis) or after mechanical transfer across the skin (arthropod-borne infections, schistosomiasis).

Several types of fungi (the dermatophytes) infect the non-living keratinous structures (stratum corneum, hair, nails) produced by the skin. Infection is established as long as the parasites' rate of downward growth into the keratin exceeds the rate of shedding of the keratinous product. When the latter is very slow, as in the case of nails, the infection is more likely to become chronic.

Wounds, abrasions or burns are more common sites of infection. Even a small break in the skin can be a portal of entry if virulent microorganisms such as streptococci, water-borne leptospira or blood-borne hepatitis B virus are present at the site. A few pathogens, such as leptospira or the larvae of *Ancylostoma* and *Schistosoma*, are able to traverse the unbroken skin by their own activity.

Biting arthropods

Biting arthropods such as mosquitoes, ticks, fleas and sandflies (see Ch. 28) penetrate the skin during feeding and can thus introduce infectious agents or parasites into the body. The arthropod transmits the infection and is an essential part of the life cycle of the microorganism. Sometimes the transmission is mechanical, the microorganism contaminating the mouth parts without multiplying in the arthropod. In most cases, however, the infectious agent multiplies in the arthropod and, as a result of millions of years of adaptation, causes little or no damage to that host. After an incubation period, it appears in the saliva or faeces and is transmitted during a blood feed. The mosquito, for instance, injects saliva directly into host tissues as an anticoagulant, whereas the human body louse defecates as it feeds, and *Rickettsia rickettsii*, which is present in the faeces, is introduced into the bite wound when the host scratches the affected area.

The conjunctiva

The conjunctiva can be regarded as a specialized area of skin. It is kept clean by the continuous flushing action of tears, aided every few seconds by the windscreen wiper action of the eyelids. Therefore, the microorganisms that infect the normal conjunctiva (chlamydia, gonococci) must have efficient attachment mechanisms (see Ch. 26). Interference with local defences due to decreased lacrimal gland secretion or conjunctival or eyelid damage allows even non-specialist microorganisms to establish themselves. Contaminated fingers, flies, or towels carry infectious material to the conjunctiva, examples including herpes simplex virus infections leading to keratoconjunctivitis or chlamydial

infection resulting in trachoma. Antimicrobial substances in tears, including lysozyme, an enzyme, and certain peptides have a defensive role.

Respiratory tract

Some microorganisms can overcome the respiratory tract's cleansing mechanisms

Air normally contains suspended particles, including smoke, dust and microorganisms. Efficient cleansing mechanisms (see Chs. 19 and 20) deal with these constantly inhaled particles. With about 500–1000 microorganisms / m^3 inside buildings, and a ventilation rate of 6 L / min at rest, as many as 10 000 microorganisms / day are introduced into the lungs. In the upper or lower respiratory tract, inhaled microorganisms, like other particles, will be trapped in mucus, carried to the back of the throat by ciliary action and swallowed. Those that invade the normal healthy respiratory tract have developed specific mechanisms to avoid this fate.

Interfering with cleansing mechanisms

The ideal strategy is to attach firmly to the surfaces of cells forming the mucociliary sheet. Specific molecules on the organism (often called adhesins) bind to receptor molecules on the susceptible cell (Fig. 14.2). Examples of such respiratory infections are given in Table 14.2.

Inhibiting ciliary activity is another way of interfering with cleansing mechanisms. This helps invading microorganisms establish themselves in the respiratory tract. *B. pertussis*, for instance, not only attaches to respiratory epithelial cells, but also interferes with ciliary activity; other bacteria (Table 14.3) produce various ciliostatic substances of generally unknown nature.

Avoiding destruction by alveolar macrophages

Inhaled microorganisms reaching the alveoli encounter alveolar macrophages, which remove foreign particles and keep the air spaces clean. Most microorganisms are destroyed by these macrophages, but one or two pathogens have learnt either to avoid phagocytosis or to avoid destruction after phagocytosis. Tubercle bacilli, for instance, survive in the macrophages, and respiratory tuberculosis is thought to be initiated in this way. Inhalation of as few as 5–10 bacilli is enough. The vital role of macrophages in antimicrobial defences is dealt with more thoroughly in Chapter 15. Alveolar macrophages are damaged following inhalation of toxic asbestos particles and certain dusts, and this leads to increased susceptibility to respiratory tuberculosis.

Gastrointestinal tract

Some microorganisms can survive the intestine's defences of acid, mucus and enzymes

Apart from the general flow of intestinal contents, there are no particular cleansing mechanisms in the intestinal tract,

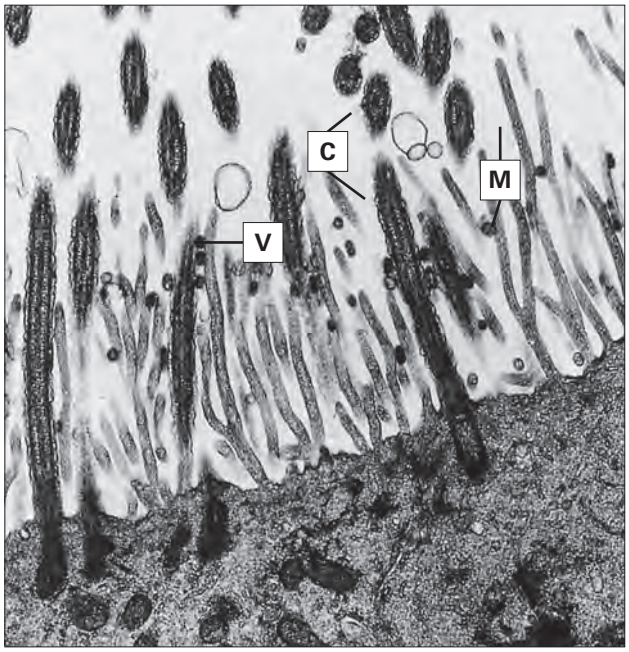


Figure 14.2 Influenza virus attachment to ciliated epithelium. Influenza virus particles (V) attached to cilia (C) and microvilli (M). Electron micrograph of thin section from organ culture of guinea pig trachea 1 h after addition of the virus. (Courtesy of R.E. Dourmashkin.)

Table 14.2 Microbial attachment in the respiratory tract

Microorganisms	Disease	Microbial adhesion	Receptor on host cell
Influenza A virus	Influenza	Haemagglutinin	Sialyloligosaccharides
Rhinovirus	Common cold	Capsid protein	ICAM-1 (CD54)
Coxsackie A viruses	Common cold, oropharyngeal vesicles	Capsid protein	Integrin or ICAM-1
Parainfluenza virus type 1, respiratory syncytial virus	Respiratory illness	Envelope protein	Sialoglycolipids
Mycoplasma pneumoniae	Atypical pneumonia	Mediated by the terminal organelle, a membrane bound extension of the mycoplasma-infected cell	Neuraminic acid
Haemophilus influenza, *Strep. pneumonia*, *Klebsiella pneumoniae*	Respiratory disease	Surface molecule	Carbohydrate sequence in glycolipid
Measles virus	Measles	Haemagglutinin	CD46

CD46, membrane cofactor protein involved in complement regulation; ICAM-1, intercellular adhesion molecule-1; integrins, family of adhesion receptors (e.g. laminin receptor) expressed on many cell types.

Table 14.3 Interference with ciliary activity in respiratory infections

Cause	Mechanisms	Importance
Infecting bacteria interfere with ciliary activity (*B. pertussis*, *H. influenzae*, *P. aeruginosa*, *M. pneumoniae*)	Production of ciliostatic substances (tracheal cytotoxin from *B. pertussis*, at least two substances from *H. influenzae*, at least seven from *P. aeruginosa*)	+ +
Viral infection	Ciliated cell dysfunction or destruction by influenza, measles	+ + +
Atmospheric pollution (automobiles, cigarette smoking)	Acutely impaired mucociliary function	? +
Inhalation of unhumidified air (indwelling tracheal tubes, general anaesthesia)	Acutely impaired mucociliary function	+
Chronic bronchitis, cystic fibrosis	Chronically impaired mucociliary function	+ + +

Although pathogens can actively interfere with ciliary activity (first item), a more general impairment of mucociliary function also acts as a predisposing cause of respiratory infection.

Table 14.4 Microbial attachment in the intestinal tract

Microorganism	Disease	Attachment site	Mechanism
Poliovirus	Poliomyelitis	Intestinal epithelium	Viral capsid protein attaches to a specific receptor, called Pvr (polio virus receptor) or CD155, a cellular glycoprotein
Rotavirus	Diarrhoea	Intestinal epithelium	Viral outer capsid protein VP4 attaches to host cell glycans and then interacts with several coreceptors during post-attachment steps
Vibrio cholera	Cholera	Intestinal epithelium	Multivalent adhesion molecule (MAM) 7 is an outer membrane protein mediating host cell attachment
Escherichia coli (EPEC and EHEC)	Diarrhoea	Intestinal epithelium	Bacteria inject Tir, an effector, that inserts into host cell plasma membrane acting as a receptor for the bacterial surface protein called intimin
Salmonella typhi	Enteric fever	Ileal epithelium	Bacterial adhesins bind to host cell receptors
Shigella spp.	Dysentery	Colonic epithelium	Shigella surface protein, IscA, acts as an adhesin and interacts with host cells after activating a type III secretion system, triggering its uptake into epithelial cells
Giardia lamblia	Diarrhoea	Duodenal, jejuna epithelium	Protozoa bind to mannose-6-phosphate on host cell; also have mechanical sucker – the ventral disc
Entamoeba histolytica	Dysentery	Colonic epithelium	Lectin on surface of amoeba binds host cell
Ancylostoma duodenale	Hookworm	Intestinal epithelium	Buccal capsule

except insofar as diarrhoea and vomiting can be included in this category. Under normal circumstances, multiplication of resident bacteria is counterbalanced by their continuous passage to the exterior with the rest of the intestinal contents. Ingestion of a small number of non-pathogenic bacteria, followed by growth in the lumen of the alimentary canal, produces only relatively small numbers within 12–18 h, the normal intestinal transit time.

Infecting bacteria must attach themselves to the intestinal epithelium (Table 14.4) if they are to establish themselves and multiply in large numbers. They will then avoid being carried straight down the alimentary canal to be excreted with the rest of the intestinal contents. The concentration of microorganisms in faeces depends on the balance between the production and removal of bacteria in the intestine. *Vibrio cholerae* (Figs 14.3 and 14.4) and rotaviruses both establish specific binding to receptors on the surface of intestinal epithelial cells. For *V. cholerae*, establishment in surface mucus may be sufficient for infection and pathogenicity. The fact that certain pathogens infect mainly the large bowel (*Shigella* spp.)

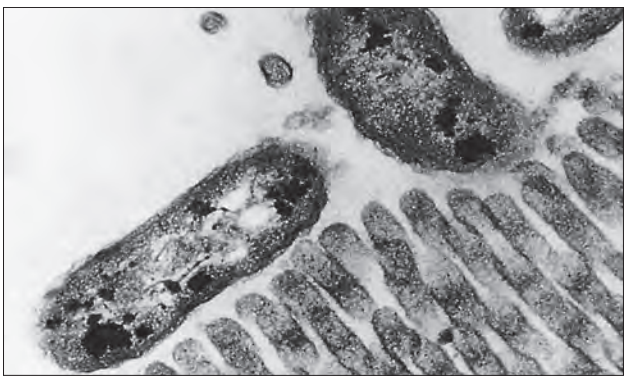


Figure 14.3 Attachment of *Vibrio cholerae* to brush border of rabbit villus. Thin section electron micrograph (×10 000). (Courtesy of E.T. Nelson.)

or small intestine (most salmonellae, rotaviruses) indicates the presence of specific receptor molecules on mucosal cells in these sections of the alimentary canal.

Infection sometimes involves more than mere adhesion to the luminal surface of intestinal epithelial cells. *Shigella flexneri*, for example, can only enter these cells from the basal surface. Initial entry occurs after uptake by M cells, and the bacteria then invade local macrophages. This gives rise to an inflammatory response with an influx of polymorphs, which in turn causes some disruption of the epithelial barrier. Bacteria can now enter on a larger scale from the intestinal lumen and invade epithelial cells from below. The bacteria enhance their entry by exploiting the host's inflammatory response.

Crude mechanical devices for attachment

Crude mechanical devices are used for the attachment and entry of certain parasitic protozoans and worms. *Giardia lamblia*, for example, has specific molecules for adhesion to the microvilli of epithelial cells, but also has its own microvillar-sucking disk. Hookworms attach to the intestinal mucosa by means of a large mouth capsule containing hooked teeth or cutting plates. Other worms (e.g. *Ascaris*) maintain their position by 'bracing' themselves against peristalsis, while tapeworms adhere closely to the mucus covering the intestinal wall, the anterior hooks and sucker playing a relatively minor role for the largest worms. A number of worms actively penetrate into the mucosa as adults (*Trichinella*, *Trichuris*) or traverse the gut wall to enter deeper tissues (e.g. the embryos of *Trichinella* released from the female worm and the larvae of *Echinococcus* hatched from ingested eggs).

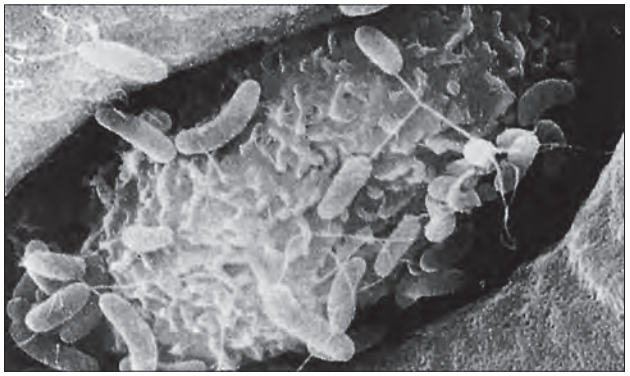


Figure 14.4 Adherence of *Vibrio cholerae* to M cells in human ileal mucosa. (Courtesy of T. Yamamoto.)

Mechanisms to counteract mucus, acids, enzymes and bile

Successful intestinal pathogens must counteract or resist mucus, acids, enzymes and bile. Mucus protects epithelial cells, perhaps acting as a mechanical barrier to infection. It may contain molecules that bind to microbial adhesins, therefore blocking attachment to host cells. It also contains pathogen-specific secretory IgA antibodies, which protect the immune individual against infection. Motile microorganisms (*V. cholerae*, salmonellae and certain strains of *E. coli*) can propel themselves through the mucus layer and are therefore more likely to reach epithelial cells to make specific attachments; *V. cholerae* also produces a mucinase, which probably helps its passage through the mucus. Non-motile microorganisms, in contrast, rely on random and passive transport in the mucus layer.

As might be expected, microorganisms that infect by the intestinal route are often capable of surviving in the presence of acid, proteolytic enzymes and bile. This also applies to microorganisms shed from the body by this route (Table 14.5).

All organisms infecting by the intestinal route must run the gauntlet of acid in the stomach. *Helicobacter pylori* has evolved

Table 14.5 Microbial properties that aid success in the gastrointestinal tract

Property	Examples	Consequence
Specific attachment to intestinal epithelium	Poliovirus, rotavirus, *Vibrio cholerae*	Microorganism avoids expulsion with other gut contents and can establish infection
Motility	*V. cholerae*, certain *E. coli* strains	Bacteria travel through mucus and are more likely to reach susceptible cell
Production of mucinase	*V. cholerae*	May assist transit through mucus (neuraminidase)
Acid resistance	*Mycobacterium tuberculosis*	Encourages intestinal tuberculosis (acid-labile microorganisms depend on protection in food bolus or in diluting fluid) increased susceptibility in individuals with achlorhydria
	Helicobacter pylori	Establish residence in stomach
	Enteroviruses (poliovirus, coxsackieviruses, echoviruses), hepatitis A virus	Infection and shedding from gastrointestinal tract
Bile resistance	*Salmonella, Shigella,* enteroviruses	Intestinal pathogens
	Enterococcus faecalis, E. coli, Proteus, Pseudomonas	Establish residence
Resistance to proteolytic enzymes	Reoviruses in mice	Permits oral infection
Anaerobic growth	*Bacteroides fragilis*	Most common resident bacteria in anaerobic environment of colon

Box 14.1 Lessons in Microbiology

How to survive stomach acid: the neutralization strategy of *Helicobacter pylori*

This bacterium was discovered in 1983, and was shown to be a human pathogen when two courageous doctors, Warren and Marshall in Perth, Western Australia, drank a potion containing the bacteria and developed gastritis. The infection spreads from person to person by the gastro–oral or fecal–oral route, and 150 years ago, nearly all humans were infected as children. Today, in countries with improved hygiene, this is put off until later in life, until at the age of 50 more than half of the population has been infected. The clinical outcome includes peptic ulcer, gastric cancer and gastric mucosa-associated lymphoid tissue (MALT) lymphoma and host, bacterial and environmental factors are thought to be involved. Genetic susceptibility is implicated in both acquiring and clearing *H. pylori* (HP) infection. After being eaten, the bacteria have a number of strategies resulting in adaptation to the host gastric mucosa having attached by special adhesins to the stomach wall. These include host mimicry leading to evasion of the host response and genetic variation. Most pathogens (e.g. *V. cholerae*) are soon killed at the low pH encountered in the stomach. *H. pylori*, however, protects itself by releasing large amounts of urease, which acts on local urea to form a tiny cloud of ammonia round the invader. The attached bacteria induce apoptosis in gastric epithelial cells, as well as inflammation, dyspepsia and occasionally a duodenal or gastric ulcer, so that treatment of these ulcers is by antibiotics rather than merely antacids. Some 90% of duodenal ulcers are due to HP infection, and the rest to aspirin or non-steroidal anti-inflammatory drugs (NSAIDs). The bacteria do not invade tissues, and they stay in the stomach for years, causing asymptomatic chronic gastritis. Up to 3% of infected individuals develop chronic active gastritis and progress to intestinal metaplasia, which can lead to stomach cancer. *H. pylori* was the third bacterium for which the entire genome was sequenced; several gene products have been characterized and key developments include understanding the genetic variation of genes encoding the outer membrane proteins and host adaptation.

a specific defence (Box 14.1). The fact that tubercle bacilli resist acid conditions favours the establishment of intestinal tuberculosis, but most bacteria are acid sensitive and prefer slightly alkaline conditions. For instance, volunteers who drank different doses of *V. cholerae* contained in 60 mL saline showed a 10 000-fold increase in susceptibility to cholera when 2 g of sodium bicarbonate was given with the bacteria. The minimum disease-producing dose was 10^8 bacteria without bicarbonate and 10^4 bacteria with bicarbonate. Similar experiments have been carried out in volunteers with *Salmonella typhi*, and the minimum infectious dose of 1000–10 000 bacteria was again significantly reduced by the ingestion of sodium bicarbonate. Infective stages of protozoa and worms resist stomach acid because they are protected within cysts or eggs. Unenveloped viruses are also at an advantage as they resist hot, acidic and dry environments.

When the infecting microorganism penetrates the intestinal epithelium (*Shigella*, *S. typhi*, hepatitis A and other enteroviruses) the final pathogenicity depends upon:

- subsequent multiplication and spread
- toxin production
- cell damage
- inflammatory and immune responses.

Microbial exotoxin, endotoxin and protein absorption

Microbial exotoxins, endotoxins and proteins can be absorbed from the intestine on a small scale. Diarrhoea generally promotes the uptake of protein, and absorption of protein also takes place more readily in the infant, which in some species needs to absorb antibodies from milk. As well as large molecules, particles the size of viruses can also be taken up from the intestinal lumen. This occurs in certain sites in particular, such as those where Peyer's patches occur. Peyer's patches are isolated collections of lymphoid tissue lying immediately below the intestinal epithelium, which in this region is highly specialized, consisting of so-called M cells (see Fig. 14.4). M cells take up particles and foreign proteins and deliver them to underlying immune cells with which they are intimately associated by cytoplasmic processes.

Urogenital tract

Microorganisms gaining entry via the urogenital tract can spread easily from one part of the tract to another

The urogenital tract is a continuum, so microorganisms can spread easily from one part to another, and the distinction between vaginitis and urethritis, or between urethritis and cystitis, is not always easy or necessary (see Chs. 21 and 22).

Vaginal defences

The vagina has no particular cleansing mechanisms, and repeated introductions of a contaminated, sometimes pathogen-bearing foreign object (the penis), makes the vagina particularly vulnerable to infection, forming the basis for sexually transmitted diseases (see Ch. 22). Nature has responded by providing additional defences. During reproductive life, the vaginal epithelium contains glycogen owing to the action of circulating estrogens, and certain lactobacilli colonize the vagina, metabolizing the glycogen to produce lactic acid. As a result, the normal vaginal pH is about 5.0, which inhibits colonization by all except the lactobacilli and certain other streptococci and diphtheroids. Normal vaginal secretions contain up to 10^8 / mL of these commensal bacteria. If other microorganisms are to colonize and invade they must either have specific mechanisms for attaching to vaginal or cervical mucosa or take advantage of minute local injuries during coitus (genital warts, syphilis) or impaired defences (presence of tampons, estrogen imbalance). These are the microorganisms responsible for sexually transmitted diseases.

Urethral and bladder defences

The regular flushing action of urine is a major urethral defence, and urine in the bladder is normally sterile.

The bladder is more than an inert receptacle, and in its wall there are intrinsic, but poorly understood, defence mechanisms.

These include a protective layer of mucus and the ability to generate inflammatory responses and produce secretory antibodies and immune cells.

Mechanism of urinary tract invasion

The urinary tract is nearly always invaded from the exterior via the urethra, and an invading microorganism must first and foremost avoid being washed out during urination. Specialized attachment mechanisms have therefore been developed by successful invaders (e.g. gonococci, Fig. 14.5). A defined peptide on the bacterial pili binds to a syndecan-like proteoglycan on the urethral cell, and the cell is then induced to engulf the bacterium. This is referred to as parasite-directed endocytosis and also occurs with chlamydia.

The foreskin is a handicap in genitourinary infections. This is because sexually transmitted pathogens often remain in the moist area beneath the foreskin after detumescence, giving them increased opportunity to invade. All sexually transmitted infections are more common in uncircumcised males.

Intestinal bacteria (mainly *E. coli*) are common invaders of the urinary tract, causing cystitis. The genitourinary anatomy is a major determinant of infection (Fig. 14.6). Spread to the bladder is no easy task in the male, where the flaccid urethra is 20 cm long. Therefore, urinary infections are rare in males unless organisms are introduced by catheters or when the flushing activity of urine is impaired (see Ch. 21). The foreskin causes trouble, again, in urinary tract infection by faecal bacteria. These infections are more common in uncircumcised infants because the prepuce may harbour faecal bacteria on its inner surface.

Things are different in females. Not only is the urethra much shorter (5 cm), but it is also very close to the anus (Fig. 14.6), which is a constant source of intestinal bacteria. Urinary infections are about 14 times more common in women, and at least 20% of women have a symptomatic urinary tract infection at some time during their life. The invading bacteria often begin their invasion by colonizing the mucosa around the urethra and probably have special attachment mechanisms to cells in this area. Bacterial invasion is favoured by the mechanical deformation of the urethra and surrounding region that occurs during sexual intercourse, which can lead to urethritis and cystitis. Bacteriuria is about 10 times more common in sexually active women than in nuns.

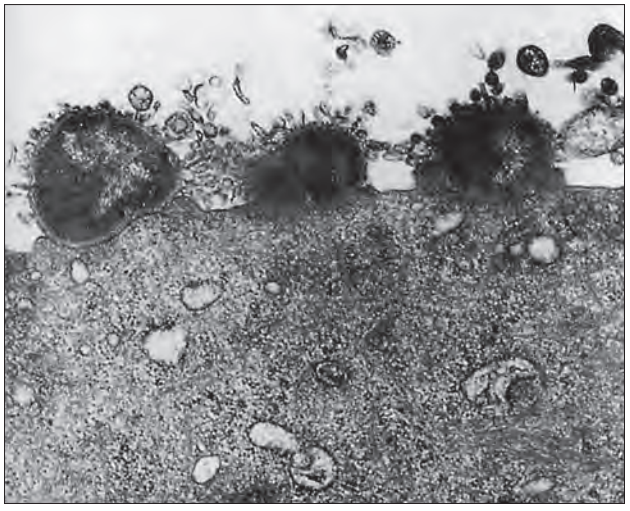


Figure 14.5 Adherence of gonococci to the surface of a human urethral epithelial cell. (Courtesy of P.J. Watt.)

Oropharynx

Microorganisms can invade the oropharynx when mucosal resistance is reduced

Commensal microorganisms in the oropharynx are described in Chapter 19.

Oropharyngeal defences

The flushing action of saliva provides a natural cleansing mechanism (about 1 L / day is produced, needing 400 swallows), aided by masticatory and other movements of the tongue, cheek and lips. On the other hand, material borne backwards from the nasopharynx is firmly wiped against the pharynx by the tongue during swallowing, and pathogens therefore have an opportunity to enter the body at this site. Additional defences include secretory IgA antibodies, antimicrobial substances such as lysozyme, the normal flora, and the antimicrobial activities of leukocytes present on mucosal surfaces and in saliva.

Mechanisms of oropharyngeal invasion

Attaching to mucosal or tooth surfaces is obligatory for both invading and resident microorganisms. For instance, different types of streptococci make specific attachments via lipoteichoic acid molecules on their pili to the buccal epithelium and tongue (resident *Streptococcus salivarius*), to teeth (resident *Strep. mutans*), or to pharyngeal epithelium (invading *Strep. pyogenes*).

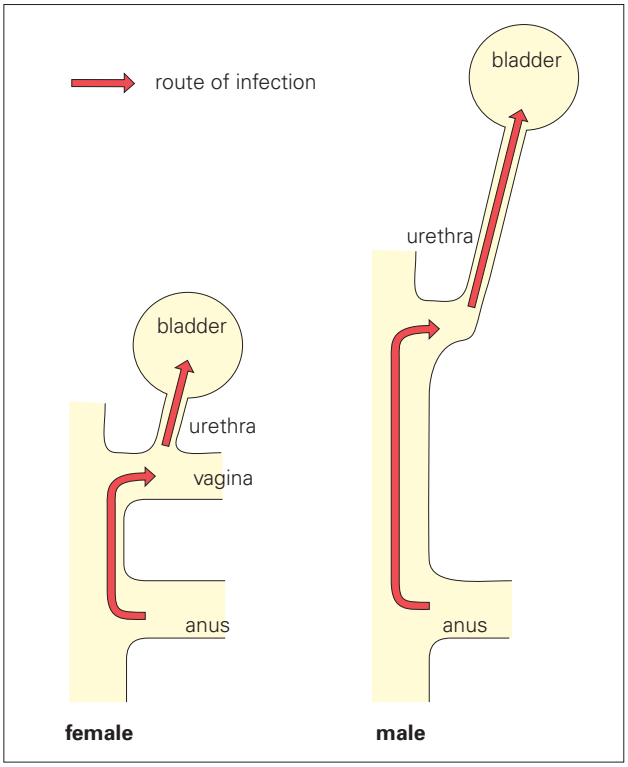


Figure 14.6 The female urogenital tract is particularly vulnerable to infection with faecal bacteria, mainly because the urethra is shorter and nearer to the anus.

Table 14.6 Types of infection and their role in transmission

Type of infection	Host defences	Evasion mechanism of pathogen	Examples	Value of evasion mechanism in transmission
Respiratory tract	Mucociliary clearance	Adhere to epithelial cells, interfere with ciliary action	Influenza viruses, pertussis	Essential
	Alveolar macrophage	Replicate in alveolar macrophage	*Legionella*, tuberculosis	Essential
Intestinal tract	Mucus, peristalsis	Adhere to epithelial cells	Rotavirus, *Salmonella*	Essential
	Acid, bile	Resist acid, bile	Poliovirus	Essential
Reproductive tract	Flushing action of urine and genital secretions, mucosal defences	Adhere to urethral/vaginal epithelial cells	Gonococcus, *Chlamydia*	Essential
Urinary tract	Flushing action of urine	Adhere to urethral/epithelial cells	*E. coli*	No value
		Reach urine from tubular epithelium	Polyomavirus	Valuable
Central nervous system	Enclosed in bony 'box' of skull and vertebral column	Reach CNS via nerves or blood vessels that enter skull or vertebral column	Bacterial meningitis, viral encephalitis	No value
Skin, mucosa	Layers of constantly shed cells (mucosa)	Invade skin/mucosa from below	Varicella, measles	Essential
	Dead keratinized cell layers (skin)	Infect basal epidermal layer	Papillomaviruses	Essential
		Infect via minor abrasions	Staphylococci, streptococci	Essential
		Penetrate intact skin	Schistosomiasis, ancyclostomiasis, leptospirosis	Essential
Vascular system	Skin	Infection of host by biting vector, replication in blood cells or in vascular endothelial cells	Malaria, yellow fever	Essential

For each type of host defence, the successful pathogen has an answer, which may or may not be important for transmission.

Factors that reduce mucosal resistance allow commensal and other bacteria to invade, as in the cases of gum infections caused by vitamin C deficiency, or of *Candida* invasion (thrush) promoted by changed resident flora after broad-spectrum antibiotics. When salivary flow is decreased for 3–4 h, as between meals, there is a fourfold increase in the number of bacteria in saliva (see Ch. 19). In dehydrated patients, salivary flow is greatly reduced and the mouth soon becomes overgrown with bacteria. As at all body surfaces, there is a shifting boundary between good behaviour by residents and tissue invasion according to changes in host defences.

EXIT AND TRANSMISSION

Microorganisms have a variety of mechanisms to ensure exit from the host and transmission

Successful pathogens must leave the body and then be transmitted to fresh hosts. Highly pathogenic microbes (e.g. Ebola virus, *Legionella pneumophila*) will have little impact on host populations if their transmission from person to person is uncommon or ineffective. Nearly all pathogens are shed from body surfaces, this being the route of exit to the outside world. Some, however, are extracted from inside the body by vectors (e.g. the blood-sucking arthropods that transmit yellow fever, malaria and filarial worms). Table 14.6 lists the types of infection and their role in the transmission of the pathogen and provides a summary of the host defences and the ways in which they are evaded. Transfer from one host to another forms the basis for the epidemiology of infectious disease (see Ch. 32).

Transmission depends upon three factors:

- the number of microorganisms shed
- the microorganism's stability in the environment
- the number of microorganisms required to infect a fresh host (the efficiency of the infection).

Number of microorganisms shed

Obviously, the more virus particles, bacteria, protozoa and eggs that are shed, the greater is the chance of reaching a fresh host. There are, however, many hazards. Most of the shed microorganisms die, and only an occasional one survives to perpetuate the species.

Stability in the environment

Microorganisms that resist drying spread more rapidly in the environment than those that are sensitive to drying (Table 14.7).

Table 14.7 Microbial resistance to drying as a factor in transmission

Stability on drying	Examples	Consequence
Stable	Tubercle bacilli Staphylococci	Spread more readily in air (dust, dried droplets)
	Clostridial spores Anthrax spores *Histoplasma* spores	Spread readily from soil
Unstable	*Neisseria meningitides* Streptococci *Bordetella pertussis* Influenza virus Measles virus	Require close (respiratory) contact
	Gonococci HIV *Treponema pallidum*	Require close (sexual) contact
	Vibrio cholerae Leptospira	Spread via water, food
	Yellow fever virus Malaria Trypanosomes	Spread via vectors (i.e. remain in a host)
	Larvae/eggs of worms	Need moist soil (except pinworms)

Pathogens that are already dehydrated such as spores are also more resistant to thermal inactivation. Spores can survive for years in soil.

Microorganisms also remain infectious for longer periods in the external environment when they are resistant to thermal inactivation. Certain microorganisms have developed special forms (e.g. clostridial spores, amoebic cysts) that enable them to resist drying, heat inactivation and chemical insults, and this testifies to the importance of stability in the environment. If still alive, microorganisms are more thermostable when they have dried. Drying directly from the frozen state (freeze drying) can make them very resistant to environmental temperatures. The fact that spores and cysts are dehydrated accounts for much of their stability. Microorganisms that are sensitive to drying depend for their spread on close contact, vectors, or contamination of food and water for spread.

Number of microorganisms required to infect a fresh host

The efficiency of the infection varies greatly between microorganisms, and helps explain many aspects of transmission. For instance, volunteers ingesting 10 *Shigella dysenteriae* bacteria (from other humans) will become infected, whereas as many as 10^6 *Salmonella* spp. (from animals) are needed to cause food poisoning. The route of infection also matters. A single tissue culture infectious dose of a human rhinovirus instilled into the nasal cavity causes a common cold and, although this dose contains many virus particles, about 200 such doses are needed when applied to the pharynx. As few as 10 gonococci can establish an infection in the urethra, but many thousand times this number are needed to infect the mucosa of the oropharynx or rectum.

Other factors affecting transmission

Genetic factors in microorganisms also influence transmission. Some strains of a given microorganism are therefore more readily transmitted than others, although the exact mechanism is often unclear. Transmission can vary independently of the ability to do damage and cause disease (pathogenicity or virulence).

Activities of the infected host may increase the efficiency of shedding and transmission. Coughing and sneezing are reflex activities that benefit the host by clearing foreign material from the upper and lower respiratory tract, but they also benefit the microorganism. Strains of microorganism that are more able to increase fluid secretions or irritate respiratory epithelium will induce more coughing and sneezing than those less able and will be transmitted more effectively. Similar arguments can be applied to the equivalent intestinal activity: diarrhoea. Although diarrhoea eliminates the infection more rapidly (prevention of diarrhoea often prolongs intestinal infection), from the pathogen's point of view it is a highly effective way of contaminating the environment and spreading to fresh hosts.

TYPES OF TRANSMISSION BETWEEN HUMANS

Microorganisms can be transmitted to humans by humans, vertebrates and biting arthropods. Transmission is most effective when it takes place directly from human to human. The most common worldwide infections are spread by the respiratory, faecal–oral or sexual route. A separate set of infections is acquired from animals, either directly from vertebrates (the zoonoses) or indirectly from biting arthropods. Infections acquired from other species are either not transmitted or transmit very poorly from human to human. Types of transmission are illustrated in Fig. 14.7.

Transmission from the respiratory tract

Respiratory infections spread rapidly when people are crowded together indoors

An increase in nasal secretions with sneezing and coughing promotes effective shedding from the nasal cavity. In a sneeze (Fig. 14.8) up to 20 000 droplets are produced, and during a

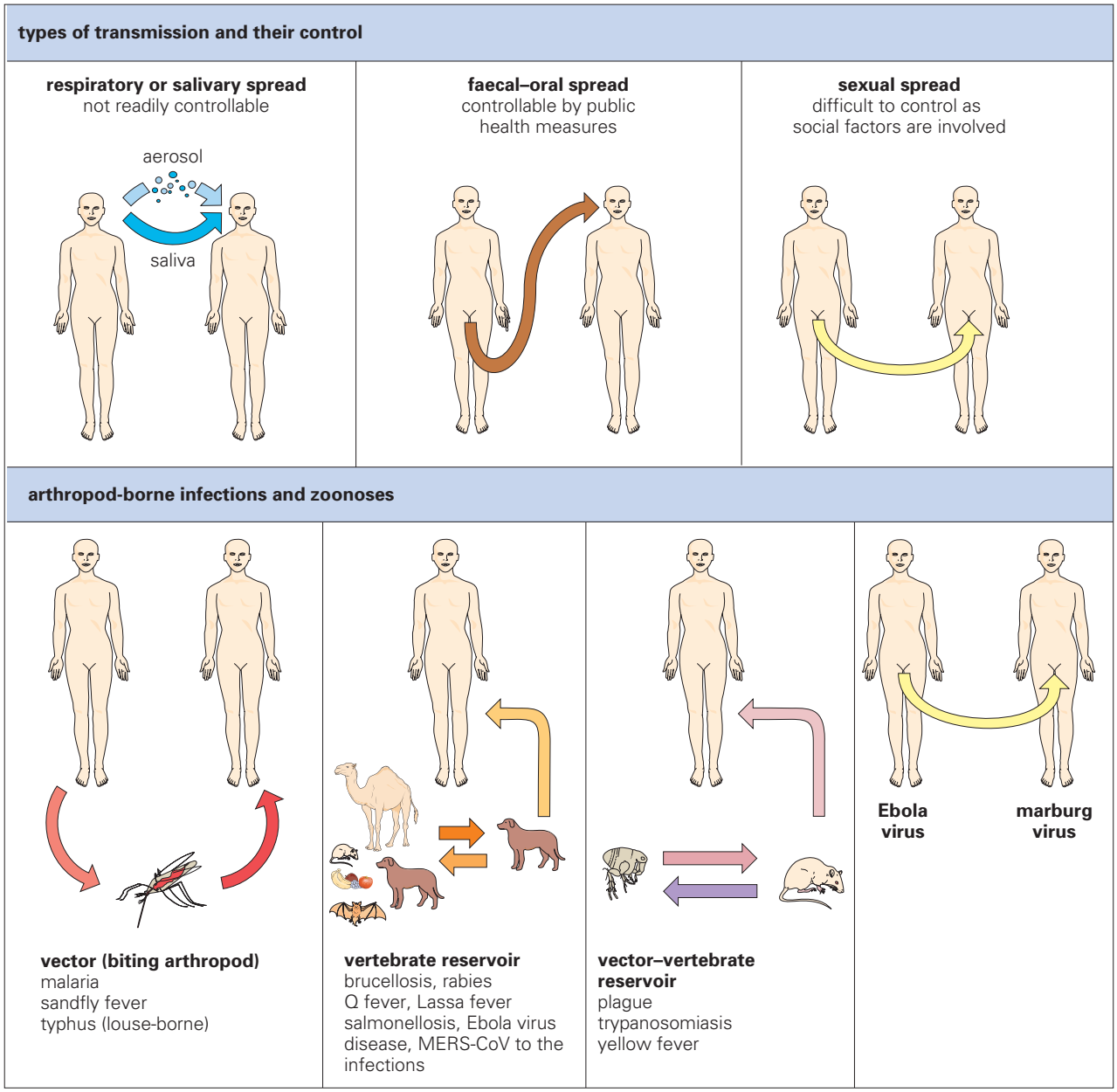


Figure 14.7 Types of transmission and their control. Arthropod-borne infections and zoonoses can be controlled by controlling vectors or by controlling animal infection; there is virtually no person-to-person transmission of these infections (except for pneumonic plague and Ebola virus infection, see Ch. 29).

common cold, for instance, many of them will contain virus particles.

A smaller number of microorganisms (hundreds) are expelled from the mouth, throat, larynx and lungs during coughing (whooping cough, tuberculosis). Talking is a less important source of air-borne particles, but does produce them, especially when the consonants 'f, p, t and s' are used. It is surely no accident that many of the most abusive words in the English language begin with these letters, so that a spray of droplets (possibly infectious) is delivered with the abuse!

The size of inhaled droplets determines their initial localization. The largest droplets fall to the ground after travelling approximately 4 m, and the rest settle according to size. Those 10 µm or so in diameter can be trapped on the nasal mucosa. The smallest (1–4 µm diameter) are kept suspended for an indefinite period by normal air movements, and particles of this size are likely to pass the turbinate baffles in the nose and reach the lower respiratory tract.

When people are crowded together indoors, respiratory infections spread rapidly – for example, the common cold in schools and offices and meningococcal infections in military recruits. This is perhaps why respiratory infections are common in winter. The air in ill-ventilated rooms is also more humid, favouring survival of suspended microorganisms such as streptococci and enveloped viruses. Air conditioning is another factor, as the dry air leads to impaired mucociliary activity. Respiratory spread is, in one sense, unique. Material from one person's respiratory tract can be taken up almost immediately

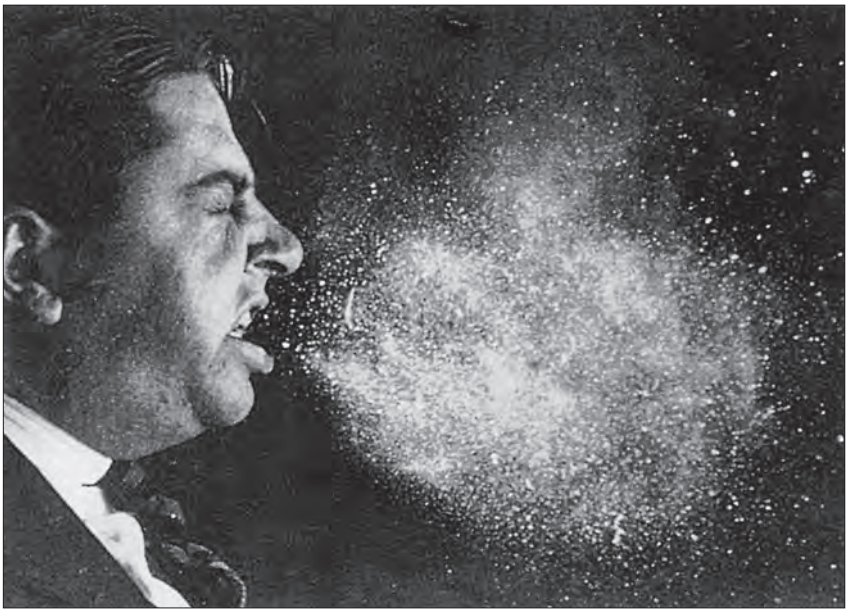


Figure 14.8 Droplet dispersal following a violent sneeze. Most of the 20 000 particles seen are coming from the mouth. (Reprinted with permission from: Moulton F.R. [ed.] [1942] *Aerobiology*. American Association for the Advancement of Science.)

into the respiratory tract of other individuals. This is in striking contrast to the material expelled from the gastrointestinal tract, and helps explain why respiratory infections spread so rapidly when people are indoors.

Handkerchiefs, hands and other objects can carry respiratory infection such as common cold viruses from one individual to another, although coughs and sneezes provide a more dramatic route. Transmission from the infected conjunctiva is referred to in Chapter 26.

The presence of receptors (see Table 14.2) and local temperature as well as initial localization can determine which part of the respiratory tract is infected. For instance, it can be assumed that rhinoviruses arrive in the lower respiratory tract on a large scale, but fail to grow there because, like leprosy bacilli, they prefer the cooler temperature of the nasal mucosa.

Transmission from the gastrointestinal tract

Intestinal infection spreads easily if public health and hygiene are poor

The spread of an intestinal infection is assured if public health and hygiene are poor, the pathogen appears in the faeces in sufficient numbers and there are susceptible individuals in the vicinity. Diarrhoea gives it an additional advantage, and the key role of diarrhoea in transmission has been referred to above. During most of human history, there has been a large-scale recycling of faecal material back into the mouth, and this continues in resource-poor countries. The attractiveness of the faecal–oral route for microorganisms and parasites is reflected in the great variety that are transmitted in this way.

Intestinal infections have been to some extent controlled in resource-rich countries. The great public health reforms of the nineteenth century led to the introduction of adequate sewage disposal and a supply of purified water. For instance, in England 200 years ago, there were no flushing toilets and no sewage disposal and much of the drinking water was contaminated. Cholera and typhoid spread easily, and in London, the Thames became an open sewer. Today, as in other cities, a complex underground disposal system separates sewage from drinking water. Intestinal infections are still transmitted in resource-rich countries, but via food and fingers rather than by water and flies. Therefore, although each year in the UK there are dozens of cases of typhoid acquired on visits to resource-poor countries, the infection is not transmitted to others.

The microorganisms that appear in faeces usually multiply in the lumen or wall of the intestinal tract, but there are a few that are shed into bile. For instance, hepatitis A enters bile after replicating in liver cells.

Transmission from the urogenital tract

Urogenital tract infections are often sexually transmitted

Urinary tract infections are common, but most are not spread via urine. Urine can contaminate food, drink and living space. Examples of some infections that are spread by urine are listed in Table 14.8.

Sexually transmitted infections (STIs)

Microorganisms shed from the urogenital tract are often transmitted as a result of mucosal contact with susceptible individuals, typically as a result of sexual activity. If there is a discharge, organisms are carried over the epithelial surfaces and transmission is more likely. Some of the most successful sexually transmitted microorganisms (gonococci, chlamydia) therefore induce a discharge. Other microorganisms are transmitted effectively from mucosal sores (ulcers) – for example, *Treponema pallidum* and herpes simplex virus. The human papillomaviruses are transmitted from genital warts or from foci of infection in the cervix where the epithelium, although apparently normal, is dysplastic and contains infected cells (see Ch. 22).

The transmission of STIs is determined by social and sexual activity. Changes in the size of the human population and way of life have had a dramatic effect on the epidemiology of STIs. More opportunities to have sexual encounters have arisen owing to increasing population density, increased movement

Table 14.8 Human infections transmitted via urine

Infection	Details	Value in transmission
Schistosomiasis	Parasite eggs excreted in bladder	+++
Typhoid	Bacterial persistence in bladder scarred by schistosomiasis	+
Polyomavirus infection	Commonly excreted in urine	?
Cytomegalovirus infection	Commonly excreted in infected children	?
Leptospirosis	Infected rats and dogs excrete bacteria in urine	++
Lassa fever (and South American haemorrhagic fevers)	Persistently infected rodent excretes virus in urine	+++

Schistosomiasis is the major infection transmitted in this way, the eggs undergoing development in snails before re-infecting humans. Viruses are shed in the urine after infecting tubular epithelial cells in the kidney.

of people, the decline of the idea that sexual activity is sinful, social media and the internet, and the knowledge that STIs are treatable and pregnancy is avoidable. In addition, the contraceptive pill has favoured the spread of STIs by reducing the use of mechanical barriers to conception. Condoms have been shown to reliably retain and reduce the transmission potential of herpes simplex virus, HIV, chlamydia and gonococci in simulated coital tests of the syringe and plunger type (see Ch. 22).

STIs are, however, transmitted with far less speed and efficiency than respiratory or intestinal infections. Influenza can be transmitted to a multitude of others during 1 h in a crowded room, or a rotavirus to a score of children during a morning at kindergarten, but STIs can spread to each person only by a separate sexual act. Multiple partners are therefore essential. Frequent sexual activity is not enough without involving multiple partners because those in a stable partnership can do no more than infect each other. Changes in sexual practices have led to a dramatic rise in the incidence of STIs.

As almost all mucosal surfaces of the body can be involved in sexual activity, microorganisms have had increasing opportunity to infect new body sites. The meningococcus, a nasopharyngeal resident, has therefore sometimes been recovered from the cervix, the male urethra, and the anal canal, while occasionally gonococci and chlamydia infect the throat and anal canal. It is no surprise that genito–oro–anal contacts have sometimes allowed intestinal infections such as *Salmonella*, *Giardia*, hepatitis A virus, *Shigella* and pathogenic amoebae to spread directly between individuals despite good sanitation and sewage disposal.

Semen as a source of infection

It might be expected that semen is involved in the transmission of infection, and this is the case in viral infections of animals such as blue tongue and foot and mouth disease. In humans, cytomegalovirus that is shed from the oropharynx is also often present in large quantities in semen, and the fact that it is also recoverable from the cervix suggests that it is sexually transmitted. Hepatitis B virus and HIV are also present in semen.

Perinatal transmission

The female genital tract can also be a source of infection for the newborn child (see Ch. 24). During passage down an infected birth canal, microorganisms can be wiped onto the conjunctiva of the infant or inhaled, leading to a variety of conditions such as conjunctivitis, pneumonia and bacterial meningitis.

Transmission from the oropharynx

Oropharyngeal infections are often spread in saliva

Saliva is often the vehicle of transmission. Microorganisms such as streptococci and tubercle bacilli reach saliva during upper and lower respiratory tract infections, while certain viruses infect the salivary glands and are transmitted in this way. Paramyxovirus, herpes simplex virus, cytomegalovirus and human herpes virus type 6 are shed into saliva. In young children, fingers and other objects are regularly contaminated by saliva, and each of these infections is acquired by this route. Epstein–Barr virus is also shed into saliva, but is transmitted less effectively, perhaps because it is present only in cells or in small amounts. In resource-rich countries, people often escape infection during childhood, and become infected as adolescents or adults during the extensive salivary exchanges (mean 4.2 mL / h) that accompany oral encounters of the deep and meaningful kind (see Ch. 19). Saliva from animals is the source of a few infections, and these are included in Table 14.9.

Transmission from the skin

Skin can spread infection by shedding or direct contact

Dermatophytes (fungi such as those that cause ringworm) are shed from skin and also from hair and nails, the exact source depending on the type of fungus (see Ch. 27). Skin is also an important source of certain other bacteria and viruses, as outlined in Table 14.10.

Shedding to the environment

The normal individual sheds desquamated skin scales into the environment at a rate of about 5×10^8 / day, the rate depending upon physical activities such as exercise, dressing and undressing. The fine white dust that collects on indoor surfaces, especially in hospital wards, consists largely of skin scales. Staphylococci are present, and different individuals show great variation in staphylococcal shedding, but the reasons are unknown.

Transmission by direct contact or by contaminated fingers is much more common than following release into the environment, and microorganisms transmitted in this way include potentially pathogenic staphylococci and human papillomaviruses.

Table 14.9 Human infections transmitted via saliva

Microorganism	Comments
Herpes simplex virus	Infection generally during childhood
Cytomegalovirus, Epstein–Barr virus	Adolescent/adult infection is common
Rabies virus	Shed in saliva of infected dogs, wolves, jackals, vampire bats
Pasteurella multocida	Bacteria in upper respiratory tract of dogs, cats appear in saliva and are transmitted via bites, scratches
Streptobacillus moniliformis	Present in rat saliva and infects humans (rat bite fever)

Table 14.10 Human infections transmitted from the skin

Microorganism	Disease	Comments
Staphylococci	Boils, carbuncles, neonatal skin sepsis	Pathogenicity varies, skin lesions or nose picking are common sources of infection
Treponema pallidum	Syphilis	Mucosal surfaces more infectious than skin
Treponema pertenue	Yaws	Regular transmission from skin lesions
Streptococcus pyogenes	Impetigo	Vesicular (epidermal) lesions crusting over, common in children in hot, humid climates
Staphylococcus aureus	Impetigo	Less common; bullous lesions, especially in newborn
Dermatophytes	Skin ringworm	Different species infect skin, hair, nails
Herpes simplex virus	Herpes simplex, cold sore	Up to 10^6 infectious units/mL of vesicle fluid
Varicella-zoster virus	Varicella, zoster	Vesicular skin lesions occur but transmission is usually respiratory[a]
Coxsackievirus A16	Hand, foot and mouth disease	Vesicular skin lesions but transmission faecal and respiratory
Papillomaviruses	Warts	Many types[b]
Leishmania tropica	Cutaneous leishmaniasis	Skin sores are infectious
Sarcoptes scabei	Scabies	Eggs from burrow transmitted by hand (also sexually)

[a]Except in zoster, where a localized skin eruption occurs.
[b]Generally direct contact, but plantar warts are commonly spread following walking barefoot on contaminated floors such as swimming pool surrounds.

Transmission in milk

Milk is produced by a skin gland. Microorganisms are rarely shed into human milk, and examples include HIV, cytomegalovirus and human T-cell lymphotropic virus 1 (HTLV-1), but milk from cows, goats and sheep can be important sources of infection (Table 14.11). Bacteria can be introduced into milk after collection.

Transmission from blood

Blood can spread infection via arthropods or needles

Blood is often the vehicle of transmission. Microorganisms and parasites spread by blood-sucking arthropods (see below) are effectively shed into the blood. Infectious agents present in blood (hepatitis B and C viruses, HIV) are also transmissible by needles, either in transfused blood or when contaminated needles are used for injections or intravenous drug misuse. Intravenous drug misuse is a well-known factor in the spread of these infections. In addition, at least 12000 million injections are given each year, worldwide, about 1 in 10 of them for vaccines. Unfortunately, in parts of the resource-poor world, disposable syringes tend to be used more than once, without being properly sterilized in between ('If it still works, use it again'). The prolonged outbreak of hepatitis C virus genotype 4 infection in Egypt was thought to have originated from the time when parenteral antischistosomal treatment with injectable antimony was given in mass campaigns, involving reused syringes, from the 1950s to the 1980s. To prevent this, the World Health Organization (WHO) encouraged the use of syringes in which, for instance, the plunger cannot be withdrawn once it has been pushed in.

Blood is also the source of infection in transplacental transmission and this generally involves initial infection of the placenta (see Ch. 24).

Vertical and horizontal transmission

Vertical transmission takes place between parents and their offspring

When transmission occurs directly from parents to offspring via, for example, sperm, ovum, placenta (Table 14.12), milk or blood, it is referred to as vertical. This is because it can be represented as a vertical flow down a page (Fig. 14.9), just like a family pedigree. Other infections, in contrast, are said to be horizontally transmitted, with an individual infecting other individuals by contact, respiratory or faecal–oral spread. Vertically transmitted infections can be subdivided as shown in Table 14.13. Strictly speaking, these infections are able to maintain themselves in the species without spreading horizontally, as long as they do not affect the viability of the

Table 14.11 Human infections transmitted via milk

Microorganism	Type of milk	Importance in transmission
Cytomegalovirus	Human	–
HIV	Human	+
HTLV-1	Human	+
Brucella	Cow, goat, sheep	+ +
Mycobacterium bovis	Cow	+ +
Coxiella burnetii (Q fever)	Cow	+
Campylobacter jejuni	Cow	+ +
Salmonella spp. *Listeria monocytogenes* *Staphylococcus* spp. *Streptococcus pyogenes* *Yersinia enterocolitica*	Cow	+

Human milk is rarely a significant source of infection. All pathogens listed are destroyed by pasteurization.

Table 14.12 Human infections transmitted via the placenta

Transplacental transmission of infection	
Microorganism	**Effect**
Rubella virus, cytomegalovirus	Placental lesion, abortion, stillbirth, malformation
HIV	Childhood HIV and AIDS
Hepatitis B virus	Antigen carriage in infant, but most of these infections are perinatal or postnatal
Treponema pallidum	Stillbirth, congenital syphilis with malformation
Listeria monocytogenes	Meningoencephalitis
Toxoplasma gondii	Stillbirth, CNS disease

host. Various retroviruses are known to maintain themselves vertically in animals (e.g. mammary tumour virus in milk, sperm and ovum of mice), but this does not appear to be important in humans, except possibly for HTLV-1, where milk transfer is important. There are, however, many retrovirus sequences present in the normal human genome known as endogenous retroviruses. These DNA sequences are too incomplete to produce infectious virus particles, but can be regarded as amazingly successful parasites. In addition, some of them may confer benefit, for example, by coding for proteins that help coordinate early stages of fetal development. They presumably do no harm and survive within the human species, watched over, conserved and replicated as part of our genetic constitution.

TRANSMISSION FROM ANIMALS

Humans and animals share a common susceptibility to certain pathogens

Humans live in daily contact, directly or indirectly, with a wide variety of other animal species, both vertebrate and invertebrate, sharing not only a common environment, but also a common susceptibility to certain pathogens. The degree to which animal contacts transmit infection depends upon the type of environment (e.g. urban / rural, tropical / temperate, hygienic / insanitary) and on the nature of the contact. Close contact is made with vertebrate animals used for food or as pets, and with invertebrate animals adapted to live or feed on the human body. Less intimate contact is made with many other species, which nevertheless may transmit pathogens equally well. For convenience, animal-transmitted infections can be divided into two categories:

- those involving arthropod and other invertebrate vectors
- those transmitted directly from vertebrates (zoonoses).

More detailed accounts of these infections are given in Chapters 28 and 29.

Invertebrate vectors

Insects, ticks and mites – the bloodsuckers – are the most important vectors spreading infection

By far the most important vectors of disease belong to these three groups of arthropods. Many species are capable of transmitting infection, and a wide range of organisms is transmitted (Table 14.14). In the past, insects have been responsible for some of the most devastating epidemic diseases, for example, fleas and plague and lice and typhus. Even today, one of the world's most important infectious diseases – malaria – is transmitted by the *Anopheles* mosquito. The distribution and epidemiology of these infections are determined by the climatic conditions that allow the vectors to breed and the organism to complete its development in their bodies. Some diseases are therefore purely tropical and subtropical (e.g. malaria, sleeping sickness and yellow fever), whereas others are much more widespread (e.g. plague and typhus). However, with climate change and increased travel, some viral infections are now being seen in previously unaffected regions – for example, West Nile virus and chikungunya infections reported in Italy.

Passive carriage

Insects may carry pathogens passively on their mouth parts, on their bodies, or within their intestines. Transfer onto food

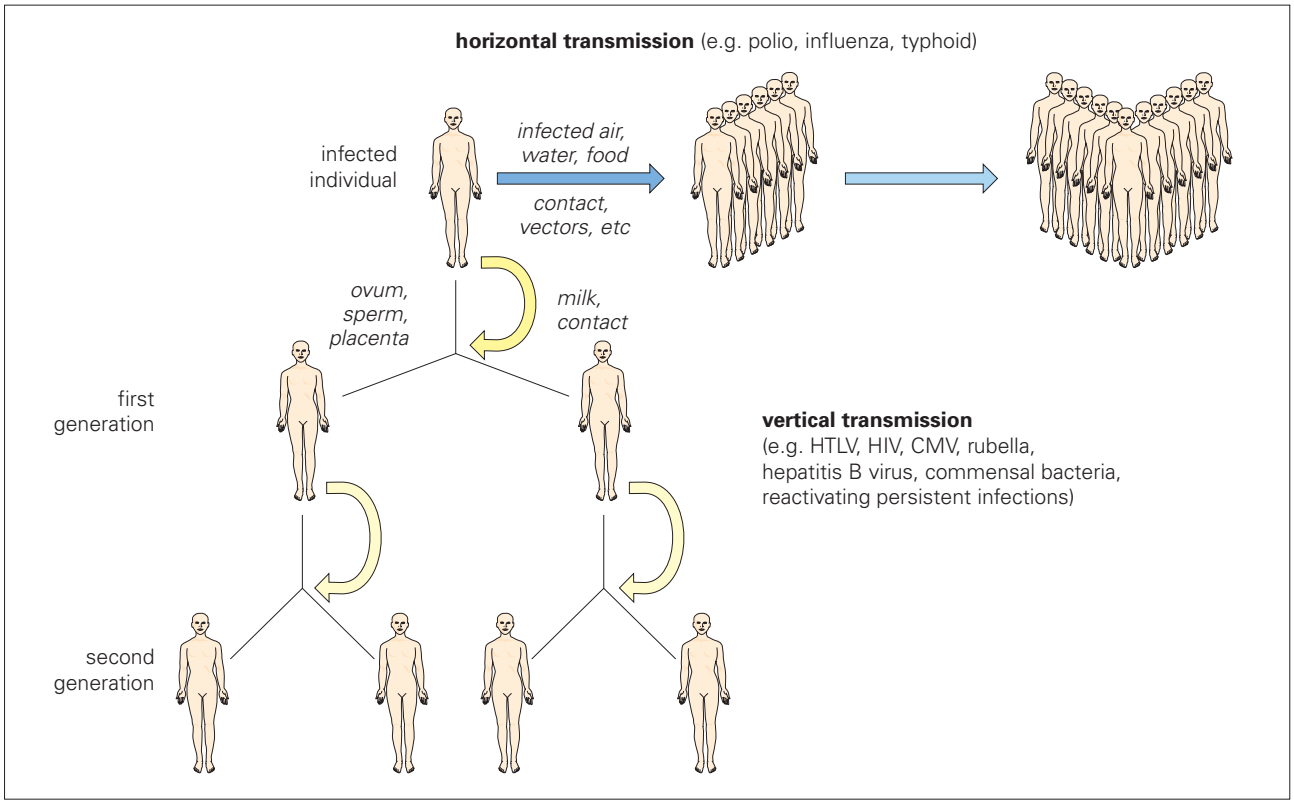


Figure 14.9 Vertical and horizontal transmission by infection. Most infections are transmitted horizontally, as might be expected in crowded human populations. Vertical transmission becomes more important in small isolated communities (see Ch. 18). CMV, cytomegalovirus; HIV, human immunodeficiency virus; HTLV, human T-cell lymphotropic virus.

Table 14.13 Types of vertical transmission

Type	Route	Examples
Prenatal	Placenta	Rubella, cytomegalovirus, syphilis, toxoplasmosis, hepatitis B
Perinatal	Infected birth canal	Gonococcal / chlamydial conjunctivitis, herpes simplex
Postnatal	Milk Direct contact with blood at delivery	Cytomegalovirus Hepatitis B virus, HIV, HTLV-1
Germline	Viral DNA sequences in human genome	Many ancient retroviruses

HIV, human immunodeficiency virus; HTLV, human T-cell lymphotropic virus.

or onto the host occurs directly as a result of the insect feeding, regurgitating or defecating. Many important diseases, such as trachoma, can be transmitted in this way by common species such as houseflies and cockroaches.

Blood-feeding species have mouth parts adapted for penetrating skin in order to reach blood vessels or to create small pools of blood (Fig. 14.10). The ability to feed in this way provides access to organisms in the skin or blood. The mouth parts can act as a contaminated hypodermic needle, carrying infection between individuals.

Biological transmission

This is much more common, the blood-sucking vector acting as a necessary host for the multiplication and development of the pathogen. Almost all of the important infections (listed in Table 14.14) are transmitted in this way. The pathogen is re-introduced into the human host, after a period of time, at the next blood meal. Transmission can be by direct injection, usually in the vector's saliva (malaria, yellow fever), or by contamination from faeces or regurgitated blood deposited at the time of feeding (typhus, plague).

Other invertebrate vectors spread infection either passively or by acting as an intermediate host

Many invertebrates used for food convey pathogens (Fig. 14.11). Perhaps the most familiar are the shellfish (molluscs and crustaceans) associated with food poisoning and acute gastroenteritis. These filter feeders accumulate viruses and bacteria in their bodies, taking them in from contaminated waste, and transferring them passively. In other cases, the relationship between the pathogen and the invertebrate is much closer. Many parasites, especially worms, must undergo

Table 14.14 Arthropod-borne pathogens[a]

Arthropod-borne pathogens			
Arthropods		**Pathogens**	**Diseases**
Insects Houseflies Sandflies Mosquitoes Blackflies Lice Fleas Hemiptera bugs Midges Tabanids **Acarids** Ticks Mites	Viruses Bacteria Protozoa Helminths	Flaviviruses	Yellow fever Dengue Zika microcephaly West Nile Japanese encephalitis
		Bunyaviruses	Haemorrhagic fevers
		Yersinia	Plague, tularemia
		Rickettsias	Q fever, spotted fevers, typhus, rickettsial pox
		Spirochaetes	Relapsing fever, Lyme disease
		Trypanosomes	Sleeping sickness, Chagas disease
		Leishmania	Leishmaniasis
		Plasmodium	Malaria
		Plasmodium nematodes	Lymphatic filariases, loiasis, onchocerciasis

[a]Mosquitoes are a major source of infection. (Note that, with the exception of pneumonic plague and Ebola virus, none is transmitted from human to human.)

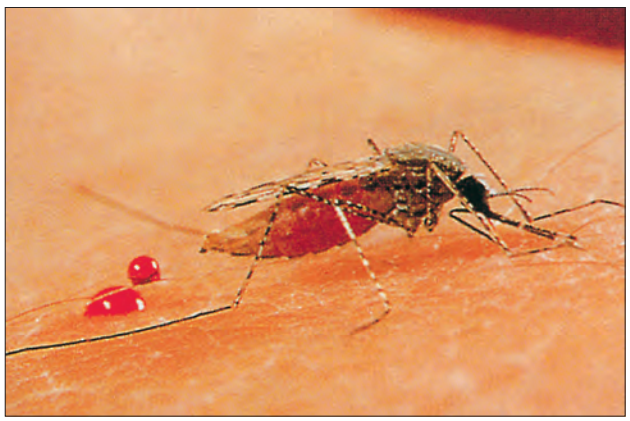


Figure 14.10 Female *Anopheles* mosquito feeding. (Courtesy of C.J. Webb.)

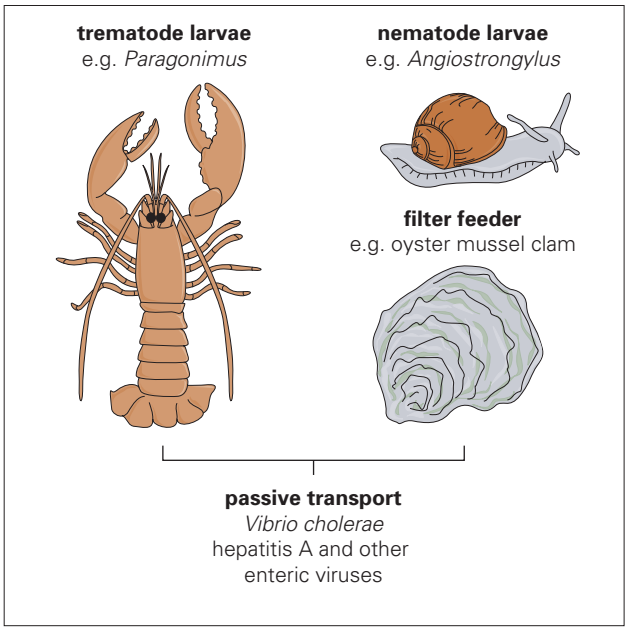


Figure 14.11 Microorganisms transmitted via invertebrates used for food. Filter-feeding molluscs living in estuaries near sewage outlets are a common source of infection.

part of their development in the invertebrate before being able to infect a human. Humans are infected when they eat the invertebrate (intermediate) host. Dietary habits are therefore important in infection.

Aquatic molluscs (snails) are necessary intermediate hosts for schistosomes – the blood flukes. They become infected by larval stages, which hatch from eggs passed into water in the urine or faeces of infected people. After a period of development and multiplication, large numbers of infective stages (cercariae) escape from the snails. These can rapidly penetrate through human skin, initiating the infection that will result in adult flukes occupying visceral blood vessels (see Ch. 31).

Transmission from vertebrates

Many pathogens are transmitted directly to humans from vertebrate animals

Strictly, the term zoonoses can apply to any infection transmitted to humans from infected animals, whether this is direct (by contact or eating) or indirect (via an invertebrate vector). Here, however, zoonoses are used to describe infections of vertebrate animals that can be transmitted directly. Many

Table 14.15 Zoonoses: human infections transmitted directly from vertebrates (birds and mammals)

Pathogens	Vertebrate vector	Diseases
Viruses		
Arenaviruses	Mammals	Lassa fever, lymphocytic choriomeningitis, Bolivian haemorrhagic fever
Poxviruses	Mammals	Cowpox, orf
Hepatitis E virus	Pigs	Hepatitis E
Rhabdoviruses	Mammals	Rabies
SARS coronavirus[a]	Monkeys, Himalayan palm civets, raccoon dogs, cats, dogs, rodents	SARS (severe acute respiratory syndrome)
MERS coronavirus[a]	Camels	MERS (Middle East respiratory syndrome)
Avian influenza viruses[a]	Chickens	Influenza A H5N1 and other strains
Bacteria		
Bacillus anthracis	Mammals	Anthrax
Brucella	Mammals	Brucella
Chlamydia	Birds	Psittacosis
Leptospira	Mammals	Leptospirosis (Weil's disease)
Listeria	Mammals	Listeriosis
Salmonella	Birds, mammals	Salmonellosis
Mycobacterium tuberculosis	Mammals	Tuberculosis
Fungi		
Cryptococcus	Birds	Meningitis
Dermatophytes	Mammals	Ringworm
Protozoa		
Cryptosporidium	Mammals	Cryptosporidiosis
Giardia	Mammals	Giardiasis
Toxoplasma	Mammals	Toxoplasmosis
Helminths		
Ancylostoma	Mammals	Hookworm disease
Echinococcus	Mammals	Hydatid disease
Taenia	Mammals	Tapeworms
Toxocara	Mammals	Toxocariasis (visceral larval migrans)
Trichinella	Mammals	Trichinellosis

[a]Poor transmission from person to person, but they may at any time change and develop the capacity for efficient transmission.

pathogens are transmitted in this way (Table 14.15) by a variety of different routes including contact, inhalation, bites, scratches, contamination of food or water and ingestion as food.

The epidemiology of zoonoses depends upon the frequency and the nature of contact between the vertebrate and the human hosts. Some are localized geographically, being dependent, for example, on local food preferences. Where these involve eating uncooked animal products such as fish or amphibia, a variety of parasites (especially tapeworms and nematodes) can be acquired. Others are associated with occupation – for example, if this involves contact with raw animal products (butchers in the case of toxoplasmosis and Q fever), or frequent contact with domestic stock (farm workers in the case of brucellosis and dermatophyte fungi). In urban areas, zoonoses are most likely to be acquired by eating or drinking infected animal products or by contact with dogs, cats and other domestic pets. Hepatitis E virus infections were regarded as a sporadic cause of hepatitis in Europe until studies reported in 2015 that blood transfusion recipients developed the infection having received blood from asymptomatic hepatitis E viraemic donors. Eating undercooked pork sausages, as well as other more exotic meat products, was associated with transmission and it was reported as a zoonotic infection of pigs (see Ch. 23).

Domestic pets or pests?

Dogs and cats are the most common domestic pets, and both are reservoirs of infection for their owners (Fig. 14.12). The

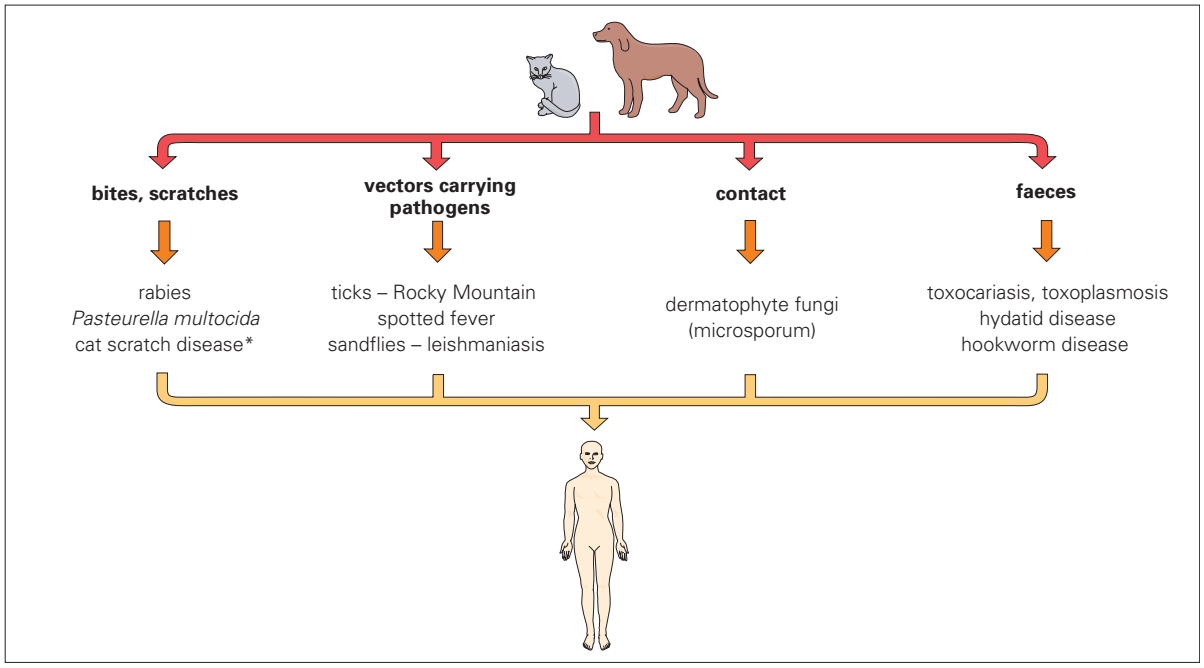


Figure 14.12 Man's best friends? Zoonoses transmitted from dogs and cats. (* A benign infection, with skin lesions and lymphadenopathy, shown to be due to a bacterium, *Bartonella henselae*.)

pathogens concerned are spread by contact, bites and scratches, by vectors, and by contamination with faecal material. Major infections transmitted in these ways include:

- toxocariasis from dogs
- toxoplasmosis from cats.

Both are almost universal in their distribution.

Humans may acquire hydatid disease from tapeworm eggs passed in dog faeces where dogs are used for herding domestic animals and have access to infected carcasses. In rural areas of many countries this has been, or remains, an important infection.

Many species of birds are kept as pets and some can pass on serious infections to those in contact with them. Contact is usually through inhalation of infected particulate material. Perhaps the most important of these is psittacosis caused by *Chlamydophila* (formerly *Chlamydia*) *psittaci*, which despite the common name 'parrot fever' can be acquired from many avian species.

The recent trend in resource-rich countries towards keeping unusual or exotic pets (especially reptiles, exotic birds and mammals) raises new risks of zoonotic infection. Many reptiles, for example, pass human-infective *Salmonella* spp. in their droppings. Exotic birds and mammals can carry a range of viruses that could be transmitted under the correct conditions. Diagnosis of infections under these circumstances can be difficult if the physician does not know of the existence of such pets.

KEY FACTS

- To establish infection in the host, pathogens must attach to, or pass across, body surfaces.
- Many pathogens have developed chemical or mechanical mechanisms to attach themselves to the surface of the respiratory, urogenital or alimentary tracts. In the skin, they generally depend upon entry via small wounds or arthropod bites.
- Pathogens must exit from the body after replication in order to be transmitted to fresh hosts. This also takes place across body surfaces.
- Efficient shedding of pathogens from the skin or respiratory, urogenital or alimentary tracts, and delivery into the blood or dermal tissues for uptake during arthropod feeding, are vital stages in their life cycles.
- Many human infections come from animals, either directly (zoonoses) or indirectly (via blood-sucking arthropods), and the incidence of these infections depends upon exposure to infected animals or arthropods.

15 Immune defences in action

Introduction

The immune system has a number of defence strategies at its disposal with which to attack and neutralize the threats from invading pathogens. As discussed in Chapters 10–12, these include both innate and adaptive cells and soluble cytokine mediators. Although they lack the dramatic specificity and memory of adaptive (i.e. T- and B cell-based) immune mechanisms, the innate defences are vital to survival – particularly in invertebrates, where they are the only defence against infection.

In addition to these non-specific mechanisms, the immune system enables the specific recognition of antigens by T and B cells as part of adaptive immunity. Broadly speaking, antibodies are particularly important in combating infection by extracellular microbes, particularly pyogenic bacteria, while T-cell immunity is required to control intracellular infections with bacteria, viruses, fungi or protozoa. Their value is illustrated by the generally disastrous results of defects in T and/or B cells, or their products, discussed in more detail in Chapter 31. This chapter gives examples of how these different types of immunity contribute to and collaborate in the body's defences against pathogens.

Antimicrobial peptides protect the skin against invading bacteria

A number of proteins that are expressed at epithelial surfaces, and by polymorphonuclear leukocytes (PMNs), can have a direct antibacterial effect. These include beta defensins, dermicidins and cathelicidins. Defensins form 30–50% of neutrophil granules, and disrupt the lipid membranes of bacteria. Dermicidin is made by sweat glands and secreted into sweat; it is active against *Escherichia coli*, *Staphylococcus aureus* and *Candida albicans*. Cathelicidin has potent anti-microbial effects against most Gram-positive and Gram-negative bacteria. The precursor cathelicidin protein is cleaved into two peptides, one of which, LL37, is not only toxic to microorganisms, but also binds lipopolysaccharide (LPS). Cathelicidin is active against methicillin-resistant *Staph. aureus*, showing it has a therapeutic potential. Mice whose PMNs and keratinocytes are unable to make cathelicidin become susceptible to infection with group A *Streptococcus*. Cathelicidin also plays a role in immunity to *Mycobacterium tuberculosis*, through its action on vitamin D.

Another interesting innate defence mechanism is the formation of neutrophil extracellular traps (NETS). NETS are formed from decondensed unwound DNA, together with neutrophil granule proteins and myeloperoxidase, and can bind both Gram-positive and Gram-negative bacteria, although they may not always be killed (Fig. 15.1). NETS can damage fungal hyphae that are too large to be phagocytosed; the key molecule here seems to be calprotectin, which is released from the NETS. Of course, the bacteria can fight back, in this case through secreting DNAases or by having capsules to prevent entrapment.

Lysozyme is one of the most abundant antimicrobial proteins in the lung. Genetically engineered transgenic mice with a lot more lysozyme activity than control mice in their bronchoalveolar lavage were much better at killing group B streptococci, and *Pseudomonas aeruginosa* (Fig. 15.2).

COMPLEMENT

The alternative pathway and lectin-binding pathways of complement activation are part of the early defence system

The basic biology of the complement system and its role in inducing the inflammatory response and promoting chemotaxis, phagocytosis and vascular permeability have been described in Chapter 10. Complement can also directly damage microorganisms as part of the early response to infection. Lack of the central complement component C3 leads to infection with a wide range of pyogenic bacteria. Patients deficient in later complement components C5, C6, C7, C8 or C9 are unable to eliminate *Neisseria* (gonococci and meningococci), with the increased risk of developing septicaemia or becoming a carrier. This suggests that these bacteria require the extracellular lytic pathway for elimination.

All three pathways can be activated by the innate system, but activation through the classical pathway is the only one for which antibodies improve the response. It should be recognized that the complement classical pathway activation is most efficiently activated by IgM.

ACUTE PHASE PROTEINS AND PATTERN RECOGNITION RECEPTORS

C-reactive protein is an antibacterial agent produced by liver cells in response to cytokines

Among the acute phase proteins produced during inflammatory reactions, C-reactive protein (CRP) is particularly interesting in being an antibacterial agent, although most of this activity has so far been shown against *Streptococcus pneumoniae*. CRP is a pentameric beta globulin, somewhat resembling a miniature

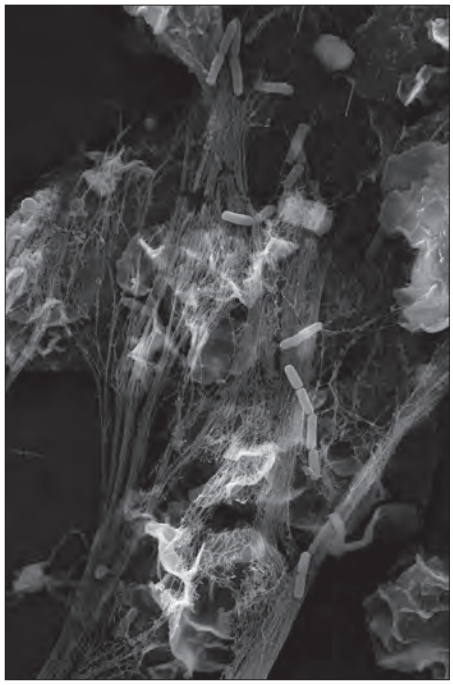


Figure 15.1 Neutrophil extracellular traps can trap bacteria. These chromatin-containing complexes can trap bacteria such as *Shigella* (illustrated). (Photograph courtesy of Dr Volker Brinkmann, Max Planck Institute for Infection Biology, Berlin.)

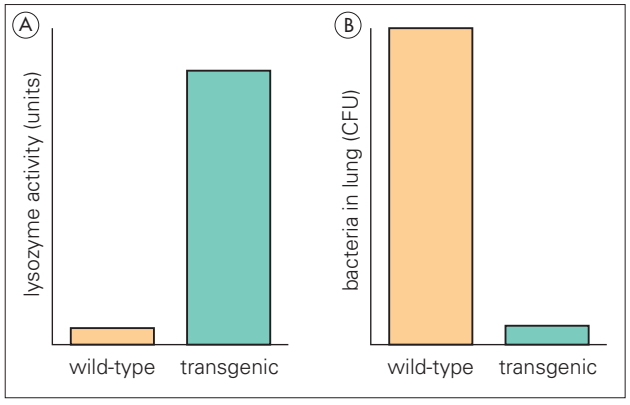


Figure 15.2 Transgenic mice making greater amounts of lysozyme are more resistant to infection with *Pseudomonas aeruginosa*. (A) The transgenic mice have 18-fold more lysozyme activity than the wild-type control mice. (B) The transgenic mice showed much greater killing of *P. aeruginosa* in the lungs following intratracheal infection than did the wild-type mice. (Redrawn with data from Akinbi, H.T. et al. [2000] Bacterial killing is enhanced by expression of lysozyme in the lungs of transgenic mice. *J Immunol* 165:5760–5766.)

version of IgM (molecular weight 130 000 compared with 900 000 for IgM). It reacts with phosphorylcholine in the wall of some streptococci and subsequently activates both complement and phagocytosis. CRP is produced by liver cells in response to cytokines, particularly interleukin 6 (IL-6, see Ch. 12), and levels can rise as much as 1000-fold in 24 h – a much more rapid response than that of antibody production. Therefore, CRP levels are often used to monitor inflammation, for example, in rheumatic diseases. The other acute phase proteins are also produced in increased amounts early in infection and have not just antimicrobial activity but can also act as opsonins or antiproteases, be involved in the fibrinolytic or anticoagulant pathways, or play an immunomodulatory role. For example, many of the complement components are acute phase proteins. Those with a role in protection against infection are also termed pattern recognition receptors, such as mannose-binding lectin. The important acute phase protein splA$_2$ (a member of the secretory phospholipase A$_2$ family) is important in protection against Gram-positive bacteria in serum. Some acute phase proteins such as lipopolysaccharide (LPS)-binding protein may reduce pathology by binding toxic bacterial products such as LPS.

Collectins and ficolins

Collectins are proteins that bind to carbohydrate molecules expressed on bacterial and viral surfaces. This results in cell recruitment, activation of the alternative complement cascade, and macrophage activation. Two collectins, the surfactant proteins A and D, are able to inhibit bacterial growth and opsonize bacteria directly, leading to phagocytosis and activation of complement. Surfactant protein A has been shown to play a role in the innate defence of the lung against infection with group B streptococci. Mice deficient in surfactant protein A were much more susceptible to infection, developing greater pulmonary infiltration and dissemination of bacteria to the spleen, compared with those able to produce the collectin. Polymorphisms in the surfactant A and D genes have also been linked to susceptibility to respiratory syncytial virus (RSV), as these surfactants act as opsonins for the virus.

Mannose-binding lectin (MBL) is another collectin found in serum. Binding of MBL to carbohydrates containing mannose on microorganisms leads to complement activation, through the mannan-binding lectin pathway. Bacteria opsonized by MBL bind to the C1q receptor on macrophages, leading to phagocytosis. Many individuals have low serum concentrations of MBL due to mutations in the *MBL* gene or its promoter. A recent study of children with malignancies showed that MBL deficiency increased the duration of infections. Lung surfactant proteins A and D, and MBL, bind to the surface spikes or S protein of the SARS virus (see Ch. 20), and so people with low MBL genotypes may be at increased risk of SARS infection.

Ficolins are plasma proteins with a similar structure to collectins, and bind *N*-acetyl glucosamine and lipotechoic acid from the cell walls of Gram-positive bacteria.

Macrophages can recognize bacteria as foreign using Toll-like receptors

Toll-like receptors (TLRs), on macrophages and other cells, bind conserved microbial molecules such as lipopolysaccharide (endotoxin), bacterial DNA, double-stranded RNA or bacterial flagellin pathogen-associated molecular patterns (PAMPs) (see Ch. 10) leading to the release of proinflammatory cytokines and increased expression of major histocompatibility complex (MHC) molecules and co-stimulatory molecules, thus enhancing antigen presentation and usually leading to the activation of T-helper-1 (Th1) cells. It was recently suggested that a number of rare single nucleotide polymorphisms within the *TLR4* gene (TLR4 binds endotoxin) were more common in people with meningococcal disease compared with controls.

Microbes in the cytosol of a cell can also be recognized as foreign, using another family of pattern recognition receptors called nucleotide-binding and oligomerization leucine-rich repeat receptors (NLRs). Some NLRs can sense bacterial or viral DNA, leading to activation of inflammasomes, which are complexes of proteins, and ultimately leading to the secretion of IL-1β and other proinflammatory cytokines. NLRs can also induce a process called autophagy, in which normal cytoplasmic contents are degraded after fusion with autolysosmes.

FEVER

A raised temperature almost invariably accompanies infection (see Ch. 30). In many cases, the cause can be traced to the release of cytokines such as IL-1 or IL-6, which play important roles in both immunity and pathology (see Ch. 10).

It is probably unwise to generalize about the benefit or otherwise of fever

Several microorganisms have been shown to be susceptible to high temperature. This was the basis for the 'fever therapy' of syphilis by deliberate infection with blood-stage malaria for which Julius Wagner-Jaurgg won the Nobel Prize for Medicine in 1927, and the malaria parasite itself may also be damaged by high temperatures, though it is obviously not totally eliminated. In general, however, one would predict that successful parasites would be adapted to survive episodes of fever; indeed the 'stress' or 'heat-shock' proteins produced by both mammalian and microbial cells in response to stress of many kinds, including heat, are thought to be part of their protective strategy. On the other hand, several host immune mechanisms might also be expected to be more active at slightly higher temperatures; examples are complement activation, membrane function, lymphocyte proliferation and the synthesis of proteins such as antibody and cytokines.

NATURAL KILLER CELLS

Natural killer cells are a rapid but non-specific means of controlling viral and other intracellular infections

Natural killer (NK) cells provide an early source of cytokines and chemokines during infection, until there is time for the activation and expansion of antigen-specific T cells. NK cells can provide an important source of interferon gamma (IFNγ) during the first few days of infection. NK cell cytokine production can be induced by cytokines such as IL-12 and IL-18, which are induced by macrophages in response to LPS or other microbial components. As well as IFNγ, NK cells can make TNFα and, under some conditions, the down-regulatory cytokine IL-10. Some tissues such as the gut need their own special populations of innate lymphoid cells (ILC3 cells), which make large amounts of the cytokine IL-22 to help defend the gut against certain intestinal pathogens.

NK cells can also act as cytotoxic effector cells, lysing host cells infected with viruses and some bacteria, as they make both cytotoxic granules and perforin. They recognize their targets by means of a series of activating and inhibitory receptors that are not antigen specific. The main NK-cell-activating receptors are called killer cell immunoglobulin (Ig)-like receptors (KIRs); others are carbohydrate-binding C-type lectins such as NKG2D, which bind the MHC-like MIC-A and MICB molecules that are expressed on virus-infected cells as well as tumour cells. The inhibitory receptors recognize the complex of MHC class I and self-peptide; if both this inhibitory receptor and another NK-cell-activating receptor are engaged, the NK cell will not be activated, and a healthy cell will not be killed. However, if there is insufficient MHC class I on the cell surface, the inhibitory receptor is not engaged and the NK cell is activated to kill the target cell. This is an effective strategy, as some viruses inhibit MHC class I expression on the cells they infect. NK cells are therefore a more rapid but less specific means of controlling viral and other intracellular infections. The importance of NK cells is highlighted by the ability of mice lacking both T and B cells (severe combined immunodeficiency, SCID) to control some virus infections, and humans with NK cell defects are also susceptible to certain viruses (Table 15.1). NK cells can also lyse red cells containing malaria parasites (Fig. 15.3).

NK cells and the other ILCs form a bridge between the innate and adaptive immune responses, and their function may be enhanced by components of adaptive immunity. Some recent work even suggests that some NK cells can show some immunological memory, so perhaps their full abilities are not yet appreciated!

NKT cells and γδT cells

Two further small populations of cells may play a role in infection by responding to non-protein antigens from pathogens. A small group of cells express both NK cell and T cell markers – and so are called NKT cells; they can also recognize lipid antigens presented by CD1 molecules, that are similar to MHC class I molecules but less polymorphic. The γδ T cells are classical T cells that express a T-cell receptor (TCR) with γ and δ chains rather than an αβ TCR; they respond to microbial lipids and small phosphorylated antigens also presented by MHC class I-like molecules that have limited polymorphism. γδ T cells are often found at epithelial surfaces and make up about 10% of the intraepithelial lymphocytes in the human gut.

Table 15.1 Natural killer cells play an important role in controlling infections

Infections where NK cells have been shown to help control infection	
Human	**Mouse**
Human cytomegalovirus (HCMV; human herpes virus 5)	Mouse cytomegalovirus (MCMV)
Vesicular stomatitis virus (VSV)	Herpes simplex virus
Herpes simplex virus (HSV)	Vaccinia virus
Human papilloma virus (HPV)	Influenza virus
Human immunodeficiency virus (HIV)	*Toxoplasma gondii*
Epstein–Barr virus (EBV)	EBV in human reconstituted mice
	Malaria
	Trypanosoma cruzi

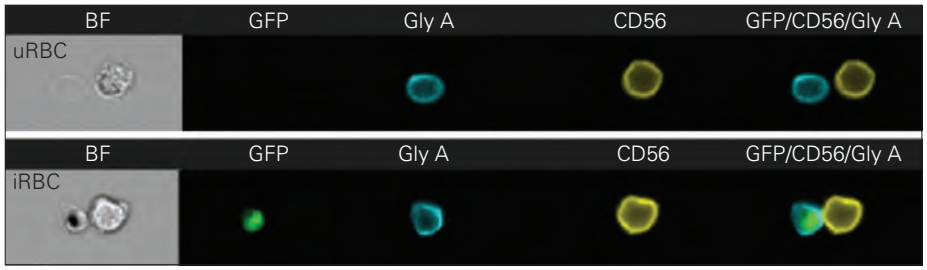


Figure 15.3 NK cells can bind to and kill malaria-infected erythrocytes. The upper panels show uninfected human red cells (uRBC) and the bottom ones show red cells infected with *Plasmodium falciparum* (iRBC). The transgenic malaria parasites are labelled with green fluorescent protein (GFP), the red cell membrane with phycoerythrin-labelled glycophorin A (Gly A), and the NK cell membrane with yellow phycoerythrin–cyanine 7 tandem protein. The NK cells expressing the NK cell marker CD56 bind only to the malaria-infected erythrocytes, as shown in the merged images. BF, bright field; CD56, cluster of differentiation 56. (Images courtesy of Samuel Sherratt, London School of Hygiene & Tropical Medicine.)

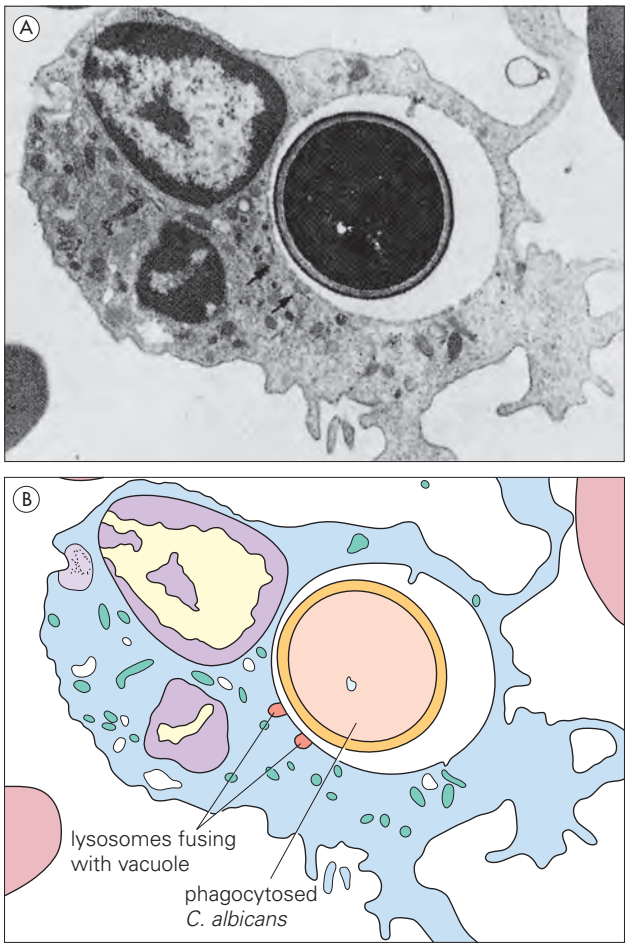


Figure 15.4 (A) Electron micrograph and (B) diagrammatic representation of neutrophil containing phagocytosed *Candida albicans* (×7000). (Courtesy of H. Valdimarsson.)

PHAGOCYTOSIS

Phagocytes engulf, kill and digest would-be parasites

Perhaps the greatest danger to the would-be parasite is to be recognized by a phagocytic cell, engulfed, killed and digested (Fig. 15.4). A description of the various stages of phagocytosis is given in Chapter 10. Phagocytes (principally macrophages) are normally found in the tissues where invading microorganisms are more likely to be encountered. In addition, phagocytes present in the blood (principally the PMNs) can be rapidly recruited into the tissues when and where required. Only about 1% of the normal adult bone marrow reserve of 3×10^{12} PMNs is present in the blood at any one time, representing a turnover of about 10^{11} PMNs / day. Most macrophages remain within the tissues, and well under 1% of our phagocytes are present in the blood as monocytes. PMNs are short lived, but macrophages can live for many years (see below).

Intracellular killing by phagocytes

Phagocytes kill organisms using either an oxidative or a non-oxidative mechanism

The mechanisms by which phagocytes kill the organisms they ingest are traditionally divided into oxidative and non-oxidative, depending upon whether the cell consumes oxygen in the process. Respiration in PMNs is non-mitochondrial and anaerobic, and the burst of oxygen consumption, the so-called 'respiratory burst' (Fig. 15.5) that accompanies phagocytosis represents the generation of microbicidal reactive oxygen intermediates (ROIs).

Oxidative killing

Oxidative killing involves the use of ROIs

The importance of ROIs in bacterial killing was revealed by the discovery that PMNs from patients with chronic granulomatous disease (CGD) did not consume oxygen after phagocytosing staphylococci. Patients with CGD have one of three kinds of genetic defect in a PMN membrane enzyme system involving nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, *PHOX* (see Ch. 31). The normal activity of this system is the progressive reduction of atmospheric oxygen to water with the production of ROIs such as the superoxide ion, hydrogen peroxide and free hydroxyl radicals, all of which can be extremely toxic to microorganisms (Table 15.2).

CGD patients are unable to kill staphylococci and certain other bacteria and fungi, which consequently cause deep chronic abscesses. They can, however, deal with catalase-negative bacteria such as pneumococci because these produce, and do not destroy, their own hydrogen peroxide in sufficient amounts to interact with the cell myeloperoxidase, producing the highly toxic hypochlorous acid. The defective PMNs from CGD patients can be readily identified in vitro by their failure to reduce the yellow dye nitroblue tetrazolium to a blue compound (the 'NBT test', see Ch. 32).

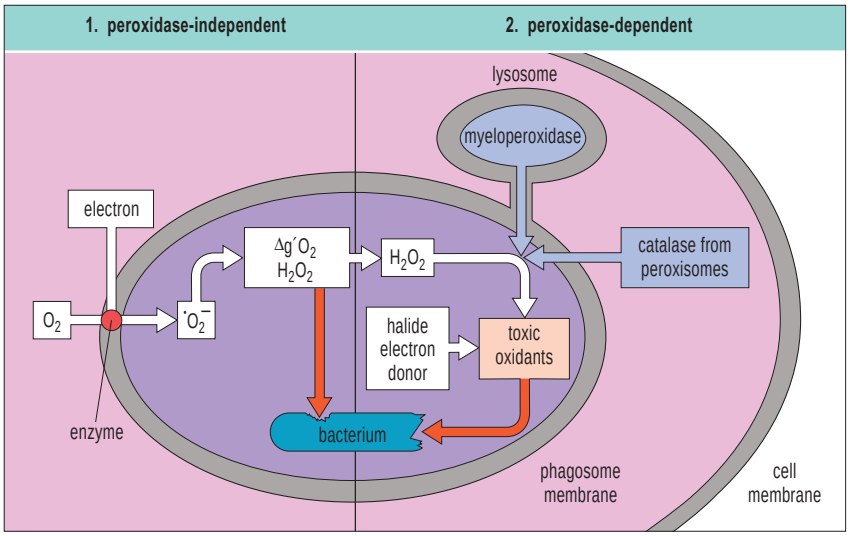


Figure 15.5 Oxygen-dependent microbicidal activity during the respiratory burst. The enzyme nicotinamide adenine dinucleotide phosphate (NADPH) oxidase in the phagosome membrane reduces oxygen by the addition of electrons to form superoxide anion ($^{\cdot}OH_2^-$). This can then give rise to hydroxyl radicals ($^{\cdot}OH$), singlet oxygen ($\Delta g'O_2$) and hydrogen peroxide (H_2O_2), all of which are potentially toxic. If lysosome fusion occurs, myeloperoxidase or in some cases, catalase from peroxisomes, acts on peroxides in the presence of halides to generate toxic oxidants such as hypohalite. (Reproduced from: Male, D., Brostoff, J., Roth, D.B., Roitt, I. [2006] *Immunology*, 7th edn. Mosby Elsevier, with permission.)

Table 15.2 Some organisms killed by reactive oxygen and nitrogen species

Bacteria	Fungi	Protozoa
Staph. aureus	*Candida albicans*	*Plasmodium*
E. coli	*Aspergillus*	*Leishmania* (nitric oxide)
Serratia marcescens		

Antimicrobial effects of ROIs

ROIs can damage cell membranes (lipid peroxidation), DNA and proteins (including vital enzymes), but in some cases it may be the altered pH that accompanies the generation of ROIs that does the damage. Killing of some bacteria and fungi (e.g. *E. coli*, *Candida*) occurs only at an acid pH, while killing of others (e.g. staphylococci) occurs at an alkaline pH. There may also be a need for protease activity (e.g. cathepsins, elastase), with enzyme solubilization occurring as a result of the influx of H^+ and K^+ into the phagocytic vesicle.

Cytotoxic lipids prolong the activity of ROIs

As already mentioned, one of the targets of the toxic ROIs is lipid in cell membranes. ROIs are normally extremely short lived (fractions of a second), but their toxicity can be greatly prolonged by interaction with serum lipoproteins to form lipid peroxides. Lipid peroxides are stable for hours and can pass on the oxidative damage to cell membranes, both of the parasite (e.g. malaria-infected red cell) and of the host (e.g. vascular endothelium). The cytotoxic activity of normal human serum to some blood trypanosomes has been traced to the high-density lipoproteins.

Non-oxidative killing

Non-oxidative killing involves the use of the phagocyte's cytotoxic granules

Oxygen is not always available for killing microorganisms; indeed, some bacteria grow best in anaerobic conditions (e.g. the *Clostridia* of gas gangrene), and oxygen would in any case be in short supply in a deep tissue abscess or a TB granuloma. Phagocytic cells therefore also contain other cytotoxic molecules. The best studied are the proteins in the various PMN granules (Table 15.3), which act on the contents of the phagosome as the granules fuse with it. Note that the transient fall in pH accompanying the respiratory burst enhances the activity of the cationic microbicidal proteins and defensins. Neutrophil serine proteinases have homology to the cytotoxic granzymes released by cytotoxic T cells.

Another phagocytic cell, the eosinophil, is particularly rich in cytotoxic granules (Table 15.3). The highly cationic (i.e. basic) contents of these granules give them their characteristic acidophilic staining pattern. Five distinct eosinophil cationic proteins are known and seem to be particularly toxic to parasitic worms, at least in vitro. Because of the enormous difference in size between parasitic worms and eosinophils, this type of damage is limited to the outer surfaces of the parasite. The eosinophilia typical of worm infections is presumably an attempt to cope with these large and almost indestructible parasites. Both the production and level of activity of eosinophils is regulated by T cells and macrophages and mediated by cytokines such as interleukin 5 (IL-5) and tumour necrosis factor alpha (TNFα).

Monocytes and macrophages also contain cytotoxic granules. Unlike PMNs (Table 15.4), macrophages contain little or no myeloperoxidase, but secrete large amounts of lysozyme. Lysozyme is an antibacterial molecule that attacks peptidoglycan in the cell wall of bacteria, which is particularly effective against Gram-positive bacteria where it has easier access to the peptidoglycan. Macrophages are extremely sensitive to activation by bacterial products (e.g. LPS) and T-cell cytokines (e.g. IFNγ). Activated macrophages have a greatly enhanced ability to kill both intracellular and extracellular targets.

Nitric oxide

A major secreted product of the activated macrophage is nitric oxide (NO), one of the reactive nitrogen intermediates (RNIs) generated during the conversion of arginine to citrulline by

Table 15.3 Contents of polymorphonuclear leukocyte (PMN) and eosinophil granules

PMN and eosinophil granule contents		
PMN		**Eosinophil**
Primary (azurophil)	***Specific (heterophil)***	***Cationic***
Myeloperoxidase	Lysozyme	Peroxidase
Acid hydrolases	Lactoferrin	Cationic proteins
Cathepsins G, B, D	Alkaline phosphatase	ECP
Defensins	NADPH oxidase	MBP
BPI	Collagenase	Neurotoxin
Cationic proteins	Histaminase	Lysophospholipase
Lysozyme		

BPI, bactericidal permeability increasing protein; ECP, eosinophil cationic protein; MBP, major basic protein; NADPH, nicotinamide adenine dinucleotide phosphate.

Table 15.4 The major phagocytic cells – PMNs and macrophages – differ in a number of important respects

Polymorphonuclear leukocytes and macrophages compared		
	PMN	**Macrophage**
Site of production	Bone marrow	Bone marrow or tissues
Duration in blood	7–10 h	20–40 h (monocyte)
Average life span	4 days	Months–years
Numbers in blood	$(2.5–7.5)\times10^9$/L	$(0.2–0.8)\times10^9$/L
Numbers in tissues	(Transient)	100×blood
Principal killing mechanisms	Oxidative, non-oxidative	Oxidative, nitric oxide, cytokines
Activated by	TNFα, IFNγ, GM-CSF, microbial products	TNFα, IFNγ, GM-CSF, microbial products (e.g. LPS)
Important deficiencies	CGD Myeloperoxidase Chemotactic Chediak–Higashi	Lipid storage diseases
Major secretory products	Lysozyme	Over 80, including: lysozyme, cytokines (TNFα, IL-1), complement factors

CGD, chronic granulomatous disease; GM-CSF, granulocyte-macrophage colony-stimulating factor; IFN, interferon; IL, interleukin; LPS, lipopolysaccharide; TNFα, tumour necrosis factor alpha.

arginase. NO is strongly cytotoxic to a variety of cell types, and RNIs are generated in large amounts during infections (e.g. leishmaniasis, malaria).

CYTOKINES

Cytokines contribute to both infection control and infection pathology

Early studies with supernatants from cultures of lymphocytes and macrophages revealed a family of non-antigen-specific molecules with diverse activities, which were involved in cell-to-cell communication. These are now collectively known as 'cytokines'. Cytokines play many crucial roles in protection against infectious diseases. The way in which these molecules acquired their sometimes rather misleading names, and the bewildering overlap of function between molecules of quite different structure, are described in detail in Chapter 12.

Cytokines are of importance in infectious disease for two contrasting reasons:

- They can contribute to the control of infection.
- They can contribute to the development of pathology.

The latter harmful aspect, of which TNFα in septic shock is a good example, is discussed in Chapter 18. The beneficial effects can be direct or more often indirect via the induction of some other antimicrobial process.

Interferons

The best-established antimicrobial cytokines are the interferons (IFNs) (Table 15.5). The name is derived from the demonstration in 1957 that virus-infected cells secreted a molecule that interfered with viral replication in bystander cells. IFNs of all three types (α, β and γ) interact with specific receptors on most cells, one for α and β and another for γ, following which they induce an antiviral state via the generation of at least two types of enzyme: a protein kinase and a 2′,5′-oligoadenylate synthetase. Both of these enzymes result in the inhibition of viral RNA translation and therefore of protein synthesis (Fig. 15.6).

Table 15.5 Human interferons (IFNs)

Human interferons			
	IFNα	**IFNβ**	**IFNγ**
Alternative name	'Leukocyte' IFN	'Fibroblast' IFN	'Immune' IFN
Principal source	All cells	All cells	T lymphocytes (NK cells)
Inducing agent	Viral infection (or dsRNA)	Viral infection (or dsRNA)	Antigen (or mitogen)
Number of species	22[a]	1	1
Chromosomal location of gene(s)	9	9	12
Antiviral activity	+++	+++	+
Immunoregulatory activity			
Macrophage action	–	–	++
MHC I up-regulation	+	+	+
MHC II up-regulation	-	-	+

Infα and IFNβ are also called Type I interferons and IFNγ called Type II interferon.
dsRNA, double-stranded ribonucleic acid; MHC, major histocompatibility complex.
[a]Each species coded by a different gene.

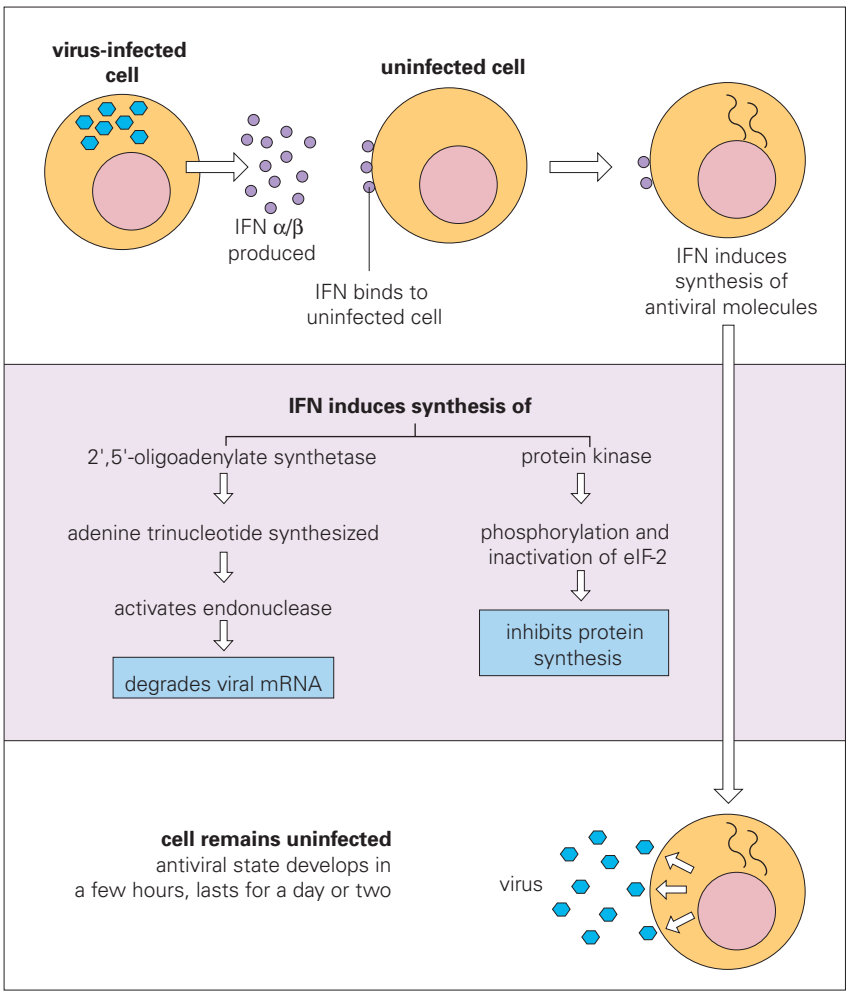


Figure 15.6 The molecular basis of type I interferon–α/β (IFN) action. eIF-2, eukaryotic initiation factor 2.

IFNα and IFNβ constitute a major part of the early response to viruses

IFNα and IFNβ (type I interferons) are produced rapidly within 24 h of infection, and constitute a major part of the early response to viruses.

Type I IFNs can also inhibit virus assembly at a later stage (e.g. retroviruses), while many of their other effects contribute to the antiviral state, for example, by the enhancement of cellular MHC expression and the activation of NK cells and macrophages (Fig. 15.7). Unlike cytotoxic T cells, type I IFN normally inhibits viruses without damaging the host cell. In animal experiments, treatment with antibodies to IFNα greatly increases susceptibility to viral infection; treatment with IFNα has proved useful for some human virus infections, notably chronic hepatitis B (see Ch. 23). Cleverly, it seems that once viral DNA has been sensed in the cytoplasm of the infected cell, by cyclic guanosine monophate–adenosine monophosphate synthase, the heterodinucleotide cyclic GMP–AMP (called cGAMP) can not only trigger a protein that stimulates interferon expression (STING), but also the cGAMP can be packaged into newly produced viruses – which means the virus itself carries stimulators of antiviral interferons into the next cell it infects!

Although best known for their antiviral activity, type I IFNs have recently been shown to be induced by, and active against, infections with a wide range of organisms, including rickettsia, mycobacteria and several protozoa. A study of gene expression in patients with tuberculosis identified that many genes induced by type I interferons as well as by type II IFNγ were activated.

IFNγ (type II, immune interferon) is mainly a T-cell product and is therefore produced later, although an early IFNγ response may be mounted by NK cells and type 1 ILCs. The role of IFNγ is discussed further under T cells, below. Some intracellular organisms (e.g. *Leishmania*) can counteract the effect of IFNγ on MHC expression, thereby facilitating their own survival.

Other cytokines

TNFα production can be good or bad

A striking example of a potentially useful role for TNFα in infection is illustrated by what happened when a humanized antibody against TNFα was used to treat patients with rheumatoid arthritis and Crohn's disease. A number of treated patients developed tuberculosis soon after starting therapy (Fig. 15.8); others developed *Listeria*, *Pneumocystis* or *Aspergillus* infections. Patients should now be tested for latent tuberculosis before starting treatment with a TNF-blocking antibody. However, TNF is also thought to contribute to the pathology of tuberculosis, as well as that of malaria (see Ch. 18). The requirement to have not too little and not too much of a mediator that would induce damaging pathology, but rather an amount that is just right, is sometimes referred to as the 'Goldilocks' principle. Paradoxically, TNF concentration is raised in HIV infection and has been found to enhance the replication of HIV in T cells – a 'positive feedback' with worrying potential. The role of T-cell-derived cytokines such as IFNγ in immunity to infection is discussed below.

ANTIBODY-MEDIATED IMMUNITY

The key property of the antibody molecule is to bind specifically to antigens on the foreign microbe. In many cases, this is followed by secondary binding to other cells or molecules of the immune system (e.g. phagocytes, complement). These are discussed below, but first some general features that

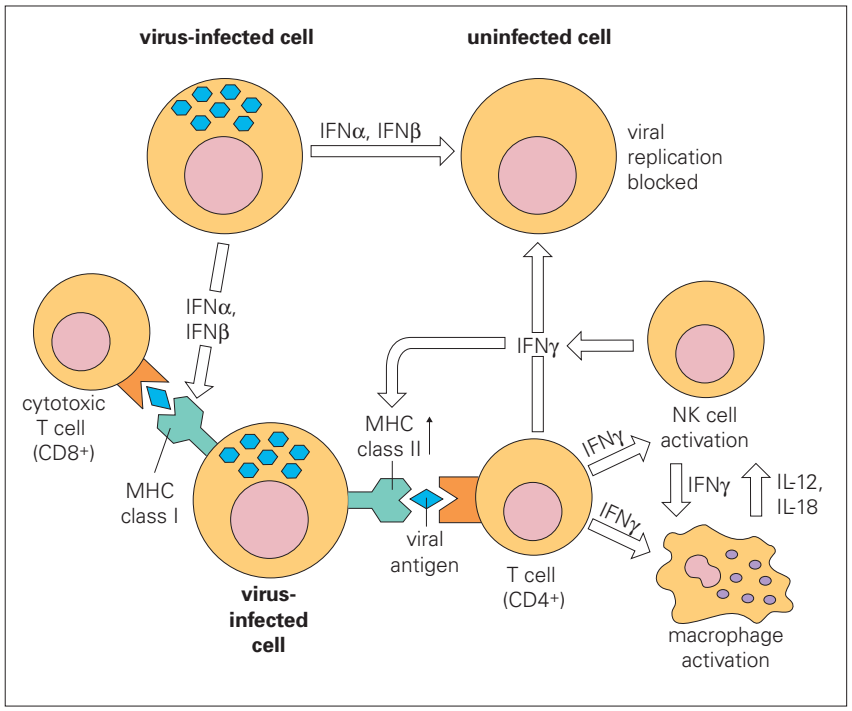


Figure 15.7 The multiple activities of interferons (IFNs) in viral immunity. CD, cluster of differentiation; IL, interleukin; MHC, major histocompatibility complex; NK, natural killer.

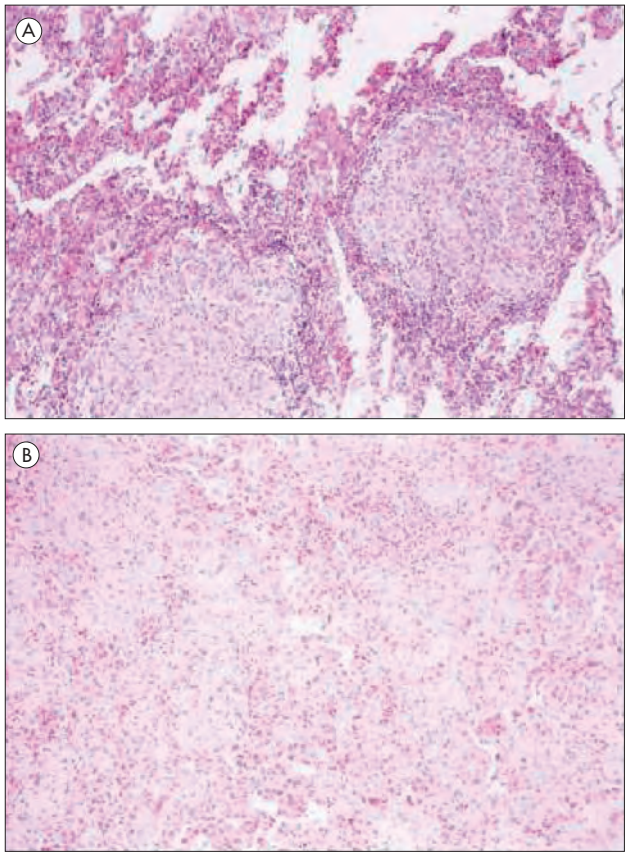


Figure 15.8 Photomicrographs of lung specimens from patients with tuberculosis (A) who did not (×100) or (B) who did (×100) receive infliximab, a humanized antibody to TNFα. In the patient without infliximab treatment, there are well-formed granulomas; in the patient with anti-TNF treatment, there is minimal granuloma formation but much fibrosis and inflammation. (Reproduced from Keane, J. et al. [2001] Tuberculosis associated with infliximab, a tumor necrosis factor alpha-neutralizing agent. *N Engl J Med* 345:1098–1104, with permission.)

influence the effectiveness of the antibody response should be mentioned.

Speed, amount and duration

Because of the cell interactions involved and the need for proliferation of a small number of specific precursor lymphocytes, a primary antibody response can be dangerously slow in reaching protective levels. The classic example, before penicillin, was lobar pneumonia, where the race between bacterial multiplication and antibody production was 'neck-and-neck' for about 1 week, at which point one side or the other dramatically won. Nowadays, of course, vaccines and antibiotics have intervened to improve the patient's chances. Experiments with specially bred lines of mice suggest that the speed and size of an antibody response is under the control of a large number of genes, and the same is undoubtedly true in humans. To help provide cover while specific antibodies are produced, there are some pre-existing natural antibodies that are usually low-affinity and cross-reactive IgM antibodies.

The rate of replication of the microorganism must also be considered. Replication rates, as indicated by doubling times (see Ch. 16) vary from <1 h (most viruses, many bacteria) to days or even weeks (mycobacteria, *T. pallidum*). Microorganisms tend to grow more slowly in vivo than in vitro, which shows that the host environment is generally hostile. When the incubation period is only a few days (e.g. rhinovirus, rotavirus, cholera) the antibody response is too slow to affect the initial outcome, and rapidly produced cytokines such as interferons are more important.

Usually the antibody response continues as long as antigen is present, although some down-regulation may occur in very prolonged responses, presumably in an effort to limit immunopathology (see Ch. 18). The lifelong immunity that follows many virus infections may often be due to regular boosting by viruses in the community, but sometimes (e.g. yellow fever) there is no obvious boost yet antibodies persist for decades. Such persistence of immunological memory may be due to the non-specific stimulation of memory B and T cells by cytokines during responses to other antigens, a process called bystander activation.

Affinity

It seems self-evident that a higher antigen-binding affinity would render antibody more useful, and passive protection experiments have confirmed this. Affinity is determined by both the germline antibody gene pool and somatic mutation in individual B lymphocytes, and appears to be under genetic control which is separate from that controlling the total amount of antibody made. A tendency towards a low antibody affinity to the tetanus toxoid vaccine has been found in some subjects, particularly those with predominantly IgG4 responses, and there is strong evidence from mouse experiments that failure to develop high-affinity antibody responses can predispose to immune complex disease.

Antibody classes and subclasses (isotypes)

The different Fc portions of the antibody molecule are responsible for most of the differences in antibody function (see Ch. 11). Switching from one to another while preserving the same Fab portion allows the immune system to 'try out' different effector mechanisms against the microbial invader. This flexibility is not total. For example, T-independent antigens such as some polysaccharides induce mainly IgM antibodies, T cells being required for the switch to IgE and helpful for IgG switching. IgG antibodies to polysaccharides tend to be mainly IgG2, whereas IgG antibodies to protein are mainly IgG1. The poor development of IgG2 in children below the age of about 2 years explains their lack of response to bacteria with polysaccharide capsules (e.g. *Strep. pneumoniae*, *Haemophilus influenzae*). Antibodies to viruses are predominantly IgG1 and IgG3, and those to helminths are IgG4 and IgE. Antigens encountered via the digestive tract induce mainly IgA, which is processed during its passage through epithelial cells to sIgA, the only type of antibody that can function in this protease-rich intestinal environment; the gut microbiota may induce T-independent IgA class switching through interactions with intestinal epithelial cells.

Blocking and neutralizing effects of antibody

Simple binding of antibody molecules to a microbial surface is often enough to protect the host. It may physically interfere with the receptor interaction necessary for microbial entry (e.g. of a virus into a cell) or with the binding of a toxin to its host

receptor. This is the basis of many life-saving vaccines against viruses or bacterial toxins. Such vaccines need to generate high-affinity antibodies, and T-cell help will be needed.

Blocking of attachment and entry can be effective against all organisms that use specific attachment sites, whether viral, bacterial or protozoal (see Ch. 16). An important exception is those organisms that parasitize the macrophage, such as the virus of dengue fever; here the presence of a low concentration of IgG antibody can actually enhance infection by promoting attachment to Fc receptors (see Ch. 18).

A more subtle blocking effect of antibody is interference with essential surface components of the parasite, particularly if these are enzymes or transport molecules. Needless to say, the successful pathogen takes steps to protect such components whenever possible, as described in Chapter 17.

Immobilization and agglutination

Immunoglobulin antibodies, particularly the large, pentameric IgM, are the same order of size as some of the smaller viruses, and larger than the thickness of a bacterial flagellum (Fig. 15.9), so the simple physical attachment of antibody can considerably restrict the activities of motile organisms. In addition, the multivalent design of the antibody molecules enables it to link together two or more organisms, as can readily be demonstrated in the bacterial agglutination tests (Fig. 15.10). The protective value of agglutination in vivo is hard to assess; once clumped, most organisms are probably rapidly phagocytosed, but clumps of still-motile trypanosomes can be seen in the blood of infected animals with enough serum antibodies. Agglutination reactions in vitro are very useful in diagnosis (see Ch. 32).

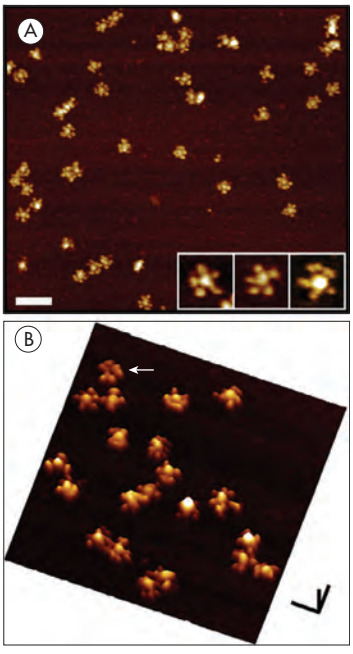


Figure 15.9 (A, B) The IgM molecule. The free form of IgM adopts a star-like configuration (arrow), as shown in the image obtained with low temperature atomic force microscopy. (Reproduced from Daniel M. Czajkowsky. The human IgM pentamer is a mushroom-shaped molecule with a flexural bias. 2009;106:14960-5 http://www.pnas.org/content/106/35/14960.)

Lysis

Lysis of bacteria in the presence of complement provides another convenient assay for the presence of antibody (IgG and IgA). However, lysis probably plays a major protective role in only a restricted range of infections, notably those caused by *Neisseria* and some viruses (see Ch. 18).

Opsonization

Whether by the direct binding of the immunoglobulin CH2 and CH3 regions to Fc receptors, or via the activation of complement to allow C3b to bind to its receptor, opsonization represents the most important overall function of the antibody molecule. Telling evidence for this is the general similarity in the effects on the patient of defects in antibody, complement (up to and including C3) and phagocytic cells (see Ch. 31). It is estimated that the rate of phagocytosis is enhanced by up to 1000-fold by antibody and complement acting together (Fig. 15.11). Lobar pneumonia due to *Strep. pneumoniae* again provides a good example: IgG antibody against the capsule allows neutrophils to phagocytose the organisms, converting overnight a lung virtually solid with fluid, fibrin and phagocytic cells into the normal breathing apparatus. Note

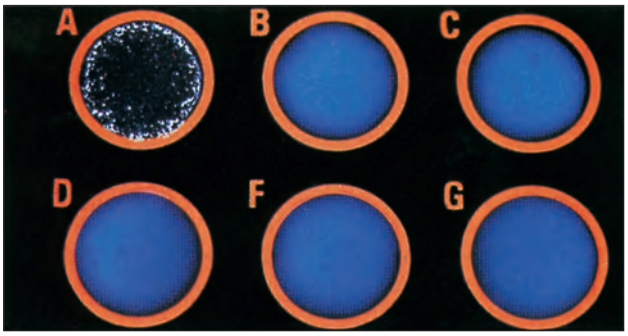


Figure 15.10 Bacterial agglutination. Well A shows agglutination of group A streptococci with latex particles coated with anti-group A antibodies. (Courtesy of D.K. Banerjee.)

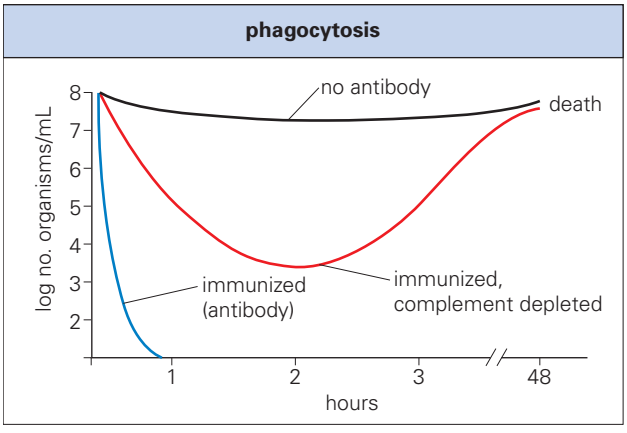


Figure 15.11 Opsonization enhances the clearance of bacteria. Antibody and complement together accelerate the clearance of pneumococci from the blood of mice (blue line); depletion of complement allows some opsonization if antibody is present but fails to control the infection (red line).

that the later complement components C5–9 are not required, so that deficiencies of these do not predispose to bacterial infection in general (see Ch. 31). Of course, the effectiveness of opsonization depends on the phagocytic cell being capable of finishing off the ingested organism. This is not the case, however, with organisms that inhibit or avoid the normal intracellular killing processes, of which mycobacteria are a typical example (see Ch. 17).

Antibody-dependent cellular cytotoxicity

In the case of larger organisms (worms being the most obvious example), phagocytosis is clearly not a possibility. However, several types of cell, having made contact with the parasite through antibody and Fc receptors in the same way as phagocytes do, can inflict damage extracellularly through antibody-dependent cellular cytotoxicity. These include most conventional phagocytes as well as eosinophils and platelets.

Indeed, the precise way in which antibody protects against infection is, in the majority of cases, still unknown. For example, the enormous production of IgA in the intestine, which may amount to half of all antibody produced in the body, suggests the vital importance of mucosal protection, and yet deficiency of IgA is relatively common and not particularly serious.

Table 15.6 gives some examples of common infections normally controlled by antibody. Once again, it must be emphasized that the presence of antibody by no means denotes a protective role. It may be directed against irrelevant or non-critical microbial antigens, or the infection may be of a type that is not primarily controlled by antibody, as with many intracellular infections (e.g. tuberculosis, typhoid, herpes virus). The best indication of the value of antibody comes from antibody-deficiency syndromes (Ch. 31).

CELL-MEDIATED IMMUNITY

T cells form the second main component of the adaptive immune response (see Chs. 11 and 12). Some act by producing cytokines that induce macrophage activation or help antibody production, others by their direct cytotoxic action on infected target cells. In both cases, the T cell needs to 'see' the combination of specific peptide and MHC molecule that is recognized by its T-cell receptor. Some examples of the importance of antibody and cell-mediated immunity in resistance to systemic infections are given in Table 15.6.

T-cell immunity correlates with control of bacterial growth in leprosy

In leprosy, there is a spectrum of disease, ranging from the paucibacillary tuberculoid form to the multibacillary lepromatous disease. *Mycobacterium-leprae*-specific T-cell immunity, as measured by lymphocyte proliferation, secretion of Th1 cytokines such as IFNγ, or delayed-type hypersensitivity skin testing, is found in patients with tuberculoid leprosy, but absent in patients with lepromatous leprosy (see Ch. 27). The value of T-cell stimulation leading to macrophage activation and bacterial killing is clearly illustrated by experiments in which lepromatous leprosy patients' skin lesions were injected with IFNγ. This resulted in an influx of T cells and macrophages into the skin lesions, and a reduction in the number of bacteria. Another good example of the protective role of IFNγ and Th1 immunity is seen in animal models of *Leishmania* infection: that is, some mouse strains such as C57BL / 6 are resistant to disease, controlling the infection and making a good Th1 cytokine response, whereas other susceptible strains such as BALB / c cannot control parasite growth and fail to make IFNγ (Table 15.7).

Further evidence for the protective effects of IFNγ

The protective effects of making IFNγ, which then binds to its specific receptor on macrophages and induces macrophage activation and the production of antimicrobial molecules, are illustrated very clearly by the consequences of a failure in IFNγ synthesis or of binding to its receptor. Mice in which the gene for IFNγ has been inactivated ('knocked-out') become

Table 15.6 Antibody and cell-mediated immunity (CMI) in resistance to systemic infections

Antibody and CMI in resistance to systemic infections		
Type of resistance	**Antibody**	**CMI**
Recovery from primary infection	Yellow fever, polioviruses, coxsackieviruses Streptococci, staphylococci *Neisseria meningitides* *Haemophilus influenza* *Candida* spp. *Giardia lamblia* Malaria[a]	Poxviruses: e.g. ectromelia (mice), vaccinia (humans) Herpes-type viruses: herpes simplex, varicella-zoster, cytomegalovirus LCM virus (mice) Tuberculosis Leprosy Systemic fungal infections Chronic mucocutaneous candidiasis[b]
Resistance to re-infection	Nearly all viruses including measles, most bacteria	Tuberculosis Leprosy
Resistance to reactivation of latent infection		Varicella-zoster, cytomegalovirus, herpes simplex, tuberculosis, *Pneumocystis jiroveci*[c]

Either antibody or CMI is known to be the major factor in these examples. But in many other infections there is no information, and sometimes both types of immunity are important. It is likely that CMI is also involved in resistance to activation of latent TB infection.
LCM, lymphocytic choriomeningitis.
[a]Protection is incomplete and short lived.
[b]Both Th1 and Th17 cells may be involved.
[c]Formerly *P. carinii*.

Table 15.7 Cytokine production in the spleens of mice infected with *Leishmania major*

Protective influence of IFNγ in *Leishmania* infection			
Mouse strain	**Phenotype**	**IFNγ**	**IL-4**
C57BL/6	Resistant	+	-
BALB/c	Susceptible	-	+

The resistant phenotype (C57BL/6 mice) was associated with the production of the Th1 cytokine interferon gamma (IFNγ), whereas the susceptible phenotype was associated with the production of the Th2 cytokine interleukin 4 (IL-4).
(From Heinzel, F.P. et al. [1989] Reciprocal expression of interferon gamma or interleukin 4 during the resolution or progression of murine leishmaniasis. *J Exp Med* 169:59, with permission.)

very susceptible to intracellular infections. Rare individuals with mutations in the genes for the IFNγ receptor have been identified. Such individuals are susceptible to infections with mycobacteria, or to disseminated infections following bacille Calmette-Guérin (BCG) vaccination (Fig. 15.12).

Some bacteria evade protective Th1 responses by inducing antigen-specific regulatory T cells that produce TGFβ or IL-10, that down-regulate IFNγ production.

Cytokine signatures

T cells can make a variety of cytokines but it may be most useful if particular combinations of cytokines are made by the same cell. For example, polyfunctional T cell that make IFNγ, TNFα and IL-2 make greater quantities of IFNγ than do those T cells that make only IFNγ, and are associated with control of the size of *Leishmania* lesions in the mouse.

During viral infections, the pattern or biosignature of cytokines produced by T cells may vary with clearance of infection, or antigen load during a chronic infection (Fig. 15.13). For example, primary infection with human immunodeficiency virus (HIV) or cytomegalovirus (CMV) induces mainly IFNγ-producing T cells; in influenza IL-2-producing cells predominate after viral clearance; chronic viral infection such as Epstein–Barr virus (EBV) or HIV in non-progressors seem to lead to a mixed IFNγ and IL-2 signature, but with progressive HIV infection and a higher antigen load this shifts to dominant IFNγ production. The balance between effector T cells and resting memory T cells will also change from acute to chronic disease with HIV. Healthy people have balanced populations of naive, effector and memory T cells in both the CD4 and CD8 compartments; in acute HIV, the effector CD8 T cells expand, but with chronic infection the naive and memory CD4 T cells are lost. The phenotype of the memory cells will also differ in the different types of infection (see Fig. 12.14).

Th17 T cells

The division of CD4 T cells into Th1 and Th2 T cells aided our understanding of immunity to many infections. However, another CD4 subset making IL-17, and so called Th17, and induced by the cytokine IL-23, also contributes to antimicrobial immunity. Th17 cells play a role in immunity against a number of bacterial infections including *Klebsiella pneumoniae*, *E. coli*, *Staph. aureus*, *Listeria monocytogenes* and *Candida albicans*. One way in which IL-17 works is by inducing neutrophil recruitment. Some patients with chronic mucocutaneous candidiasis have signalling defects leading to problems with production of Th17

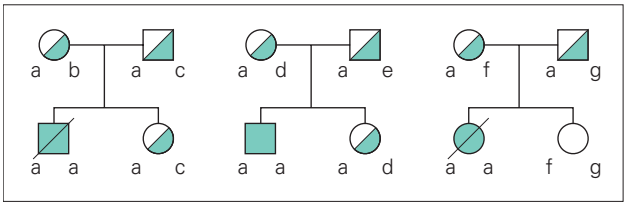


Figure 15.12 Genetic mutations in the IFNγ receptor cause susceptibility to mycobacterial infections. Three Maltese families had children who were susceptible to atypical mycobacterial infection (solid symbols), two of whom died (slashed symbols). Individuals with carrier status are shown with half-filled symbols. All of the affected children were homozygous for the disease locus on chromosome 6q22-q23, with a point mutation in the gene for the IFNγ receptor. This mutation introduces a stop codon resulting in a non-functional truncated protein. (From: Newport, M.J. et al. (1996) A mutation in the interferon-gamma-receptor gene and susceptibility to mycobacterial infection. *N Engl J Med* 335:1941–1949, with permission. ©Massachusetts Medical Society.)

cells and increased susceptibility to *Candida*. Another recently recognized role for Th17 cells is to secrete IL-26, a cytokine that forms pores in the membranes of Gram-negative bacteria leading to lysis of *E. coli, Pseudomonas aeruginosa* and *Klebsiella pneumoniae*. IL-17 along with IL-22 may also help restrict tissue damage during episodes of inflammation.

T-cell responses can be exploited in diagnostic tests for tuberculosis (TB)

There are two types of test that measure T-cell responses to *Mycobacterium tuberculosis*: the tuberculin skin test and the newer interferon gamma release assays (IGRA). The tuberculin skin test (Mantoux test) is a delayed-type hypersensitivity (DTH) skin test, in which induration induced by the intradermal injection of purified protein derivative from *M. tuberculosis* is measured 2–3 days later. However, the Mantoux skin test is positive in those with either latent infection or active TB disease. Worse, many of the antigens in the purified protein derivative preparation of *M. tuberculosis* used as the antigen in this test are cross-reactive with those in other mycobacteria, including BCG and non-tuberculous environmental mycobacteria. This means that BCG-vaccinated subjects and those not exposed to *M. tuberculosis* itself may have a positive Mantoux skin test, so a higher cut-off is used to exclude those with such cross-reactivity. Those with a large skin test response are at increased risk of developing tuberculosis, showing that strong T-cell responses can be induced during disease progression and that a strongly positive skin test can indicate infection rather than immunity. Skin tests can also be used to screen for T-cell anergy (e.g. by using candidin), as most individuals will have been exposed to *Candida*.

More specific interferon-gamma release assays that measure IFNγ release in response to peptides from antigens present in *M. tuberculosis* and not found in BCG or most environmental mycobacteria are now available. However, again these tests will be positive in those with latent tuberculosis or with active tuberculosis disease.

Cytotoxic T lymphocytes kill by inducing 'leaks' in the target cell

The well-known cytotoxic T lymphocyte (CTL) is unusual in that both antigen-specific recognition and killing of the

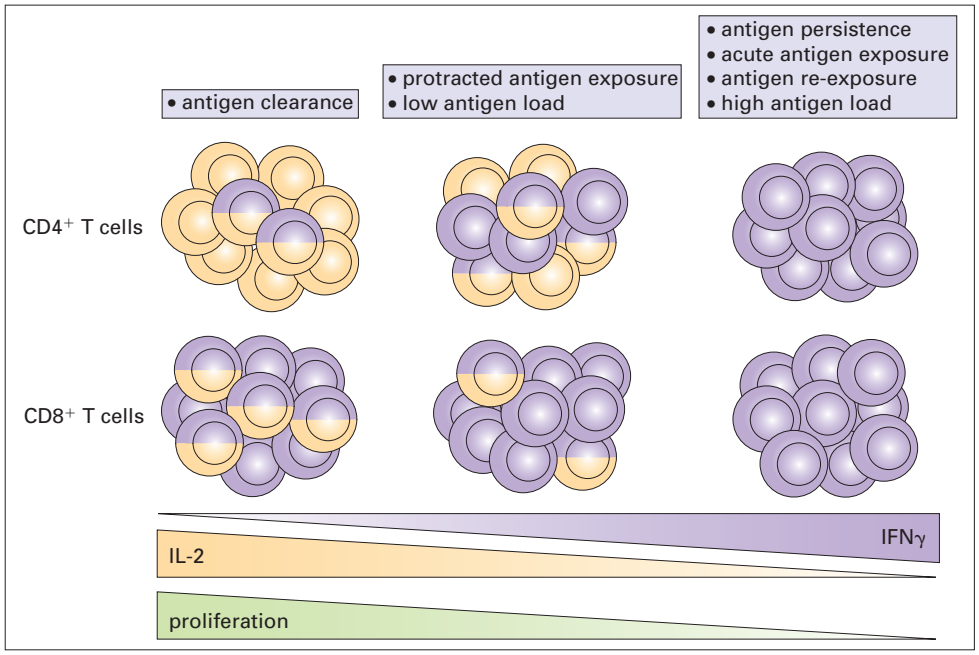


Figure 15.13 Cytokine signatures. The balance between virus-specific T cells secreting interferon gamma (IFNγ, purple cells) and T cells secreting interleukin 2 (IL-2, yellow cells) that proliferate better varies with the antigen load and the type of viral infection. Acute infections such influenza have a high antigen load; following clearance of virus the antigen load falls and IL-2-producing cells predominate particularly in the CD4 T cell population; in a chronic controlled infection such as Epstein-Barr virus (EBV), chronic cytomegalovirus (CMV) or HIV-1 in long-term non-progressors there is production of both IL-2 and IFNγ-secreting T cells as well as polyfunctional cells that make both cytokines; in chronic infection with high antigen load such as progressive HIV-1 infection, IFNγ-producing cells with a limited ability to proliferate predominate. CD, cluster of differentiation. (Redrawn from: Pantaleo, G., Harari, A. [2006] Functional signatures in antiviral T-cell immunity for monitoring virus-associated diseases. *Nat Rev Immunol* 6:417–423.)

target are carried out by the same cell. The recognition step, involving a peptide from an antigen that becomes associated with a class I MHC molecule, is discussed in Chapter 12, and displays the high degree of specificity characteristic of all adaptive responses. Antigenic stimulation is necessary to induce the formation of cytotoxic granules, which are not present in naïve CD8 T cells. The granule contents are delivered into the contact zone between the cytotoxic T cell and its target cell, and nearby cells are spared. A useful marker called CD107 of lysosomal-associated membrane protein-1 (LAMP-1) is left on the surface of the cytotoxic T cell, once a T cell has released its granules.

The killing mechanism, however, is relatively non-specific. It involves the induction of 'leaks' or pores in the target cell membrane by the insertion of perforin, a 66 kDa molecule that when polymerized is structurally and functionally similar to the terminal complement component C9 (80 kDa; Fig. 15.14). Granzymes are found as proenzymes in acidic granules where they bind to serglycin; once cleaved by cathepsin they enter the target cell and induce apoptosis. A recent study has shown that, once inside a target cell infected with bacteria or protozoa such as *Listeria* or *Toxoplasma*, granulysin, a third component of human lytic granules, enables granzymes to kill the bacteria and parasites in a process similar to apoptosis. Target cell death can also be caused by apoptosis, a 'suicide' programme built into all cells that is induced by Fas / FasL interactions, granzymes and TNFα. Leakage of cell contents may also contribute to cell death.

These mechanisms are thought to operate principally against virus-infected cells (such as EBV, hepatitis, HIV, influenza, CMV), but can also kill cells infected with other intracellular pathogens, including *Listeria* or *Toxoplasma*.

Most cytotoxic T cells are CD8 positive, recognizing MHC class- I-restricted peptide epitopes, but cytotoxicity can also be mediated by CD4 T cells, and by γδ T cells. It may seem unexpected that CD8 T cells are activated in some intracellular bacterial infections such as TB, where the mycobacteria should be within phagosomes, but microbial antigens or even the bacteria may escape into the cytoplasm of the host cell, allowing the antigens to be picked up and presented by MHC I molecules. CD8 activation may also result from a process called cross-priming, where bacterial antigens taken up by a dendritic cell are processed for MHC class I presentation as well as MHC class II presentation. In some cases, apoptotic blebs released by apoptotic, infected macrophages may be taken up by dendritic cells. Unfortunately the lysis of an infected target cell may not always kill the intracellular pathogen, but its release from its hideaway could lead to subsequent killing by a more highly activated macrophage (Fig. 15.15).

Another interesting recent finding is that not all CD8 T cells can act as effector cytotoxic T cells. More human CD8 T cells express the granzyme A than the preformed effector molecule perforin. In HIV infection, two-thirds of the CD8 T cells express granzymes but only one-third express perforin. This may explain why virus-infected cells escape killing by antigen-specific CD8 T cells in HIV infection.

The cytotoxic molecules used by cytotoxic T cells are shown in Table 15.8. Of course these cytotoxic cells are also important in dealing with tumour cells.

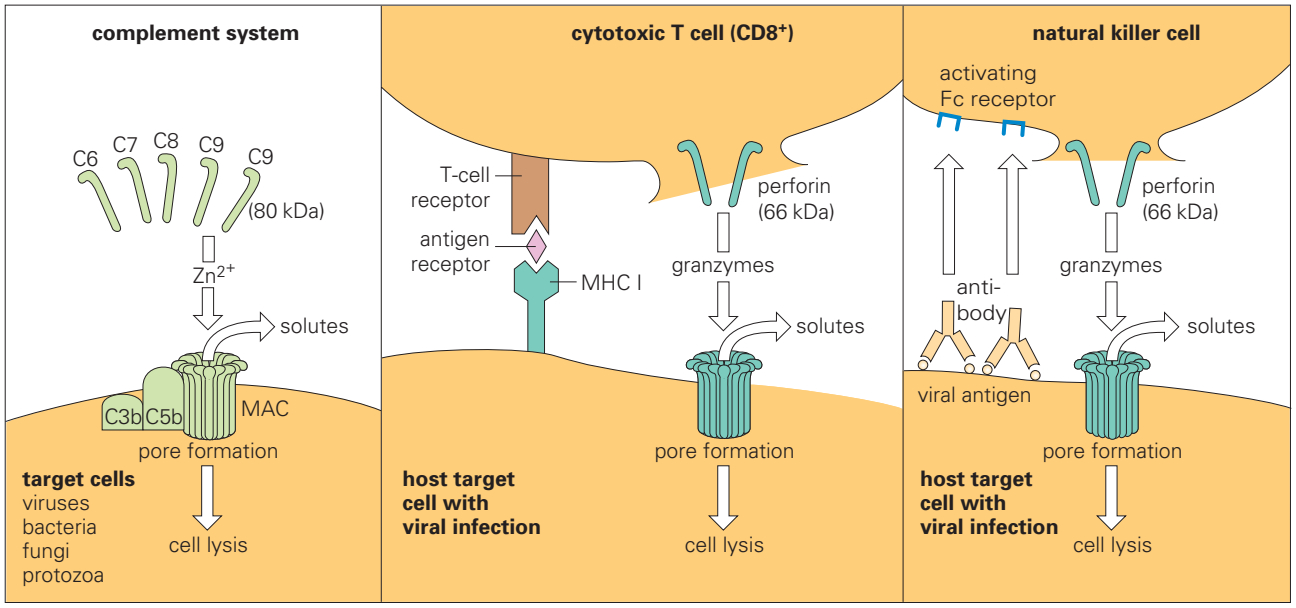


Figure 15.14 Comparison of the lytic mechanisms of cytotoxic cells and the complement system. The FcγRIII receptor (CD16) on the natural killer (NK) cell binds immunoglobulin IgG1 and IgG3 antibodies; other activating receptors are NKG2C and NKG2D. CD, cluster of differentiation; MAC, membrane attack complex; MHC, major histocompatibility complex; Zn^{2+}, zinc ion.

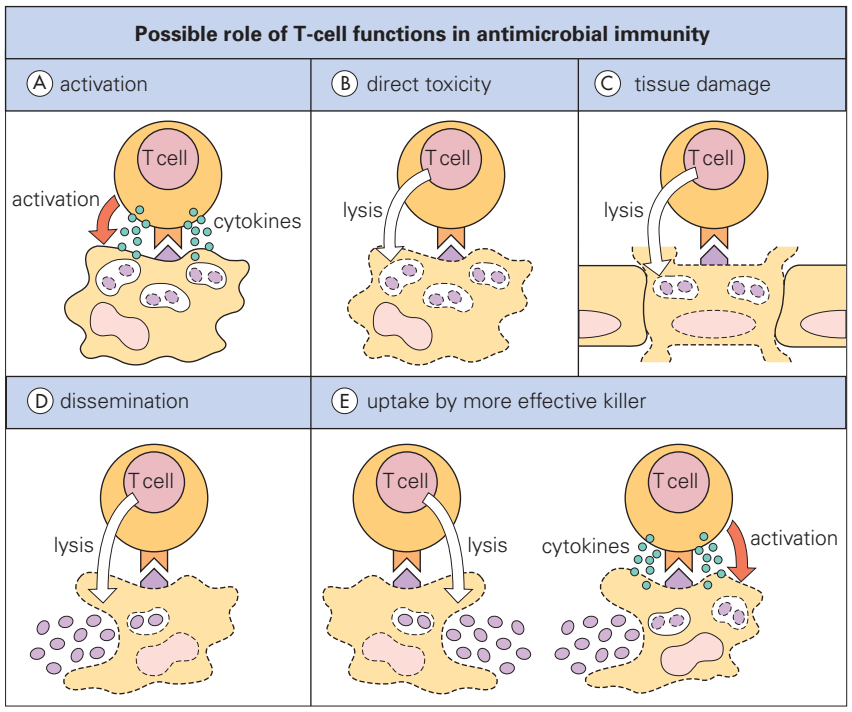


Figure 15.15 Possible roles for T cells in immunity to intracellular pathogens. (A) The T cell activates intracellular killing mechanisms by secretion of cytokines such as IFNγ (e.g. in a macrophage). (B) The T cell directly kills cell and parasite. (C) The T cell destroys vital tissue in the process of killing the parasite. (D) By lysing cells the T cell allows still-living parasites to disseminate. (E) Parasites released in this way may be phagocytosed by a host cell that is better at intracellular killing. (Redrawn from: Kaufmann, S.H. [1989] In vitro analysis of the cellular mechanisms involved in immunity to tuberculosis. *Rev Infect Dis* 11(Suppl 2):S448–S454.)

Table 15.8 Some important cytotoxic molecules in cytotoxic T-cell and NK-cell granules that operate against infectious organisms

Cytotoxic molecules	Properties	Effect
Perforin	Monomer; forms pore once polymerized	Pore allows entry of granzymes into cell
Granzymes*	Proenzymes cleaved by cathepsin	Induce apoptosis
Granulysin (human cells only)	Alters membrane permeability	Delivers granzymes to intracellular bacteria and protozoa

*Human granzyme B can also play a beneficial role in wound healing.

RECOVERY FROM INFECTION

The everyday concept of an infectious disease is one where the patient is ill for a period of days to months and then recovers. In many cases, they are subsequently immune to the disease. In such circumstances, one can be fairly certain that adaptive (lymphocyte-based) mechanisms have been at work, since: (1) the existence of disease symptoms implies that natural defence mechanisms, which act rapidly, did not succeed in eliminating the parasite; (2) a period of days or weeks is typical of the time that adaptive immune mechanisms take to reach maximal levels; and (3) subsequent immunity is a sign of the immunological memory exclusive to T and B cells, which possess the ability to specifically recognize antigens, to proliferate into clones, and to survive as memory cells. Thus, the older individuals are, the better they are adapted to the environment, until old age begins to weaken the immune system itself.

In the early stages of an infection, however, adaptive immunity can need some assistance. Since the lymphocytes are programmed to recognize the shapes of antigenic epitopes, they cannot distinguish virulent from harmless parasites, and must rely on recognizing 'danger' signals – nor can they 'know' which type of immune response will be most effective. Often, one mechanism is responsible for recovery and another for resistance to re-infection (e.g. cytotoxic cells and interferon in recovery from measles, antibody in prevention of a second attack). In many infections, there is still controversy as to which of the numerous responses that can be detected are useful, harmful or neutral. The reason for an individual's failure to recover from, or to suffer from, an infection can also be hard to pinpoint. If the infection is one from which most people recover (e.g. measles), or from which they do not suffer at all (e.g. *Pneumocystis*), an immunodeficiency should be considered (see Ch. 31). Infections that are rapidly fatal in normal individuals (e.g. Lassa fever) are frequently those to which the human immune system has not been exposed, as they are normally maintained in animals and only accidentally infect humans (see Zoonoses, Ch. 29). But if the infection normally runs a prolonged course without either being eliminated or killing the host, the parasite can be considered to be successful, and this success will be due to one or more survival strategies. These are the subject of Chapter 17.

Nutrition may have more subtle effects on immunity to infection

Even if an immunodeficiency state is not present, other factors may affect how a person copes with an infection. For example, during starvation or malnutrition, concentrations of the hormone leptin (which is produced by adipocytes and among other functions induces PMN activation) fall. Mice fasted for 2 days had higher numbers of *Strep. pneumoniae* in their lungs than did normally fed animals, but if the fasted animals were given leptin the number of PMNs in the lungs increased and the bacterial counts fell (Fig. 15.16). Leptin-deficient mice are highly susceptible to bacterial infections such as *Klebsiella* and *Listeria*. However, being obese is not good either – it is worth noting that obese people seem to be more susceptible to many more types of infections than those of a normal weight.

Other factors that affect how well the immune system works include stress, the microbiome, exercise, seasonality and genetics.

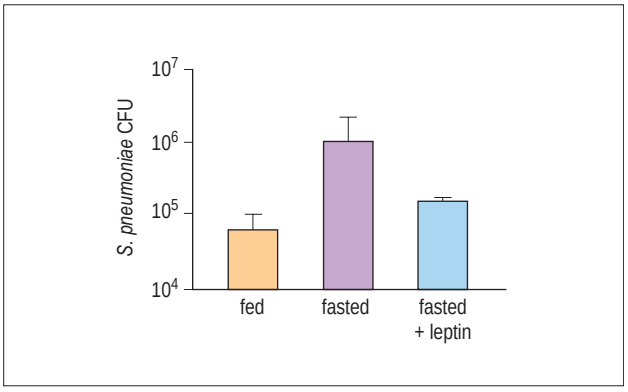


Figure 15.16 Leptin can restore host defence against *Strep. pneumoniae* in fasted mice. Colony-forming units (CFU) of bacteria in the lung were measured after normal feeding (orange column), in animals fasted for 48 h (purple column), or fasted but given leptin (blue column), 24 h after infection with *Strep. pneumoniae*. (Redrawn from: Mancuso, P. et al. [2006] Leptin corrects host defense defects after acute starvation in murine pneumococcal pneumonia. *Am J Respir Crit Care Med* 173:212–218.)

KEY FACTS

- Protection against infectious organisms that penetrate the outer barriers of the skin and mucous membranes is mediated by a variety of early defence mechanisms, which constitute innate immunity.
- These early defence mechanisms occur more rapidly but are less specific than the adaptive mechanisms based on antigen-specific lymphocyte (T- and B-cell) responses.
- Important early defence mechanisms include the acute phase response, the complement system, IFNs, phagocytic cells, NK cells and other innate lymphoid cells. Together, these act as a first line of defence during the initial hours or days of infection.
- Adaptive immunity, mediated by antibody and T cells, is responsible for recovery from infection in many cases, although these mechanisms take days to weeks to reach peak efficiency.
- Sometimes, as in the common viral infections, cell-mediated immunity is responsible for recovery from infection, and antibody for the maintenance of immunity.
- Failure to recover from infection may be due to some deficiency of host immunity or to successful evasion strategies used by the microorganism.

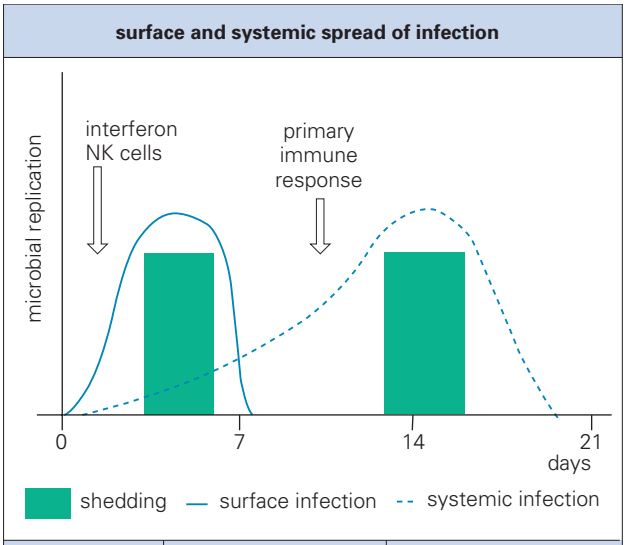
16 Spread and replication

Introduction

An infection may be a surface infection or a systemic infection

Many successful microorganisms multiply in epithelial cells at the site of entry on the body surface, but fail to spread to deeper structures or through the body. Local spread takes place readily on a fluid-covered mucosal surface, often aided by ciliary action, and large-scale movements of fluid spread the infection to more distant areas on the surface. This is obvious in the gastrointestinal tract. In the upper respiratory tract, high 'winds' (coughing, sneezing) can splatter infectious agents onto new areas of mucosa, or into the openings of sinuses or the middle ear, while the gentler downward trickle of mucus during sleep may seed an infectious agent into the lower respiratory tract. As a result, large areas of the body surface can be involved within a few days, with shedding to the exterior. There is not enough time for a primary immune response to be generated, and therefore non-adaptive responses – interferon, natural killer cells – are more important in controlling the infection. These surface infections therefore show a 'hit-and-run' pattern.

In contrast, other microorganisms spread systemically through the body via lymph or blood. They often undergo a complex or stepwise invasion of various tissues before reaching the final site of replication and shedding to the exterior (e.g. measles, typhoid). Surface and systemic infections and their consequences are compared in Fig. 16.1.



	surface	systemic
examples	common cold gonorrhoea bacillary dysentery	typhoid measles
incubation period	<1 week	>1 week
recovery mechanism	non-adaptive* (IFN, NK)	adaptive (immune response)

*if there is pre-existing immunity (memory), a secondary immune response comes into operation within 1–2 days

Figure 16.1 Surface and systemic infections. IFN, interferon; NK, natural killer.

FEATURES OF SURFACE AND SYSTEMIC INFECTIONS

A variety of factors determine whether an infection is a surface or a systemic infection

What prevents surface infections from spreading more deeply? Why do the pathogens that cause systemic infections leave the relatively safe haven of the body surface to spread through the body, where they will bear the full onslaught of host defences? These are important questions. For instance, what are the factors that persuade meningococci residing harmlessly on the nasal mucosa to invade deeper tissues, reach the blood and meninges, and cause meningitis? The answer is not known.

Temperature is one factor that can restrict pathogens to body surfaces. Rhinovirus infections, for instance, are restricted to the upper respiratory tract because they are temperature sensitive, replicating efficiently at 33°C, but not at the temperatures encountered in the lower respiratory tract (37°C). *Mycobacterium leprae* is also temperature sensitive, which accounts for its replication being more or less limited to nasal mucosa, skin and superficial nerves.

The site of budding is a factor that can restrict viruses to body surfaces. Influenza and parainfluenza viruses invade surface epithelial cells of the lung, but are liberated by budding from the free (external) surface of the epithelial cell, not from the basal layer from where they could spread to deeper tissues (Fig. 16.2).

Many microorganisms are obliged to spread systemically because they fail to spread and multiply at the site of initial infection, the body surface. In the case of measles or typhoid, there is, for unknown reasons, next to no replication at the site of initial respiratory or intestinal infection. Only after spreading through the body systemically are large numbers of microorganisms delivered back to the same surfaces, where they multiply and are shed to the exterior. Other microorganisms need to spread systemically because they have committed themselves to infection by one route, while major replication and shedding occurs at a different site. The pathogen must reach the replication site, and there is then no need for extensive replication at the site of initial infection. For instance, mumps and hepatitis A viruses infect via the respiratory and alimentary routes, respectively, but must spread through the body to invade and multiply in salivary glands (mumps) and liver (hepatitis A).

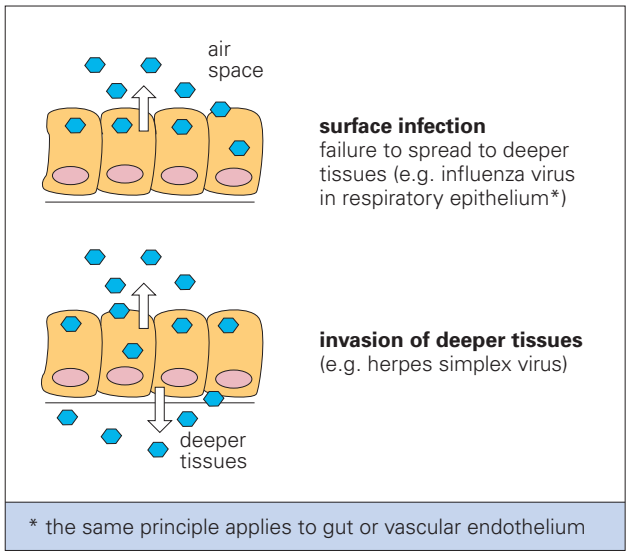


Figure 16.2 Topography of virus release from epithelial surfaces can determine the pattern of infection.

In systemic infections, there is a stepwise invasion of different tissues of the body

This stepwise invasion is demonstrated by measles (Fig. 16.3) and typhoid (Fig. 16.4) infections. Although the final sites of

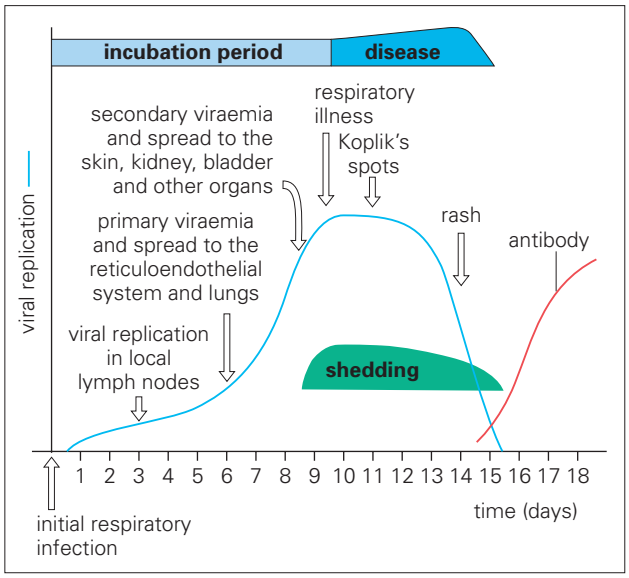
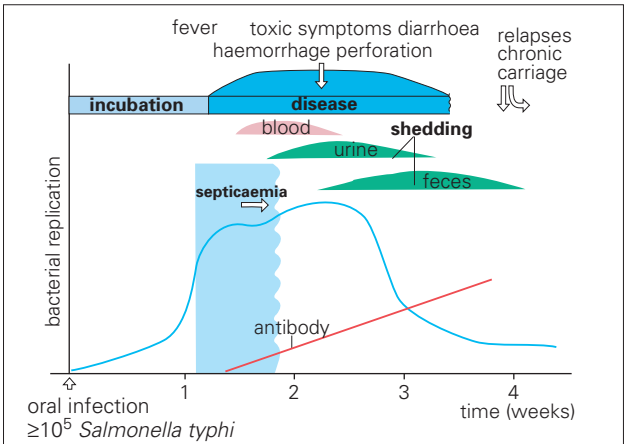


Figure 16.3 The pathogenesis of measles. Virus invades body surfaces from the blood, traversing blood vessels to reach surface epithelium first in the respiratory tract where there are only 1–2 layers of epithelial cells and then in mucosae (Koplik's spots) and finally in the skin (rash).



week	invasion of:
1	small intestine, lymphatics, spread to blood (bacteraemia)
2	reticuloendothelial system (liver, spleen and bone marrow macrophages)
3	kidney, gall bladder, gut (Peyer's patches)

Figure 16.4 The pathogenesis of typhoid fever.

Table 16.1 Replication rates of different microorganisms

Microorganisms	Situation	Mean doubling time
Most viruses	In cell[a]	<1 h
Many bacteria, e.g. *Escherichia coli*, staphylococci	In vitro	20–30 min
Salmonella typhimurium	In vitro In vivo	30 min 5–12 h
Mycobacterium tuberculosis	In vitro In vivo	24 h Many days
Mycobacterium leprae[b]	In vivo	2 weeks
Treponema pallidum[b]	In vivo	30 h
Plasmodium falciparum	In vitro/in vivo (erythrocyte or hepatic cell)	8 h

[a]But some viruses show greatly delayed replication or delayed spread from cell to cell.
[b]But cannot be cultivated in vitro.

multiplication may be essential for pathogen shedding and transmission (e.g. measles), they are sometimes completely unnecessary from this point of view (e.g. meningococcal meningitis, paralytic poliomyelitis). These pathogens are not shed to the exterior after multiplying in the meninges or spinal cord.

For the pathogen, systemic spread is fraught with obstacles and a major encounter with immune and other defences is inevitable. Microorganisms have therefore been forced to develop strategies for bypassing or countering these defences (see Ch. 17).

Rapid replication is essential for surface infections

The rate of replication of the infecting microorganism is of central importance, and doubling times vary from 20 min to several days (Table 16.1). Hit-and-run (surface) infections need to replicate rapidly, whereas a microorganism that divides every few days (e.g. *Mycobacterium tuberculosis*) is likely to cause a slowly evolving disease with a long incubation period. Microorganisms nearly always multiply faster in vitro than they do in the intact host, as might be expected if host defences are performing a useful function. In the host, microorganisms are phagocytosed and killed and the supply of nutrients may be limited. The net increase in numbers is slower than in laboratory cultures where pathogens are not only free from attack by host defences, but also every effort has been made to supply them with optimal nutrients, susceptible cells, and so on.

MECHANISMS OF SPREAD THROUGH THE BODY

Spread to lymph and blood

Invading pathogens encounter a variety of defences on entering the body

After traversing the epithelium and its basement membrane at the body surface, invading pathogens face the following defences:

- *Tissue fluids* containing antimicrobial substances (antibody, complement).
- *Local macrophages (histiocytes).* Subcutaneous and submucosal macrophages are a threat to microbial survival.
- *The physical barrier of local tissue structure.* Local tissues consist of various cells in a hydrated gel matrix; although viruses can spread by stepwise invasion of cells, invasion is more difficult for bacteria, and those that spread effectively sometimes possess special spreading factors (e.g. streptococcal hyaluronidase).
- *The lymphatic system.* The rich network of the lymphatic system soon conveys microorganisms to the battery of phagocytic and immunological defences awaiting them in the local lymph node (Fig. 16.5). Macrophages, strategically placed in the marginal and other lymph sinuses, constitute an efficient filtering system for lymph.

The infection may be halted at any stage, but by multiplying locally or in lymph nodes and by evading phagocytosis the microorganism can ultimately reach the bloodstream. Therefore, a minor injury to the skin, followed by a red streak (inflamed lymphatic) and a tender, swollen local lymph node are classic signs of streptococcal invasion. Most bacteria cause a great deal of inflammation when they invade in this way. In the early stages, lymph flow increases, but eventually, if there is enough inflammation and tissue damage in the node itself, the flow of the lymph may cease. In contrast, viruses and other intracellular microorganisms often invade lymph and blood silently and asymptomatically during the incubation period; this is facilitated when they infect monocytes or lymphocytes without initially damaging them.

Spread from blood

The fate of microorganisms in the blood depends upon whether they are free or associated with circulating cells

Viruses or small numbers of bacteria can enter the blood without causing a general body disturbance. For instance, transient bacteraemia are fairly common in normal individuals (e.g. they may occur after defecation or brushing teeth), but the bacteria are usually filtered out and destroyed in macrophages lining the liver and spleen sinusoids. Under certain circumstances, the same bacteria have a chance to localize in less well-defended sites, such as congenitally abnormal heart valves in the case of viridans streptococci causing infective endocarditis, or in the ends of growing bones in the case of *Staphylococcus aureus* osteomyelitis.

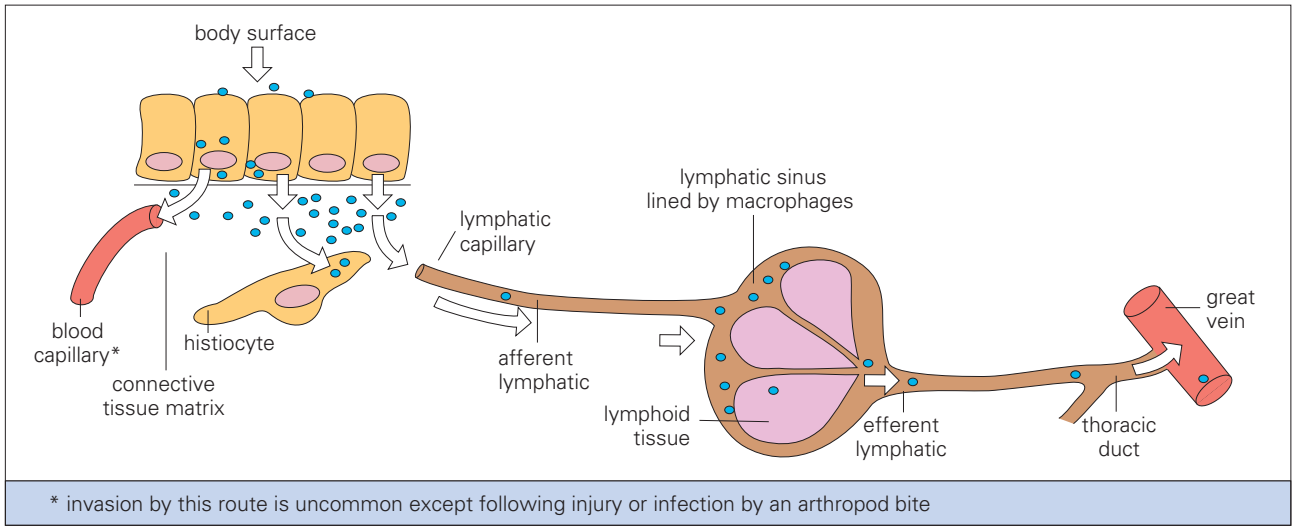


Figure 16.5 Microbial invasion and spread to lymph and blood. Pathogens (or other particles) beneath surface epithelium readily enter local lymphatics.

If microorganisms are free in the blood, they are exposed to body defences such as antibodies and phagocytes. However, if they are associated with circulating cells, these cells can protect them from host defences and carry them around the body. For example, many viruses, such as Epstein–Barr virus (EBV) and rubella, and intracellular bacteria (*Listeria*, *Brucella*), are present in lymphocytes or monocytes and, if not damaged or destroyed, these 'carrying cells' protect and transport them. Malaria infects erythrocytes.

On entering the blood, microorganisms are exposed to macrophages of the reticuloendothelial system. Here, in the sinusoids, where blood flows slowly, they are often phagocytosed and destroyed. But certain microorganisms survive and multiply in these cells (*Salmonella typhi*, *Leishmania donovani*, yellow fever virus). The microorganism may then:

- spread to adjacent hepatic cells in the liver (hepatitis viruses), or splenic lymphoid tissues (measles virus)
- re-invade the blood (*S. typhi*, hepatitis viruses).

Each circulating microorganism invades characteristic target organs and tissues

If uptake by reticuloendothelial macrophages is not complete within a short time, or if large numbers of microorganisms are present in the blood, there is an opportunity for localization elsewhere in the vascular system. Why each circulating microorganism invades characteristic target organs and tissues (Table 16.2) is not completely understood, but may be due to:

- specific receptors for the microorganism, leading to localization on the vascular endothelium of certain target organs
- subsequent colonization and replication
- accumulation of circulating pathogens in sites where there is local inflammation, because of the slower flow and sticky endothelium in inflamed vessels.

After localization and organ invasion, the replicating pathogen is shed from the body if the organ has a surface with access to the outside world. It may also be shed back into the bloodstream, either directly or via the lymphatic system.

Spread via nerves

Certain viruses spread via peripheral nerves from peripheral parts of the body to the central nervous system and vice versa

Tetanus toxin reaches the central nervous system (CNS) by this route. Rabies, herpes simplex virus (HSV) and varicella-zoster virus (VZV) travel in axons and although the rate is slow, being accounted for by axonal flow (up to 10 mm / h), this movement is important in the pathogenesis of these infections. Rabies not only reaches the CNS largely by peripheral nerves, but also takes the same route from the CNS when it invades the salivary glands. Few, if any, host defences are in a position to control this type of viral spread once nerves are invaded. Routes of invasion of the CNS are illustrated in Fig. 16.6.

An uncommon route of spread to the CNS is via olfactory nerves with axons terminating on olfactory mucosa. For instance, certain free-living amoebae (e.g. *Naegleria* spp.) found in sludge at the bottom of freshwater pools may take this route and cause meningoencephalitis in swimmers. Viruses and bacteria in the nasopharynx (e.g. meningococci, poliovirus) generally spread to the CNS via the blood.

Spread via cerebrospinal fluid

Once microorganisms have crossed the blood–cerebrospinal barrier, they spread rapidly in the cerebrospinal fluid spaces

Such microorganisms can then invade neural tissues (e.g. echoviruses, mumps virus) as well as multiply locally (*Neisseria meningitidis*, *Haemophilus influenzae*, *Streptococcus pneumoniae*) and possibly infect ependymal and meningeal cells.

Spread via other routes

Rapid spread from one visceral organ to another can take place via the pleural or peritoneal cavity

Both the pleural and peritoneal cavities are lined by macrophages, as if in expectation of such invasion, and the peritoneal cavity contains an antimicrobial armoury, consisting of the omentum (the 'abdominal policeman'), and many

Table 16.2 Circulating microorganisms that invade organs via small blood vessels

Pathogen	Disease	Principal organs invaded[a]
Viruses		
Hepatitis B virus	Hepatitis B	Liver
Rubella virus	Congenital rubella	Placenta (fetus)
Varicella-zoster virus	Chickenpox	Skin, respiratory tract
Polio virus	Poliomyelitis	Brain, spinal cord
Mumps virus	Mumps	Parotid, testes, ovaries, CNS, pancreas
Zika virus	Microcephaly	Brain
Bacteria		
Rickettsia rickettsii	Rocky Mountain spotted fever	Skin
Treponema pallidum	Secondary syphilis	Skin, mucosae
Neisseria meningitidis	Meningitis	Meninges
Protozoa		
Trypanosoma cruzi	Chagas disease	Heart, skeletal muscle
Plasmodium spp.	Malaria	Liver
Helminths		
Schistosoma spp. (larvae)	Schistosomiasis	Veins of bladder, bowel
Ascaris lumbricoides (larvae)	Ascariasis	Lung
Ancylostoma duodenale (larvae)	Hookworm	Lung

[a]In liver, sinusoids; elsewhere, capillaries, venules.

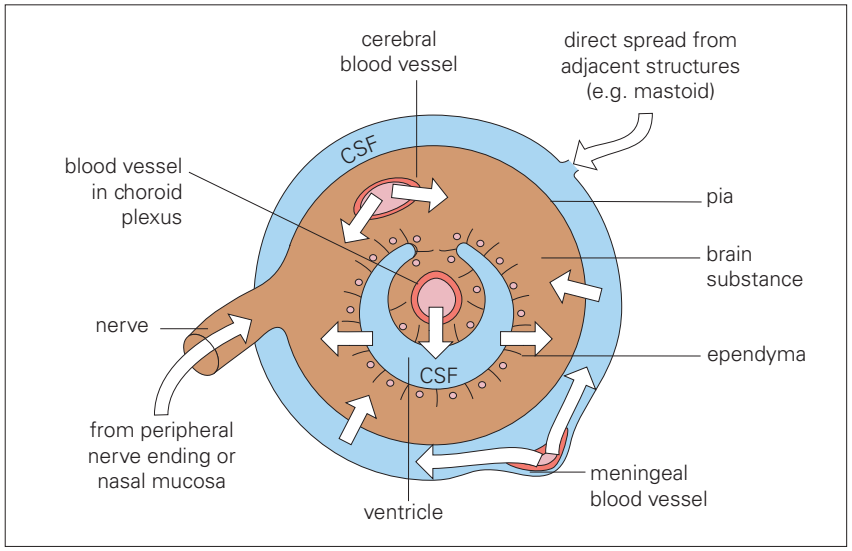


Figure 16.6 Routes of microbial invasion of the central nervous system. CSF, cerebrospinal fluid.

lymphocytes, macrophages and mast cells. Injury or disease in an abdominal organ provides a source of infection for peritonitis, as do chest wounds or lung infections for pleurisy.

GENETIC DETERMINANTS OF SPREAD AND REPLICATION

The pathogenicity of a microorganism is determined by the interplay of a variety of factors

These factors are referred to in Chapters 13 and 17. A distinction is sometimes made between pathogenicity and virulence: virulence implies a quantitative measure of pathogenicity. For instance, it can be expressed as the number of organisms necessary to cause death in 50% of individuals: lethal dose 50 (LD50). Nearly all pathogenicity factors are controlled by host and microbial genes. It has long been known that there are host genetic influences on susceptibility to infectious disease, and that mutations in microorganisms affect their pathogenicity. A number of these genetic factors have been revealed by the application of molecular genetics techniques, and as a result it is increasingly possible to identify the specific gene products involved. Progress has also been made, though

with greater difficulty, in understanding the mode of action of these gene products.

Genetic determinants in the host

The ability of a microorganism to infect and cause disease in a given host is influenced by the genetic constitution of the host

At a relatively gross level, some human pathogens either do not infect other species or infect only closely related primates (e.g. measles, trachoma, typhoid, hepatitis B, warts), whereas others infect a very wide range of hosts (e.g. rabies, anthrax). Also, within a given host species, there are genetic determinants of susceptibility. The best examples are found in animals, but there are examples for human disease (see below).

One example at the molecular level is the sickle cell gene and susceptibility to malaria. Malaria merozoites (see Ch. 28) parasitize red blood cells and metabolize haemoglobin, freeing haem and using globin as a source of amino acids. The sickle cell gene causes a substitution of the amino acid valine for glutamic acid at one point in the b-polypeptide chain of the haemoglobin molecule. The new haemoglobin (haemoglobin S) becomes insoluble when reduced, and precipitates inside the red cell envelope, distorting the cell into the shape of a sickle. In homozygous individuals, there are two of these genes and the individual has the disease sickle cell anaemia, because the red cells are so fragile they sickle under normal circumstances. But in the heterozygote (sickle cell trait), the gene is less harmful, and provides resistance to severe forms of *P. falciparum* malaria, which ensures its selection in endemic malarial regions. The gene would be eliminated from populations after 10–20 generations unless it conferred some advantage. Restriction endonuclease analyses of the gene in Indian and West African populations have revealed that it arose independently in these malarious countries. Homozygotes, however, show increasing susceptibility to other infections, particularly *Strep. pneumoniae*, as a result of splenic dysfunction following repeated splenic infarcts.

Other examples are individuals whom are non-secretors of ABO blood groups, due to homozygosity for a fucosyl-transferase 2 (FUT2) variant that is critical for AB antigen synthesis, who are completely resistant to norovirus infections that cause diarrhoea. Almost total resistance to HIV-1 infection and new variant Creutzfeldt–Jakob disease is seen in individuals homozygous for a 32-base pair deletion in the *CCR5* chemokine receptor gene and those homozygous for valine at codon 129 of the prion protein gene, respectively.

The P blood group antigens are attachment sites for bacteria. Uropathogenic *E. coli* binds the Pk antigen and people with the P1k phenotype are at high risk of urinary tract infections and pyelonephritis. In addition, the *P. vivax* malarial parasite binds to Duffy-positive red blood cells and people whom are Duffy negative are relatively resistant to infection. Those with the p phenotype cannot develop parvovirus B19 infection as the receptor for B19 is the P antigen or globoside on the red blood cell. So, heterogeneity in blood-group antigens could have developed to protect humans against a variety of pathogens.

Susceptibility often operates at the level of the immune response

A poor immune response to a given infection can lead to increased susceptibility to disease, whereas an immune response that is too vigorous may lead to immunopathological disease (see Ch. 18). Of particular importance are the major histocompatibility complex (MHC) genes on chromosome 6, coding for MHC class II (HLA DP, DQ, DR) antigens and controlling specific immune responses. For example, susceptibility to leprosy is strongly influenced by MHC class II genes. People with the HLA DR3 antigen are more susceptible to tuberculoid leprosy, whereas those with HLA DQ1 are more susceptible to lepromatous leprosy.

Studies of identical twins (Box 16.1) provide evidence that genetic determinants affect susceptibility to tuberculosis. The present-day European population shows considerable resistance to this disease. During the great epidemics of pulmonary tuberculosis in Europe in the seventeenth, eighteenth and nineteenth centuries, genetically susceptible individuals were weeded out. In 1850, mortality rates in Boston, New York, London, Paris and Berlin were over 500 / 100 000, but with improvements in living conditions these fell to 180 / 100 000 by 1900, and they have fallen even more since then. However, previously unexposed populations, especially in Africa and the Pacific Islands, show much greater susceptibility to respiratory tuberculosis. In the Plains Indians living in the Qu'Appelle Valley reservation in Saskatchewan, Canada, in 1886, tuberculosis spread through the body to infect glands, bones, joints and meninges, giving a death rate of 9000 / 100 000.

Genetic determinants in the pathogen

Virulence is often coded for by more than one microbial gene

Virulence is determined by numerous factors such as adhesion, penetration into cells, antiphagocytic activity, production of toxins and interaction with the immune system. Consequently, different genes and gene products are involved in different ways and at different stages in pathogenesis.

Under natural circumstances, microorganisms are constantly undergoing genetic change (i.e. mutations). The single-stranded RNA viruses in particular show very high mutation rates. Mutations affecting surface antigens undergo rapid selection in the host under immune pressure (antibody, cell-mediated immunity), as in the case of the rapidly evolving M proteins of streptococci, and the capsid proteins of picornaviruses. In addition, genetic changes in bacteria are often due to acquisition or loss of genetic elements such as integrins, pathogenicity islands, transposons and plasmids (see Chs. 3 and 34).

Changes in the virulence of a microorganism take place during artificial culture in the laboratory. For instance, in the classic procedure for obtaining a live vaccine, a microorganism is repeatedly grown (passaged) in vitro, and this generally leads to reduced pathogenicity in the host. The new strain is then referred to as 'attenuated' (Table 16.3).

Our understanding of the genetic basis for microbial pathogenicity has advanced owing to genetic manipulation techniques such as cloning and site-specific mutagenesis. For instance, by introducing or deleting / inactivating genome segments, the virulence genes can be identified. Major advances in genomics including high-throughput rapid DNA sequencing and bioinformatics have made major contributions to our understanding of virulence genes and conditions affecting their expression. This has allowed sequencing of

Box 16.1 ■ Lessons in Microbiology

Genetically determined susceptibility to infection

There are several classic examples of susceptibility to infectious disease determined by unidentified but presumably genetic factors in the human host.

The Lubeck disaster due to vaccination with virulent tubercle bacilli

In Lubeck, Germany, between December 1929 and April 1930, three oral doses of living tubercle bacilli, instead of attenuated (vaccine) bacilli were inadvertently given to 251 infants <10 days old. There were 72 deaths, 135 developed clinical tuberculosis but recovered and were alive and well 12 years later, while 44 became tuberculin positive but remained well. Each received the same inoculum, and it seems likely that the differences in outcome were largely due to genetic factors in the host. This disaster was a setback for early BCG enthusiasts. Dr George Deycke, in whose laboratory the contaminated batch of BCG had been produced (but never tested for virulence before use), was tried, found guilty of manslaughter and injury by negligence, and sent to prison, together with the Director of Lubeck Health Office.

A military misfortune due to contamination of yellow fever vaccine with hepatitis B virus

In 1942, >45 000 US military personnel were vaccinated against yellow fever, but were inadvertently injected at the same time with hepatitis B virus present as a contaminant in the human serum used to stabilize the vaccine. There were 914 clinical cases of hepatitis, of which 580 were mild, 301 moderate and 33 severe. Even with a given batch of vaccine, the incubation period varied in the range 10–20 weeks. Serological tests were not then available, so the number of subclinical infections is unknown. In this case, both physiological and genetic influences on susceptibility may have played a part.

Identical twins are affected similarly by respiratory tuberculosis

A study of tuberculosis in twins when at least one twin had the disease showed that, for identical twins, the other twin was affected in 87% of cases. With non-identical twins, the equivalent figure was only 26%. In addition, the identical twins had a similar type of clinical disease.

Table 16.3 Examples of attenuation of pathogens following repeated passage in vitro

Pathogen	Passage	Attenuated (live) product
Mycobacterium bovis	10 years of repeated passage in glycerin–bile–potato medium	Bacille Calmette–Guérin (BCG) vaccine
Rubella virus	27 passages in human diploid cells	Rubella vaccine (Wistar RA 27/3)

the entire genome for many infectious agents (bacteria and viruses). This information has facilitated the assignment of virulence functions to specific loci and greatly improved our understanding of the way microorganisms sense and respond to the host environment.

OTHER FACTORS AFFECTING SPREAD AND REPLICATION

Various other factors have an influence on susceptibility to infectious disease (Table 16.4). In most cases, it is not known whether this involves differences in microbial spread and replication or differences in host immune and inflammatory responses. Infections in hosts with immunological and other defects are described in Chapter 31.

The brain can influence immune responses

When stress (a loosely used word) is associated with malnutrition or crowding, it may be difficult to disentangle the separate influences of these various factors on susceptibility to infection, as in the case of tuberculosis. The brain can, however, influence immune responses, acting via the hypothalamus, pituitary and adrenal cortex. It has long been known that glucocorticoids, which have powerful actions on immune cells, are needed for resistance to infection and trauma. A shortage of glucocorticoids, as in Addison's disease, or an excess, as with steroid therapy, results in increased susceptibility to infection (see Table 16.4). In addition, the brain, the endocrine and the immune systems often use the same molecular messengers: cytokines, peptide hormones and neurotransmitters. Neural cells, for instance, have receptors for interferons and for interleukins IL-1, IL-2, IL-3, and IL-6, and thymic lymphocytes can produce prolactin and growth hormone. Immune–neuroendocrine cross-talk now has molecular respectability and provides an acceptable basis for the influence of the brain on immunity and infectious disease.

Table 16.4 Host factors influencing susceptibility to infectious diseases

Factor	Example	Alteration in susceptibility	Mechanism
Pregnancy	Hepatitis E virus	Maternal mortality 10–30% in third trimester	?Increased metabolic burden for liver in pregnancy / immune response
	Zika virus	Congenital Zika Syndrome, especially fetal microcephaly	Zika virus antigen found in cytoplasm of degenerating and necrotic neurons and glial cells
	Urinary infections	Pyelonephritis more common	Reduced peristalsis in ureter
Malnutrition	Measles	More severe; more lethal	Vitamin A deficiency; depressed CMI
Age	Respiratory syncytial virus	More severe; more lethal in infant	Small diameter of airways
	Mumps, chickenpox, Epstein–Barr virus infection	More severe in adult	?Increased immunopathology
Atmospheric pollution	Raised sulphur dioxide levels	Excess acute respiratory disease	?Interference with mucociliary defences
	Silicosis	Increased susceptibility to tuberculosis	?Damage to lung macrophages
Foreign bodies	Necrotic bone fragments	Chronic osteomyelitic more common	Antimicrobial defences less effective in necrotic tissue
	Necrotic tissues	Increased susceptibility to *Clostridium perfringens*	Anaerobic necrotic tissues favour bacterial growth
Stress, hormones	Glucocorticoid production:		
	Decreased (Addison's disease)	Increased susceptibility to infection	Hypersensitivity to inflammatory / immune responses?
	Increased (steroid therapy)	Increased susceptibility to infection	Reduction in protective immune / inflammatory responses

CMI, cell-mediated immunity.

KEY FACTS

- Infections restricted to the body surfaces (e.g. common cold, *Shigella* dysentery) have shorter incubation periods than do systemic infections (e.g. measles, typhoid), and adaptive (immune) host responses tend to be less important.
- Pathogens with a slow growth rate (e.g. *M. tuberculosis*) tend to cause diseases which evolve slowly.
- Spread through the body takes place primarily via lymph and blood. The fate of circulating pathogens depends upon whether they are free or present in circulating blood cells.
- Uptake by reticuloendothelial cells in liver and spleen focuses infection into these organs, but specific localization in the vascular bed of other organs (e.g. mumps virus in salivary glands, meningococci in meninges) is not understood.
- Viruses can spread in either direction along nerve axons, and this is important in the pathogenesis of recurrent herpes simplex virus infection, zoster and rabies.
- Pathogenicity and virulence are strongly influenced by genetic factors in the host (e.g. tuberculosis in identical twins) and by genetic factors in the pathogen (e.g. sickle cell trait in falciparum malaria).
- Our understanding of virulence has been greatly enhanced by advances in molecular biology which have allowed sequence analysis of entire microbial genomes and a clearer view of microbial response to the host environment.

17 Parasite survival strategies and persistent infections

Introduction

The most common pathogens have developed 'answers' to host defences

So far, we have concentrated on the battery of mechanisms available to the host, both innate and adaptive, to keep out and destroy pathogens. Powerful as these are, they are obviously not 100% effective; otherwise healthy people would never have infections. In fact, most of the common infectious organisms described in this book have developed 'answers' to host defences that enable them to survive as human parasites. They successfully infect humans and are of concern to the physician precisely because they have developed strategies for evading or actively interfering with host defences.

Strategies to evade innate non-adaptive defences such as the phagocyte

These are many and include the following:

- *Avoiding being killed by phagocytes*. Successful parasites have evolved numerous ingenious antiphagocytic devices (Fig. 17.1). These range from avoiding being phagocytosed, not being killed if phagocytosed, and killing or inhibiting the phagocyte itself. Some bacteria such as *Mycobacterium tuberculosis* inhibit phagosome-lysosome fusion; others such as *Listeria* break out of the phagolysosome by punching holes in the membranes and escaping into the cytoplasm. If a microorganism can survive within the phagocyte, this poses a very serious challenge to the host.
- *Interfering with ciliary action* (see Table 14.3).
- *Interfering with the activation of complement*. Microorganisms can acquire or mimic complement regulators, actively inhibit complement components, or enzymatically destroy complement components. A variety of pathogens can bind complement regulators, including *E. coli*, streptococci and *Candida albicans*. The smallpox and vaccinia viruses produce proteins that mimic host complement regulators. *Staphylococcus aureus*, streptococci, herpes simplex virus (HSV), *Schistosoma* and *Trypanosoma* express complement inhibitors. *Psuedomonas* bacteria produce an elastase that inactivates the C3b and C5a components of complement; other proteases that destroy complement components are produced by *Pseudomonas*, *Serratia marcescans* and *Schistosoma mansoni*. *Staph. aureus* has a surface immunoglobulin banding protein and an extracellular fibrinogen banding protein that generate plasmin to degrade C3 and C3b. Another strategy is to physically block complement lysis – the insertion of the C567 complex is prevented by the long side chains of the cell wall polysaccharides of smooth strains of *Salmonellae* and by the capsules of staphylococci, which do not activate complement, and the cell wall of Gram-positive bacteria prevents lysis by the complement membrane attack complex (Fig. 17.2). However, some pathogens take the opposite approach, choosing to enter host cells by exploiting opsonization with complement components – HIV-1 and *M. tuberculosis* exploit the CR3 receptor in this way.
- *Producing iron-binding molecules*. Nearly all bacteria need iron, but the host's iron-binding proteins such as transferrin limit the availability of this element. Accordingly, certain bacteria (e.g. *Neisseria*) produce their own powerful iron-binding proteins to circumvent the shortage.
- *Blocking type I interferons*. Host cells respond to double-stranded DNA (dsRNA) from infecting pathogens (including all viruses), by forming interferons alpha and beta. These are produced rapidly, within 24 h after infection, and are part of the innate immune response (see Ch. 10). Certain viruses are either poor inducers of interferons (hepatitis B) or produce molecules that block the action of interferons in cells (hepatitis B, HIV, adenoviruses, Epstein–Barr virus (EBV), rotavirus, vaccinia virus.

Many pathogens target the TLR signalling pathway

Signalling through the macrophage Toll-like receptors (TLR, see Ch. 10) is essential for activation of many antimicrobial defences. The TLR provide a series of cell surface and intracellular receptors with which the cell can sense infectious organisms. These receptors are clearly very important to host defence, as there are now over 70 examples of bacteria and viruses interfering with signalling through the TLR receptors (Table 17.1). Following TLR binding a complex series of intracellular events take place, with the TLR cytoplasmic tail interacting with adaptor molecules, recruiting serine threonine kinases, ubiquitin ligases, production of a polyubiquitin scaffold, further phosphorylation events in protein complexes and the eventual activation and translocation to the nucleus of transcription factors such as nuclear factor (NF)-κB. Most of these steps can be targeted by bacteria or viruses. For example hepatitis C virus can cleave the adaptor molecule TRIF that interacts with TLR3 and 4, blocking signalling.

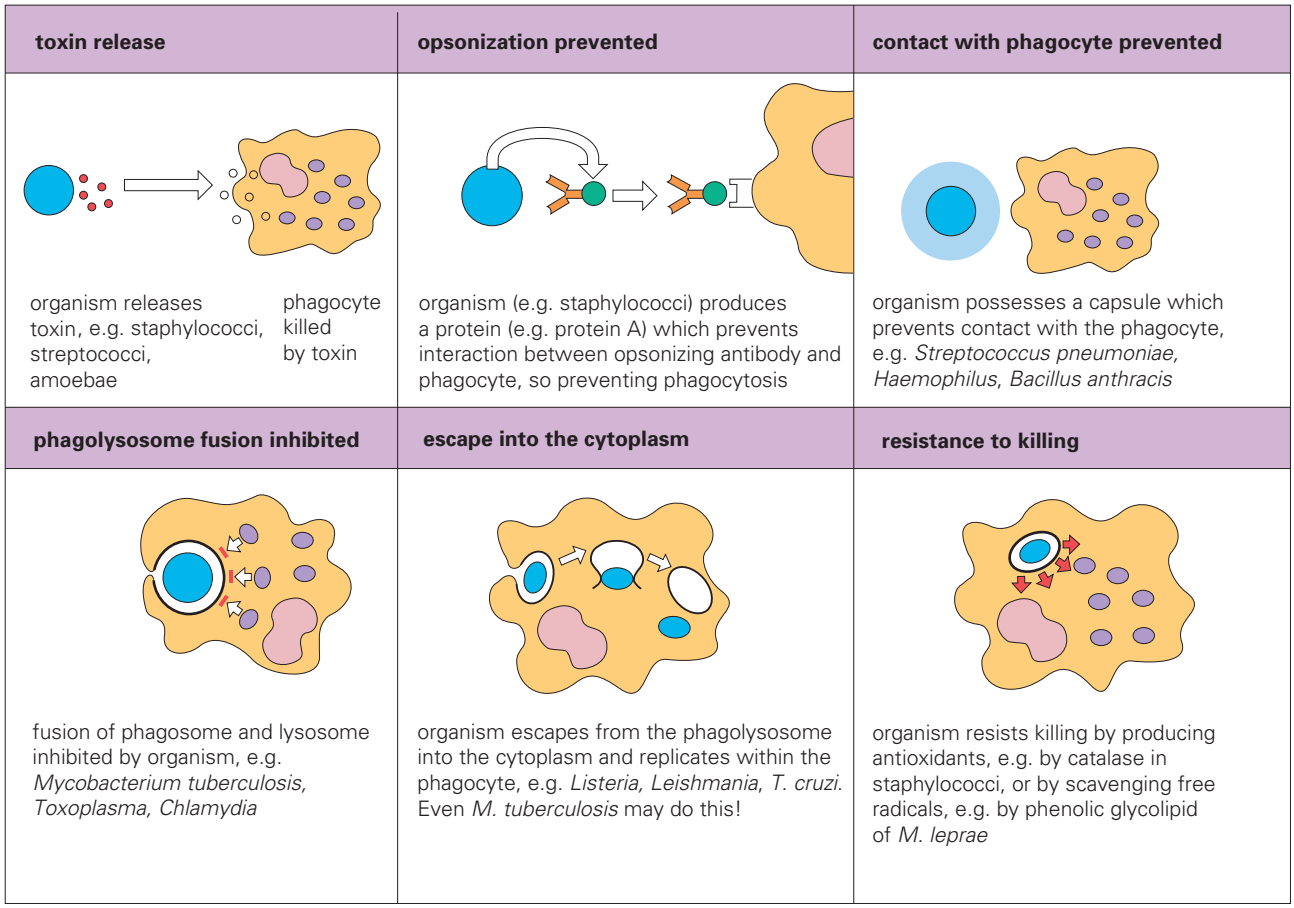


Figure 17.1 Various mechanisms adopted by microorganisms to avoid phagocytosis.

Table 17.1 Many pathogens interfere with the Toll-like receptor (TLR) signalling pathways

Step targeted	Pathogen	Result
TLR binding	*Staph. aureus*	SSL3 and SSL4 proteins bind TLR2 ectodomain
Supramolecular organizing centre (SMOC)	*E.coli*	Blocks TIR–TIR interactions
TRAF6 and TAK1 protein ubiquitination and activation	Epstein–Barr virus	Deubiquinates TRAF6
Mitogen-activated protein kinases (MAPKs)	Ebola virus	Blocks p38 phosphorylation
IKK complex	Vaccinia virus	Binds IKKβ
Transcription factors	*Shigella flexneri*	Inhibits nuclear translocation of NFκB

The complex signalling pathways that follow TLR binding are targeted by a range of pathogens. TLRs interact first with intracellular sorting adaptor proteins that recruit signalling adaptor proteins with formation of a supramolecular organizing centre. These activate an E3 ubiquitin ligase and further proteins activate mitogen-activated protein kinases (MPKs). The IKK complex (I kappa kinase) enables ubiquinization that promotes further activation and signaling, which ultimately leads to activation of transcription factors that translocate to the nucleus (see Fig. 10.11). *SSL*, staphylococcal superantigen-like; *TIR*, Toll/IL-1 Receptor/Resistance; *TAK*, TGFβ-activated kinase; *TRAF*, TNF receptor associated family.
(For more details see Rosadini, C.V. and Kagan, J.G. *Curr Opin Immunol* 2015; 32:61–70.)

Strategies to evade adaptive defences

Strategies to evade adaptive defences are more sophisticated than those for evading innate defences

The strategies that pathogens use to evade or interfere with adaptive (immune) defences are more sophisticated than those for evading innate defences, because antigen-specific lymphocytes have cell receptors that can recognize virtually any shape (B cells) or amino acid sequence (T cells), provided it is not identical to self. For example:

- The polysaccharide capsules of bacteria prevent non-immune contact between phagocytes and the bacterial cell wall, but are quickly recognized as foreign by B-cell surface receptors (immunoglobulin), leading to the formation of antibody with consequent opsonization and phagocytosis of the bacteria.
- Many microorganisms such as bacteria and fungi can resist intracellular destruction by macrophages, but if their peptides are presented in association with major histocompatibility

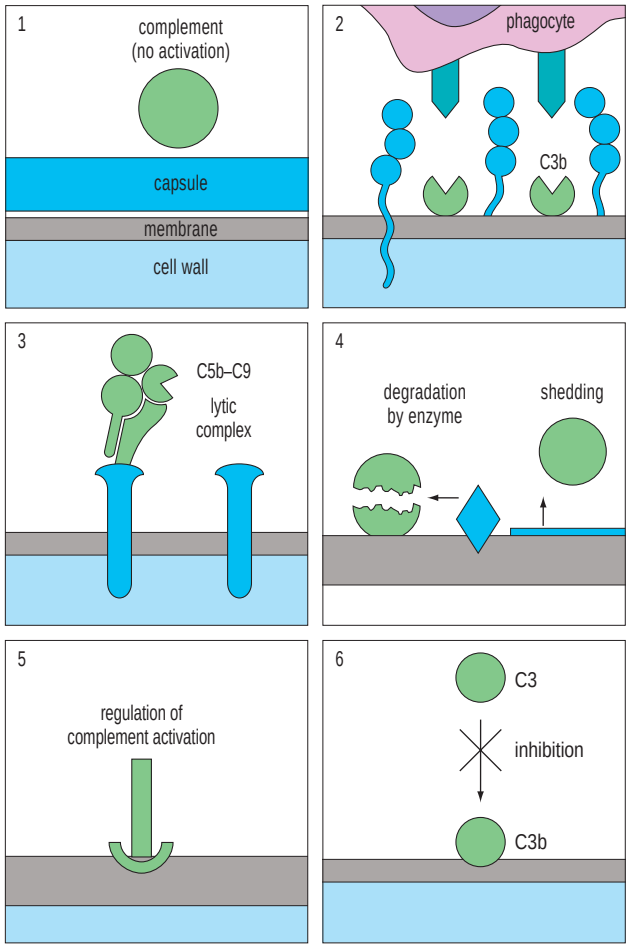


Figure 17.2 Bacteria avoid complement-mediated damage by a variety of strategies. (1) An outer capsule or coat prevents complement activation. (2) An outer surface can be configured so that complement receptors on phagocytes cannot obtain access to fixed C3b. (3) Surface structures can be expressed that divert attachment of the lytic membrane attack complex (MAC) from the cell membrane. (4) Membrane-bound enzyme can degrade fixed complement or cause it to be shed. (5) Complement inhibitors can be captured onto the surface. (6) Direct inhibition of the C3 and C5 convertases blocks complement activation. (Panels 1–4 reproduced from: Male, D., Brostoff, J., Roth, D.B., Roitt, I. [2006] *Immunology*. Mosby Elsevier, with permission.)

complex (MHC) molecules on the macrophage surface, their presence is detected by T cells. This enables T-helper (Th) cells to produce macrophage-activating cytokines like interferon gamma (IFNγ), and cytotoxic T cells to kill the infected cell.

In both these examples, the T and B cells are behaving like a highly specialized and sharply observant secret police force that is more effective than the cells of the innate immune system.

PARASITE SURVIVAL STRATEGIES

Parasite survival strategies can take as many forms as there are parasites, but they can be usefully classified by the immune component that is evaded and the means selected to do this. They enable the pathogen to undergo what are often quite lengthy periods of growth and spread during the incubation period before being shed and transmitted to the next host, as occurs in hepatitis B and tuberculosis. Shedding of the pathogen for just a few extra days after clinical recovery also gives more extensive transmission in the community, and this is a worthwhile result for the pathogen. A person who has recovered from norovirus or rotavirus may remain infectious for up to 2 weeks after recovery from the clinical symptoms.

Viruses are particularly good at hindering immune defences

Viruses are able to impede immune defences for a number of reasons:

- Their invasion of tissues and cells is often 'silent'. Unlike most bacteria, they do not form toxins, and as long as they do not cause extensive cell destruction there is no sign of illness until the onset of immune and inflammatory responses, sometimes several weeks after infection, as occurs in hepatitis B virus and EBV infections.
- Viruses such as rubella virus, wart viruses, hepatitis B virus and EBV can infect cells for long periods without adverse effects on cell viability.

Some pathogens are able to persist in the host for even longer

Certain pathogens can remain (persist) in the host for many years, or often for life. From the pathogen's point of view, persistence is worthwhile only if shedding occurs during the persistence (see Box 17.1).

Virus latency is a type of persistence and is based on an intimate molecular relationship with the infected cell. The viral genome continues to be present in the host without continuously producing antigens or infectious material, until the virus reactivates (becomes patent).

Strategies for evading host defences include causing a rapid 'hit-and-run' infection

One evasion strategy for microorganisms is to cause a rapid 'hit-and-run' infection. The pathogen invades, multiplies and is shed within a few days, before adaptive immune defences have had time to come into action. Infections of the body surfaces (rhinoviruses, rotaviruses) come into this category. Otherwise, the principal strategies employed by parasites to elude the lymphocyte adaptive responses are:

- concealment of antigens
- antigenic variation
- immunosuppression.

CONCEALMENT OF ANTIGENS

A spy in a foreign country can conceal his presence from the police by hiding, by never venturing out of doors, or by adopting the disguise of a local. Parasites have the same options. Places to hide include the interior of host cells (though the MHC molecules act as 'informers' for this compartment, picking up and transporting microbial peptides to the cell surface where they will be recognized) and particular sites in the body where lymphocytes do not normally circulate ('privileged sites', the equivalent of 'no-go' areas or 'safe houses').

Box 17.1 ■ Lessons in Microbiology

Trail of illness from a slippery cook

In 1901, Mary Mallon, from Long Island, New York, took a job as cook with a family in New York City. Soon afterwards, the family washerwoman and a visitor to the house became ill with enteric fever (typhoid). Mary moved to another job and a few weeks later all seven family members plus two of the servants went down with enteric fever. Similar infections followed her movements as a cook and in 1906 the authorities tried to dissuade her from such work. She was indignant at the suggestion that she was carrying a dangerous germ, knowing that she was healthy, and failed to keep promises to have regular checks and give up work. She was suspicious of officials and aggressive, on one occasion advancing towards the questioner brandishing a carving knife. She was later arrested and put in an isolation hospital. After appealing to the US Supreme Court, she was released in 1910 promising not to work as a cook. Then in 1914 typhoid epidemics broke out in a hospital and in a sanatorium where she had worked as a cook. She was traced, living under a false name, and in the interests of public safety, she was detained permanently on North Brother Island, where she died in 1938. In her cooking career she had been responsible for about 200 cases of typhoid in eight different families and had started seven epidemics of the disease. Her favourite recipe, an iced peaches dessert, may have been a good source of infection.

Mary had recovered fully from an attack of typhoid earlier in life, but she had gallstones and this enabled the bacteria to persist in her gallbladder for many years, appearing intermittently in the faeces. About 5% of cases become carriers, in either the gallbladder or the urinary bladder, and they play a central role as foci of infection. Nowadays, Mary would have to have acquired her original infection in a region such as the Indian subcontinent where typhoid is endemic. Each year, there about 21 million typhoid cases worldwide, 300 of which are confirmed cases in the USA, most being travellers to the Indian subcontinent (see also Ch. 23).

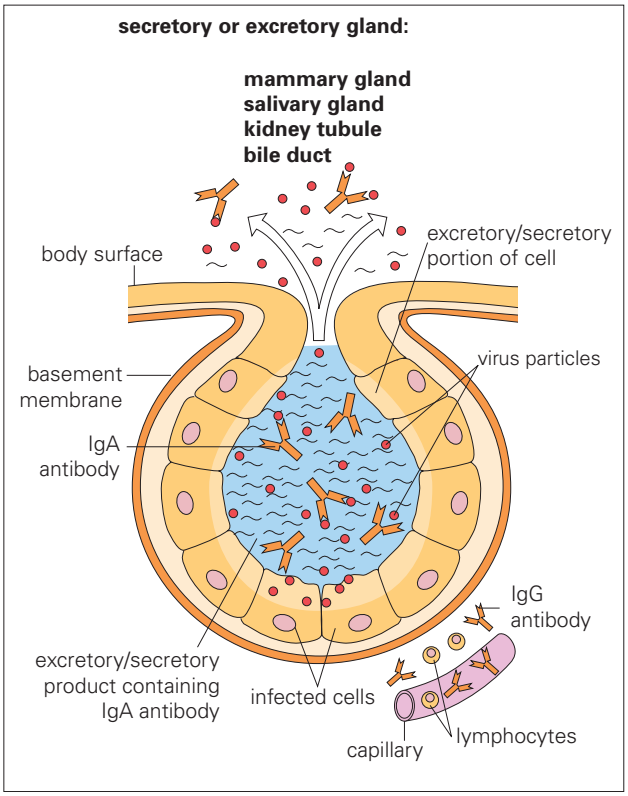


Figure 17.3 Viral infection of cell surfaces facing the external world. Infection of the surface epithelium of, for instance, a secretory or excretory gland allows direct shedding of the virus to the exterior, as well as avoidance of host immune defences.

Remaining inside cells without their antigens being displayed on the surface prevents recognition

If a pathogen can remain inside cells without allowing its antigens to be displayed on the cell surface, it will remain unrecognized ('incognito') as far as immune defences are concerned. Even if specific antibody and T-cell responses have been induced, the pathogen inside such a cell is unaffected. Persistent latent viruses such as HSV in sensory neurones behave in this way. During reactivation, antigenic boosting of immune defences is, however, inevitable.

Other strategies are possible. Several viruses (HIV in macrophages, coronaviruses) display their proteins 'secretly' on the walls of intracellular vacuoles instead of at the cell surface, and bud into these vacuoles. Adenoviruses have taken more active steps to avoid antigen display. One of the adenoviral proteins (E19) combines with class I MHC molecules and prevents their passage to the cell surface so that infected cells are not recognized by cytotoxic T cells.

Colonizing privileged sites keeps the pathogen out of reach of circulating lymphocytes

The vast numbers of pathogens that colonize the skin and the intestinal lumen, together with those that are shed directly into external secretions, are effectively out of reach of circulating lymphocytes. They are exposed to secretory antibodies, which although able to bind to the microbe (e.g. influenza virus) and render it less infectious, are generally unable to kill the pathogen or control its replication in or on the epithelial surface (Figs 17.3 and 17.4). A local inflammatory response, however, can enhance host defences.

Within the body, it is more difficult to avoid lymphocytes and antibodies, but certain sites are safer than others. These include the central nervous system, joints, testes and placenta. Here, lymphocyte circulation is less intense, and access of antibodies and complement is more restricted. However, as soon as inflammatory responses are induced, then lymphocytes, monocytes and antibodies are rapidly delivered and the site loses its privilege.

Additional privileged sites can be created by the infectious organism itself. A good example is the hydatid cyst that develops in liver, lung or brain around growing colonies of the tapeworm *Echinococcus granulosus* (Fig. 17.5), inside which the worms can survive even though the blood of the host contains protective concentrations of antibody.

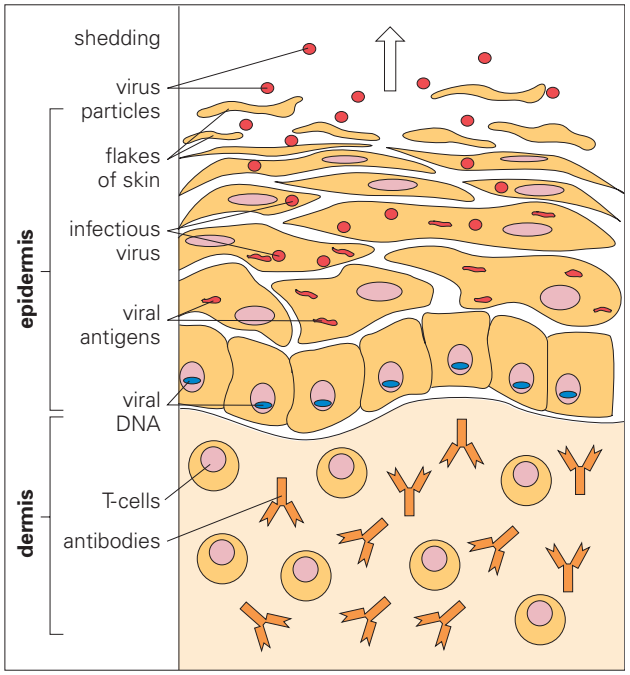


Figure 17.4 Wart virus replication in epidermis – a privileged site? Cell differentiation such as keratinization controls virus replication, and as a result virus matures when it is physically removed from immune defences.

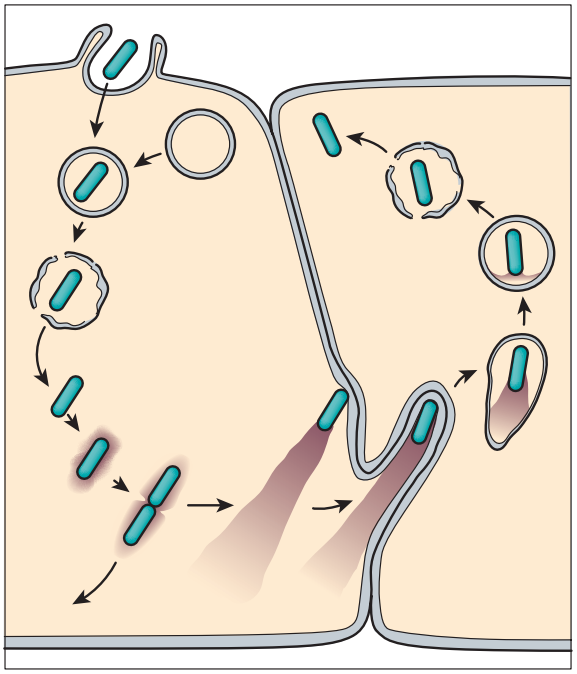


Figure 17.6 Listeria can move directly from cell to cell. Moving directly from one cell into another enables the bacteria to evade any harmful antibodies they might otherwise be exposed to. (Redrawn after Portnoy, D, *Mol Biol Cell* 2012; 23:1141–1145.)

Figure 17.5 Hydatid cysts. Multiple, thin-walled, fluid-filled cysts in a surgical specimen. The lung is a common site. Growing within a cyst is a survival strategy for *Echinococcus granulosus*. (Courtesy of J.A. Innes.)

Perhaps the most highly privileged site of all is host DNA, and this is occupied by the retroviruses. Retroviral RNA is transcribed by the reverse transcriptase into DNA as a necessary part of the replicative cycle, and this then becomes integrated into the DNA of the host cell (see Ch. 22). Once integrated, and as long as there is no cell damage and viral products are not expressed on the cell surface where they can be recognized by immune defences, the virus enjoys total anonymity. This is what makes complete removal of virus from a patient infected with HIV such a daunting task. The intragenomic site becomes even more privileged if the egg or sperm is infected. The viral genome will then be present in all embryonic cells and transferred from one generation to another as if it were the host's own DNA. Luckily, this does not happen with HIV or with human T-cell lymphotropic virus (HTLV) 1 and 2. However, the 'endogenous' retroviruses of humans present in profusion as DNA sequences in our genome, but not expressed as antigens, come into this category. They are part of our inheritance. This surely represents the ultimate step in parasitism, at the borderline between infection and heredity.

Cell-to-cell spread is another effective way of avoiding exposure to harmful extracellular molecules

Listeria exploits its listeriolysin molecule to punch a hole in the phagosome membrane and escape into the cytoplasm of the cell. It can also move from cell to cell with minimal exposure to the exterior. It acts on actin regulatory factors inducing the production of actin-rich protrusions, which can essentially inject the *Listeria* bacteria directly into the next cell without the immune system getting a chance to attack it (Fig. 17.6).

Mimicry sounds like a useful strategy, but does not prevent the host from making an antimicrobial response

If the pathogen can in some way avoid inducing an immune response, this can be regarded as a 'concealment' of its antigens. One method is by mimicking host antigens, as such self antigens are not recognized as foreign. Some parasite-derived molecules resemble those of the host (Table 17.2). In the case of viral proteins, mimicry based on amino acid sequence homology (sharing of 8–10 consecutive amino acids) is seen to be common when sequence comparisons

Table 17.2 Some examples of mimicry or uptake of host antigens by parasites

Pathogen's strategy	Parasite	Corresponding host antigen
Mimicry	Streptococci (M protein and *N*-acetyl-beta-D-glucosamine)	Cardiac myosin
	Mycobacterium tuberculosis	65 kDa heat shock protein
	Treponema	Cardiolipin[a]
	Plasmodium falciparum	Vitronectin, thrombospondin[b]
	Trypanosoma cruzi	Heart myosin, nerve
Antigen uptake	*Neisseria meningitides*	Complement factor H
	Cytomegalovirus	β_2-microglobulin
	Schistosoma	Glycolipids, HLA I, HLA II
	Ascaris	Blood group A, B antigens

[a]Basis for the original Wasserman-antibody test for syphilis developed in 1906.
[b]The homologous malaria protein thrombospondin-related anonymous protein (TRAP) shares an 18 amino acid sequence with the circumsporozoite protein that mediates binding to hepatocytes. HLA, human leukocyte antigen (MHC or major histocompatibility antigens).

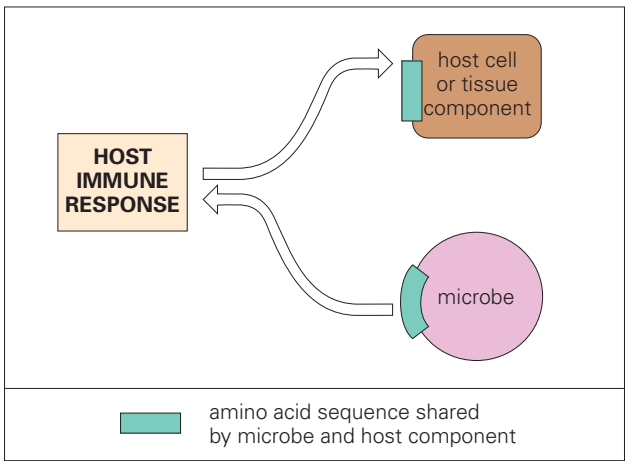


Figure 17.7 Molecular mimicry. Molecular mimicry by the pathogen can induce host cell damage; for example, rheumatic heart disease following streptococcal infection is caused by antibodies reacting with meromyosin, the cross-reacting determinant.

are made between viral and host proteins. Perhaps the most celebrated example is the cross-reaction between group A beta-haemolytic streptococci and human myocardium. This cross-reaction underlies the development of rheumatic heart disease following repeated streptococcal infection because of antibody made against the cross-reacting determinant meromyosin (Fig. 17.7). The fact that the host makes such autoantibodies shows that in this case mimicry does not prevent an antimicrobial host response.

Pathogens can conceal themselves by taking up host molecules to cover their surface

This is illustrated in Table 17.2. A superb example of this is the blood fluke *Schistosoma*, as the schistosomula acquire a surface coat of host blood group glycolipids and MHC antigens from the plasma. Such a parasite must indeed be virtually invisible to a T or B cell. For unknown reasons, this strategy is essentially restricted to worms.

The uptake of immunoglobulin molecules by a pathogen seems to be a more widespread phenomenon. A number of viruses and bacteria produce immunoglobulin-binding proteins, which are displayed on their surface and bind immunoglobulin molecules of all specificities in an immunologically useless upside-down position (Fig. 17.8). This prevents the access of specific antibodies or T cells to the pathogen or the infected cell. *Mycoplasma* produce a unique immunoglobulin-binding protein that was recognized only in 2014; a better-known bacterial protein that binds immunoglobulins is staphylococcal protein A, a cell wall protein excreted from virulent staphylococci that inhibits the phagocytosis of antibody-coated bacteria. Certain herpesviruses (HSV, varicella-zoster virus [VZV], cytomegalovirus [CMV]) code for molecules that act as Fc receptors for IgG, and streptococci produce an Fc receptor for IgA.

Immune modulation

Modulation of the host immune response by the pathogen can prevent this response being an effective one. An alternative strategy for the pathogen is to avoid inducing an immune response or to induce a poor and ineffective response. Possible methods include:

- infection during early embryonic life
- the production of large quantities of the microbial antigen or of antigen–antibody complexes
- upsetting the balance between cell and antibody-mediated immune responses – or between T-helper cell (Th) 1 and 2 responses
- inducing regulatory T cells or molecules that down-regulate protective immunity.

Infection during early embryonic life

Before full development of the immune system, a time when antigens present are regarded as 'self', infection could possibly result in immune tolerance. However, in intrauterine infection with CMV, rubella virus and syphilis, the fetus does eventually produce IgM antibody, which is detectable in umbilical cord blood, but cell-mediated responses are more seriously impaired. Children with congenital CMV or rubella fail to develop lymphoproliferative responses to CMV or rubella antigens and consequently take years to clear the virus from the body (see Ch. 24). In some cases, infection in the neonatal period is more likely to result in tolerance than infection in later life. Therefore, neonatal infection with hepatitis B virus frequently results in permanent carriage of the virus.

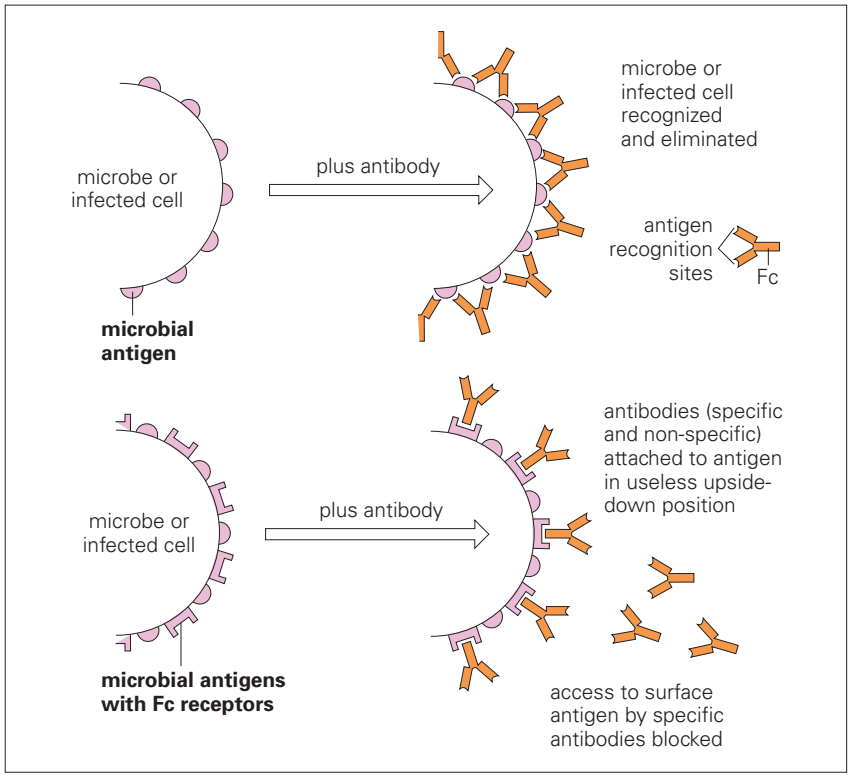


Figure 17.8 Evasion through production of Fc receptors. The production of Fc receptors is of some benefit to pathogens, for example staphylococci, streptococci, herpes simplex virus, varicella-zoster virus and cytomegalovirus.

Production of large quantities of microbial antigen or antigen–antibody complexes

Large quantities of microbial antigen or antigen–antibody complexes circulating in the body can cause immune tolerance to that antigen. Anergy, as evidenced by normal antibody but depressed cell-mediated immune responses to the invading pathogen, is seen in disseminated coccidioidomycosis and cryptococcosis, and in visceral and diffuse cutaneous leishmaniasis, in each case associated with large amounts of microbial antigen in the circulation.

Upsetting the balance between Th1 and Th2 responses

Resistance to infection often depends upon a suitable balance between Th1 and Th2 responses (see Ch. 11). Good defence against tuberculosis and herpesviruses needs cell-mediated immunity, whereas antibody is required to protect us against polioviruses or *Streptococcus pneumoniae*. In active tuberculosis, T cells making IL-4 can be detected, with a reduction in the beneficial Th1 cytokine response. By inducing an ineffective type of Th2 response rather than effective Th1 activation a pathogen can promote its own survival.

An altered Th1 : Th2 balance can also drive macrophages to be classically activated or alternatively activated. Saliva from the *Leishmania* sandfly vector can reduce Th1 and increase Th2 cytokine production and IL-10, thus reducing macrophage activation by inducing alternatively activated macrophages. In helminth infections, as well as allergy, Th2 cytokines such as IL-4 and IL-13 induce alternatively activated macrophages (classically activated macrophages are those activated by IFNγ). Alternatively activated macrophages are thought to play a role in worm expulsion from the gut.

Table 17.3 Infections where regulatory T cells are induced

Bordetella pertussis	Inhibit Th1 immunity
Mycobacterium tuberculosis	Inhibit Th1 immunity
Helicobacter pylori	Control peptic ulcer disease
Hepatitis B virus	Associated with viral load in serum
Hepatitis C virus	Suppress cytotoxic T-cell responses but also pathology
Herpes simplex virus	Reduce extent of inflammation
Plasmodium falciparum	Associated with higher parasitaemia

Regulatory T cells

Some bacteria evade protective Th1 responses by inducing antigen-specific regulatory T cells, that were originally called suppressor T cells. Regulatory T cells (Tregs) can be found in healthy individuals but can also be induced by exposure to antigen in the presence of transforming growth factor beta (TGFβ) and IL-10. *Bordetella pertussis* infection induces regulatory T cells specific for its filamentous haemagglutinin and pertactin. These regulatory T cells produce TGFβ and IL-10, and thus suppress Th1 immunity to two vital bacterial components which help the bacteria attach to host cells. Regulatory T cells are induced by many other bacteria including *M. tuberculosis*, during malaria infection and by some helminth antigens (Table 17.3).

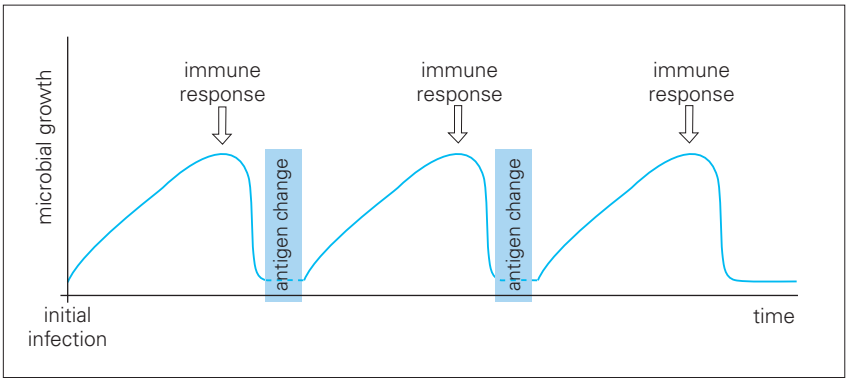


Figure 17.9 Antigenic variation as a microbial strategy. The change in antigens may take place in the originally infected individual, enabling the pathogen to undergo renewed growth (e.g. trypanosomiasis, relapsing fever – see Ch. 28) or it may take place as the pathogen passes through the host population, enabling it to re-infect a given individual (e.g. influenza).

ANTIGENIC VARIATION

Reverting to the metaphor of a spy in foreign territory, there is another way to confuse the enemy and that is by repeated changes in appearance. The African trypanosome, the causative organism of sleeping sickness, does this, and so do a wide range of viruses, bacteria and protozoa. Antigenic variation can occur during:

- the course of infection in a given individual
- spread of the pathogen through the host community (Fig. 17.9).

As a strategy for evading host immune responses, antigenic variation depends upon variation occurring in antigens whose recognition is involved in protection. Antigenic variation is common as the pathogen passes through the host community. It tends to be more important in longer-lived hosts, such as humans in whom microbial survival is favoured by multiple re-infections during the lifetime of an individual. It is more common in infections limited to respiratory or intestinal epithelium where the incubation period is <1 week and the pathogen can infect, multiply and be shed from the body before a significant secondary immune response is generated. During systemic infections (e.g. measles, mumps, typhoid), the incubation period is longer and secondary responses have more opportunity to be mobilized and control an infection by an antigenic variant. Accordingly, antigenic variation is not an important feature of these systemic infections.

At the molecular level, there are three main mechanisms for antigenic variation:

- mutation
- recombination
- gene switching.

The best-known example of mutation is the influenza virus

As the influenza virus spreads through the community there are repeated mutations in the genes coding for haemagglutinin and neuraminidase (see Ch. 20), causing small antigenic changes that are sufficient to reduce the effectiveness of B- and T-cell memory built up in response to earlier infections. This is called 'antigenic drift'. Human rhinoviruses and enteroviruses also evolve rapidly and show a similar antigenic drift. Antigenic drift could account for the wealth of antigenic types of staphylococci, streptococci and pneumococci. During earlier poliovirus epidemics, mutations occurred at the rate of about two base substitutions per week, some of them involving the main antigenic sites on the virus. HIV (see Ch. 22) undergoes antigenic drift, but in this case it occurs during infection of a given individual, which is why this infection is difficult for the immune system to control. Mutations affecting the epitopes recognized by cytotoxic T (Tc) cells are the source of 'escape mutants'.

The classic example of antigenic variation using gene recombination involves influenza A virus

More extensive and sudden alterations in antigens can take place by the exchange of genetic material between two different pathogens. The classic example is genetic 'shift' in influenza A virus (Fig. 17.10, and see Ch. 20). When human and avian virus strains recombine, a completely new strain of influenza A virus suddenly emerges, expressing a haemagglutinin or neuraminidase of avian origin. This new virus, not previously experienced by the present population, gives rise to an influenza pandemic. The 2009 / 2010 'swine flu' epidemic which originated in Mexico was caused by a H1N1 virus – segments of its genome were identified in flu isolates almost 20 years earlier but gene reassortment led to the new pandemic strain (Fig. 17.10). Surprisingly, it now seems that the 1918 Spanish flu pandemic may not have been caused by antigenic shift, but instead by an avian flu that became able to infect humans.

Gene switching was first demonstrated in African trypanosomes

Gene switching represents the most dramatic form of antigenic variation and was first demonstrated in the African trypanosomes, *Trypanosoma gambiense* and *T. rhodesiense* (see Ch. 28). These organisms carry genes for about 1000 quite distinct surface molecules known as variant-specific glycoproteins, which cover almost the entire surface and are immunodominant. The trypanosome can switch from using one gene to another, much as a B cell does with the immunoglobulin heavy-chain-constant genes. This explains why a sequence of antigenically unrelated infections occurs at approximately weekly intervals. This enables the trypanosome to persist while the immune system is constantly trying to catch up with it. The main stimulus for each gene switch is possibly the antibody response itself, but the exact mechanism is not clear. About 10% of the trypanosome genome consists of surface coat genes, but this is a worthwhile investment for the parasite.

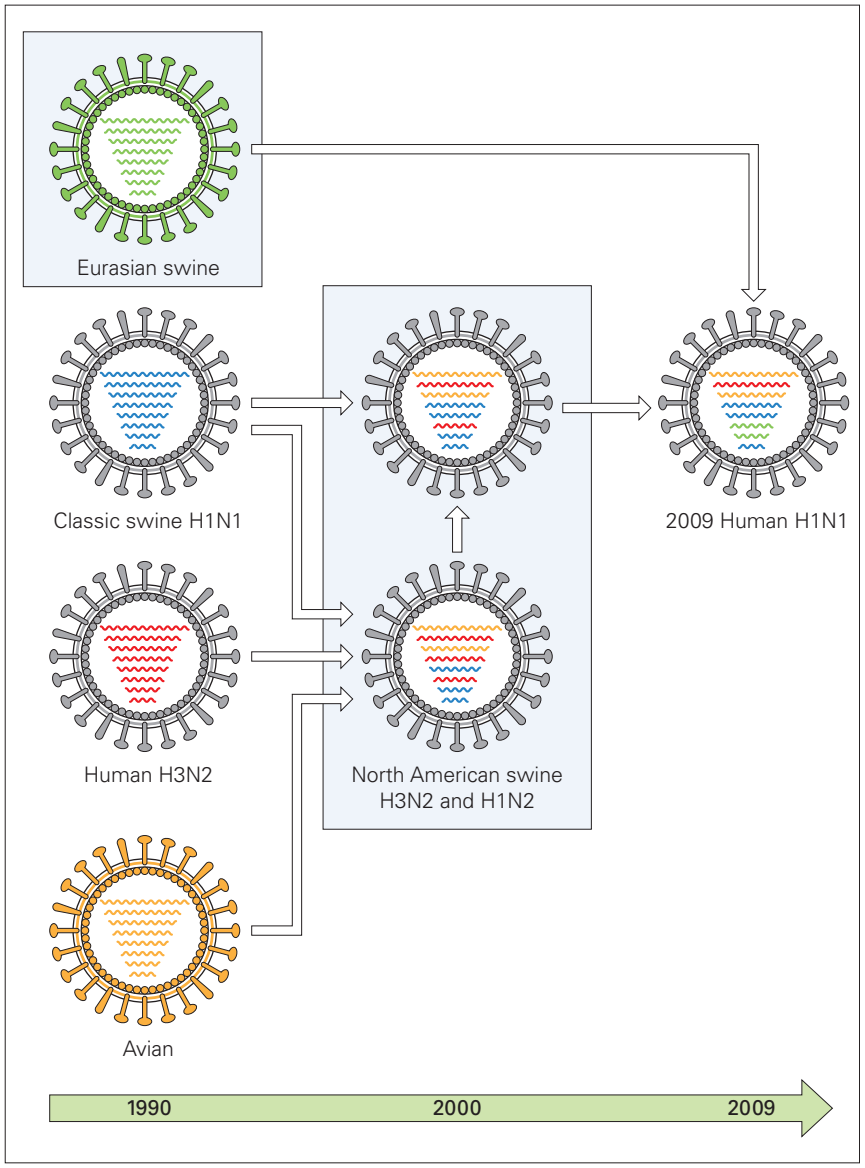


Figure 17.10 Antigenic shift in influenza. The major surface antigens of influenza virus are haemagglutinin and neuraminidase. Haemagglutinin is involved in attachment to cells, and antibodies to haemagglutinin are protective. Antibodies to neuraminidase are much less effective. The influenza virus can change its antigenic properties slightly (antigenic drift) or radically (antigenic shift). Pandemics can arise when there is antigenic shift with reassortment of genes. The diagram shows the origins of the 2009 pandemic influenza A (H1N1). The official influenza antigen nomenclature is based on the type of haemagglutinin (H_1, H_2, etc.) and neuraminidase (N_1, N_2, etc.) expressed on the surface of the virion. Note that, although new strains replace old strains, the internal antigens remain largely unchanged. (Redrawn from Trifonof V. et al., *N Engl J Med* [2009] 361: 115–119.)

Gene conversion can result in the relapsing infections

Gene conversion is thought to be responsible for the relapsing persistent course of certain other infections, including that by *Borrelia. B. borgdorferi* moves genes from 15 silent cassettes into the surface-bound lipoprotein gene, *vlsE*, in a process called segmental gene conversion, which results in diversity in six central regions of the *vlsE* gene. Gonococci also show great antigenic variation as they circulate through the host community, including through genetic recombination in their pilin genes. Here, there is recombination with a sequence from one of the many silent copies of *pilS* being transferred to the *pilE* gene using a gene conversion system involving a number of DNA repair genes including *RecA* and *RecF*.

Stage-specific antigens provide another useful strategy to evade immunity

Some parasites have more complicated life cycles than bacteria or viruses, and need to display state-specific molecules that are restricted to each part of the life cycle. So while the immune system is busy trying to recognize and respond to malaria antigens expressed on the invading sporozoites in the liver, parasites in the blood stage of infection express different antigens on the surface of infected red cells and merozoites. The sexual forms of the parasite that will continue the life cycle once back in the mosquito express further novel gametocyte antigens. Some of these stage-specific antigens are being targeted as malaria vaccines, although protection against the liver stage must be complete to prevent blood-stage infection.

IMMUNOSUPPRESSION

Many virus infections cause a general temporary immunosuppression

A large variety of microorganisms cause immunosuppression in the infected host. The mechanism is not fully understood, but it often involves invasion of the immune system by the pathogen – in other words, 'to evade, invade'. The host shows a depressed immune response to antigens of the infecting

Table 17.4 Depressed immune responses in microbial infections

Parasite	Feature of immunosuppression	Mechanism
Viruses		
HIV	↓Ab ↓CMI, long-lasting	↓CD4[a] T cells; immunosuppression by gp41; reduced antigen presentation by infected APC
Epstein–Barr virus	↓CMI, temporary	Includes polyclonal activation of infected B cells[a]
Measles	↓CMI, temporary[b]	Differentiation blocked in infected T and B cells[c]
Varicella-zoster virus, mumps	↓CMI, temporary	Infection of T cells
Bacteria		
M. leprae (lepromatous leprosy)	↓CMI	Polyclonal activation of B cells, production of IL-4 and IL-10
Protozoa		
Trypanosoma	↓Ab ↓CMI	Regulatory T cells, production of IL-10, ↓T cell proliferation
Plasmodia		Regulatory T cells, ↓antigen presentation
Toxoplasma		↓CD4 T cells, IFNγ
Leishmania		Production of IL-10 and TGFβ

[a]For HIV, the depressed responses are seen later, after initial neutralizing antibody and cytotoxic cell responses. There are many possible mechanisms involved in HIV immunosuppression, but decreased numbers of $CD4^+$ T cells is probably the most important.
[b]The *BCRF-1* gene of the virus codes for an IL-10-like molecule that enhances antibody rather than protective CMI responses.
[c]Patients with a positive tuberculin skin test become temporarily negative during measles infection. Measles also stops macrophages producing IL-12, a molecule needed for the Th1-type (protective) immune response.
Ab, antibody; APC, antigen-presenting cell; CMI, cell mediated immunity.

pathogen (antigen-specific suppression) or, more commonly, to both antigens of the infecting pathogen and unrelated antigens. HIV is one of the most spectacular, but by no means the only pathogen that interferes with the immune system in this way (Table 17.4). HIV causes death of $CD4^+$ T cells, resulting in a disastrous loss of T-cell function.

To induce antigen-specific immunosuppression would bring most benefit to the invading pathogen, but a general immunosuppression, as long as it is temporary, may give the pathogen enough time to grow, spread and be shed before being eliminated. This is what happens in many virus infections. A lasting general immunosuppression would be detrimental to the pathogen because susceptibility to other infections would cause unnecessary damage to the host species. From this point of view, HIV certainly overstepped the mark.

Different pathogens have different immunosuppressive effects

Immunosuppression by pathogens often involves actual infection of immune cells:

- T cells (HIV, measles)
- B cells (EBV)
- macrophages (HIV, CMV, leishmania)
- dendritic cells (HIV).

This may result in impaired cell function, (e.g. blocking of cell division, or of release of interleukin 2 (IL-2) (or other cytokines) or in cell death.

Additional immunosuppressive actions taken by pathogens include the release of immunosuppressive molecules. For instance, the gp41 polypeptide formed by HIV acts as an 'immunological anaesthetic', temporarily blocking T-cell function. Other pathogens (poxviruses, herpesviruses, *Trypanosoma cruzi*) release molecules that interfere with the action of complement or with immunologically important cytokines such as IL-2, IFNs (see above) or tumour necrosis factor (TNF).

Certain pathogen toxins are immunomodulators

A particularly dramatic form of immune interference is practised by the staphylococci. Many strains liberate exotoxins (staphylococcal enterotoxin, epidermolytic toxin and toxic shock syndrome toxin) that are responsible for disease. At first sight, producing these toxins seems to be of no advantage to the staphylococci, but it is now recognized that they have extremely powerful immunomodulatory actions – they are the most potent T-cell mitogens known, and act at picomolar concentrations. They function as 'superantigens' and, after binding to MHC class II molecules on antigen-presenting cells, act as polyclonal activators of T cells bearing particular families of genes in their T cell receptors (Fig. 17.11). A large proportion (2–20%) of all T cells then respond by dividing and releasing cytokines; only 0.001–0.01% are capable of doing this in response to a regular antigen. Similar molecules are produced by certain streptococci and mycoplasmas.

Possible mechanisms by which the staphylococcal toxins may interfere with immune defences include:

- excessive local liberation of cytokines by activated cells in a 'cytokine storm'
- killing of T cells or other immune cells
- diversion of T cells of all specificities into immunologically unproductive activity by polyclonal activation.

The fungus *Candida albicans* has recently been shown to produce a secreted toxin called candidalysin, which is necessary for

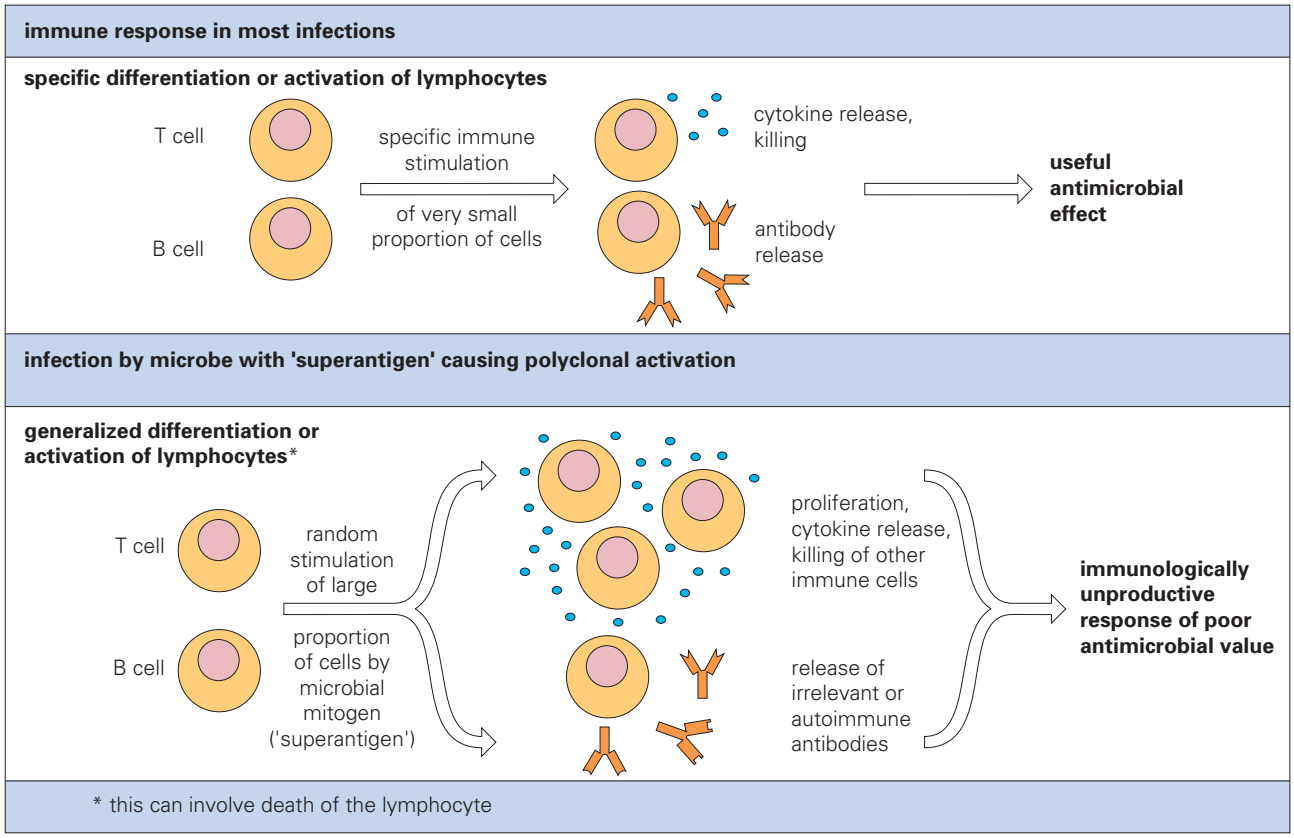


Figure 17.11 Microbial interference with the immune system by production of T-cell superantigens. Some microbial toxins will induce the proliferation of families of T cells resulting in activation of much larger number of cells than is seen with antigens. This leads to excessive release of cytokines and severe illness.

mucosal invasion by fungal hyphae. The toxin causes epithelial cell damage but also triggers a 'danger signal' alert to the immune system that this normally surface-colonizing yeast has become invasive.

Less dramatic polyclonal activation is seen in many other infections. Pathogens may cause polyclonal activation of B cells as well as T cells (e.g. in EBV and HIV infections), and this can be interpreted as an 'immunodiversion' by the infecting pathogen, or in the case of EBV as production of a supply of B cells in which the virus can grow. One consequence is that a range of 'irrelevant', sometimes autoimmune, antibodies are formed (e.g. heterophil antibodies in EBV infection). Finally, some persistent infections just wear out the immune system resulting in T-cell exhaustion, or immune senescence where the T cells become less good at proliferating, and lose the ability to make IL-2. CMV does this, as does HIV.

Successful pathogens often interfere with signalling between immune cells, with cytotoxic T-cell recognition or with host apoptotic responses

Many pathogens interfere with host molecules such as cytokines, chemokines, MHC, and apoptotic and complement receptors, all of which are essential components of host defence. Many DNA viruses code for fake molecules or fake cell receptors for the host molecules, and this disrupts the antimicrobial response. HSV produces a molecule, glycoprotein C, that functions as a receptor for C3b. It is present on the virus particle and on the infected cell and interferes with complement activation, protecting both the virus and the infected cell from destruction by antibody and complement.

EBV produces a homologue of IL-10, a cytokine initially called 'cytokine synthesis inhibitory factor'. Virulent strains of *Mycobacterium tuberculosis* induce IL-10 production by infected macrophages, which again favours the infecting pathogen. Furthermore, *M. tuberculosis*, as well as other intracellular organisms (*Leishmania major*, *Histoplasma capsulatum*) inhibit IL-12 production by the infected macrophages. Th1 T-cells are therefore not activated by IL-12 to form IFNγ, and the immune response is again diverted from the protective Th1 response.

Adenoviruses and herpesviruses prevent cytotoxic T cells from killing infected cells by reducing MHC class I expression and so antigen expression on the target cell.

A strategy useful for one pathogen is not necessarily good for others. For example, a local cell infected with a virus can commit suicide by undergoing apoptosis, a useful defence if it takes place before virus replication is complete. So certain viruses (HSV, EBV, HIV) code for proteins that interfere with apoptosis, permitting long-term infection of the cell. Other viruses, however, such as measles, induce apoptosis, as do certain bacteria (*Shigella flexneri*, *Salmonella*) after encountering macrophages, enabling them to escape destruction. It may be useful to induce apoptosis in one cell but not in another.

Thus, HIV inhibits apoptosis in the infected immune cell, but induces apoptosis in neighbouring uninfected cells.

Some pathogens interfere with the local expression of the immune response in tissues

Some pathogens do not interfere with the development of an immune response, but instead actively interfere with its expression in tissues; for instance *N. gonorrhoeae*, *Strep. pneumoniae* and many strains of *Haemophilus influenzae* liberate a protease that cleaves human IgA antibody. These bacteria are residents or invaders of mucosal surfaces where IgA antibodies operate, and the ability to produce such an enzyme seems unlikely to be mere coincidence.

PERSISTENT INFECTIONS

Persistent infections represent a failure of host defences

One way of looking at persistent infections (Table 17.5) is to regard them as failures of host defences that should control

Table 17.5 Examples of persistent infections in humans

Microorganism	Site of persistence	Infectiousness of persistent microorganism	Consequence	Shedding of microorganism to exterior
Viruses				
Herpes simplex	Dorsal root ganglia	–	Activation, cold sore	+
	Salivary glands	+	Not known	+
Varicella-zoster	Dorsal root ganglia	–	Activation, zoster	+
Cytomegalovirus	Lymphoid tissue	–	Activation ± disease	+
Epstein–Barr virus	Lymphoid tissue	–	Lymphoid tumour	–
	Epithelium	–	Nasopharyngeal carcinoma	–
	Salivary glands	+	Not known	+
Hepatitis B and C	Liver (virus shed into blood)	+	Chronic hepatitis: liver cancer	+
Adenoviruses	Lymphoid tissue	–	Not known	+
Polyomaviruses BK and JC (humans)	Kidney	–	Activation (pregnancy, immunosuppression)	+
T-cell leukaemia viruses	Lymphoid and other tissues	±	Late leukaemia, neurological disease	–
Paramyxovirus	Brain	±	Subacute sclerosing panencephalitis	–
HIV	Lymphocytes, macrophages	+	Chronic disease	+
Chlamydia				
Chlamydia trachomatis	Conjunctiva	+	Chronic disease and blindness	+
Rickettsia				
Rickettsia prowazekii	Lymph node	?	Activation	+
Bacteria				
Salmonella typhi	Gallbladder Urinary tract	+	Intermittent shedding in urine, faeces	+
Mycobacterium tuberculosis	Lung		Reactivation (immunosuppression, old age)	+
Treponema pallidum	Disseminated	±	Chronic disease	–
Parasites				
Plasmodium vivax	Liver	?	Activation, clinical malaria	+
Toxoplasma gondii	Lymphoid tissue, muscle, brain	±	Activation, neurological disease	–
Trypanosoma cruzi	Blood, macrophages	±	Chronic disease	–
Schistosoma mansoni	Gut	+	Chronic disease	Eggs
Filaria	Lymphatics, lymph nodes	+	Chronic disease	+

Shedding to the exterior takes place either directly, for example via skin lesions, saliva or urine, or indirectly via the blood (hepatitis B, malaria).

microbial growth and spread and eliminate the pathogen from the body. The pathogen may persist in a:

- defiant infectious form, as with hepatitis B in the blood or the schistosome in the blood vessels of the alimentary tract or bladder
- form with low or partial infectivity, for instance adenoviruses in the tonsils and adenoids
- metabolically altered state, such as latent *M. tuberculosis*
- completely non-infectious form.

Latent virus infections are classic examples of this type of persistence. For example, HSV DNA persists in sensory neurones in the dorsal root ganglia. During latency a single latency-associated transcript is highly expressed in the neurones, in contrast to the 80 or more proteins produced in the lytic cycle in epithelial cells. This latency-associated transcript plays a role in silencing the HSV lytic genes. The viral genome is not integrated with host DNA, and instead of being linear it is circular, and exists in free episomal form. It now seems that epigenetics (through which DNA transcription can be regulated) plays a major role in controlling viral latency. This impacts on the frequency of reactivation and in maintenance of the latent reservoir of virus.

Latent infections can become patent

Latent infections are so-called because they can become patent. This is where they become of great medical interest, and the legacy of latent herpesvirus infections in humans is described in Chapter 27. Different patterns of persistent infections are illustrated in Fig. 17.12. Persistent infections are important for four main reasons:

1. They can be reactivated and represent a reservoir of infection within the individual and community.
2. They are sometimes associated with chronic disease, as in the case of chronic hepatitis B infections, subacute sclerosing panencephalitis following measles, and AIDS.
3. They are sometimes associated with cancers, such as hepatocellular carcinoma with hepatitis B virus, and Burkitt's lymphoma and nasopharyngeal carcinoma with EBV.
4. From the microbial viewpoint, they enable the infectious agent to persist in the host community (Box 17.2).

Box 17.2 Lessons in Microbiology

Persistence is of survival value for the pathogen

Persistence without any further shedding, as occurs in subacute sclerosing panencephalitis and progressive multifocal leukoencephalopathy (see Ch. 25), is of no survival value, but there are obvious advantages if the pathogen is also shed, either continuously or intermittently. This is especially true when the host species consists of small isolated groups of individuals (Fig. 17.13). Measles, for instance, is not normally a persistent infection. It infects only humans, does not survive for long outside the body and has nowhere else to go (i.e. there is no animal reservoir). Without a continued supply of fresh susceptible humans, the virus could not maintain itself and would become extinct. At all times there must be an individual acutely infected with measles. From studies of island communities it is clear that a minimum of about 500 000 humans is needed to maintain measles without reintroduction from outside. In Palaeolithic times, when humans lived in small, isolated groups, measles could not have existed in its present form.

In contrast, persistent and latent infections are admirably adapted for survival under these circumstances. VZV can maintain itself in a community of <1000 individuals. Children get chickenpox, the virus persists in latent form in sensory neurones, and later in life the virus reactivates to cause shingles. By this time, a new generation of susceptible individuals has appeared and the shingles vesicles provide a fresh source of virus.

Serological studies show that the viral infections prevalent in small, completely isolated Indian communities in the Amazon basin are persistent or latent (e.g. due to adenoviruses, polyomaviruses, papillomaviruses, herpesviruses) rather than non-persistent (e.g. due to influenza, measles, poliovirus). The same principles apply to non-viral infections. Those present in small communities are either persistent/latent (e.g. typhoid, respiratory tuberculosis) or have an animal reservoir for maintenance of the pathogen.

Reactivation of latent infections

Reactivation is clinically important in immunosuppressed individuals

Reactivation occurs in immunocompromised patients, and is of major clinical importance in those immunosuppressed as a result of chronic infection and disease, such as HIV and AIDS, tumours, including leukaemias and lymphomas, or in those immunosuppressed following transplantation. Reactivation also occurs during naturally occurring periods of immunocompromise, the most important of these being pregnancy and old age. From the pathogen's point of view, latency is an adaptation that allows reactivation with renewed growth and shedding of the infectious agent during these naturally occurring periods.

Features of reactivation in herpesvirus infections are described in Chapters 22 and 27.

New imaging techniques have shown that latency in tuberculosis is a more active state than was previously recognized, with some granulomas in the lung showing metabolic activity while others are more silent. *M. tuberculosis* needs to make certain proteins to keep itself in a latent state. Other products – called resuscitation-promoting factors – are needed to reactivate latent *M. tuberculosis*. There is interest in identifying gene expression signatures that would predict those individuals progressing from latent to active tuberculosis, so that they could be treated before they infect others.

It is useful to distinguish two stages in viral reactivation

The first event in reactivation (Fig. 17.14), the resumption of viral activity in the latently infected cell, involves transcription of immediate-early genes. In the case of HSV, this can be triggered by sensory stimuli arriving in the neurone from skin areas responding to sunlight, by trauma such as dental procedures, by other infections or by hormonal influences.

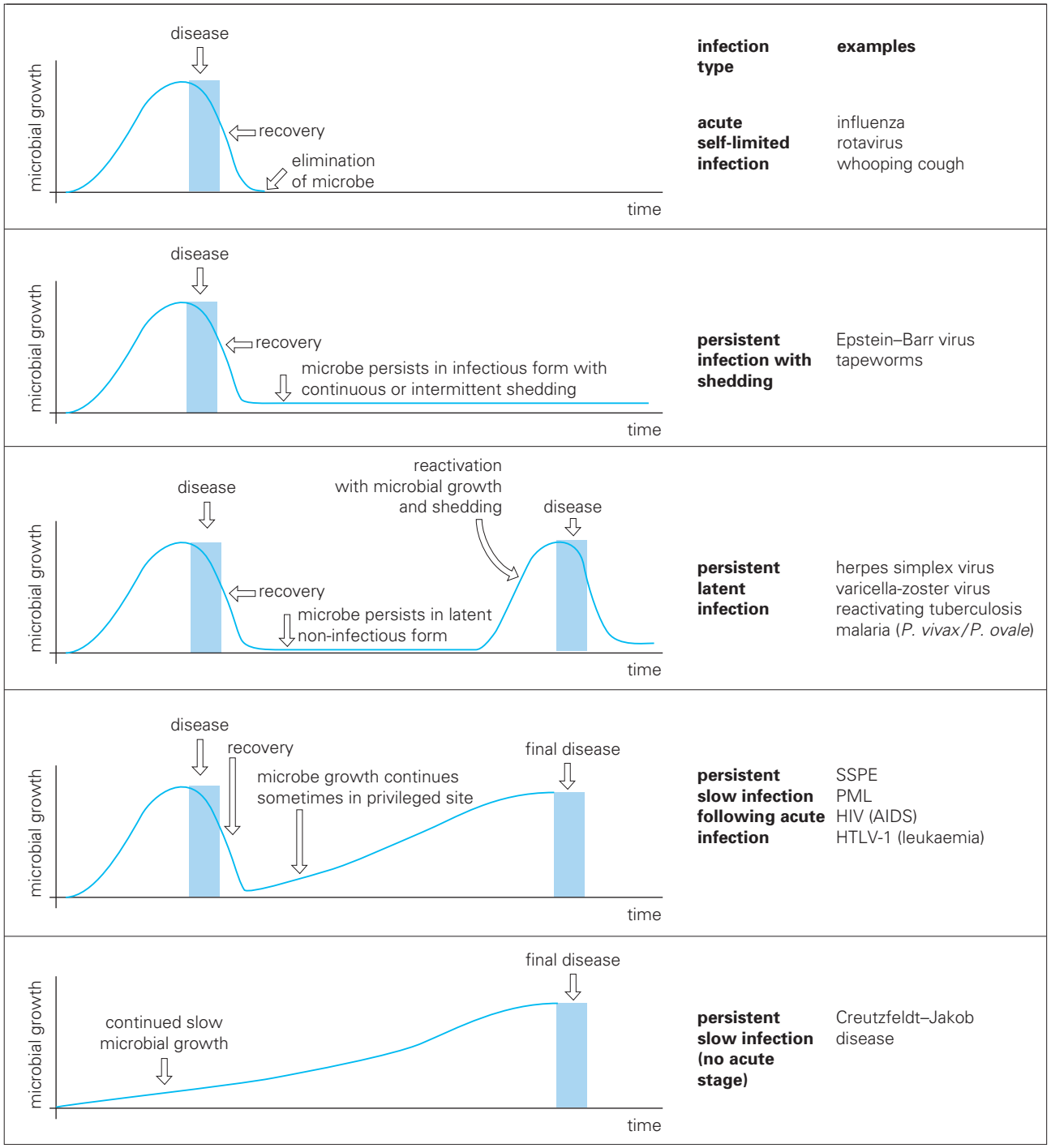


Figure 17.12 Patterns of acute and persistent infections. For some pathogens (e.g. cytomegalovirus), the distinction between persistence in infectious form and true latency is not clear. HIV, human immunodeficiency virus; HTLV-1, human T-cell leukaemia virus 1; PML, progressive multifocal leukoencephalopathy; SSPE, subacute sclerosing panencephalitis.

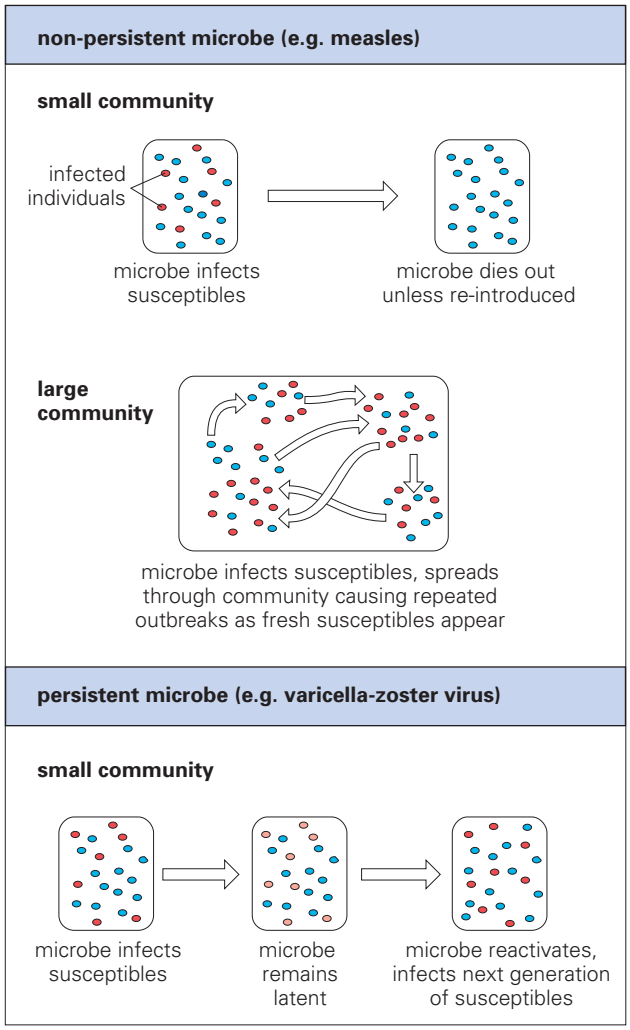


Figure 17.13 Persistence is a microbial survival strategy.

The second event involves the spread and replication of the reactivated virus. HSV must travel down the sensory axon to the skin or mucosal surface, infect and spread in subepithelial tissues and then in the epithelium, finally forming a virus-rich vesicle (>1 million infectious units / mL of vesicle fluid). All this takes at least 3–4 days. This second stage is less mysterious than the first and can be controlled by the immune system. Therefore, cold sores may be associated with poor lymphocyte responses to HSV antigens, and zoster with declining cell-mediated responses to VZV antigens in older people.

The first stage probably occurs more frequently, because immune defences often arrest the process during the second stage before final production of the lesion. As many as 10–20% of HSV reactivation episodes are thought to be 'non-lesional' with burning, tingling and itching at the site, but no signs of a cold sore. Also, zoster may involve no more than the sensory prodrome associated with virus reactivation and replication in sensory neurones; skin lesions are prevented by host defences.

Reactivation of EBV and CMV with appearance of the virus in saliva or blood is generally asymptomatic. In immunologically deficient individuals, however, reactivation may progress to cause clinical disease: either hepatitis and pneumonitis in the case of CMV, or post-transplant lymphoma and the rarer hairy tongue leukoplakia due to EBV (see Ch. 31).

Pathogens are clever and often use a number of these evasion strategies

The most successful pathogens have evolved multiple ways by which to interfere with what would otherwise be damaging immune responses. Cytomegalovirus can interfere with dendritic cell maturation, and so antigen presentation, antibody-mediated immunity, cytokine function, and with apoptosis. CMV infection may mostly be silent, but is quietly pushing otherwise useful T cells towards exhaustion and senescence. The only good thing is that through understanding these evasion mechanisms we have learnt more about how the immune system works.

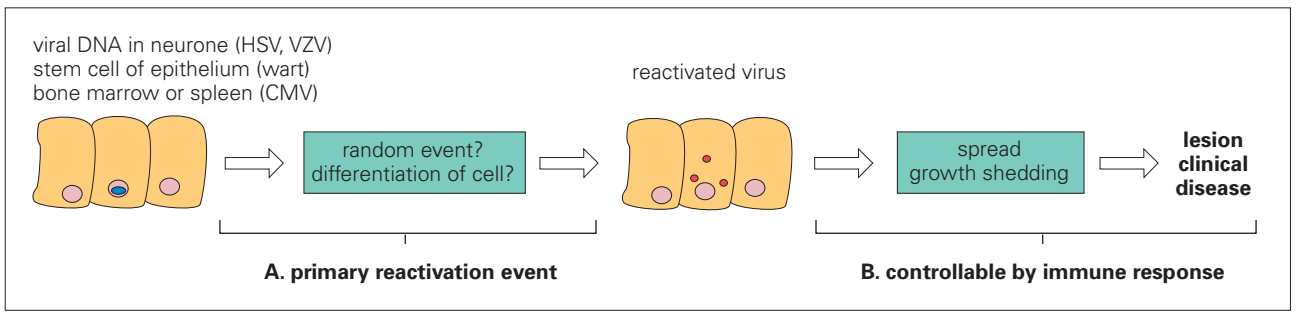


Figure 17.14 Two stages in reactivation of latent viruses. CMV, cytomegalovirus; HSV, herpes simplex virus; VZV, varicella-zoster virus.

KEY FACTS

- Many successful parasites have adopted strategies for evading immune responses. These enable them to stay in the body long enough to complete their business of infection and shedding to fresh hosts. Some parasites persist indefinitely in the body.
- Mechanisms of immune evasion include:
  - concealing parasite antigens from the host (staying inside host cells, infecting 'privileged sites')
  - changing parasite antigens, either in the infected individual (e.g. trypanosomiasis) or during spread through the host population (e.g. influenza)
  - direct action on immune cells (e.g. HIV on $CD4^+$ T cells) or on immune signalling systems (e.g. production of fake cytokine molecules)
  - local interference with immune defences (production of IgA proteases, Fc receptors).
- During some persistent infections, the pathogen may continue to multiply and be able to infect others (e.g. HIV, hepatitis B).
- In other persistent infections, the pathogen enters into a latent state and later in life reactivates with renewed multiplication and the ability to infect others (e.g. herpesviruses, *M. tuberculosis*).

18 Pathological consequences of infection

Introduction

Infections can cause a range of unwanted symptoms, sometimes caused by the microbe itself and sometimes by the immune response in response to infection. Inflammation is beneficial but also unpleasant. A range of other hypersensitivity responses can be damaging to the host. Some viruses can even cause cancer. We will now review these unwanted results of infections and how the immune system responds.

Symptoms of infections are produced by the microorganisms or by the host's immune responses

Symptoms that appear rapidly after the acquisition of an infection are usually due to the direct action of molecules secreted by the invading microbe. A virus in a cell may cause metabolic 'shut-down' or lyse the cell. Bacteria, however, provoke most of their acute effects by releasing toxins, but may also cause distress by inducing inflammation. The inflammatory response is, of course, an important component of host protection, vascular permeability being vital for the rapid mobilization of cells such as neutrophils, and serum components such as complement and antibody. Inflammation is therefore intrinsically a healthy sign, and it is interesting that some virulent bacteria (e.g. staphylococci) try to inhibit the inflammatory response.

Pathological changes are often secondary to the activation of immunological mechanisms that are normally thought of as protective

These may involve the innate or the adaptive immune system or, more usually, both (Fig. 18.1). Tissue damage resulting from adaptive immune responses is usually referred to as 'immunopathology' and is quite common in infectious diseases, particularly those that are chronic and persistent. The immunological basis of these mechanisms of tissue damage is described in Chapter 15.

Certain viruses can cause permanent malignant changes in cells as a result of direct, indirect and a mixture of both types of mechanisms. Seven viruses that infect humans cause up to 15% of human cancers around the world. These include human T-cell lymphotropic virus type 1 (HTLV-1; lymphomas, leukaemias), Epstein–Barr virus (EBV; nasopharyngeal carcinoma and Burkitt's lymphoma), human papillomaviruses (cervical cancer), hepatitis B and C virus infections (liver cancer), HIV (immunosuppression leads to the development of cancers associated with Kaposi's sarcoma-associated herpes virus [KSHV] and EBV) and Merkel cell polyomavirus (Merkel cell carcinoma of the skin). Co-factors may be involved. Immunization programmes for hepatitis B and human papillomaviruses should now reduce the incidence of liver and cervical cancer, respectively.

PATHOLOGY CAUSED DIRECTLY BY MICROORGANISMS

Direct effects may result from cell rupture, organ blockage or pressure effects

Organisms that multiply in cells and subsequently spread usually do so by rupturing the cell. Many viruses and some intracellular bacteria and protozoa behave in this way (Table 18.1) but many others do not. For example, viruses or bacteria may remain latent (e.g. herpes simplex virus and varicella-zoster virus in nerve ganglia, and *Mycobacterium tuberculosis* in macrophages), and many viruses can bud from a cell without disrupting it. The type of cell infected may also have an influence on survival of the organism. Thus, although HIV causes lysis of CD4 T cells, macrophages are more resistant to both infection and lysis. Other direct effects include:

- blockage of major hollow viscera by worms
- blockage of lung alveoli by dense growth of e.g. *Pneumocystis*
- mechanical effects of large cysts (e.g. hydatid).

Mode of action of toxins and consequences

These can be considered under five headings (Fig. 18.2).

Exotoxins are a common cause of serious tissue damage, especially in bacterial infection

The pathogen may actively secrete 'exotoxins' (Table 18.2). In some cases, these are clearly part of its strategy for entry, spread or defence against the host, but sometimes they seem to be of little or no benefit to the pathogen.

Most exotoxins are proteins and are often coded not by the bacterial DNA, but in plasmids (e.g. *E. coli*) or phages (e.g. botulism, diphtheria, scarlet fever). In some cases, they consist of two or more subunits, one of which is required for binding and entry to the cell while the other switches on or inhibits some cellular function.

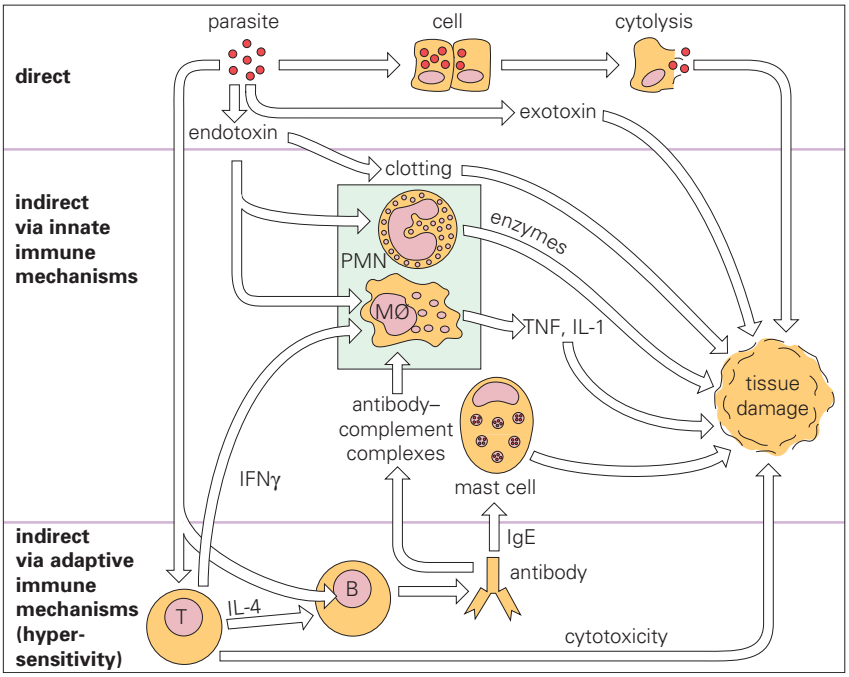


Figure 18.1 Pathological effects of infection: a general scheme. Infectious parasitic organisms can cause disease directly (top) or indirectly via overactivation of various immune mechanisms, either innate (middle) or adaptive (bottom). IFN, interferon; IgE, immunoglobulin E; IL, interleukin; Mϕ, macrophage; PMN, polymorphonuclear leukocyte; TNF, tumour necrosis factor.

Table 18.1 Examples of organisms that directly damage tissue

Organism	Cell or tissue damaged	Mechanism
Viruses		
Poliovirus	Neurones	Cytopathic
Rhinovirus	Lower respiratory tract epithelium	
HIV	CD4 T cells, macrophages	
Coxsackievirus	Pancreatic beta cells, cardiac cells	
Rotavirus	Enterocytes	
Bacteria		
Streptococcus mutans	Teeth	Acid production
Fungi		
Histoplasma	Macrophages	Damaged macrophage releases cytokines
Protozoa		
Plasmodium	Erythrocytes	Damaged erythrocyte removed
Helminths		
Ascaris	Intestinal occlusion	Mechanical
	Biliary occlusion	Mechanical, inflammation
Echinococcus	Hydatid cyst	Pressure effects

Many organisms directly damage or destroy the tissues they infect. This is especially common with cytopathic viruses.

Powerful toxins are generally secreted from extracellular pathogens. Microbes that multiply in cells cannot afford to cause serious damage at too early a stage, and such toxins therefore tend to be less prominent in intracellular infections due to *Mycobacteria*, *Chlamydia* or *Mycoplasma*. For example, leprosy patients with lepromatous disease can live with huge bacterial loads for many years. Although many toxins can kill host cells, lower concentrations may be important by causing dysfunction in immune or phagocytic cells. For example, concentrations of streptolysin well below the cell-killing level will inhibit leukocyte chemotaxis, and the staphylococcal enterotoxin and epidermolytic toxins also have

Table 18.2 Some important exotoxins in disease

Organism	Exotoxin	Tissue damaged	Action	Disease
Bacteria				
Clostridium tetani	Tetanus toxin	Neurones	Spastic paralysis	Tetanus
Clostridium botulinum	Neurotoxin	Nerve–muscle junction	Flaccid paralysis	Botulism
Corynebacterium diphtheriae	Diphtheria toxin	Throat, heart, peripheral nerve	Inhibits protein synthesis	Diphtheria
Shigella dysenteriae	Enterotoxin	Intestinal mucosa	Destroys mucosal cells	Dysentery
E. coli (EHEC)	Enterotoxin	Intestinal epithelium	Fluid loss from intestinal cell	Gastroenteritis
Vibrio cholerae	Enterotoxin	Intestinal epithelium	Fluid loss from intestinal cell	Cholera
Staphylococcus aureus	α-haemolysin	Red and white cells (via cytokines)	Haemolysis	Abscesses
	Enterotoxins[a]	Intestinal cells	Induces vomiting, diarrhoea	Food poisoning
	TSST1	T cells	Release of cytotoxins	Toxic shock syndrome
Streptococcus pyogenes	Streptolysin O and S	Red and white cells	Haemolysis	Haemolysis, pyogenic lesions
	Erythrogenic	Skin capillaries	Skin rash	Scarlet fever
Bacillus anthracis	Cytotoxin	Lung	Pulmonary oedema	Anthrax
Bordetella pertussis	Pertussis toxin	Trachea	Kills epithelium	Whooping cough
Listeria monocytogenes	Haemolysin	Leukocytes, monocytes	Cell lysis	Listeriosis
Fungi				
Aspergillus fumigatus	Aflatoxin	Liver	Carcinogenic	? Liver damage / cancer[b]
Protozoa				
Entamoeba histolytica	Enterotoxin	Colonic epithelium	Cell lysis	Amoebic dysentery

Many bacteria and a few other organisms damage host tissues by secreting exotoxins, some examples of which are shown here. Some bacterial exotoxins are among the most powerful toxins known. Vaccination, by inducing antibody, is often very effective in protection.

[a] *Staph. aureus* has five enteroxins: SEA, SEB, SEC, SED and SEE. TSST1, toxic shock syndrome toxin. Staphylococcal entertoxins and TSST-1 are superantigens that activate T cells expressing particular Vβ genes in their T-cell receptors.

[b] In turkeys and pigs from *A. fumigatus*-contaminated ground nuts, but not so far in humans.

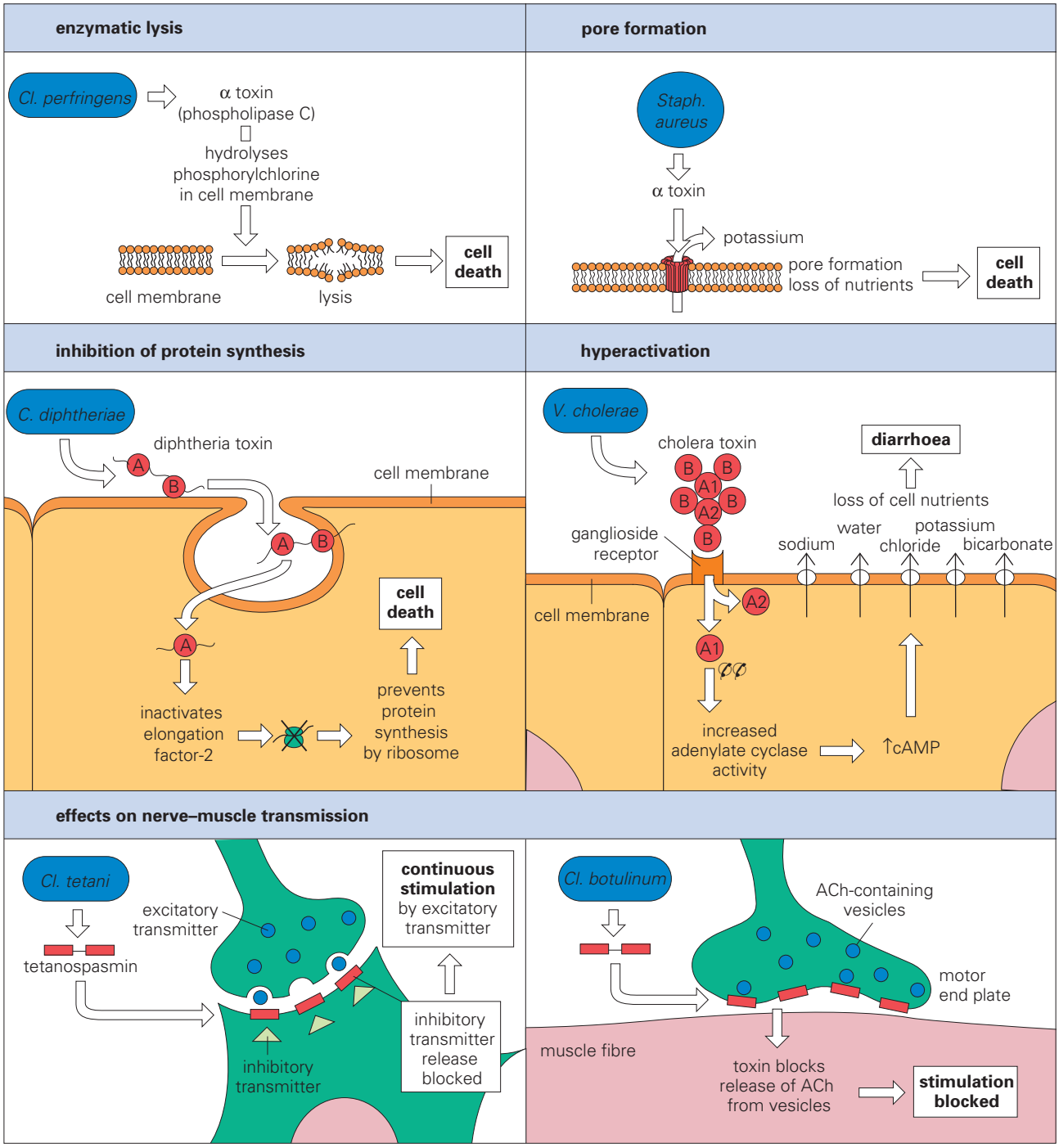


Figure 18.2 The mode of action of some exotoxins. Bacterial toxins act in a variety of ways. Often the toxin is a two-chain molecule, one chain being concerned with entry into cells while the other has inhibitory activity against some vital function. ACh, acetylcholine; cAMP, cyclic adenosine monophosphate; C, *Corynebacterium*; Cl, *Clostridium*; Staph, *Staphylococcus*; V, *Vibrio*.

immunomodulatory activity at exceedingly low (nanogram to picogram) levels.

Bacteria may produce enzymes to promote their survival or spread

A number of bacteria release enzymes that break down the tissues or the intercellular substances of the host, allowing the infection to spread freely. Among these enzymes are hyaluronidase, collagenase, DNase and streptokinase. Some staphylococci release a coagulase, which deposits a protective layer of fibrin onto and around the cells, thus localizing them.

Toxins may damage or destroy cells and are then known as haemolysins

Cell membranes can be damaged enzymatically by lecithinases or phospholipases, or by insertion of pore-forming molecules, which destroy the integrity of the cell. The collective term

for such toxins is 'haemolysins', although many cells other than red blood cells can be affected. Both staphylococci and streptococci produce pore-forming toxins; pseudomonads release enzymatic haemolysins. The staphylococcal alpha haemolysin is secreted as a soluble monomer but binds to a membrane protein to form a heptamer, making a beta-barrel pore in the membrane.

Toxins may enter cells and actively alter some of the metabolic machinery

Characteristically, these toxin molecules have two subunits. The A subunit is the active component, while the B subunit is a binding component needed to interact with receptors on the cell membrane. When binding occurs, the A subunit, or the whole toxin–receptor complex, is taken into the cell by endocytosis, and the A subunit becomes activated. Two well-studied toxins of this type are those of diphtheria (see Ch. 20) and cholera.

Diphtheria toxin blocks protein synthesis

Diphtheria toxin is synthesized as a single polypeptide and binds by the B subunit to target cells (Fig. 18.2). The polypeptide is partially cleaved and then the entire toxin–receptor complex is internalized. The A subunit then splits off and passes into the cytosol, where it inactivates the transfer of amino acids from transfer RNA to the polypeptide chain during translation of mRNA by ribosomes. It does this by catalysing attachment of adenosine diphosphate (ADP) ribose to the elongation protein (ADP ribosylation), effectively blocking protein synthesis.

Cholera toxin results in massive loss of water from intestinal epithelial cells

Cholera toxin is released as a complex of five B subunits surrounding the A subunit. The latter is cleaved into two fragments: A1 and A2, held by disulphide bonds. The B subunits bind to ganglioside receptors on intestinal epithelial cells, leading to internalization of the A subunits, which then separate from one another (Fig. 18.2). The Al portion then ADP-ribosylates one of the regulatory molecules involved in the production of cyclic adenosine monophosphate (cAMP). As a result, the regulatory molecule is unable to turn off cAMP production. The increased levels of cAMP in the cell change the sodium / chloride flux across the cell membrane, resulting in a massive outflow of water and electrolytes from the cell and causing the profuse diarrhoea of cholera. The exotoxins of *E. coli* and salmonella have similar actions, as does pertussis toxin.

Tetanus and botulinum toxins are among the most potent affecting nerve impulses

These toxins are extremely potent and active at low doses. Tetanus and botulinum toxins have the characteristic A+B structure, the B subunit binding to ganglioside receptors on nerve cells. The internalized A subunit of tetanus is carried by axonal transport from the point of production to the central nervous system (CNS), where it interferes with synaptic transmission in inhibitory neurones by blocking neurotransmitter release. This allows the excitatory transmitter to continuously stimulate the motor neurones, causing spastic paralysis. Botulinum toxin enters the body via the intestine, escaping digestion and crossing the gut wall. The toxin affects peripheral nerve endings at the neuromuscular junction, blocking presynaptic release of acetylcholine. This prevents muscle contraction, causing flaccid paralysis.

Inactivation of toxins without altering antigenicity results in successful vaccines

Toxins can often be inactivated (e.g. by formaldehyde) without altering their antigenicity, and the resulting toxoids are among the most successful of all vaccines (see Ch. 35), the classic examples being diphtheria and tetanus toxoids. Toxins are generally more highly conserved in their structure than the surface antigens of the organism secreting them. This allows for more effective cross-immunity and explains, for example, why scarlet fever (caused by streptococcal erythrotoxin) usually occurs only once, whereas streptococcal infections recur almost indefinitely.

Toxins as magic bullets

An interesting offshoot of the two-subunit structure of toxins is that, by changing the specificity of the part responsible for attachment, the specificity of the toxin for a particular cell type can be changed. An example is the plant toxin ricin – the A subunit can be attached to a monoclonal antibody to make it a specific poison for tumour cells, and the toxin could also be delivered to cancer cells by nanoparticles. The same strategy could obviously be used against parasites if desired.

DIARRHOEA

Diarrhoea is an almost invariable result of intestinal infections

Diarrhoea is one of the major causes of death in children worldwide, with rotavirus as the main culprit (see Ch. 23). In industrialized regions, bacterial pathogens such as *Campylobacter* and non-typhoidal *Salmonella* are increasingly important, and *Clostridium difficile* and norovirus infections are a problem in hospitals, particularly in the elderly. Another culprit is the enterotoxigenic strains of *E.coli*, which can produce a heat-stable toxin (ST) and a heat-labile toxin (LT-I); diarrhoea can be caused by *E.coli* strains that produce one or both of these toxins.

Diarrhoea can be considered as:

- a means for the host to rid itself rapidly of the infectious organism
- a means for the infection to spread to other hosts.

Diarrhoea is a feature of a wide range of organisms, but in only a few cases is the exact mechanism understood. While toxins are often the cause (e.g. cholera, shigella), microbial invasion and damage to epithelial cells may also be important. The pathophysiology, with changes in electron transport or loss of enterocytes, has been elucidated in some cases. Many of the organisms causing diarrhoea can be 'picked up' from food, but the term 'food poisoning' is usually reserved for those cases where toxins are already present in the food rather than being generated during the growth of organisms in the intestine (Fig. 18.3). As would be expected, 'food poisoning' causes symptoms earlier – that is, hours after exposure rather than days (Table 18.3). Some viruses, especially norovirus infections, sometimes referred to as causing 'winter vomiting disease',

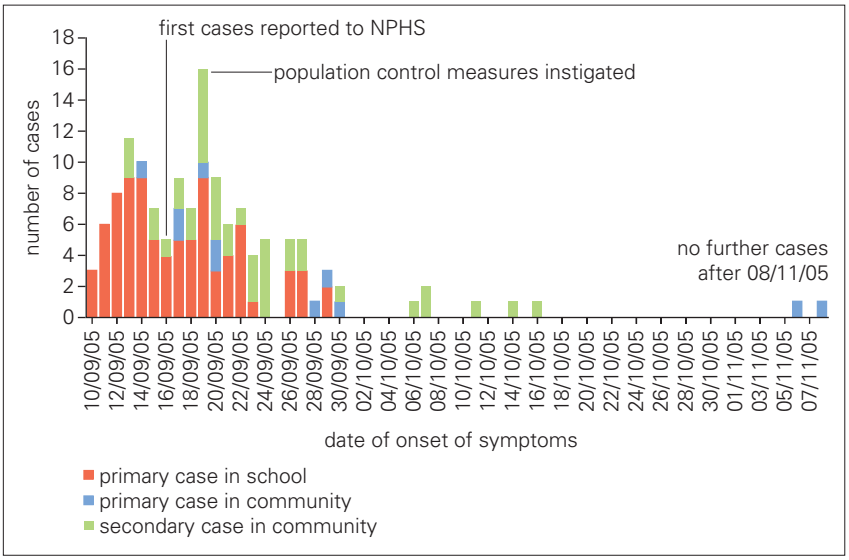


Figure 18.3 Outbreak of bloody diarrhoea caused by enterohaemorrhagic *E. coli* (EHEC) 0157 in South Wales, in 2005. The verotoxin produced by EHEC causes diarrhoea and is similar to the *Shigella* toxin. The first cases had all eaten school dinners containing cooked meats from a single supplier. Of the total 157 reported cases, 65% were in school-aged children. Thirty-one people were admitted to hospital and one child died. NPHS, National Public Health Service. (Redrawn from: The Public Inquiry into the September 2005 Outbreak of *E. coli* O157 in South Wales. Chairman H. Pennington, March 2009. http://wales.gov.uk/ecolidocs/3008707/reporten.pdf?skip=1&lang=en.)

Table 18.3 Infectious causes of diarrhoea

	Onset	Source
Food poisoning (due to pre-formed toxin in food)		
Staphylococcus aureus	1–6 h	Cream, meat, poultry
Clostridium perfringens	8–20 h	Reheated meat
Clostridium botulinum	12–36 h	Canned food
Bacillus cereus	1–20 h	Reheated foods
Intestinal infections		
Rotavirus	2–5 days	Faecal–oral
Norovirus	1–2 days	Faecal–oral
Salmonella	1–2 days	Eggs, food
Clostridium difficile	1–2 days	Faecal–oral
Shigella	1–4 days	Faecal–oral
Campylobacter	1–4 days	Poultry, domestic animals
Vibrio cholerae	2 days	Faecal–oral
Escherichia coli	1–4 days	Food
Yersinia enterocolitica	Days–weeks	Pets (e.g. dogs)
Giardia lamblia *Entamoeba histolytica*	1–2 weeks days–weeks	Contaminated water
Cryptosporidium *Isospora belli*	Days–weeks	Faecal–oral opportunistic (e.g. in AIDS)

Worldwide, infectious diarrhoea is the major cause of infant mortality.

cause outbreaks of diarrhoea and vomiting, particularly in closed groups or communities – such as in hospitals or on cruise ships; in England in 2015 / 2016 there were 490 reported hospital outbreaks, 95% of which led to ward or bay closures.

PATHOLOGICAL ACTIVATION OF NATURAL IMMUNE MECHANISMS

Overactivity can damage host tissues

The very potent innate immune mechanisms discussed in Chapter 15 have inbuilt safety as far as specificity is concerned. They have had to evolve in the constant presence of the host's 'self' antigens, to which they do not therefore respond. However, they are not so well controlled quantitatively, and there are many cases when overactivity damages not only an invading parasite, but also innocent host tissues. The expression of natural immunity often causes a certain amount of inflammation – and this can be severe, with tissue damage. Complement, polymorphs and tumour necrosis factor (TNF) play important roles.

Microbial endotoxin activates the immune system and induces cytokines, causing a bewildering variety of biological

effects (Fig. 18.4). At the clinical level, it can be responsible for septic shock.

Endotoxins are typically lipopolysaccharides

'Endotoxins' of bacteria and other microorganisms have a deceptively similar name to exotoxins, but are profoundly different in their significance. Unlike exotoxins, these are integral parts of the microbial cell wall and are normally released only when the cell dies. Endotoxins are particularly characteristic of Gram-negative bacteria. A typical lipopolysaccharide (LPS) endotoxin is composed of:

- a conserved lipid portion (lipid A) inserted into the cell wall, responsible for much of the toxic activity
- a conserved core polysaccharide
- the highly variable *O*-polysaccharide, responsible for the serological diversity which is a feature of organisms such as salmonellae and shigellae.

LPSs stimulate an extraordinary range of host responses – or perhaps one should say a wide range of responses have evolved to respond to LPSs. These include LPS-binding protein (the LPS–LPS binding protein complex then binds to CD14 on macrophages and dendritic cells) and TLR4 (see Ch.10). In the words of Lewis Thomas, 'when we sense lipopolysaccharide, we are likely to turn on every defence at our disposal' (Fig. 18.4). Evidently, the body needs to be aware of invading Gram-negative bacteria at the earliest possible stage.

Clinically, the most important effects of LPS are:

- fever
- vascular collapse (or shock).

As mentioned in Chapter 15, fever may benefit host or parasite, or both, and is currently considered to be mainly due to the action of two cytokines, interleukin 1 (IL-1) and TNF, on the hypothalamus. Both these cytokines are produced by macrophages in response to LPS (and to analogous molecules from other organisms; see below and Box 18.1).

Endotoxin shock is usually associated with systemic spread of organisms

The commonest example of endotoxin (or 'septic') shock is septicaemia with Gram-negative bacteria such as *E. coli* or *Neisseria meningitidis*. However, many other organisms also release molecules that stimulate TNFα and / or IL-1 production (Table 18.4) and therefore function in part like LPS, although they are more or less unrelated in structure. In the 'toxic shock syndrome' of young women with staphylococcal infections of the genital tract, toxic shock syndrome toxin (TSST1) is the mediator; it acts as a superantigen, activating a large proportion of all T cells (up to 1 in 5, see Ch. 17) that express particular Vβ genes in their T-cell receptors. Activating an enormous number of T cells produces enough cytokine to cause the toxic effect.

Septic shock, however, is a complex phenomenon, and other bacterial components, such as peptidoglycans, may also play a part. Disseminated intravascular coagulation (DIC), hypoglycaemia and cardiovascular failure are all features of septic shock. In streptococcal infections, the culprits are pyrogenic (erythrogenic) exotoxins released by the bacteria.

The involvement of cytokines in the pathogenesis of shock is by no means a purely academic concern, because it suggests the possibility of treatment by antagonists of a small number of cytokines (e.g. by monoclonal antibodies or inhibitors), rather than by antibodies to the toxins themselves, which are of enormous antigenic diversity. Anti-TNF monoclonal antibodies are now used to treat rheumatoid arthritis.

The cytokine most closely linked to disease is TNF

Raised concentrations of TNFα in the serum have been shown to correlate with severity in patients with meningococcal

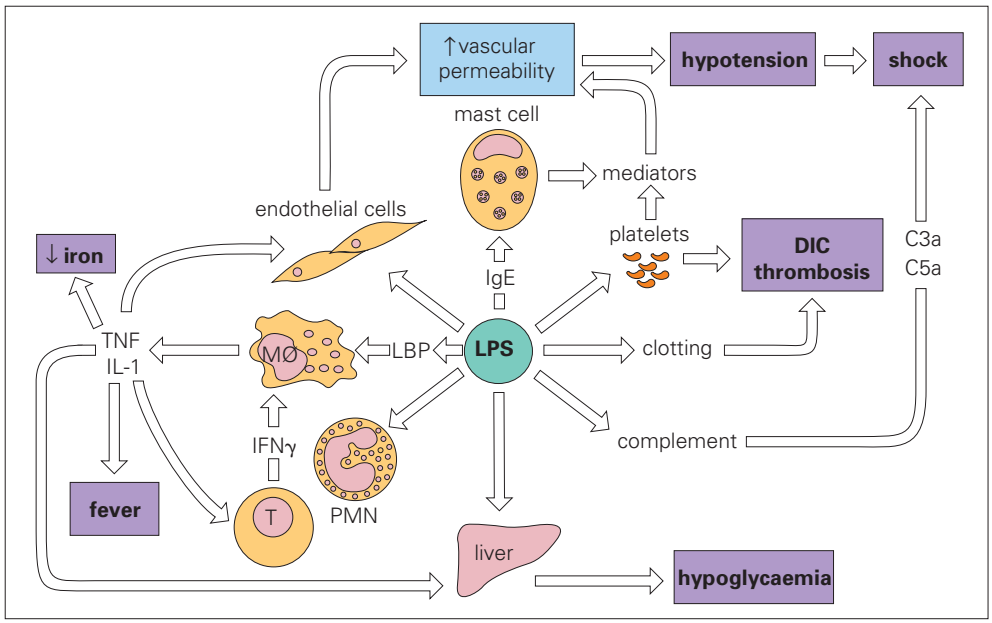


Figure 18.4 The many activities of bacterial endotoxin. Lipopolysaccharide (LPS) activates almost every immune mechanism as well as the clotting pathway and, as a result, LPS is one of the most powerful immune stimuli known. DIC, disseminated intravascular coagulation; IFN, interferon; IL, interleukin; LBP, LPS binding protein; Mϕ, macrophage; PMN, polymorphonuclear leukocyte; TNF, tumour necrosis factor.

Box 18.1 Lessons in Microbiology

Is it a cold – or is it flu?

The common cold is usually caused by a rhinovirus, or a coronavirus. Real influenza, caused by the influenza virus, usually has a more sudden onset and the combination of fever and a cough has a predictive value of around 80%. But what causes the symptoms of sore throat, sneezing, nasal discharge and nasal congestion?

Sore throat symptoms are thought to be caused by prostaglandins and bradykinin acting on sensory nerve endings in the airway. Sneezing is triggered by inflammatory mediators in the nose and nasopharynx acting on the trigeminal nerves. The plasma-rich exudate that forms part of the nasal discharge can change from clear to yellow / green during an upper respiratory infection. The colour reflects the recruitment of leukocytes into the airway lumen. If large numbers of leukocytes are present, the green protein myeloperoxidase found in the azurophil granules of neutrophils gives the discharge a green colour. Nasal congestion occurs later in infection, when inflammatory mediators such as bradykinin cause the large veins in the nasal epithelium to dilate. Common cold viruses do not cause such damage to the airway epithelium and infection may not create a cough – but influenza usually causes serious damage to the respiratory epithelium. Fever is mainly caused by the interleukins IL-1 and IL-6. It also seems that cytokines are responsible for muscle aches and pains, by causing the breakdown of muscle proteins. Of course, tumour necrosis factor was originally called cachexin, because of its ability to cause muscle wasting or cachexia.

Sometimes, in past flu epidemics, such as the Spanish flu epidemic in 1918, people died very quickly, within a few days of infection – which seems too fast for secondary infections to be responsible. Reconstructed viruses with the same haemagglutinin and neuraminidase seem to cause severe inflammation and it is possible that excessive cytokine release, in a 'cytokine storm', caused the pathology.

Table 18.4 Important endotoxins and functionally related molecules that induce TNF

Organisms	Toxin
Bacteria	
Gram-negative	
Salmonella *Shigella* *Escherichia coli* *Neisseria meningitidis*	LPS
Gram-positive	
Staphylococcus aureus	TSST1
Mycobacteria	Lipoarabinomannan
Bordetella pertussis	Endotoxin
Fungi	
Yeasts	Zymosan
Protozoa	
Plasmodium	Phospholipids (exoantigens)

Most endotoxins are lipopolysaccharides (LPS) and exert their main effects by stimulating cytokine release. LPS can also induce the secretion of other cytokines such as interleukin 1. TSST1, toxic shock syndrome toxin.

septicaemia and with *Plasmodium falciparum* malaria. However, animal experiments indicate that, in such cases, TNFα probably synergizes with other cytokines such as IL-1 and interferon gamma (IFNγ), to produce its full effects. In meningococcal disease, TNFα concentrations in blood and cerebrospinal fluid (CSF) can change independently, the former being raised in septicaemia and the latter in meningitis; it therefore appears that the production and / or effects of TNFα can be restricted to a particular body compartment.

In some cases, it may be worth suppressing inflammation with steroids (e.g. a randomized trial in which dexamethasone was given to patients with acute bacterial meningitis showed that corticosteroid reduced mortality). The immune system itself also tries to control inflammation during sepsis by producing anti-inflammatory mediators such as IL-10 and TGFβ.

There may also be strain differences in the ability of bacteria to induce inflammation; *Haemophilus influenzae* strains isolated from patients with chronic obstructive pulmonary disease exacerbations induce more inflammation than do colonizing strains not associated with the worsening of symptoms (Fig. 18.5).

Complement is involved in several tissue-damaging reactions

The activation of complement is a vital part of immunity to many bacteria, viruses and protozoa (see Ch. 15). Complement can, however, be involved in tissue-damaging reactions, such as immune complex disease, which also involves antibody and, usually, polymorphonuclear leukocytes (PMNs). Complement also plays an important role in the acute inflammatory response by generating the chemotactic factors C3a and C5a (see Ch. 10). Animal experiments suggest that C5a contributes to cardiac problems during sepsis, as it binds to C5a receptors on cardiomyocytes (cardiomyocytes are also damaged by LPS itself and by inflammatory cytokines such as IL-1β, TNFα and IL-6).

Direct activation of complement by LPS may contribute to the shock induced by toxic amounts of this endotoxin, in which the levels of complement components (e.g. C3) drop profoundly; this response appears to involve both the classic and the alternative complement pathways, which are activated by the lipid and polysaccharide components, respectively. C3a and C5a are produced in large amounts, and there is frequently a severe decrease in the number of PMNs because of aggregation of these cells, adherence to vessel walls and their activation to release toxic molecules, both oxidative and non-oxidative. When this occurs in the pulmonary capillaries, severe pulmonary oedema may result – the 'acute respiratory distress syndrome' (ARDS).

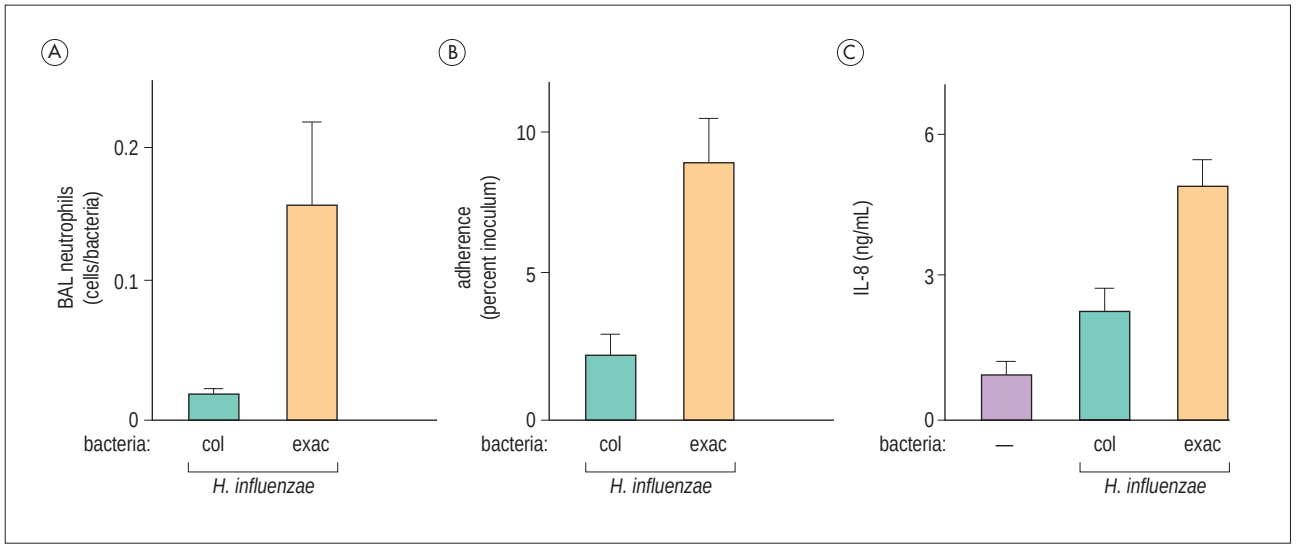


Figure 18.5 *Haemophilus influenzae* strains isolated from patients with chronic obstructive pulmonary disease (COPD) induce more inflammation than colonizing strains not associated with worsening of symptoms. *H. influenzae* strains from patients with COPD exacerbations (exac) induce greater numbers of neutrophils (A), more adherence to airway epithelial cells (B) and more IL-8 (C) than do isolates associated with colonization (col). BAL, bronchoalveolar lavage; IL-8, interleukin 8. (Redrawn from: Chin, C.L. et al. [2005] *Haemophilus influenzae* from patients with chronic obstructive pulmonary disease exacerbation induce more inflammation than colonizers. *Am J Respir Crit Care Med* 172:85–91.)

Disseminated intravascular coagulation is a rare but serious feature of bacterial septicaemia

Disseminated intravascular coagulation (DIC) can be a feature of bacterial (e.g. meningococcal) septicaemia, but is also seen in some virus infections such as Ebola fever (Ch. 29). The relative contributions of immune complexes, platelets and direct activation of the clotting pathway via the effect of LPS on Hageman factor remain controversial. For example, the haemorrhagic phenomena of yellow fever are probably secondary to coagulation defects due to the extensive liver damage, whereas in dengue ('haemorrhagic') fever it has been suggested that there is immune complex deposition in blood vessels. However, in all these haemorrhagic syndromes the role of cytokines such as TNF also needs to be considered.

PATHOLOGICAL CONSEQUENCES OF THE IMMUNE RESPONSE

Overreaction of the immune system is known as 'hypersensitivity'

Adaptive immune responses are vital to defence against infection, as witnessed by the increased susceptibility to infectious disease of immunodeficient patients (see Ch. 31). The antimicrobial effects of lymphocyte responses act mainly by focusing or enhancing non-specific effector mechanisms (see Ch. 11). This may also enhance the pathological effects outlined above. The tissue-damaging effects of hypersensitivity are referred to as 'immunopathological'. Coombs and Gell in 1958 classified hypersensitivity into four types, based on the immunological mechanism underlying the tissue-damaging reaction.

Each of the four main types of hypersensitivity can be of microbial or non-microbial origin

Hypersensitivity of microbial origin includes some of the most serious of these responses (Table 18.5). Organisms of many sorts can be involved, but one common feature is that the infection is prolonged, with continuous or repeated antigenic stimulation.

Type I hypersensitivity

These reactions are often called 'immediate', as they can occur within minutes, when the allergen triggers the degranulation of mast cells precoated with specific IgE antibodies.

Allergic reactions are a feature of worm infections

The most dramatic allergic (type I) reaction is that following the rupture of a hydatid cyst. Slow leakage of worm antigens ensures that the patient's mast cells are sensitized with specific IgE, and the massive flood of antigens on rupture may cause acute fatal anaphylaxis, with vascular collapse and pulmonary oedema. Even the small amount of antigen used in diagnostic skin tests can have this effect, although this is rare.

Another worm associated with high levels of IgE is *Ascaris*, but here the pathological consequences are mainly respiratory, with eosinophilic infiltrates and asthmatic episodes corresponding to passage of the parasite through the lung. The itching rashes characteristic of helminth infections when the worms die in the skin are probably also of this type, an example being 'swimmer's itch' due to cercariae released from snails infected with human, animal or avian schistosomes.

Why allergic reactions are such a feature of worm infections is not really clear, but they may be due to some feature of the antigens; in addition, it has been suggested that IgE plays a role in protection against worms. One would hope so, as in all other respects this class of antibody appears to be nothing but a nuisance.

Some insect venoms cause severe and life-threatening systemic reactions called anaphylaxis. One-third of beekeepers are sensitized to bee venom, and have venom-specific IgE; the

Table 18.5 Hypersensitivity of microbial origin

Coombs and Gell classification	Principal mechanism	Examples
Type I (allergic/anaphylactic)	IgE, mast cells	Helminths *Ascaris* Hydatid (ruptured cyst) ? Viral skin rash ? Upper respiratory tract Viral infections
Type II (cytotoxic)	IgG to surface antigens Complement Cytotoxic cells	Virus-infected cells Malaria-infected erythrocytes Autoantibodies in: *Mycoplasma* Streptococci *Trypanosoma cruzi*
Type III (immune complex-mediated)	Immune complexes Complement PMN	In tissues: Allergic alveolitis Actinomycosis In blood vessels: Glomerulonephritis Malaria Streptococci Hepatitis B Syphilis
Type IV (cell-mediated)	T lymphocytes Cytokines Macrophages (and other non-specific cells)	Granuloma Tuberculosis Leprosy (tuberculoid) Schistosomiasis (eggs) *Histoplasma* Mononuclear infiltration ± cell damage in many virus infections with CD4 and CD8 T cell-derived cytokines and macrophages playing roles Viral rashes
Autoimmunity	Cross-reaction with host	Streptococcal myocarditis
	Polyclonal B-cell activation	African trypanosomiasis

All four classic types of hypersensitivity can be induced by infectious organisms, types II and III being the most commonly encountered. Note that some mechanisms mediating hypersensitivity also take part in protective immunity.
PMN, polymorphonuclear leukocyte.

main honey bee allergen is Api m 1. Some insect allergens are enzymes such as hyaluronidases or di-peptidylpeptidases that are cross-reactive between species such as bees and wasps. Hypersensitivity can be demonstrated through skin prick or basophil activation tests. Venom immunotherapy can induce tolerance through desensitization, reducing the risk of a future systemic reaction by 90%.

Type II hypersensitivity

Type II reactions are mediated by antibodies to the infectious organism or autoantibodies

Strictly speaking, type II reactions are mediated by antibody (usually IgG) leading to cytotoxicity, either extracellular or intracellular (e.g. after phagocytosis). Antibody binds to the cell and, if complement is activated, the cell is lysed. An important distinction can be made between antibodies to the (foreign) infectious organism and autoantibodies; the former kill host cells because they display foreign antigens, whereas the latter bind to unaltered host antigens, and both types of response occur in infectious disease (see Table 18.5).

In blood-stage malaria, malarial antigens attach themselves to host cells

It has been shown that the haemolytic anaemia of blood-stage malaria is due not to autoantibody, as previously thought, but to antibodies to parasite-derived antigens that have been picked up by red cells. In some cases, it may be the antigen–antibody complex that binds to the cell. A similar reaction can occur following quinine treatment of *P. falciparum* malaria – blackwater fever.

Antimyocardial antibody of group A β-haemolytic streptococcal infection is the classic autoantibody triggered by infection

This reaction is due to the presence of the same cross-reacting carbohydrate antigen on the bacterium and the myocardium. However, as more protein sequences are obtained and compared, numerous other similar examples have come to light, and it is possible that cross-reaction between microbial and human antigens may underlie a number of diseases of currently unknown origin. Whether this mimicry of host

antigens has any survival value to the microbe is discussed in Chapter 17.

Type III hypersensitivity

Immune complexes cause disease when they become lodged in tissues or blood vessels

Immune complexes cause pathology if they are made in excess, if they are not removed properly from the circulation and if they deposit in tissues.

The formation of immune complexes can lead to phagocytosis and removal of antigen, but also to complement activation. Complications occur when the complexes escape removal by the phagocytes of the reticuloendothelial system and become lodged in the tissues or blood vessels, attracting complement and neutrophils. Release of lysosomal enzymes then results in local damage, which is particularly serious in small blood vessels, especially in the renal glomeruli. Immune complex disease is a major cause of both acute and chronic glomerulonephritis, and the majority of cases are probably the result of infection. There is also an important group in which autoantigen–autoantibody complexes are responsible (e.g. DNA–anti-DNA in systemic lupus erythematosus [SLE]), but even these may ultimately be the consequence of viral infection or reactivation – there is an association between EBV infection and SLE, particularly in younger patients.

Like most other immunopathological conditions, immune complex deposition is usually a feature of chronic infection (e.g. malaria). However, a persistent antigenic stimulus is not the only prerequisite, indicated by the fact that the most serious form of malarial nephropathy is found in *Plasmodium malariae* (quartan) malaria, which progresses despite successful treatment of the infection, whereas the nephropathy of *P. falciparum* (malignant tertian) malaria typically recovers after the infection has been cured. Predisposing factors may include a poor antibody response (in terms of amount or affinity), a particular tendency of the antigen itself to bind to vascular endothelium, or inhibition of the normal function of phagocytes or complement in removing circulating complexes.

Acute glomerulonephritis occurs as a serious complication of streptococcal infection (see Ch. 19) and is at least partly due to localization in glomeruli of immune complexes containing streptococcal antigens (see Fig. 18.6). Polymorph infiltration and alterations in the basement membrane cause leakage of albumin, even red cells, into the urine. The glomerulonephritis appears a few weeks after the infection has been terminated. When complexes are deposited over a long period (malarial nephropathy), the mesangial cell intrusions and fusion of foot processes cause a more irreversible impairment of glomerular function (chronic glomerulonephritis).

Occupational diseases associated with inhalation of fungi are the classic examples of immune complex deposition in the tissues

Immune complex deposition in the tissues, made famous by the work of Arthus on antigens injected into the skin of animals with pre-existing antibody (mainly IgG), manifests as a combination of thrombosis in small blood vessels and

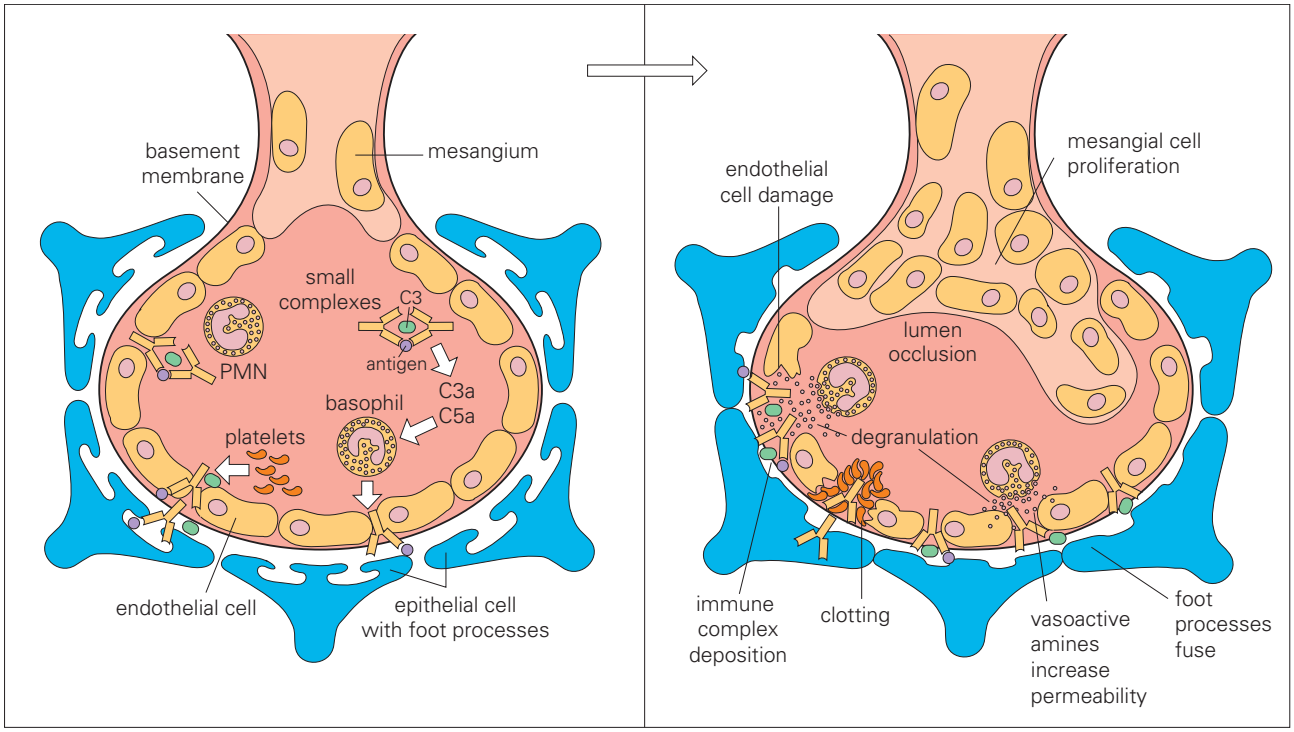


Figure 18.6 Glomerulonephritis caused by immune-complex-mediated tissue damage. Type III hypersensitivity results in the deposition of immune complexes in the blood vessel walls, particularly at sites of high pressure, filtration or turbulence such as the kidney. Large complexes deposit on the glomerular basement membrane, whereas small ones pass through the basement membrane and then deposit on the epithelial side of the glomerulus. PMN, polymorphonuclear leukocyte.

necrosis in the tissues due to PMN degranulation (Fig. 18.7). The best-studied examples are the occupational diseases associated with inhalation of fungi (e.g. farmer's lung, pigeon-fancier's disease, maple bark stripper's disease) in which chronic inflammation of the lung can lead to a state of destruction and fibrosis known as 'extrinsic allergic alveolitis', an unfortunate name as classic (IgE-mediated) allergy does not seem to be involved.

Another well-known example of immune complex disease was serum sickness

Serum sickness follows repeated injections of foreign protein, leading to circulating immune complexes, which deposit in the kidneys (Fig. 18.6), skin and joints. This was common in the pre-antibiotic days of passive serotherapy with horse serum for diphtheria (see Ch. 36). To prevent a similar reaction to monoclonal antibodies used as immunotherapy, antibodies are now genetically engineered so that as much of the molecule as possible is humanized, and some are now fully human.

Type IV hypersensitivity

Cell-mediated immune responses invariably cause some tissue destruction, which may be permanent

Despite the examples of antibody-mediated tissue damage discussed above, the antibody response generally achieves its purpose in eliminating invading organisms without any trace of damage to the host. Cell-mediated (type IV) responses with the activation of both T cells and macrophages invariably cause some tissue destruction, which may be reparable if not too prolonged, but that over time can lead to fibrosis and even calcification (Table 18.6).

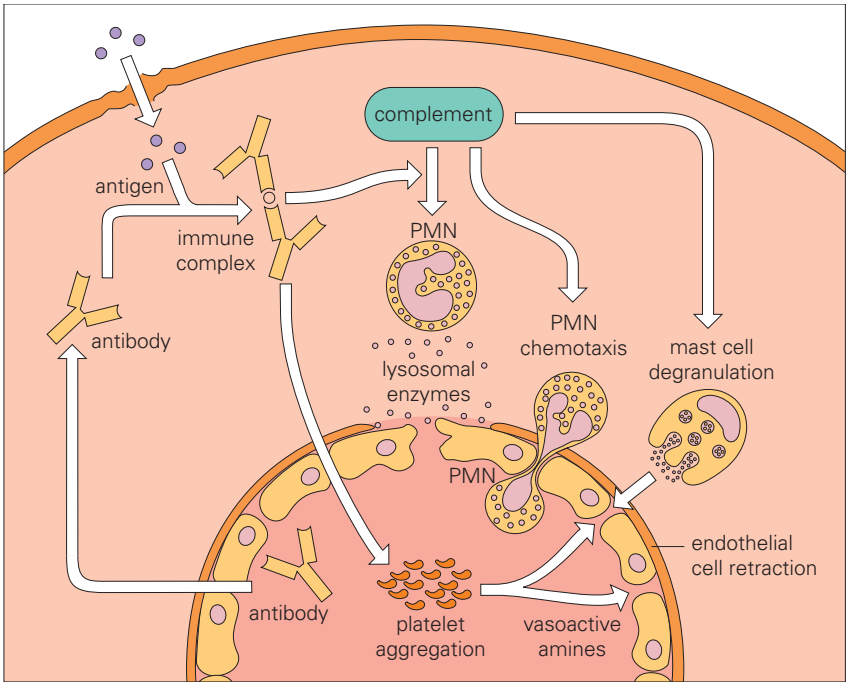


Figure 18.7 The Arthus reaction. Microbial antigens that enter the tissues (e.g. fungal particles in the lung) encounter antibodies and form immune complexes. These activate complement and initiate chemotaxis of polymorphonuclear leukocytes (PMNs), and degranulation of these and tissue mast cells. The resulting inflammatory response is further potentiated by damage induced by PMN-derived lysosomal enzymes.

Table 18.6 Cell-mediated immunity in protection and disease

Cell-mediated responses			
Immune cells or molecules	**Protective effect against**	**Pathological effect**	**Skin test**
Cytotoxic T cells (CD8)	Virus infections *Theileria*, (Mycobacteria)[a]	Local tissue loss	
Basophils, T cells	?	Inflammation	24 h (Jones–Mote reaction)
T cells (CD4) Macrophages Cytokines Giant cells Epithelioid cells Eosinophils	Intracellular organisms Viruses Bacteria Fungi Protozoa Worms	Mononuclear cell infiltration Granuloma Fibrosis Calcification	Delayed/tuberculin type (>2 days)

[a]A role for CD8 T cells in protection against *M. tuberculosis* has been proposed.

From the medical viewpoint, granuloma formation is the most important type IV hypersensitivity response

The cell-mediated response to microbial antigen is responsible for granuloma formation and plays a major role in diseases such as tuberculosis, tuberculoid leprosy, lymphogranuloma inguinale, and in *Toxocara* infection. Some granulomas have a tendency to undergo necrosis (e.g. caseation in tuberculosis) whereas others do not (e.g. leprosy, sarcoidosis) which may be explained in terms of the different pattern of cytokines involved. TNF, often in association with some microbial products, is especially likely to cause necrosis through its effects on vascular endothelium.

The clinical features of schistosomiasis are produced by cell-mediated immunity

The price paid for protective cell-mediated immunity is particularly well illustrated by the helminth disease schistosomiasis. *Schistosoma mansoni* (the blood fluke) lays eggs in the mesenteric venous system, some of which become lodged in small portal vessels in the liver. Strong cell-mediated reactions to secreted enzymes lead to granulomatous reactions around each egg, resulting in egg destruction and sparing of liver parenchyma from the toxic effects of the egg enzymes. However, the coalescent calcified granulomas ultimately cause portal cirrhosis, with portal hypertension, oesophageal varices and haematemesis (see Ch. 23).

The rather unexpected effect of malnutrition in reducing the incidence and severity of certain diseases (e.g. typhus, malaria) may be attributable to a reduction in immunopathology, though in the majority of diseases (e.g. measles, meningococcal infection, tuberculosis), the reverse is true. Indeed, poor nutrition may be a major factor predisposing to the greater severity of many common infections in tropical countries.

Antibodies can also cause enhancement of pathology, as in dengue infection

Most cases of dengue haemorrhagic fever occur in people who get a second infection with the dengue virus. Neutralizing antibodies bind to the envelope-dimer epitope. The problem is that there are four dengue serotypes that can differ by as much as 30% in the amino acid sequence of their envelope proteins. After infection with a second serotype, the concentration and avidity of the antibodies that have been generated to the first serotype will not be sufficient to prevent the new infection, but will enhance virus uptake as antibody binding to the virus leads to greater internalization through Fc binding (Fig. 18.8). The viral load falls as the fever falls, but this is when the most severe symptoms and pathology appear, including leakage of plasma from capillaries, haemorrhage and shock, at least partly due to excessive inflammatory cytokine release in a 'cytokine storm'. There are recent concerns that such antibody-dependent enhancement of infection might occur if individuals previously infected with dengue become infected with the Zika virus, due to cross-reactivity between the envelope proteins of these two Flaviviruses.

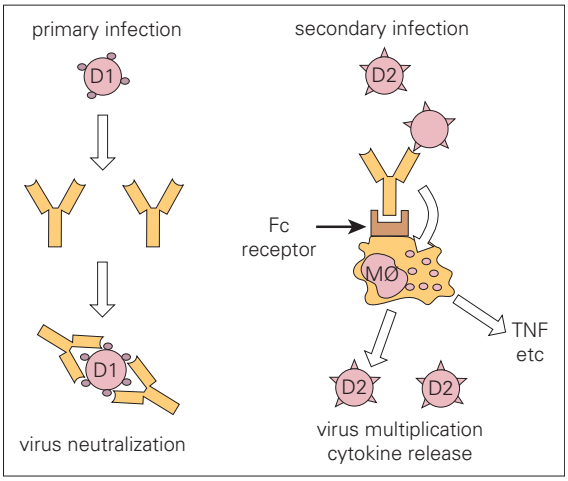


Figure 18.8 Antibody-dependent enhancement in dengue (D) infection. A primary infection with one of the four dengue serotypes generates protective neutralizing antibodies to the E protein. A subsequent infection with a different serotype can lead to severe pathology with vascular leakage as a result of virus multiplication and cytokine release. It is thought that cross-reactive antibodies from the first infection are insufficient in quantity or avidity to neutralize the second serotype, but do enable the virus to enter monocytes/macrophages through Fc receptor binding. Mϕ, macrophage; TNF, tumour necrosis factor.

SKIN RASHES

A variety of skin rashes have an immunological origin

The ways in which infections can affect the skin are detailed in Chapter 27, but some rashes are considered to be immunologically mediated. For example, the characteristic skin rash of measles is absent in children with T-cell deficiency (e.g. thymic aplasia or DiGeorge syndrome), who instead develop a fatal systemic infection, indicating that the skin lesions are T-cell mediated and are associated with cell-mediated immunity. In contrast, if children with T-cell deficiency are vaccinated with live vaccinia virus, they develop an inexorable spreading skin lesion, which is clearly a direct and not an immunopathological effect.

Table 18.7 lists the more common skin conditions of immunological origin in which an infectious organism is thought to be involved.

The SARS coronavirus caused lung immunopathology and T-cell loss

The SARS coronavirus infected more than 8000 people with >750 fatalities in around 29 countries in an epidemic in 2002–2003. Acute respiratory distress syndrome (ARDS) was seen in patients with severe disease, mostly elderly, resulting in around 50% mortality. The lungs of patients with SARS viral pneumonia show diffuse damage to the alveoli, with the presence of multinucleate giant cells and many macrophages. Acute pulmonary edema, extensive inflammatory cell infiltration and multi-organ failure were hallmarks and the virus could be found in other organs such as the intestine, liver and kidney of patients.

During the acute phase of infection, lymphopenia occurs with loss of both CD4 and CD8 T cells. Studies have suggested that alterations in antigen-presenting cell function and dendritic cell migration could reduce T-cell priming resulting in fewer virus-specific T cells. In addition, T-cell apoptosis could be induced by the high glucocorticoid levels seen in stress responses as well as the explosive type 1 interferon response.

Table 18.7 Skin rashes and their immunological basis

Organism	Disease	Character	Pathogenic basis
Viruses			
Measles	Measles	Maculopapular rash	T cells, immune complexes, allergy
Rubella	German measles	Maculopapular rash	? Immune mediated
Enterovirus	Hand, foot and mouth	Vesicular Erythematous	Viral cytopathic Viral ? immune mediated
Varicella-zoster	Chickenpox / zoster	Vesicular rash	Viral cytopathic
HIV	AIDS	Maculopapular	Lymphocytic infiltrate
EBV	Glandular fever	Erythematous rash Transient erythematous rash	Idiosyncratic antibody development to ampicillin Viral ? immune mediated
Parvovirus B19	Erythema infectiosum	Erythematous rash	Immune complexes
Bacteria			
Streptococcus pyogenes	Scarlet fever	Erythematous rash	Erythrogenic toxin
Treponema pallidum *Treponema pertenue*	Syphilis Yaws	Disseminated infectious rash in secondary stage	Immune complexes
Salmonella typhi	Typhoid, enteric fever	Sparse rose spots	Immune complexes
Neisseria meningitidis	Meningitis, spotted fever	Petechial or maculopapular lesions	Immune complexes
Mycobacterium leprae	Tuberculoid leprosy	Hypopigmented skin lesions	T cells, macrophages
Rickettsia prowazeki and others	Typhus	Maculopapular or haemorrhagic rash	Thrombosis
Fungi			
Dermatophytes	Dermatophytid or allergic rash		Immune complexes?
Blastomyces dermatitidis	Blastomycosis	Papule or pustule developing into granuloma	Hypersensitivity to fungal antigens, T cells
Protozoa			
Leishmania tropica	Cutaneous leishmaniasis	Papules ulcerating to form crusted infectious sores	T cells, macrophages

Many skin rashes represent immunological reactions occurring in the skin. It is suspected that several skin diseases of unknown origin are in fact caused by viruses, either directly or indirectly.

Severe lung and systemic inflammation is probably a result of innate cytokine dysregulation, with higher concentrations of cytokines such as TNFα, IL-6 and IL-8 being present. This may be due to massive activation of monocytes / macrophages. In those with severe infection, high concentrations of type I interferon and a dysregulated interferon-stimulated gene response are present.

Overall, it is still not known whether the lung pathology seen in SARS was mostly due to a type I interferon-independent exaggerated proinflammatory reaction or whether both interferon-dependent and -independent anomalous cytokine production contributed. The SARS-CoV acute antibody response was found to last less than 6 months, whereas virus-specific IgG antibodies had fallen 12 months post-infection. Despite this poor virus-specific memory B-cell response, SARS-CoV-specific memory T cells persisted in SARS survivors for up to 6 years post-infection, suggesting T cells are important in survival.

The hygiene hypothesis – are we too clean?

Allergic diseases are more common than they used to be, and it has been proposed that this may be because most of us now grow up in an environment that is too clean. The hygiene hypothesis proposes that, if we are exposed to a range of bacterial and viral infections in infancy, this may prevent the development of more harmful allergies by promoting a bias towards Th1 cytokine production. A more informative name might be 'microbial exposure deficiency hypothesis'. Certainly, people living in Africa seem to have had more exposure to antigenic stimulation, age for age, than those living in Europe, with more memory T cells and fewer naive T cells, although this may also be due to earlier infections with viruses like cytomegalovirus. Slightly surprisingly, it seems that infections with helminths, which induce copious Th2 responses, also protect against development of atopy, possibly because they out-compete the allergen-specific IgE on mast

cells. Other factors, such as innate immunity and regulatory T cells that act to reduce harmful immune responses causing immunopathology, may be involved.

VIRUSES AND CANCER

A variety of RNA and DNA viruses can cause permanent malignant changes within cells (Table 18.8). An account of proviruses and oncogenes (genes causing malignancy) is included in Chapter 3. Various human cancers have been shown to be associated with such oncogenic viruses (Table 18.9). Some of these include cancers associated with HIV infection. Three types of cancer, namely Kaposi sarcoma (KS), non-Hodgkin's lymphoma (NHL) and cervical cancer, are part of the classification of an acquired immunodeficiency syndrome (AIDS) defining diagnosis, consistent with advanced HIV infection. The resulting immunosuppression and loss of immune control over HHV-8 (KS-associated herpes virus), Epstein–Barr virus (EBV)-associated diffuse large B-cell and central nervous system NHL and human papillomavirus (HPV)-associated cervical cancer, leads to these cancers developing. There are latent and lytic components to the life cycles of these viruses. Few genes are expressed in latent infections, allowing the virus to reside in specific sites with the potential for reactivation but enabling infected cells to undergo future malignant change. The virus can disseminate in the lytic stage by release from infected cells. Some of the genes expressed can also promote tumour development. This part of the viral replicative cycle may therefore be of more importance in virus-associated malignancy. HIV-infected individuals are also at higher risk of developing HPV-associated anal cancer and hepatitis-B-associated liver cancer.

Table 18.8 Malignant transformation

Changes	Details
Morphology	Loss of shape; rounding Decreased adhesion to surface
Growth, contact	Loss of contact inhibition of growth and movement Increased ability to grow from a single cell Increased ability to grow in suspension Capacity for continued growth (immortalization)
Cellular properties	DNA synthesis induced Chromosomal changes Appearance of new antigens (viral or cellular in origin)
Biochemical properties	Loss of fibronectin Reduced cAMP

These changes occur when tumour viruses cause transformation of cultured cells. Many of these changes are obviously relevant for tumour production in vivo. cAMP, cyclic adenosine monophosphate.

Human T-cell lymphotropic virus type 1 (HTLV-1) is associated with adult T-cell leukaemia / lymphoma

HTLV-1 and HTLV-2 are retroviruses that have no oncogenes (see Ch. 3). HTLV-1 proviral DNA is detectable in the cellular DNA of individuals with adult T-cell leukaemia / lymphoma (ATLL). Although reported around the world, HTLV-1 infection is endemic in Southern Japan, the Caribbean islands, West and Central Africa and parts of South America. Less is known about the geographic distribution of HTLV-2,

Table 18.9 Viruses and human cancer

Viruses	Cancer	Strength of association	Viral genome in cancer cells	Cofactor
Epstein–Barr virus	Burkitt's lymphoma	++	+	Malaria
	Nasopharyngeal carcinoma	++	+	Nitrosamines
	Hodgkin's disease	–	–	–
Human papillomavirus	Cervical cancer	++	+	? Sexual practices
	Oropharyngeal cancer	++	+	Sexual practices
	Skin cancer	+	+	Genetic predisposition ? UV light
HHV-8 (KSHV)	Kaposi sarcoma	++	+	HIV immunosuppression
Hepatitis B virus	Liver cancer	++	+	? Aflatoxin
Hepatitis C virus	Liver cancer	++	–	? Hepatocyte regeneration
HTLV-1	T-cell leukaemia	++	+	–

Many viruses transform cells in culture, but only a few are important in human cancer. The associations are strongly supported by studies of naturally occurring or experimentally induced cancers in animals. HHV-8, human herpes virus 8; HTLV-1, human T-cell lymphotropic virus 1; KSHV, Kaposi's sarcoma-associated herpes virus; UV, ultraviolet.

which can be isolated from hairy T-cell leukaemia but has no association with malignancy. This virus can be found in certain Amerindian tribes and is associated with neurological and other chronic inflammatory conditions.

The carcinogenic nature of HTLV-1 is not due to activation of a cellular oncogene, but rather to the Tax accessory gene product enhancing transcription of host genes involved in cell division. This transactivation by *Tax* and stimulation of T-cell proliferation by HTLV-1 are thought to be central to oncogenesis. ATLL cells contain the integrated HTLV-1 proviral DNA but the latter is not transcribed very actively. The Tax and HBZ oncoproteins play key roles in immortalizing T cells and / or leading to leukemia by down-regulating various host cell functions, in particular repressing a tumour-suppressor protein. These infections are described in more detail in Chapter 27.

Epstein–Barr virus (EBV) is associated with nasopharyngeal carcinoma and lymphoma including post-transplant lymphoproliferative disease

Epstein–Barr virus is closely linked with the development of nasopharyngeal carcinoma (NPC) (see Ch. 19), which is common in Southern China and other parts of Asia (8–30 cases / 100 000 people / year, but higher in men than women), less common in parts of North Africa, and rare elsewhere in the world. The reason for this restricted geographical distribution is unknown. There is no convincing evidence for specific carcinogenic EBV strains, but these effects could be due to local co-carcinogens such as nitrosamines in salted fish. EBV DNA can be demonstrated in the cancer cells, but the precise mechanism for tumorigenicity is unknown; cellular oncogenes have not been implicated. People at high risk of developing NPC show high IgA titres to EBV capsid antigen a year or more before clinical symptoms appear.

Epstein–Barr virus is associated with Burkitt's lymphoma

Burkitt's lymphoma (BL), a tumour of immature B cells, occurs in parts of East Africa, such as Uganda, and in Papua New Guinea in 6–14-year-old children, especially boys. EBV DNA is present in the tumour cells, but most of the many copies of the EBV genes are not integrated into the host cell DNA. The tumour is probably caused by the action of EBV on B cells, causing them to proliferate and making activation of cellular oncogenes more likely. The cellular oncogene c-*myc* is translocated from chromosome 8 to the immunoglobulin heavy chain locus on chromosome 14, where it is expressed. As a result, the B cell may be prevented from entering the resting stage. There is also down-regulation of adhesion and human leukocyte antigen (HLA) molecules, so that the EBV-containing cells, which are normally subject to immune control, develop into tumour cells. The BL cells also show other chromosomal abnormalities, but their role in tumorigenesis is unclear.

The fact that EBV is a common worldwide infection, whereas BL, like NPC, is restricted geographically, points once again to the involvement of local co-factors, perhaps chemical or infectious co-carcinogens. Malaria is a recognized co-factor in BL, perhaps operating by altering the balance of the host's immune response, inducing polyclonal B-cell expansion and lytic cycle EBV reactivation. Expansion of latently infected B cells increases the chance of a *c-myc* translocation, seen in all BL. Another hypothesis is that malaria co-infection impairs EBV-specific T-cell responses with loss of viral immune surveillance and control.

Epstein–Barr virus is also associated with Hodgkin's lymphoma and lymphomas in immunosuppressed individuals

Epstein–Barr virus has been shown to be associated with classical Hodgkin's lymphoma, in particular as seen in childhood and older adulthood. In addition, bearing in mind that cytotoxic T cells police EBV infection, when host immunosuppression reduces T-cell surveillance then uncontrolled lymphoproliferation can result. Reducing immunosuppression in EBV-driven post-transplant lymphoproliferative may, however, result in graft rejection. Alternative treatment is usually required involving targeted monoclonal antibodies, namely rituximab, which acts on the B-cell CD20 receptor used for EBV entry and cytotoxic chemotherapy.

EBV-associated primary cerebral lymphoma may occur in HIV-infected individuals. The role of HIV is mostly indirect and is related to immunosuppression or B-cell activation. About 30% of AIDS-related lymphomas are Burkitt's lymphomas. Counterintuitively, since the advent of combined antiretroviral therapy (cART) there has been an increased risk for developing Hodgkin's lymphoma, which may be partly due to the increase in the age of the population living with HIV due to cART, (a 'downside effect' of immune reconstitution). This is because Hodgkin's lymphoma is associated with EBV infection and immune reconstitution increases the stimulation of B cells in which EBV exists episomally.

Certain human papillomavirus infections are associated with cervical cancer

Papillomavirus infections are ubiquitous, transmitted by direct contact and associated with a number of epithelial hyperproliferative diseases. The viral life cycle is intertwined with the host keratinocyte cells' differentiation cycles. After small epithelial abrasions in skin or mucosal surfaces, these cells in the basal skin layer are exposed to HPV and the viral DNA becomes episomal and replicates with the host DNA using host synthetic machinery.

There are clear associations between the development of cervical cancer and infection with certain of the around 200 subtypes of human papillomavirus (HPV; see Chs. 3, 22 and 27). It has been suggested that the vaginal microbiome (VM), the microbial flora in that site, could play both a protective as well as a destructive role in HPV persistence. Using high-throughput gene sequencing, higher microbial diversity was detected in the VM of HPV-positive compared with HPV-negative women. It is possible that the composition of the VM could influence the host's innate immune response, susceptibility to infection and the development of cervical disease.

Most HPV infections resolve within 24 months, but those that do not account for more than 80% of cervical cancers, the remainder being penile, vulval, rectal and oropharyngeal cancers, which are also associated with HPV. The HPV high-risk types include 16 and 18; low-risk types include 6 and 11. The latter cause cervical lesions but have a lower risk of progression to malignancy. HPV vaccine programmes were started in 2009 (see Ch. 35).

In most primary and metastatic cancer cells, the HPV genomes are present in integrated form, within the host genome and certain viral oncoprotein genes referred to as E6 and E7 are transcribed and translated. Integration occurs at different chromosomal locations and the E6 and E7 open-reading frames seem to be involved in transformation of epithelial cells and in maintenance of the transformed state, probably by binding to and inactivating tumour-suppressing cellular proteins concerned with regulation of the cell cycle. E6 is involved in up-regulating telomerase activity, maintaining telomere integrity during cell division and mediating degradation of p53, a tumour-suppressor protein; E7 binds and inactivates the retinoblastoma proteins (pRb). Both these activities are critical in HPV-induced oncogenesis and result in genome instability, accumulation of oncogene mutations, uncontrolled cell growth and eventually cancer. The viral E6 and E7 proteins drive cell proliferation in the nasal and parabasal cell layers at sites such as the cervix, where neoplastic changes can occur. There are functional differences in E6 and E7 that may explain the presence of high-risk and low-risk HPV types. For example, the low-risk E7 proteins differ from the high-risk ones in the way they associate with the pRb, while the high-risk E7 protein binds to and degrades other proteins that control cell cycle entry and re-entry in basal and upper epithelial cell layers. Cervical cancer is an uncommon sequel to infection with the low-risk types of HPV, and co-carcinogens such as cigarette smoke and herpes simplex virus (HSV) have been implicated.

Human papillomavirus infection is also associated with squamous cell carcinoma of the skin

It is possible that ultraviolet light acts as a co-carcinogen, as is known to be the case with papillomaviruses and skin cancers in sheep and cattle. People with the rare autosomal recessive disease epidermodysplasia verruciformis (EV) are infected with up to 20 different but less common types of HPV, and 30–60% of EV patients between 20 and 40 years of age develop multiple squamous cell carcinomas (SCCs) of the skin. Of these tumours, 90% contain HPV-5, -8, -14 and -20 DNA. These HPV types may act as co-carcinogens with ultraviolet light or immunosuppression in the development of non-melanoma skin cancers, the most common form of skin tumours in populations with fair skin.

HPVs may also play a role in the genesis of 90% of the skin cancers that appear in immunosuppressed organ transplant recipients, and cutaneous warts are common in these patients. In addition, there are reports that skin cancers in healthy individuals may be associated with HPV infection.

Head and neck SCC are caused mostly by exposure to tobacco and alcohol. It was thought that oropharyngeal SCC (OPSCC) would fall as public health programmes were increasingly successful in reducing smoking rates. However, the incidence of OPSCC plateaued and then increased, associated with HPV-16. HPV-positive OPSCC was associated with exposure to high-risk HPV and was found to have wild-type p53 and high levels of p16, a marker of HPV DNA integration into nuclear DNA. In the USA, 5% annual increases in the rate of OPSCC diagnosis have been reported particularly in males with multiple sexual partners and / or orogenital partners. Increases in incidence of OPSCC have also been seen in Europe and Australia.

Hepatitis B and hepatitis C viruses are major causes of hepatocellular carcinoma

The results, in sequential order, of chronic active hepatitis (CAH) include hepatocyte necrosis, chronic inflammation, cytokine production, fibrosis and finally cirrhosis. Therefore, CAH is a major driver for the development of hepatocellular carcinoma (HCC).

Individuals with active hepatitis B infections are 20-fold more likely to develop HCC than are uninfected individuals. The oncogenic process depends on a number of predisposing factors that are both viral and host derived (Fig. 18.9). HCC is the outcome of chronic necroinflammatory liver disease associated with higher levels of HBV replication as well as the host immune response. Moreover, some HBV mutant strains and specific genotypes may be associated with HCC development. Integrated HBV sequences found in HCC tumour cells may activate cellular oncogenes that encode proteins linked with controlling cell signalling, proliferation and viability, such as the *myc* family. Chronic inflammation, associated with increased liver cell proliferation, induces several rearrangements of the integrated HBV genome that can generate chromosomal instability. Moreover, there is evidence for the involvement of occult HBV infections, in which hepatitis B surface antigen cannot be detected but HBV DNA is integrated in the hepatocytes. The relationship between HBV DNA integration and either activation or inactivation of specific genes in the pathogenesis of HCC is still unclear. Specific HBV proteins such as the intriguingly named HBx, as well as the L envelope protein, may have important roles in cellular transformation are being investigated. Finally, HBV and hepatitis C virus (HCV) co-infection may act in concert with chronic alcohol consumption in liver carcinogenesis.

HCC is more common in certain parts of the world, such as Africa and South-East Asia, and this may be due to the presence of co-carcinogens (e.g. aflatoxin). However, the closely related hepadnavirus of woodchucks (Box 18.2) causes the same tumour in these animals in the apparent absence of co-carcinogens.

The mechanism by which HCV causes HCC is considered to be indirect, as HCV sequences are not integrated into tumour cells. It is thought that the persistent hepatocyte damage and inflammation in HCV carriers, together with

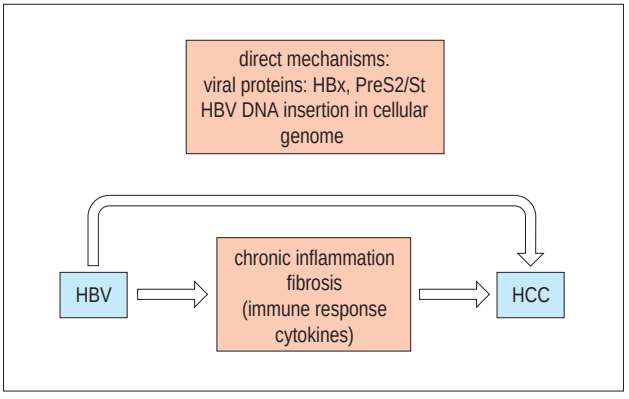


Figure 18.9 Development of hepatocellular carcinoma (HCC). HBV, hepatitis B virus; HBx, hepatitis B virus x protein.

Box 18.2 Lessons in Microbiology

The many faces of hepatitis B

Classic epidemiological studies on hepatitis B virus in Taiwan showed two things. First, 90% of those infected in infancy became carriers, as did 23% of those infected at 1–3 years, but only 3% of those infected as university students. Second, among 3454 HBsAg carriers, there were 184 cases of hepatocellular carcinoma, whereas there were only 10 cases among 19 253 non-carriers. Some 80% of all liver cancers are due to hepatitis B.

Worldwide, there are about 350 million carriers of this virus and, therefore, with liver cancer causing up to 2 million deaths each year, hepatitis B virus is second only to tobacco as a human carcinogen.

Very similar viruses infect woodchucks, ground squirrels and Pekin ducks. In northwest USA, 30% of woodchucks are carriers and most develop liver cancer in later life. In this host, the virus infects not only liver cells but also lymphoid cells in the spleen, peripheral blood and thymus, and pancreatic acinar cells and bile duct epithelium.

Transmission by hepatitis B carriers has been reported in a number of different healthcare settings, but hepatitis B immunization and advances in antiviral therapy will reduce these incidents.

the effects of cytokines on the development of fibrosis and hepatocyte proliferation, results in HCC. It is also thought that HCV may have a direct action via specific viral proteins interacting with host cell factors modulating pathways such as cell signalling and proliferation and apoptosis that result in malignant transformation of liver cells. Once cirrhosis is established, the annual incidence of HCC is 1–7% per year. In addition, HCV is associated with mixed cryoglobulinaemia, a lymphoproliferative disorder that can develop into B-cell non-Hodgkin's lymphoma.

Several DNA viruses can transform cells in which they are unable to replicate

Extensive studies have been carried out concluding that despite high oncogenicity in vitro and in laboratory animals, these viruses do not seem to be important in human cancer. For instance:

- Human adenoviruses transform cells in culture and cause sarcomas experimentally in hamsters. About 10% of the adenovirus genome integrates, and the T antigen is expressed. However, adenoviruses are not associated with human cancer.
- Polyomavirus (Latin: *poly*, many; *oma*, tumours), a mouse papovavirus, and simian vacuolating virus 40 (SV40), a monkey papovavirus, both cause tumours in experimentally inoculated hamsters. The viral DNA is integrated into the DNA of tumour cells, and T antigens are expressed. Are these viruses, or their human equivalents (BK and JC viruses), linked with human cancers? An incident occurred about 30 years ago, when thousands of children were accidentally inoculated with SV40 virus present in certain batches of poliovirus vaccine. The formalin inactivation procedure had failed to kill the SV40 virus present in the monkey kidney cells in which the polio vaccine had been grown. There was, however, no consequent increase in tumour incidence in the SV40-infected individuals. Nevertheless, evidence is accumulating that JC, BK and SV40 viruses are associated with certain cancers of the brain, with certain lymphomas and with other tumours, although a causative role has not been established.

Kaposi's sarcoma is caused by HHV-8

Kaposi's sarcoma (KS) is a multicentric tumour that involves massive proliferation of endothelial cells. It is 300 times more common among patients with AIDS than among other immunosuppressed groups, but is seen almost entirely in those who acquired HIV by sexual contact. It was identified in 1994 from a lesion in an individual with AIDS-associated KS. Human herpes virus 8 (HHV-8), referred to originally as the Kaposi's sarcoma-associated herpes virus (KSHV), appears to be sexually transmitted and is present in the tumours.

HHV-8 latently infects most tumour cells in lymphomas and KS. As a result, they are resistant to antiviral drugs targeting herpesviruses in the lytic cycle of replication. One of the HHV-8 lytic genes encodes the viral G-protein-coupled receptor (vGPCR), a constitutively active cellular chemokine receptor. vGPCR signalling can result in cell proliferation, the production of angiogenic factors and, in an animal model, can lead to KS-like lesions (Fig. 18.10). Moreover, there are indirect mechanisms involved in oncogenesis relating to altered T-cell responses and HHV-8 immunoregulation.

KSHV has developed strategies to evade innate and specific immunity, affect cell signalling, induce proliferation and prevent apoptosis of infected cells, thus promoting oncogenesis and angiogenesis.

The incidence of KS fell sharply in HIV-infected individuals after the advent of combined antiretroviral therapy (cART), However, as the number of HIV-infected individuals increases and ages, a stabilization of incident KS is likely and may be followed by an increase in numbers. KS treatment is aimed at immune reconstitution by giving cART. Localized treatment is usually avoided and systemic treatment involves chemotherapy using liposomal anthracycline agents.

HHV-8 is also associated with other lymphomatous conditions, namely multicentric Castleman's disease and primary effusion lymphoma.

Bacteria associated with cancer

The association between *Helicobacter pylori* and stomach and duodenal cancer, including gastric mucosa-associated lymphoid tissue (MALT) lymphoma, is discussed in Chapter 23. It is thought that a number of inflammatory reactions are triggered as a result of *H. pylori* colonizing the stomach mucosa leading to chronic atrophic gastritis (CAG). This sets off a cascade of mucosal changes resulting in intestinal metaplasia, dysplasia and carcinoma. The question is whether there are other environmental or genetic co-factors involved in oncogenesis. The tumour is associated with chronic inflammation secondary to *H. pylori* colonization, but it is thought that the bacterium alone is not sufficient for cancer to develop.

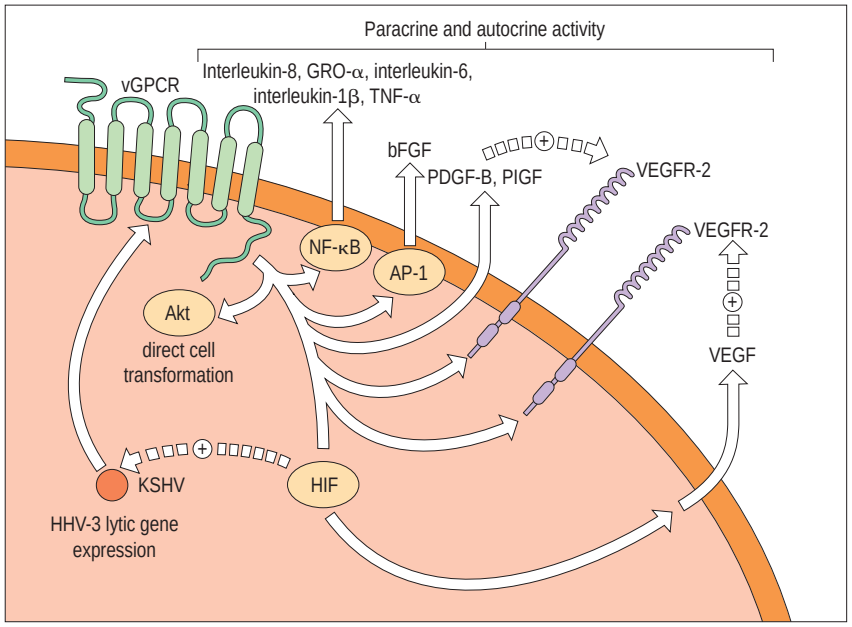


Figure 18.10 Activities of the vGPCR protein in human herpes virus 8 (HHV-8). The constitutively active viral G-protein-coupled receptor (vGPCR) of HHV-8 may promote the development of Kaposi's sarcoma by means of a variety of mechanisms. Signalling by vGPCR activates Akt, an activated protein kinase which directly induces cell transformation. The vGPCR also results in the production of a variety of other factors, including the nuclear factor (NF)-κB-dependent factors interleukin (IL)-8, growth-related protein alpha (GRO-α), IL-6, IL-1β, tumour necrosis factor alpha (TNFα), AP-1-dependent basic fibroblast growth factor (bFGF), platelet-derived growth factor B (PDGF-B) and placental growth factor (PlGF). Some, but not all, studies have found that vGPCR induces secretion of vascular endothelial growth factor (VEGF) and there is evidence that this secretion may be mediated by hypoxia-inducible factor (HIF). Also, vGPCR up-regulates the expression of the VEGF receptors 1 and 2 (VEGFR-1 and VEGFR-2, respectively). PlGF, VEGF and other factors can act in an autocrine or paracrine fashion to promote Kaposi's sarcoma. KSHV, Kaposi's sarcoma-associated herpes virus. (Redrawn from: Yarchoan, R. [2006] Key role for a viral lytic gene in Kaposi's sarcoma. *N Engl J Med* 355:1383–1385, with permission.)

KEY FACTS

- Tissue damage or disease can be caused by infectious organisms in several ways.
- Infectious organisms may destroy cells directly (e.g. cytopathic viruses), release toxins that destroy cells or their cellular function (e.g. staphylococcal or tetanus toxins), overstimulate normal defence systems (e.g. LPS) or stimulate excessive or prolonged adaptive responses.
- Such effects of infectious organisms on defence systems may be antibody or T-cell mediated and are collectively known as 'hypersensitivity reactions' or 'immunopathology'.
- Some viruses have been shown to be involved in the initiation of tumours, with the viral genome being found in the cancer cells. The restricted geographic distribution of some of these tumours may be due to the local presence of co-carcinogens.

19 Upper respiratory tract infections

Introduction

The air we inhale contains millions of suspended particles, including microorganisms, most of which are harmless. However, the air may contain large numbers of pathogenic microorganisms if someone is near an individual with a respiratory tract infection. Efficient cleansing mechanisms (see Chs. 10 and 14) are therefore vital components of the body's defence against infection of both the upper and lower respiratory tract. Infection takes place against the background of these natural defence mechanisms, and it is then appropriate to ask why the defences have failed. For the upper respiratory tract, the flushing action of saliva is important in the oropharynx and the mucociliary system in the nasopharynx traps invaders. As on other surfaces of the body (see Ch. 9), a variety of microorganisms live harmoniously in the upper respiratory tract and oropharynx (Table 19.1); they colonize the nose, mouth, throat and teeth and are well adapted to life in these sites. Normally they are well-behaved guests, not invading tissues and not causing disease. However, as in other parts of the body, resident microorganisms can cause trouble when host resistance is weakened. In addition, a host of invaders cause upper respiratory tract symptoms that may progress to the lower respiratory tract, depending on the pathogen.

The upper and lower respiratory tracts form a continuum for infectious agents

We distinguish between upper and lower respiratory tract infections, but the respiratory tract from the nose to the alveoli is a continuum as far as infectious agents are concerned (Fig. 19.1). There may, however, be a preferred 'focus' of infection (e.g. the nasopharynx for coronaviruses and rhinoviruses); but parainfluenza viruses, for instance, can infect the nasopharynx to give rise to a cold, as well as the larynx and trachea resulting in laryngotracheitis (croup), and occasionally the bronchi and bronchioles (bronchitis, bronchiolitis or pneumonia).

Generalizations can be made about upper and lower respiratory tract infections:

1. Although many microorganisms are restricted to the surface epithelium, some spread to other parts of the body before returning to the respiratory tract, oropharynx and salivary glands (Table 19.2).
2. Two groups of pathogens can be distinguished: 'professional' and 'secondary' invaders (Table 19.3).
3. Professional invaders are those that successfully infect the normally healthy respiratory tract. They generally possess specific properties that enable them to evade local host defences, such as the attachment mechanisms of respiratory viruses (Table 19.4). Secondary invaders cause disease only when host defences are already impaired (see Table 19.3).
4. The symptoms of an upper respiratory tract infection include fever, rhinitis and pharyngitis or sore throat. It is not just respiratory pathogens that cause these symptoms, Cytomegalovirus (CMV) and Epstein–Barr virus (EBV) infections are included in this chapter but are associated only with the fever and pharyngitis components. They are both part of a glandular fever differential diagnosis.

RHINITIS

Molecular diagnostic tests have demonstrated a much wider range of viruses that cause colds compared with older techniques

Viruses are the most common invaders of the nasopharynx, and a great variety of types (see Table 19.4) are responsible for the symptoms referred to as the common cold. They induce a flow of virus-rich fluid, called rhinorrhea, from the nasopharynx, and when the sneezing reflex is triggered then large numbers of virus particles are discharged into the air. Transmission is therefore by aerosol and also by virus-contaminated hands (see Ch. 14). Most of these viruses possess surface molecules that bind them firmly to host cells or to cilia or microvilli protruding from these cells. As a result, they are not washed away in secretions and are able to initiate infection in the normally healthy individual. Virus progeny from the first-infected cell then spread to neighbouring cells and via surface secretions to new sites on the mucosal surface. After a few days, damage to epithelial cells and the secretion of fluid containing inflammatory mediators such as bradykinin lead to common cold-type symptoms (Fig. 19.2).

Viral co-infections are being detected using more sensitive tests

In view of the large variety of viruses and because common colds are generally mild and self-limiting with no systemic spread in healthy individuals, determination of the aetiology is helpful both from a management as well as from an

epidemiological perspective. In particular, the advent of molecular diagnostic tests of higher sensitivity and specificity has meant that, as well as detecting a wider range of viruses, co-infections have been seen where before only one pathogen was identified. This has had an impact on diagnosis, especially when the lower respiratory tract is involved, as for instance with influenza viruses or in children with respiratory syncytial virus (RSV) infection.

Table 19.1 The normal flora of the respiratory tract

Type of resident[a]	Microorganism
Common residents (>50% of normal people)	Oral streptococci *Neisseria* spp. *Moraxella* Corynebacteria *Bacteroides* Anaerobic cocci (*Veillonella*) Fusiform bacteria[b] *Candida albicans*[b] *Streptococcus mutans* *Haemophilus influenzae*
Occasional residents (<10% of normal people)	*Streptococcus pyogenes* *Streptococcus pneumoniae* *Neisseria meningitidis*
Uncommon residents (<1% of normal people)	*Corynebacterium diphtheria* *Klebsiella pneumoniae* *Pseudomonas* *E. coli*[c] *C. albicans*[c]
Residents in latent state in tissues:[d] Lung Lymph nodes Sensory neurone / dorsal root ganglia	*Pneumocystis jirovecii* *Mycobacterium tuberculosis* Cytomegalovirus (CMV) Epstein–Barr virus (EBV) Herpes simplex virus (HSV) Varicella-zoster virus (VZV)

[a]All except tissue residents are present in the oronasopharynx or on teeth.
[b]Present in mouth; also *Entamoeba gingivalis, Trichomonas tenax*, micrococci, *Actinomyces* spp.
[c]Especially after antibiotic treatment.
[d]All except *M. tuberculosis* are present in most humans.

There has been a revolution in laboratory diagnosis. The older methods of cell culture, looking for a cytopathic effect or adding red cells to detect haemagglutinating viruses, as well as immunofluorescence detecting viral antigens in exfoliated cells in samples such as nasopharyngeal aspirates or throat swabs (see Fig. 19.5), have been superseded in many parts of the world by molecular diagnostic tests. These include detecting genomic material by methods including real-time multiplex polymerase chain reaction (PCR), which may be performed in laboratories or as point-of-care tests, and microarrays.

Alternatively, collecting an acute and convalescent serum sample and looking for a rise in virus-specific antibodies can confirm the diagnosis retrospectively.

Due to the increased sensitivity in detecting respiratory viruses by PCR, together with automated sample extraction

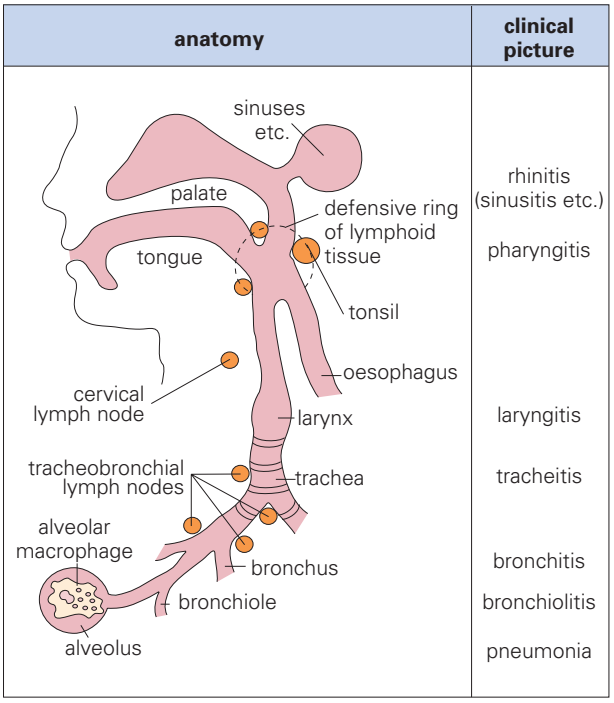


Figure 19.1 The respiratory tract as a continuum.

Table 19.2 Pathogens that gain entry via the upper respiratory tract

Type	Examples	Consequences
Restricted to surface	Rhinoviruses Influenza *Streptococci* in throat *Chlamydia* (conjunctivitis) Diphtheria Pertussis *Candida albicans* (thrush)	Local spread Local (mucosal) defences important Adaptive (immune) response sometimes too late to be important in recovery Short incubation period (days)
Spread through body	Measles, mumps, rubella EBV, CMV *Chlamydophila psittaci*[a] Q fever *Cryptococcosis*	Little or no lesion at entry site Pathogen spreads through body, returns to surface for final multiplication and shedding, e.g. salivary gland (mumps, CMV, EBV), respiratory tract (measles) Adaptive immune response important in recovery Longer incubation period (weeks)

After entry via the respiratory tract, pathogens either stay on the surface epithelium or spread through the body. CMV, cytomegalovirus; EBV, Epstein–Barr virus.
[a]Formerly *Chlamydia psittaci*.

Table 19.3 The two types of respiratory invader – professional or secondary

Type	Requirement	Examples
Professional invaders (infect healthy respiratory tract)	Adhesion to normal mucosa (in spite of mucociliary system)	Respiratory viruses (influenza, rhinoviruses) *Streptococcus pyogenes* (throat) *Strep. pneumoniae* *Chlamydia* (psittacosis, chlamydial conjunctivitis and pneumonia, trachoma)
	Ability to interfere with cilia	*Bordetella pertussis* *Mycoplasma pneumoniae* *Strep. pneumoniae* (pneumolysin)
	Ability to resist destruction in alveolar macrophage	*Legionella* *Mycobacterium tuberculosis*
	Ability to damage local (mucosal, submucosal) tissues	*Corynebacterium diphtheriae* (toxin) *Strep. pneumoniae* (pneumolysin)
Secondary invaders (infect when host defences impaired)	Initial infection and damage by respiratory virus (e.g. influenza virus)	*Staphylococcus aureus* *Strep. pneumoniae,* pneumonia-complicating influenza
	Local defences impaired (e.g. cystic fibrosis)	*Staph. aureus* *Pseudomonas*
	Chronic bronchitis, local foreign body or tumour	*Haemophilus influenzae* *Strep. pneumoniae*
	Depressed immune responses (e.g. AIDS, neoplastic disease)	*Pneumocystis jirovecii* Cytomegalovirus *M. tuberculosis*
	Depressed resistance (e.g. elderly, alcoholism, renal or hepatic disease)	*Strep. pneumonia* *Staph. aureus* *H. influenzae*

Table 19.4 Respiratory viruses and their mechanisms of attachment

Virus	Types involved	Attachment mechanism	Disease
Rhinoviruses (>100 types)[a]	All	Capsid protein binds to ICAM-1 type molecule on cell[b]	Common cold
Enteroviruses including: Coxsackie virus A (24 types) Echoviruses (34 types) Enteroviruses (116 serotypes)	Many	Capsid protein binds to ICAM-1 type molecule on cell[b]	Common cold; also oropharyngeal vesicles (herpangina) and hand, foot and mouth disease (A16, EV71)
Influenza virus	A, B and C	Haemagglutinin binds to neuraminic acid-containing glycoprotein on cell surface	May also invade lower respiratory tract
Parainfluenza virus (4 types)	1, 2, 3, 4	Viral envelope protein binds to glycoside on cell	May also invade larynx
Respiratory syncytial virus	A and B	G protein on virus attaches to receptor on cell	May also invade lower respiratory tract
Coronaviruses (several types)	All	Viral envelope protein binds to glycoprotein receptors on cell	Common cold Severe acute respiratory syndrome (SARS) Middle East respiratory syndrome coronavirus (MERS CoV)
Adenovirus (41 types)	5–10	Penton fibre binds to cell receptor	Mainly pharyngitis; also conjunctivitis, bronchitis

[a]A given type shows little or no neutralization by antibody against other types.
[b]ICAM-1: intercellular adhesion molecule expressed on a wide variety of normal cells; member of immunoglobulin superfamily, coded on chromosome 19.

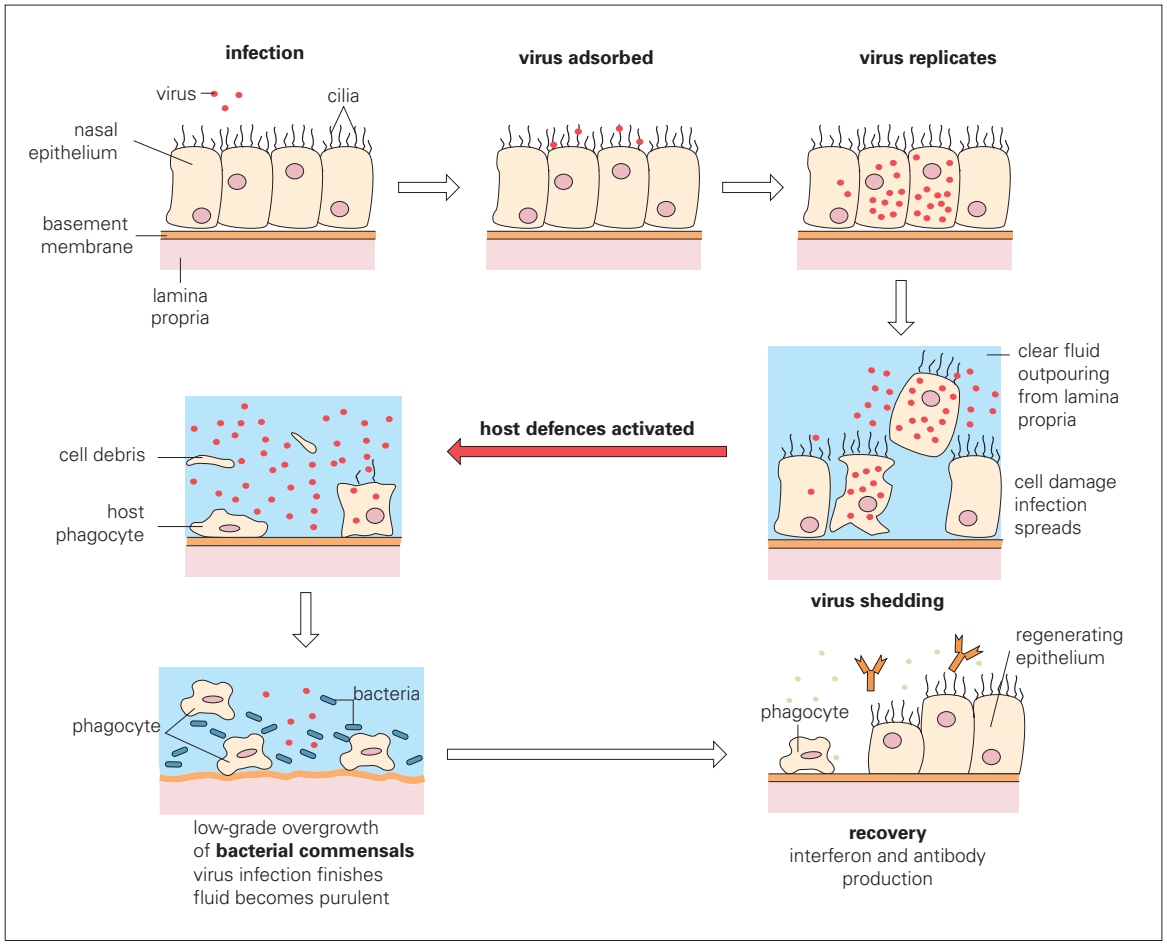


Figure 19.2 The pathogenesis of the common cold. For simplification, the epithelium is represented as one cell thick.

and detection methods, many laboratories use molecular methods for making a diagnosis using combined nose and throat swab samples. These swabs can be used instead of collecting nasopharyngeal aspirates that are more invasive and lead to aerosol production – an infection control issue.

Treatment of the common cold is symptomatic

It is often said that a common cold will resolve within 48 h if vigorous treatment with anticongestants, analgesics and antibiotics is undertaken. There are no vaccines to protect against the common cold viruses as the vaccines would have to be polyvalent to cover this antigenically diverse group of viruses, and treatment is for the most part symptomatic.

PHARYNGITIS AND TONSILLITIS

About 70% of acute sore throats are caused by viruses

Microorganisms that cause sore throats (acute pharyngitis) are listed in Table 19.5. Those viruses that infect the upper respiratory tract inevitably encounter the submucosal lymphoid tissues that form a defensive ring around the oropharynx (see Fig. 19.1). The throat becomes sore either because the overlying mucosa is infected or because of inflammatory and immune responses in the lymphoid tissues themselves. Adenoviruses are common causes, often infecting the conjunctiva as well as the pharynx to cause pharyngoconjunctival fever. Epstein–Barr virus (EBV) and cytomegalovirus (CMV) multiply locally in the pharynx (Fig. 19.3), and herpes simplex virus (HSV) and certain coxsackie A viruses multiply in the oral mucosa to produce a painful local lesion or ulcer. Certain enteroviruses (e.g. coxsackie A16) can cause additional vesicles on the hands and feet and in the mouth (hand, foot and mouth disease; Fig. 19.4).

Cytomegalovirus infection

Cytomegalovirus can be transmitted by saliva, urine, blood, semen and cervical secretions

Cytomegalovirus is the largest human herpesvirus (Fig. 19.5) and is species specific; humans are the natural hosts. Cytomegalovirus refers to the multinucleated cells, which together with the intranuclear inclusions are characteristic responses to infection with this virus. CMV was originally called 'salivary gland' virus and is transmitted by saliva and other secretions. In addition, it can be transmitted by sexual contact, as semen and cervical secretions may also contain this virus, and by blood transfusions (although leukodepletion reduces the risk significantly) and organ transplants from CMV antibody positive donors. The CMV load will be high in the urine from babies with congenital CMV infection and careful hand washing and disposal of nappies will reduce the risk of transmission to susceptible individuals. CMV

Table 19.5 Microorganisms that cause acute pharyngitis

Organisms	Examples	Comments
Viruses	Rhinoviruses, coronaviruses	A mild symptom in the common cold
	Adenoviruses (types 3, 4, 7, 14, 21)	Pharyngoconjunctival fever
	Parainfluenza viruses	More severe than common cold
	Influenza viruses, CMV, EBV	Not always present
	Coxsackie A and other enteroviruses	Small vesicles (herpangina)
	Epstein–Barr virus	Occurs in 70–90% of glandular fever patients
	Herpes simplex virus type 1	Can be severe, with palatal vesicles or ulcers
Bacteria	*Streptococcus pyogenes*	Causes 10–20% of cases of acute pharyngitis; sudden onset; mostly in 5- to 10-year-old children
	Neisseria gonorrhoeae	Often asymptomatic; usually via orogenital contact
	Corynebacterium diphtheriae	Pharyngitis often mild, but toxic illness can be severe
	Haemophilus influenzae	Epiglottis
	Borrelia vincentii plus fusiform bacilli	Vincent's angina; commonest in adolescents and adults

CMV, cytomegalovirus; EBV, Epstein–Barr virus.

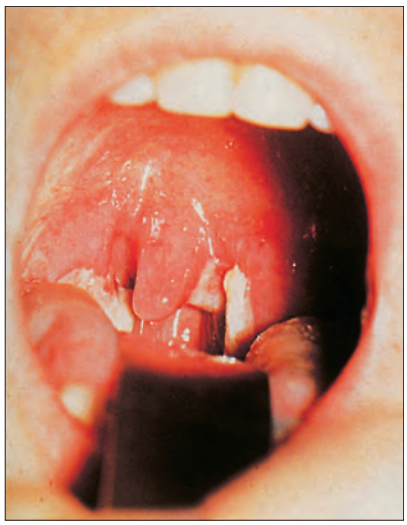


Figure 19.3 Infectious mononucleosis caused by Epstein–Barr virus. The tonsils and uvula are swollen and covered in white exudate. There are petechiae on the soft palate. (Courtesy of J.A. Innes.)

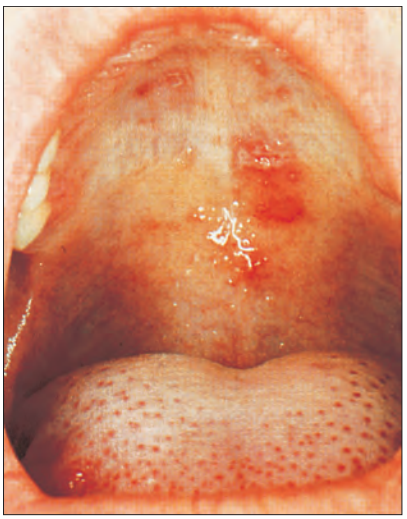


Figure 19.4 Ulcers on the hard palate and tongue in hand, foot and mouth disease due to coxsackie A virus. (Courtesy of J.A. Innes.)

can be detected in breast milk, which is another route of transmission.

Cytomegalovirus infection is often asymptomatic, but can reactivate and cause disease when cell-mediated immunity (CMI) defences are impaired

After clinically silent infection in the upper respiratory tract, CMV spreads locally to lymphoid tissues and then systemically in circulating lymphocytes and monocytes to involve lymph nodes and the spleen. The infection then localizes in epithelial cells in salivary glands and kidney tubules, and in the cervix, testes and epididymis, from where the virus is shed to the outside world (Table 19.6).

Infected cells may be multinucleated or bear intranuclear inclusions, but pathological changes are minor. The virus

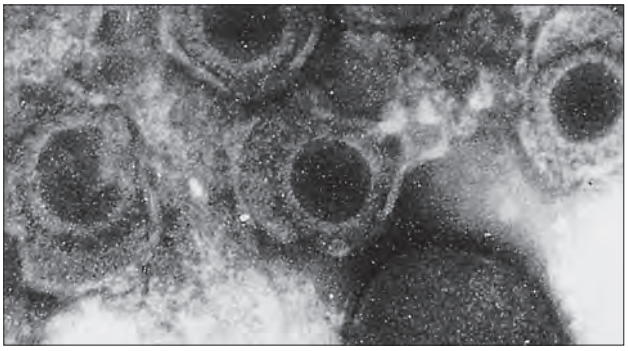


Figure 19.5 Electron micrograph of cytomegalovirus particles. This is the largest human herpes virus, with a diameter of 150–200 nm, and a dense DNA core. (Courtesy of D.K. Banerjee.)

Table 19.6 The effects of cytomegalovirus infection

Site of infection	Result	Comment
Salivary glands	Salivary transmission	Via kissing and contaminated hands
Tubular epithelium of kidney	Virus in urine	Probable role in transmission by contaminating environment
Cervix, testis /epididymis	Sexual transmission	Up to 10^7 infectious doses /mL of semen in an acutely infected male
Lymphocytes, macrophages	Virus spreads through body via infected cells Mononucleosis may occur Immunosuppressive effect	Probable site of persistent infection
Placenta, fetus	Congenital abnormalities	Greatest damage in fetus after primary maternal infection rather than reactivation

inhibits T-cell responses, and there is a temporary reduction in their immune reactivity to other antigens.

Although specific antibodies and CMI responses are generated, these fail to clear the virus (see Ch. 17), which often continues to be shed in saliva and urine for many months. The infection is, however, eventually controlled by CMI mechanisms, although infected cells remain in the body throughout life and can be a source of reactivation and disease when CMI defences are impaired.

CMV owes its success in our species to its ability to evade immune defences. For instance, it presents a poor target for cytotoxic T (Tc) cells by interfering with the transport of major histocompatibility complex (MHC) class I molecules to the cell surface (see Ch. 11), and it induces Fc receptors on infected cells (see Ch. 17).

Cytomegalovirus infection can cause fetal malformations and pneumonia in immunodeficient patients

In the natural host, the human infant or child, CMV causes no illness, and in general it causes a mild illness in adults. However, as with all infections, there is a spectrum of clinical disease ranging from asymptomatic to severely ill. A glandular fever type illness can occur in adolescents, which is similar to Epstein–Barr virus infection, with fever, lethargy and abnormal lymphocytes and mononucleosis in blood smears. Primary infection during pregnancy allows the spread of virus from the blood to the placenta and then to the fetus, resulting in symptomatic CMV infection at birth in 18%, and detection of other sequelae in 25% by just under 5 years of age, as described in Chapter 24. Reactivation of infection during pregnancy also occurs, which may be asymptomatic at birth but up to 8% of children will have symptoms by 5 years of age. CMV is second only to Down's syndrome as a cause of intellectual disability.

In immunodeficient patients such as bone marrow or solid organ transplant recipients (see Ch. 31), CMV infection can cause an interstitial pneumonitis with infiltrating infected mononuclear cells. Other sites affected include the CNS, with focal cerebral 'micronodular' lesions with infected mononuclear cells, together with a variety of other complications, including retinitis in HIV-infected individuals with AIDS. This was a major complication before the advent of combined antiretroviral therapy. In addition, the gastrointestinal tract may be involved, with a colitis and hepatitis.

Clinical diagnosis of primary infection is rarely possible, because it is often asymptomatic. However, in symptomatic immunocompetent individuals, the diagnosis is made by detecting CMV IgM in blood samples. In those with possible CMV pneumonitis, a bronchoalveolar lavage sample is collected by passing a bronchoscope into the lungs and collecting washings, and CMV DNA or CMV antigen detection methods are used to make the diagnosis. Multinucleated cells or cells with prominent intranuclear inclusions may be seen in lung biopsy material. CMV IgM and IgG serology is available but is unlikely to be of diagnostic help in immunosuppressed patients. The management of post-transplant recipients involves CMV DNA monitoring of whole blood or plasma samples and giving pre-emptive therapy, having detected a CMV viraemia (see Ch. 31).

Antiviral treatment options in CMV infection

While ganciclovir, foscarnet or cidofovir (although the latter is a third line agent and used infrequently) are effective treatments, aciclovir is ineffective. These antiviral drugs reduce viral replication, do not eliminate the virus and can be used in specific clinical situations as pre-emptive therapy (see Ch. 31). As CMV pneumonitis is an immunopathological disease, CMV-specific or human normal immunoglobulin is given in addition to the antiviral agent to potentially block the Tc-cell response to pneumocytes expressing the target antigens.

Prevention of CMV infection

There is no vaccine, but trials of live, inactivated and recombinant vaccines have been carried out. Bearing in mind that it is the second most common cause of intellectual disability in babies, immunization is a major consideration once a number of practical issues have been resolved. The results of a recombinant CMV glycoprotein B vaccine trial reported in 2011 involving solid organ transplant recipients suggested that antibody levels generated in response to vaccine led to reduced viraemia and duration of antiviral use. Transmission can be reduced in various settings by avoiding contact between congenitally infected children and susceptible pregnant women, or maintaining good hand hygiene if this is not possible. Blood for transfusion of newborns, and solid organ and bone marrow transplants, should preferably come from CMV antibody negative donors.

Epstein–Barr virus infection

Epstein–Barr virus is transmitted in saliva

Epstein–Barr virus (EBV), like CMV, is species specific. EBV is structurally and morphologically identical to other herpesviruses (see Ch. 3), but is antigenically distinct. Major antigens include the viral capsid antigen (VCA) and the EBV-associated nuclear antigens (EBNA) that are used in diagnostic tests. Humans are the natural hosts.

EBV is transmitted by the exchange of saliva, for instance during kissing, and is a ubiquitous infection. In resource-poor countries, infection probably occurs via close contact in early childhood and is subclinical. Elsewhere, infection occurs in two peaks at 1–6 years and 14–20 years of age, and in most cases, causes illness.

The clinical features of EBV infection are immunologically mediated

Clinical and immunological events in EBV infection are illustrated in Fig. 19.6. EBV replicates in B lymphocytes, after making a specific attachment to the C3d receptor (CD21) on these cells, and also in certain epithelial cells. The pathogenesis of the disease and the clinical features can be accounted for on this basis. Virus is shed in saliva from infected epithelial cells and possibly lymphocytes in salivary glands, and from the oropharynx, with clinically silent spread to B lymphocytes in local lymphoid tissues and elsewhere in the body (lymph nodes, spleen).

T lymphocytes respond immunologically to the infected B cells (outnumbering the latter by about 50 to 1) and appear in peripheral blood as 'atypical lymphocytes' (Fig. 19.7). Much of the disease is attributable to an immunological civil war, as specifically activated T cells respond to the infected B cells. In the naturally infected infant or small child, these immune responses are weak and there is generally no clinical disease. Older children, however, become unwell, and young adults develop infectious mononucleosis or glandular fever 4–7 weeks after initial infection. This is characterized by fever, sore throat, often with petechiae on the hard palate (see Fig. 19.3), lymphadenopathy and splenomegaly, with anorexia and lethargy as prominent features. Hepatitis may occur, with mild elevations of hepatocellular enzymes in 90% of cases and jaundice in 9%. Splenic rupture may occur.

Complications are seen in about 1% of acute EBV infections and may be due to virus invading the tissue or to immune-mediated damage. These include aseptic meningitis and encephalitis, nearly always with complete recovery, haemolytic anaemia, airway obstruction due to oropharyngeal swelling, haemophagocytic syndrome and splenic rupture.

The symptoms are presumably due to the action of cytokines released during the intense immunological activity. High levels of interferon gamma (IFNγ), produced by activated

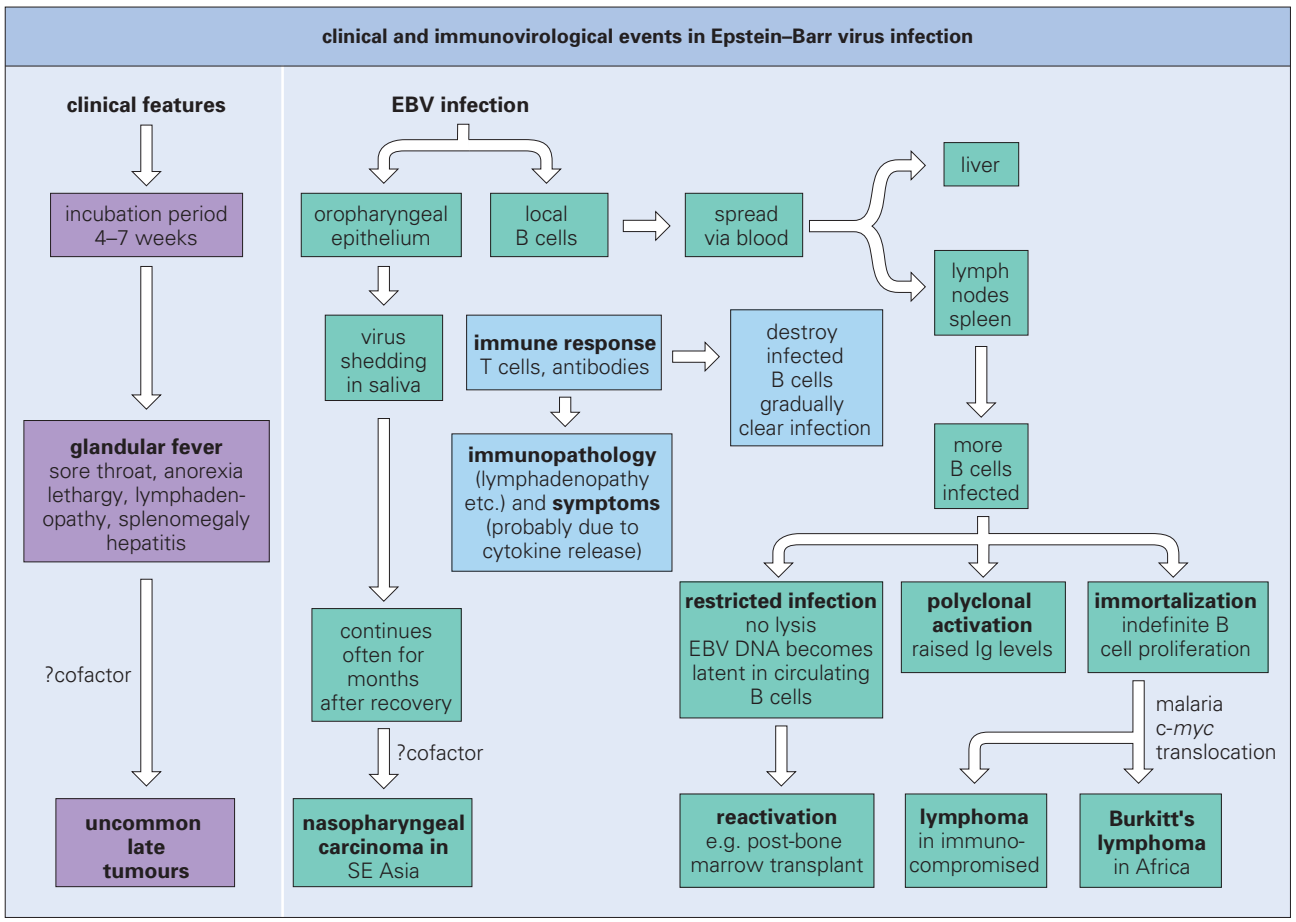


Figure 19.6 Clinical and immunovirological events in Epstein–Barr virus (EBV) infection in adolescents or adults. A milder, often subclinical, infection occurs in children.

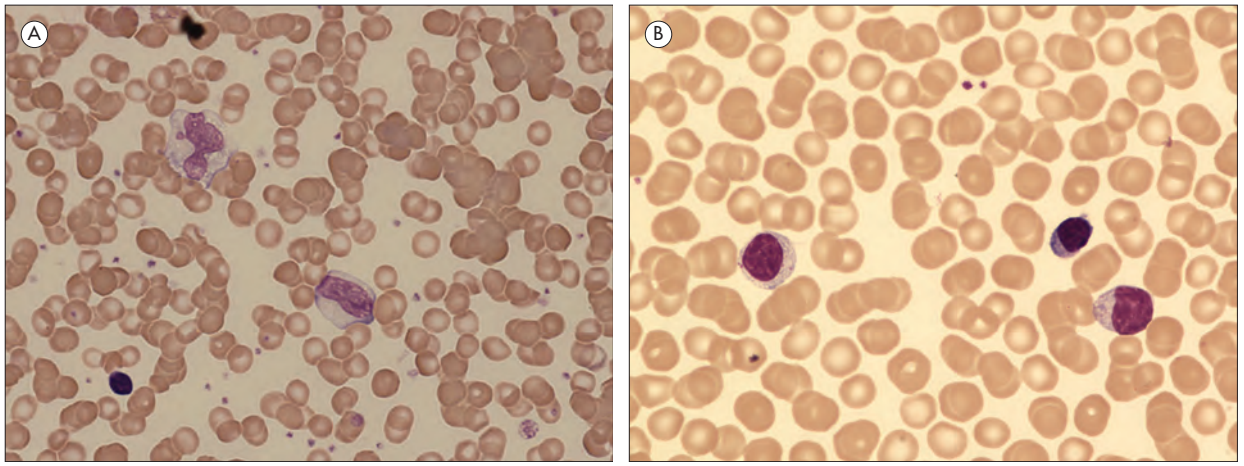


Figure 19.7 (B) Atypical lymphocytes characteristic of Epstein–Barr virus infection and (B) a normal lymphocyte for comparison. (Courtesy of Dr Sue Height, Paediatric Haematology, King's College Hospital NHS Foundation Trust, London.)

T cells and NK cells, are likely to contribute to the symptoms as it causes headache, tiredness and fever. The infected B cells are stimulated to differentiate and produce antibodies; this polyclonal activation of B cells is responsible for the production of heterophil antibodies (reacting with erythrocytes of sheep or horses) and a variety of autoantibodies. Spontaneous recovery usually occurs in 2–3 weeks, but the symptoms may persist for a few months. The virus remains as a latent infection in spite of antibody and CMI responses, and saliva often remains infectious for months after clinical recovery.

The autoantibodies produced in response to EBV infection include IgM antibodies to erythrocytes (cold agglutinins), which are present in most cases. About 1% of cases develop an autoimmune haemolytic anaemia, which subsides within 1–2 months.

A 'hairy tongue' condition caused by EBV replication in squamous epithelial cells in the tongue occurs in immunodeficient patients.

Epstein–Barr virus remains latent in a small proportion of B lymphocytes

Epstein–Barr virus is well equipped to evade immune defences (see Ch. 17). It acts against complement and interferon, and produces a fake interleukin 10 (IL-10) molecule that interferes with the action of the host's own IL-10 (an important immunoregulatory cytokine). EBV also prevents apoptosis (lysis) of infected cells, and the boldness of its strategy has enabled it to take up permanent residence within the immune system.

EBV DNA is present in episomal form in a small proportion of B lymphocytes, and a few copies may be integrated into the cell genome. Later in life, immunodeficiency can lead to reactivation of infection so that EBV reappears in the saliva, usually with no clinical symptoms.

Laboratory tests for diagnosing infectious mononucleosis should include viral capsid antigen IgM detection

Infectious mononucleosis is diagnosed clinically by the characteristic syndrome and the appearance of palatal petechiae in the throat. Laboratory diagnosis is made by detecting VCA IgM in the serum. However, there are other tests that help and these include the following:

- Demonstrating atypical lymphocytes, comprising up to 30% of nucleated cells, in a blood smear. However, a number of viral infections cause an atypical lymphocytosis; therefore this is not specific to EBV.
- Demonstrating heterophil antibodies to horse (or sheep) erythrocytes in the 'monospot' test. These are present in 90% of cases, but may not be detected in those less than 14 years of age and the response is also short lived.
- EBV-specific antibody is the mainstay of diagnosis; in particular, detecting VCA IgM indicates current infection. VCA IgG can be detected soon after VCA IgM, and EBNA IgG appears a few weeks later after symptom onset.

Treatment of EBV infection is limited

Antiviral agents are not used to treat EBV-infected immunocompetent individuals. In immunosuppressed people in specific clinical settings there are some data on using specific antivirals to reduce viral replication, but they are effective only in the lytic part of the life cycle. In addition, an anti-CD20 receptor humanized monoclonal antibody called rituximab has been used to target EBV-infected B cells in specific clinical settings. There is no licensed vaccine, but placebo-controlled clinical trials have been carried out involving an envelope glycoprotein subunit vaccine and a CD8 T-cell peptide vaccine. The subunit vaccine was shown to have a significant effect on clinical disease but did not prevent infection.

Cancers associated with EBV

Epstein–Barr virus is closely associated with Burkitt's lymphoma in African children

Burkitt's lymphoma (Fig. 19.8) is virtually restricted to parts of Africa and Papua New Guinea, so it is clear that EBV alone is not enough to cause the lymphoma. The most likely co-carcinogen is malaria, which acts by weakening T-cell control of EBV infection and perhaps by causing polyclonal activation of B cells, the increased turnover rendering them more susceptible to neoplastic transformation.

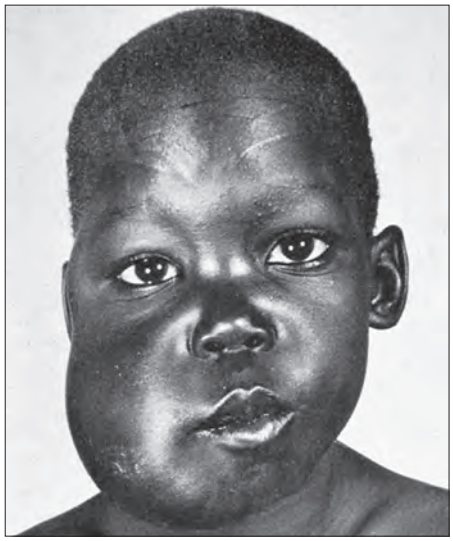


Figure 19.8 Burkitt's lymphoma affecting the maxilla in an African child. (Courtesy I. Magrath, MD, Bethesda, Md. From Zitelli B., Davis H. *Atlas of Pediatric Physical Diagnosis,* 2007, Mosby Elsevier.)

Epstein–Barr virus is closely associated with other B-cell lymphomas in immunodeficient patients

For example, B-cell lymphomas occur in 1–10% of solid organ transplant recipients, especially children, when primary EBV infection occurs post-transplantation. EBV DNA and RNA transcripts are found in the tumour cells, which also show a translocation of the *c-myc* oncogene on chromosome 8 to the immunoglobulin heavy chain locus on chromosome 14 (see Ch. 18). Post-transplant lymphoproliferative disorders (PTLD) are due to uncontrolled B-cell proliferation. In addition, there is the rare X-linked lymphoproliferative disease (XLP) that is associated with EBV infection. This inherited disorder involves mutations in the gene that codes for the signalling lymphocyte activation-molecule-associated protein. The latter is key to B-cell activation of T cells and NK cells which control EBV-infected B cells. Therefore, individuals with this X-linked disorder can develop fatal infectious mononucleosis and lymphomas and these can be prevented only by having an allogeneic bone marrow transplant.

Epstein–Barr virus infection is also closely associated with nasopharyngeal carcinoma

Nasopharyngeal carcinoma (NPC) is a very common cancer in China and South-East Asia. EBV DNA is detectable in the tumour cells, and a co-carcinogen is likely – possibly ingested nitrosamines from preserved fish. Host genetic factors controlling human leukocyte antigens (HLA) and immune responses may confer susceptibility to NPC.

Bacterial infections

Bacteria responsible for pharyngitis include:

- *Streptococcus pyogenes* (Fig. 19.9), which is a group A streptococcus (GAS), is beta haemolytic and colonizes the throat, skin and anogenital tract. It is a common infection, transmitted by respiratory droplets and direct skin contact, and diagnosing it is important because it can lead to complications (see below), but can be readily treated with penicillin
- *Corynebacterium diphtheriae*
- *Haemophilus influenzae* (type B), which occasionally causes severe epiglottitis with obstruction of the airways, especially in young children
- *Borrelia vincentii* together with certain fusiform bacilli, which can cause throat or gingival ulcers
- *Neisseria gonorrhoeae*.

Each of these types of bacteria attach to the mucosal surface, sometimes invading local tissues.

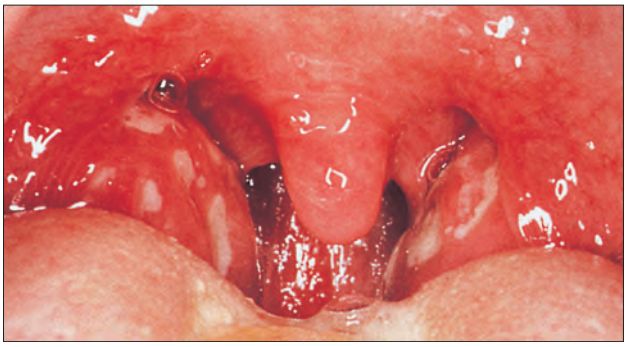


Figure 19.9 Streptococcal tonsillitis due to group A beta haemolytic *Streptococcus pyogenes* with intense erythema of the tonsils and a creamy-yellow exudate. (Courtesy of J.A. Innes.)

Complications of *Strep. pyogenes* infection

Complications of *Strep. pyogenes* throat infection include quinsy, scarlet fever and rarely, rheumatic fever, rheumatic heart disease and glomerulonephritis

These complications are important enough to be listed separately and can be associated with streptococcal toxic shock syndrome, although most are uncommon in resource-rich countries where there is good access to medical care and probably less exposure to streptococci. Invasive GAS (iGAS) is a severe infection in which the streptococci are isolated in sterile sites, including the bloodstream. The complications include the following:

- Peritonsillar abscess ('quinsy') is an uncommon complication of untreated streptococcal sore throat.
- Otitis media, sinusitis and mastoiditis (see below) are caused by local spread of *Strep. pyogenes*.
- Scarlet fever – certain strains of *Strep. pyogenes* produce an erythrogenic toxin coded for by a lysogenic phage. The toxin spreads through the body and localizes in the skin to induce a punctate erythematous rash (scarlet fever; Fig. 19.10). The tongue is initially furred, but later red. Symptoms include a rash, sore throat, red cheeks and swollen tongue. It is a notifiable disease and is highly contagious. The rash begins as facial erythema and then spreads to involve most of the body except the palms and soles. The face is generally flushed with circumoral pallor. The rash fades over the course of 1 week and is followed by extensive desquamation. The skin lesions themselves are not serious, but they signal infection by a potentially harmful streptococcus, which in pre-antibiotic days could sometimes spread through the body to cause cellulitis and septicaemia.

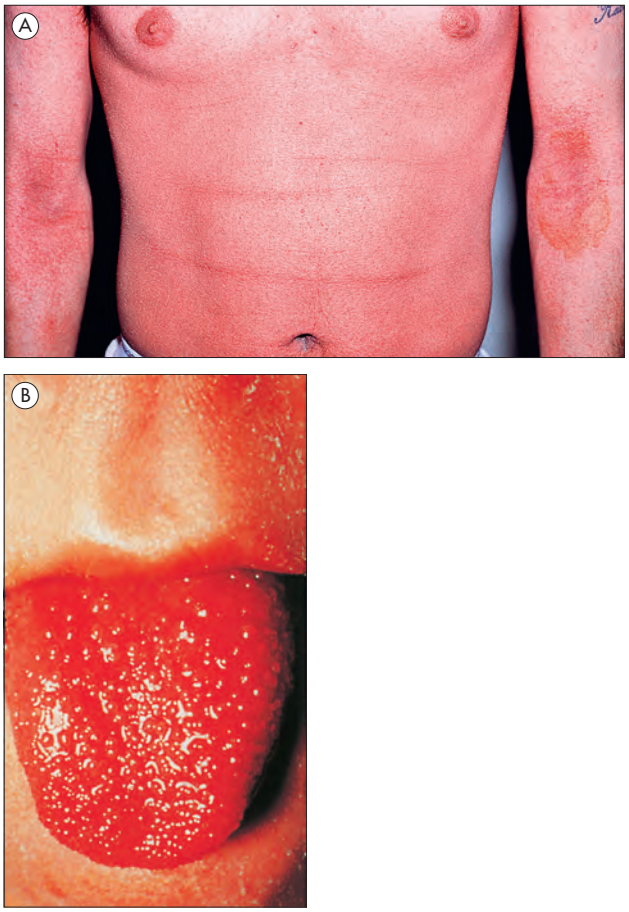


Figure 19.10 Scarlet fever. (A) Punctate erythema is followed by peeling for 2–3 weeks. (B) The tongue is furred at first and then becomes raw with prominent papillae. ([A] From James W.D., Berger T. *Andrews' Diseases of the Skin*, 2006, Saunders Elsevier. [B] Courtesy of W.E. Farrar.)

Table 19.7 Revised Jones Criteria for the diagnosis of rheumatic fever (RF) in people with evidence of a preceding GAS infection

Acute RF	2 Major manifestations or 1 major plus 2 minor manifestations
Recurrent RF	2 Major or 1 major and 2 minor or 3 minor
Major criteria	
Low-risk populations[a]	**Moderate- and high-risk populations**
Carditis • Clinical and /or subclinical	Carditis • Clinical and /or subclinical
Arthritis • Polyarthritis only	Arthritis • Monoarthritis or polyarthritis • Polyarthralgia
Chorea	Chorea
Erythema marginatum	Erythema marginatum
Subcutaneous nodules	Subcutaneous nodules
Minor criteria	
Low-risk populations	**Moderate- and high-risk populations**
Polyarthralgia	Monoarthralgia
Fever (≥38.5°C)	Fever (≥38°C)
ESR ≥60 mm in the first hour and /or CRP ≥3.0 mg /dL	ESR ≥30 mm /h and /or CRP ≥3.0 mg /dL
Prolonged PR interval, after accounting for age variability (unless carditis is a major criterion)	Prolonged PR interval, after accounting for age variability (unless carditis is a major criterion)

CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; GAS, group A streptococcal infection

[a]Low-risk populations are those with ARF incidence ≤2 per 100 000 school-aged children or all-age rheumatic heart disease prevalence of ≤1 per 1000 population per year.

Based on Gewitz MH et al. Revision of the Jones Criteria for the diagnosis of acute rheumatic fever in the era of Doppler echocardiography – a scientific statement from the American Heart Association. *Circulation* 2015; 131:1806–1818.

- Impetigo, erysipelas and cellulitis (see Ch. 27).
- Pneumonia.
- Rheumatic fever – this is an indirect complication. Antibodies formed to antigens in the streptococcal cell wall cross-react with the sarcolemma of the heart, and with tissues elsewhere. Granulomas are formed in the heart (Aschoff's nodules) and 2–4 weeks after the sore throat the patient (usually a child) develops myocarditis or pericarditis, which may be associated with subcutaneous nodules, polyarthritis and, rarely, chorea. Chorea is an involuntary movement disorder and disease of the central nervous system resulting from streptococcal antibodies reacting with neurones. Dr T. Duckett-Jones produced the Jones criteria for the diagnosis of rheumatic fever with major and minor manifestations and evidence of a recent GAS infection as an essential criterion. These were modified by the American Heart Association in 2015 (Table 19.7).
- Rheumatic heart disease – repeated attacks of *Strep. pyogenes* with different M types can lead to damage to the heart valves. Certain children have a genetic predisposition to this immune-mediated disease. If a primary attack is accompanied by rising or high anti-streptolysin O (ASO) antibody levels, future attacks must be prevented by penicillin prophylaxis throughout childhood. In many resource-poor countries, rheumatic heart disease is the most common type of heart disease, seen where there is poverty and overcrowding.
- Acute glomerulonephritis – this is an immune-complex-mediated disease in which antibodies to streptococcal components combine with them to form circulating immune complexes, which are then deposited in glomeruli. Immune cells are recruited and cytokines and chemical mediators produced, together with local complement and coagulation systems being activated, resulting in inflammation in the glomeruli. Blood appears in the urine (red cells, protein) and there are signs of an acute nephritis syndrome (oedema, hypertension) 1–2 weeks after the sore throat. ASO antibodies are usually elevated. There are seven of

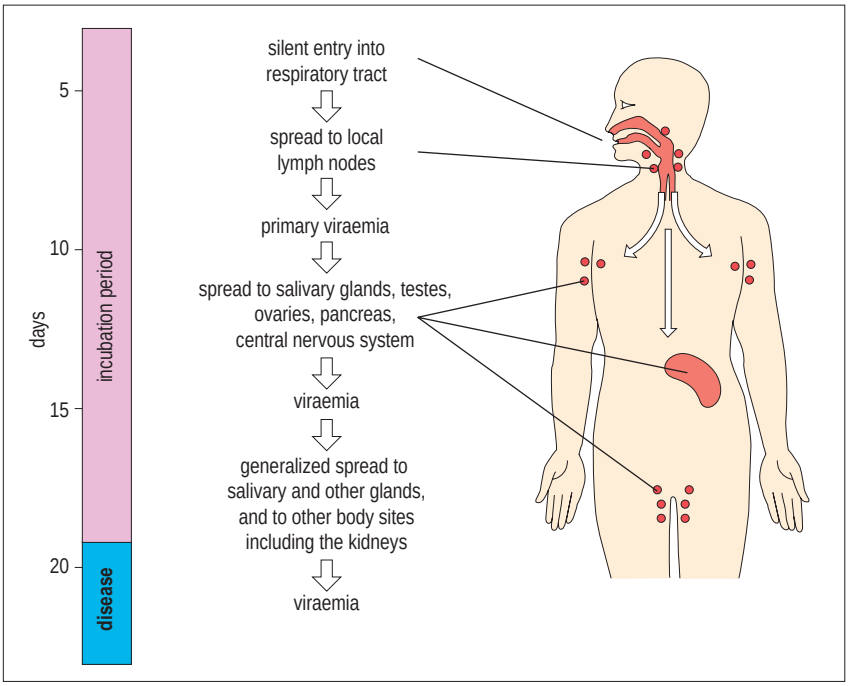


Figure 19.11 The pathogenesis of mumps. Understanding the pathogenesis of this infection helps to explain the disease picture, sites of shedding and the complications that can arise.

at least 80M types of *Strep. pyogenes* that give rise to this condition, this being a protein that is a primary virulent factor, but nephritis may also follow group C streptococcal infection. Penicillin prophylaxis is therefore not given. In contrast to rheumatic fever, second attacks are rare.

Diagnosis

A laboratory diagnosis is not generally necessary for pharyngitis and tonsillitis

There are many possible viral causes of pharyngitis and tonsillitis, and the clinical condition is generally not serious enough to seek laboratory help. The diagnosis of EBV or CMV infection is helped by detection of a lymphocytosis and atypical lymphocytes in a blood film. EBV is distinguished from CMV by detection of the VCA IgM, although the less-specific tests such as the Paul–Bunnell or monospot test may be used in some laboratories, whereas CMV diagnosis is made by detection of CMV IgM. HSV is readily isolated or the DNA detected in swabs from the lesions sent to the laboratory, but clinical diagnosis is usually adequate. Bacteria are identified by culture of throat swabs (see Ch. 33). It is especially important to diagnose *Strep. pyogenes* infection by culture because of the possible complications (see above) and because, unlike *Streptococcus pneumoniae*, it remains susceptible to penicillin. Resistance to erythromycin and tetracycline, however, is increasing. Although during the winter months up to 16% of schoolchildren carry group A streptococci in the throat without symptoms, treatment is recommended.

PAROTITIS

Mumps virus is spread by air-borne droplets and infects the salivary glands

There is only one serotype of this single-stranded RNA paramyxovirus. It spreads by air-borne droplets, salivary secretions and possibly urine. Close contact is necessary, for example, at school, as the peak incidence is at 5–14 years of age. However, susceptible adults are at risk of complications of mumps such as orchitis.

After entering the body, the primary site of replication is the epithelium of the upper respiratory tract or eye. The virus spreads, undergoing further multiplication in local lymphoid tissues (lymphocytes and monocytes) and reticuloendothelial cells. After approximately 7–10 days the virus enters the blood, a primary viraemia, and localizes in salivary and other glands and body sites including the central nervous system, testis, pancreas and ovary (Fig. 19.11) and is excreted in the urine. Infected cells lining the parotid ducts degenerate and finally, after an incubation period of 16–18 days, the inflammation, with lymphocyte infiltration and often oedema, results in disease. After a prodromal period of malaise and anorexia lasting 1–2 days, the parotid gland becomes painful, tender and swollen, and is sometimes accompanied by submandibular gland involvement (Fig. 19.12). This is the classic sign of mumps, and parotitis is the most common clinical sign. Other sites may be invaded, with clinical consequences such as inflammation of the testis and pancreas, resulting respectively in orchitis and pancreatitis (Table 19.8). CMI as well as antibody responses appear, and the patient usually recovers within 1 week. Mumps reinfection can occur after both natural infection or having received MMR vaccine.

Laboratory diagnosis is made:

- by detecting viral RNA in throat swabs, cerebrospinal fluid (CSF) or urine or by isolating virus in cell culture
- by detecting mumps-specific IgM antibody.

Treatment and prevention

There is no specific treatment, but mumps is prevented by using the attenuated live virus vaccine, which is safe and effective. This is usually given in combination with measles and rubella vaccines (MMR vaccine).

Combined MMR has been a controversial issue in the UK after autism and bowel disorders were reported as being possibly associated with immunization. However, despite a series of epidemiological studies showing no association with immunization, there was a fall in MMR uptake rates and subsequent outbreaks of mumps and measles around the UK. These rates had improved by 2017: in the United Kingdom the coverage was above 90%. However, outbreaks of measles were being reported in other parts of Europe.

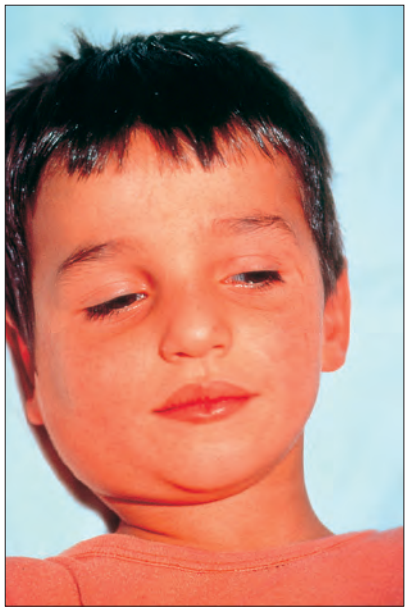


Figure 19.12 Enlarged submandibular glands in a child with mumps. (From Heumann et al. *Klinische Infektiologie,* 2008, Elsevier.)

OTITIS AND SINUSITIS

Otitis and sinusitis can be caused by many viruses and a range of secondary bacterial invaders

Many viruses are capable of invading the air spaces associated with the upper respiratory tract (sinuses, middle ear, mastoid). Mumps virus or respiratory syncytial virus (RSV), for instance, can cause vestibulitis or deafness, which is generally temporary. The range of secondary bacterial invaders is the same as for other upper respiratory tract infections (i.e. *Strep. pneumoniae*, *H. influenzae* and *Moraxella catarrhalis* and sometimes anaerobes, such as *Bacteroides fragilis*). Brain abscess is a major complication (see Ch. 25). Blockage of the eustachian (auditory) tube or the opening of sinuses, caused by allergic swelling of the mucosa, prevents mucociliary clearance of infection, and the local accumulation of inflammatory bacterial products causes further swelling and blockage.

Acute otitis media

Common causes of acute otitis media are viruses, *Strep. pneumoniae* and *H. influenzae*

This condition is extremely common in infants and small children, partly because the eustachian (auditory) tube is open more widely at this age. A study in Boston showed that 83% of 3-year-olds had had at least one episode, and 46% had had three or more episodes since birth. At least 50% of the attacks are viral in origin (especially RSV), and the bacterial invaders are nasopharyngeal residents, most commonly *Strep. pneumoniae*, *H. influenzae* and *M. catarrhalis* and sometimes *Strep. pyogenes* or *Staphylococcus aureus*. There may be general symptoms, and acute otitis media should be considered in any child with unexplained fever, diarrhoea or vomiting. The ear drum shows dilated vessels with bulging of the drum at a later stage (Fig. 19.13). Fluid often persists in the middle ear for weeks or months ('glue ear'), regardless of therapy,

Table 19.8 Clinical consequences of mumps virus invasion of different body tissues

Site of growth	Result	Comment
Salivary glands	Inflammation, parotitis Virus shed in saliva (from 3 days before to 6 days after symptoms)	Often absent; can be unilateral
Meninges Brain	Meningitis Encephalitis	Common (in about 10% cases) Less common; complete recovery is the rule, deafness is a rare complication Both may occur up to 7 days after parotitis
Kidney	Virus present in urine	No clinical consequences
Testis, ovary	Epididymo-orchitis; rigid tunica albuginea around testis make orchitis more painful and more damaging in male	Common in adults (20% in adult males); often unilateral; not a significant cause of sterility
Pancreas	Pancreatitis	Rare complication (possible role in juvenile diabetes mellitus)
Mammary gland	Virus detectable in milk; mastitis in 10% post-pubertal females	
Thyroid	Thyroiditis	Rare
Myocardium	Myocarditis	Rare
Joints	Arthritis	Rare

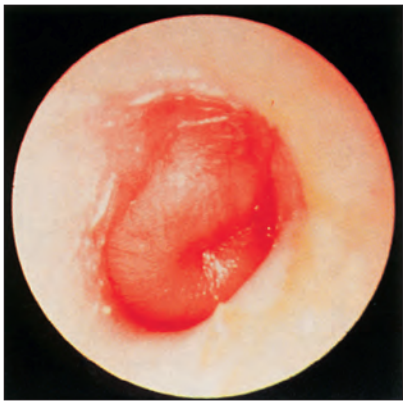


Figure 19.13 Acute otitis media with bulging ear drum. (Courtesy of M. Chaput de Saintonge.)

and contributes to impaired hearing and learning difficulties in infants and small children. Most uncomplicated infections resolve with oral analgesics, but if there is no improvement then systemic antibiotics should be started.

If acute attacks are inadequately treated, there may be continued infection with a chronic discharge through a perforated drum and impaired hearing. This is 'chronic suppurative otitis media'.

Otitis externa

Causes of otitis externa are *Staph. aureus*, *Candida albicans* and Gram-negative opportunists

Infections of the outer ear can cause irritation and pain, and must be distinguished from otitis media. In contrast to the middle ear, the external canal has a bacterial flora similar to that of the skin (staphylococci, corynebacteria and, to a lesser extent, propionibacteria), and the pathogens responsible for otitis media are rarely found in otitis externa. The warm moist environment favours *Staph. aureus*, *C. albicans* and Gram-negative opportunists such as *Proteus* and *Pseudomonas aeruginosa*.

Ear drops containing neomycin or chloramphenicol are usually an effective treatment.

Acute sinusitis

The aetiology and pathogenesis of acute sinusitis are similar to those of otitis media. Clinical features include facial pain and localized tenderness. It may be possible to identify the causative bacteria by microscopy and culture of pus aspirated from the sinus, but sinus puncture is not often carried out. In addition, the patient can be treated empirically with amoxicillin, or co-amoxiclav to deal with beta-lactamase-producing organisms.

ACUTE EPIGLOTTITIS

Acute epiglottitis is generally due to *H. influenzae* capsular type B infection

Acute epiglottitis is most often seen in young children. For unknown reasons, *H. influenzae* capsular type B spreads from the nasopharynx to the epiglottis, causing severe inflammation and oedema. There is usually a bacteraemia.

Acute epiglottitis is an emergency and necessitates intubation and treatment with antibiotics

Acute epiglottitis is characterized by difficulty in breathing because of respiratory obstruction and, until the airway has been secured by intubation, extreme care must be taken when examining the throat in case the swollen epiglottis is sucked into the oedematous airway and causes total obstruction. Treatment is begun immediately with antibiotics effective against *H. influenzae* such as cefotaxime. The clinical diagnosis is confirmed by isolating bacteria from the blood and possibly the epiglottis. The *H. influenzae* type B (Hib) vaccine greatly reduces the frequency of this and other infections due to *H. influenzae* type B.

Respiratory obstruction due to diphtheria (see below) is rare in resource-rich countries, but the characteristic false membrane and local swelling can extend from the pharynx to involve the uvula.

ORAL CAVITY INFECTIONS

Saliva flushes the mouth and contains a variety of antibacterial substances

The oral cavity is continuous with the pharynx, but is dealt with separately because of the presence of teeth, which are subject to a particular set of microbiological problems. The normal mouth contains commensal microorganisms, some of which are to a large extent restricted to the mouth (see Table 19.1). Most of them make specific attachments to teeth or mucosal surfaces and are shed into the saliva as they multiply. The litre or so of saliva secreted each day mechanically flushes the mouth. It also contains secretory antibodies, polymorphs, desquamated mucosal cells and antibacterial substances such as lysozyme and lactoperoxidase. When salivary flow is decreased for a few hours, as between meals, there is a fourfold increase in the number of bacteria in saliva, and, in dehydrated patients or in severe illnesses such as typhoid or pneumonia, the mouth becomes foul because of microbial overgrowth.

Oral candidiasis

Changes in the oral flora produced by broad-spectrum antibiotics and impaired immunity predispose to thrush

The presence of commensal bacteria in the mouth makes it difficult for invading microorganisms to become established, but changes in oral flora upset this balance. For instance, prolonged administration of broad-spectrum antibiotics allows the normally harmless *C. albicans* to flourish, penetrating the epithelium with its pseudomycelia, and causing thrush. Oral thrush (candidiasis, Fig. 19.14) is also seen when immunity is impaired, as in HIV infection and after cytotoxic chemotherapy to treat various cancers, and occasionally in newborn infants and the elderly. It sometimes spreads to involve the oesophagus. The diagnosis is readily confirmed by Gram stain and culture of scraped material, which shows large Gram-positive budding yeasts.

Topical antifungal agents such as nystatin, clotrimazole or oral fluconazole (see Ch. 34) are effective treatments for thrush, together with attention to any predisposing factors.

Another example of the shifting boundary between harmless coexistence and tissue invasion by resident microbes

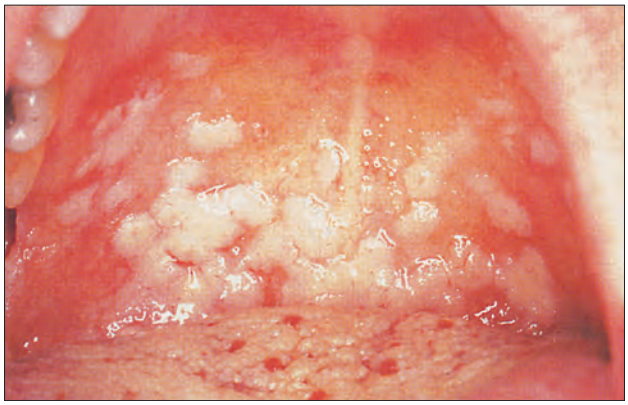


Figure 19.14 Oral candidiasis. (Courtesy of J.A. Innes.)

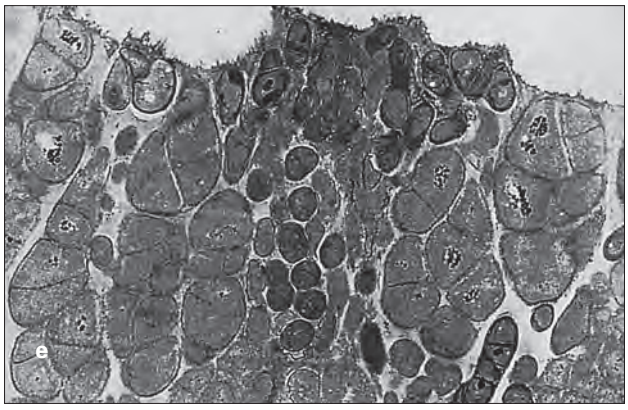


Figure 19.15 Dental plaque on the deep surface of a child's tooth. e, enamel (×20 000). (Courtesy of H.N. Newman.)

is seen with vitamin C deficiency, which reduces mucosal resistance and allows oral residents to cause gum infections.

Caries

In the USA and Western Europe, 80–90% of people are colonized by *Streptococcus mutans*, which causes dental caries

The microorganisms specifically adapted for life on teeth form a film called dental plaque on the tooth surface. This is a complex mass containing about 10^9 bacteria / g embedded in a polysaccharide matrix (Fig. 19.15). The film, visible as a red layer when a dye such as Erythrocin is taken into the mouth, is largely removed by thorough brushing, but re-establishes itself within a few hours. The clean teeth become covered with salivary glycoproteins to which certain streptococci (especially *Strep. mutans* and *Strep. sobrinus*) become attached and multiply. In the USA and Western Europe, 80–90% of people are colonized by *Strep. Mutans*, which itself synthesizes glucan (a sticky high-molecular-weight polysaccharide) from sucrose and this forms a matrix between these streptococci. Certain other bacteria, including anaerobic filamentous fusobacteria and actinomycetes, are also present. When the teeth are not cleaned for several days, plaque becomes thicker and more extensive – a tangled forest of microorganisms.

The bacteria in plaque use dietary sugar and form lactic acid, which decalcifies the tooth locally. Proteolytic enzymes from the bacteria help to break down other components of the enamel to give rise to a painful cavity in the tooth (caries). Infection may then spread into the pulp of the tooth to form a pulp or root abscess, and from here to the maxillary or mandibular spaces.

The pH in an active caries lesion may be as low as 4.0. Therefore, caries usually develops in crevices on the tooth when suitable bacteria (*Strep. mutans*) are in the plaque and there is a regular supply of sucrose. Acid tolerance is a primary ecological advantage for the bacteria involved in caries. *Strep. mutans*, lactobacilli and *Bifidobacteria* spp. are very acid tolerant and break down carbohydrates in the diet to a pH far lower than that in which the commensal bacteria are able to survive. In addition, the commensals do not have a chance as the competitive advantage is bolstered even more by the bacteriocins that are produced by *Strep. mutans*, which are active against bacteria associated with oral health. It may legitimately be regarded as an infectious disease – one of the most prevalent infectious diseases in resource-rich countries due to closely placed bacteria-coated teeth and a sugary, often fluoride-deficient, diet.

Periodontal disease

Actinomyces viscosus, *Actinobacillus* and *Bacteroides* spp. are commonly involved in periodontal disease

A space (the gingival crevice) readily forms between the gums and tooth margin, and it may be considered as an oral backwater. It contains polymorphs, complement and IgG and IgM antibodies, and easily becomes infected. Gingival crevices normally contain an average of 2.7×10^{11} microbes / g, and 75% of them are anaerobes. The oral microbiome contains hundreds of bacterial species, of which a smaller number is associated with progressive periodontal disease, with Gram-negative anaerobic rods and spirochetes predominant. Bacteria such as *Actinomyces viscosus*, *Actinobacillus, Porphyromonas gingivalis, Fusobacterium* spp. and *Bacteroides* spp. are commonly involved. In periodontal disease, the space enlarges to become a 'pocket', with local inflammation, an increasing number of polymorphs and a serum exudate. The inflamed gum bleeds readily and later recedes, while the multiplying bacteria cause halitosis. Finally, the structures supporting the teeth are affected, with reabsorption of ligaments and weakening of bone, causing the teeth to loosen. The interplay between bacterial factors and host response, once again, is key as bacterial lipopolysaccharides activate macrophages to produce cytokines that include interleukins and tumor necrosis factor. These cytokines activate periodontal fibroblasts and matrix metalloproteinases inducing collagen degradation. This is a local disaster as collagen is the main constituent of the periodontal matrix and the result is bone resorption. Periodontal disease with gingivitis is highly prevalent, although its severity varies greatly. It is a major cause of tooth loss in adults. Periodontal disease is multifactorial as there are other risk factors including genetic risk factors, diet, smoking and diabetes.

KEY FACTS

- The respiratory tract from the nose to the alveoli is a continuum, and any given pathogen can cause disease in more than one segment.
- Some respiratory infections (e.g. influenza, diphtheria, pertussis) are restricted to the surface epithelium, whereas others (e.g. measles, rubella, mumps, CMV, EBV) enter via the respiratory tract, causing local symptoms such as pharyngitis, but then spread throughout the body and are associated with a range of non-respiratory symptoms.
- 'Professional' invaders (e.g. common cold viruses, influenza viruses, mumps, CMV, EBV, *M. tuberculosis*) infect the healthy respiratory tract, whereas 'secondary' invaders (e.g. *Staph. aureus*, *Pneumocystis jirovecii*, *Pseudomonas*) cause disease only when host defences are impaired.
- Common diseases of the teeth and neighbouring structures – caries, periodontal disease – are of microbial aetiology.

20 Lower respiratory tract infections

Introduction

Although the respiratory tract is continuous from the nose to the alveoli, it is convenient to distinguish between infections of the upper and lower respiratory tract, even though the same microorganisms might be implicated in infections of both. Infections of the upper respiratory tract and associated structures are the subject of Chapter 19. Here, we discuss infections of the lower respiratory tract. These infections tend to be more severe than infections of the upper respiratory tract and the choice of appropriate antimicrobial therapy is important and may be life saving. In addition, immunization is important to protect those at particular risk of complications.

LARYNGITIS AND TRACHEITIS

Parainfluenza viruses are common causes of laryngitis

Laryngeal infection (laryngitis) and tracheitis cause hoarseness and a burning retrosternal pain. The larynx and trachea have non-expandable rings of cartilage in the wall, and are easily obstructed in children, due to their narrow passages, leading to hospital admission. Swelling of the mucous membrane may lead to a dry cough and inspiratory stridor ('crowing') known as croup. Viral infections of the upper respiratory tract may spread downwards to involve the larynx and the trachea. With the advent of more sensitive molecular diagnostic tests, a broader range of viruses causing laryngitis and tracheitis are being detected and include rhinovirus, parainfluenza virus, influenza virus, adenovirus and respiratory syncytial virus (RSV) infections. Diphtheria (see below) may involve the larynx or trachea.

Bacteria such as group A streptococci, *Haemophilus influenzae* and *Staphylococcus aureus* are less common causes of laryngitis and tracheitis.

DIPHTHERIA

Diphtheria is caused by toxin-producing strains of *Corynebacterium diphtheriae* and can cause life-threatening respiratory obstruction

Diphtheria is now rare in resource-rich countries owing to widespread immunization with toxoid and as a result may be difficult to diagnose clinically, but it is still common in resource-poor countries. It can be respiratory or cutaneous in nature, due to the exotoxin-producing *C. diphtheria* and *C. ulcerans*, respectively. Non-toxigenic strains occur in the normal pharynx, but bacteria producing the extracellular toxin must be present to cause disease. They can colonize the pharynx (especially the tonsillar regions), the larynx, the nose and occasionally the genital tract and in the tropics or in indigent people with poor skin hygiene, the skin.

Adhesion is mediated by pili or fimbriae covalently attached to the bacterial cell wall. The bacteria multiply locally without invading deeper tissues or spreading through the body. The toxin destroys epithelial cells and polymorphs and an ulcer forms, which is covered with a necrotic exudate forming a 'false membrane'. This soon becomes dark and malodorous and bleeding occurs on attempting to remove it. The onset of membranous pharyngitis and fever is accompanied by extensive inflammation and swelling of the soft tissue (Fig. 20.1) and enlarged cervical lymph nodes giving a 'bull-neck' appearance.

Nasopharyngeal diphtheria is the most severe form of the disease. When the larynx is involved, increasing hoarseness and stridor can result in life-threatening respiratory obstruction. Anterior nasal diphtheria is a mild form of the disease if it occurs on its own, because the toxin is less well absorbed from this site, and a nasal discharge may be the main symptom. The patient will, however, be highly infectious.

The incubation period is 2–5 days, but may be up to 10 days. It is most commonly spread by droplets, but direct contact

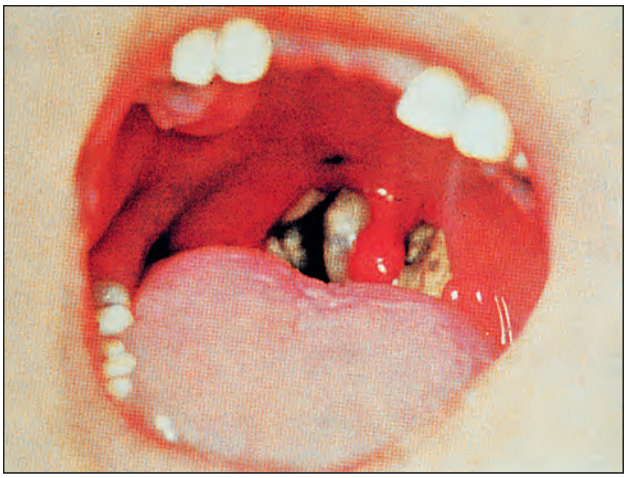


Figure 20.1 Pharyngeal diphtheria. Characteristic diphtheria 'false membrane' in a child, with local inflammation. (Courtesy of Norman Begg.)

Box 20.1 Lessons in Microbiology

Diphtheria toxin

The genes encoding toxin production are carried by a temperate bacteriophage which, during the lysogenic phase, is integrated into the bacterial chromosome. The toxin is synthesized as a single polypeptide (molecular weight 62 000; 535 amino acids) consisting of:

- fragment B (binding) at the carboxy terminal end, which attaches the toxin to the host cells (or to any eukaryotic cell)
- fragment A (active) at the amino terminal end, which is the toxic fragment.

Toxic fragment A is formed only by protease cleavage and reduction of disulphide bonds after cellular uptake of the toxin. Fragment A inactivates elongation factor-2 (EF-2) by adenosine diphosphate (ADP) ribosylation and thereby inhibits protein synthesis (see Fig. 20.2). Prokaryotic and mitochondrial protein synthesis is not affected because a different EF is involved. A single bacterium can produce 5000 toxin molecules / h and the toxic fragment is so stable within the cell that a single molecule can kill a cell. For unknown reasons, myocardial and peripheral nerve cells are particularly susceptible.

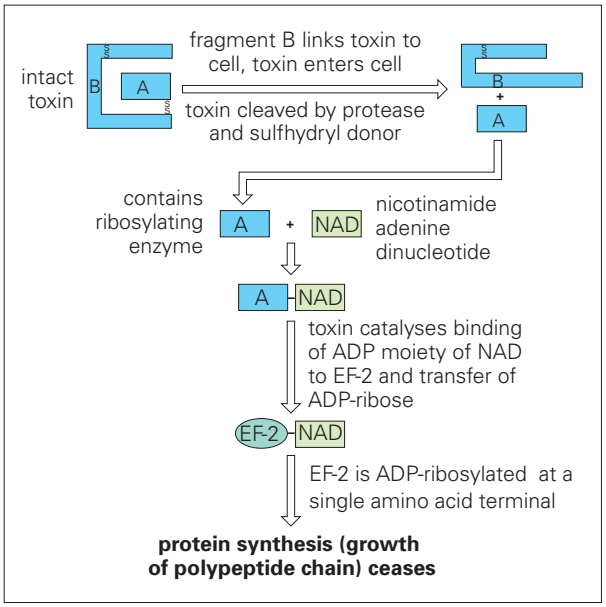


Figure 20.2 Mechanism of action of diphtheria toxin. ADP, adenosine diphosphate; EF-2, elongation factor-2.

with cutaneous diphtheria lesions or infected secretions can also result in transmission.

Diphtheria toxin can cause fatal heart failure and a polyneuritis

Although there are four biovars of *C. diphtheria*, the management from both a clinical and a public health perspective is the same. The exotoxin causes local tissue necrosis and has several effects when absorbed into the lymphatics and blood (Box 20.1 and Fig. 20.2):

- Constitutional upset, with fever, pallor, exhaustion.
- Myocarditis, usually within the first 2 weeks. Electrocardiographic changes are common and cardiac failure can occur. If this is not lethal, complete recovery is usual.
- Polyneuritis, which may occur after the onset of illness, due to demyelination. It may, for instance, affect the 9th cranial nerve, resulting in paralysis of the soft palate and regurgitation of fluids.

Diphtheria is managed by immediate treatment with antitoxin and antibiotic

Diphtheria is a life-threatening disease and clinical diagnosis is a matter of urgency. As soon as the diagnosis is suspected clinically, the patient is isolated to reduce the risk of the toxigenic strain spreading to other susceptible individuals and antitoxin and antibiotic treatment is started. The antitoxin is produced in horses and tests for hypersensitivity to horse serum should be carried out. Until the patient can swallow properly, parenteral benzylpenicillin or erythromycin is also given. Laryngeal diphtheria may result in an obstructed airway and require a tracheotomy to assist with respiration. Patients should also be immunized with a diphtheria-toxoid-containing vaccine once they have recovered as the antitoxin level may be inadequate post-infection.

The diagnosis is confirmed in the laboratory by culture on standard agar and identification by biochemical tests or, depending on availability, matrix-assisted laser desorption / ionization – time of flight (MALDI-TOF) analysis. PCR can be carried out in some reference laboratories to detect the *tox* gene responsible for producing the toxin and toxin production is demonstrated by a gel-diffusion precipitin reaction (Elek test).

Contacts may need chemoprophylaxis or immunization

Close contacts of diphtheria patients should have a nasopharyngeal and throat swab collected and tested for carriage of toxigenic *C. diphtheriae* before chemoprophylaxis to see if they are asymptomatic carriers. They should then be given antibiotic prophylaxis with erythromycin and immunized. Toxigenic bacteria may be carried and transmitted by asymptomatic convalescents or by apparently healthy individuals.

Diphtheria is prevented by immunization

Diphtheria has almost disappeared from resource-rich countries as a result of the immunization of children with a safe, effective toxoid vaccine. However, the disease reappears when immunization is neglected. In 1990, epidemics began in the Russian Federation and by 1994 all 15 of the newly independent states of the former Soviet Union were involved, with 157 000 reported cases by 1997. The World Health Organization (WHO) website reported in 2011 that the incidence of diphtheria ranged from 0.5 to 1 / 100 000 population in Armenia, Estonia, Lithuania and Uzbekistan, to 27–32 / 100 000 in Russia and Tajikistan. Case fatality rates ranged from 2–3% in Russia to 17–23% in Azerbaijan, Georgia and Turkmenistan. Worldwide, in 2015, WHO reported that

there were 4778 cases and the immunization coverage globally was 86%.

WHOOPING COUGH

Whooping cough is caused by the bacterium *Bordetella pertussis*

Whooping cough or pertussis is a severe disease of childhood. Infants, especially if not immunized, are at the highest risk of severe complications. *Bordetella pertussis*, first described by Bordet and Gengou in 1906, is confined to humans and is spread from person to person by airborne droplets. The bacteria attach to, and multiply in, the ciliated respiratory mucosa, but do not invade deeper structures. Surface components such as filamentous haemagglutinin and fimbriae play an important role in specific attachment to respiratory epithelium and / or suppressing the initial inflammatory response to infection, helping persistence.

B. pertussis infection is associated with the production of a variety of toxic factors

Some of these virulence factors affect inflammatory processes, whereas others damage ciliary epithelium. They are:

- Pertussis toxin, sometimes called lymphocytosis-promoting factor as it induces lymphocytosis, resembles diphtheria and other toxins in being a subunit toxin with an active (A) catalytic subunit and membrane-binding (B) subunits. The A unit is an adenosine diphosphate (ADP) ribosyl transferase, which catalyses the transfer of ADP-ribose from nicotinamide adenine dinucleotide (NAD) to the host cell inhibitory G proteins. The functional consequence of this is a disruption of signal transduction to the affected cell as the modification stops the G proteins inhibiting adenylate cyclase activity, thereby increasing the cyclic adenosine monophosphate (cAMP) levels in the cell, causing dysregulation of the immune response, in addition to other effects the toxin has on the cell surface.
- Adenylate cyclase toxin has a C-terminal domain that mediates binding to target cells and forms pores in the plasma membrane and an N-terminal domain which is adenylate cyclase that converts ATP to cAMP. It affects host cells, on entry, by causing ion permeability, increased cAMP levels, which has an effect on cell signalling, and reduces intracellular ATP. In neutrophils, this results in an inhibition of defence functions such as chemotaxis, phagocytosis and bactericidal killing. This toxin may also be responsible for the haemolytic properties of *B. pertussis*.
- Tracheal cytotoxin, which is a cell wall component derived from the peptidoglycan of *B. pertussis* that specifically kills tracheal epithelial cells.
- Endotoxin, which differs from the classic endotoxin of other Gram-negative rods, but has functional similarities and may play a role in the pathogenesis of infection.

B. pertussis infection is characterized by paroxysms of coughs followed by a 'whoop'. There are three phases, the catarrhal, paroxysmal and convalescent phase. After an incubation period of 7–10 days (range 5–21 days), *B. pertussis* infection is manifest first as a catarrhal illness with little to distinguish it from other upper respiratory tract infections. This is followed up to 1 week later by a dry non-productive cough, which becomes paroxysmal. A paroxysm is characterized by a series of short coughs producing copious mucus, followed by a 'whoop', which is a characteristic sound produced by an inspiratory gasp of air. Despite the severity of the cough, the symptoms are confined to the respiratory tract, and lobar or segmental collapse of the lungs can occur (Fig. 20.3).

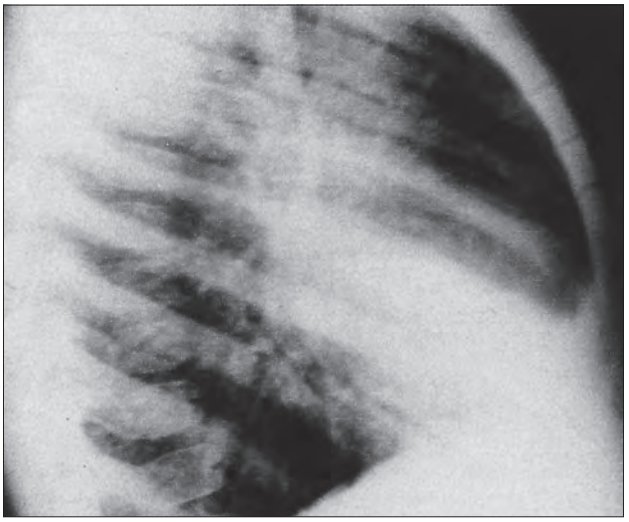


Figure 20.3 Chest radiograph showing patchy consolidation and collapse of the right middle lobe in whooping cough. (Courtesy of J.A. Innes.)

Complications include central nervous system (CNS) anoxia, exhaustion and secondary pneumonia due to invasion of the damaged respiratory tract by other pathogens.

The early clinical picture is non-specific, and the true diagnosis may not be suspected until the paroxysmal phase. The organisms can be isolated on suitable media from nasopharyngeal or per nasal swabs, not throat swabs as the bacteria are most likely to be found on the posterior wall of the nasopharynx or on 'cough plates', but they are fastidious and do not survive well outside the host. Polymerase chain reaction (PCR) is usually more sensitive than culture but may not be positive if the symptoms have lasted more than 3 weeks.

Whooping cough is managed with supportive care and erythromycin

Supportive care is of prime importance. Infants are at greatest risk of complications, and admission to hospital should be considered for children less than 1 year of age. For specific antibacterial treatment to be effective it must penetrate the respiratory mucosa and inhibit or kill the infecting organism. Treatment with macrolide antibiotics such as erythromycin, clarithromycin or azithromycin is recommended. Although the treatment is often started only when the disease is recognized in the paroxysmal phase, it does appear to reduce its severity and duration. It also reduces the bacterial load in the throat, thereby helping to reduce both the infectivity of the patient and the risk of secondary infections.

Prophylaxis with macrolide antibiotics of close contacts of active cases is helpful in controlling the spread of infection.

Whooping cough can be prevented by active immunization

For many years, a whole cell vaccine comprising a killed suspension of *B. pertussis* cells was used, combined with

purified diphtheria and tetanus toxoids and administered as 'DPT' or 'triple' vaccine. Although an effective vaccine, there were major concerns about side effects. These included fever, malaise and pain at the site of administration in up to 20% of infants; convulsions, thought to be associated with the vaccine in about 0.5% of vaccinees; and encephalopathy and permanent neurological sequelae associated with vaccination, with an estimated rate of 1 in 100 000 vaccinations (<0.001%).

Concern about side effects led to a marked fall in uptake of the vaccine and subsequently to a marked increase in the incidence of whooping cough.

Acellular pertussis vaccines became the dominant vaccine preparation as they provide the same or better protection against whooping cough, and cause fewer side effects as they are highly purified with much reduced levels of endotoxin compared with whole cell vaccines. The acellular vaccines contain pertussis toxoid and other bacterial components, including the filamentous haemagglutinin and fimbriae, and are given in combination with other vaccines such as diphtheria, tetanus and inactivated polio. In 2012, surveillance in the UK detected the highest rise in pertussis infections in 20 years. These were seen in young adults and adolescents, but morbidity and mortality occurred in unimmunized infants. Pertussis immunization in pregnancy was introduced that year and extended to at least 2019, as babies born to immunized mothers were 90% less likely to develop pertussis than were those born to unimmunized mothers. This was due to passively transferred maternal antibody to the baby. In 2015, about 86% of all infants worldwide received three doses of pertussis vaccine. WHO estimated that there were about 89 000 deaths due to pertussis in 2008 and 123 210 reported infections worldwide in 2015 (Fig. 20.4).

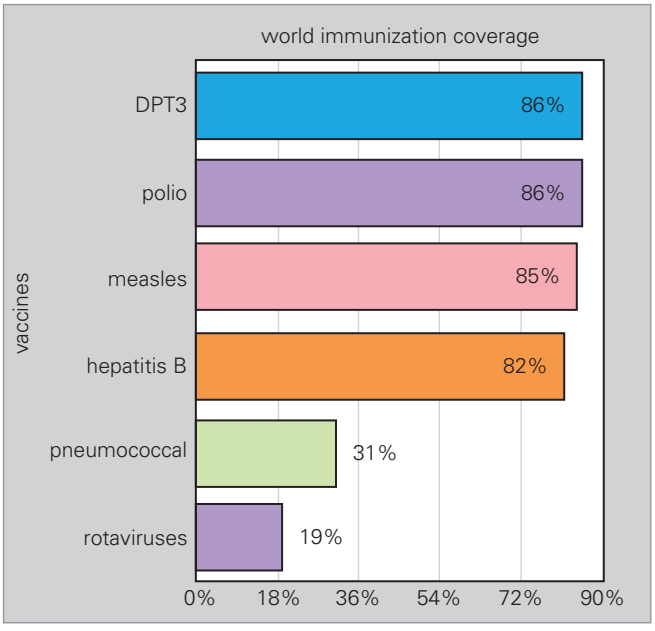


Figure 20.4 World Immunization coverage of diphtheria–tetanus–pertussis (DTP3) vaccines in infants (from <50%), 2015. (From WHO/UNICEF coverage estimates, 2015 version (July 2016), with permission.)

ACUTE BRONCHITIS

Acute bronchitis is an inflammatory condition of the tracheobronchial tree, usually due to infection

Causative agents include rhinoviruses and coronaviruses, which also infect the upper respiratory tract, and lower tract pathogens such as influenza viruses, adenoviruses and *Mycoplasma pneumoniae*. Secondary bacterial infection with *Streptococcus pneumoniae* and *H. influenzae* may also play a role in pathogenesis. The degree of damage to the respiratory epithelium varies with the infecting agent:

- With influenza virus infection, it may be extensive and leave the host prone to secondary bacterial invasion (post-influenza pneumonia; see below).
- With *M. pneumoniae* infection, a cause of community-acquired pneumonia, specific attachment of the organism to receptors on the bronchial mucosal epithelium, evading the host's attempts at mucociliary clearance (Fig. 20.5) and the release of community-acquired respiratory distress toxin that causes airways inflammation and sloughing of affected cells are key components. It was considered an atypical bacterium due to the pneumonia not responding to antibiotics that act on the cell wall, a result of it not having a cell wall! There is a 4-yearly epidemic cycle that normally occurs 2 years after the Olympic Games. A dry cough is the most prominent presentation, with fever, and treatment is largely symptomatic. However, it can cause pneumonia and complications involving other organs, such as hepatitis, encephalitis, arthralgia, haemolytic anaemia and skin lesions known as erythema multiforme and Stevens–Johnson syndrome, which is a toxic epidermal necrolysis. Treatment involves antibiotics such as tetracyclines or macrolides.

ACUTE EXACERBATIONS OF CHRONIC BRONCHITIS

Infection is only one component of chronic bronchitis

Chronic bronchitis is a condition characterized by cough and excessive mucus secretion in the tracheobronchial tree that is not attributable to specific diseases such as

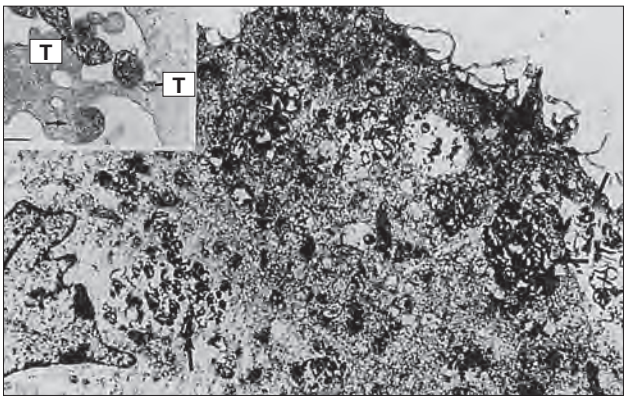


Figure 20.5 Opsonized *Mycoplasma pneumoniae* cells (arrowed) phagocytosed by an alveolar macrophage (bar, 2 μm). The insert shows *M. pneumoniae* cells adhering with the tip organelle (T) to macrophage surfaces. (From Jacobs E. *Rev Med Microbiol* 2:83–90, ©1991, with permission.)

bronchiectasis, asthma or tuberculosis. Infection appears to be only one component of the syndrome, the others being cigarette smoking and inhalation of dust or fumes from the workplace. Bacterial infection does not appear to initiate the disease, but is probably significant in perpetuating it and in producing the characteristic acute exacerbations. *S. pneumoniae* and unencapsulated strains of *H. influenzae* are the organisms most frequently isolated, but interpretation of the significance of their presence in sputum is difficult because they are also commonly found in the normal throat flora and can therefore contaminate expectorated sputum. Other bacteria such as *Staph. aureus* and *M. pneumoniae* are less commonly associated with infection and exacerbation. Viruses are frequent causes of acute infection and cause the initial damage that results in secondary bacterial infections.

Antibiotic therapy may be helpful in the treatment of acute exacerbations, although its efficacy is difficult to assess.

BRONCHIOLITIS

Around 75% of bronchiolitis presentations are caused by RSV infection

Bronchiolitis is a disease restricted to childhood, and usually to children less than 2 years of age. The bronchioles of a young child have such a fine bore that if their lining cells are swollen by inflammation the passage of air to and from the alveoli can be severely restricted. Infection results in necrosis of the epithelial cells lining the bronchioles and leads to peribronchial infiltration, which may spread into the lung fields to give an interstitial pneumonia (see below). As many as 75% of these infections are caused by RSV and the rest are also of viral aetiology, including parainfluenza viruses, human metapneumovirus and influenza viruses.

RESPIRATORY SYNCYTIAL VIRUS (RSV) INFECTION

RSV is the most important cause of bronchiolitis and pneumonia in infants

Respiratory syncytial virus is a typical paramyxovirus, and two major strains have been identified: group A and group B. Its surface spikes bear G protein (not haemagglutinin or neuraminidase) for attachment to the cell, and fusion (F) protein. The latter initiates viral entry by fusing the viral envelope to the cell membrane, and also fuses host cells to form syncytia.

RSV infection is transmitted by droplets and to some extent by hands. Outbreaks occur each winter and, during the RSV season, infection can spread in hospitals as well as in the community. Nearly all individuals have been infected by 2 years of age. About 1 in every 100 infants with RSV bronchiolitis or pneumonia requires admission to hospital.

RSV infection can be particularly severe in young infants

After inhalation, the virus establishes infection in the nasopharynx and lower respiratory tract. Clinical illness appears after an incubation period of 4–5 days. The illness can be particularly severe in babies, with peak mortality at 3 months of age, the virus invading the lower respiratory tract by direct surface spread to cause bronchiolitis or pneumonia. They develop a cough, rapid respiratory rate and cyanosis. In young children and adults, however, the virus may be restricted to the upper respiratory tract, causing a less severe common cold-type illness. Otitis media is quite common. Secondary bacterial infection is thought to be rare, but with more sensitive diagnostic tests, over time it may become apparent that it is more frequent than was recognized previously.

The manifestations of RSV infection appear to have an immunopathological basis

Maternal antibodies in the infant react with virus antigens, perhaps with the liberation of histamine and other mediators from the host's cells. In early trials, a killed vaccine was used and during subsequent natural RSV infection the vaccinees had more frequent and severe lower respiratory tract disease compared with unimmunized children, supporting an immune-mediated pathogenesis.

Neutralizing antibodies are formed, at lower levels in younger infants, but cell-mediated immunity (CMI) is needed to terminate the infection. The virus continues to be shed from the lungs of children lacking CMI for many months. Apparently healthy children may continue to show depressed pulmonary function or wheeze even 1–2 years after apparent recovery.

Recurrent infections are common, but are less severe. The reason for recurrence, which is also a feature of parainfluenza virus infection, is unknown.

RSV RNA is detectable in throat swab specimens and ribavirin is indicated for severe disease

Molecular methods, such as PCR, used to detect RSV RNA in throat swab specimens, have a higher diagnostic sensitivity than the older diagnostic tests such as immunofluorescence (Fig. 20.6) and virus isolation.

In most children, treatment is supportive, involving rehydration, bronchodilators and, if needing admission to hospital, oxygen. The antiviral agent ribavirin, given as an aerosol or orally, has been used successfully in a number of clinical settings, including children with severe infection and immunosuppressed individuals at risk of severe disease. A monoclonal antibody, palivizumab, can be used as prophylaxis to prevent RSV infection in infants less than 2 years old at risk of severe disease such as those with chronic lung disease, congenital heart disease or those born at less than 32 weeks of age. As of 2017, there is no vaccine available but a number of vaccines are being developed.

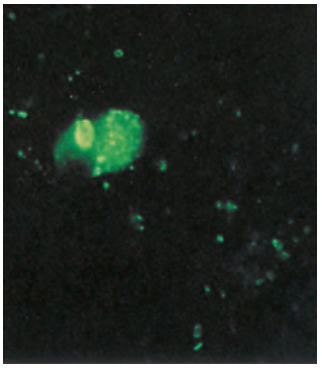


Figure 20.6 Immunofluorescent preparation from the nasopharynx showing respiratory syncytial virus-infected cells (bright green). (Courtesy of H. Stern.)

HANTAVIRUS PULMONARY SYNDROME (HPS)

The reservoir host for Sin Nombre virus (SNV), a New World hantavirus, is the deer mouse found commonly in North America. In 1993, individuals were infected in southwestern USA and developed severe cardiopulmonary disease. HPS followed flu-like symptoms, such as fever and myalgia, followed around 10 days later by cough and dyspnoea as viral invasion of the pulmonary capillary endothelium results in fluid pouring into the lungs owing to increased vascular permeability. At least 26 deaths were reported secondary to pulmonary oedema, hypotension and cardiogenic shock. The route of transmission is by inhaling SNV-infected rodent faeces, saliva or urine. The Old World hantaviruses cause haemorrhagic fever with renal syndrome. The pathogenesis of both diseases is thought to involve aberrant immune responses by SNV-infected endothelial cells that are also involved in regulating vascular permeability. By 2016, 690 individuals with HPS had been reported in the USA, with a 36% mortality rate. HPS has also been reported in South America. There are other hantaviruses that cause HPS in other areas of the USA with different rodents hosts including the white-footed mouse and cotton rat. Person-to-person transmission has not been reported. Treatment is mainly supportive in an intensive care unit setting. Ribavirin treatment has not been shown to be effective despite success treating patients with haemorrhagic fever with renal syndrome.

PNEUMONIA

Pneumonia has long been known as 'the old person's friend' as it is the most common cause of infection-related death in the USA and Europe. It is caused by a wide range of microorganisms and the challenge lies not in the clinical diagnosis of pneumonia, except perhaps in children, in whom it may be more difficult to diagnose, but in the laboratory identification of the microbial cause.

Microorganisms reach the lungs by inhalation, aspiration or via the blood

Microorganisms gain access to the lower respiratory tract by inhalation of aerosolized material or by aspiration of the normal flora of the upper respiratory tract. The size of inhaled particles is important in determining how far they travel down the respiratory tract; only those less than about 5 mm in diameter reach the alveoli. Less frequently, the lungs become seeded with organisms as a result of spread via the blood from other infected sites. Healthy individuals are susceptible to infection by a range of pathogens possessing adhesins, which allow the pathogens to attach specifically to the respiratory epithelium. In addition, people with impaired defences, for example, if immunocompromised, with preceding viral damage, or with cystic fibrosis, may develop infections with organisms that do not cause infections in healthy individuals. An example is *Pneumocystis jirovecii*, a fungal infection that is an important cause of pneumonia in individuals with AIDS.

The respiratory tract has a limited number of ways in which it can respond to infection

The host's response can be defined by the pathological and radiological findings. Four descriptive terms are in common use (Fig. 20.7):

- Lobar pneumonia refers to involvement of a lobe, a distinct region of the lung. The polymorph exudate formed in response to infection clots in the alveoli and renders them solid. Infection may spread to adjacent alveoli until constrained by anatomical barriers between segments or lobes of the lung. Thus one lobe may show complete consolidation.
- Bronchopneumonia refers to a more diffuse patchy consolidation, which may spread throughout the lung as a result of the original pathological process in the small airways.
- Interstitial pneumonia or pneumonitis involves invasion of the lung interstitium and is particularly characteristic of viral infections of the lungs, but is also seen in atypical bacterial causes of pneumonia and *Pneumocystis* infection.
- Lung abscess, sometimes referred to as necrotizing pneumonia, is a condition in which there is cavitation and destruction of the lung parenchyma.

The outcomes common to all these conditions are respiratory distress resulting from the interference with air exchange in the lungs, and systemic effects as a result of infection in any part of the body.

A wide range of microorganisms can cause pneumonia

Age is an important determinant (Table 20.1):

- Most childhood pneumonia is caused either by viruses or by bacteria invading the respiratory tract secondary to a viral infection, such as influenza. Neonates born to mothers with genital *Chlamydia trachomatis* infection may develop a chlamydial interstitial pneumonitis resulting from colonization of the respiratory tract during birth.
- In the absence of an underlying disorder such as cystic fibrosis, pneumonia is unusual in older children. Children and young adults with cystic fibrosis are very prone to lower respiratory tract infection, caused characteristically by *Staph. aureus*, *H. influenzae* and *Pseudomonas aeruginosa*.
- The cause of pneumonia in adults depends upon a number of risk factors such as older age, underlying disease and exposure to pathogens through occupation, travel or contact with animals.

Pneumonia acquired in hospital tends to be caused by a different spectrum of organisms, particularly Gram-negative bacteria. The causative agents of adult pneumonia are summarized in Fig. 20.8. Although clinical and epidemiological clues help

Table 20.1 Causes of pneumonia related to age

Children	Adults
Mainly viral (e.g. respiratory syncytial virus, parainfluenza) or bacterial secondary to viral respiratory infection (e.g. after influenza, measles)	Bacterial causes more common than viral
Neonates may develop interstitial pneumonitis caused by *Chlamydia trachomatis* acquired from the mother at birth	Aetiology varies with age, underlying disease, occupational and geographic risk factors

Pneumonia in children is more often viral in origin or bacterial secondary to a viral respiratory infection. In adults, bacterial pneumonia is more common.

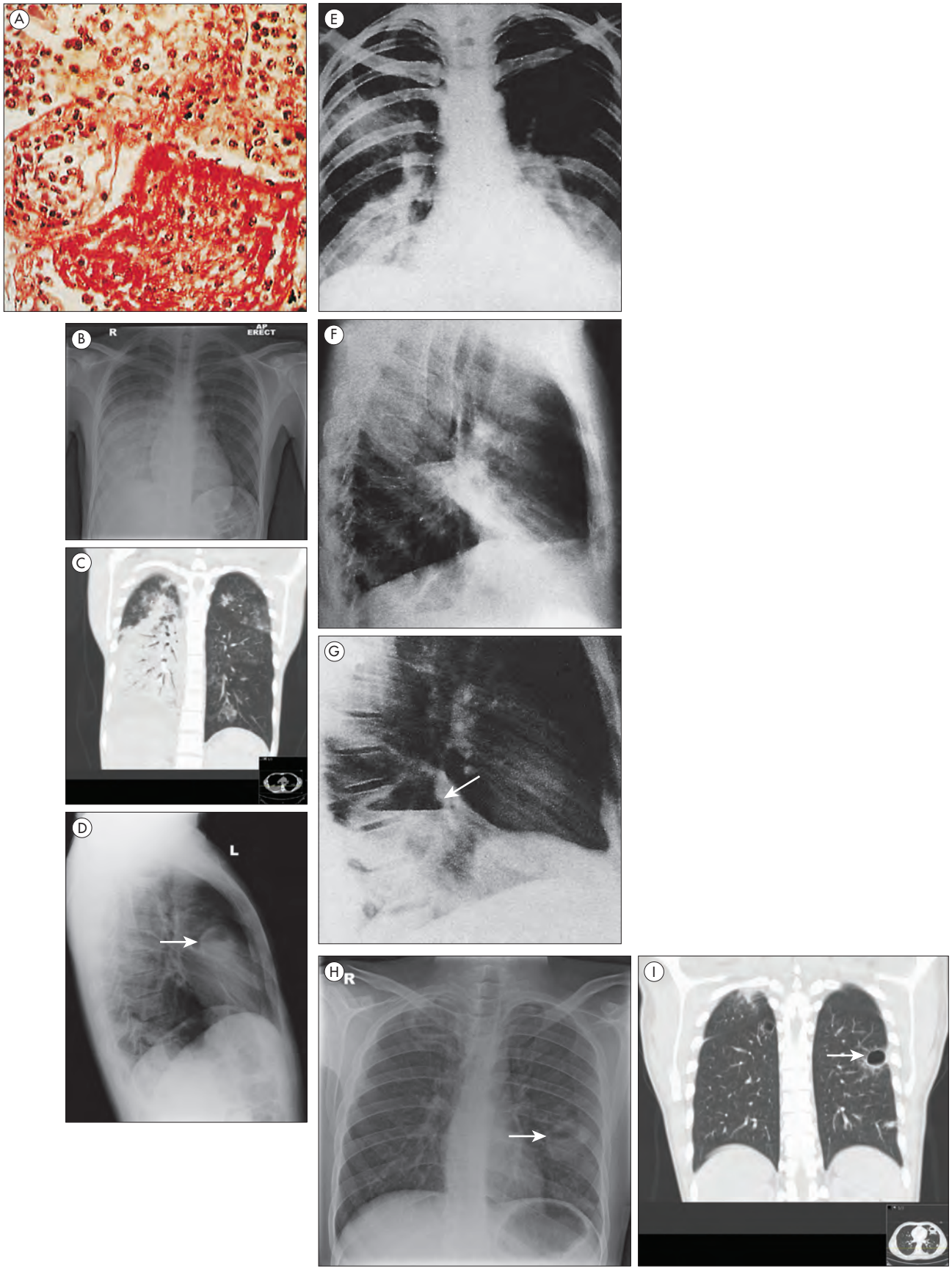


Figure 20.7 Four types of pneumonia. (A) Pneumococcal lobar pneumonia, showing consolidated alveoli filled with neutrophils and fibrin (H&E stain). (B) Right lower lobe pneumonia – white out on chest X-ray and chest CT. (E) Mycoplasma bronchopneumonia, with patchy consolidation in several areas of both lungs. (F) Interstitial pneumonia due to influenza virus. (G) Lung abscess, showing an abscess cavity (arrowed) on chest X-ray (H) and chest CT (I). ([A] Courtesy of I.D. Starke and M.E. Hodson. [B]–[D] Courtesy of G. Bain, London North West Healthcare Trust). [E] Courtesy of J.A. Innes. [F] Courtesy of I.D. Starke and M.E. Hodson. [G]–[I] Courtesy of G. Bain, London North West Healthcare Trust.)

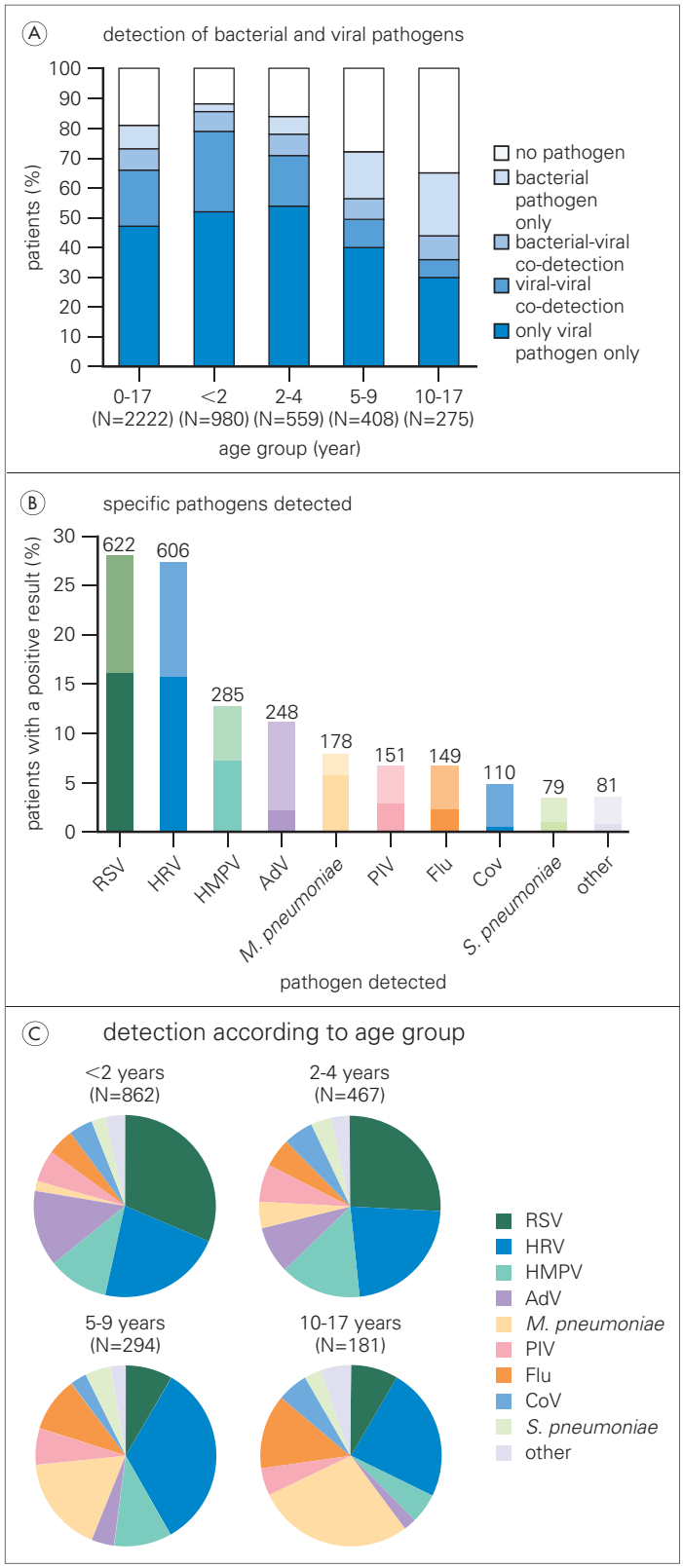


Figure 20.8 (A)–(C) Pathogens detected in US children with community-acquired pneumonia requiring hospitalization 2010–2012.

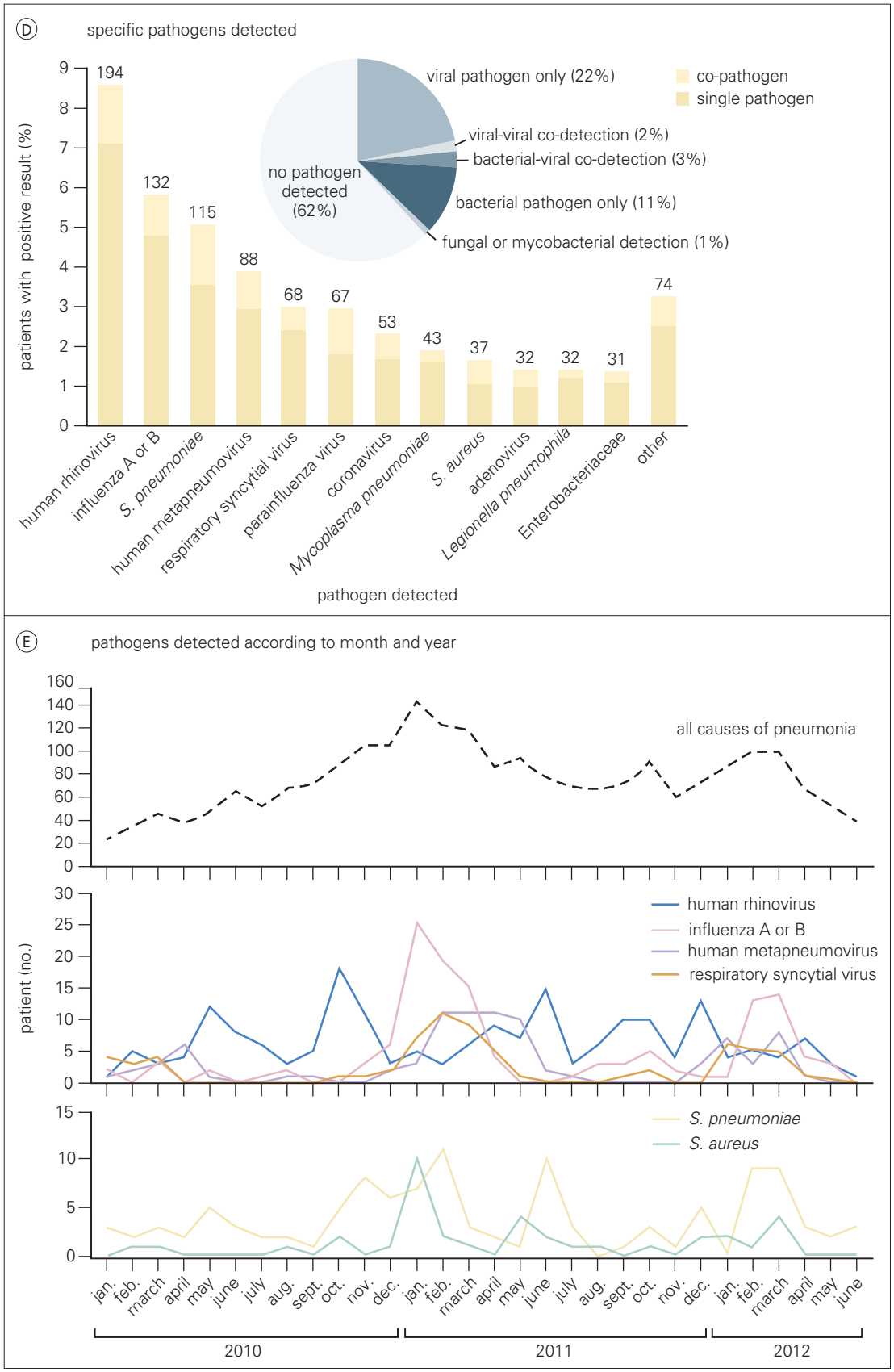


Figure 20.8—Cont'd (D)–(E) Pathogens detected in US adults with community-acquired pneumonia requiring hospitalization 2010–2012. ([A] – [C] from Jain S., Williams D.J., Arnold S.R. et al. Community-acquired pneumonia requiring hospitalization among U.S. children. *NEJM* 2015; 372:835–845, with permission. [D] – [E] From Jain S., Self W.H., Wunderink R.G. et al. Community-acquired pneumonia requiring hospitalization among U.S. adults. *NEJM* 2015; 373:415–427, with permission.)

to suggest the likely cause, microbiological investigations are essential to confirm the diagnosis and ensure optimal antimicrobial therapy.

Viral pneumonias show a characteristic interstitial pneumonia on chest radiography more often than bacterial pneumonias (see Fig. 20.7F), and for the sake of clarity are described separately below. Infections with RSV have been described earlier in this chapter and opportunist pathogens, such as *Pneumocystis jirovecii*, associated specifically with pneumonia in the immunocompromised, are described in Chapter 31.

BACTERIAL PNEUMONIA

Streptococcus pneumoniae is the classic bacterial cause of acute community-acquired pneumonia

In the past, 50–90% of pneumonias were caused by *Strep. pneumoniae* (the 'pneumococcus') and it is still the most common cause of bacterial pneumonia in children worldwide, with *H. influenzae* being the second most common cause. WHO reported that pneumonia causes 15% of all deaths of children under 5 years old, accounting for nearly one million deaths in 2015. The CDC Etiology of Pneumonia in the Community (EPIC) study was a prospective, multicentre, population-based, active surveillance study involving adults and children with community-acquired pneumonia who were admitted to hospital from January 2010 to June 2012. A viral or bacterial pathogen was detected in 81% of the 2638 children, one or more viruses in 66%, bacteria in 8% and bacterial and viral co-infection in 7% (Fig. 20.8A). Of 2488 adults, a pathogen was detected in 38%, one or more viruses in 23%, bacteria in 11%, bacterial and viral co-infections in 3% and a fungal or mycobacterial (should have been included in bacterial!) pathogen in 1% (Fig. 20.8B).

A variety of bacteria causes primary atypical pneumonia

When penicillin, an effective antibiotic treatment for pneumococcal infection, became widely available, a significant proportion of patients with pneumonia failed to respond to this treatment and were labelled 'primary atypical pneumonia'. 'Primary' referred to pneumonia occurring as a new event, not secondary to influenza, for example, and 'atypical' to the fact that *Strep. pneumoniae* was not isolated from sputum from such patients, the symptoms often general as well as respiratory, and the pneumonia did not to respond to penicillin or ampicillin. The causes of atypical pneumonia include *M. pneumoniae*, *Chlamydophila pneumoniae* and *C. psittaci*, *Legionella pneumophila* and *Coxiella burnetii*. Infection with *C. pneumoniae* is common, CDC in the USA estimates it causes at least 300 000 cases of pneumonia each year in adults. *Mycoplasma pneumoniae* and *C. pneumoniae* appear to be solely human pathogens, whereas *C. psittaci* and *C. burnetii* are acquired from infected animals, and *L. pneumophila* is acquired from contaminated environmental sources (see Fig. 20.9).

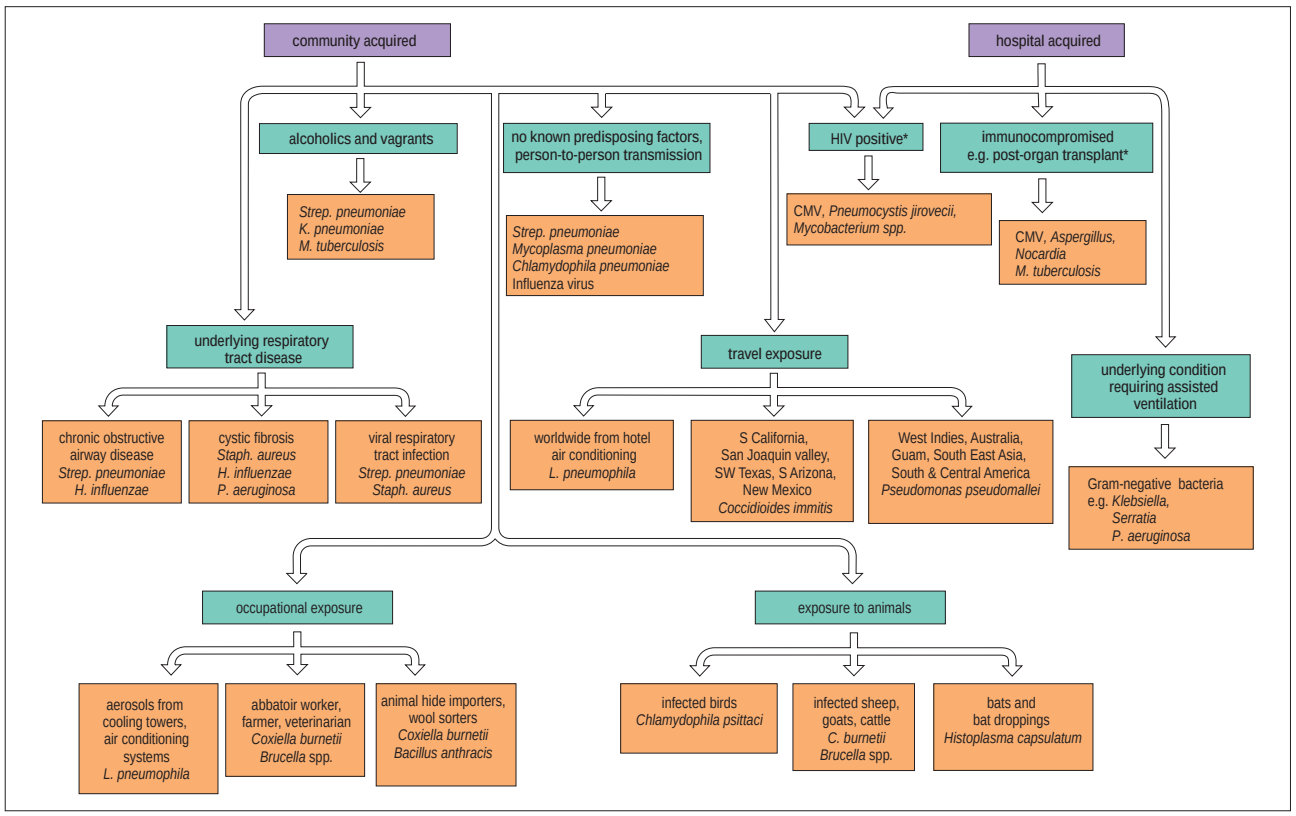


Figure 20.9 Many pathogens are capable of causing pneumonia in adults, and the aetiology is related to risk factors such as the exposure to pathogens through occupation, travel and contact with animals. The elderly are more likely to be infected and tend to have a more severe illness than young adults. *These infections are often reactivating endogenous infections rather than community or hospital acquired. *C.*, *Coxiella*; CMV, cytomegalovirus; *H.*, *Haemophilus*; *K.*, *Klebsiella*; *L.*, *Legionella*; *M.*, *Mycobacterium*; *P.*, *Pseudomonas*; *Staph.*, *Staphylococcus*; *Strep.*, *Streptococcus*.

Moraxella catarrhalis is recognized increasingly as a cause of pneumonia, particularly in patients with carcinoma of the lung or other underlying lung disease. Other aetiological agents of pneumonia associated with particular underlying diseases, occupations or exposure to animals and travel are summarized in Fig. 20.9 and described in other chapters. It is important to note that a causative organism is not isolated in up to 35% of lower respiratory tract infections.

Patients with pneumonia usually present feeling unwell and with a fever

Signs and symptoms of a chest infection include:

- chest pain, which may be pleuritic in nature (pain on inspiration)
- a cough, which may produce sputum
- shortness of breath (dyspnoea).

Some infections result in symptoms confined mainly to the chest, whereas others such as Legionnaires' disease caused by *L. pneumophila* have a much wider systemic involvement, and the patient may present with confusion, diarrhoea and evidence of renal or liver dysfunction. However, the distinction between localized and systemic symptoms is not usually reliable enough for an accurate diagnosis.

Chest examination may reveal abnormal crackling sounds, called 'rales', and evidence of consolidation, even before changes become evident on radiography.

Patients with pneumonia usually have shadows in one or more areas of the lung

Imaging the chest with chest X-rays, computed tomography (CT) and magnetic resonance imaging (MRI) is an important adjunct to the clinical diagnosis. Patients with pneumonia usually have shadows indicating consolidation (see above for descriptions of lobar, broncho- and interstitial pneumonia). However, careful interpretation is required to differentiate between infection and non-infective processes such as tumours.

Pneumonia is the most common cause of death from infection in the elderly

It is also an important cause of death in the young and previously healthy. Complications of infection include spread of the infecting organisms:

- directly, to extrapulmonary sites such as the pleural space, giving rise to empyema (see below)
- indirectly, via the blood to other parts of the body.

For example, the majority of patients with pneumococcal pneumonia have positive blood cultures, and pneumococcal meningitis may follow pneumonia in the elderly.

Sputum samples are best collected in the morning and before breakfast

Microscopic examination and culture of expectorated sputum remain the mainstays of respiratory bacteriology, despite doubts about the value of these procedures. Collection of sputum is non-invasive, but more invasive techniques, such as transtracheal aspiration, bronchoscopy and bronchoalveolar lavage and open lung biopsy, may yield more useful results.

Sputum samples are best collected in the morning because sputum tends to accumulate while the patient is lying in bed, and before breakfast to reduce contamination by food particles and bacteria from food. It is important that the specimen submitted for examination is truly sputum and not simply saliva. A physiotherapist can be of great assistance to ill patients who may be unable to cough unaided.

The usual laboratory procedures on sputum specimens from patients with pneumonia are Gram stain and culture

Examination of the Gram-stained sputum can give a presumptive diagnosis within minutes if the film reveals a host response in the form of abundant polymorphs and the putative pathogen, e.g. Gram-positive diplococci characteristic of *Strep. pneumoniae* (Fig. 20.10). The presence of organisms in the absence of polymorphs is suggestive of contamination of the specimen rather than infection, but it is important to remember that immunocompromised patients may not be able to mount a polymorph leukocyte response. Also, remember that the causative agents of atypical pneumonia, with the exception of *L. pneumophila* (Fig. 20.11), will not be seen in Gram-stained smears.

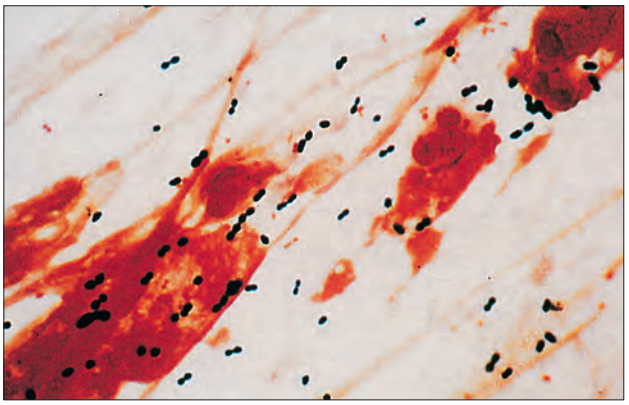


Figure 20.10 Gram-stained smears of sputum can help the physician make a rapid diagnosis if, like this, they contain abundant Gram-positive diplococci characteristic of pneumococci, as well as polymorphs. However, many of the important causes of pneumonia will not be stained by Gram stain.

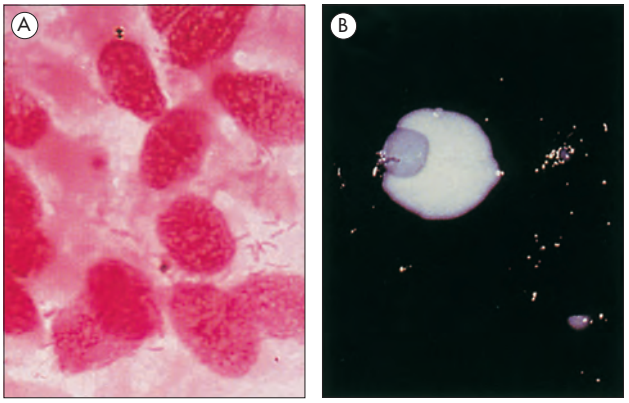


Figure 20.11 *Legionella pneumophila.* (A) Gram stain of a bronchial biopsy specimen in a patient with fulminant Legionnaires' disease. (B) Culture plate showing white colonies on buffered charcoal yeast extract medium. ([A] Courtesy of S. Fisher-Hoch. [B] Courtesy of I. Farrell.)

Standard culture techniques will allow the growth of the bacterial pathogens such as *Strep. pneumoniae*, *Staph. aureus*, *H. influenzae* and *Klebsiella pneumoniae* and other non-fastidious Gram-negative rods. Special media or conditions are required for the causative agents of atypical pneumonia, including *L. pneumophila* (Fig. 20.11).

Rapid non-cultural techniques have been applied successfully to the diagnosis of pneumococcal pneumonia. Detection of pneumococcal antigen by agglutination of antibody-coated latex particles can be used with both sputum and urine specimens, as antigen is excreted in the urine. Use of this technique means the result is available within 1 h of receipt of the specimen, but antibiotic susceptibility tests cannot be performed unless the organisms are isolated.

Microbiological diagnosis of atypical pneumonia is usually confirmed by serology

As mentioned above, several important causes of pneumonia will not be revealed in Gram-stained sputum smears and cannot be grown on simple routine culture media. For these reasons, the diagnosis is usually confirmed by serological tests rather than by culture. In some infections, IgM, antigen or genome detection is being used to make the diagnosis at an early stage. The classic techniques involve detection of a single high titre of specific antibodies, or preferably demonstration of a rising titre between the acute and convalescent phase of the disease, but the diagnosis is often made retrospectively. The serological tests are shown in Table 20.2.

Pneumonia is treated with appropriate antimicrobial therapy

Once the cause of the pneumonia has been identified, appropriate antimicrobial therapy can be given, although there are different guidelines around the world and the incidence of penicillin and other antibiotics resistance in pneumococci has increased in some countries (Table 20.3).

Table 20.2 Serological diagnosis of 'atypical' pneumonia

Pathogen	Test
Mycoplasma pneumoniae	Complement fixation test (CFT), IgM by latex agglutination or ELISA
Legionella pneumophila	Urinary antigen test or rapid microagglutination test
Chlamydophila pneumonia *Chlamydophila psittaci*	Microimmunofluorescence or ELISA using species-specific antigens
Coxiella burnetii	CFT (phase I and phase II antigens)

Several of the bacterial causes of pneumonia are difficult to grow in the laboratory, so examination of the patient's serum for specific antibodies is the usual method of diagnosis. It is always better to demonstrate a rising titre between acute- and convalescent-phase sera than to rely on a single sample. ELISA, enzyme-linked immunosorbent assay. However, molecular diagnostic tests on respiratory samples have been developed that are more sensitive and rapid.

Table 20.3 Antibiotic treatment for bacterial pneumonia

Initial treatment of community-acquired pneumonia	
First choice	Amoxicillin or co-amoxiclav + doxycycline
Pneumonia secondary to viral respiratory tract infection	Co-amoxiclav
Pneumonia in chronic bronchitis	Co-amoxiclav or cefuroxime
Pneumonia in an alcoholic, drug user or a patient who may have aspirated	Co-amoxiclav + gentamicin
Treatment of choice when pathogen has been identified	
Streptococcus pneumoniae	Amoxicillin (erythromycin if allergic to beta-lactams)
Mycoplasma pneumonia *Legionella pneumonia* *Chlamydophila pneumonia* *Chlamydophila psittaci* *Coxiella burnetii*	Doxycycline
Staphylococcus aureus	Flucloxacillin
Haemophilus influenzae	Co-amoxiclav or cefuroxime
Klebsiella pneumoniae	Gentamicin, chloramphenicol or ciprofloxacin

Amoxicillin remains the agent of choice for pneumococcal infections as long as the isolates are susceptible. Penicillin-resistant pneumococci now occur in many countries, and in some it is no longer safe to assume susceptibility to amoxicillin. Many of the resistant strains are still susceptible to cephalosporins, and in countries with a high incidence of resistance these agents may replace amoxicillin, at least until the results of antibiotic susceptibility are known. It is important to recognize that amoxicillin and cephalosporins are not active against the other common causes of pneumonia. Therefore, a combination is often recommended for initial therapy.

[a]If non-beta-lactamase producer.

The choice of treatment is more difficult when sputum is not produced or does not reveal the pathogen. It is therefore important to take a full history and use invasive diagnostic techniques if appropriate to help establish the cause.

Prevention of pneumonia involves measures to minimize exposure and pneumococcal immunization post-splenectomy and for those with sickle cell disease

Respiratory infections are usually transmitted by airborne droplets, so person-to-person spread is virtually impossible to prevent, although less crowding and better ventilation help to reduce the chances of acquiring infection. Infections acquired from sources other than humans may be more amenable to prevention, for example, by avoiding contact with sick animals (Q fever) or birds (psittacosis). The contamination of cooling systems and hot-water supplies by *Legionella* has been the subject of intense study, and regulations are now in force in the UK and elsewhere to provide guidance for maintenance engineers.

Immunization is available for a few respiratory pathogens. A pneumococcal vaccine incorporating the polysaccharide capsular antigens of the most common types of *Strep. pneumoniae* is recommended for those at particular risk (e.g. post-splenectomy or individuals with sickle cell disease who are unable to deal effectively with capsulate organisms).

VIRAL PNEUMONIA

Viruses can invade the lung from the bloodstream as well as directly from the respiratory tract

Many viruses cause pneumonia (Table 20.4) in the face of normal host defences. Healthy individuals are susceptible, and most of these viruses have surface molecules that attach specifically to the respiratory epithelium.

Even when viruses of this group do not themselves cause pneumonia, they may damage respiratory defences, laying the ground for secondary bacterial pneumonia. Sometimes the virus fails to spread significantly to air spaces, but remains in interstitial tissues to cause interstitial pneumonitis. An example is cytomegalovirus (CMV) pneumonitis in immunodeficient patients, particularly allogeneic bone marrow transplant recipients.

PARAINFLUENZA VIRUS INFECTION

As with RSV, parainfluenza viruses are most likely to cause lower respiratory tract disease, croup and pneumonia, in children.

There are four types of parainfluenza viruses with differing clinical effects

The surface spikes of parainfluenza viruses are composed of haemagglutinin plus neuraminidase on one type of spike and fusion proteins on another. The four types of virus have different antigens. After infection by respiratory droplets, these viruses spread locally on respiratory epithelium.

Parainfluenza viruses 1–3 cause pharyngitis, croup, otitis media, bronchiolitis and pneumonia. Croup is seen in children less than 5 years of age, and consists of acute laryngotracheobronchitis with a harsh cough and hoarseness. Parainfluenza virus 4 is less common and generally causes a common-cold-type illness.

Table 20.4 Viral pneumonia

Virus	Clinical condition	Comments
Influenza A or B	Primary viral pneumonia or pneumonia associated with secondary bacterial infection	Pandemics (type A) and epidemics (type A or B); increased susceptibility in elderly or in certain chronic diseases; antivirals and vaccine available
Parainfluenza (types 1–4)	Croup, pneumonia in children <5 years of age; upper respiratory illness (often subclinical) in older children and adults	No treatment available (no published evidence of ribavirin being effective), supportive care, vaccines not available
Measles	Secondary bacterial pneumonia common; primary viral (giant cell) pneumonia in those with immunodeficiency	Adult infection rare but severe; ribavirin may be used as treatment, the King and Queen of Hawaii both died of measles when they visited London in 1824; vaccine available
Respiratory syncytial virus	Bronchiolitis (infants); common cold syndrome (adults)	Peak mortality in 3- to 4-month-old infants; ribavirin treatment available, palivizumab prophylaxis if at high risk
Adenovirus	Pharyngoconjunctival fever, pharyngitis, atypical pneumonia (military recruits)	Cidofovir or ribavirin could be used in specific clinical settings, vaccine available for military
Cytomegalovirus	Interstitial pneumonitis	In immunocompromised patients (e.g. bone marrow transplant recipients); antivirals (e.g. ganciclovir, valganciclovir, foscarnet, cidofovir) and immunoglobulin available
Herpes simplex	Interstitial pneumonitis	In immunocompromised patients; antivirals (e.g. aciclovir, valaciclovir, foscarnet)
Varicella-zoster virus	Pneumonia in young adults with chickenpox	Uncommon; recognized 1–6 days after rash; lung lesions may eventually calcify; antivirals (e.g. aciclovir, valaciclovir, foscarnet) and vaccine available

Several different groups of viruses cause infection of the lower respiratory tract, particularly in children. Some, such as influenza and measles, leave the patient particularly susceptible to secondary bacterial infection. Since PCR has been used to make a diagnosis, more viral co-infections have been detected and more secondary bacterial infections too.

Real-time PCR methods detecting parainfluenza RNA in throat swabs have revolutionized the diagnosis of these and other respiratory virus infections owing to the increased sensitivity and rapid diagnosis using these tests. Virus-specific antigens can be detected in cells from respiratory washings, and virus culture can be carried out, in settings where molecular analysis is not available. Treatment involves supportive care as no antiviral drugs have been shown to be effective and there is no vaccine.

ADENOVIRUS INFECTION

Adenoviruses cause about 5% of acute respiratory tract illness overall

There are 41 antigenic types of adenovirus, some of which cause upper respiratory tract infections such as pharyngoconjunctival fever and sore throat (see Ch. 19) and lower respiratory tract infections.

Types 3, 4 and 7 have caused outbreaks of respiratory illness ranging from pharyngitis to atypical pneumonia in military recruits, with crowding and stress as possible co-factors.

Recovery is generally uneventful, but adenoviruses may persist in the body as latent infections and in the 1950s were detected in tissue extracts from surgically removed tonsils and adenoids. An enteric-coated vaccine for types 4 and 7 has been used to prevent outbreaks of infection in military recruits. In 2011, the FDA approved a new version of this vaccine that is offered to all military trainees in the USA.

HUMAN METAPNEUMOVIRUS INFECTION

Human metapneumovirus (hMPV), discovered in Holland in 2001, is a respiratory pathogen closely related to RSV and peaks in the winter months. In a prospective hMPV surveillance study in the USA in 2013 in children under 5 years old, hMPV was detected in 6% of hospitalized children, 7% of children seen in outpatients and 7% of those seen in emergency departments. It is associated with a spectrum of illness from mild infection to bronchiolitis and pneumonia. Symptoms may include a fever, runny nose, cough, sore throat and wheeze. Infection occurs in infants and young children, with some reports that by 5 years of age most children have had an hMPV infection. In addition, hMPV has also been detected in older children and adults, suggesting that re-infection may occur. Archived sera have been tested and demonstrated that humans have been exposed to hMPV for at least 50 years.

HUMAN BOCAVIRUS INFECTION

Human bocavirus (hBoV), discovered in 2005, is a member of the Parvoviridae family. Of the four hBoV species, hBoV1 has been detected in respiratory samples from patients with upper and lower respiratory tract infections and hBoV2-4 in faecal specimens from patients with gastroenteritis. The clinical importance of hBoV has been difficult to determine, especially as it can be detected in ill as well as in healthy control subjects. However, when quantifying the hBoV load, it has been shown to be significantly higher in those patients with hBoV alone compared with those co-infected.

INFLUENZA VIRUS INFECTION

Influenza viruses are classic respiratory viruses and cause endemic, epidemic and pandemic influenza

The structure of a typical orthomyxovirus single-stranded RNA is shown in Fig. 20.12, and the budding process in Fig. 20.13.

There are four types of influenza virus: A, B, C and D

Antigenic differences between the nucleocapsid and matrix proteins distinguishes influenza A, B, C and D viruses:

- Influenza A viruses cause epidemics and occasionally pandemics, and there is an animal reservoir, notably in birds.
- Influenza B viruses cause only epidemics and do not involve animal hosts.
- Influenza C viruses do not cause epidemics and give rise to only minor respiratory illness.
- Influenza D viruses mostly affect cattle.

The influenza virus envelope has haemagglutinin and neuraminidase spikes

These are shown in Fig. 20.12. In the case of influenza A, the haemagglutinin (H) and neuraminidase (N) are type-specific antigens and are used to characterize different strains of influenza A virus (Table 20.5). Circulating strains are H3N2, H1N1 and H1N2. In giving the full nomenclature, the influenza antigenic type, the host origin if not human, geographical origin, strain number and year of isolation is also included (e.g. A / Philippines / 82 / H3N2).

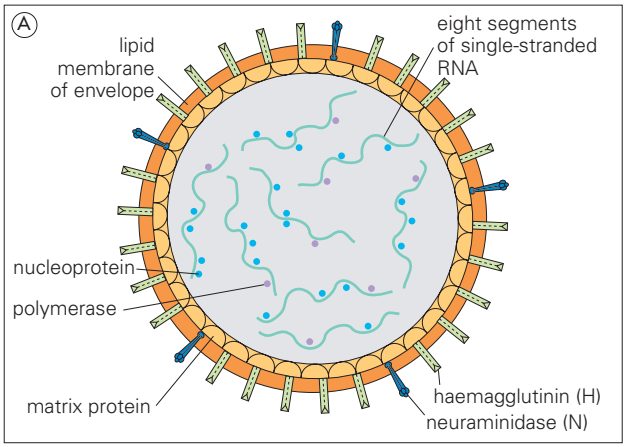
The single-stranded RNA genome is segmented, and these eight segments can be reassorted during virus replication to give a progeny virus with a novel combination of H and N antigens when virus particles of more than one strain infect a cell simultaneously. Two different influenza A viruses can simultaneously infect one cell and reassort resulting in a new influenza virus strain.

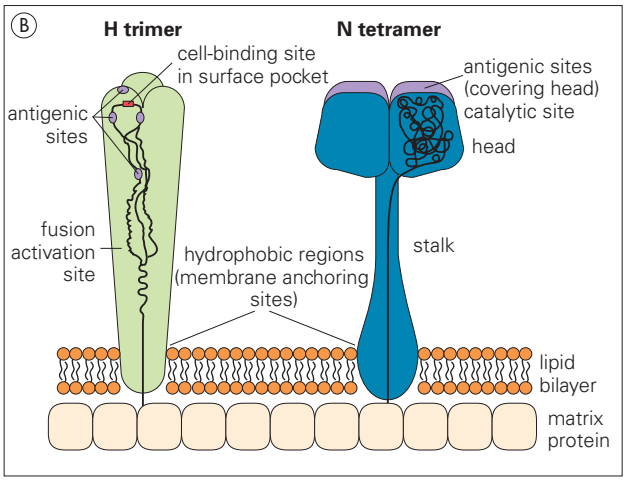
Influenza viruses undergo genetic change as they spread through the host species

These changes are of two types:

1. *Antigenic drift*. Small mutations affecting the H and N antigens occur constantly. When changes in these antigens enable the virus to multiply significantly in individuals with immunity to preceding strains, the new subtype can re-infect the community. Antigenic drift is seen with all types of influenza.
2. *Antigenic shift*. Less commonly, and only with influenza A, there is a sudden major change referred to as shift, in the antigenicity of the H or N antigens. This is based on recombination between different virus strains when they infect the same cell. The major change in H or N means that the new strain can spread through populations immune to pre-existing strains and the stage is set for a new pandemic (Table 20.5). Associated with the change in H and N are other genetic changes, which may or may not confer increased pathogenicity or change the ability to spread rapidly from person to person.

However, the H1N1 virus pandemic in 2009 demonstrated that antigen shift alone may not be required for a global outbreak. Epidemiological data revealed that a younger age group, under





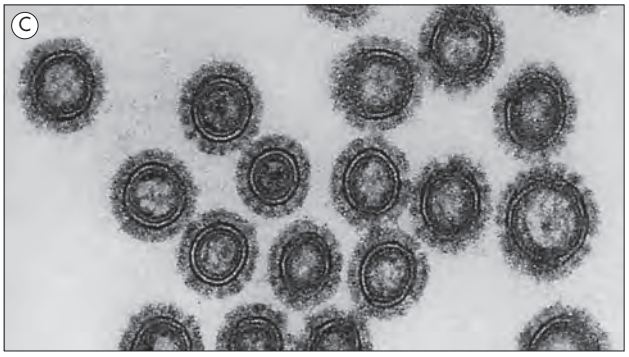


Figure 20.12 The influenza A virus particle (A), with detail enlarged (B) to show surface haemagglutinin (H) and neuraminidase (N). Each particle has approximately 500 H spikes, which bind to the host cell and fuse the viral envelope to the cell's plasma membrane to initiate infection, and approximately 100 N spikes, which release the virus from the cell surface. Nucleoprotein and polymerase proteins are closely associated with RNA segments to form ribonucleoprotein (RNP). The N tetramer is propeller shaped as viewed from the end. Detail of only one unit of H trimer and N tetramer is shown. The three-dimensional structure is known from X-ray crystallographic analysis. Electron micrograph (C) shows sectioned influenza virus particles (×300 000). ([C] Courtesy of D. Hockley.)

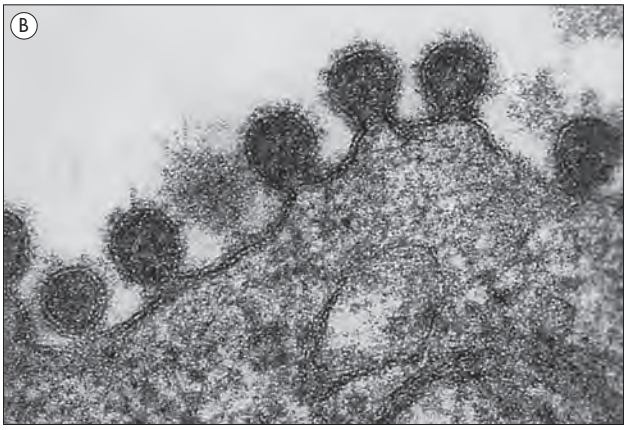


Figure 20.13 Influenza virus budding from the surface of an infected cell. (A) Scanning electron micrograph (×27 000). (B) In section (×350 000). (Courtesy of D. Hockley.)

35 years of age, were more susceptible to infection than those who were 65 years old. Therefore, pre-existing immunity and host factor adaptations can affect the pathological potential of influenza A virus infections.

Influenza is a highly infectious, acute viral infection that has affected both humans and animals over the centuries. It was so named after an outbreak of a respiratory disease in Italy in the fifteenth century that was thought to have developed under the influence of the stars, therefore influenza.

The mixing vessel hypothesis for the production of new influenza strains came about as a result of influenza A viruses infecting pigs, horses, seals and other mammals, and the ability of the virus to reassort. For example, pigs in some countries live in the same dwellings as the farmers, allowing the potential mixing of influenza viruses and emergence of new strains.

The 1918 Spanish influenza pandemic (H1N1) was estimated to have led to 50–100 million deaths around the world and was followed in 1957 and 1968 by the less severe Asian (H2N2) and Hong Kong (H3N2) influenza pandemics, respectively. These were examples of antigenic shift, whereas antigenic drift resulted in frequent epidemics between the pandemic years. In 1976, there was a swine influenza scare in Fort Dix, USA and, in 1997, 18 people in Hong Kong became ill having had an H5N1 avian influenza A virus infection. Six of the infected people subsequently died. The outbreak

Table 20.5 Pandemic human influenza viruses

Type	Subtype[a]	Year	Clinical severity	Prototype virus
A	H3N2 (?)	1889	Moderate	Designation based on serological studies
	H1N1 (avian)[b]	1918	Severe	H1N1 virus sequenced retrospectively
	H2N2 (Asian)	1957	Severe	A/Japan/57/H2N2
	H3N2 (Hong Kong)[c]	1968	Moderate	A/Hong Kong/68/H3N2
	H1N1	1977	Mild	A/USSR/77
	H1N1pdm09	2009	Mild	H1N1 virus sequenced

Novel strains of virus arising in one continent spread rapidly to other continents, causing outbreaks during appropriate times of the year (winter months in temperate climates). There is a WHO global surveillance system for influenza involving more than 100 laboratories in 79 different countries. Novel strains affecting humans included H5N1, an avian strain which caused 18 infections in humans in Hong Kong in 1997, and by 2016, there were 856 infections in 16 countries that resulted in 452 deaths mostly in Egypt and Indonesia; H9N2, an avian strain which, by 2016, had caused 28 mild infections in humans in Hong Kong and South China; H5N6, another avian strain in China that caused 16 human infections and 6 deaths; and H7N9, by 2017 there were 808 laboratory confirmed human infections and 322 deaths in China.

[a]Antigenic shift in influenza A virus is shown by the appearance of a novel combination of H and N antigens.

[b]Reports suggest that this virus was derived from an avian source. In a remarkable experiment, viral RNA was extracted from the lung tissue of someone who died in the 1918 pandemic and was buried in the permafrost and also from formalin-fixed lung tissue. This allowed the 1918 viral genome to be reconstructed.

[c]Amino acid and base sequence analysis suggest that recombination between H3N8 (from ducks) and H2N2 gave rise to H3N2.

ceased after public health authorities ordered the slaughter of all live chickens in Hong Kong.

Five human infections were reported in 1999 in Hong Kong and South China with the avian influenza A virus, H9N2. There was no evidence of wider spread nor of human-to-human transmission with either strain although it had circulated widely among birds in Hong Kong and China.

Another avian influenza virus, H7N7, is highly pathogenic in birds and may be more transmissible between humans. During an outbreak of highly pathogenic avian influenza in Holland in 2003, an H7N7 virus infected 86 poultry workers and three family members who had no contact with chickens. They developed conjunctivitis and / or flu-like symptoms. A veterinarian who handled infected chickens died of pneumonia and acute respiratory distress.

The 16 antigenically distinct H subtypes (H1–16) of influenza A virus reservoirs include wild birds, especially waterfowl. These include the H5 and H7 subtypes. There are nine N subtypes (N1–9). Outbreaks of H5N1 avian influenza in migratory waterfowl, domestic poultry and humans in Asia have occurred (Fig. 20.14). Over time, the host range has increased, with infections in waterfowl, ferrets, members of the cat family and humans. The virus has become more virulent, as seen by the mortality rate in the human population together with neurological clinical features.

Descriptive molecular epidemiology has shown that the precursor of the 1997 Hong Kong H5N1 virus was first seen in geese in 1996 in Guangdong, China. In turn, the goose virus had RNA segments from influenza viruses found in quail and the N segment from a duck virus. Subsequent evolution of the goose virus resulted in a predecessor of the Z genotype that caused the death of many waterfowl in Hong Kong nature parks and infected humans in that area in 2002. The Z genotype then predominated and spread across South-East Asia and killed, or resulted in culling of, millions of domestic fowl.

The 1918 pandemic H1N1 strain is believed to have resulted from spontaneous mutations in an avian H1N1 virus after sequence analysis was carried out on viral RNA recovered from people who had died and had been buried in the Scandinavian permafrost. However, the other pandemic viruses mentioned above, including the 2009 pandemic H1N1 strain, were due to genetic reassortment of the viral segmented RNA genomes after a host was infected by avian and human influenza A viruses at the same time.

In April 2009, there were reports from Mexico and the USA, in southern California, of a respiratory illness caused by a novel swine influenza A H1N1 virus. These were worrying times as it was thought that the new influenza virus could cause a pandemic with high morbidity and mortality. Pandemic influenza response plans had been developed and refined in many countries for the expected and overdue influenza outbreak. Viral sequence analysis showed that it was composed of a combination of genes most closely related to North American and Eurasian swine-lineage H1N1 influenza viruses. Exposure to pigs was not seen when investigating those infected. In addition, the new virus was circulating among humans and not among pig herds. Within weeks there were reports of people with influenza in a number of American states and also Canada and other parts of the world. The influenza pandemic alert was raised to phase 4 on the basis of human-to-human spread and outbreaks in the community. This became phase 5 by the end of April and countries started to activate their pandemic response plans as the pandemic had started. Diagnostic real-time polymerase chain reaction (PCR) tests were developed in days in order to confirm the diagnosis and a vaccine virus chosen for high-yield preparation in case it was needed. National stockpiles of antiviral drugs (oseltamivir and zanamivir) and personal protective equipment were activated.

By June 2009, WHO changed its alert level to pandemic phase 6 as pandemic H1N1 was reported in more than 70 countries and community outbreaks were happening globally. This virus contained gene reassortments from Eurasian and North American swine influenza, North American avian influenza and North American human influenza virus infections. The seasonal aspect of influenza virus infections had altered as laboratories experienced huge workloads over the northern hemisphere summer months.

Confirmed and probable infections occurred mainly among 5–24-year-olds. Mostly older children and young adults were admitted to hospital as well as those in the at-risk groups identified in previous influenza pandemics, including women

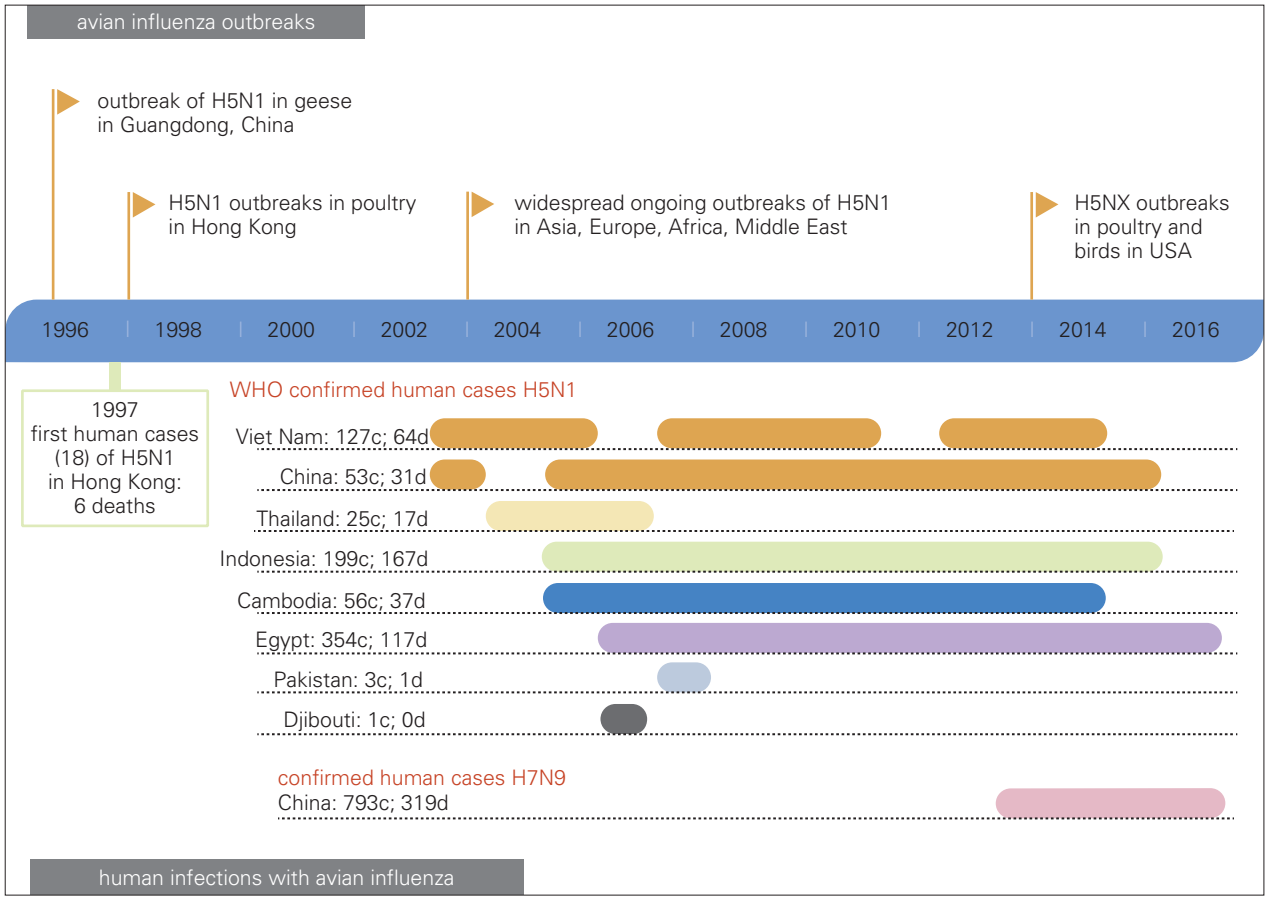


Figure 20.14 Timeline showing the spread of main countries affected by avian H5 viruses since 1996 and the human H5 and H7 infections (c=cases, d=deaths) reported to WHO. (From Barr I., Wong F. Avian influenza. Why the concern? *Microbiology Today* November 2016, https://www.microbiologysociety.org/uploads/assets/uploaded/7dbf49f8-da5f-44e2-8126ec6cd16da2a5.pdf, with permission.)

who were pregnant. In addition, increased risk of complications was seen in obese people and those with chronic neurological conditions. There were few influenza infections seen in the 65-years-and-older age group, which was unusual. Studies showed that children and young adults had no pre-existing cross-reactive antibody to the 2009 H1N1 influenza virus compared with over 30% of adults 60 years of age or older who had been exposed previously.

Networks were set up worldwide to ensure that the experiences managing influenza-infected individuals in critical care facilities and elsewhere in the southern hemisphere were shared and lessons learnt. In addition, the circulating influenza viruses were monitored closely for any antigenic variation as well as the development of antiviral resistance. Influenza-infected patients on critical care units in acute respiratory failure received mechanical ventilation with intermittent positive-pressure ventilation in which the lungs receive air enriched with oxygen at high pressure. However, another technique called extracorporeal membrane oxygenation (ECMO) treatment improved recovery by providing gas exchange outside the body using heart–lung bypass equipment and obviating the deleterious effects of providing direct oxygenation at high pressure.

Across the northern hemisphere, the 2009 H1N1 influenza A summer activity peaked and declined during the summer but levels of influenza activity remained above normal with small community outbreaks. On 10 August 2010, the WHO International Health Regulations (IHR) Emergency Committee declared an end to the 2009 H1N1 pandemic globally.

There was concern about a second wave of infection and preparations were made to offer the recently prepared vaccine to specific groups of individuals: those at risk and healthcare workers. The anticipated second wave started in the autumn and the amount of influenza activity fell quite quickly and remained at lower levels until the spring. By 2016, the H1N1 and H3N2 viruses were circulating around the world.

The WHO Global Influenza Surveillance and Response System monitoring circulating influenza viruses detected an avian influenza A H5N1 virus that was reported as H5N1 clade 2.3.2.1 that circulated in poultry in parts of Asia in February 2011. This was not detected in humans and was not seen as a public health threat, more as a marker of the continual evolution of these viruses.

Between 2003 and October 2016, 856 human infections with H5N1 were reported to WHO of whom 452 (53%) died. Most infections were reported in Egypt, Indonesia, Viet Nam and Cambodia.

Epidemics and pandemics are due to the appearance of new strains of viruses so that a given individual is regularly

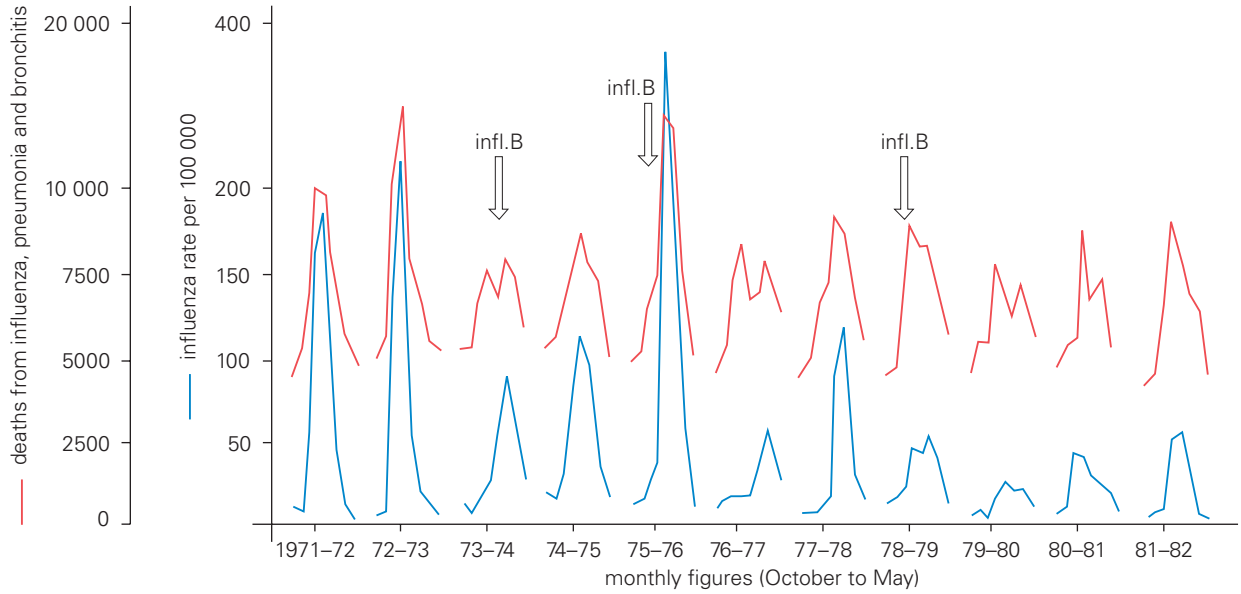


Figure 20.15 Outbreaks of influenza within a community are reflected by a general increase in deaths from acute respiratory disease. Notifications of new cases of clinical influenza are paralleled by an increase in deaths attributed to influenza, pneumonia and bronchitis. Monthly figures from October to May for England and Wales (1971–1983) are shown. The peaks are due to the spread of different strains of influenza A (H3N2 and H1N1) and influenza B (arrows) viruses in the community. (Data from the Office of Population, Censuses and Surveys.)

re-infected with different strains. This is in contrast to viruses that undergo minimal antigenic variation (monotypic viruses), such as hepatitis A. Monitoring avian influenza viruses such as H5N1 and H7N7 is therefore critical in determining their potential to become more pathogenic and spread. Reassortment between H5N1 or H7N7 and human H1N1 or H3N2 influenza viruses may result in efficient transmissibility together with retention of viral pathogenicity. An influenza pandemic could then evolve.

Transmission of influenza is by droplet inhalation

Influenza infections occur throughout the world. Except in the tropics, the infection is almost entirely restricted to the coldest months of the year. This is largely because, during cold weather, people spend more time inside buildings with limited air space, which favours transmission by droplet inhalation, and perhaps also because of decreased host resistance. Influenza activity within a community is reflected not only in the numbers of people becoming ill and consulting doctors, but also in excess mortality due to acute respiratory disease, such as pneumonia, which particularly affects the elderly (Fig. 20.15).

With respect to the avian influenza viruses, they are spread by movement of poultry and poultry products, live poultry markets and unhygienic practices, and backyard flocks that are not controlled.

The initial symptoms of influenza are due to direct viral damage and associated inflammatory responses. The virus enters the respiratory tract in droplets and attaches to sialic acid receptors on epithelial cells via the H glycoprotein of the virus envelope. Just 1–3 days after infection, the cytokines liberated from damaged cells and from infiltrating leukocytes cause symptoms such as chills, malaise, fever and muscular aches. There are also respiratory symptoms such as a runny nose and cough. Most people feel better within 1 week. The direct viral damage and associated inflammatory responses can be severe enough to cause bronchitis and interstitial pneumonia.

Influenzal damage to the respiratory epithelium predisposes to secondary bacterial infection

Secondary bacterial invaders include staphylococci, pneumococci and *H. influenzae*. Life-threatening influenza is often due to secondary bacterial infection, especially with *Staph. aureus*, the viral infection being brought under control by antibody and cell-mediated immune responses to the infecting virus. Although antiviral antibodies may not be detected within the serum for 1–2 weeks, they are produced at an earlier stage, but are complexed with viral antigens in the respiratory tract.

Mortality due to secondary bacterial pneumonia is higher in apparently healthy individuals over 60 years of age and in those with impaired resistance due to, for example, chronic cardiorespiratory disease or renal disease. Pregnant women are also more vulnerable.

Rarely, influenza causes CNS complications

Central nervous system (CNS) complications include meningitis, encephalomyelitis and polyneuritis. These appear to be indirect immunopathological complications rather than due to CNS invasion by the virus. Guillain–Barré syndrome, a polyneuropathy with proximal, distal or generalized motor weakness, occurred as a significant but rare (1 / 100000) sequel to the widespread vaccination of citizens in the USA with inactivated H3N2 influenza virus in 1976. However, subsequent vaccines have not been associated with this syndrome.

During influenza epidemics a diagnosis can generally be made clinically

Rapid diagnosis can be made by collecting samples from the respiratory tract, such as throat swabs that can be tested by real-time PCR for influenza viral RNA, and the viruses can be typed simultaneously. Antiviral resistance can also be detected using PCR as well as sequence analysis. Alternatively, if these methods are not available, influenza-infected cells can be detected using immunofluorescence techniques, but nasopharyngeal aspirates are usually required to improve the yield of cellular material for testing. Virus isolation can also be used but there may be a delay of at least 7 days until identification. Finally, a rise in specific antibodies can be detected by complement fixation test or ELISA in paired serum samples, taken within a few days of illness and 7–10 days later. However, this is helpful only retrospectively.

Vaccines can be used to prevent influenza

The aim of immunization is to help prevent infection, and those at risk of complications from influenza infection should be offered vaccine before the 'flu season'. The vaccines may be trivalent or quadrivalent and the viruses inactivated or live attenuated.

Influenza virus vaccines in regular use are:

- those consisting of egg-grown virus, which are then purified, formalin-inactivated and extracted with ether
- the less reactogenic purified H and N antigens prepared from virus that has been disrupted ('split') by lipid solvents
- live attenuated egg-grown virus.

Studies investigating the protective efficacy of cell culture-derived influenza virus vaccines have demonstrated similar results to the egg-grown vaccine. A cell-based quadrivalent inactivated influenza vaccine was approved for use in the USA in 2016.

Influenza A (H3N2 and H1N1 strains) and influenza B are included in the vaccine. The exact virus strains are reviewed annually in relation to the viruses circulating the previous year. The vaccines are given by parenteral injection, and provide protection against disease in up to 70% of individuals for about 1 year. Vaccination of individuals at high risk, especially those over 65 years of age and those with chronic cardiopulmonary disease, is recommended. It might be expected that the respiratory route would be a better way of inducing respiratory immunity and on this basis live attenuated virus vaccines were developed and are administered intranasally. They are offered to specific age groups of children, providing there are no contraindications, as part of the annual immunization programme. Some data demonstrating concerns regarding low effectiveness against influenza A(H1N1)pdm09 in 2016 resulted in only the inactivated vaccine being used in some countries.

Antiviral drugs can be used to treat and prevent influenza

Oseltamivir and zanamivir are neuraminidase inhibitor antiviral agents that act on both influenza A and B viruses. They superseded rimantadine and amantadine, M2 ion channel blockers that stop hydrogen ion efflux by altering the pH as they are basic compounds and affect intracellular viral uncoating. They inhibit only the replication of influenza A viruses. Oseltamivir (Tamiflu) is easier to administer as it is given orally, as opposed to zanamivir that is given by inhaler. These antivirals can reduce the severity of the infection, but should be given within 1–2 days of disease onset. They have also been shown to be effective when used for prophylaxis if given within 48 hours of symptom onset.

Oseltamivir resistance has been widely reported and transmission of oseltamivir resistance has occurred without direct selective drug pressure. This did not affect virulence or viral replication. During the 2009 influenza A H1N1 pandemic, intravenous oseltamivir and zanamivir preparations were made available together with peramivir and laninamivir, also neuraminidase (NA) inhibitors that had been developed, the latter has a longer half-life.

Finally, another therapeutic option from last century involved using hyperimmune plasma made from blood collected from human donors who had recovered from the 1918 Spanish influenza pandemic. This was given to patients with severe influenza infections who subsequently recovered. Some individuals with severe pandemic H1N1 infections recovered, having received hyperimmune plasma infusions collected from individuals with pandemic H1N1 infection or from vaccinated donors.

With an eye to a future pandemic, nations developed stockpiles of anti-influenza drugs. New drug targets focusing on entry, replication, and maturation as well as novel approaches to rapid vaccine production are being investigated.

Culling domestic poultry has contained the spread of the H5N1 virus as well as other avian influenza viruses including H5N8 and H7N9. However, rapid detection and increased biosecurity together with the use of vaccines are critical in controlling the infection. In addition, after the SARS-associated coronavirus (SARS CoV) outbreak (see next section) there are questions as to what lessons were learnt for any future influenza epidemic or pandemic. The problem is that influenza viruses are more easily transmitted than SARS CoV. Together with the reduced transmissibility, early detection and containment that were successful in controlling the SARS CoV may not be effective in preventing an influenza pandemic.

SEVERE ACUTE RESPIRATORY SYNDROME AND MIDDLE EAST RESPIRATORY SYNDROME CORONAVIRUS INFECTIONS

By the first twelve years of the 21st century, two previously unknown coronaviruses had been identified as causes of severe respiratory infections. An outbreak of severe respiratory disease with no identifiable cause was reported from Guangdong Province in the People's Republic of China in November 2002. The agent spread to mainly parts of East and South-East Asia, as well as Toronto in Canada, and was eventually reported in 30 countries. WHO issued a global health alert in March 2003 concerning severe acute respiratory syndrome (SARS). The main symptoms were high fever (>38°C), cough, shortness of breath or difficulty in breathing. Chest X-rays consistent with pneumonia were also seen. Close contact with someone infected with the SARS agent was the highest risk of the infection spreading from person to person and occurred mostly in family members and hospital staff caring for SARS patients. The incubation period was generally between 2 and 7 days, with a 10-day maximum.

The SARS-associated coronavirus (SARS-CoV), a new member of the coronavirus family, was identified by virus isolation in cell culture and electron microscopy in conjunction

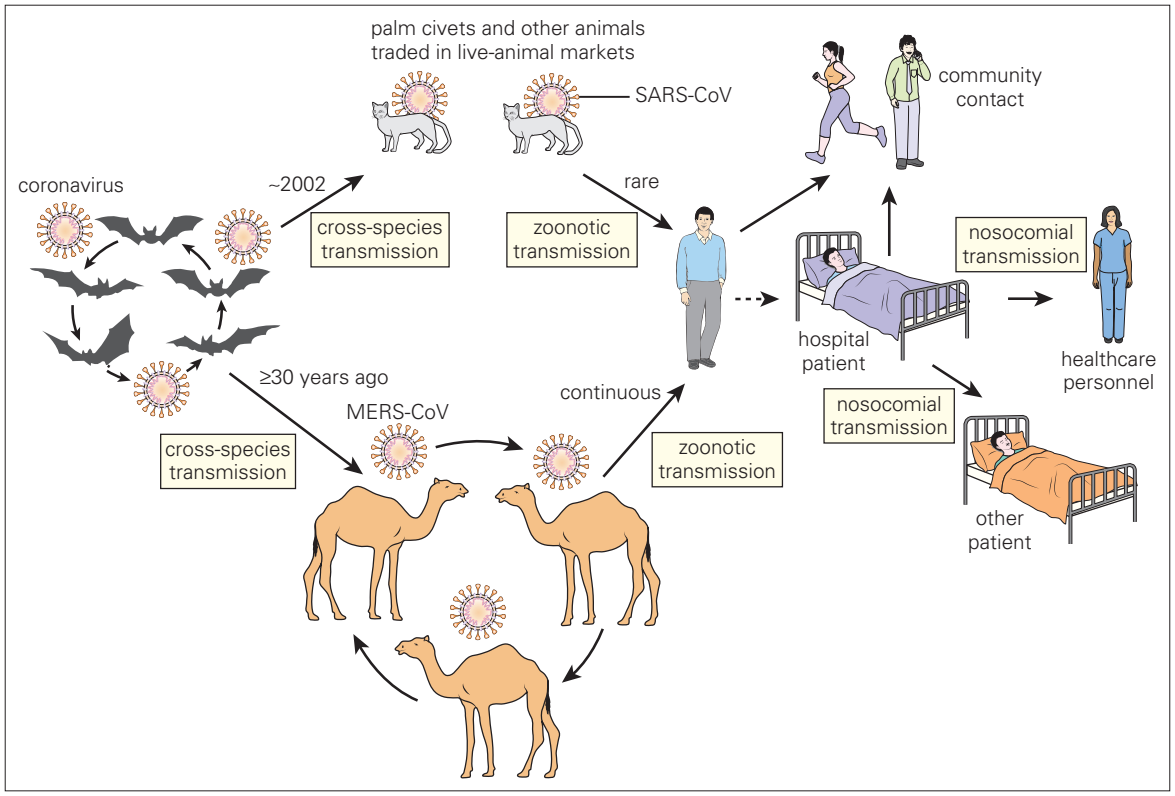


Figure 20.16 Emergence of SARS-CoV and MERS-CoV. (From de Wit F., van Doremalen N., Falzarano D., Munster V.J. SARS and MERS: recent insights into emerging coronaviruses. *Nat Rev Microbiol* 2016; 14[8]:523–534, with permission.)

with molecular methods. Diagnostic methods included PCR detection and serology. The rapid identification of the SARS-CoV, implementation of infection control on a scale not seen previously involving face masks, checking for fever in the community and at airports, which resulted in rapid isolation on detecting symptom onset, international scientific networking and immediate availability of data set a global standard for investigation of disease outbreaks.

By July 2003, just slightly more than 4 months since the virus began moving between countries via international air travel, WHO reported that all known chains of person-to-person transmission of the SARS virus had been broken. The largest outbreaks occurred in mainland China, with 5327 cases and 348 deaths, and Hong Kong, where 1755 cases and 298 deaths were reported. Overall, there were 8437 SARS diagnoses in 29 countries and nearly 10% case fatality rate.

Reservoirs of infection

The predecessor of the SARS-CoV crossed species barriers over the years when changes in the viral reservoir and humans' eating habits resulted in an ability to transmit to, and between, humans. In China, the quality of the food is considered to be best if it is prepared freshly from live animals in wet-markets found close to residential areas. In addition, eating a range of exotic wild animals, including bats and civet cats, is popular in south China as it is thought to improve both health and sexual performance. A number of SARS-CoV-like viruses were detected in various wildlife species including Himalayan masked palm civet cats, Chinese ferret badgers, raccoon dogs and horseshoe bats. These animals were incidental hosts and bats were found to be the reservoir of not just SARS-CoV, but a number of other coronaviruses (Fig. 20.16).

Pathogenesis may be viral as well as immune mediated

Angiotensin-converting enzyme 2 (ACE2) is the SARS-CoV receptor on host cells that binds the viral spike protein. Once bound, the receptor is down-regulated resulting in lung injury due to massive production of angiotensin 2, which may stimulate an angiotensin 2 receptor that then increases lung blood vessel permeability and respiratory distress.

Immune mechanisms may play a part as it was shown that the SARS-CoV RNA load fell whilst there was a clinical deterioration. Increases in proinflammatory cytokines and chemokines have also been noted in patients with acute respiratory distress syndrome as a result of SARS-CoV infection. However, if those levels dropped demonstrating an adaptive immune response, the patients were more likely to survive.

With respect to transmissibility, it is interesting to note that there is a large difference in the binding affinity of the palm civet and human SARS-CoV strains spike proteins to the human ACE2 receptor despite there being only four amino acid differences between them. Sequencing studies showed that, during the outbreaks, various genes evolved quite rapidly in the animal reservoirs, which would have improved the transmissibility between animals and humans and between humans as well (Fig. 20.17).

From a management perspective, the relatively poor transmissibility of the virus spreading mainly by respiratory

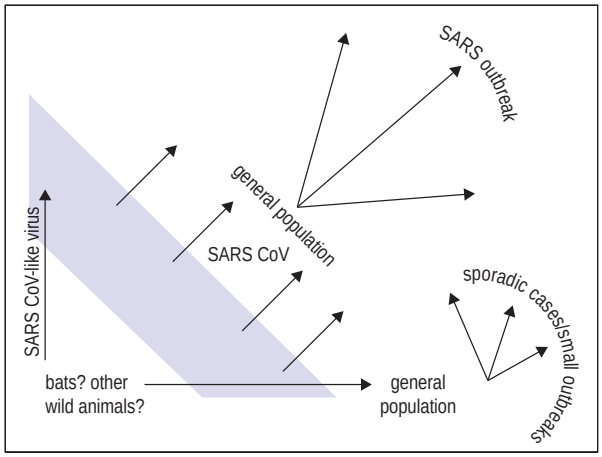


Figure 20.17 Chinese wet-markets and SARS. (Redrawn from Woo PC et al. Infectious diseases emerging from Chinese wet-markets: zoonotic origins of severe respiratory viral infections. *Curr Opin Infect Dis* 2006; 19:401–407.)

Figure 20.18 The N95 mask is recommended in this setting and is fit tested to ensure maximum protection to that individual. (Courtesy of A Letters, King's College Hospital, London.)

droplets over a short distance was helpful in controlling infections.

However, transmission also occurred by direct and indirect contact with respiratory secretions, faeces or infected animals. The virus was shown to be stable at room temperature, surviving for up to 2 days on surfaces and up to 4 days in faeces. Protection was afforded by face masks, including the N95 masks (Fig. 20.18). SARS-CoV spread more efficiently in hospitals, especially in intensive care unit settings, and clusters of cases occurred in hotel and apartment buildings in Hong Kong. Attack rates as high as 50% or more were seen. Isolation of infected individuals and stringent infection control measures were observed. By 2016, no other people were infected with SARS-CoV after four patients developed SARS in China between December 2003 and January 2004.

Laboratory diagnosis was carried out using methods including SARS-CoV RNA detection by PCR in clinical specimens including respiratory samples and faeces.

No specific antiviral treatment was available, although ribavirin was used to treat some individuals, although little effect was seen in vitro unless ribavirin was used at a high concentration. Corticosteroids damped down the effect of virally induced cytokine responses that could damage lung tissue. Interferons were reported to inhibit the virus in vitro. Protease inhibitors, used to treat HIV infections, were shown to improve the outcome of patients with SARS-CoV infection when combined with ribavirin, but there have been no clinical trials.

Finally, with regard to potential vaccines and the correlates of protection, neutralizing antibodies are found in convalescent human serum. As these antibodies to the viral spike protein prevent virus entry and neutralize virus infectivity in vitro, whole inactivated virus and recombinant protein vaccines have been developed that elicit neutralizing antibody responses. These have been shown to prevent SARS although cell-mediated immunity may also assist viral clearance and disease resolution.

In June 2012, another new coronavirus, Middle East respiratory syndrome coronavirus (MERS-CoV) was isolated from a sputum sample collected from a man in Saudi Arabia who died of acute pneumonia and kidney failure. By April, there was a report of a cluster of patients with a severe respiratory disease in Jordan, diagnosed as MERS-CoV infections and further spread was seen in other countries associated with travel to those areas. The largest nosocomial outbreak was reported in South Korea, after someone with MERS-CoV infection travelled from Saudi Arabia and resulted in 186 patients being infected in 16 hospitals in a 4-week period. By November 2016, 27 countries had reported 1826 patients with MERS-CoV infections, of whom 649 had died. Pneumonia is the most common finding, although gastrointestinal symptoms have also been reported.

Most infections have been due to person-to-person transmission, but it is a zoonosis and camels are likely to be a major reservoir host, but their exact role and route of transmission is still not known. A survey found a high prevalence of MERS-CoV antibodies in dromedary camels (one hump, in case you were wondering) together with MERS-CoV RNA in respiratory swabs collected from camels in a farm in Qatar linked to some human infections.

With molecular clock analyses, one can answer the question about whether these viruses had been around for ages or had just appeared. The analysis, based on sequencing the genome, showed that SARS-CoV crossed the species barrier into masked palm civets and other animals in the markets of China in late 2002. A MERS-CoV seems to have done the same, but into dromedary camels, around the mid 1980s. As humans and camels are in close contact, MERS-CoV infections are still happening, in contrast to SARS-CoV.

There are in vitro data demonstrating the effectiveness of interferons against MERS-CoV and some case reports have indicated that combination therapy using ribavirin, interferon and protease inhibitors may be beneficial in patients with MERS.

MEASLES VIRUS INFECTION

Secondary bacterial pneumonia is a frequent complication of measles in developing countries

Measles is dealt with in detail as a multisystem infection in Chapter 27. It is mentioned here because:

- it can cause 'giant cell' pneumonia in those with impaired immune responses

- the virus replicates in the lower respiratory tract and, under certain circumstances, causes sufficient damage to lead to secondary bacterial pneumonia.

Secondary bacterial pneumonia is now uncommon in resource-rich countries, but is a frequent complication among children in resource-poor countries and measles remains a major cause of death in childhood. Depressed immune responsiveness, inadequate vaccination programmes, malnutrition (especially vitamin A) and poor medical care to deal with complications tip the host–parasite balance markedly in favour of the virus.

After an incubation period of 10–14 days, symptoms include fever, a runny nose, conjunctivitis and cough. Koplik's spots and then the characteristic rash appear 1–2 days later. The virus replicates in the epithelium of the nasopharynx, middle ear and lung, interfering with host defences and enabling bacteria such as pneumococci, staphylococci and meningococci to establish infection. Pneumonia generally results in those with measles being admitted to hospital, but otitis media is also common. Virus replication continues unchecked in children with severely impaired cell-mediated immune responses, giving rise to a giant cell pneumonia, which is a rare and usually fatal manifestation (Fig. 20.19). Other complications are referred to in Chapter 27, and the neurological complications in Chapter 25.

Measles is diagnosed clinically, but detection of specific IgM responses and measles viral RNA detection and sequence analysis are important to confirm the diagnosis and for surveillance purposes.

Antibiotics are needed for secondary bacterial complications of measles, but the disease can be prevented by immunization

If severe, ribavirin treatment is available, but antibiotics are needed for bacterial complications. Children with severe measles in resource-poor countries generally have very low levels of serum retinol, the predominant circulating form of vitamin A in the blood. Therefore, vitamin A supplements improve clinical outcome, reducing the number of deaths from measles by half.

Measles is prevented by a highly effective, live, attenuated vaccine, given with mumps and rubella vaccines (MMR, see Ch. 35). Since immunization began, the number of cases has declined by 70%. In the USA, after a rise to nearly 30 000 cases in 1990, the number fell to 488 (47 of them imported) in 1996. It was planned to eliminate the disease in the Americas by the year 2000, by which time a group of scientists convened by the Centers for Disease Control (CDC) decided that measles was no longer endemic in the USA. Due to an unfounded MMR vaccine scare in the UK, the number of individuals with measles rose considerably owing to a fall in vaccine uptake. By 2016, vaccine uptake had improved to 95% and the number of notifications of measles infection had dropped.

Before the vaccine was available in the 1960s, there were 135 million infections and 7–8 million deaths each year worldwide. The WHO hoped for global eradication by 2010–2015 but the goal changed to measles elimination by 2010. By 2015, a 79% reduction in deaths had been reported owing to improved vaccine coverage. During 2015, about 183 million children received the vaccine in 41 countries.

CYTOMEGALOVIRUS INFECTION

Cytomegalovirus (CMV) infection can cause an interstitial pneumonitis in immunocompromised patients

CMV does not normally replicate in respiratory epithelium or cause respiratory illness; however, in immunocompromised patients, and in particular allogeneic bone marrow transplant recipients, it can give rise to an interstitial pneumonia. CMV monitoring in specific groups of immunosuppressed patients is critical, especially in the first few months post-transplantation. In a number of different types of sample, CMV DNA can be detected and quantified and characteristic inclusions demonstrated in lung tissue (Fig. 20.20).

TUBERCULOSIS

Tuberculosis is one of the most serious infectious diseases of the resource-poor world

Mycobacterium tuberculosis causes tuberculosis (TB), which is one of the top 10 causes of death globally. In 2015, WHO reported 10.4 million people were infected with TB and 1.8 million died, with over 95% of deaths occurring in low- and middle-income countries, wherever poverty, malnutrition and poor housing prevail. It affects the apparently healthy as well as being a serious disease of the immunocompromised and is one of the AIDS-defining illnesses. TB is primarily a disease of the lungs, but may spread to other sites or proceed to a generalized infection ('miliary' TB).

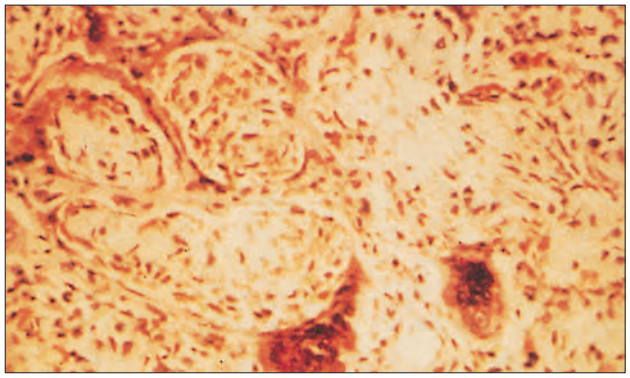


Figure 20.19 Lung biopsy in measles pneumonia showing inflammatory cell infiltrate, proliferation of the alveolar lining cells and large, darkly staining, multinucleate giant cells (H&E stain). (Courtesy of I.D. Starke and M.E. Hodson.)

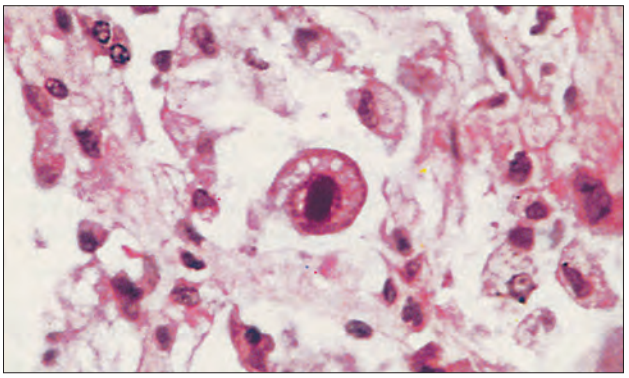


Figure 20.20 Owl's eye inclusion body in cytomegalovirus infection. Large numbers of virus particles accumulate in the nucleus of the enlarged infected cell to produce a single dense inclusion (H&E stain). (Courtesy of I.D. Starke and M.E. Hodson.)

Table 20.6 Mycobacteria associated with human disease

Species	Clinical disease
Slow growers[a]	
M. tuberculosis	Tuberculosis
M. bovis	Bovine tuberculosis
M. leprae	Leprosy
M. avium[b] *M. intracellulare*[b]	Disseminated infection in AIDS patients M. avium complex (MAC)
M. kansasii	Lung infections
M. marinum	Skin infections and deeper infections (e.g. arthritis, osteomyelitis) associated with aquatic activity
M. scrofulaceum	Cervical adenitis in children
M. simiae	Lung, bone and kidney infections
M. szulgai	Lung, skin and bone infections
M. ulcerans	Skin infections
M. xenopi	Lung infections
M. paratuberculosis	? Association with Crohn's disease
Rapid growers[a]	
M. fortuitum *M. chelonae*	Opportunist infections with introduction of organisms into deep subcutaneous tissues; usually associated with trauma or invasive procedures

Many species of mycobacteria are associated with occasional disease, but the major pathogens of the genus are *M. tuberculosis*, *M. bovis* and *M. leprae*.
[a]Slow growers require >7 days for visible growth from a dilute inoculum; rapid growers require <7 days from a dilute inoculum.
[b]*M. avium* complex; the two species are distinct. Of the *M. avium* complex, serotypes 1–6 and 8–11 are assigned to *M. avium*, serotypes 7, 12–17, 19, 20 and 25 are assigned to *M. intracellulare*.

Other species of mycobacteria, referred to as atypical mycobacteria, mycobacteria other than tuberculosis (MOTT) or non-tuberculous mycobacteria (NTM) also cause infection in the lungs (Table 20.6).

Infection is acquired by inhalation of *M. tuberculosis* in aerosols and dust. Airborne transmission of TB is efficient because infected people cough up enormous numbers of mycobacteria, projecting them into the environment, where their waxy outer coat allows them to withstand drying and therefore survive for long periods of time in air and house dust.

The pathogenesis of TB depends upon the history of previous exposure to the organism

In primary infection (i.e. infection in individuals encountering *M. tuberculosis* for the first time), the organisms are engulfed by the alveolar macrophages, in which they can both survive and multiply. Non-resident macrophages are attracted to the site, ingest the mycobacteria and carry them via the lymphatics to the local hilar lymph nodes. In the lymph nodes, the immune response, predominantly a CMI response, is stimulated. This T-cell response can be detected in the tuberculin skin test, also called the Mantoux test, 4–6 weeks after infection by injecting a small amount of purified protein derivative (PPD) of *M. tuberculosis* into the skin to assess whether someone is sensitive to tuberculin protein. A positive result is shown by local induration and erythema, 48–72 h later. However, as for the other commercial interferon-gamma (IFNγ) test for TB, a positive response could mean that the person has been infected previously, has latent TB infection or has an active TB infection. A strong skin test reaction would lead to referral to a respiratory clinic for further assessment and treatment.

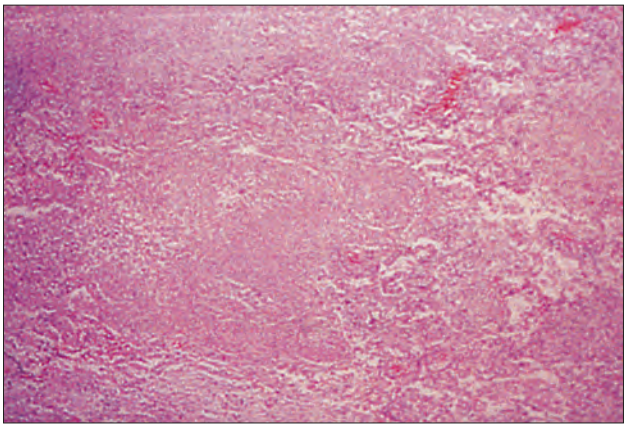


Figure 20.21 Histopathology showing dense inflammatory infiltration, granuloma formation and caseous necrosis in pulmonary tuberculosis. (Courtesy of R. Bryan.)

The CMI response helps to curb further spread of *M. tuberculosis*

However, some *M. tuberculosis* organisms may have already escaped to set up foci of infection in other body sites. Sensitized T cells release lymphokines that activate macrophages and increase their ability to destroy the mycobacteria. The body reacts to contain the organisms within 'tubercles', which are small granulomas consisting of epithelioid cells and giant cells (Fig. 20.21). The lung lesion plus the enlarged lymph nodes (Fig. 20.22) is often called the Ghon or primary complex. After a time, the material within the granulomas becomes necrotic and caseous or cheesy in appearance.

The tubercles may heal spontaneously, become fibrotic or calcified, and persist as such for a lifetime in people who are otherwise healthy. They will show up on a chest radiograph as radio-opaque nodules. However, in a small percentage of people with primary infection, and particularly in the immunocompromised, the mycobacteria are not contained within the tubercles, but invade the bloodstream and cause disseminated disease ('miliary' tuberculosis, Fig. 20.23).

Secondary tuberculosis is due to reactivation of dormant mycobacteria, and is usually a consequence of impaired immune function resulting from some other cause such as malnutrition, infection (e.g. advanced HIV and AIDS), chemotherapy for treatment of malignancy, or corticosteroids for the treatment of inflammatory diseases.

TB illustrates the dual role of the immune response in infectious disease

On the one hand, the CMI response controls the infection and, when it is inadequate, the infection disseminates or reactivates. On the other hand, nearly all the pathology and disease is a consequence of this CMI response, as *M. tuberculosis* causes little or no direct or toxin-mediated damage.

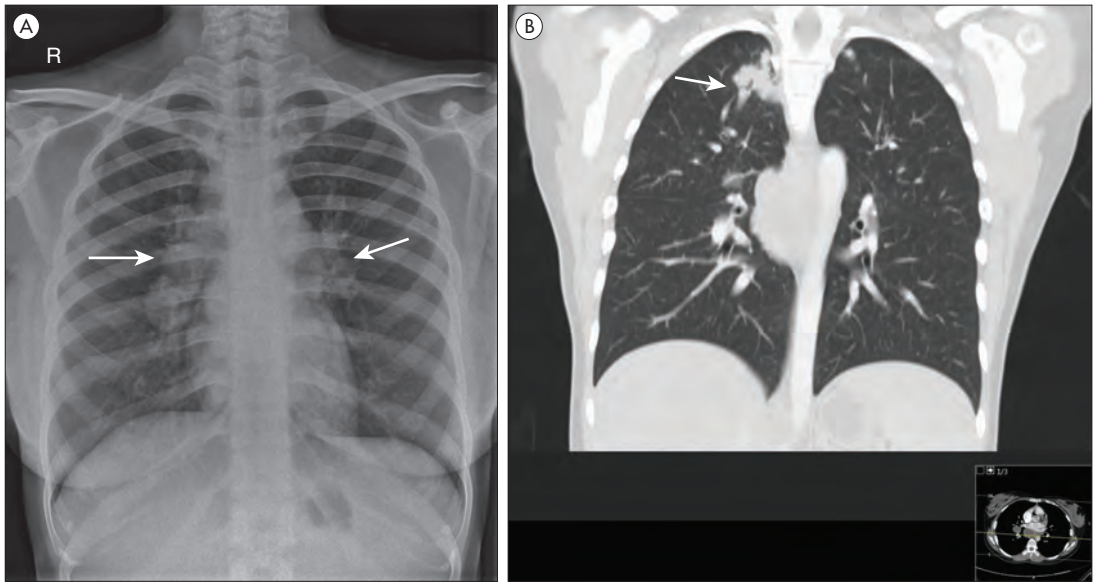


Figure 20.22 (A) Chest radiograph showing bilateral hilar and paratracheal lymphadenopathy. (B) CT of patchy parenchymal consolidation in both upper lobes. (Courtesy of G. Bain, London North West Healthcare Trust.)

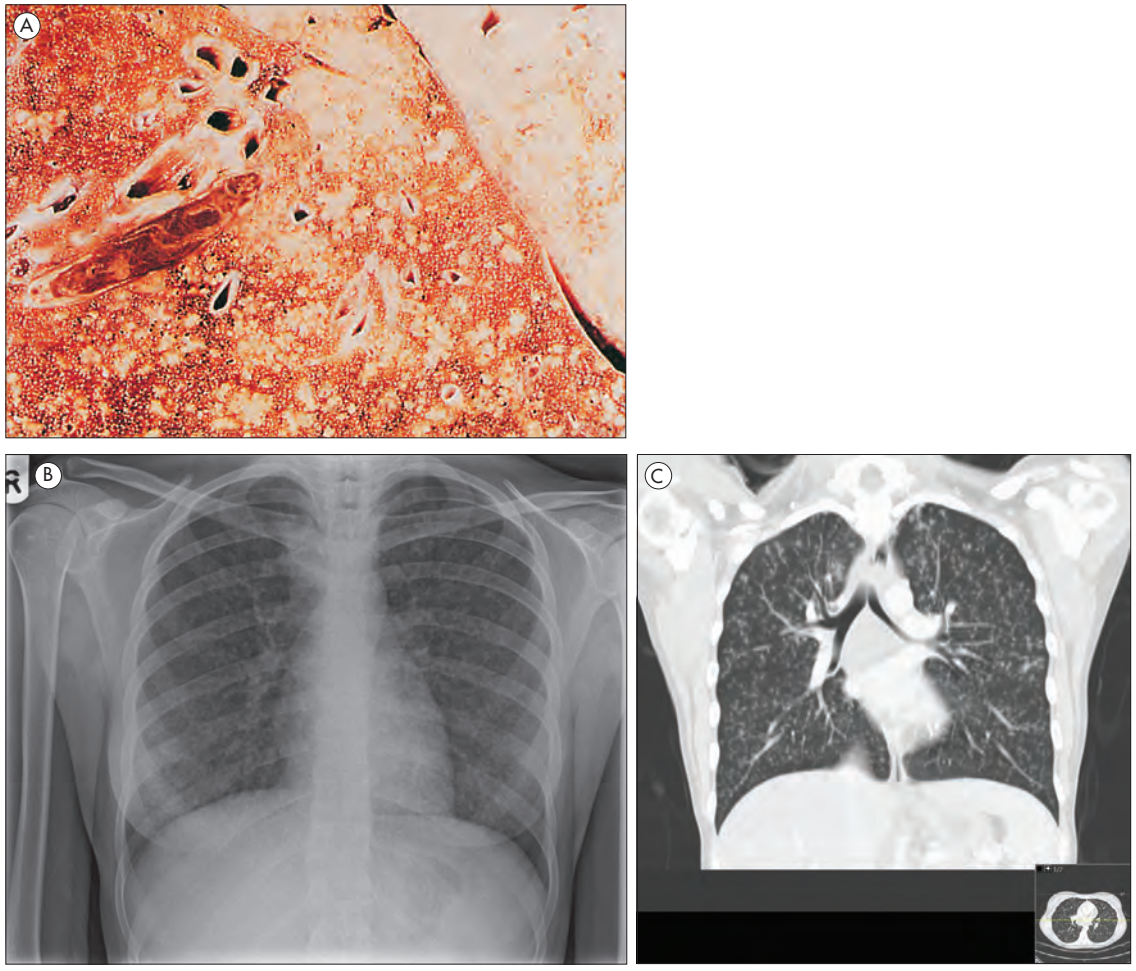


Figure 20.23 Miliary tuberculosis. (A) Gross specimen of lung showing the cut surface covered with white nodules, which are the miliary foci of tuberculosis. (B) Miliary TB chest X-ray and (C) CT. ([A] Courtesy of J.A. Innes. [B] and [C] Courtesy of G. Bain, London North West Healthcare Trust.)

Reactivation occurs most commonly in the apex of the lungs. This site is more highly oxygenated than elsewhere, allowing the mycobacteria to multiply more rapidly to produce caseous necrotic lesions, which spill over into other sites in the lung, and from where organisms spread to more distant sites in the body.

Primary TB is often asymptomatic

In contrast to pneumonia, which is usually an acute infection, the onset of TB is insidious, the infection proceeding for some time before the patient becomes sufficiently ill to seek medical attention. Primary TB is usually mild and asymptomatic and in 90% of cases does not proceed further. However, clinical disease develops in the remaining 10%.

Mycobacteria have the ability to colonize almost any site in the body. The clinical manifestations are variable: fatigue, weight loss, weakness and fever are all associated with TB. Infection in the lungs characteristically causes a chronic productive cough, and the sputum may be blood stained as a result of tissue destruction. Necrosis may erode blood vessels, which can rupture and cause death through haemorrhage.

Complications of *M. tuberculosis* infection arise from local spread or dissemination

The organism may disseminate via the lymphatics and bloodstream to other parts of the body. This usually occurs at the time of primary infection, and in this way chronic foci are established, which may proceed to necrosis and destruction in, for example, the kidney. Alternatively, spread may be by extension to a neighbouring part of the lung, for instance when a tubercle erodes into a bronchus and discharges its contents, or into the pleural cavity, resulting in a pleural effusion.

Although the number of cases of pulmonary TB has been declining in resource-rich countries since the beginning of the twentieth century, hastened by the advent of specific antimicrobial drugs, the incidence of extrapulmonary TB has stayed roughly constant for many years and therefore makes up a greater proportion of the TB caseload in resource-rich countries than in resource-poor countries.

The Ziehl–Neelsen stain of sputum can provide a diagnosis of TB within 1 h, whereas culture can take 6 weeks

A diagnosis of TB is suggested by the clinical signs and symptoms referred to above, supported by characteristic changes on chest radiography (see Fig. 20.23A and B) and positive skin test reactivity in the tuberculin (Mantoux) test. These tests are confirmed by microscopic demonstration of acid-fast rods and culture of *M. tuberculosis*. Microscopic examination of a smear of sputum stained by Ziehl–Neelsen's method or by auramine often reveals acid-fast rods (Fig. 20.24). This result can be obtained within 1 h of receipt of the specimen in the laboratory. This is important because *M. tuberculosis* can take up to 6 weeks to grow in culture, although radiometric methods may reduce the time required for detection, and therefore confirmation of the diagnosis is necessarily delayed. Rapid non-culture tests to detect *M. tuberculosis* are PCR and the automated Xpert MTB-RIF molecular test that detects TB and rifampicin resistance. Further tests are required to identify the mycobacterial species and to establish susceptibility to antituberculosis drugs.

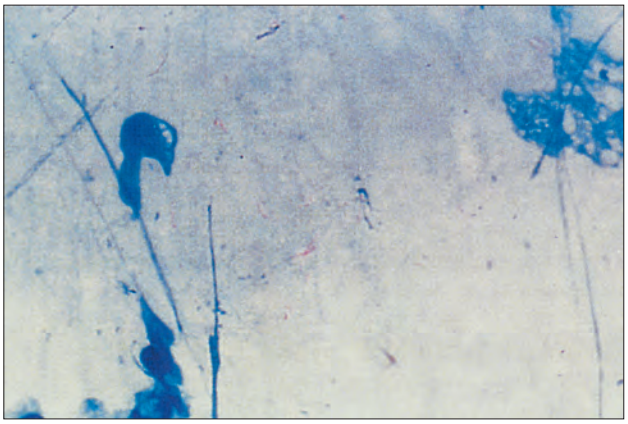


Figure 20.24 Pulmonary tuberculosis. Sputum preparation showing pink-stained, acid-fast tubercle bacilli (Ziehl–Neelsen stain). (Courtesy of J.A. Innes.)

Specific antituberculosis drugs and prolonged therapy are needed to treat TB

Mycobacteria are innately resistant to most antibacterial agents, and specific antituberculosis drugs have to be used; these are reviewed in Chapter 34. The key features of treatment are the use of:

- combination therapy – usually four drugs such as isoniazid, rifampicin, ethambutol and pyrazinamide to prevent emergence of resistance
- prolonged therapy – minimum 6 months period, which is necessary to eradicate these slow-growing intracellular organisms.

The number of strains resistant to the first-line antituberculosis drugs has increased as these antibiotics have been used for decades and may appear if there are compliance problems due to the number of drugs and lengthy treatment period. Other factors can include variable quality of the drugs and poor prescribing practices. Treatment is monitored carefully with directly observed treatment and shorter courses. Multidrug-resistant TB (MDR-TB) occurs when there is little response to the first-line drugs, isoniazid and rifampicin. In 2015, there were nearly 500 000 people around the globe with MDR-TB, mostly in China, India and Russia. Extremely drug-resistant TB, referred to as XDR TB, does not respond to the second-line drugs and WHO reported that about 10% of patients with MDR-TB had XDR-TB in 2015.

Tuberculosis is prevented by improved social conditions, immunization and chemoprophylaxis

The steady decline in incidence of TB since the beginning of the twentieth century, and before specific preventive measures were available, underlines the importance of improvements in social conditions in the prevention of this and many other infectious diseases. However, there has been an increase in the number of cases associated with AIDS; in some countries in the resource-poor world, HIV infection and AIDS are threatening to overwhelm TB control programmes. In 2015, over 30% of HIV positive individuals worldwide had TB, of whom nearly 400 000 died. To further emphasize the magnitude of the problem, in 2015, it was estimated that there were over 1 million new TB infections in HIV-positive people, 700 000 of whom were living in Africa.

Immunization with a live attenuated BCG (bacille Calmette–Guérin) vaccine has been used effectively in situations where TB is prevalent. It was introduced in the UK in 1953 and the programme changed along sociodemographic lines as it was initially targeted at 14-year-olds as most TB was seen in young adults. This was modified in the 1960s when a selective neonatal immunization programme was instituted aimed at infants born to parents whom had emigrated from high-prevalence countries as those groups were found to have higher rates of TB than those born in the UK. Immunization, which confers positive skin test reactivity, does not prevent infection, but it does allow the body to react quickly to limit proliferation of the organisms. In areas where there is a low prevalence of disease, immunization has been largely replaced by chemoprophylaxis. Those immunized have been reported to be up to 8% less likely to develop the most severe complications of TB.

In the UK, prophylaxis with rifampicin and isoniazid for 3 months is recommended for people who have had close contact with a case of TB (unless isoniazid resistant). It is also advocated for individuals who show recent conversion to skin test positivity, when it is essentially early treatment of subclinical infection rather than prophylaxis.

CYSTIC FIBROSIS

Individuals with cystic fibrosis are predisposed to develop lower respiratory tract infections

Cystic fibrosis is the most common lethal inherited disorder among Caucasians, with an incidence of approximately 1 in 2500 live births. The disease is characterized by pancreatic insufficiency, abnormal sweat electrolyte concentrations and production of very viscid bronchial secretions. The latter tend to lead to stasis in the lungs and this predisposes to infection.

P. aeruginosa colonizes the lungs of almost all 15- to 20-year-olds with cystic fibrosis

The respiratory mucosa of individuals with cystic fibrosis presents a different environment for potential pathogens from that found in healthy individuals, and the common infecting organisms and the nature of infections differ from other lung infections. These invaders include:

- *Staph. aureus*, which causes respiratory distress and lung damage, but can be well controlled by specific antistaphylococcal chemotherapy
- *P. aeruginosa*, which is the main pathogen
- *Burkholderia cepacia*, which is aggressive and hard to treat
- *H. influenzae*, typically non-encapsulated strains, which may be found in association with *Staph. aureus* and *P. aeruginosa*; their pathogenic significance is unclear, but they appear to contribute to respiratory exacerbations
- *Aspergillus fumigatus*, which is a fungus in the environment that may cause symptoms
- non-tuberculous mycobacteria may also cause symptoms.

P. aeruginosa infection is uncommon in those under 5 years of age, but colonizes the lungs of almost all patients aged 15–20 years, often encouraged by its intrinsic resistance to antistaphylococcal agents. Early in the course of infection, normal colony types are grown from sputum cultures, but as infection progresses, the organism changes to a highly mucoid form, almost mimicking the mucoid secretions of the patient (Fig. 20.25). These mucoid forms are thought to grow in microcolonies in the lung, but most of the lung damage is due to immunological responses to the organisms and to the alginate, which forms the mucoid material (Fig. 20.26). *P. aeruginosa* rarely invades beyond the lung even in the most severely infected individuals. Inhaled antibiotics are recommended for bacterial eradication and to try to prevent chronic infection.

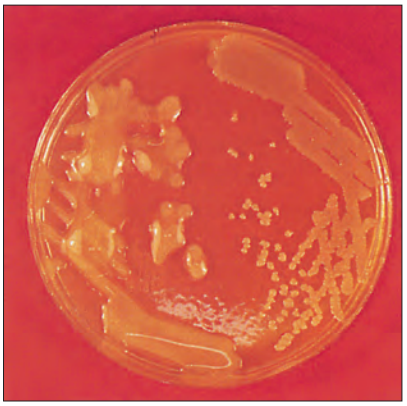


Figure 20.25 *Pseudomonas aeruginosa* isolated from the sputum of patients with cystic fibrosis characteristically grows in a very mucoid colonial form, shown here on the left of the picture, with the normal colonial form on the right for comparison.

Although specific antibacterial chemotherapy can reduce the symptoms of infection and improve the quality of life, infections, particularly with *P. aeruginosa* and *B. cepacia*, are difficult to eradicate and are still a major cause of morbidity and mortality.

LUNG ABSCESS

Lung abscesses usually contain a mixture of bacteria including anaerobes

This is a suppurative infection of the lung, sometimes referred to as 'necrotizing pneumonia'. The most common predisposing cause is aspiration of respiratory or gastric secretions as a result of altered consciousness. The infection is therefore endogenous in origin and cultures often reveal a mixture of bacteria, with anaerobes such as *Bacteroides* and *Fusobacterium* playing an important role (Fig. 20.27).

Patients with lung abscesses may be ill for at least 2 weeks before presentation, with possible swinging fever, and usually produce large amounts of sputum, which, if foul smelling, gives a strong hint of the presence of anaerobes and often suggests the diagnosis. Most diagnoses are made from chest radiographs (see Fig. 20.7G) and the cause confirmed by microbiological investigation.

Treatment of lung abscess should include an antianaerobic drug and last 2–4 months

Because of the likely presence of anaerobes, a suitable antianaerobic agent such as metronidazole should be part of the treatment regimen and treatment may be needed for 2–4 months to prevent relapse. If diagnosis and treatment are delayed, infection may spread to the pleural space, giving rise to empyema (see below).

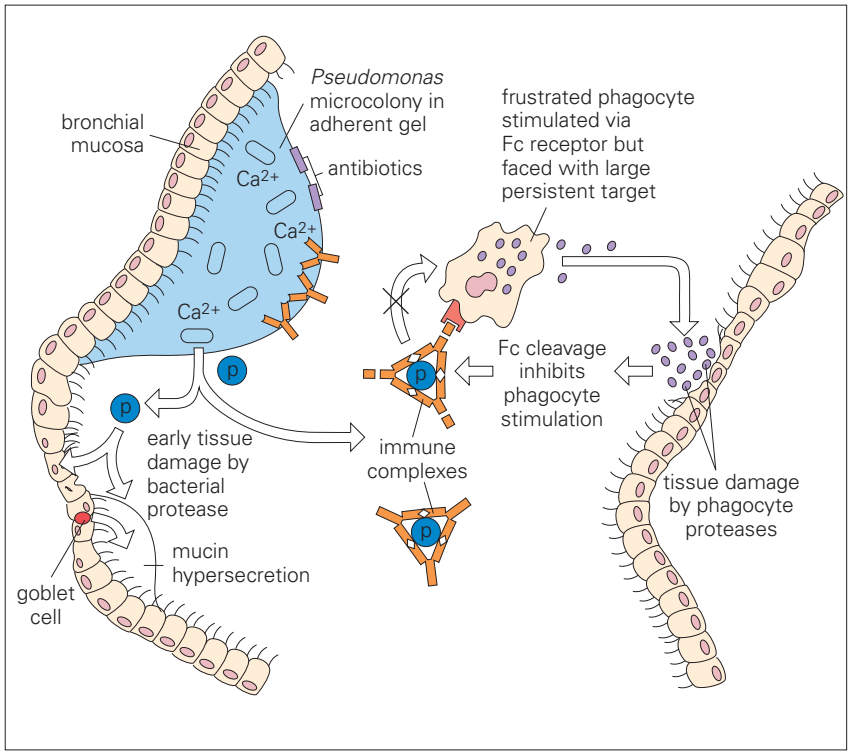


Figure 20.26 *Pseudomonas* infection in the lung of cystic fibrosis is chronic, but rarely invasive beyond the bronchial mucosa. The organisms are thought to grow in microcolonies embedded in a calcium (Ca^{2+})-dependent mucoid alginate gel, which contains DNA and tracheobronchial mucin, and attaches to the bronchial mucosa. This protects the organisms from the host defences and provides a physical and electrolyte barrier to antibiotics. Much of the damage to tissue is thought to be due to the slow release of bacterial proteases (which disrupt the mucosa and cause mucin hypersecretion), immunopathological mechanisms exacerbated by the size, antigenicity and persistence of the alginate matrix, and the indirect action of immune complexes associated with *Pseudomonas antigens* (p). Tissue damage is also caused by phagocyte proteases. Intermittent exacerbations can be explained by the cleavage of the Fc of immune complexes by these proteases and consequent inhibition of further phagocyte stimulation. (Redrawn from Govan J.R.W. *Rev Med Microbiol* 1:19–28, ©1990.)

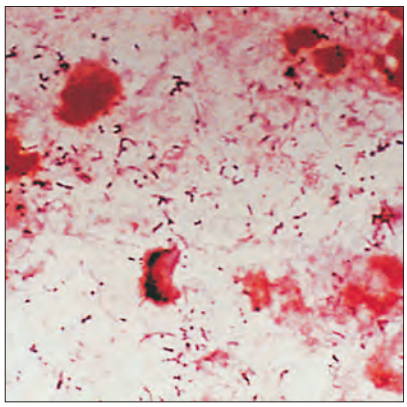


Figure 20.27 Gram stain of pus from a lung abscess showing Gram-positive cocci and both Gram-negative and Gram-positive rods. (Courtesy of J.R. Cantey.)

Pleural effusion and empyema

Up to 50% of patients with pneumonia have a pleural effusion

Pleural effusions arise in a variety of different diseases. Sometimes the organisms infecting the lung spread to the pleural space and give rise to a purulent exudate or 'empyema'.

Pleural effusions can be demonstrated radiologically, but detection of empyema can be difficult, particularly in a patient with extensive pneumonia.

Aspiration of pleural fluid provides material for microbiological examination, and *Staph. aureus*, Gram-negative rods and anaerobes are commonly involved.

Treatment should be directed at drainage of pus, eradication of infection and expansion of the lung.

FUNGAL INFECTIONS

Disease associated with fungal infection is most commonly seen in patients with defective immunity, as a consequence either of immune suppressive treatment or of concomitant disease. A number of species can cause opportunistic infections, and two are of particular importance: *Aspergillus fumigatus* and *Pneumocystis jirovecii*.

Aspergillus

The most important species are *A. fumigatus* and *A. flavus*.

Aspergillus can cause allergic bronchopulmonary aspergillosis, aspergilloma or disseminated aspergillosis

The genus *Aspergillus* contains many species and these are ubiquitous in the environment. They do not form part of the normal flora. Their spores are regularly inhaled without harmful consequences, but some species, notably *A. fumigatus*, are able to cause a range of diseases, including:

- Allergic bronchopulmonary aspergillosis (ABPA), which is, as its name suggests, an allergic response to the presence of *Aspergillus* antigen in the lungs and occurs in patients

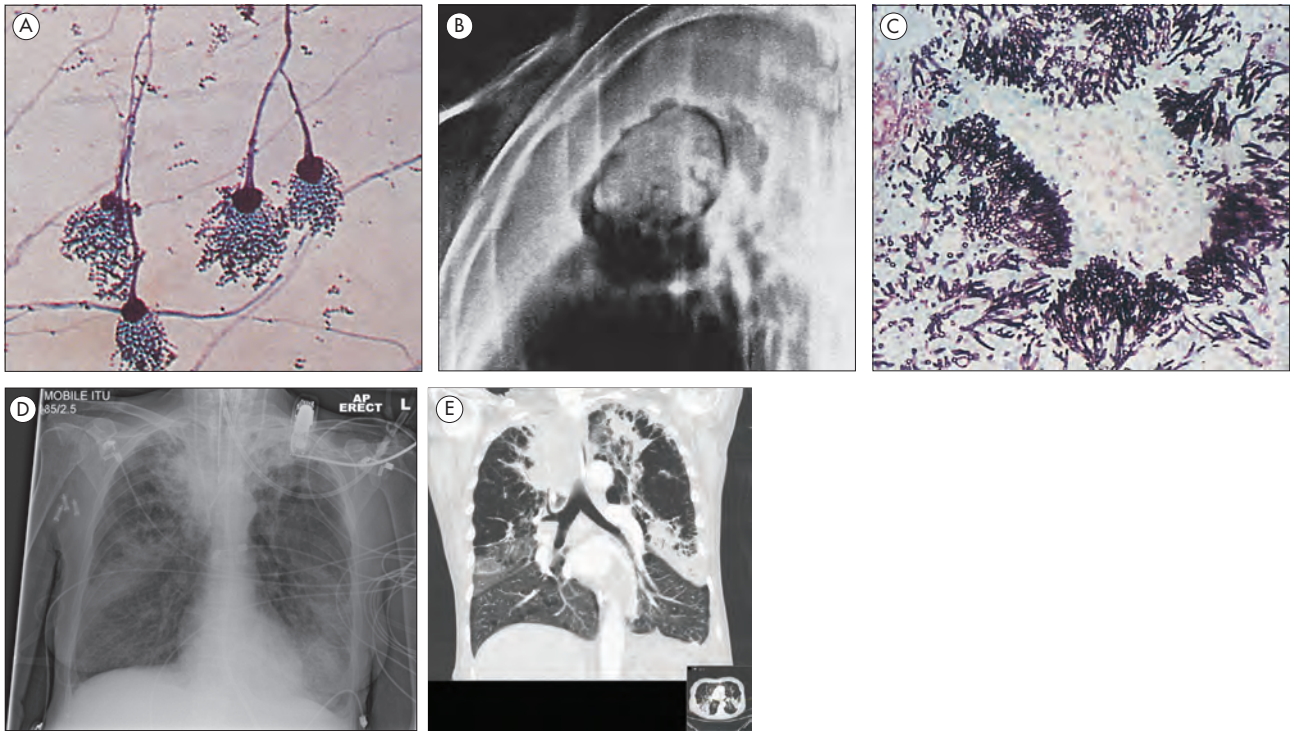


Figure 20.28 (A) *Aspergillus fumigatus*. Lactophenol cotton blue stained preparation showing the characteristic conidiophores. (B) Aspergilloma. Tomogram showing fungus ball contained within the lung cavity, outlined by air space. (C)–(E) Invasive aspergillosis: (C) histological section showing fungal hyphae invading the lung parenchyma and blood vessels (Grocott stain); (D) chest X-ray and (E) chest CT scan. ([A] and [B] Courtesy of J.A. Innes. [C] Courtesy of C. Kibbler. [D] and [E] Courtesy of G. Bain, London North West Healthcare Trust).

with asthma. ABPA occurs in some 10% of cystic fibrosis patients.

- Aspergilloma in patients with pre-existing lung cavities or chronic pulmonary disorders. *Aspergillus* colonizes a cavity and grows to produce a fungal ball, a mass of entangled hyphae – the aspergilloma (Fig. 20.28). In this case, fungi do not invade the lung tissue, but the presence of a large aspergilloma can cause respiratory problems. Aspergillomas can be related, however, to chronic pulmonary aspergillosis where invasion of lung tissue does occur.
- Disseminated disease in the immunosuppressed patient when the fungus spreads from the lungs.

Invasive aspergillosis carries a high mortality as treatment is very difficult owing to the limited number and toxic nature of antifungal agents active against *Aspergillus* plus the lack of functional host defences. Treatment is with an intravenous lipid formulation of amphotericin B. Voriconazole or caspofungin are alternatives. A primary aim of therapy is to improve the neutrophil count.

Pneumocystis jirovecii (formerly *P. carinii*)

Pneumocystis pneumonia is an important opportunistic infection in AIDS

P. jirovecii is a fungus commonly found in immunocompetent humans and in rodents. There is strong host specificity, so *Pneumocystis* infection of humans is not a zoonosis. Infection probably spreads by droplet though airborne transmission has been directly demonstrated only in animal models. Disease occurs in debilitated and immune-deficient individuals. Before the advent of combined active antiretroviral therapy, a high proportion of patients with advanced HIV infection developed *Pneumocystis* pneumonia, which could be fatal.

Pneumocystis occurs as three developmental forms: a trophozoite, up to 5 µm diameter, a precyst and a cyst. Spores are released when the cysts rupture. Disease is associated with an interstitial pneumonitis (Fig. 20.29), with plasma cell infiltration. Infections of internal organs other than the lung (e.g. lymph nodes, spleen, liver) have also been reported at post-mortem examination.

Treatment is with co-trimoxazole or pentamidine.

PROTOZOAL INFECTIONS

A variety of protozoa localizes to the lung or involve the lung at some stage in their development

These include:

- Nematodes such as *Ascaris, Strongyloides* and the hookworms (see Chs. 7 and 23), which migrate through the lungs as they move to the small intestine, breaking out of the capillaries around the alveoli to enter the bronchioles. The damage caused by this process, and the development of inflammatory responses, can lead to a transient pneumonitis with cough, wheeze, dyspnoea and pulmonary infiltrates.
- Schistosome larvae, which may cause mild respiratory symptoms as they migrate through the lungs. Heavy acute infections may produce pneumonitis with poorly defined nodular lesions or reticulonodular appearances.
- The microfilariae of filarial nematodes such as *Wuchereria* or *Brugia*, which appear in the peripheral circulation

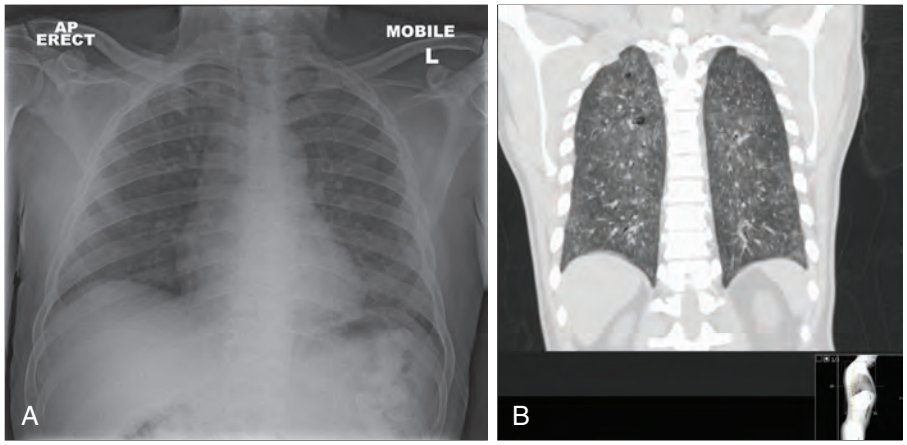


Figure 20.29 (A) Chest X-ray and (B) chest CT scan of *Pneumocystis* pneumonia. (Courtesy of G. Bain, London North West Healthcare Trust.)

with a regular diurnal or nocturnal periodicity, their appearance coinciding with the time at which the vector blood-sucking insects are likely to feed. Outside these periods, the larvae become sequestered in the capillaries of the lung. Under certain conditions, as yet undefined, and in certain individuals, the presence of the larvae triggers a condition known as 'tropical pulmonary eosinophilia' (TPE or Weingarten's syndrome). This is characterized by the onset over several months of cough, dyspnoea and wheeze, which is worse at night, and marked peripheral blood eosinophilia. Microfilariae are absent from the peripheral blood. Antifilarial antibody tests are strongly positive. Chest X-ray examination shows bilateral fine nodular or reticulonodular shadowing.

- *Ascaris* and *Strongyloides* infections, which may also trigger a pulmonary eosinophilia, although the condition is distinct from TPE.
- *Echinococcus granulosus* infection, which leads to the development of hydatid cysts in a proportion (20–30%) of cases owing to localization of the larvae of the tapeworm in the lungs. These cysts may reach a considerable size, causing respiratory distress, largely as a consequence of the mechanical pressure exerted on lung tissue. Spontaneous rupture may occur and result in acute anaphylaxis.
- *Entamoeba histolytica* infection, which may rarely involve the lung.
- *Paragonimus westermani*, the oriental lung fluke, which is the most important example of one of the very few adult parasites that live in the lung, infecting an estimated 22 million people, mainly in Asia. Infection is acquired by eating crustaceans containing the infective metacercariae. These migrate from the intestine across the body cavity and penetrate into the lungs. The adults develop within fibrous cysts, which connect with the bronchi to provide an exit for the eggs (Fig. 20.30). Infections cause chest pain and difficulty in breathing, and can cause bronchopneumonia when large numbers of parasites are present. Single lesions can be confused with lung cancer, TB and fungal lesions. Praziquantel is an efficient anthelmintic for paragonimiasis.

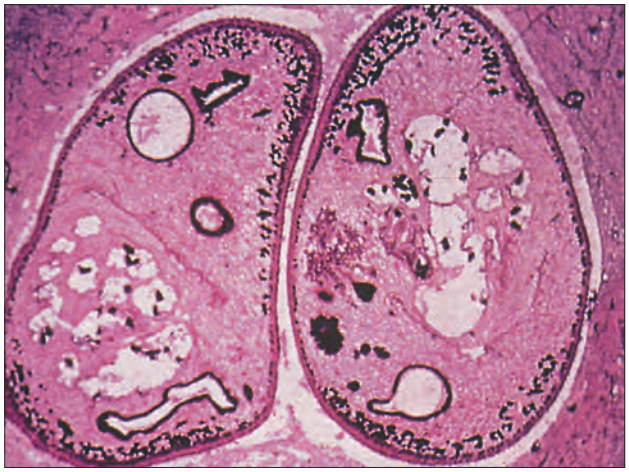


Figure 20.30 Two adult *Paragonimus* contained within a fibrous cyst in the lung. (Courtesy of H. Zaiman.)

KEY FACTS

- Although continuous from nose to alveoli, the respiratory tract is divided into 'upper' and 'lower' from the viewpoint of infection.
- Infections in the lower respiratory tract are spread by the airborne route (except parasites), are acute or chronic, tend to be severe and may be fatal without correct treatment. They are caused by a wide range of organisms – usually bacteria or viruses, but also fungi and parasites.
- Bronchitis, an inflammatory condition of the tracheobronchial tree, is usually chronic with acute exacerbations associated with infection by viruses and bacteria. The disease is characterized by cough and excessive mucus production, and the diagnosis is clinical. Antibiotics are often given, but their efficacy is uncertain.
- Bronchiolitis, usually caused by RSV, is especially acute and severe in infants. RSV causes outbreaks in the community and in hospitals. The disease has an immunopathological basis, and specific treatment (ribavirin) may be considered. Vaccines are in development.
- Pneumonia is caused by a variety of pathogens depending upon patients' age, previous or underlying disease, and occupational and geographical factors. Correct microbiological diagnosis is essential to optimize therapy. Mortality from pneumonia remains significant.
- *B. pertussis* colonizes the ciliated respiratory epithelium causing the specifically human infection whooping cough. Pertussis toxin and other toxic factors are important for virulence. Diagnosis is clinical, alerted by the characteristic paroxysmal cough. Supportive care is paramount; antibiotics play a peripheral role. Prevention by immunization is effective, and new safer vaccines are becoming available.
- Influenza viruses cause endemic, epidemic and pandemic infections as a result of the capacity of the virus for antigenic drift and shift. The disease is acute in onset and can be clinically severe. Viral damage to the respiratory mucosa predisposes to secondary bacterial pneumonia. Antiviral agents are available. Immunization is important, but needs to be kept up to date owing to the frequent antigenic changes in the circulating virus.
- The TB epidemic is larger than was previously estimated, after WHO had collected new surveillance data from India. However, the number of TB deaths and the TB incidence rate continue to fall globally. In 2015, there were an estimated 10.4 million new (incident) TB cases worldwide, and people with HIV made up 1.2 million (11%) of all new TB infections. Six countries accounted for 60% of the new cases: India, Indonesia, China, Nigeria, Pakistan and South Africa. TB was one of the top 10 causes of death worldwide in 2015.
- Primary infection with *M. tuberculosis* results in a localized pulmonary lesion, while secondary disease arises from reactivation as a result of an impairment of immune function. Clinical diagnosis is supported by demonstrating the acid-fast *M. tuberculosis* in sputum. Effective treatment is available, but long courses of drug combinations are essential. Chemoprophylaxis and BCG immunoprophylaxis are important in prevention.
- *Aspergillus* causes disease in the lung ranging from invasive disease in the immunocompromised to allergic conditions in the otherwise healthy. Effective treatment is difficult because of the limited number of active antifungals and lack of host defences.
- Cystic fibrosis is an inherited disease that predisposes to a particular pattern of lung disease characterized by infection with *P. aeruginosa*. Infection can be controlled by antibacterials, but is rarely eradicated.
- Various species of parasites pass through or localize in the lungs at some stage in their life cycle. Damage is limited unless the parasite load is high, and is usually immunopathological in nature.

21 Urinary tract infections

Introduction

Urinary tract infections are common, especially among women

The urinary tract is one of the most common sites of bacterial infection, particularly in females; 20–30% of women have recurrent urinary tract infections (UTIs) at some time in their life. UTIs in men are less common and primarily occur after 50 years of age. Although the majority of infections are acute and short lived, they contribute to a significant amount of morbidity in the population. Severe infections result in a loss of renal function and serious long-term sequelae. In females, a distinction is made between cystitis, urethritis and vaginitis, but the genitourinary tract is a continuum and the symptoms often overlap.

ACQUISITION AND AETIOLOGY

Bacterial infection is usually acquired by the ascending route from the urethra to the bladder

The infection may then proceed to the kidney. Occasionally, bacteria infecting the urinary tract invade the bloodstream to cause septicaemia. Less commonly, infection may result from haematogenous spread of an organism to the kidney, with the renal tissue being the first part of the tract to be infected.

From an epidemiological viewpoint, UTIs occur in two general settings: community acquired and hospital (nosocomially) acquired, the latter most often being associated with catheterization. Hospital-acquired UTIs, although less common than community acquired, contribute significantly (about 40%) to overall nosocomial infection rates.

The Gram-negative rod *Escherichia coli* is the commonest cause of ascending UTI

Other members of the Enterobacteriaceae are also implicated (Fig. 21.1). *Proteus mirabilis* is often associated with urinary stones (calculi), probably because this organism produces a potent urease, which acts on urea to produce ammonia, rendering the urine alkaline. *Citrobacter*, *Klebsiella*, *Enterobacter*, *Proteus* and *Pseudomonas aeruginosa* are more frequently found in hospital-acquired UTI because their resistance to antibiotics favours their selection in hospital patients (see Ch. 34).

Among the Gram-positive species, *Staphylococcus saprophyticus* has a particular propensity for causing infections, especially in young sexually active women. *Staphylococcus epidermidis* and *Enterococcus* species are more often associated with UTI in hospitalized patients (especially those with AIDS), where multiple antibiotic resistance can cause treatment difficulties. In some instances, capnophilic species (organisms that grow better in air enriched with carbon dioxide), including corynebacteria and lactobacilli, have been implicated as possible causes of UTI. Obligate anaerobes are very rarely involved.

When there has been haematogenous spread to the urinary tract, other species may be found, e.g. *Salmonella typhi*, *Staphylococcus aureus* and *Mycobacterium tuberculosis* (renal tuberculosis).

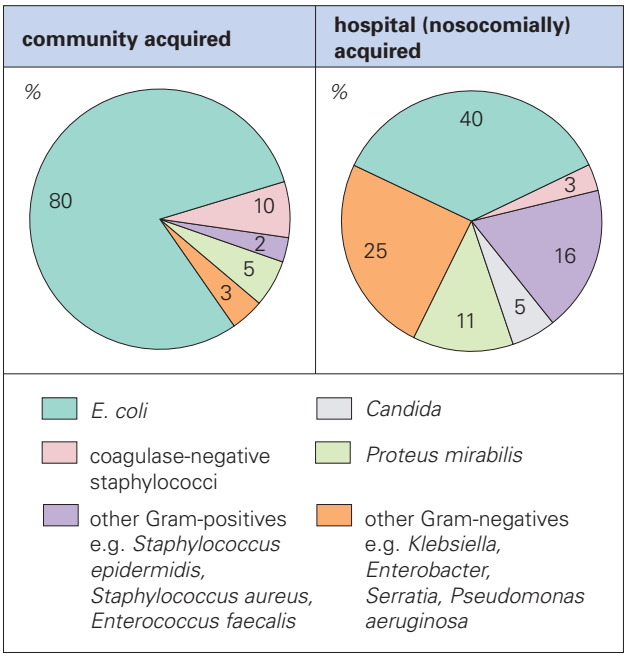


Figure 21.1 Common causes of urinary tract infection. The percentages of infections caused by different bacteria in outpatients and hospital inpatients are shown. *E. coli* is by far the commonest isolate in both groups of patients, but note the difference in the percentage of infections caused by other Gram-negative rods. These isolates often carry multiple antibiotic resistance and colonize patients in hospital, especially those receiving antibiotics.

Viral causes of UTI appear to be rare, although there are associations with haemorrhagic cystitis and other renal syndromes

Certain viruses may be recovered from the urine in the absence of urinary tract disease and include the following:

- The human polyomaviruses, JC and BK, enter the body via the respiratory tract, spread through the body and infect epithelial cells in the kidney tubules and ureter, where they establish latency with persistence of the viral genome.

About 35% of kidneys from healthy individuals contain polyomavirus DNA sequences. However, during normal pregnancy, the viruses may reactivate asymptomatically, with the appearance of large amounts of virus in the urine. Reactivation also occurs in immunocompromised patients (see Ch. 31) and may lead to haemorrhagic cystitis.

- High titres of cytomegalovirus (CMV) and rubella may be shed asymptomatically in the urine of congenitally infected infants (see Ch. 24).
- In contrast to asymptomatic shedding, some serotypes of adenovirus have been implicated as a cause of haemorrhagic cystitis.
- The rodent-borne hantavirus responsible for Korean haemorrhagic fever infects capillary blood vessels in the kidney and can cause a renal syndrome with proteinuria.
- Finally, a number of other viruses can infect the kidneys, including mumps and HIV.

Urine samples are commonly investigated by virus isolation, immunological and genomic detection methods.

Very few parasites cause UTIs

Other causes of UTI include:

- the fungi *Candida* spp. and *Histoplasma capsulatum*
- the protozoan *Trichomonas vaginalis* (see Ch. 22), which can cause urethritis in both males and females, but is most often considered as a cause of vaginitis
- infections with *Schistosoma haematobium* (see Ch. 28), which result in inflammation of the bladder and commonly haematuria. The eggs penetrate the bladder wall, and in severe infections large granulomatous reactions can occur and the eggs may become calcified. Bladder cancer is associated with chronic infections, although the mechanism is uncertain. Obstruction of the ureter as a result of egg-induced inflammatory changes can also lead to hydronephrosis.

PATHOGENESIS

A variety of mechanical factors predispose to UTI

Anything that disrupts normal urine flow or complete emptying of the bladder or facilitates access of organisms to the bladder will predispose an individual to infection (Fig. 21.2). The shorter female urethra is a less effective deterrent to infection than the male urethra (see Ch. 14). Sexual intercourse facilitates the movement of organisms up the urethra, particularly in females, so the incidence of UTI is higher among sexually active women than among celibate women. Preceding bacterial colonization of the periurethral area of the vagina is perhaps important (see below).

In male infants, UTIs are more common in the uncircumcised, and this is associated with colonization of the inside of the prepuce and urethra with faecal organisms.

Pregnancy, prostatic hypertrophy, renal calculi, tumours and strictures are the main causes of obstruction to complete bladder emptying

Increased volumes of post-void residual urine are associated with a greater likelihood of infection. Infection, superimposed on urinary tract obstruction, may lead to ascent of infection to the kidney and rapid destruction of renal tissue.

Loss of neurological control of the bladder and sphincters (e.g. in spina bifida, paraplegia or multiple sclerosis), and the resultant large residual volume of urine in the bladder, causes a functional obstruction to urine flow, and such patients are particularly prone to recurrent infections.

Vesicoureteral reflux (reflux of urine from the bladder cavity up the ureters, sometimes into the renal pelvis or parenchyma) is common in children with anatomical abnormalities of the urinary tract and may predispose to ascending infection and kidney damage. Reflux may also occur in association with infection in children without underlying abnormalities, but tends to disappear with age.

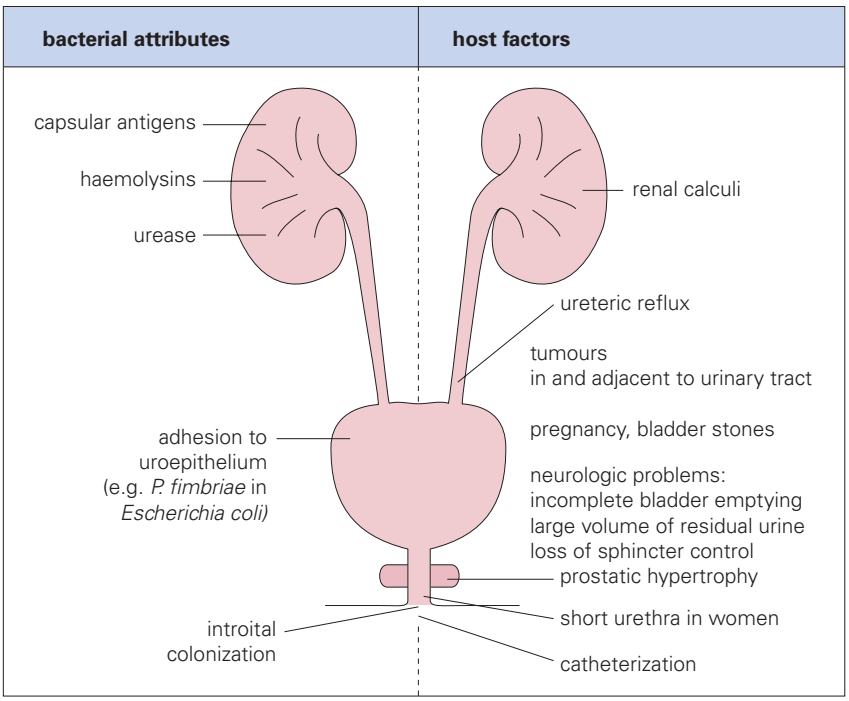


Figure 21.2 Bacterial attributes and host factors favouring urinary tract infection. Abnormalities of the urinary tract tend to predispose to infection. Bacterial adherence factors have been studied in detail, but relatively little is known about other bacterial virulence factors in UTI.

Clinical studies including reports that pyelonephritis (infection of the kidney) is commonly found in people with diabetes mellitus at post-mortem suggest an increased propensity for UTI in individuals with diabetes mellitus. People with diabetes mellitus may have more severe UTIs, and if diabetic neuropathy interferes with normal bladder function then persistent UTIs are common.

Catheterization is a major predisposing factor for UTI

During insertion of the catheter, bacteria may be carried directly into the bladder and, while in situ, the catheter facilitates bacterial access to the bladder either via the lumen of the catheter or by tracking up between the outside of the catheter and the urethral wall. The catheter disrupts the normal bladder's protective function action and allows bacterial introduction into the bladder as the catheter is inserted and, while the catheter is in place, bacteria reach the bladder by tracking up between the outside of the catheter and the urethra. Contamination of the catheter drainage system by bacteria from other sources can also result in infection. The duration of catheterization is directly associated with increased probability of infection, due in part to the formation of biofilms (see Ch. 2) which protect the organisms from antimicrobials and host defence mechanisms. Thus, the risk of UTI increases by about 3–10% each day of catheterization.

A variety of virulence factors are present in the causative organisms

The conflict between host and parasite in the urinary tract has been discussed in Chapter 14. Most urinary tract pathogens originate in the faecal flora, but only the aerobic and facultative species such as *E. coli* possess the attributes required to colonize and infect the urinary tract. The ability to cause infection of the urinary tract is limited to certain serogroups of *E. coli* such as O (semantic) serotypes (e.g. O1, O2, O4, O6, O7, O8 and O75) and K (capsular) serotypes (e.g. K1, K2, K3, K5, K12 and K13). These serotypes differ from those associated with gastrointestinal tract infection (see Ch. 23), which has led to use of the term 'uropathogenic *E. coli*' (UPEC). The success of these strains is attributable to a variety of genes in chromosomal pathogenicity islands (see Ch. 2) which are not found in faecal *E. coli*. For example, UPEC typically contains genes associated with colonization of the periurethral areas. A prime example is the adhesion known as *P. fimbriae* (pyelonephritis-associated pili [PAP]), which allows UPEC to adhere specifically to urethral and bladder epithelium. Studies with other species of urinary tract pathogens have confirmed the presence of similar adhesins for uroepithelial cells (Fig. 21.3).

Other features of *E. coli* which appear to assist in the localization of organisms in the kidney and in renal damage include the following:

- The capsular acid polysaccharide (K) antigens are associated with the ability to cause pyelonephritis and are known to enable *E. coli* strains to resist host defences by inhibiting phagocytosis.
- Haemolysin production by *E. coli* is linked with the capacity to cause kidney damage; many haemolysins act more generally as membrane-damaging toxins.

The production of urease by organisms such as *Proteus* spp. has been correlated with their ability to cause pyelonephritis and stones.

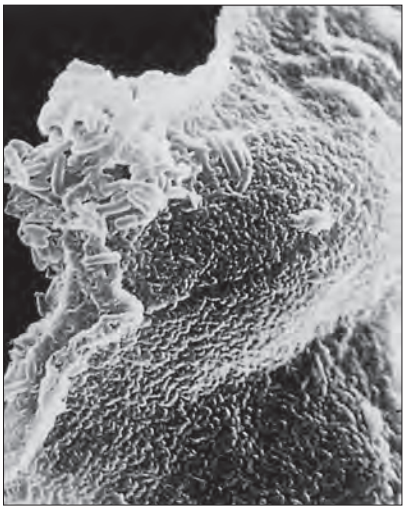


Figure 21.3 Scanning electron micrograph showing bacteria attached to an exfoliated uroepithelial cell from a patient with acute cystitis. (Courtesy of T.S.J. Elliot and the editor of *British Journal of Urology*.)

The healthy urinary tract is resistant to bacterial colonization

With the exception of the urethral mucosa, the urinary tract usually eliminates microorganisms rapidly and efficiently (see Ch. 14). The pH, chemical content and flushing mechanism of urine help to dispose of organisms in the urethra. Although urine is a good culture medium for most bacteria, it is inhibitory to some, and anaerobes and other species (non-haemolytic streptococci, corynebacteria and staphylococci), which comprise most of the normal urethral flora, do not readily multiply in urine.

Although the inflammatory response to urinary tract infection involves leukocytic, chemokine, and cytokine response, the role of humoral immunity in the host's defence against infection of the urinary tract is poorly understood. After infection of the kidney, IgG and secretory IgA antibodies can be detected in urine but do not appear to protect against subsequent infection. Infection of the lower urinary tract is usually associated with a low or undetectable serological response, reflecting the superficial nature of the infection; the bladder and urethral mucosa are rarely invaded in UTIs.

CLINICAL FEATURES AND COMPLICATIONS

Acute lower UTIs cause dysuria, urgency and frequency

Acute infections of the lower urinary tract are characterized by a rapid onset of:

- dysuria (burning pain on passing urine)
- urgency (the urgent need to pass urine)
- frequency of micturition.

However, UTIs in the elderly and those with indwelling catheters are usually asymptomatic.

The urine is cloudy owing to the presence of pus cells (pyuria) and bacteria (bacteriuria), and may contain blood (haematuria). Examination of urine specimens in the laboratory is essential to confirm the diagnosis. Patients with genital tract infections such as vaginal thrush or chlamydial urethritis may present with similar symptoms (see Ch. 22).

Pyuria in the absence of positive urine cultures can be due to chlamydiae or tuberculosis and is also seen in patients receiving antibacterial therapy for UTI, as the bacteria are inhibited or killed by the antibacterial agent before the inflammatory response dies away.

Recurrent infections of the lower urinary tract occur in a significant proportion of patients. They may be:

- relapses, caused by the same strain of organism
- re-infections by different organisms.

Recurrent infections can result in chronic inflammatory changes in the bladder, prostate and periurethral glands.

Acute bacterial prostatitis causes systemic symptoms (fever) and local symptoms (perineal and low back pain, dysuria and frequency)

Acute bacterial prostatitis may arise from ascending or haematogenous infection, and people lacking the antibacterial substances normally present in prostatic fluid are perhaps more susceptible. Chronic bacterial prostatitis, however, although usually caused by *E. coli*, is difficult to cure and can be a source of relapsing infection within the urinary tract.

Upper UTIs

Although it may be important to know whether an infection is restricted to the bladder (lower urinary tract) or has ascended to the upper urinary tract and kidney, distinguishing the two can be difficult (e.g. examining urine directly from the ureter by ureteric catheterization).

Pyelonephritis causes a fever and lower urinary tract symptoms

Patients with pyelonephritis (infection of the kidney, Fig. 21.4) present with lower urinary tract symptoms and usually have a fever. Staphylococci are a common cause and renal abscesses are generally present. Recurrent episodes of pyelonephritis result in a loss of function of renal tissue, which may in turn cause hypertension, itself a cause of renal damage. Infection associated with stone formation can result in obstruction of the renal tract and septicaemia.

Haematuria is a feature of endocarditis and a manifestation of immune complex disease, as well as a result of infections of the kidney, and its presence warrants careful investigation.

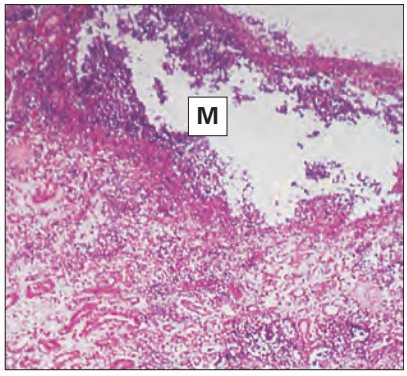


Figure 21.4 Histological appearance of the kidney in acute pyelonephritis, showing the intense inflammatory reaction and microabscesses (M). (H&E stain.) (Courtesy of M.J. Wood.)

Pyuria may be associated with kidney infection by *M. tuberculosis*. This organism cannot be grown by normal urine culture methods and therefore the patient may appear to have a sterile pyuria.

Asymptomatic infection (i.e. significant numbers of bacteria in the urine in the absence of symptoms, see below) can be detected only by screening urine samples in the laboratory. It is important in instances such as:

- pregnant women and young children, where failure to treat may result in chronic renal damage
- people undergoing instrumentation of the urinary tract, in whom bacteriuria may proceed to bacteraemia
- the elderly and those with diabetes (both risk factors for asymptomatic bacteriuria).

LABORATORY DIAGNOSIS

A key feature is the detection of significant bacteriuria.

Infection can be distinguished from contamination by quantitative culture methods

Historically, the urinary tract has been considered to be sterile. However, modern molecular methods indicate that low levels of harmless organisms may be present. The distal region of the urethra is colonized with commensal organisms, which may include periurethral and faecal organisms. As urine specimens are usually collected by voiding a specimen into a sterile container, they may become contaminated with the periurethral flora during collection. However, infection is traditionally distinguished from contamination by quantitative culture methods. Bacteriuria is defined as 'significant' when a properly collected midstream urine (MSU) specimen is shown to contain over 10^5 organisms / mL. Infected urine usually contains a single predominant bacterial species. Contaminated urine usually has $<10^4$ organisms / mL and often contains more than one bacterial species (Fig. 21.5). Distinguishing infection from contamination when counts are 10^4–10^5 organisms / mL can be difficult. Careful

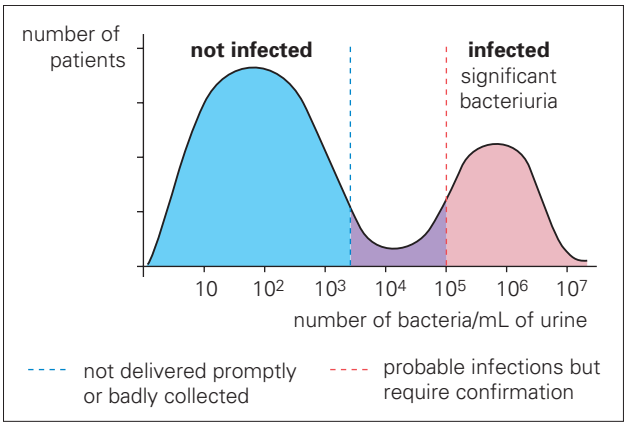


Figure 21.5 Significant bacteriuria. Voided specimens of urine are rarely sterile because the urine is contaminated with organisms from the periurethral area during collection. Even well-collected specimens from healthy individuals may contain up to 10^3 bacteria / mL of urine. A count of 10^5 bacteria / mL is considered a reliable indicator of infection. However, there are various reasons why lower counts may sometimes be significant (e.g. acute dysuria, ureteral obstruction, etc.).

collection and rapid transport of urine specimens to the laboratory are essential (see below).

It is important to recognize that the criteria for 'significant bacteriuria' do not apply to urine specimens collected from catheters or nephrostomy tubes or by suprapubic aspiration directly from the bladder, in which any number of organisms may be significant because the specimen is not contaminated by periurethral flora. In addition, infection of sites in the urinary tract below the bladder, and by organisms that are not members of the normal faecal flora, may not lead to the presence of significant numbers in the urine.

The usual urine specimen for microbiological examination is an MSU sample

An MSU sample should be collected into a sterile wide-mouthed container after careful cleansing of the labia or glans with soap (not antiseptic) and water, and after allowing the first part of the urine stream to be voided, as this helps to wash out contaminants in the lower urethra. After suitable instruction, the majority of adult patients can collect satisfactory samples with minimum supervision, though collection may be difficult for elderly and bedridden patients and consideration should be given to these difficulties when interpreting results.

Collection of MSU samples from babies and young children is difficult. 'Bag urine' may be collected by sticking a plastic bag to the perineum in girls or to the penis in boys, but such specimens are frequently heavily contaminated with faecal organisms. These problems can be overcome by suprapubic aspiration of urine directly from the bladder.

Urine specimens should be transported to the laboratory with minimum delay because urine is a good growth medium for many bacteria and multiplication of organisms in the specimen between collection and culture will distort the results, giving much greater numbers than those present in the patient.

Ideally, samples should be collected before antimicrobial therapy is started. If the patient is receiving, or has received, therapy within the previous 48 h, this should be stated clearly on the request form.

For patients with a catheter, a catheter specimen of urine is used for microbiological examination

Patients should not be catheterized simply to obtain a urine sample. Urine is obtained from patients who have a catheter in situ by withdrawing a sample with a syringe and needle from the catheter tube.

Special urine samples are required to detect *M. tuberculosis* and *Schistosoma haematobium*

These include:

- three early-morning urine samples on consecutive days for *M. tuberculosis*; these do not require the same precautions during collection as an MSU sample, because the culture technique prohibits the growth of organisms other than mycobacteria. Molecular tests are also helpful for *M. tuberculosis* diagnosis.
- the last few millilitres of a urine sample collected early afternoon after exercise for detection of *S. haematobium*.

Laboratory investigations

Urine specimens should be examined macroscopically and microscopically and should be cultured by quantitative or semiquantitative methods (see Ch. 32).

Microscopic examination of urine allows a rapid preliminary report

Bacteria may be seen on microscopy when present in the specimen in large numbers. However, they are not necessarily indicative of infection, but may indicate that the specimen has been poorly collected or left at room temperature for a prolonged period of time.

The presence of red and white blood cells, although abnormal, is not necessarily indicative of UTI. Haematuria may be present in association with:

- infection of the urinary tract and elsewhere (e.g. bacterial endocarditis)
- renal trauma
- calculi
- urinary tract carcinomas
- clotting disorders
- thrombocytopenia.

Occasionally, red blood cells may contaminate urine specimens of menstruating women.

White blood cells are present in the urine in very small numbers (e.g. <10 / mL) in health; a count of over 10 / mL is considered abnormal, but is not always associated with bacteriuria. Sterile pyuria is an important finding and may reflect:

- concurrent antibiotic therapy
- other diseases such as neoplasms or urinary calculi
- infection with organisms not detected by routine urine culture methods.

Renal tubular cells, seen in the urine of aspirin misusers, may be confused with white blood cells. Urinary casts are also indicative of renal tubular damage.

A laboratory diagnosis of significant bacteriuria requires quantification of the bacteria

Conventional culture methods produce results within 18–24 h, but rapid methods (e.g. based on bioluminescence, turbidimetry, leukocyte esterase / nitrate reductase test, etc.) are also available. In some laboratories, direct antibiotic susceptibility tests may be initiated upon detection of abnormal numbers of white blood cells or bacteria on microscopy, so that both culture and susceptibility results are available within 24 h.

Interpretation of the significance of bacterial culture results depends upon a variety of factors

These factors relate to:

- *collection* – specimen collection must be carried out properly
- *storage* – the urine must be cultured within 1 h of collection or held at 4°C for not more than 18 h before culture
- *antibiotic treatment* – in a patient receiving antibiotics, smaller numbers of organisms may be significant and may represent an emerging resistant population; laboratory methods are available to detect antibacterial substances
- *fluid intake* – the patient may be taking more or less fluid than usual, and this will clearly influence the quantitative result

- *the specimen* – the quantitative guidelines are valid for MSU specimens; they do not apply to catheter specimens, suprapubic aspirates or nephrostomy samples.

TREATMENT

Depending on clinical evaluation of the patient and local antimicrobial resistance trends, uncomplicated UTI is typically treated with an oral antibacterial for 3 days

Uncomplicated UTI (cystitis) generally resolves spontaneously within 4 weeks in up to 40% of patients; however, treatment with antibacterial agents reduces symptoms and ensures bacterial eradication. Administration of oral antimicrobial chemotherapy depends upon the drug and clinical evaluation of the patient. Examples of commonly prescribed agents are shown in Table 21.1. The choice of agent should be based on the results of susceptibility tests. However, for uncomplicated UTIs in patients in the community, therapy is often 'best guess', at least until laboratory results are available. This requires knowledge of the likely pathogens and their antibiotic susceptibility patterns in the locality. Follow-up cultures should be carried out after treatment has been completed (at least 2 days later) to confirm eradication of the infecting organism. In addition to antibacterial therapy, the patient should be advised to drink large volumes of fluid to help the normal flushing process.

Children and pregnant women with asymptomatic bacteriuria should be treated with antibacterials and followed up to check for eradication of the infection. Instrumentation of the urinary tract should be delayed in patients with significant bacteriuria until appropriate treatment has eliminated the infection.

Initial treatment of complicated UTI (pyelonephritis) usually involves a systemic antibacterial agent

The organism should be known to be susceptible to the antibacterial, and systemic treatment should continue until the signs and symptoms subside. It can then be replaced by oral therapy. The usual length of treatment is at least 10 days, but longer treatment may be necessary to clear the infection.

Hospital-acquired infections or recurrent infections, particularly in catheterized patients, may be caused by antibiotic-resistant organisms, and the agent of choice will depend upon the antibacterial susceptibility pattern. If possible, the catheter should be removed, as eradication of infection is extremely difficult to achieve in catheterized patients, and some would advocate treatment only when the patient complains of symptoms or before invasive procedures. Guidelines for catheter care and for the prevention of catheter-associated UTIs are shown in Box 21.1.

Infections acquired by haematogenous spread require specific antibacterial therapy, as described in Chapter 34 for tuberculosis, Chapter 23 for *S. typhi*, Chapter 27 for *Staph. aureus* and Chapter 28 for schistosomiasis.

Box 21.1 Guidelines for Catheter Care

- Avoid catheterization whenever possible.
- Keep duration of catheterization to a minimum.
- Use intermittent rather than continuous catheterization when feasible.
- Insert catheters with good aseptic technique.
- Use a closed sterile drainage system.
- Maintain a gravity drain.
- Use topical antiseptics around the meatus in women.
- Wash hands before and after inserting catheters and collecting specimens, and after emptying drainage bags.
- Catheters that drain into open collecting vessels are highly conducive to infection. Thus, closed drainage systems are now used in most hospitals but, even then, bacteriuria occurs in a significant number of patients.

Table 21.1 Examples of oral antibacterials for urinary tract infections (UTIs)

Common oral antibacterials for UTIs		
Antibacterial	**Class of agent**	**Comments**
Trimethoprim	Antimetabolite / nucleic acid synthesis inhibitor	Incidence of resistant strains increasing
TMP-SMX	Combination of trimethoprim with sulphamethoxazole (also antimetabolite nucleic acid synthesis inhibitor)	One of the most common 'first-line' therapeutic approaches; may be useful in 'blind' treatment but more toxic than trimethoprim alone; resistance is also an issue
Nitrofurantoin	Urinary antiseptic	For uncomplicated UTI caused by *E. coli* and *Staphylococcus saprophyticus*; not active in alkaline pH (therefore not useful for *Proteus* infections)
Ciprofloxacin, levofloxacin, ofloxacin, etc.	Fluoroquinolone	Very broad spectrum; not highly active against enterococci; increasing resistance an issue

Several different classes of antibacterial are available in oral formulations and suitable for treatment of UTI. Nitrofurantoin is useful only for lower UTIs as it does not achieve adequate serum and tissue concentrations to treat upper UTIs.

PREVENTION

Many of the features of the pathogenesis of UTI and host predispositions are not clearly understood

Recurrent infections in otherwise healthy women can be prevented by regularly emptying the bladder. This washes bacteria out of the urinary tract and is particularly important following intercourse. The prophylactic use of antibiotics may also prevent recurrent infections, but in the presence of underlying abnormalities, there is a tendency to select antibiotic-resistant strains, which subsequently cause infections that are more difficult to treat.

Infection in catheterized patients is very common, but can be reduced by good catheter care procedures (see Box 21.1, also Ch. 37). Catheterization should be avoided if possible or kept to a minimum duration.

KEY FACTS

- UTIs are among the commonest bacterial infections, especially in women.
- Most UTIs are acute episodes without sequelae.
- UTIs are usually endogenously acquired, with colonizing bacteria ascending the urinary tract from the periurethral area. *E. coli* is the predominant pathogen; other Gram-negative rods are also responsible, especially in hospitalized patients. Viruses are not important causes of UTI.
- Structural or mechanical factors in the host, or catheterization, predispose to infection.
- Bacterial attributes such as adhesions and capsular polysaccharides may be important in the development of UTI. Specific toxins are not implicated, but haemolysins (cytotoxins) may be.
- Lower UTI usually presents with acute frequency and dysuria. Asymptomatic infection is common in pregnancy and in children. Infection is recurrent in a significant proportion of people.
- Pyelonephritis (upper UTI) has a more severe presentation than does lower UTI, with fever and loin pain; recurrent infection results in renal damage.
- Bacteriological confirmation of the diagnosis requires quantitative methods. Pyuria also implies infection.
- Short-course treatment with oral antibacterials is effective for lower UTI; pyelonephritis needs longer treatment, often commencing with systemically administered drugs.
- Hospital-acquired UTI is often caused by multiple-resistant Gram-negative bacteria, and treatment should be based on the results of antibiotic susceptibility tests.

22 Sexually transmitted infections

Introduction

Sexually transmitted infections usually cause diseases

In some instances, sexually transmitted infections (STIs) may not result in overt disease symptoms, such as in the early stages of human immunodeficiency virus (HIV) infection and asymptomatic gonorrhoea in females. This is particularly concerning as people with asymptomatic or unreported STIs are unlikely to receive treatment, thus facilitating further cycles of infection and spread. While STIs are of major medical importance throughout the world, HIV infection has had the greatest global impact, estimated to affect nearly 37 million people in 2015. In addition to HIV, new cases of other STIs occur globally with alarming frequency (hundreds of millions of new cases) each year.

STIs are difficult to control

This is typified by the situation in the UK where there were 472 038 new STD cases reported by genitourinary medicine clinics in 2014 / 2015. A similar situation exists in other countries, including the USA. The reasons for this increase include:

- increasing density and mobility of human populations
- the difficulty of engineering changes in human sexual behaviour
- the absence of vaccines for almost all STIs, except for the human papillomavirus (HPV) vaccine.

HIV infection, syphilis, gonorrhoea, chlamydia and genital wart infections are some of the STIs included in national and global surveillance programmes.

The most common STIs are listed in Table 22.1. Table 22.2 gives examples of the strategies used by the pathogens to overcome host defences.

STIS AND SEXUAL BEHAVIOUR

The general principles of entry, exit and transmission of the pathogens that cause STIs are set out in Chapter 14.

The spread of STIs is inextricably linked with sexual behaviour

There are therefore many more opportunities for controlling STIs than, for instance, respiratory infections. Infected but asymptomatic individuals play an important role, and important determinants are promiscuity and sexual practices involving contact between different orifices and mucosal surfaces (see Ch. 14). For example, transmission between heterosexuals or men who have sex with men (MSM) can take place following oral or anal intercourse. The gonococcus, for instance, causes pharyngitis and proctitis, although it infects stratified squamous epithelium less readily than columnar epithelium. As described more fully in Chapter 33, calculations regarding the number of infected secondary cases resulting from each primary STD case depends on a variety of behavioural factors since the number of sexual partners acquired by a given individual (i.e. the level of promiscuity) varies considerably. Those who have many sexual partners are both more likely to acquire and to transmit infection and play a key role in the persistence of such infections in the community of sexually active individuals. People with many sexual partners are therefore an obvious target for treatment and education about safer sex practices, such as condom use.

Various host factors influence the risk of acquiring an STI

It is not surprising that the type of sexual activity is important or that genital lesions or ulcers increase the risk of acquiring infections such as HIV. In addition, it is well reported that uncircumcised men have a higher risk of infection.

STIs do not necessarily occur singly, and the possibility of multiple infections must always be borne in mind. For instance, syphilis can accompany gonorrhoea, and there is evidence that genital herpes may be reactivated during an attack of gonorrhoea.

SYPHILIS

Syphilis is caused by the spirochete *Treponema pallidum*

Treponema pallidum is closely related to the treponemes that cause the non-venereal infections of pinta and yaws (Table 22.3; Fig. 22.1). *T. pallidum* has a worldwide distribution, and syphilis remains a serious problem not only in resource-rich countries but also especially in resource-poor areas, due to the serious sequelae and the risk of congenital infection. In the USA during 2014–2015 the rate of syphilis in women increased by almost 30% and congenital syphilis increased by 6%, a trend that continues currently and has also been seen in the UK.

Table 22.1 The most common sexually transmitted infections (STIs)

Organism	Disease	Comment	Treatment
Papillomaviruses (types 6, 11, 16 and 18)	Genetic warts, dysplasias	Vaccines available; most common STI in the US	Podophyllin Imiquimod Cryotherapy Cidofovir gel
Chlamydia trachomatis	D-K serotypes (non-specific urethritis); L serotypes (lymphogranuloma venereum)	Most common easily cured STI in the US; urethritis very common; lymphogranuloma venereum primarily in resource-poor countries	Azithromycin, doxycycline
Candida albicans	Vaginal thrush	Predisposing factors	Clotrimazole, fluconazole
Trichomonas vaginalis	Vaginitis, urethritis	Often asymptomatic; causes ca. 50% of curable vaginal infections worldwide	Metronidazole
Herpes simplex virus types 1 and 2	Genital herpes	Problem of latency and reactivation	Aciclovir, valaciclovir, famciclovir
Neisseria gonorrhoeae	Gonorrhoea	2nd most most commonly reported notifiable disease in the in the US; quinolone resistance common	Cephalosporin (e.g. ceftriaxone)
HIV	AIDS	Worldwide problem	Antiretroviral drugs
Treponema pallidum	Syphilis	Incidence increasing in the US	Penicillin
Hepatitis B virus	Hepatitis B	Vaccine available	Antivirals include lamivudine, tenofovir, entecavir, adefovir, interferon alpha
Haemophilus ducreyi	Chancroid	Mainly tropical	Azithromycin, ceftriaxone
Sarcoptes scabiei	Genital scabies	Human mite burrows into upper skin layer	Permethrin cream
Phthirus pubis	Pubic lice	No. 1 louse infestation in US adults	Permethrin cream

Table 22.2 Strategies adopted by sexually transmitted microorganisms to combat host defences

Host defences	Microbial strategies	Examples
Integrity of mucosal surface	Specific attachment mechanism	Gonococcus or chlamydia to urethral epithelium
Urine flow (for urethral infection)	Specific attachment; induce own uptake and transport across urethral epithelial surface in phagocytic vacuole	Gonococcus
	Infection of urethral epithelial or subepithelial cells	Herpes simplex virus (HSV), chlamydia
Phagocytes (especially polymorphs)	Induce negligible inflammation	*Treponema pallidum*, mechanism unclear, perhaps poorly activates alternative complement pathway due to sialic acid coating
	Resist phagocytosis	Gonococcus (capsule), *T. pallidum* (absorbed fibronectin)
Complement	C3d receptor on pathogen binds C3b/d and reduces C3b/d-mediated polymorph phagocytosis	*Candida albicans*
Inflammation	Induce strong inflammatory response, yet evade consequences	Gonococcus, *C. albicans*, HSV, chlamydia
Antibodies (especially IgA)	Produce IgA protease	Gonococcus
Cell-mediated immune response (T cells, lymphokines, natural killer cells, etc.)	Antigenic variation; allows re-infection of a given individual with an antigenic variant	Gonococcus, chlamydia
	Poorly understood factors cause ineffective cell-mediated immune response	*T. pallidum*, HIV

Table 22.3 Spiral organisms of medical importance

Family	Genus	Species	Subspecies	Disease
Spirochaetaceae	*Treponema*	*pallidum*	*pallidum*	Syphilis
		pallidum	*pertenue*	Yaws
		carateum		Pinta
	Borrelia	*recurrentis*		Relapsing fever
		burgdorferi		Lyme disease
Leptospiraceae	*Leptospira*	*interrogans*	(serovar) Icterohaemorrhagiae	Leptospirosis (Weil's disease)

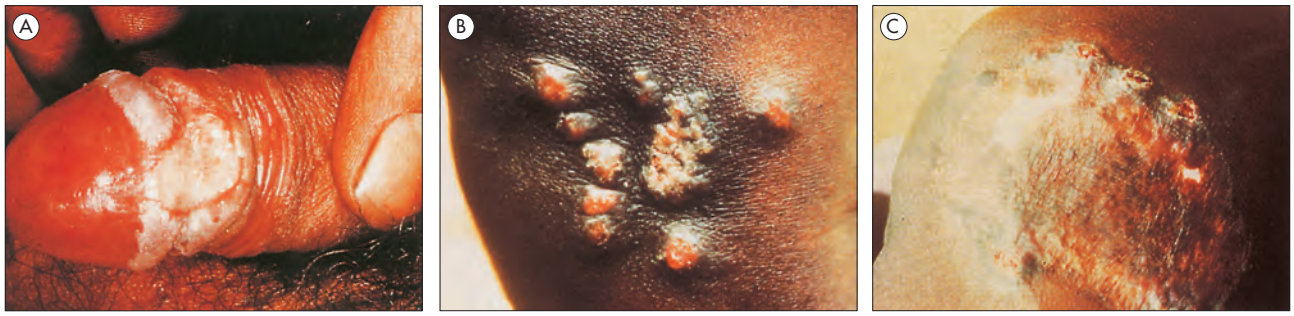


Figure 22.1 (A) Typical penile chancre of primary syphilis. (B) Yaws and (C) pinta are endemic in tropical and subtropical countries and are spread by direct contact. ([A] Courtesy of R.D. Catterall. [B] and [C] Courtesy of P.J. Cooper and G. Griffin.)

T. pallidum enters the body through minute abrasions on the skin or mucous membranes. Transmission of *T. pallidum* requires close personal contact because the organism does not survive well outside the body and is very sensitive to drying, heat and disinfectants. Horizontal spread (see Ch. 14) occurs through sexual contact, and vertical spread via transplacental infection of the fetus (see Ch. 24).

Local multiplication leads to plasma cell, polymorph and macrophage infiltration, with later endarteritis. The bacteria multiply very slowly, and the average incubation period is 3 weeks.

Classically, *T. pallidum* infection is divided into three stages

The three classical stages of syphilis are primary, secondary and tertiary syphilis (Table 22.4). However, not all patients go through all three stages; a substantial proportion remains permanently free of disease after suffering the primary or secondary stages of infection. The lesion of primary syphilis is illustrated in Fig. 22.1. The secondary stage may be followed by a latent period of some 3–30 years, after which the disease may recur – the tertiary stage. Unlike most bacterial pathogens, *T. pallidum* can survive in the body for many years despite a vigorous immune response. It has been suggested that the healthy treponeme evades recognition and elimination by the host by maintaining a cell surface rich in lipid. This layer is antigenically unreactive and the antigens are uncovered only in dead and dying organisms when the host is then able to respond. Tissue damage is mostly due to the host response.

Despite many years of effort, *T. pallidum* still cannot be cultivated in the laboratory in artificial media. It has therefore been difficult to study possible virulence factors at a molecular level until more recent advances in whole genome sequencing, which have allowed molecular characterization.

An infected woman can transmit *T. pallidum* to her baby in utero

Congenital syphilis is acquired after the first 3 months of pregnancy. The disease may manifest as:

- serious infection resulting in intrauterine death
- congenital abnormalities, which may be obvious at birth
- silent infection, which may not be apparent until about 2 years of age (facial and tooth deformities).

Laboratory diagnosis of syphilis

As *T. pallidum* cannot be grown in vitro, laboratory diagnosis hinges on microscopy and serology.

Microscopy

Exudate from the primary chancre should be examined by either:

- dark-field microscopy immediately after collection
- ultraviolet (UV) microscopy after staining with fluorescein-labelled antitreponemal antibodies.

The organisms have tightly wound, slender coils with pointed ends and are sluggishly motile in unstained preparations. *T. pallidum* is very thin (about 0.2 mm in diameter, compared with *E. coli*, which is about 1 mm) and cannot be seen in Gram-stained preparations. Silver impregnation stains can be used to demonstrate the organisms in biopsy material.

Serology

Serological tests for syphilis are the mainstay of diagnosis. They are divided into non-specific and specific tests for the detection of antibodies in patients' serum.

Non-specific tests (non-treponemal tests) for syphilis are the VDRL and RPR tests

The term non-specific is used because the antigens are not treponemal in origin, but are from extracts of normal

Table 22.4 The pathogenesis of syphilis

Stage of disease	Signs and symptoms	Pathogenesis
Initial contact		Multiplication of treponemas at site of infection; associated host response
↓ 2–10 weeks (depends on inoculum size)	Primary chancre[a] at site of infection	
↓ Primary syphilis	Enlarged inguinal nodes, spontaneous healing	Proliferation of treponemas in regional lymph nodes
↓ 1–3 months		
↓ Secondary syphilis		
↓ 2–6 weeks	Flu-like illness; myalgia, headache, fever; mucocutaneous rash[a]; spontaneous resolution	Multiplication and production of lesion in lymph nodes, liver, joints, muscles, skin and mucous membranes
↓ Latent syphilis		Treponemas dormant in liver or spleen
↓ 3–30 years		Re-awakening and multiplication of treponemas
Tertiary syphilis	Neurosyphilis; "general paralysis of the insane", tabes dorsalis Cardiovascular syphilis; aortic lesions, heart failure Progressive destructive disease	Further dissemination and invasion and host response (cell-mediated hypersensitivity) Gummas in skin, bones, testis

A feature of *Treponema pallidum* infection is its chronic nature, which seems to involve a delicately balanced relationship between pathogen and host.
[a]Chancre: initially a papule; forms a painless ulcer; heals without treatment within 2 months. Live treponemas can be seen in dark-ground microscopy of fluid from lesions; patient highly infectious.

mammalian tissues. Cardiolipin, from beef heart, allows the detection of anti-lipid IgG and IgM formed in the patient in response to lipoidal material released from cells damaged by the infection, as well as to lipids in the surface of *T. pallidum*. Two tests in common use today are:

- the Venereal Disease Research Laboratory (VDRL) test
- the rapid plasma reagin (RPR) test.

Both are available in kit form.

Non-specific tests show up as positive within 4–6 weeks of infection (or 1–2 weeks after the primary chancre appears) and decline in positivity in tertiary syphilis or after effective antibiotic treatment of primary or secondary disease. Therefore, these tests are useful for screening. However, they are non-specific and may give positive results in conditions other than syphilis (biological false positives, Table 22.5). All positive results should therefore be confirmed by a specific test. However, treatment (e.g. especially during the primary and secondary stages) tends to result in seroreversion to these tests. Thus, with confirmed disease (see below), these tests can provide at least an indication of therapeutic efficacy.

Table 22.5 Serological tests for syphilis and conditions associated with false-positive results

Test	Conditions associated with false-positive results
Non-specific (non-treponemal) VDRL RPR	Viral infection, collagen vascular disease, acute febrile disease, post-immunization, pregnancy, leprosy, malaria, drug misuse
Specific (non-treponemal) FTA-ABS TP-PA TPHA	Diseases associated with increased or abnormal globulins, lupus erythematosus, Lyme disease, autoimmune disease, diabetes mellitus, alcoholic cirrhosis, viral infections, drug misuse, and pregnancy

FTA-ABS, fluorescent treponemal antibody absorption test; MHA-TP, microhaemagglutination assay for *T. pallidum*; RPR, rapid plasma reagin test; TPHA, *T. pallidum* haemagglutination test; TP-PA, *T. pallidum* particle agglutination test; VDRL, Venereal Disease Research Laboratory test.

Commonly used specific tests for syphilis include the treponemal antibody test, FTA-ABS test and the MHA-TP

These tests use recombinant proteins or treponemal antigens extracted from *T. pallidum*. Tests in common use include:

- the enzyme-linked immunosorbent assay (ELISA), which detects IgM and IgG
- the fluorescent treponemal antibody absorption (FTA-ABS, Fig. 22.2) test in which the patient's serum is first absorbed with non-pathogenic treponemes to remove cross-reacting antibodies before reaction with *T. pallidum* antigens

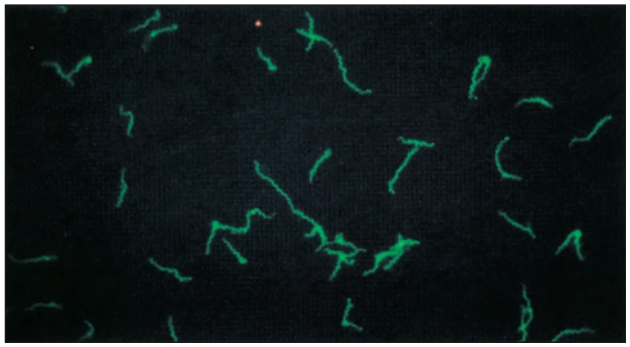


Figure 22.2 The fluorescent treponemal antibody absorption test for syphilis. Antibody in the patient's serum binds to bacteria and is visualized by a fluorescent dye.

- the microhaemagglutination assay for *T. pallidum* (MHA-TP).

These tests should be used to confirm that a positive result with a non-specific test is truly due to syphilis. Also, because they become positive earlier in the course of the disease, they can be used for confirmation when the clinical picture is strongly indicative of syphilis. They tend to remain positive for many years and may be the only positive test in patients with late syphilis. However, they remain positive after appropriate antibiotic treatment and cannot therefore be used as indicators of therapeutic response. They can also give false-positive reactions (see Table 22.5).

Confirmation of a diagnosis of syphilis depends upon several serological tests

Positive serological test results for babies born to infected mothers may represent passive transfer of maternal antibody or the baby's own response to infection. These two possibilities can be distinguished by testing for IgM and retesting at 6 months of age, by which time maternal antibody levels have waned. Antibody titres remain elevated in babies with congenital syphilis.

At present, several serological tests are needed to confirm a diagnosis of syphilis. None of these tests distinguishes syphilis from the non-sexually transmitted treponematoses, yaws and pinta. Western blot assays using whole *T. pallidum* cells as antigen are an important newer confirmatory test.

Treatment

Penicillin is the drug of choice for treating people with syphilis and their contacts

Penicillin is very active against *T. pallidum* (see Table 22.1). For patients who are allergic to penicillin, treatment with doxycycline should be given. Only penicillin therapy reliably treats the fetus when administered to a pregnant mother.

Prevention of secondary and tertiary disease depends upon early diagnosis and adequate treatment. Contact tracing with screening and treatment are also important. Several STIs may be present in one patient concurrently, and patients with other STIs should be screened for syphilis.

Congenital syphilis is completely preventable if women are screened serologically early in pregnancy (<3 months) and those who are positive are treated with penicillin.

GONORRHOEA

Gonorrhoea is caused by the Gram-negative coccus *Neisseria gonorrhoeae* (the 'gonococcus')

This bacterium is a human pathogen and does not cause natural infection in other animals. Therefore its reservoir is human and transmission is direct, usually through sexual contact, from person to person. The organism is sensitive to drying and does not survive well outside the human host, so intimate contact is required for transmission. It is thought that a woman has a 50% chance of becoming infected after a single sexual intercourse with an infected man, whereas a man has a 20% chance of acquiring infection from an infected woman.

Asymptomatically infected individuals (almost always women, see below) form the major reservoir of infection. Infection may also be transmitted vertically from an infected mother to her baby during childbirth. Infection in babies is usually manifest as ophthalmia neonatorum (see Ch. 24).

The gonococcus has special mechanisms to attach itself to mucosal cells

The usual site of entry of gonococci into the body is via the vagina or the urethral mucosa of the penis, but other sexual practices may result in the deposition of organisms in the throat or on the rectal mucosa. Special adhesive mechanisms (Fig. 22.3) prevent the bacteria from being washed away by urine or vaginal discharges. Following attachment, the gonococci rapidly multiply and spread through the cervix in women, and up the urethra in men. Spread is facilitated by various virulence factors (Fig. 22.3), although the organisms do not possess flagella and are non-motile. Production of an IgA protease helps to protect them from the host's secretory antibodies.

Host damage in gonorrhoea results from gonococcal-induced inflammatory responses

The gonococci invade non-ciliated epithelial cells, which internalize the bacteria and allow them to multiply within intracellular vacuoles, protected from phagocytes and antibodies. These vacuoles move down through the cell and fuse with the basement membrane, discharging their bacterial contents into the subepithelial connective tissues. *Neisseria gonorrhoeae* does not produce a recognized exotoxin. Damage to the host results from inflammatory responses elicited by the organism (e.g. lipopolysaccharide and other cell wall components; see Ch. 2). Persistent untreated infection can result in chronic inflammation and fibrosis.

Infection is usually localized, but in some cases bacteria isolates (e.g. resistant to the bactericidal action of serum, etc.) can invade the bloodstream and so spread to other parts of the body.

Gonorrhoea is initially asymptomatic in many women, but can later cause infertility

Symptoms develop within 2–7 days of infection and are characterized:

- in the male by urethral discharge (Fig. 22.4) and pain on passing urine (dysuria)
- in the female by vaginal discharge.

At least 50% of all infected women have only mild symptoms or are completely asymptomatic. They do not therefore seek

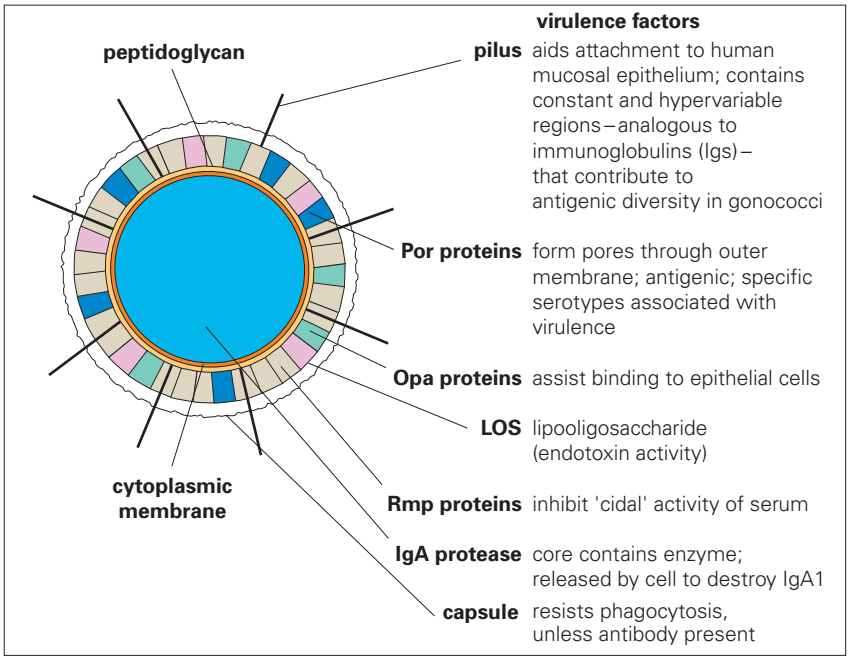


Figure 22.3 The spread of *Neisseria gonorrhoeae* is facilitated by various virulence factors. Changes in the surface structure of the gonococcus render the organism avirulent.

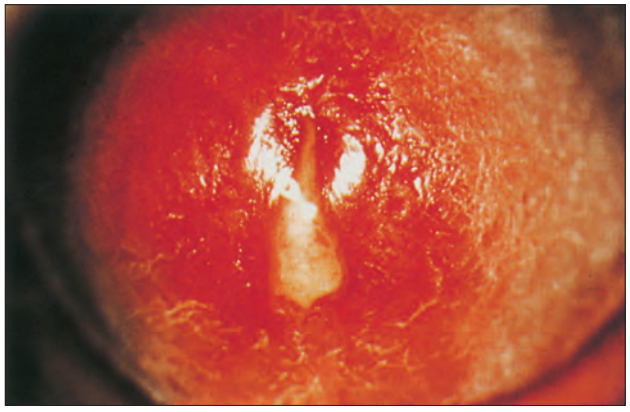


Figure 22.4 Gonococcal urethritis. Typical purulent meatal discharge with inflammation of the glans. (Courtesy of J. Clay.)

treatment and will continue to infect others. Asymptomatic infection, however, is not the usual course of events in men. Women may not be alerted to their infection unless or until complications arise, such as:

- pelvic inflammatory disease (PID)
- chronic pelvic pain
- infertility resulting from damage to the fallopian tubes.

Ophthalmia neonatorum is characterized by a sticky discharge (see Fig. 24.6).

Gonococcal infection of the throat may result in a sore throat (see Ch. 19), and infection of the rectum also results in a purulent discharge.

In men, local complications of urethral infection are rare (Fig. 22.5). Invasive gonococcal disease is much more common in infected women than in men, but prompt treatment is important in containing local infection. The common occurrence of asymptomatic infection in women is an important factor in the occurrence of complications (i.e. the infection is unrecognized and untreated). In 10–20% of untreated women, infection spreads up the genital tract to cause pelvic inflammatory disease (PID) and damage to the fallopian tubes.

Disseminated infection occurs in 1–3% of women, but is less common in men (see above and Fig. 22.6). It is a function not only of the strain of gonococcus (see above), but also host factors (e.g. about 5% of people with disseminated infection have deficiencies in the late-acting components of complement [C5–C8]).

A diagnosis of gonorrhoea is made from microscopy and culture of appropriate specimens

Urethral and vaginal discharges and other specimens where indicated are used for microscopy and culture. Although a purulent discharge is characteristic of local gonococcal infection, it is not possible to distinguish reliably between gonococcal discharge and that caused by other pathogens such as *Chlamydia trachomatis* on clinical examination.

With experience, the finding of Gram-negative intracellular diplococci in a smear of urethral discharge from a symptomatic male patient is a highly sensitive and specific test for the diagnosis of gonorrhoea.

Culture is essential in the investigation of infection in women and asymptomatic men, and for specimens taken from sites other than the urethra. Specimens from symptomatic men should also be cultured:

- to confirm the identity of the isolate; misinterpretation of microscopy or culture results can cause severe distress and may result in litigation
- to perform antibiotic susceptibility tests (see Ch. 34)
- to aid in the distinction between treatment failure and re-infection.

Because of the organism's sensitivity to drying, cultures should be made on warmed selective (i.e. modified Thayer Martin) and non-selective (chocolate blood agar) medium to ensure

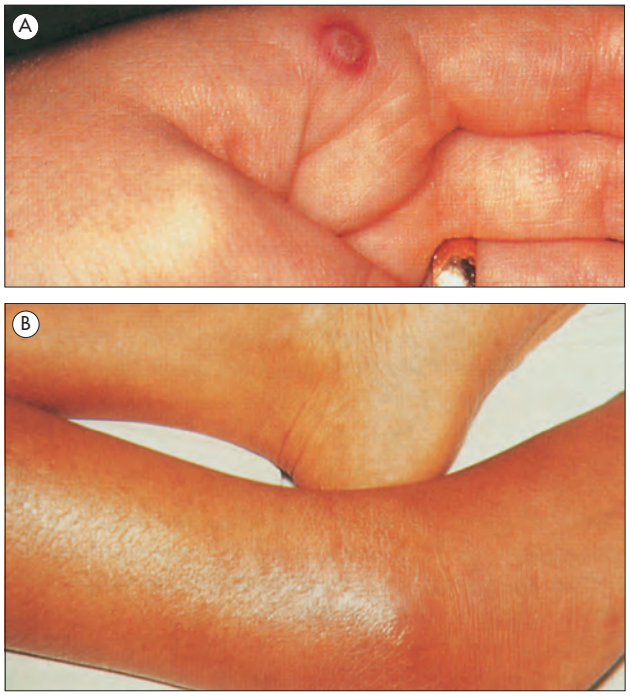


Figure 22.5 Local and systemic complications of gonococcal infection. (A) Skin lesions start as erythematous papules, which often become pustular and haemorrhagic with necrotic centres. (B) Septic arthritis of the ankle with marked erythema and swelling of the ankle and leg. ([A] Courtesy of J.S. Bingham. [B] Courtesy of T.F. Sellers, Jr.)

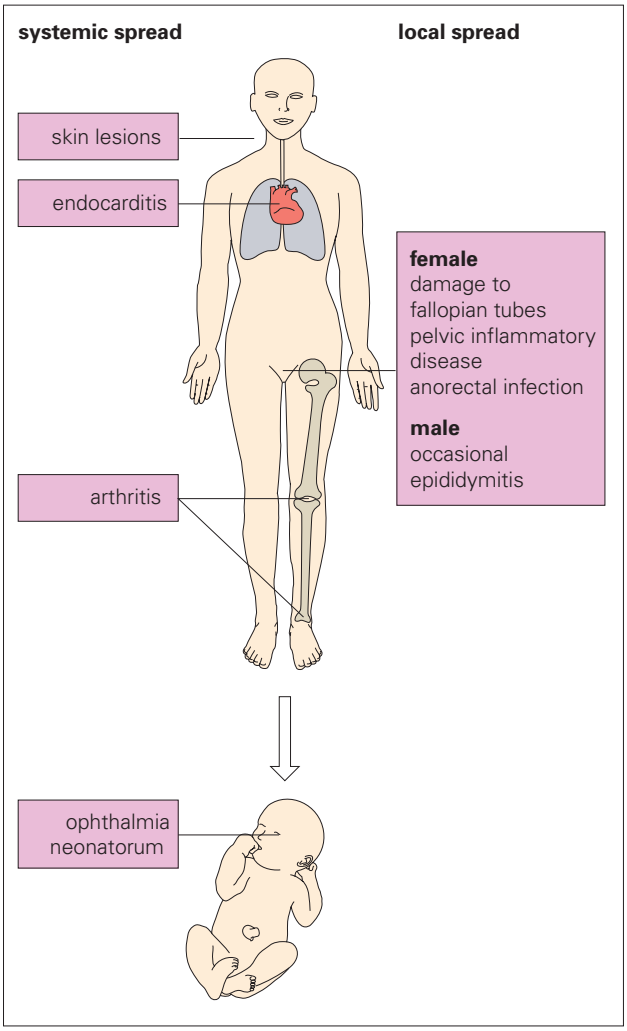


Figure 22.6 Local and systemic spread of gonococcal infection and complications.

recovery. Inoculation into appropriate transport medium is required if transfer to the laboratory will be delayed (no more than 48 h). Blood cultures should be collected if disseminated disease is suspected, and joint aspirates may yield positive cultures.

Serological tests are unsatisfactory. Commercial molecular approaches (specific probes, amplification, etc.) are now available, providing reliable results within a few hours.

Antibacterials used to treat gonorrhoea are cefixime or ceftriaxone

The antibacterial agents of choice are shown in Table 22.1. Penicillinase-producing *N. gonorrhoeae* were first observed in 1976 with increasing resistance that has severely compromised the effective treatment of gonorrhoea in many parts of the world, especially SE Asia. Resistance to fluoroquinolones has also occurred leading to recommendation of dual therapy (ceftriaxone and azithromycin), which has the added benefit of targeting chlamydia (see below), which may also be infecting the patient. Early treatment of a significant proportion of sexually promiscuous patients achieves a striking reduction in the duration of infectiousness and transmission rates. Prophylactic use of antibacterials has no effect in preventing sexually acquired gonorrhoea, but the application of antibacterial eye drops to babies born to mothers with gonorrhoea or suspected gonorrhoea is effective. Infection can be prevented by the use of condoms.

Follow-up of patients and contact tracing are vital to control the spread of gonorrhoea. At present, effective vaccines are not available, but the possibility of using some of the pilus proteins or other outer membrane components of the gonococcal cell as antigens has been under investigation. However, immunization may prevent symptomatic disease without preventing infection, and the dangers of asymptomatic infection have been discussed above.

Repeated infections can occur with strains of bacteria with different pilin proteins (e.g. antigenic variation; see Ch. 17).

CHLAMYDIAL INFECTION

C. trachomatis serotypes D–K cause sexually transmitted genital infections

The chlamydiae are very small bacteria that are obligate intracellular parasites. They have a more complicated life cycle than free-living bacteria because they can exist in different forms:

- The elementary body (EB) is adapted for extracellular survival and for initiation of infection.
- The reticulate body (RB) is adapted for intracellular multiplication (Fig. 22.7).

Three species of *Chlamydia* are recognized: *C. trachomatis*, *C. psittaci* and *C. pneumonia* (Table 22.6). *C. psittaci* and

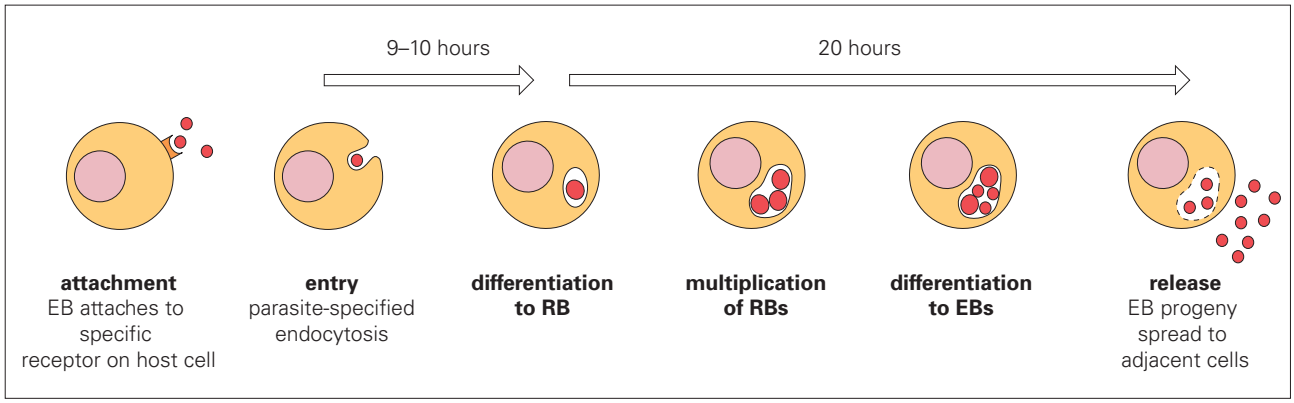


Figure 22.7 The life cycle of *Chlamydia*. EB, elementary body; RB, reticulate body.

Table 22.6 Medically important species of Chlamydiaceae

Species	Serotype	Natural host	Disease in humans
Chlamydia trachomatis	A, B, C	Humans	Trachoma
	D–K	Humans	Cervicitis, urethritis, proctitis, conjunctivitis, pneumonia (in neonates)
	L1, L2, L3	Humans	Lymphogranuloma venereum
C. psittaci	Primarily A	Birds and non-human mammals	Pneumonia
C. pneumoniae	?	Humans	Acute respiratory disease

C. trachomatis is the species associated with sexually transmitted disease.

C. pneumoniae infect the respiratory tract (see Ch. 20). The species *C. trachomatis* can be subdivided into different serotypes (also known as serovars) and these have been shown to be linked characteristically with different infections:

- Serotypes A, B and C are the causes of the serious eye infection trachoma (see Ch. 26).
- Serotypes D–K are the cause of genital infection and associated ocular and respiratory infections (Table 22.7).
- Serotypes L1, L2 and L3 cause the systemic disease lymphogranuloma venereum (LGV) (see below).

C. trachomatis serotypes D–K have a worldwide distribution, whereas the distribution of LGV serotypes is more restricted.

Most infections are genital and are acquired during sexual intercourse. Asymptomatic infection is common, especially in women. Ocular infections in adults are probably acquired by autoinoculation from infected genitalia or by ocular–genital contact. Ocular infections in neonates are acquired during passage through an infected maternal birth canal, and the infant is also at risk of developing *C. trachomatis* pneumonia (see Ch. 20).

Chlamydiae enter the host through minute abrasions in the mucosal surface

They bind to specific receptors on the host cells and enter the cells by 'parasite-induced' endocytosis (see Ch. 14). Once inside the cell, fusion of the chlamydia-containing vesicle with lysosomes is inhibited by an incompletely understood mechanism and the EB begins its developmental cycle (see Fig. 22.7). Within 9–10 h of cell invasion, the EBs differentiate into metabolically active RBs, which divide by binary fission and produce fresh EB progeny. These are then released into the extracellular environment within a further 20 h.

Table 22.7 Clinical syndromes and complications caused by *C. trachomatis,* serotypes D–K

Infection in	Clinical syndromes	Complications
Men	Urethritis, epididymitis, proctitis, conjunctivitis	Systemic spread, Reiter's syndrome[a]
Women	Urethritis, cervicitis, bartholinitis, salpingitis, conjunctivitis	Ectopic pregnancy, infertility, systemic spread: perihepatitis arthritis dermatitis
Neonates	Conjunctivitis	Interstitial pneumonitis

[a]Urethritis, conjunctivitis, polyarthritis, mucocutaneous lesions.

The clinical effects of *C. trachomatis* infection appear to result from cell destruction and the host's inflammatory response

The released EBs invade adjacent cells or cells distant from the site of infection if carried in lymph or blood.

Growth of *C. trachomatis* serotypes D–K seems to be restricted to columnar and transitional epithelial cells, but serotypes L1, L2 and L3 cause systemic disease (LGV). The site of infection determines the nature of clinical disease (see Table 22.7). Genital tract infection with serotypes D–K is

locally asymptomatic in most women, but usually symptomatic in men.

C. trachomatis can be detected directly on microscopy using the direct fluorescent antibody test

C. trachomatis can be detected directly in smears of clinical specimens made on microscope slides stained with fluorescein conjugated monoclonal antibodies and viewed by UV microscopy – the direct fluorescent antibody (DFA) test. The EBs stain as bright yellow-green dots (Fig. 22.8). Results can be obtained within a few hours but this method is not sensitive enough for asymptomatic infections.

A variety of nucleic-acid-based tests are commercially available for chlamydial detection

Chlamydial urethritis and cervicitis cannot be reliably distinguished from other causes of these conditions on clinical grounds alone. Traditional methods of detection (e.g. cell culture and direct antigen detection) have been largely replaced by rapid molecular tests.

Nucleic acid probe and amplification-based tests are capable of directly detecting *C. trachomatis* in specimens from infected individuals (e.g. cervix, urethra, urine, etc.). As mentioned previously, these commercially available kits can provide rapid (2–4 h) and specific detection of both *N. gonorrhoeae* and *Chlamydia* DNA, which is important as patients are often co-infected with both organisms.

Chlamydial infection is treated or prevented with doxycycline or azithromycin

It is important to remember that chlamydiae are not susceptible to the beta-lactam antibiotics, which are important for the treatment of gonorrhoea and syphilis. It is recommended that patients receiving treatment for gonorrhoea also be treated with azithromycin for possible concurrent chlamydial infection (see Table 22.1). In addition, patients with clinically diagnosed chlamydial genital infections, their sexual contacts and babies born to infected mothers should be treated. Erythromycin should be used for babies.

Prevention depends upon recognizing the importance of asymptomatic infections. Early diagnosis and treatment of cases and of their sexual partners is important in order to avoid complications and reduce opportunities for transmission.

Remember that STIs are not mutually exclusive, and patients may have concurrent infections with quite different pathogens.

OTHER CAUSES OF INGUINAL LYMPHADENOPATHY

Genital infections are common causes of inguinal lymphadenopathy (swelling of lymph nodes in the groin) among sexually active people. Syphilis and gonorrhoea have been discussed above. Lymphogranuloma venereum, chancroid and donovanosis are more common in tropical and subtropical countries than in Europe and the USA but may be imported by travellers who have acquired the disease through sexual contact in these areas.

Lymphogranuloma venereum

Lymphogranuloma venereum is caused by *C. trachomatis* serotypes L1, L2 and L3

Lymphogranuloma venereum (LGV) is a serious disease especially common in Africa, Asia and South America. It occurs sporadically in Europe, Australia and North America, particularly among men who have sex with men. The prevalence appears to be higher among males than females, probably because symptomatic infection is more common in men.

Lymphogranuloma venereum is a systemic infection involving lymphoid tissue and is treated with doxycycline or erythromycin

The clinical picture can be contrasted with the more restricted infection seen with *C. trachomatis* serotypes (see above). The primary lesion is an ulcerating papule at the site of inoculation (after an incubation period of 1–4 weeks) and may be accompanied by fever, headache and myalgia. The lesion heals rapidly, but the chlamydiae proceed to infect the draining lymph nodes, causing characteristic inguinal buboes (Fig. 22.9), which gradually enlarge. Chlamydiae may disseminate from the lymph nodes via the lymphatics to the tissues of the rectum to cause proctitis. Other systemic complications include fever, hepatitis, pneumonitis and meningoencephalitis. The infection may resolve untreated, but:

- Abscesses may form in lymph nodes, which suppurate and discharge through the skin.

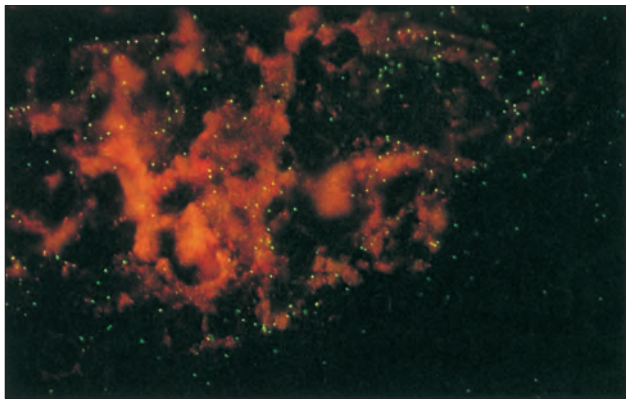


Figure 22.8 Direct fluorescent antibody test for *Chlamydia trachomatis*. Elementary bodies can be seen as bright yellow-green dots under the ultraviolet microscope. (Courtesy of J.D. Treharne.)

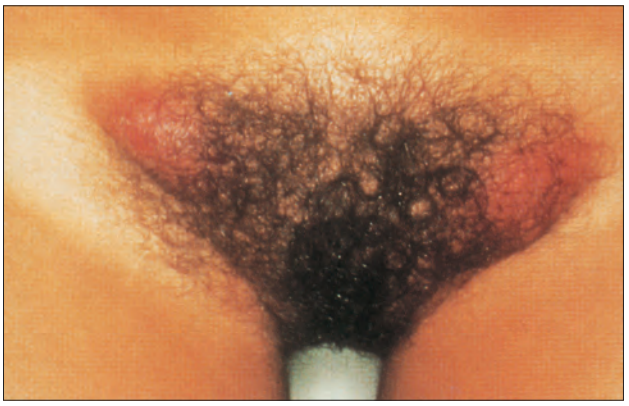


Figure 22.9 Lymphogranuloma venereum. Bilateral enlargement of inguinal glands. (Courtesy of J.S. Bingham.)

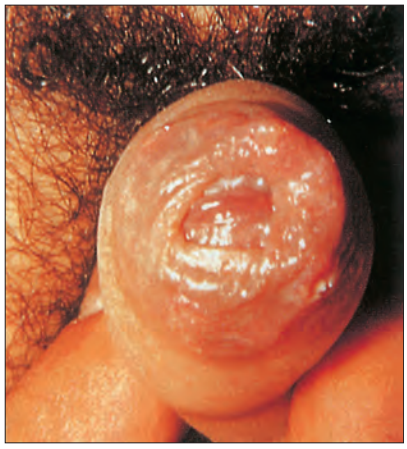


Figure 22.10 Chancroid. Several irregular ulcers on the prepuce. (Courtesy of L. Parish.)

- Chronic granulomatous reactions in lymphatics and neighbouring tissues can eventually give rise to fistula in ano or genital elephantiasis.

Cell culture methods, immunofluorescence, or nucleic acid-based tests are used for diagnosis. Treatment with doxycycline or erythromycin (see Table 22.1) is recommended. Pregnant women and children under 9 years of age should be treated with erythromycin.

Chancroid (soft chancre)

Chancroid is caused by *Haemophilus ducreyi* and is characterized by painful genital ulcers

Infection by the Gram-negative bacterium *Haemophilus ducreyi* is manifest as painful non-indurated genital ulcers and local lymphadenitis (Fig. 22.10). Note the difference between this and the chancre of primary syphilis, which is painless, but the ulcers may be confused with those of genital herpes, though they are usually larger and have a more ragged appearance. While the disease is endemic in some areas of the USA, cases generally tend to occur in distinct outbreaks. However, in Africa and Asia chancroid it is a common cause of genital ulcers. Epidemiological information is important because the diagnosis is usually clinical as the organism is difficult to grow in the laboratory. Chancroid may also be confused with donovanosis (see below).

Chancroid is diagnosed by microscopy and culture and treated with azithromycin, ceftriaxone, erythromycin or ciprofloxacin

Gram-stained smears of aspirates from the ulcer margin or enlarged lymph node characteristically show large numbers of short Gram-negative rods and chains, often described as having a 'school of fish' appearance, within or outside polymorphs. Aspirates should be cultured on a rich medium (GC agar with 1–2% haemoglobin, 5% fetal bovine serum, 10% CVA and vancomycin, 3 μg / mL) at 33°C in 5–10% carbon dioxide. *H. ducreyi* will not tolerate higher temperatures. Growth is slow, and it may take 2–9 days for colonies to appear. Treatment with a macrolide (e.g. erythromycin or azithromycin) or ceftriaxone (see Table 22.1) is generally recommended.

Donovanosis

Donovanosis is caused by *Klebsiella granulomatis* and is characterized by genital nodules and ulcers

Donovanosis (granuloma inguinale or granuloma venereum) is rare in temperate climates, but common in tropical and subtropical regions such as the Caribbean, New Guinea, India and central Australia. The infection is characterized by nodules, almost always on the genitalia, which erode to form granulomatous ulcers that bleed readily on contact. The infection may extend and the ulcers may become secondarily infected. The pathogen is a Gram-negative rod, previously called *Calymmatobacterium granulomatis* but now known as *Klebsiella granulomatis* on the basis of genomic analysis. The bacteria invade and multiply within mononuclear cells and are liberated when the cells rupture.

Donovanosis is diagnosed by microscopy and treated with doxycycline

The diagnosis of donovanosis is made by examining a smear from the lesion stained with Wright's or Giemsa stain. 'Donovan bodies' appear as clusters of blue- or black-stained organisms in the cytoplasm of mononuclear cells. Treatment with doxycycline, azithromycin or co-trimoxazole is recommended.

MYCOPLASMAS AND NON-GONOCOCCAL URETHRITIS

Mycoplasma hominis, *M. genitalium* and *Ureaplasma urealyticum* may be causes of genital tract infection

Although *Mycoplasma pneumoniae* has a proven role in the causation of pneumonia (see Ch. 20), the role of *M. hominis*, *M. genitalium* and *Ureaplasma urealyticum* (which metabolizes urea; also called 'T strains') in STIs is less certain. These organisms frequently colonize the genital tracts of healthy sexually active men and women. They are less common in sexually inactive populations, which supports the view that they may be sexually transmitted. It is difficult to prove that they cause infection of the genital tract, but *M. genitalium* may cause non-gonococcal urethritis, *M. hominis* may cause PID, postabortal and postpartum fevers, and pyelonephritis. *U. urealyticum* has also been associated with non-gonococcal urethritis and prostatitis.

M. hominis, *M. genitalium*, and *U. urealyticum* are commonly treated with doxycycline or azithromycin (depending on susceptibility testing) which is also the treatment for chlamydial infections.

OTHER CAUSES OF VAGINITIS AND URETHRITIS

Candida infection

Candida albicans causes a range of genital tract diseases, which are treated with oral or topical antifungals

These vary from mild superficial, localized infections in an otherwise healthy individual to disseminated, often fatal infections in the immunocompromised. This yeast is a normal inhabitant of the female vagina, so whilst *Candida* can be transmitted sexually, the presence of vulvovaginal candidiasis does not necessarily imply sexual transmission. In some women and in circumstances which are not clearly

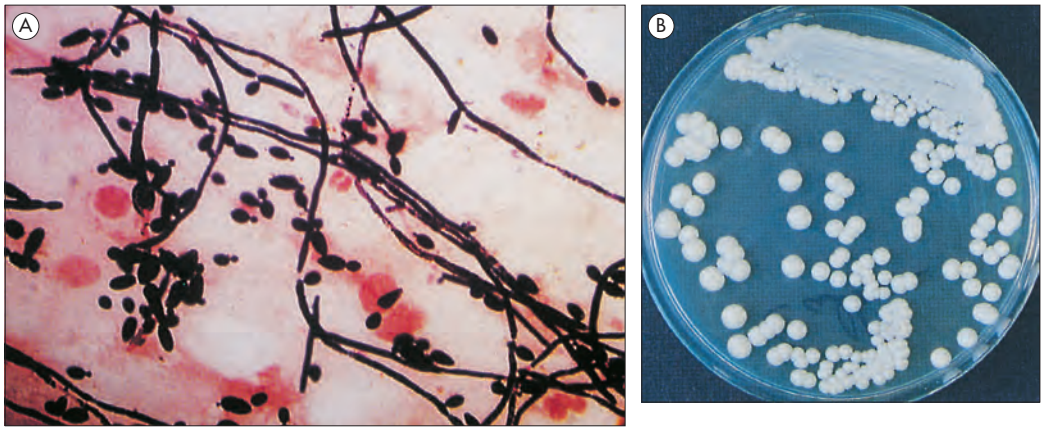


Figure 22.11 *Candida albicans*. (A) Light microscopic appearance and (B) culture of vaginal discharge.

understood, the candidal load increases and causes an intensely irritant vaginitis with a cheesy vaginal discharge. This may be accompanied by urethritis and dysuria and may present as a urinary tract infection (see Ch. 21). The diagnosis can be confirmed by microscopy and culture of the discharge (Fig. 22.11).

Treatment is with a topical antifungal such as clotrimazole or with an oral antifungal such as fluconazole. Troublesome recurrence occurs in a small proportion of women. Antibiotic therapy or diabetes mellitus predispose to recurrent infection.

Balanitis (inflammation of the glans penis) is seen in approximately 10% of male partners of females with vulvovaginal candidiasis, but urethritis is uncommon in men and is rarely symptomatic. factors for candidal balanitis include immunosuppression, diabetes mellitus and being uncircumcised.

Trichomonas infection

Trichomonas vaginalis is a protozoan parasite and causes vaginitis with copious discharge

Trichomonas vaginalis inhabits:

- the vagina in women
- the urethra (and sometimes the prostate) in men.

It is transmitted during sexual intercourse and is one of the most prevalent nonviral sexually transmitted infections in the United States. Incidence is higher in HIV-infected people and in HIV-infected women its presence is significantly associated with pelvic inflammatory disease. In pregnancy, *T. vaginalis* infection is associated with preterm delivery. In women, heavy infections cause vaginitis with a characteristic copious foul-smelling discharge, though the infection may be asymptomatic in some females. There is an associated increase in the vaginal pH. The infection should be distinguished from bacterial vaginosis (see below) by microscopic examination of the discharge, which shows actively motile trophozoites (Fig. 22.12). *Trichomonas* may be detected by wet preparation microscopy of vaginal secretions or cultured from a vaginal swab. Rapid point-of-care immunochromatographic tests are available. Highly sensitive nucleic acid detection tests (NAT) are much more sensitive than wet preparation microscopy and some centres use NAT to test microscopy-negative samples from suspected cases.

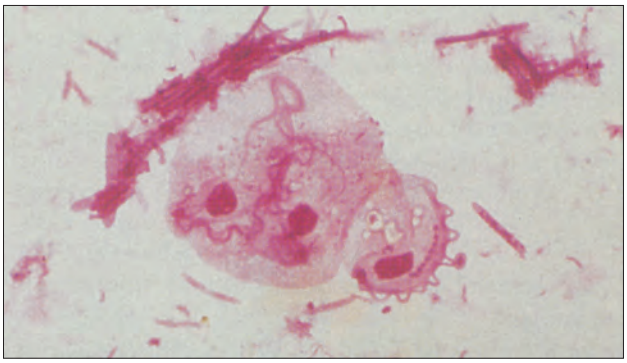


Figure 22.12 Motile trophozoites in vaginal discharge in *T. vaginalis* infection (Giemsa stain). (Courtesy of R. Muller.)

The nitroimidazoles, metronidazole or tinidazole are recommended treatment of *T. vaginalis* infections. Resistance to the nitroimidazoles is well documented so there is a clear need for orally active alternative compounds.

In men, *Trichomonas vaginalis* is frequently asymptomatic, but sometimes causes a mild urethritis. Sexual partners should be treated at the same time to prevent reinfection, reduce transmission and prevent new cases in the community.

Bacterial vaginosis

Bacterial vaginosis is associated with *Gardnerella vaginalis* plus anaerobic infection and a fishy-smelling vaginal discharge

This non-specific vaginitis is a syndrome in women characterized by at least three of the following signs and symptoms:

- excessive malodorous vaginal discharge
- vaginal pH >4.5
- presence of clue cells (vaginal epithelial cells coated with bacteria; Fig. 22.13)
- a fishy amine-like odour.

There is a significant increase in the numbers of *G. vaginalis* in the vaginal flora and a concomitant increase in the numbers of obligate anaerobes such as *Bacteroides*.

G. vaginalis is consistently found in association with vaginosis, but is also found in 20–40% of healthy women. It

is generally present in the urethra of male partners of women with vaginosis, indicating that it can be sexually transmitted. *G. vaginalis* has also been isolated from blood cultures from women with postpartum fever.

G. vaginalis has had a chequered taxonomic history, being first classified as a haemophilus, then as a corynebacterium, reflecting the fact that it tends to be Gram-variable (sometimes appearing Gram-negative, sometimes Gram-positive). It grows in the laboratory on human blood agar in a moist atmosphere enriched with carbon dioxide. The organism is treated with oral metronidazole. Species of the genus *Mobiluncus* appear to be related to *G. vaginalis* and have also been implicated in vaginosis.

The pathogenesis of bacterial vaginosis is still unclear, but appears to be related to factors that disrupt the normal acidity of the vagina and the equilibrium between the different constituents of the normal vaginal flora. Whether any of these or other unknown factors are sexually transmissible is unclear.

GENITAL HERPES

Herpes simplex virus (HSV)-2 is the most common cause of genital herpes, but HSV-1 is being detected more frequently

Herpes simplex virus (HSV) is a ubiquitous infection of humans worldwide. HSV-1 is generally transmitted via saliva, causing primary oropharyngeal infection in children, and cold sores occur after virus reactivation. However, HSV-2 emerged as a result of independent transmission by the venereal route. HSV-2 shows biological and antigenic differences from HSV-1 and can be distinguished by molecular typing methods as well as older techniques such as immunofluorescence. There is little cross-immunity. Although originally recovered from separate sites, orogenital sexual practices have obscured the topographic difference between the strains, so that HSV-1 and HSV-2 can be recovered from oral and genital sites. HSV-2 is one of the most common STIs and it has been estimated that there are over 500 million individuals with HSV-2 globally. In the USA, a Centers for Disease Control (CDC) survey from 2005 to 2008 reported that HSV-2 antibody was detected in 16% of the study population, greater among females. In addition, it is estimated there are nearly 800000 newly infected individuals in the USA annually. One of the worrying aspects surrounding HSV-2 is that most people do not know that they have this infection as up to 75% may not have symptoms and therefore will not realize that they may transmit this infection. Finally, HSV-2 infection can result in a twofold-increased risk of developing HIV infection. This is likely to be due to breaches in the mucosal barrier as a result of the HSV ulcers.

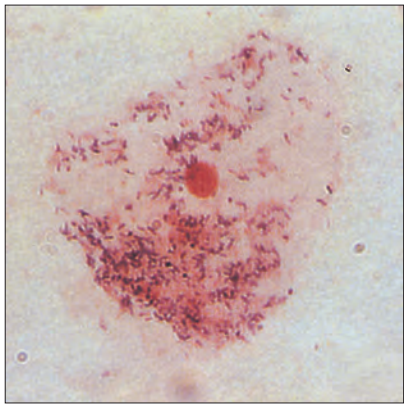


Figure 22.13 Clue cells in bacterial vaginosis.

Genital herpes is characterized by ulcerating vesicles that can take up to 2 weeks to heal

The primary genital lesion on the penis or vulva is seen 3–7 days after infection. It consists of vesicles that soon breakdown to form painful shallow ulcers (Fig. 22.14). Local lymph nodes are swollen, and there may be constitutional symptoms including fever, headache and malaise. Occasionally the lesions are on the urethra, causing dysuria or pain on micturition. Healing takes up to 2 weeks, but the virus in the lesion travels up sensory nerve endings to establish latent infection in dorsal root ganglion neurones (see Ch. 25). From this site it can reactivate, travel down nerves to the same area, and cause recurrent lesions ('genital cold sores').

Aseptic meningitis or encephalitis occurs in adults as a rare complication, and spread of infection from mother to infant at the time of delivery can give rise to neonatal disseminated herpes or encephalitis.

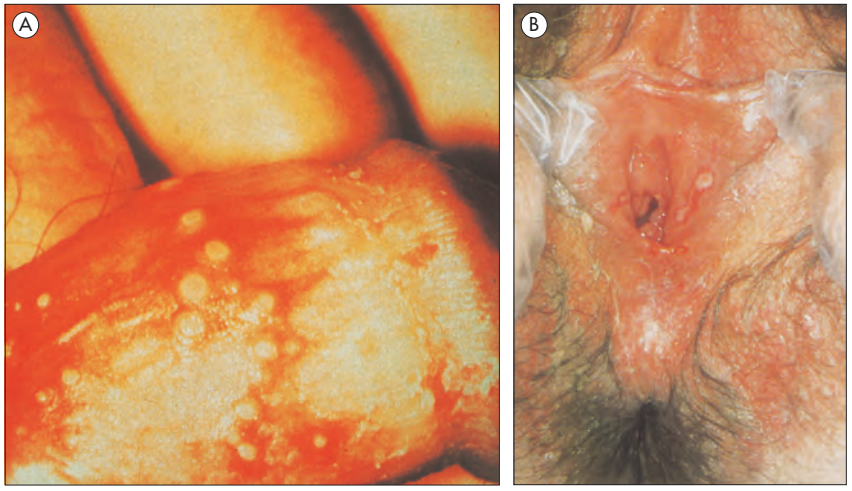


Figure 22.14 Genital herpes. Vesicles (A) on the penis and (B) in the perianal area and vulva. Those on the labia minora and fourchette have ruptured to reveal characteristic herpetic erosions. (Courtesy of J.S. Bingham.)

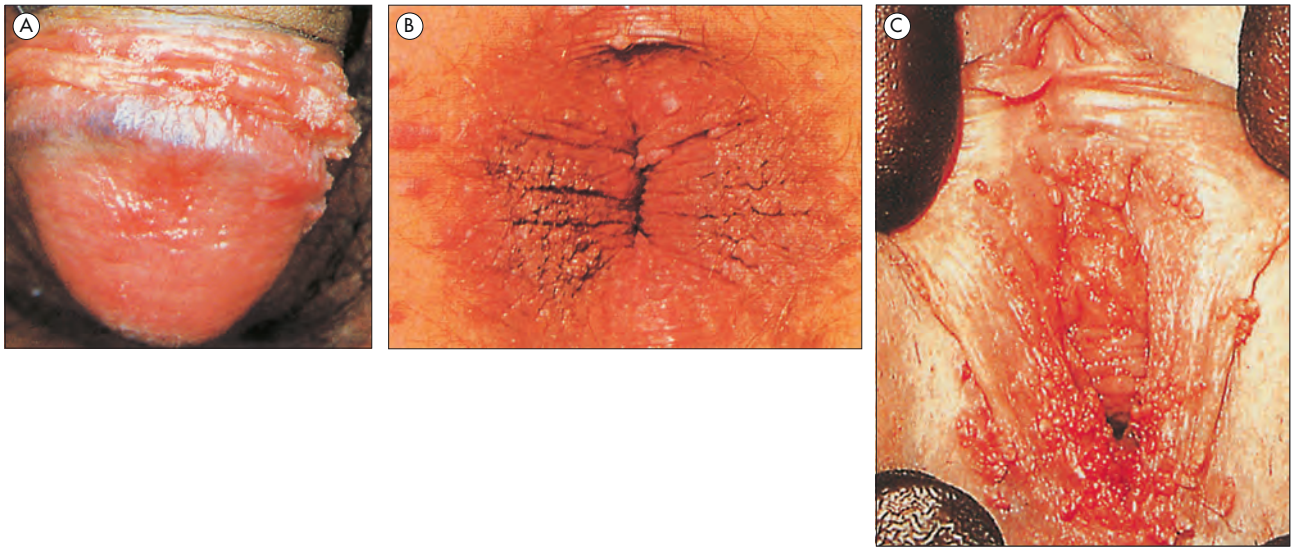


Figure 22.15 Genital warts. (A) Warts on the penis are usually multiple, and on the shaft are often flat and keratinized. (B) Warts in the perianal area often extend into the anal canal. (C) Warts in the vulvoperineal area can enlarge dramatically and extend into the vagina. (Courtesy of J.S. Bingham.)

Genital herpes is generally diagnosed from the clinical appearance and aciclovir can be used for treatment and prophylaxis

HSV DNA can be detected and typed in vesicle fluid or ulcer swabs. More classic techniques involved virus isolation and subsequently typing the isolate by immunofluorescence using type-specific monoclonal antibodies. Recurrent genital infection is more frequent with HSV-2; therefore typing is of help in determining the prognosis. The cytopathic effect is characteristic and is generally seen within 1–2 days post-inoculation, with ballooning degenerating cells and multinucleate giant cells. HSV DNA detection methods which include type differentiation may be used, and have a much greater sensitivity than virus isolation. A number of antivirals, including oral aciclovir, valaciclovir and famciclovir can be used for treatment of severe or early lesions and aciclovir may need to be given intravenously if there are systemic complications. Recurrent attacks are distressing and treatment options include starting an antiviral when prodromal symptoms occur or alternatively taking low-dose aciclover for 6 to 12 months, or one of the alternative agents, to stop or at least reduce the frequency of recurrences.

HUMAN PAPILLOMAVIRUS INFECTION

There are over 120 distinct types of human papillomaviruses, all infecting skin or mucosal surfaces, and the DNA of each showing less than 50% cross-hybridization with that of others. These are evidently ancient viral associates of humans that have evolved extensively, and many of the different types are adapted to specific regions of the body.

Many papillomavirus types are transmitted sexually and cause genital warts

Warts (condylomata acuminata) appear on the penis, vulva and perianal regions (Fig. 22.15) after an incubation period of 1–6 months (see Ch. 27). They may not regress for many months and can be treated with podophyllin. The lesion on the cervix is a flat area of dysplasia visible by colposcopy as a white plaque (Fig. 22.16) after the local application of 5% acetic acid. Because of their association with cervical cancer, especially types 16 and 18, cervical lesions are best removed by laser or loop excision.

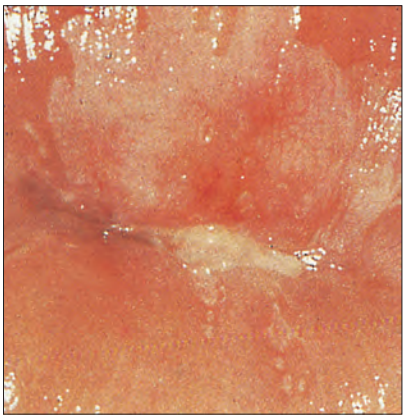


Figure 22.16 Cervical dysplasia caused by papillomavirus should be removed by laser. (Courtesy of A. Goodman.)

HUMAN IMMUNODEFICIENCY VIRUS

Human immunodeficiency virus (HIV) is a retrovirus (Table 22.8), so-called because this single-stranded RNA virus contains a *pol* gene that codes for a reverse transcriptase (Latin: *retro*, backwards).

Acquired immune deficiency syndrome (AIDS) was first recognized in 1981 in the USA

In 1981, the Communicable Disease Center, Atlanta, USA, noted an increase in requests to use pentamidine for *Pneumocystis carinii* (now classified as *P. jirovecii*) infection in previously well individuals who also suffered severe infections by other normally harmless microorganisms. These included *C. albicans* oesophagitis, mucocutaneous HSV, toxoplasma CNS infection or pneumonia, and cryptosporidial

Table 22.8 Human retroviruses

Virus	Comment
HTLV-1	Endemic in West Indies and SW Japan; transmission via blood, sexual intercourse, vertical transmission, human milk; can cause adult T-cell leukaemia, and HTLV1-associated myelopathy, also known as tropical spastic paraparesis
HTLV-2	Uncommon, sporadic occurrence; transmission via blood, sexual intercourse, vertical transmission, can cause hairy T-cell leukaemia and neurological disease
HIV-1, HIV-2	Transmission via blood, sexual intercourse; responsible for AIDS. HIV-2 West African in origin, closely related to HIV-1 but antigenically distinct
Human foamy virus	Causes foamy vacuolation in infected cells; little is known of its occurrence or pathogenic potential
Human placental virus(es)	Detected in placental tissue by electron microscopy and by presence of reverse transcriptase
Human genome viruses	Nucleic acid sequences representing endogenous retroviruses are common in the vertebrate genome, often in well-defined genetic loci; acquired during evolutionary history; not expressed as infectious virus; function unknown; perhaps should be regarded as mere parasitic DNA

Human T-cell lymphotropic virus (HTLV)-1, HTLV-2, HIV-1 and HIV-2 have been cultivated in human T cells in vitro. The human placental and genome viruses are not known as infectious agents. Retroviruses are also common in cats (FAIDS), monkeys (MAIDS), mice (mouse leukaemia) and other vertebrates. ARC, AIDS-related complex.

enteritis; Kaposi's sarcoma was also often present. Patients had evidence of impaired immune function, as shown by skin test anergies, and depletion of CD4-positive T-helper (Th) lymphocytes. This immunodeficiency syndrome appearing in an individual without a known cause such as treatment with immunosuppressive drugs was referred to as 'acquired immune deficiency syndrome' (AIDS). An internationally agreed definition of AIDS soon followed. Epidemics subsequently occurred in San Francisco, New York and other cities in the USA and in the UK and the rest of Europe a few years later.

Human immunodeficiency virus that causes AIDS, was isolated from blood lymphocytes in 1983

It was recognized as belonging to the lentivirus (slow virus) group of retroviruses and related to similar agents in monkeys and to visnavirus in sheep and goats. The structure of the viral particle and its genome are illustrated in Fig. 22.17 and its replication mechanism in Figs 22.18 and 22.19.

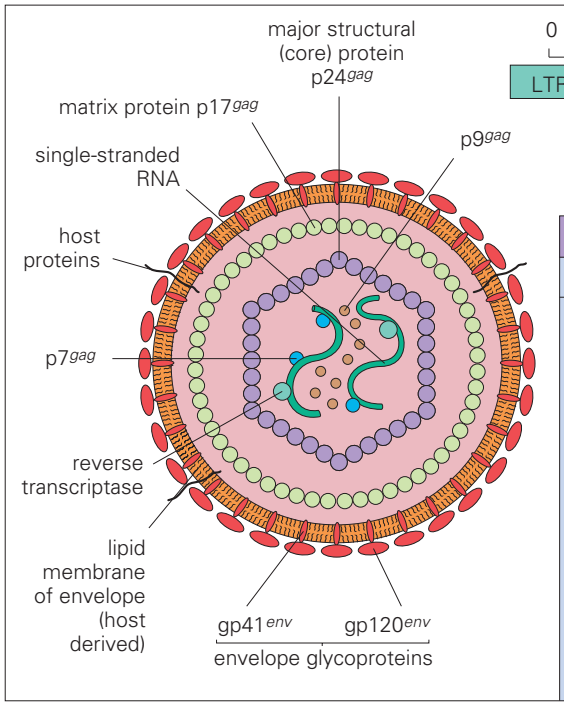
Three genes, *gag*, *pol* and *env*, encode the matrix, capsid and nucleocapsid structural proteins, reverse transcriptase, proteases and integrase enzymes and gp120 and gp41 envelope proteins, respectively. The regulatory and accessory proteins, Tat and Rev, Vif, Vpr, Vpu / x and Nef are coded by their respective genes. Overall, there are 16 proteins that are involved in a variety of pairwise interactions ensuring efficient viral replication at key parts of the HIV life cycle, such as virus entry, reverse transcription, virus integration, transcription and translation, virus assembly, budding and maturation (see Fig. 22.18).

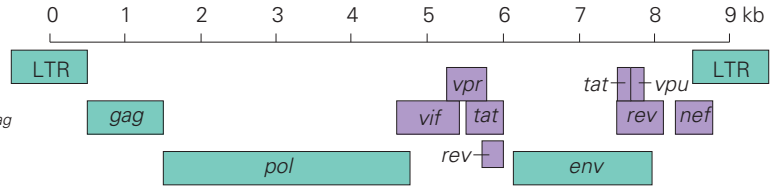
Human immunodeficiency virus infection started in Africa between 1910 and 1930

The molecular biological evidence based on nucleic acid sequencing studies has demonstrated that both HIV-1 and the closely related HIV-2 seen in West Africa arose from closely related primate viruses. HIV-1 is separated into four groups, namely M (major), N (new), O (outlier) and P, the latter was reported in 2009 after identifying an HIV strain closely related to a gorilla simian immunodeficiency virus (SIV) in a Cameroonian woman, the only person in whom it has been found. The M group comprises the HIV-1 subtypes A to K as well as circulating recombinant forms (CRF) which are due to recombination events between those subtypes, with the N and O groups focused in western central Africa. The geographical prevalence of the subtypes differs, with subtype B being most common in North America and Europe, and the non-B strains such as A and C being found more frequently in Africa. However, with increasing travel the subtype distribution is changing and, together with the potential for mixed or superinfections, i.e. an HIV-infected individual becoming infected with another strain, and viral recombination events, other subtypes are being seen such as the CRFs. The M–P groups resulted from independent cross-species human and ape contact in west-central Africa. Transmission events were most likely to have occurred through skin and mucous membrane exposure to infected ape blood and body fluids, probably when hunting. Group M was found first and is the pandemic form as it comprises the majority of HIV infections globally. This has been the subject of molecular clock analyses, a method used in evolutionary biology. The molecular clock hypothesis is that DNA and protein sequences evolve at a rate that is relatively constant over time. Due to this fact, the genetic difference between two species is proportional to the time since they shared a common ancestor.

On analyzing the HIV pandemic, the onset of the HIV-1 Group M infections was in the early 20th century. Having emerged between 1910 and 1930 in west-central Africa, HIV spread for the next 50 or so years having diversified around Kinshasa, formerly Leopoldville, the capital and largest city of the Democratic Republic of the Congo. Moreover, the rivers which serve as routes for travel and commerce would have been a link between the chimpanzee reservoir on the banks of the Congo river. In addition, the four groups cluster with a particular lineage of SIV cpz (cpz=chimpanzees), the original reservoir of human and gorilla infections. HIV-2 is mostly seen in West Africa and originated from sooty mangabeys.

HIV-1 may have been present in humans in central Africa for many years, but in the late 1970s it began to





regulatory proteins		virion proteins	
gene	**encodes**	**gene**	**encodes**
vif	virion infectivity factor	LTR	long terminal repeats, play essential role in replication (e.g. promote transcription)
vpr	weak transcriptional activator		
vpu	required for efficient budding	*gag*	group-specific antigen proteins (virion core proteins, e.g. p24, p17, p9, p7)
tat	transactivator protein regulates viral transcription		
rev	regulator virion proteins promote export of viral RNAs from nucleus	*pol*	(polymerase); protease, reverse transcriptase and integrase
		env	type-specific envelope glycoproteins; gp160 precursor cleaved by proteases to form gp120 attachment protein and gp41 fusion protein
nef	negative regulatory factor important for virulence		

Figure 22.17 The structure and genetic map of HIV. The *rev* and *tat* genes are divided into non-contiguous pieces and the gene segments spliced together in the RNA transcript. Occasional host proteins such as major histocompatibility complex (MHC) molecules are present in the envelope. (p) is protein and (gp) is glycoprotein. About 10^9 HIV-1 particles are produced each day at the peak of infection, and this, together with the low fidelity of reverse transcriptase, means that new virus variants are always appearing. Mutations are seen especially in *env* and *nef* genes. Any one patient contains many variants, and drug-resistant and immune-resistant mutants emerge.

spread rapidly (Fig. 22.20), possibly with changed biological properties, as a result of increased transmission following major socioeconomic upheavals and migrations of people from Central to East Africa. Female prostitutes and male soldiers and workers travelling around the country played a major part in transmission. The migration pathways of the different subtypes have been charted and subtype B, which predominates in Europe and the Americas, originated from one African strain that spread to Haiti in the 1960s and then to the USA and Europe.

In the late 1980s, HIV began to appear in Asian countries, beginning with Thailand, and by 1995 explosive spread was based on heterosexual transmission, with high infection rates in female sex workers and transmission among users of injected drugs in Asia.

Worldwide by 2015, about 37 million adults and children were infected with HIV including:

- 25.5 million in sub-Saharan Africa
- 5.1 million in Asia and the Pacific
- 1.5 million in Eastern Europe and Central Asia
- 2.4 million in North America, Western and Central Europe.

In 2009, nearly 3 million people were newly infected and 1.8 million died as a result of HIV infection that year. By 2015, these figures were 2.1 million newly infected and 1.1 million AIDS-related deaths.

In 2016, the UNAIDS global report stated that since 2014, 30% more people were receiving antiretroviral therapy (ART), which amounted to 17 million individuals. AIDS-related deaths had reduced by 43% since 2003. The global coverage of ART was 46% by the end of 2015, the largest gains were in east and southern Africa where the figure of 24% receiving ART in 2010 improved to 54% in 2015, just over 10 million people. These regions also had the largest reduction in new adult HIV infections, 40 000 fever in 2015 than 2010. The numbers fell more gradually or were static in most other areas, but a near 60% increase was seen in eastern Europe and central Asia.

Human immunodeficiency virus mainly infects cells bearing the CD4 glycoprotein on the cell surface and also requires chemokine co-receptors, CCR5 and CXCR4

The HIV transmission route for more than 80% of adults involves mucosal surfaces, in particular cervicovaginal, penile and rectal. The remainder may be infected by intravenous or percutaneous routes. The window period for detecting the virus is 7–21 days, as HIV multiplies in the mucosa and draining lymphoreticular tissues. The first targets are CD4 receptor-bearing cells that include Th cells, monocytes, Langerhans cells and other dendritic cells, macrophages and microglia (Figs 22.21, 22.22). The CD4 molecule acts as a high-affinity binding site for the viral gp120 envelope glycoprotein. This interacts with the gp41 transmembrane protein and leads to a conformational change that produces a fusion pore for viral entry. Productive replication and cell destruction does not occur until the Th cell is activated. Th cell activation is greatly enhanced not only in attempts to respond to HIV antigens, but also as a result of the secondary microbial infections seen in patients. Monocytes and macrophages, Langerhans cells and follicular dendritic cells also express the CD4 molecule and are infected, but are

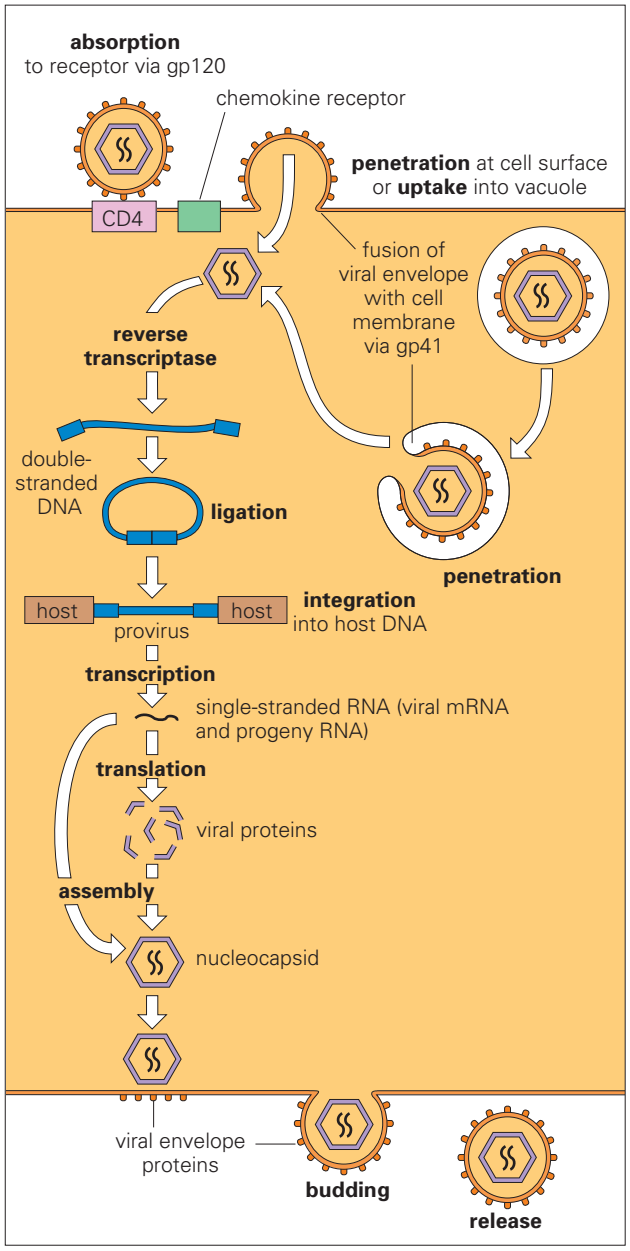


Figure 22.18 The HIV replication cycle. The virus enters the cell either by fusion with the cell membrane at the cell surface or via uptake into a vacuole and release within the cell.

not generally destroyed, potentially acting as a reservoir for infection. Langerhans cells, for example dendritic cells in the skin and genital mucosa, may be the first cells infected. Later in the disease, there is a remarkable disruption of histological pattern in lymphoid follicles as a result of the breakdown of follicular dendritic cells.

HIV-1 enters host cells by binding the viral gp120 to the CD4 receptor and a chemokine co-receptor on the host cell surface. The CCR5 beta-chemokine receptor is important in establishing the infection. Those people with CCR5 gene deletions are resistant to infection. On the other hand, disease progression has been associated with HIV variants using the CXCR4 alpha-chemokine receptor. Cell susceptibility to

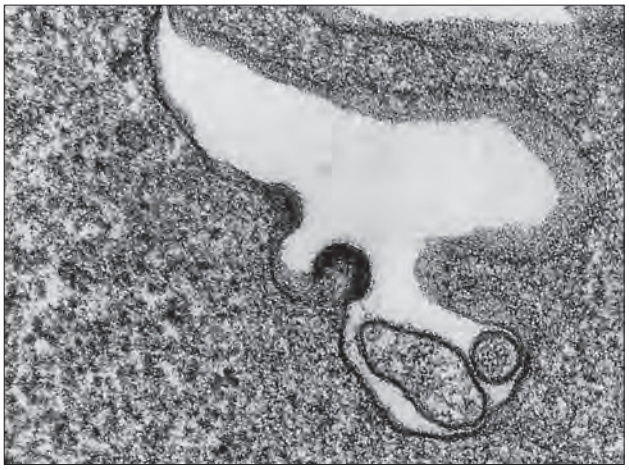


Figure 22.19 Electron micrograph showing HIV budding from the cell surface before release. (Courtesy of D. Hockley.)

infection is therefore affected by the levels of these chemokine co-receptors; for example, their expression may be up-regulated by opportunistic infections.

Productive infection of resting CD4 T cells in the lymphoreticular system of the gastrointestinal tract occurs. These cells express integrin receptors, viral attachment molecules, as well as Th cell surface markers, and HIV-1 infection rapidly expands with a rise in HIV-1 RNA levels at the same time as the irreversible depletion of reservoirs of Th cells as well as induction of an inflammatory cytokine and chemokine response. A latency state is soon established with the formation of persistent lymphoid tissue viral reservoirs.

At first the immune system fights back against HIV infection, but then begins to fail

During the first few months, virus-specific CD8-positive T cells are formed and reduce the viraemia, which is referred to as the HIV load. Innate and adaptive immune responses to HIV infection lead to this set-point of HIV replication (Fig. 22.22). This is followed by the appearance of neutralizing antibodies at around 3 months post-infection and viral escape mutants develop. Up to 10^{10} infectious virus particles and up to 10^{9} infected lymphocytes are produced daily. Then the immune system begins to suffer gradual damage, and the number of circulating CD4-positive T cells steadily falls and the HIV load rises. In addition to the loss of total CD4 T cells, T cell subsets change too including those involved in defence against bacteria. Nearly all infected CD4-positive T cells are in lymph nodes. The cell-mediated immune responses to viral antigens, as judged by lymphoproliferation, weaken, whereas responses to other antigens are normal. Perhaps the virus initially engineers a specific suppression of protective responses to itself. Eventually, the patient loses the battle to replace lost T cells, and the number falls more rapidly. Skin test delayed-type hypersensitivity (DTH) responses are absent, natural killer (NK) cell and cytotoxic T-cell (Tc) activity is reduced, and there are various other immunological abnormalities, including polyclonal activation of B cells. Functional changes in T lymphocytes – reduced responses to mitogens, reduced interleukin 2 (IL-2) and interferon gamma (IFNγ) production – are also seen. As

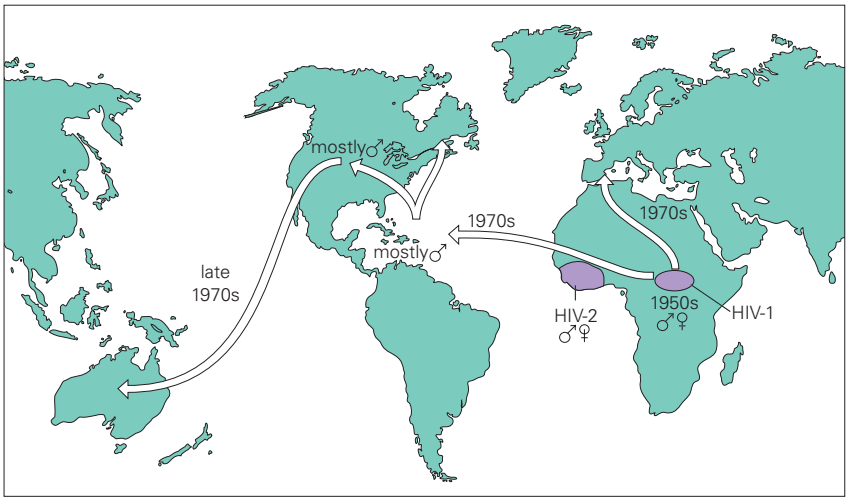


Figure 22.20 Early spread of HIV infection (now worldwide). HIV-1 may have been present in central Africa for many years before increased migration and socioeconomic upheaval caused it to begin spreading in the late 1970s. Outside Africa, most infections occurred in men.

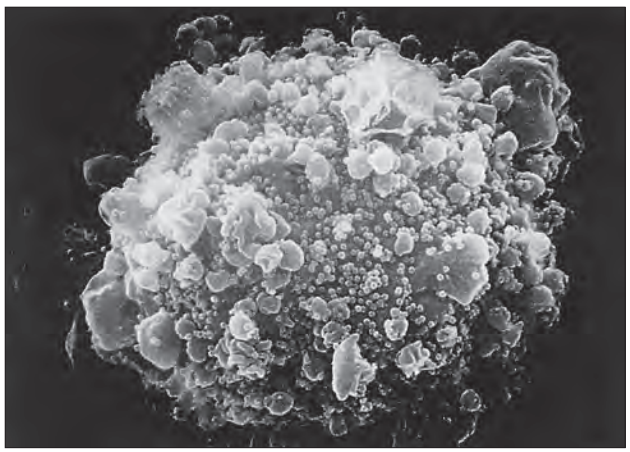


Figure 22.21 Scanning electron micrograph of an HIV-infected Th cell (×20 000). (Courtesy of D. Hockley.)

AIDS develops, responses to HIV and unrelated antigens are further depressed. The immune system has lost control. Plasma HIV-1 RNA load measurements have been shown to predict clinical outcome and are used in clinical management to help determine disease stage and progression as well as antiretroviral therapy response.

The following factors need to be considered in the development of immune suppression:

- Th cells directly killed by virus
- Th cells induced to commit suicide (apoptosis, programmed cell death) by virus
- Th cells made vulnerable to immune attack by Tc cells
- T-cell replenishment impaired by damage to the thymus and lymph nodes and by infection of stem cells
- defects in antigen presentation associated with infection of dendritic cells
- immunosuppressive virus-coded molecules (gp120, gp41).

The host response is further handicapped by the high rate of viral evolution assisted by the lack of a reverse transcriptase proofreading function. The virus exists as a quasispecies, in other words the infection comprises a number of heterogeneous strains. Some are immune escape variants and others show increased pathogenicity.

Before the advent of combined antiretroviral therapy the immunosuppression was permanent, the patient remained infectious, the virus persisted in the body and death was due to opportunist infections and tumours.

HIV-2 appears to be transmitted less easily than HIV-1, probably because the viral load is lower, and the progression to AIDS is slower. HIV-2 is endemic in West Africa, and has spread to Portugal and parts of India.

Routes of transmission

In resource-rich countries, such as western and central Europe and North America, the main route of transmission is MSM. This is due to the higher risk of transmission by receptive anal intercourse, sex networks and risk taking increasing due to effective ART. Infection is transmitted primarily from male to male and from male to female (Fig. 22.23), although not very efficiently compared with other STIs. Transmission from female to male, however, is a common and well-established feature of HIV in Africa and Asia.

Heterosexual transmission has not so far been as important in resource-rich as in resource-poor countries

One explanation for the greater heterosexual spread in resource-poor countries is that other STIs are more common, causing ulcers and discharges, which are sources of infected lymphocytes and monocytes. Genital ulcers are associated with a fourfold increase in the risk of infection. Also, viral strains from Asia and sub-Saharan Africa have been shown to infect Langerhans cells in genital mucosa more easily than do other strains. It is not clear whether HIV can infect males by the urethra or whether pre-existing genital skin breaks are necessary. As with other STIs, uncircumcised males are more likely to be infected.

A fall in plasma HIV load of 0.7 $\log_{10}$ is estimated to reduce the risk of HIV transmission by 50%, which is why pre- and post-exposure prophylaxis preventative measures have been investigated and promoted, in conjunction with safe sex messages.

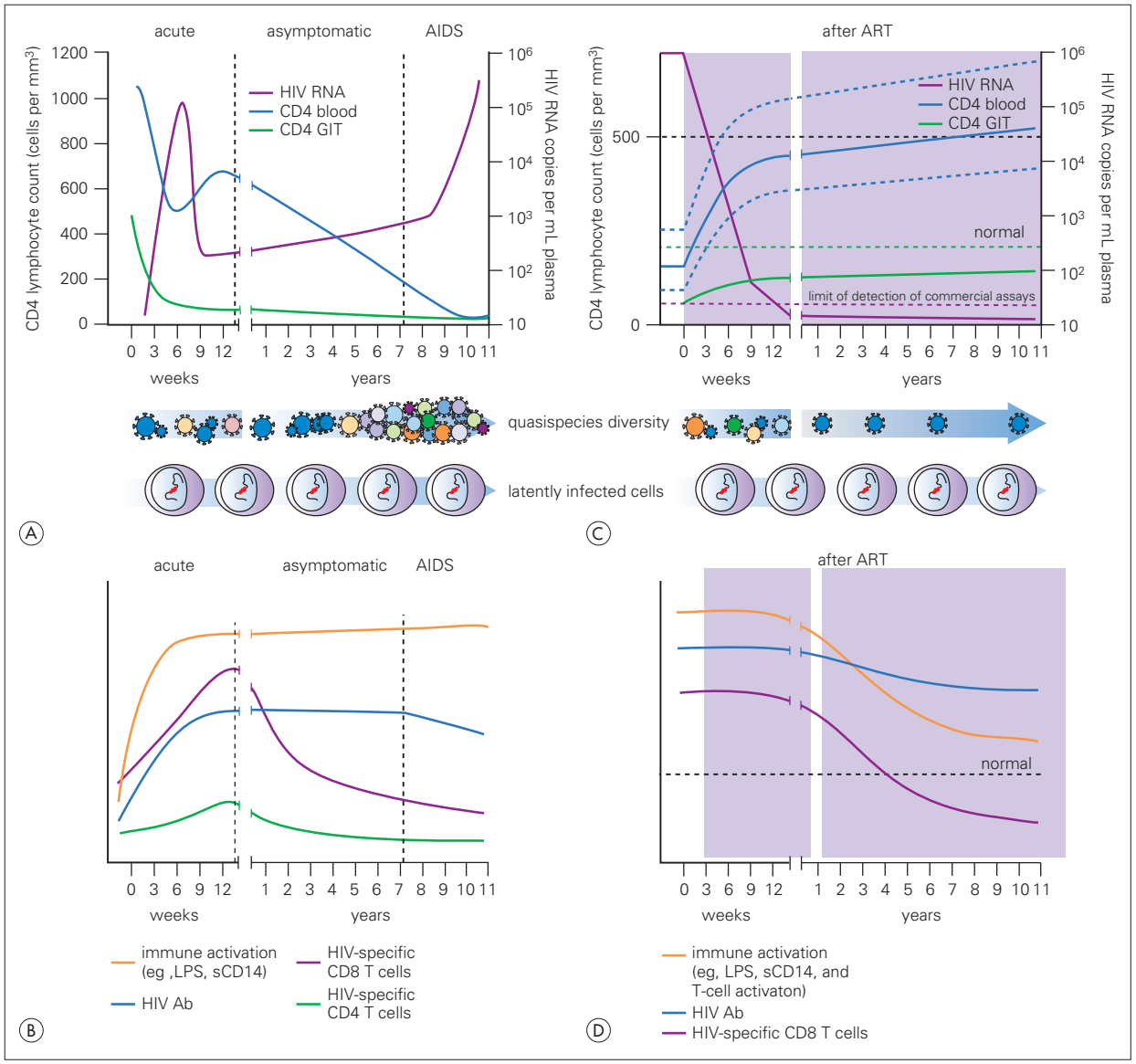


Figure 22.22 A comparison of HIV infection untreated and after antiretroviral therapy. (A) If HIV infection is untreated CD4 T cells progressively decrease in blood and are rapidly depleted early on in the gastrointestinal tract. (B) The immediate result of HIV infection is the activation of the immune response including production of non-neutralizing antibodies and HIV-specific CD4 and CD8 T cells resulting in a temporal decrease in HIV RNA in blood. (C) Antiretroviral therapy significantly decreases HIV RNA with CD4 T cell recovery varying with the individual (panel). Conversely, there is reduced recovery of CD4 T cells in the gastrointestinal tract. (D) Antiretroviral therapy is associated with decreased HIV RNA and viral antigen and a decrease in HIV-specific T cells, although antibody persists in all patients. Immune activation also decreases but remains significantly increased in most patients compared with healthy controls. *GIT*, gastrointestinal tract; *LPS*, lipopolysaccharide. (From Maartens G., Celum C., Lewin S.R. HIV infection: epidemiology, pathogenesis, treatment, and prevention. *Lancet* 2014; 384;258–271, Fig 3, with permission.)

HIV can also be transmitted vertically from infected mothers to their babies, but the infant is not infected in 55–85% of pregnancies, the upper limit being associated with avoiding breastfeeding. Overall, the infant is infected in about 20% of pregnancies in utero and intrapartum. The transmission rate peri- and postnatally is around 11–16%, the higher end of the range depending on whether the child has been breastfed for up to 24 months. In resource-rich countries, antenatal HIV screening, offering antiretroviral drugs during pregnancy and caesarean section delivery, avoiding breastfeeding, and giving antiretroviral drugs to the newborn infant have reduced the risk of HIV transmission to the child. In resource-poor countries it has been shown that giving one dose of one antiretroviral drug to both mother and child reduced HIV transmission by 47%.

By the end of 2015, there were around 150 000 children newly infected with HIV compared to 490 000 in 2000. 110 000 children died from AIDS-related illnesses in 2015 compared to the estimated 320 000 who died in 2004.

Haemophiliacs and others who received contaminated blood products were infected in the past. As with other blood-borne virus infections, using contaminated needles can lead to infection, i.e. in injecting drug use, tattooing, body piercing and acupuncture.

Finally, healthcare workers are at risk of HIV infection after sustaining needlestick or mucous membrane splash injuries

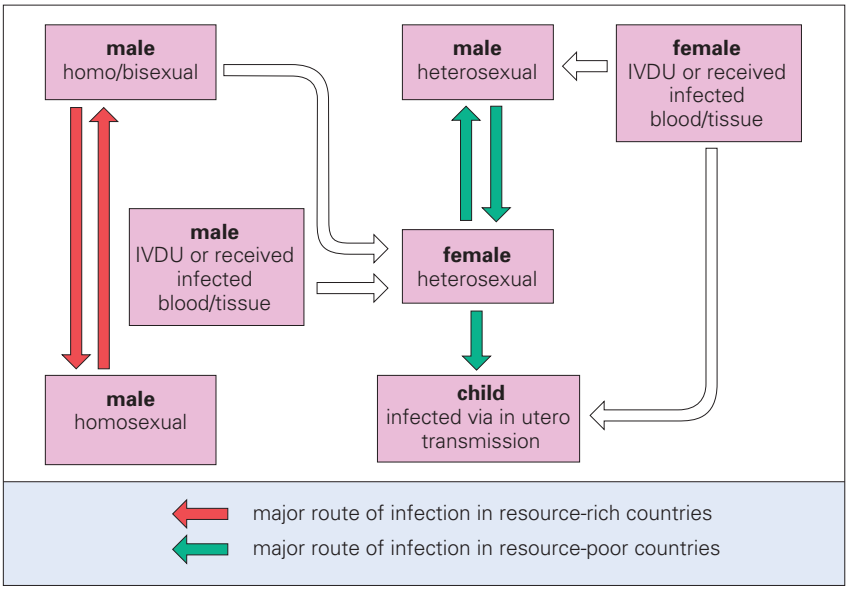


Figure 22.23 Major routes of transmission of HIV. Although the heterosexual route of transmission has so far been well established only in resource-poor countries, there is evidence that this route is becoming more important in the resource-rich countries. IVDU, intravenous drug user.

involving an HIV-infected source. The risk of infection is approximately 1 in 400 and is dependent on a number of factors, including depth of the injury and amount of blood to which the recipient has been exposed. Wearing protective clothing such as gloves and goggles is part of universal precautions to avoid exposure.

Clinical features

Primary HIV infection may be accompanied by a mild mononucleosis-type illness

Signs and symptoms of the mild mononucleosis-type illness associated with HIV infection include fever, malaise, maculopapular rash and lymphadenopathy. The acute infection and rapid, widespread viral dissemination is followed by a chronic asymptomatic stage. Viral replication is reduced in line with the immune response, and the individual usually remains well. The duration of this stage is dependent on a number of factors including the viral phenotype, host immune response and use of antiretroviral therapy (Fig. 22.24). Infected cells are, however, still present, and at a later stage the infected individual may develop weight loss, fever, persistent lymphadenopathy, oral candidiasis and diarrhoea. Further viral replication takes place until finally, some years after initial infection, full-blown AIDS develops (Fig. 22.25).

Progression to AIDS

Viral invasion of the CNS, with self-limiting aseptic meningoencephalitis as the most common neurological picture, occurs in early infection.

A progressive HIV-associated encephalopathy is seen in individuals with AIDS and is characterized by multiple small nodules of inflammatory cells; most of the infected cells appear to be microglia or infiltrating macrophages. These cells express the CD4 antigen, and it has been suggested that infected monocytes carry the virus into the brain, but the picture is complicated by the various persistent infections that are activated and give rise to their own CNS pathology. These include infections by HSV, varicella-zoster virus (VZV), *Toxoplasma gondii*, JC virus (progressive multifocal leukoencephalopathy, PML) and *Cryptococcus neoformans*.

HIV exercises complex control over its own replication (see Fig. 22.18). Replication is also affected by responses to other infections, which act as antigenic stimuli, and some of them directly as transactivating agents.

Some patients, especially in Africa, develop a wasting disease ('slim' disease), possibly due to unknown intestinal infections or infestations, and perhaps also to the direct effects of the virus infecting cells of the intestinal wall.

AIDS, symptomatic disease, consists of a large spectrum of microbial diseases acquired or reactivated as a result of the underlying immunosuppression due to HIV (Fig. 22.26; Table 22.9). The disease picture of AIDS is therefore an indirect result of infection with HIV.

Before the advent of antiretroviral therapy, one study in New York reported a mortality rate of 80%, 5 years after the onset of the disease, and the average survival time after hospital admission was 242 days.

Treatment

Antiretroviral therapy results in a dramatic improvement in disease prognosis

In the 1990s, a range of antiretroviral therapies was introduced which included the nucleoside reverse transcriptase inhibitors (NRTIs), non-nucleoside reverse transcriptase inhibitors (NNRTIs) and protease inhibitors (PIs). These were developed further over the next two decades in terms of new drugs in all classes and combinations. In 2003, a fusion inhibitor was added to the list and by 2009 two other classes were available, an integrase inhibitor and chemokine receptor antagonist (see Ch. 34 for more detail). In combination with two NRTIs, the NNRTI or PI drugs had a dramatic effect on progression to AIDS and the term highly active antiretroviral therapy (HAART) had changed by 2016 to combined antiretroviral therapy (cART). Side effects of the drugs include mitochondrial toxicity and altered fat distribution known as lipodystrophy. Treatment compliance was a problem because of the side

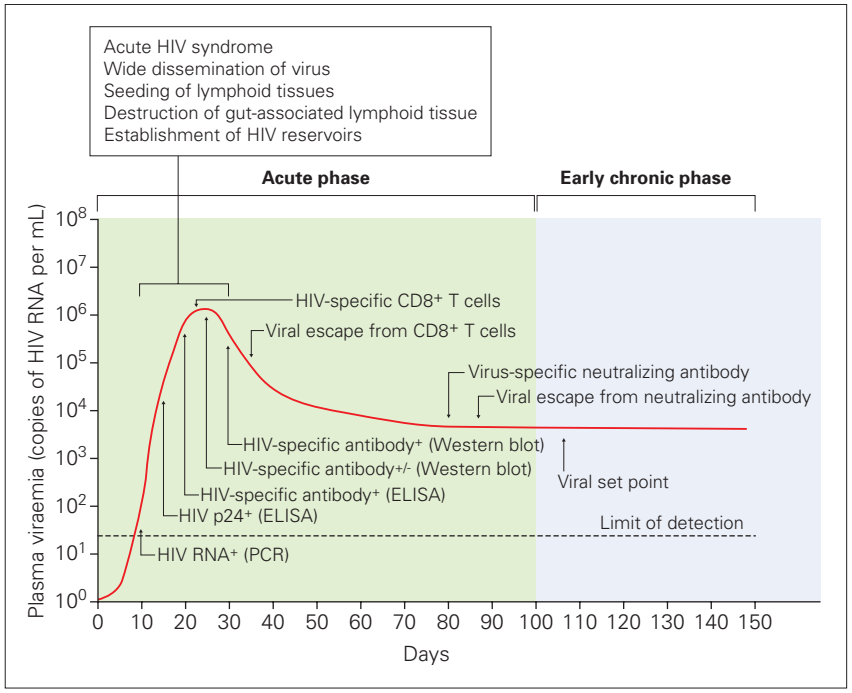


Figure 22.24 Kinetics of immunological and virological events associated with human immunodeficiency (HIV) infection during acute and early chronic phases. The schematic represents the sequence of events, including the appearance of viral antigens, HIV-specific antibodies, and HIV-specific $CD8^+$ T cells during the acute and early chronic phases of infection. HIV reservoirs are established during the acute phase of infection soon after emergence of plasma viraemia. Throughout the acute phase of infection, characterized by massive virus replication and high levels of plasma viraemia, an acute HIV syndrome develops in the majority of infected individuals, and the virus rapidly spreads to various lymphoid organs, causing extensive depletion of $CD4^+$ T cells. Although anti-HIV immunity, including virus-specific $CD8^+$ T cells and antibodies, develops during the acute phase of infection, escaped viral mutants rapidly emerge. CD, cluster of differentiation; ELISA, enzyme-linked immunosorbent assay; PCR, polymerase chain reaction. (Redrawn from Moir, S., Chun T.W., Fauci A.S. Pathogenic mechanisms of HIV disease, *Annu Rev Pathol Mech Dis* 6:223–248, 2011.)

effects and the number and frequency of pills taken each day. Missing doses can lead to the development of drug resistance, thus limiting treatment options. However, treatment has been simplified by not just combining individual classes of up to three drugs in one tablet, but also combinations of classes (see Ch. 34). Improved monitoring using plasma HIV load measurements and CD4 counts and percentages has shown the success of cART, with rapid falls in plasma HIV load and rises in CD4 cells seen after initiating therapy.

However, HIV can be detected in various compartments of the body including the CSF and genital tract. Antiretroviral drugs may not penetrate these sites, resulting in a high viral load detectable in semen despite suppression of the plasma HIV load.

As a result of improved diagnosis, surveillance, prevention and use of cART, the number of AIDS-related deaths among children and adults worldwide had fallen from 1.5 million in 2010 to 1.1 million in 2015.

Antiretroviral drug resistance and improved treatment options

Plasma HIV-1 RNA load is a good indicator of viral replication, and failure of antiretroviral therapy is seen by a rise in viral load. Antiretroviral resistance testing and therapeutic drug monitoring are part of clinical management. Drug resistance testing may be carried out when the plasma HIV-1 load is not suppressed whilst on antiretroviral therapy. Specific mutations in the HIV reverse transcriptase, protease and integrase regions associated with reduced susceptibility to one or more antiretroviral drugs have been identified by nucleic acid sequencing, known as genotypic analysis. Some drug resistance mutations confer resistance to more than one drug of the same class, whereas others appear unique to specific drugs.

Transmission of drug-resistant HIV is an important issue. Drug resistance mutations were detected in around 14% of samples tested in 2002, but had fallen to around 7% by 2013, from antiretroviral therapy naive adults infected with HIV in the UK. This fall probably reflected the introduction of improved combinations of ART, both in terms of drug classes as well as reduced pill burden improving compliance. The prevalence of drug-resistant viruses in newly infected individuals will depend on factors such as changes in testing guidance, more individuals virologically suppressed on cART and whether the individual was infected by someone failing on antiretroviral therapy. Furthermore, a huge reduction was seen in the prevalence of drug resistance mutations in ART experienced individuals, from 72% in 2002 to 33% in 2013 for reasons noted above.

Baseline antiretroviral drug resistance testing is part of the management guidelines in many countries before starting treatment, as infection with a drug-resistant virus may affect the efficacy of subsequent therapy.

Treatment of AIDS involves prophylaxis and treatment of opportunist infections as well as using antiretrovirals

Depending on the CD4 count, prophylaxis is given for specific opportunistic infections such as *Pneumocystis jirovecii* and *Cryptococcus neoformans*. When opportunist infections are diagnosed, they are treated appropriately, for example co-trimoxazole or pentamidine with or without steroids for *P. jirovecii*, ganciclovir for cytomegalovirus (CMV), and fluconazole or amphotericin for *C. neoformans* infection.

Laboratory tests

Laboratory tests for HIV infection involve both serological and molecular analysis

Acquired immune deficiency syndrome (AIDS) is a clinical definition; in the presence of antibodies to HIV, any of the conditions listed in Table 22.9, regardless of the presence of

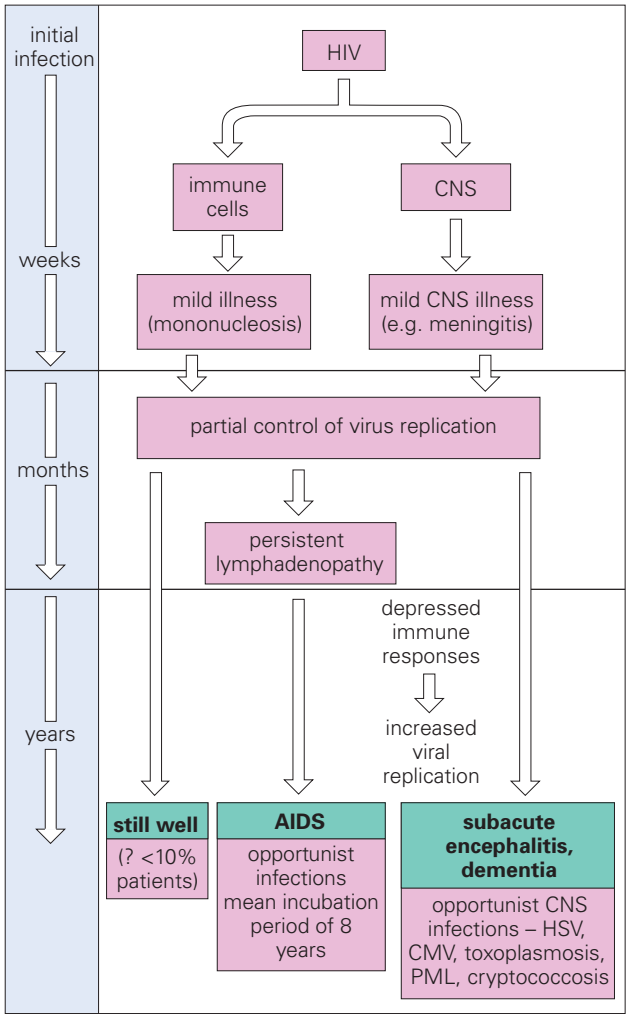


Figure 22.25 The clinical features and progression of untreated HIV infection. CMV, cytomegalovirus; CNS, central nervous system; HSV, herpes simplex virus; PML, progressive multifocal leukoencephalopathy.

other causes of immunodeficiency, indicate AIDS. The range and complexity of tests used for HIV-1 and -2 screening, diagnosis of infection, and monitoring disease progression and response to therapy have increased dramatically over time.

Viral replication occurs during the incubation period, during which time the viral genome and, briefly, viral p24 antigen but not the host's antibody response may be detected. HIV-1 and -2 diagnostic tests can be divided into combined antibody and antigen detection, antigen detection, antibody detection alone (although this is a less sensitive test) and genome detection. The last can be divided into qualitative HIV-1 or -2 proviral DNA and quantitative HIV-1 or -2 RNA detection. In addition, antiretroviral drug resistance and tropism assays are part of standard management.

Initially, an HIV-1 and -2 antibody / antigen combination assay which includes antibody and p24 antigen is carried out. These assays have been developed to reduce the diagnostic window period. Assay reactivity is confirmed using an alternative HIV test format on the original unseparated sample stored in the laboratory. This is to ensure that a specimen separation error has not occurred. HIV type differentiation may be carried out using a number of assays that may include an immunoblot, where the antigens are coated on nitrocellulose strips. A positive result is confirmed on a further blood sample, to ensure that the original sample had not been mislabelled at collection.

HIV-1 RNA or proviral DNA tests may be carried out on plasma and whole blood samples, respectively, if it is difficult to make a diagnosis because low level assay reactivity is detected when the serum sample is tested and the result is indeterminate, or the patient may have a seroconversion illness and the screening tests are negative.

Part of monitoring HIV-1-infected individuals on or off antiretroviral therapy involves measuring the plasma HIV-1 RNA load, which can be quantified using several commercial or in-house assays using different methods. The main assay formats are based on reverse transcription polymerase chain reaction (RT-PCR), branched DNA signal amplification, and RNA transcription isothermal amplification.

In addition, part of the laboratory portfolio involves antiretroviral resistance genotypic analysis by automated DNA sequencing. This is a more, specialized test and the interpretation of the results may be complicated.

Diagnosis of HIV infection in newborn infants can be difficult because passively acquired maternal IgG will be detected in the first 12 months after birth. Reference laboratories may have virus-specific IgM and IgA in-house tests, which would signify in utero infection (see Ch. 24), as part of their test portfolio. Samples from infants are tested at various time intervals up to 12–24 months for p24 antigen, HIV-1 RNA and / or HIV-1 proviral DNA and HIV antibody to assess their HIV status.

Measures to control spread

There are a number of preventative measures to reduce the spread of HIV

In resource-rich countries such as the UK, most new infections in 2014 involved MSM (Fig. 22.27A and B). The estimated annual number of new HIV infections in all ages has not substantially changed from about 2.2 million in 2010 to about 2.1 million in 2015. The largest fall in new adult HIV infections was seen in eastern and southern and western and central Africa, both recording falls of about 50 000 and 40 000 fewer new adult HIV infections, respectively, in 2015.

Much smaller reductions occurred in the Asia and Pacific region, Latin America and the Caribbean, Western and Central Europe, North America and the Middle East and North Africa. However, a near 60% rise was seen in Eastern Europe and Central Asia (UNAIDS global AIDS update 2016).

Prevention measures include reducing mother-to-child HIV transmission, where transmission may be seen in about 25% at delivery if no interventions are used. cART is a key intervention, started after the first trimester of pregnancy. Reducing plasma HIV load to below detection means vaginal delivery may be carried out, instead of caesarean section delivery and may also reduce transmission by breastfeeding in countries where infant mortality may be higher if breastfeeding is avoided. Reducing sexual transmission by changing sexual behaviour and condom use is a major public health effort, but pre-exposure prophylaxis with daily ART has been shown to reduce HIV transmission. Treating genital herpes simplex infections is another intervention.

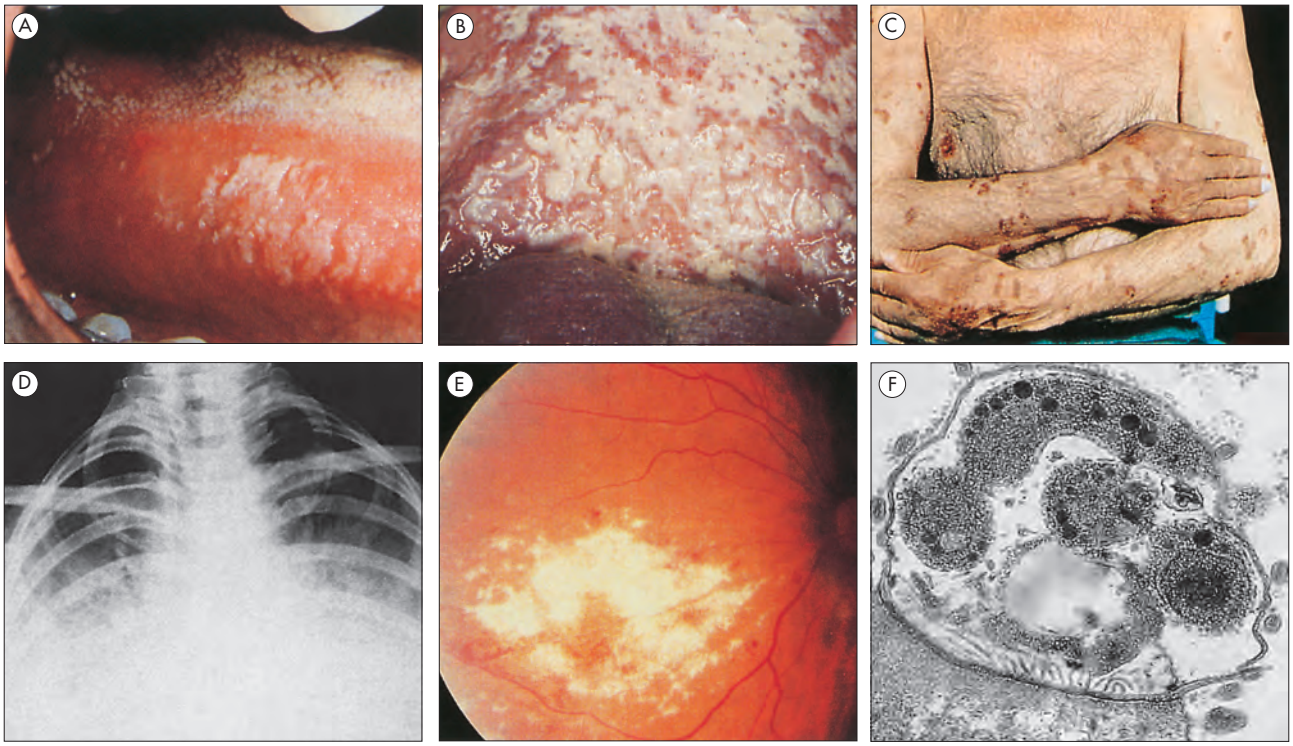


Figure 22.26 Opportunist infections and tumours associated with HIV infection. (A) Hairy leukoplakia – raised white lesions of oral mucosa, predominantly along the lateral aspect of the tongue, due to Epstein–Barr virus infection. (B) Extensive oral candidiasis. (C) Kaposi's sarcoma – brown pigmented lesions on the upper extremities. (D) *Pneumocystis* pneumonia, with extensive infiltrates in both lungs. (E) Cytomegalovirus retinitis showing scattered exudates and haemorrhages, with sheathing of vessels. (F) Cryptosporidiosis – electron micrograph showing mature schizont with several merozoites attached to intestinal epithelium. ([A] Courtesy of H.P. Holley. [B] and [F] Courtesy of W.E. Farrar. [C] Courtesy of E. Sahn. [D] Courtesy of J.A. Innes. [E] Courtesy of C.J. Ellis.)

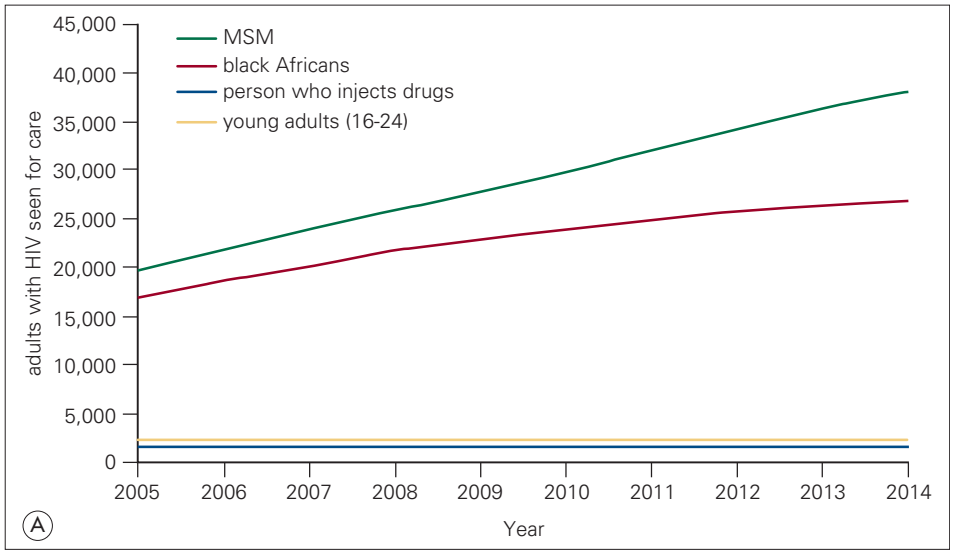
Table 22.9 Opportunist infections and tumours in AIDS

Viruses	Disseminated CMV (including retina, brain, peripheral nervous system, gastrointestinal tract) HSV (lungs, gastrointestinal tract, CNS, skin) JC virus (brain – PML) EBV (hairy leukoplakia, primary cerebral lymphoma) HHV-8 (Kaposi's sarcoma)[b]
Bacteria[a]	Mycobacteria (e.g. *Mycoplasma avium*, *M. tuberculosis* – disseminated, extrapulmonary) *Salmonella* (recurrent, disseminated) septicaemia
Protozoa	*Toxoplasma gondii* (disseminated, including CNS) *Cryptococcus neoformans* (CNS) Histoplasmosis (disseminated, extrapulmonary) *Coccidioides* (disseminated, extrapulmonary)
Other	Wasting disease (cause unknown) HIV encephalopathy

[a]Also pyogenic bacteria (e.g. *Haemophilus, Streptococcus, Pneumococcus*) causing septicaemia, pneumonia, meningitis, osteomyelitis, arthritis, abscesses, etc.; multiple or recurrent infections, especially in children.
[b]Associated with HHV-8, an independently transmitted agent; 300 times as frequent in AIDS as in other immunodeficiencies. AIDS is defined as the presence of antibodies to HIV plus one of the conditions in this table.
CMV, cytomegalovirus; CNS, central nervous system; EBV, Epstein–Barr virus; HSV, herpes simplex virus; PML, progressive multifocal leukoencephalopathy.

The risk of transmitting HIV via blood and blood products is reduced considerably by donor screening programmes and heat treatment, respectively. Those at risk of infection are advised not to donate. Heat treatment of factor VIII is a further precaution before this product is used to treat haemophiliac patients. HIV has a delicate outer envelope and is highly susceptible to heat and chemical agents. HIV is inactivated under pasteurization conditions and also by hypochlorites, even at concentrations as low as 1 in 10000 ppm; 2.5% glutaraldehyde and ethyl alcohol are also effective against the virus.

The problem of transmission between injecting drug users is being tackled in some areas by measures that were originally controversial, such as the free distribution of clean needles and syringes.



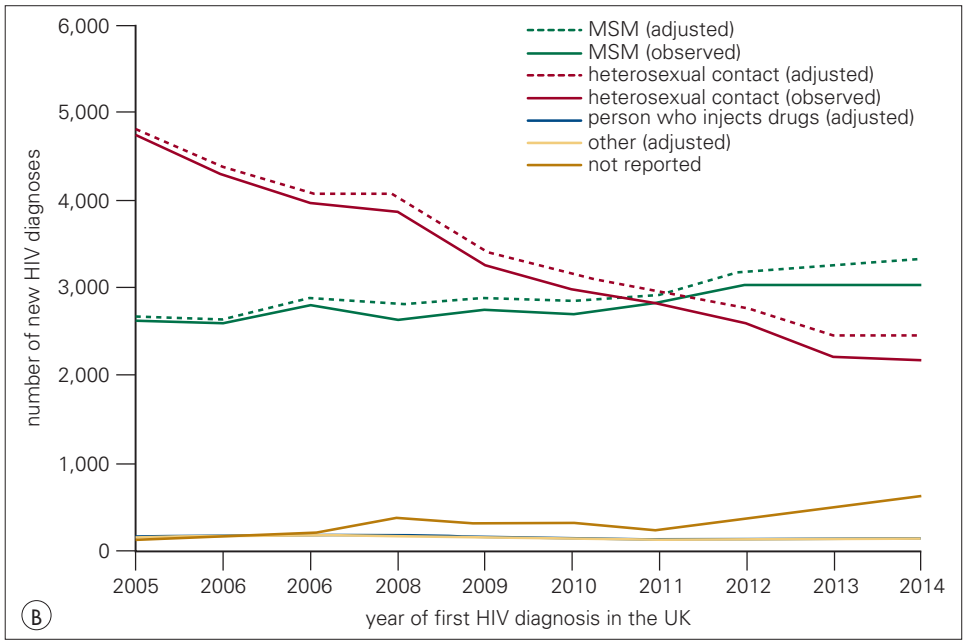


Figure 22.27 (A) Number of adults seen for HIV care over time by key prevention groups and (B) new HIV diagnoses by exposure group over time; 2005–2014. (Redrawn after Skingsley A., Kirwan P., Yin Z. et al. *HIV New Diagnoses, Treatment and Care in the UK 2015 Report: Data To End 2014*. October 2015. London: Public Health England. Figs 1 and 5.)

Public health educational programmes have been presented by various types of media in order to reduce the incidence of all STIs.

Vaccination

There are a number of challenges in developing a successful vaccine against HIV infection

More than 50 vaccine regimens have undergone clinical trials since 1999. However, only four transferred to test-of-concept or efficacy trials. The prospects are limited for a number of reasons including viral antigenic variation and sequence diversity, slow neutralizing antibody response to HIV infection, viral evasion of immune responses and establishment of latent viral reservoirs. Various subunit envelope glycoproteins, whole killed virus vaccines, plasmid DNA vaccines and virus vectors to carry HIV antigens have been investigated and tested. Trials have been carried out in animal (monkey) models and also humans.

The aim is to prevent infection or reduce the HIV load and clinical progression post-infection. The immune correlates of protection have yet to be well defined and are critical in order to protect against infection. Two vaccine candidates involved in efficacy studies were an envelope gp120 protein vaccine that resulted in type-specific but not broadly reactive neutralizing antibody responses and a replication-incompetent adenovirus vector expressing HIV-1 gag, pol and nef gene products. The latter resulted in cellular immune responses in most recipients. However, the vaccine was neither protective nor reduced HIV loads post-infection. In the 2009 RV144 Thai HIV vaccine trial, the estimated vaccine efficacy was around

31% and it was thought that antibody generated against the V1V2 loop of the envelope glycoprotein may have contributed to protection. The fact that there is a successful killed virus vaccine for a feline retrovirus (feline leukaemia), and that a similar vaccine protects monkeys from simian AIDS, does, however, give some hope for the development of an HIV vaccine. Furthermore, passively transferred neutralizing antibodies in sera or monoclonal broadly neutralizing antibodies have been shown to be protective in preventing SIV infection in macaques. Active and passive immunization approaches are being investigated.

To prevent sexual transmission, mucosal immunity is needed, and this is likely to come from a mucosally administered vaccine. The major route of HIV-1 transmission is via mucosal surfaces. Worldwide, the cervical and vaginal mucosae are the major portals but the rectal mucosa is the more common route in North America and Europe. Penile foreskin increases the risk of HIV transmission owing to the high density of HIV target Langerhans cells in addition to the inner mucosal surface not being keratinized. Circumcision has been shown to reduce the risk of transmission. Macaque SIV infection models have shown additional mucosal routes of entry, suggesting that HIV infection might also be transmitted through oropharyngeal and upper gastrointestinal mucosa. A T-cell vaccine would need to induce a long-lasting mucosal immune response that includes mucosal neutralizing IgA and IgG antibody and T-cell responses. Mucosal $CD8^+$ CTLs would limit infection and subsequent HIV viraemia as well as clearance of viral reservoirs in the gut mucosa.

OPPORTUNIST STIs

Opportunist STIs include salmonellae, shigellae, hepatitis A, *Giardia intestinalis* and *Entamoeba histolytica* infections

Although STIs are classically transmitted during heterosexual intercourse, they can also be transmitted whenever two mucosal surfaces are brought together. Anal intercourse allows the transfer of microorganisms from penis to rectal mucosa or to anal and perianal regions. Gonococcal or papillomavirus lesions, for instance, may occur in any of these sites. A few microorganisms (hepatitis B, HIV) are transmitted more often across rectal mucosa. If there is oral–anal contact, a variety of intestinal pathogens are given the opportunity to spread as STIs and can then be regarded as 'opportunistic STIs'. These include salmonellae, shigellae, hepatitis A virus, *Giardia intestinalis* and *Entamoeba histolytica* (also see Ch. 23). Together with chronic infections such as CMV and cryptosporidiosis, they contribute to intestinal symptoms and diarrhoea in AIDS patients.

Hepatitis B virus is often transmitted sexually

Hepatitis B virus is detectable in semen, saliva and vaginal secretions. HBV transmission, like HIV, is more likely when genital areas are ulcerated or contaminated with blood. Hepatitis B transmission among MSM parallels the transmission of HIV, with passive anal intercourse as a high-risk factor. Hepatitis D transmission can only follow hepatitis B as it is a defective virus that needs HBsAg to replicate. Hepatitis C is less commonly transmitted sexually; <5% of long-term sexual partners are infected.

ARTHROPOD INFESTATIONS

Infection with the pubic or crab louse causes itching and is treated with permethrin shampoo

The 'crab louse', *Phthirus pubis*, is distinct from the other human lice, *Pediculus humanus humanus* and *Pediculus humanus capitis*. The crab louse is well adapted for life in the genital region, clinging tightly to the pubic hairs (see Ch. 6) but it can infest any hair-bearing area so hairs on the eyebrows, eyelashes or in the axilla are occasionally colonized. *P. pubis* takes up to 10 blood feeds a day which results in itching at the site of the bites. Eggs known as 'nits' are seen attached to the affected hairs, and the characteristic lice, up to 2 mm long, are visible (often at the base of a hair) under a hand lens or by microscopy of a detached hair. Infestation is common (e.g. there are more than 10 000 cases / year in the UK).

Treatment is by the application of permethrin cream or malathion lotion.

Genital scabies is also treated with permethrin cream

Sarcoptes scabiei (see Ch. 27) may cause local lesions on the genitalia, and can thus be sexually transmitted. Patients may have evidence of scabies elsewhere on the body, with burrows between the fingers or toes. Genital scabies is also treated with permethrin cream. Oral ivermectin may be required in immunocompromised patients.

KEY FACTS

- Microorganisms transmitted by the sexual route in humans include representatives from all groups apart from the rickettsiae and helminths.
- STIs are found in the general community rather than being confined only to high-risk groups.
- Genital herpes, warts, chlamydial urethritis, and gonorrhoea are by far the most common of all the STIs, but HIV infection has had a major impact, although it is now considered as a long-term, chronic infection compared with the clinical situation in the late 1980s and early 1990s.
- Except for hepatitis A and B and human papillomavirus infections, there are no vaccines for these other STIs, but antimicrobial chemotherapy is often available.
- At present, the best method of control is prevention.
- Transmission depends upon human behaviour, which is notoriously difficult to influence.
- Long intervals between the onset of infectiousness and disease increase the chances of transmission.

23 Gastrointestinal tract infections

Introduction

Ingested pathogens may cause disease confined to the gut or involve other parts of the body

Ingestion of pathogens can cause many different infections. These may be confined to the gastrointestinal tract or are initiated there before spreading to other parts of the body. In this chapter, we consider the bacterial causes of diarrhoeal disease and summarize the other bacterial causes of food-associated infection and food poisoning. Viral and parasitic causes of diarrhoeal disease are discussed, as well as infections acquired via the gastrointestinal tract and causing disease in other body systems, including typhoid and paratyphoid fevers, listeriosis and viral hepatitis. For clarity, all types of viral hepatitis are included in this chapter, despite the fact that some are transmitted by other routes of infection. Infections of the liver can also result in liver abscesses, and several parasitic infections cause liver disease. Peritonitis and intra-abdominal abscesses can arise from seeding of the abdominal cavity by organisms from the gastrointestinal tract. Several different terms are used to describe infections of the gastrointestinal tract; those in common use are shown in Box 23.1.

A wide range of microbial pathogens is capable of infecting the gastrointestinal tract, and the main bacterial and viral pathogens are listed in Table 23.1. They are acquired by the faecal–oral route, from faecally contaminated food, fluids or fingers.

For an infection to occur, the pathogen must be ingested in sufficient numbers or possess attributes to elude the host defences of the upper gastrointestinal tract and reach the intestine (Fig. 23.1; see also Ch. 14). Here they remain localized and cause disease as a result of multiplication and/or toxin production, or they may invade through the intestinal mucosa to reach the lymphatics or the bloodstream (Fig. 23.2). The damaging effects resulting from infection of the gastrointestinal tract are summarized in Box 23.2.

Box 23.1 Terms Used to Describe Gastrointestinal Tract Infections

As well as many colloquial expressions, several different clinical terms are used to describe infections of the gastrointestinal tract. Diarrhoea without blood and pus is usually the result of enterotoxin production, whereas the presence of blood and/or pus cells in the faeces indicates an invasive infection with mucosal destruction.

Gastroenteritis

- A syndrome characterized by gastrointestinal symptoms including nausea, vomiting, diarrhoea and abdominal discomfort

Diarrhoea

- Abnormal faecal discharge characterized by frequent and/or fluid stool; usually resulting from disease of the small intestine and involving increased fluid and electrolyte loss

Dysentery

- An inflammatory disorder of the gastrointestinal tract often associated with blood and pus in the faeces and accompanied by symptoms of pain, fever, abdominal cramps; usually resulting from disease of the large intestine

Enterocolitis

- Inflammation involving the mucosa of both the small and large intestine

Food-associated infection versus food poisoning

Infection associated with consumption of contaminated food is often termed 'food poisoning', but 'food-associated infection' is a better term. True food poisoning occurs after consumption of food containing toxins, which may be chemical (e.g. heavy metals) or bacterial in origin (e.g. from *Clostridium botulinum* or *Staphylococcus aureus*). The bacteria multiply and produce toxins within contaminated food. The organisms may be destroyed during food preparation, but the toxin is unaffected, consumed and acts within hours. In food-associated infections, the food may simply act as a vehicle for the pathogen (e.g. *Campylobacter*) or provide conditions in which the pathogen can multiply to produce numbers large enough to cause disease (e.g. *Salmonella*).

Table 23.1 Important bacterial and viral pathogens of the gastrointestinal tract

Pathogen	Animal reservoir	Food-borne	Water-borne
Bacteria			
Escherichia coli	+?	+ (EHEC)	+ (ETEC)
Salmonella	+	+ + +	+
Campylobacter	+	+ + +	+
Vibrio cholerae	–	+	+ + +
Shigella	–	+	–
Clostridium perfringens	+	+ + +	–
Bacillus cereus	–	+ +	–
Vibrio para-haemolyticus	–	+ +	–
Yersinia enterocolitica	+	+	–
Viruses			
Rotavirus	–	–	–
Noroviruses (previously known as SRSV or Norwalk-like viruses)	–	+ +	+

Many different pathogens cause infections of the gastrointestinal tract. Some are found in both humans and animals, while others are strictly human parasites. This difference has important implications for control and prevention. EHEC, enterohaemorrhagic (verotoxin-producing) *E. coli*; ETEC, enterotoxigenic *E. coli*; SRSV, small round structured viruses.

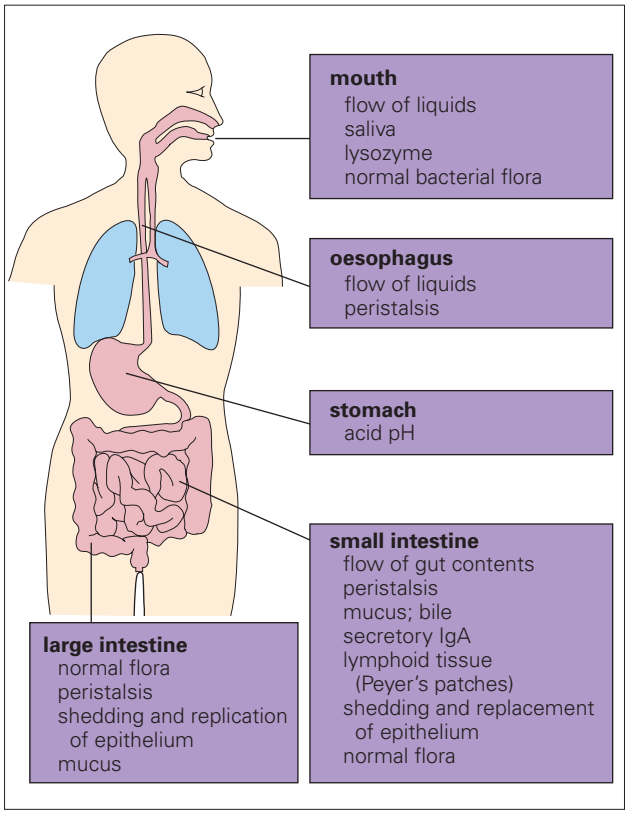


Figure 23.1 Every day we swallow large numbers of microorganisms. Because of the body's defence mechanisms, however, they rarely succeed in surviving the passage to the intestine in sufficient numbers to cause infection. IgA, immunoglobulin A.

Box 23.2 Damage Resulting From Infection of the Gastrointestinal Tract

- Pharmacological action of bacterial toxins, local or distant to site of infection (e.g. cholera, staphylococcal food poisoning)
- Local inflammation in response to superficial microbial invasion (e.g. shigellosis, amoebiasis)
- Deep invasion to blood or lymphatics; dissemination to other body sites (e.g. hepatitis A, enteric fevers)
- Perforation of mucosal epithelium after infection, surgery or accidental trauma (e.g. peritonitis, intra-abdominal abscesses)

Infection of the gastrointestinal tract can cause damage locally or at distant sites.

DIARRHOEAL DISEASES CAUSED BY BACTERIAL OR VIRAL INFECTION

Diarrhoea is the most common outcome of gastrointestinal tract infection

Infections of the gastrointestinal tract range in their effects from a mild self-limiting attack of 'the runs' to severe, sometimes fatal, diarrhoea. There may be associated vomiting, fever and malaise. Diarrhoea is the result of an increase in fluid and electrolyte loss into the gastrointestinal tract lumen, leading to the production of unformed or liquid faeces, and can be thought of as the method by which the host forcibly expels the pathogen (and in doing so, aids its dissemination). However, diarrhoea also occurs in many non-infectious conditions, and an infectious cause should not be assumed.

In the resource-poor world, diarrhoeal disease is a major cause of mortality in children

In the resource-poor world, diarrhoeal disease is a major cause of morbidity and mortality, particularly in young children (Fig. 23.3). In the resource-rich world, it remains a very common complaint, but is usually mild and self-limiting except in the very young, the elderly and immunocompromised patients. Most of the pathogens listed in Table 23.1 are found throughout the world, but some, such as *Vibrio cholerae*, have a more limited geographical distribution. However, such infections can be acquired by travellers to these areas and imported into their home countries.

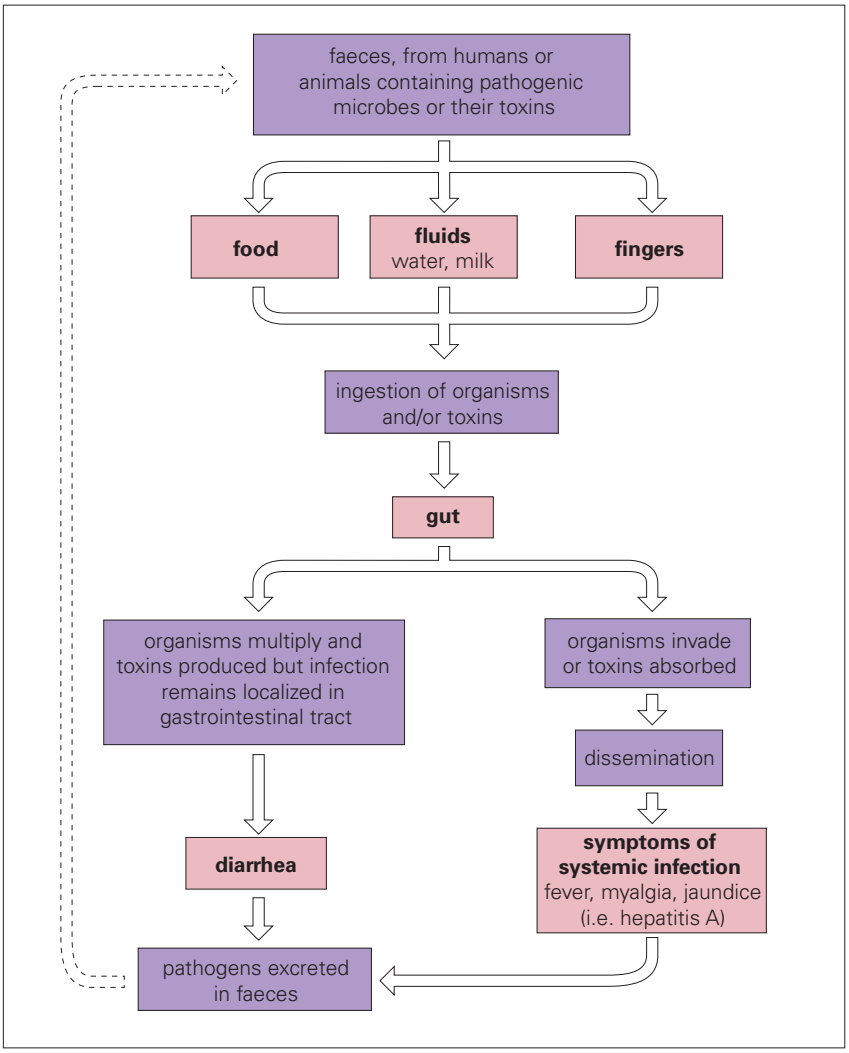


Figure 23.2 Infections of the gastrointestinal tract can be grouped into those that remain localized in the gut and those that invade beyond the gut to cause infection in other sites in the body. In order to spread to a new host, pathogens are excreted in large numbers in the faeces and must survive in the environment for long enough to infect another person directly or indirectly through contaminated food or fluids. (From World Health Organization. 2012. World Health Statistics 2012. WHO Press, Geneva, Switzerland.)

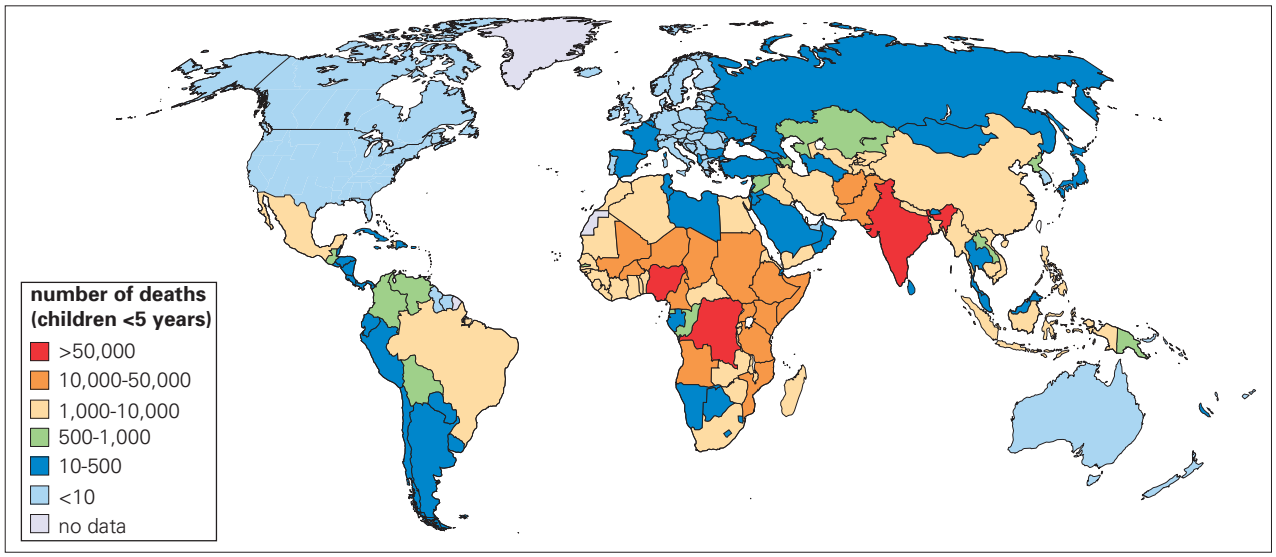


Figure 23.3 Global mortality from diarrhoea in children under the age of 5 in 2010. Estimates of diarrhoea-specific mortality among children under 5 for each country reflect high mortality in resource-poor countries. Many infectious causes, including pathogenic *E. coli*, are responsible for diarrhoea-related mortality in these children. (Reproduced from Croxen M.A., Law R.J., Scholz R. et al. Recent advances in understanding enteric pathogenic *Escherichia coli*. *Clin Mic Rev* 2013; 26[4], Fig. 1 Source data for the map: World Health Organization, with permission.)

Data from the Global Enteric Multi-Center Study, a large case–control investigation set up to determine the burden of paediatric diarrhoeal disease in South Asia and sub-Saharan Africa, showed that enterotoxigenic *Escherichia coli* and *Shigella* are in the top four causes of moderate to severe diarrhoea and therefore mortality, in children in these areas.

Many cases of diarrhoeal disease are not diagnosed, either because they are mild and self-limiting and the patient does not seek medical attention, or because medical and laboratory facilities are unavailable. It is generally impossible to distinguish on clinical grounds between infections caused by the different pathogens. However, information about the patient's recent food and travel history, and macroscopic and microscopic examination of the faeces for blood and pus, can provide helpful clues. A precise diagnosis can be achieved only by laboratory investigations. This is especially important in outbreaks, because of the need to instigate appropriate epidemiological investigations and control measures.

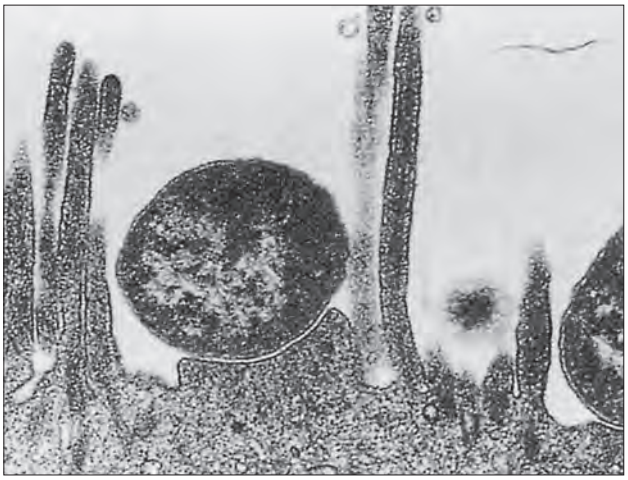


Figure 23.4 Electron micrograph of enteropathogenic *E. coli* adhering to the brush border of intestinal mucosal cells with localized destruction of microvilli. (Courtesy of S. Knutton.)

Bacterial causes of diarrhoea

Escherichia coli

E. coli is one of the most versatile of all bacterial pathogens. Named in the 1950s after Theodor Escherich after he isolated and characterized the short rods from an infant's faecal sample in 1885. Some strains are important members of the normal intestinal flora of humans and animals (see Ch. 2), whereas others possess virulence factors that enable them to cause infections in the intestinal tract or at other sites, particularly the urinary tract, bloodstream and central nervous system (see Ch. 21). Strains that cause diarrhoeal disease do so by several distinct pathogenic mechanisms and differ in their epidemiology (Table 23.2).

There are six distinct pathotypes of* E. coli *with different pathogenetic mechanisms. Initially, all diarrhoea-associated *E. coli* pathotypes were termed enteropathogenic *E. coli* (EPEC). However, greater insight into mechanisms of pathogenicity has led to specific group designations: enteropathogenic *E. coli* (EPEC), enterotoxigenic *E. coli* (ETEC), shiga-toxin-producing *E. coli* (STEC) also called enterohaemorrhagic *E. coli* (EHEC) or verocytotoxin-producing *E. coli* (VTEC), enteroinvasive *E. coli* (EIEC), enteroaggregative *E. coli* (EAEC) and diffusely aggregative *E. coli* (DAEC).

Enteropathogenic* E. coli *(EPEC) pathogens do not make any toxins. They are attaching and effacing pathogens that form distinct lesions on the surfaces of intestinal epithelial cells in the small intestine. They are classified as typical and atypical subtypes based on whether they have the adherence factor plasmid and produce bundle-forming pili (BFP), intimin (an adhesin) and an associated protein (translocated intimin receptor, Tir). These virulence factors allow bacterial attachment to epithelial cells of the small intestine, leading to disruption of the microvillus (an 'attaching–effacing' mechanism of action; Table 23.2; Fig. 23.4) leading to diarrhoea (Table 23.3).

Enterotoxigenic* E. coli *(ETEC) pathogens possess colonization factors (fimbrial adhesins). These bind the bacteria to specific receptors on the cell membrane of the small intestine (Fig. 23.5). They are a major cause of traveller's diarrhoea as well as ETEC-induced diarrhoea in the swine industry. ETEC produce powerful plasmid-associated enterotoxins, which

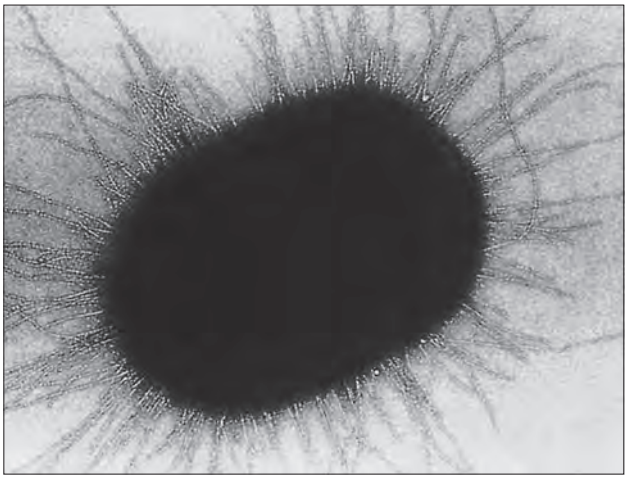


Figure 23.5 Electron micrograph of enterotoxin *E. coli*, showing pili necessary for adherence to mucosal epithelial cells. (Courtesy of S. Knutton.)

are characterized as being either heat labile (LT) or heat stable (ST):

- Heat-labile enterotoxin LT-I is very similar in structure and mode of action to cholera toxin produced by *V. cholerae*, and infections with strains producing LT-I can mimic cholera, particularly in young and malnourished children (Table 23.3).
- Other ETEC strains produce heat-stable enterotoxins (STs) in addition to or instead of LT. STs have a similar but distinct mode of action to that of LT. ST_a activates guanylate cyclase activity, causing an increase in cyclic guanosine monophosphate, which results in increased fluid secretion. Immunoassays are commercially available for the identification of ETEC but multiplex polymerase chain reaction (PCR) methods for detecting enterotoxin have been developed.

Enterohaemorrhagic* E. coli *(EHEC) isolates produce a verotoxin. EHEC is a subset of shiga-toxin-producing *E. coli* (STEC) and the verotoxin (i.e. toxic to tissue cultures of

Table 23.2 General overview of enteric *E. coli* pathotypes

Pathotype	Host(s)	Site of colonization	Disease(s)	Known reservoir(s)/source(s) of contamination	Treatment	Adhesion[a]	Genetic identifiers
tEPEC	Children <5 yrs, adults at high inocula	Small intestine	Profuse watery diarrhoea	Humans	Oral rehydration, antibiotics for persistent cases	Attaching and effacing	*eae*$^{+}$, *bfp*$^{+}$, *stx*$^{-}$
aEPEC				Humans, animals			*eae*$^{+}$, *stx*$^{-}$
STEC	Adults, children	Distal ileum, colon	Watery diarrhoea, hemorrhagic colitis, HUS	Humans, animals, food, water	Hydration, supportive for HUS	Attaching and effacing[b]	*eae*$^{+/-}$, *stx*$^{+}$
EIEC/*Shigella*	Children <5 yr, adults, travellers, immunocompromised persons	Colon	Shigellosis/bacillary dysentery, potential HUS	Humans, animals, food, water	Oral rehydration, antibiotics	NA (invasive)	*ipaH*$^{+}$, *ial*$^{+}$, *stx*$^{+}$ (*S. dysenteriae*)
EAEC	Adults	Small intestine and/or colon	Traveler's diarrhoea, HUS (*stx*+)	Food, occasionally adult carriers	Antibiotics, oral rehydration	Stacked brick and/or invasive	*aatA*$^{+}$, *aaiC*$^{+}$, other candidates
	Children		Persistent diarrhoea		Antibiotics, oral rehydration, potentially probiotics		
	Immunocompromised persons		Persistent diarrhoea		Fluoroquinolones		
ETEC	Children <5 yrs, travelers	Small intestine	Watery diarrhoea	Food, water, humans, animals	Rehydration, antibiotics	CF mediated	CFs, LT, ST
DAEC	Children (increasing in severity from 18 months to 5 yrs), adults	Intestine (uncharacterized location)	Persistent watery diarrhoea in children, speculated to contribute to Crohn's disease in adults	Unknown	Rehydration	Diffuse adherent and/or invasive	No uniform markers
AIEC	Adults, children	Small intestine	Crohn's disease	Unknown	Antibiotics, surgical resection	NA (invasive)	Uncharacterized

[a]NA, not applicable.
[b]Only for LEE-positive STEC, not for LEE-negative STEC.
(From Croxen M.A., Law R.J., Scholz R. et al. Recent advances in understanding enteric pathogenic *Escherichia coli*. *Clin Mic Rev* 2013; 26:822–880, Table 1, with permission.)

Table 23.3 The clinical features of bacterial diarrhoeal infection

Pathogen	Incubation period	Duration	Symptoms			
			Diarrhoea	Vomiting	Abdominal cramps	Fever
Salmonella	6 h–2 days	48 h–7 days	Watery	+	+	+
Campylobacter	2–11 days	3 days–3 weeks	Bloody	–	+	+
Shigella	1–4 days	2–3 days	Bloody	–	+	+
Vibrio cholerae	2–3 days	Up to 7 days	Watery	+	+	–
Clostridium perfringens	8 h–1 day	12 h–1 day	Watery	–	+	–
Bacillus cereus						
Diarrhoeal	8 h–12 h	12 h–1 day	Watery	–	+	–
Emetic	15 min–4 h	12 h–2 days	Watery	+	+	–
Yersinia enterocolitica	4–7 days	1–2 weeks	Bloody	–	+	+
Enteropathogenic *E. coli* (EPEC)	1–2 days	weeks	Watery	+	+	+
Enterotoxigenic *E. coli* (ETEC)	1–7 days	2–6 days	Watery	+	+	–
Enterohaemorrhagic *E. coli* (EHEC)	3–4 days	5–10 days	Bloody	+	+	–
Enteroinvasive *E. coli* (EIEC)	1–3 days	7–10 days	Bloody	+	+	+

It is difficult, if not impossible, to determine the likely cause of a diarrhoeal illness on the basis of clinical features alone, and laboratory investigations are essential to identify the pathogen.

'vero' cells) is essentially identical to shiga (*Shigella*) toxin. After attachment to the mucosa of the large intestine (by the 'attaching–effacing' mechanism also seen in EPEC), the produced toxin has a direct effect on intestinal epithelium, resulting in diarrhoea (Table 23.3). EHEC causes haemorrhagic colitis (HC) and haemolytic–uraemic syndrome (HUS). In HC, there is destruction of the mucosa and consequent haemorrhage; this may be followed by HUS. Verotoxin receptors have been identified on renal epithelium and may account for kidney involvement. While there are many serotypes of EHEC, the most common is O157:H7 and there are many reports worldwide of its association with severe illness. Cattle are major reservoirs for pathogenic STEC and human illness occurs after exposure to faecal material via contaminated water and food and also associated with poor hand hygiene after visiting petting farms. STEC can survive in soil for months.

Enteroinvasive* E. coli *(EIEC) pathogens attach specifically to the mucosa of the large intestine. They invade the cells by endocytosis by using plasmid-associated genes. Inside the cell, they lyse the endocytic vacuole, multiply and spread to adjacent cells, causing tissue destruction, inflammation, necrosis and ulceration, resulting in blood and mucus in stools (Table 23.3).

Enteroaggregative* E. coli *(EAEC) pathogens derive their name from their characteristic attachment pattern to tissue culture cells. The pattern is an aggregative or 'stacked brick' formation. These organisms adhere to the small intestinal mucosa to cause persistent diarrhoea, especially in children in resource-poor countries. Their aggregative adherence ability is due to plasmid-associated fimbrial adhesins. EAEC pathogens also produce heat-labile toxins (an enterotoxin and a toxin related to *E. coli* haemolysin) but their role in diarrhoeal disease is uncertain. The last stage of the EAEC pathogenesis model involves the host innate immune mechanism and strain of EAEC influencing the amount of inflammation. EAEC has caused many large outbreaks of diarrhoea across the world and is associated with traveller's diarrhoea via contaminated water and food.

Diffusely adherent* E. coli *(DAEC) pathogens produce an alpha haemolysin and cytotoxic necrotizing factor 1. They attach to cells but are not classified under localized adherence or attachment / effacing. Their role in diarrhoeal disease, especially in young children, is incompletely understood and somewhat controversial, with some studies reporting no association as DAEC was detected in healthy age-matched controls.

EPEC and ETEC are the most important contributors to global incidence of diarrhoea, whereas EHEC is more important in resource-rich countries. The diarrhoea produced by *E. coli* varies from mild to severe, depending upon the strain and the underlying health of the host. ETEC diarrhoea in children in resource-poor countries may be clinically indistinguishable from cholera. EIEC and EHEC strains both cause bloody diarrhoea (Table 23.3). Following EHEC infection, HUS is characterized by acute renal failure (Fig. 23.6), anaemia and thrombocytopenia, and there may be neurological complications. HUS is the most common cause of acute renal failure in children in the UK and USA. Although *E. coli* O157:H7 is the most commonly recognized serotype involved in HUS, *E. coli* 0104:H4, which had not been reported as causing an outbreak previously, caused a significant outbreak of HUS and bloody diarrhoea in 15 countries across Europe in 2011. Over several months starting in May 2011, 860 individuals with HUS and over 3000 with bloody diarrhoea were reported in Germany, many of whom had laboratory-confirmed *E. coli* 0104:H4 infection. More than 50 people died and the likely vehicle was sprouted beans imported from the Middle East. Detecting *E. coli* O157:H7 is a key focus; non-0157-H7 is a major contributor to both sporadic cases and outbreaks

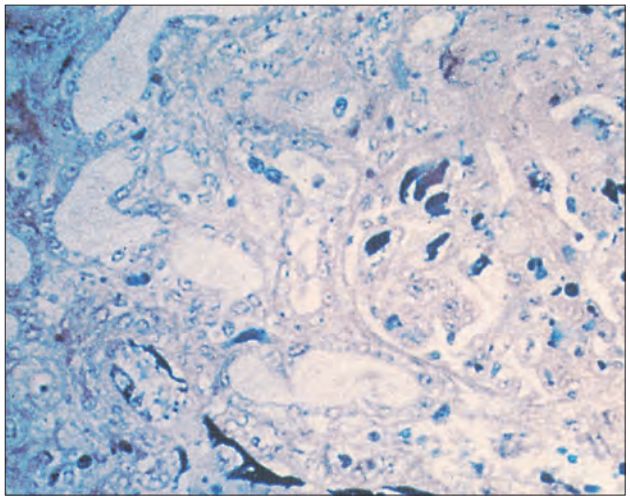


Figure 23.6 Verotoxin-producing *E. coli* infection, showing fibrin 'thrombi' in glomerular capillaries in haemolytic–uraemic syndrome (Weigert stain). (Courtesy of H.R. Powell.)

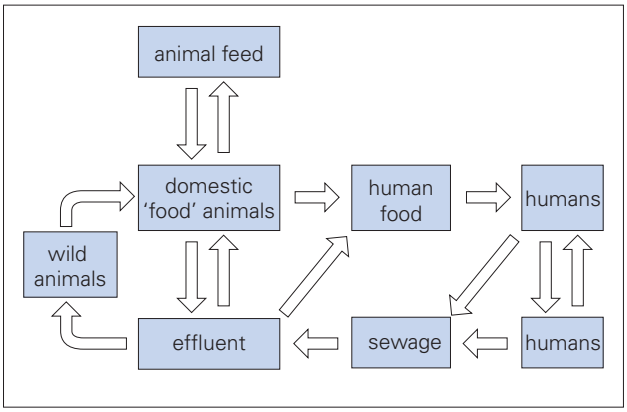


Figure 23.7 The recycling of salmonellae. With the exception of *Salmonella typhi*, salmonellae are widely distributed in animals, providing a constant source of infection for humans. Excretion of large numbers of salmonellae from infected individuals and carriers allows the organisms to be 'recycled'.

in North America, Australia and Europe. In the USA, The Foodborne Diseases Active Surveillance Network (FoodNet) has been reporting trends for infections transmitted via food since 1996. In 2014, the incidence rate of *E. coli* O157:H7 was 0.91 per 100 000 people, highest in under-5-year-olds and 16% of infections were associated with outbreaks. Non-0157 had an incidence of 1.43 per 100 000 people in mostly the same age group and 6% of infections were associated with outbreaks. Most of both STEC infections were found in July.

***Specific tests are needed to identify strains of pathogenic* E. coli.** Because *E. coli* is a member of the normal gastrointestinal flora, specific tests are required to identify strains that may be responsible for diarrhoeal disease. Infections are more common in children and are also often travel associated, and these factors should be considered when samples are received in the laboratory. It is important to note that specialized tests beyond routine stool cultures are required to identify specific diarrhoea-associated *E. coli* types. Such tests are not ordinarily performed with uncomplicated diarrhoea, which is usually self-limiting. However, concern regarding EHEC (e.g. bloody diarrhoea) has led most laboratories in resource-rich countries to screen for *E. coli* O157:H7.

Antibiotic therapy is not indicated for* E. coli *diarrhoea. Specific antibacterial therapy is not indicated. Fluid replacement may be necessary, especially in young children. Treatment of HUS is urgent and may involve dialysis.

Provision of a clean water supply and adequate systems for sewage disposal are fundamental to the prevention of disease. Food and unpasteurized milk can be important vehicles of infection, especially for EIEC and EHEC, but there is no evidence of an animal or environmental reservoir.

Salmonella

Salmonellae are the most common cause of food-associated diarrhoea in many resource-rich countries. However, in some countries such as the USA and UK, they have been relegated to second place by *Campylobacter*. FoodNet reported that, in 2014 in the USA, the incidence rate was 15.3 per 100 000 population, mostly in under-5-year-olds. The majority of serotypes were enteridis (19%), typhimurium (11%) and Newport (10%) and 6% of infections were associated with an outbreak. Like *E. coli*, the salmonellae belong to the family Enterobacteriaceae. Historically, salmonella nomenclature has been somewhat confusing, with more than 2000 serotypes defined on the basis of differences in the cell wall (O) and flagellar (H) antigens (Kauffmann–White scheme). However, DNA hybridization studies indicate that there are only two species, the most important of which, for human infection, is *Salmonella enterica*. *S. enterica* serovars Typhi, Paratyphi A, B and C are known as typhoidal *Salmonella*, restricted to humans and cause typhoid and paratyphoid fever, together called enteric fever. The rest are known as non-typhoidal *Salmonella*. To simplify discussion and comparison, past convention has been to replace this species name with the serotype designation. While technically incorrect (the serotype is not a species), this practice is helpful when discussing interrelationships between different isolates (e.g. in epidemiological analysis when tracing the source of an outbreak). This convention is thus followed here to maintain continuity with other scientific literature.

All salmonellae except for *Salmonella typhi* and *S. paratyphi* are found in animals as well as humans. There is a large animal reservoir of infection, which is transmitted to humans via contaminated food, especially poultry and dairy products (Fig. 23.7). Water-borne infection is less frequent. Salmonella infection is also transmitted from person to person, and secondary spread can therefore occur, for example, within a family after one member has become infected after consuming contaminated food. It has been estimated that, in 2010, there were nearly 12 million typhoid fever illnesses and 129 000 deaths in low- and middle-income countries.

Salmonellae are almost always acquired orally in food or drink that is contaminated with human faeces. The host barrier to infection involves gastric acid secretion, safe in the knowledge that the bacterium is acid susceptible. However, keeping one step ahead, a fluid movement really as they have flagella, gastric acid secretion has been shown to be suppressed during the acute infection. Diarrhoea is produced as a result of invasion by the salmonellae of epithelial cells in the terminal portion of the small intestine (Fig. 23.8).

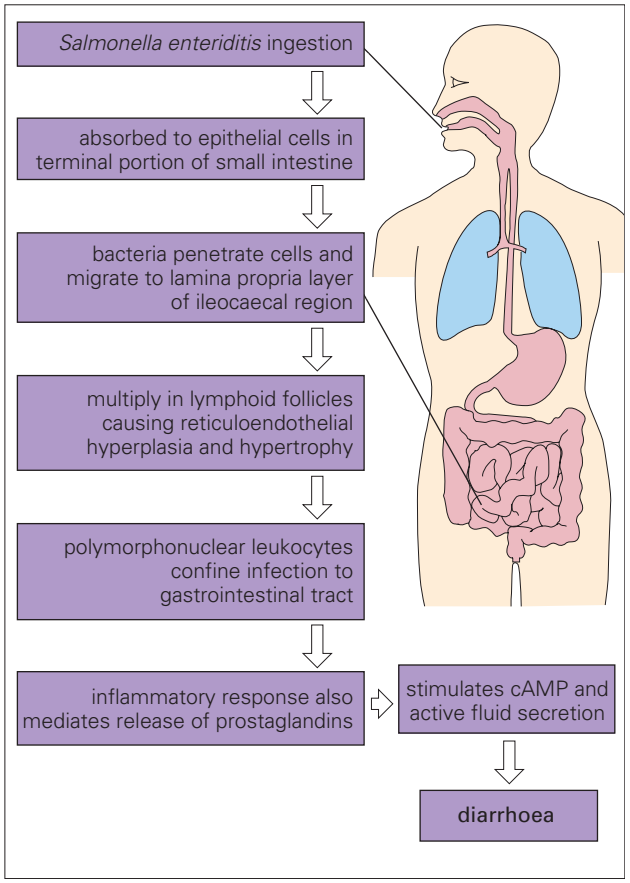


Figure 23.8 The passage of salmonellae through the body. The vast majority of salmonellae cause infection localized to the gastrointestinal tract and do not invade beyond the gut mucosa. cAMP, cyclic adenosine monophosphate.

Initial entry is probably through uptake by M cells (the 'antigenic samplers' of the bowel) with subsequent spread to epithelial cells. A similar route of invasion occurs in *Shigella*, *Yersinia* and reovirus infections. The bacteria migrate to the lamina propria layer of the ileocaecal region, where their multiplication stimulates an inflammatory response, which both confines the infection to the gastrointestinal tract and mediates the release of prostaglandins. These in turn activate cyclic adenosine monophosphate (cAMP) and fluid secretion, resulting in diarrhoea.

Species of *Salmonella* that normally cause diarrhoea (e.g. *S. enteritidis*, *S. choleraesuis*) may become invasive in patients with particular predispositions including children, immunocompromised patients or those with sickle cell anaemia. The organisms are not contained within the gastrointestinal tract, but invade the body to cause septicaemia; consequently, many organs become seeded with salmonellae, sometimes leading to osteomyelitis, pneumonia or meningitis.

In the vast majority of cases, *Salmonella* spp. cause an acute but self-limiting diarrhoea, though in the young and the elderly the symptoms may be more severe. Vomiting is also common with enterocolitis, while fever is usually a sign of invasive disease (Table 23.3). *S. typhi* and *S. paratyphi* invade the body from the gastrointestinal tract to cause systemic illness and are discussed in a later section.

Salmonella diarrhoea can be diagnosed by culture on selective media. The organisms are not fastidious and can usually be isolated within 24 h, although small numbers may require enrichment in selenite broth before culture. The best time to detect the bacteria in the bloodstream is in the first or second week of illness. Culture is diagnostic and the isolate is then tested for antibiotic sensitivity, can be typed and characterized using molecular techniques for epidemiological purposes. Isolates must be dealt with carefully as they have been a common cause of laboratory-acquired infection.

The classic antibody detection method is called the Widal test, an agglutination assay carried out on serum samples, detecting antibodies against the lipopolysaccharide (O) and flagella (H) antigens of *S. typhi*. There are time delay issues as it involves testing acute and convalescent sera, collected 10 days apart, looking for a fourfold rise in titre. Commercially available serological tests have been developed, as have molecular assays to make a rapid diagnosis.

Fluid and electrolyte replacement may be needed for* Salmonella *diarrhoea. Diarrhoea is usually self-limiting and resolves without treatment. Fluid and electrolyte replacement may be required, particularly in the very young and the elderly. Unless there is evidence of invasion and septicaemia, antibiotics should be positively discouraged because they do not reduce the symptoms or shorten the illness, and may prolong excretion of salmonellae in the faeces. There is some evidence that symptomatic treatment with drugs that reduce diarrhoea has the same adverse effect.

Salmonellae may be excreted in the faeces for several weeks after a salmonella infection. Fig. 23.7 illustrates the problems associated with the prevention of salmonella infections. The large animal reservoir makes it impossible to eliminate the organisms, and preventive measures must therefore be aimed at 'breaking the chain' between animals and humans, and from person to person. Such measures include:

- maintaining adequate standards of public health (clean drinking water and proper sewage disposal)
- education programmes on hygienic food preparation.

Following an episode of *Salmonella* diarrhoea, an individual can continue to carry and excrete organisms in the faeces for several weeks. Although in the absence of symptoms, the organisms will not be dispersed so liberally into the environment, thorough hand washing before food handling is essential. People employed as food handlers are excluded from work until three specimens of faeces have failed to grow *Salmonella*.

Campylobacter

Campylobacter *infections are among the most common causes of diarrhoea.* *Campylobacter* spp. are curved or S-shaped Gram-negative rods (Fig. 23.9). They have long been known to cause diarrhoeal disease in animals, but are also one of the most common causes of diarrhoea in humans. The delay in recognizing the importance of these organisms was due to their cultural requirements, which differ from those of the enterobacteria as they are microaerophilic and thermophilic (growing well at 42°C); they do not therefore grow on the media used for isolating *E. coli* and salmonellae. Several species of the genus *Campylobacter* are associated with human disease, but *C. jejuni* is by far the most common, together with

C. coli, and is a major cause of gastroenteritis worldwide. It may also result in Guillain–Barré syndrome, an autoimmune condition (Fig. 23.10). *Helicobacter pylori*, previously classified as *Campylobacter pylori*, is an important cause of gastritis and gastric ulcers (see below).

As with salmonellae, there is a large animal reservoir of *Campylobacter* in cattle, sheep, rodents, poultry and wild birds. Infections are acquired by consumption of contaminated food, especially poultry, milk or water. Studies have shown an association between infection and consumption of milk from bottles with tops that have been pecked by wild birds. Household pets such as dogs and cats can become infected and provide a source for human infection, particularly for young children. Person-to-person spread by the faecal–oral route is rare, as is transmission from food handlers.

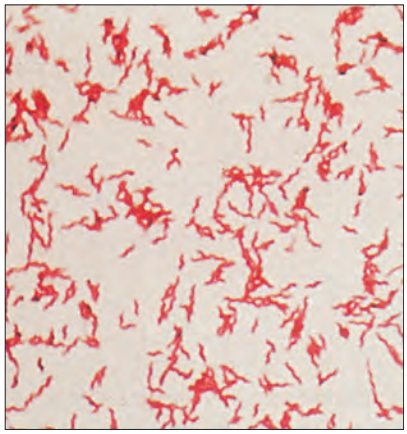


Figure 23.9 *Campylobacter jejuni* infection. Gram stain showing Gram-negative, S-shaped bacilli. (Courtesy of I. Farrell.)

Campylobacter *diarrhoea is clinically similar to that caused by other bacteria such as* Salmonella *and* Shigella. The gross pathology and histological appearances of ulceration and inflamed bleeding mucosal surfaces in the jejunum, ileum and colon (Fig. 23.11) are compatible with invasion of the bacteria, but the production of cytotoxins by *C. jejuni* has also been demonstrated. Invasion and bacteraemia are not uncommon, particularly in neonates and debilitated adults.

The clinical presentation is similar to that of diarrhoea caused by salmonellae and *Shigella*, although the disease may have a longer incubation period and a longer duration. The key features are summarized in Table 23.3.

Cultures for* Campylobacter *should be set up routinely in every investigation of a diarrhoeal illness. *Campylobacter*-selective media and conditions for growth differ from those required for the enterobacteria. Growth is often somewhat slow compared with that of the enterobacteria, but a presumptive identification should be available within 48 h of culture.

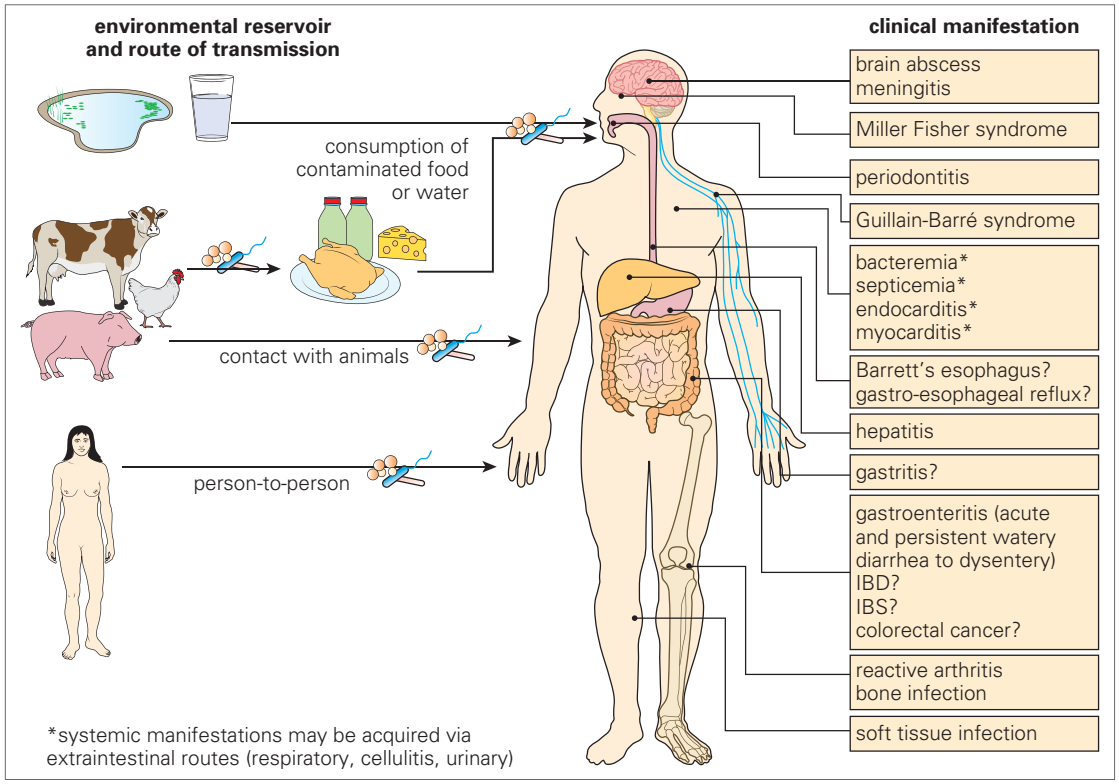


Figure 23.10 Environmental reservoirs, routes of transmission, and clinical manifestations associated with *Campylobacter* species. *Campylobacter* species can be transmitted to humans through consumption of undercooked or contaminated food or via contact with animals. Ingestion of a sufficient dose of organisms via the oral–gastric route may lead to one or more gastrointestinal and/or extragastrointestinal manifestations; the outcome is dependent on the species or strains of *Campylobacter* involved in the infection. IBD, inflammatory bowel diseases; IBS, irritable bowel syndrome. Question marks indicate conditions for which a role for *Campylobacter* is implicated but not certain. (Reproduced from Kaakoush N.O., Castaño-Rodríguez N., Mitchell H.M., Man S.M. Global epidemiology of *Campylobacter* infection, *Clin Micro Rev* 2015; 28[3]:687–720, Fig 1, with permission.)

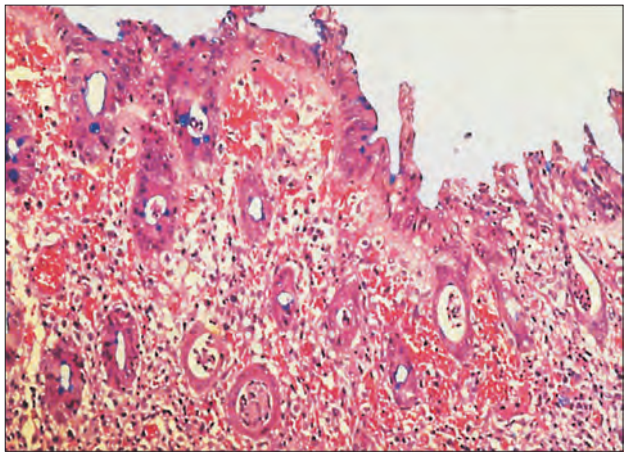


Figure 23.11 Inflammatory enteritis caused by *Campylobacter jejuni*, involving the entire mucosa, with flattened atrophic villi, necrotic debris in the crypts and thickening of the basement membrane (Cresyl-fast violet stain). (Courtesy of J. Newman.)

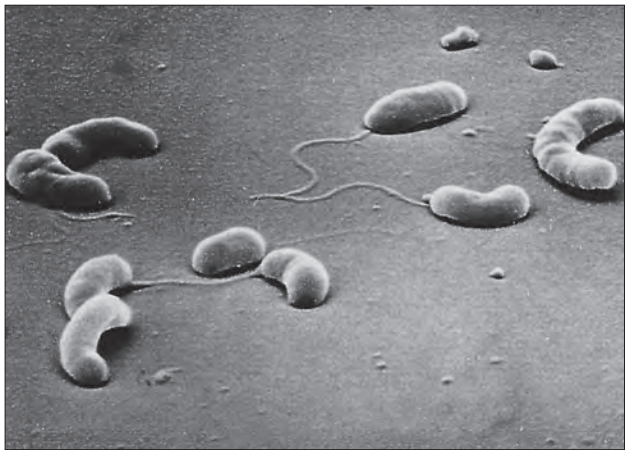


Figure 23.12 Scanning electron micrograph of *Vibrio cholerae* showing comma-shaped rods with a single polar flagellum (×13 000). (Courtesy of D.K. Banerjee.)

Azithromycin is used for severe* Campylobacter *diarrhoea. Most people with *Campylobacter* infections recover without antibiotic treatment. Macrolide antibiotics such as azithromycin can be used in severe diarrhoeal disease. Invasive infections may require treatment with an additional antibiotic such as a fluoroquinolone (e.g. ciprofloxacin), but resistance is common.

The preventive measures for *Salmonella* infections described above are equally applicable to the prevention of *Campylobacter* infections, but there are no requirements for the screening of food handlers because contamination of food by this route is very uncommon.

Cholera

Cholera is an acute infection of the gastrointestinal tract caused by the comma-shaped Gram-negative bacterium *V. cholerae* (Fig. 23.12). The disease has a long history characterized by epidemics and pandemics. The last cases of cholera acquired in the UK were in the nineteenth century following the introduction of the bacterium by sailors arriving from Europe, and in 1849 Snow published his historic essay *On the Mode of Communication of Cholera* proposing that it was a communicable disease and that infectious material was contained in the faeces.

Cholera flourishes in communities with inadequate clean drinking water and sewage disposal. The disease remains endemic in over 50 countries, especially South-East Asia and parts of Africa and South America. It is estimated there are 3–5 million people infected annually. Unlike salmonellae and *Campylobacter*, *V. cholerae* is a free-living inhabitant of fresh water, but causes infection only in humans. Asymptomatic human carriers are believed to be a major reservoir. The disease is spread via contaminated food; shellfish grown in fresh and estuarine waters have also been implicated. Direct person-to-person spread is thought to be uncommon. Therefore, cholera continues to flourish in communities where there is absent or unreliable provision of clean drinking water and sewage disposal. Natural disasters, such as floods and earthquakes, can result in a breakdown of public health facilities and cause cholera epidemics. In 2010, after a devastating earthquake in Haiti, over 7000 people died of cholera and by 2014 over 750 000 people had been infected.

V. cholerae *is classified into more than 200 serogroups based on the somatic (O) antigens of the lipopolysaccharide.* Only O1 and O139 serogroups cause epidemic cholera. O1 is the most important and is further divided into two biotypes: classical and El Tor (Fig. 23.13). The El Tor biotype, named after the quarantine camp where it was first isolated from pilgrims returning from Mecca, differs from classical *V. cholerae* in several ways. In particular, it causes only mild diarrhoea and has a higher ratio of carriers to cases than classic cholera; carriage is also more prolonged, and the organisms survive better in the environment. The El Tor biotype, which was responsible for the seventh pandemic, has now spread throughout the world and has largely displaced the classic biotype.

In 1992, a new strain, O139, arose in south India. It spread rapidly, infected O1-immune individuals, caused epidemics, and was the eighth pandemic strain of cholera. *V. cholerae* O139 appeared to have originated from the El Tor O1 biotype when the latter acquired a new O (capsular) antigen by horizontal gene transfer from a non-O1 strain, but is almost identical to O1 El Tor. This provided the recipient strain with a selective advantage in a region where a large part of the population was immune to O1 strains.

Other species of *Vibrio* cause a variety of infections in humans (Fig. 23.13). *V. parahaemolyticus* is another cause of diarrhoeal disease, but this is usually much less severe than cholera (see below).

The symptoms of cholera are caused by an enterotoxin. The symptoms of cholera are entirely due to the production of an enterotoxin in the gastrointestinal tract. This protein exotoxin has an A subunit and pentameric B subunit. The A subunit activates adenylate cyclase causing intracellular cAMP to rise resulting in chloride secretion and secretory diarrhoea. The B subunit binds to the ganglioside GM1 site on eukaryotic cells. *V. cholera* has additional virulence factors to enable it to survive the host defences. These are illustrated in Fig. 23.14 (see also Ch. 14).

The clinical features of cholera are summarized in Table 23.3. The severe watery non-bloody diarrhoea is known as rice water stool because of its appearance (Fig. 23.15) and can result in the loss of 1 litre of fluid every hour. It is this fluid

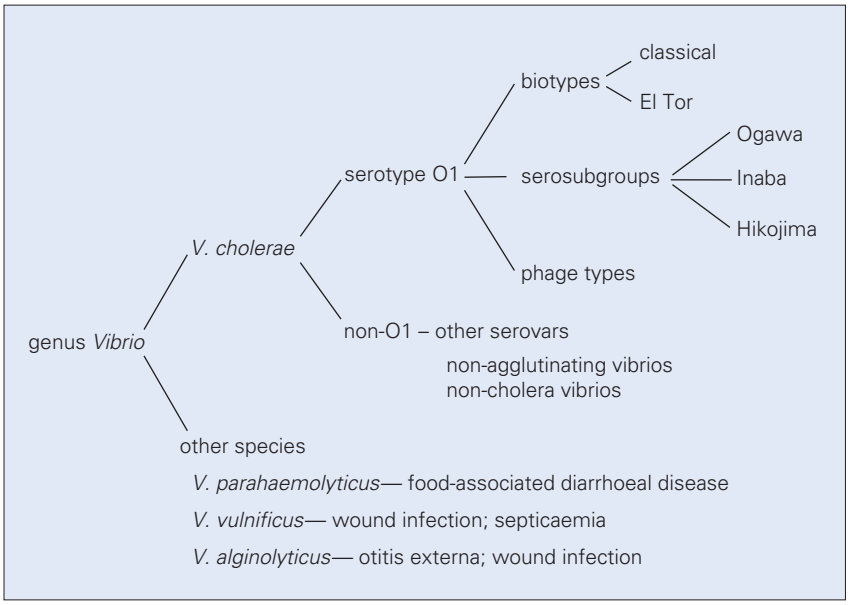


Figure 23.13 *Vibrio cholerae* serotype O1, the cause of cholera, can be subdivided into different biotypes with different epidemiological features, and into serosubgroups and phage types for the purposes of investigating outbreaks of infection. Although *V. cholerae* is the most important pathogen of the genus, other species can also cause infections of both the gastrointestinal tract and other sites.

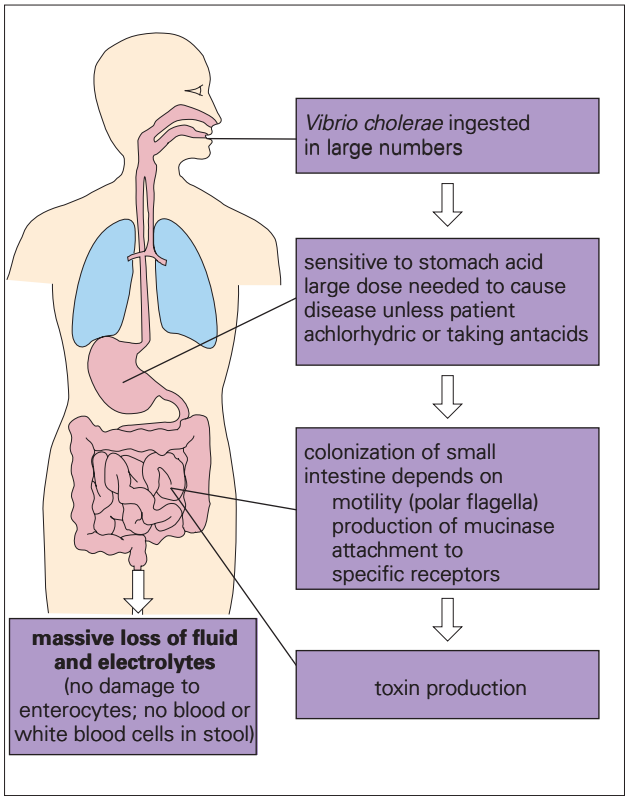


Figure 23.14 The production of an enterotoxin is central to the pathogenesis of cholera, but the organisms must possess other virulence factors to allow them to reach the small intestine and to adhere to the mucosal cells.

loss and the consequent electrolyte imbalance that results in marked dehydration, metabolic acidosis (loss of bicarbonate), hypokalaemia (potassium loss) and hypovolaemic shock resulting in cardiac failure. Untreated, the mortality from cholera is 40–60%; rapidly instituted fluid and electrolyte replacement reduce the mortality to <1%.

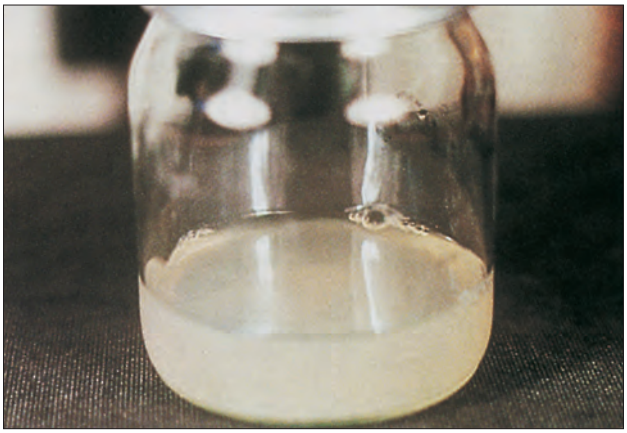


Figure 23.15 Rice water stool in cholera. (Courtesy of A.M. Geddes.)

Culture is necessary to diagnose sporadic or imported cases of cholera and carriers. In countries where cholera is prevalent, diagnosis is based on clinical grounds, and laboratory confirmation is rarely sought. It is worth remembering that ETEC infection can resemble cholera in both its severity and the management of infected individuals, as fluid and electrolyte replacement are of paramount importance.

Prompt rehydration with fluids and electrolytes is central to the treatment of cholera. Oral or intravenous rehydration is critical in the management of those affected. Antibiotics are helpful in moderate to severe dehydration, as they reduce the duration of excretion of *V. cholerae* thereby reducing the risk of transmission as well as shortening the duration and volume of diarrhoea. The antibiotics must be chosen on the basis of antimicrobial resistance patterns locally. Tetracycline-resistant *V. cholerae* is common, susceptibility to quinolones has become common in endemic areas and macrolides such as azithromycin and erythromycin may be more effective.

As with other diarrhoeal disease, a clean drinking water supply and adequate sewage disposal are fundamental to the prevention of cholera. As there is no animal reservoir, it should in theory be possible to eliminate the disease. However, carriage in humans, albeit for only a few weeks, occurs in 1–20% of previously infected patients, making eradication difficult to achieve.

Cholera vaccines are not recommended for most travellers. A killed whole-cell vaccine is available and is given parenterally, but is effective in only about 50% of those vaccinated, with protection lasting for only 3–6 months. It is no longer recommended by the World Health Organization (WHO) for travellers to cholera-endemic areas, although it may be required in certain countries. A number of live attenuated oral vaccines have been developed and are being evaluated.

Shigellosis

Symptoms of* Shigella *infection range from mild to severe gastroenteritis, depending upon the infecting species. *Shigella* and *E. coli* are similar genetically and are Gram-negative rods. *Shigellosis* is also known as bacillary dysentery (in contrast to amoebic dysentery; see below) because in its more severe form it is characterized by an invasive infection of the mucosa of the large intestine, causing inflammation and resulting in the presence of pus and blood in the diarrhoeal stool. However, symptoms range from mild to severe depending upon the species of *Shigella* involved and on the underlying state of health of the host. There are four species (also referred to as subgroups):

- *Shigella sonnei* causes most infections at the mild end of the spectrum.
- *S. flexneri* and *S. boydii* usually produce more severe disease.
- *S. dysenteriae* is the most serious.

Shigellosis is primarily a paediatric disease. Globally, the incidence of shigellosis is estimated at around 165 million infections but there has been a significant reduction in the mortality rate over the last 30 years. When associated with severe malnutrition it may precipitate complications such as the protein deficiency syndrome 'kwashiorkor'. Like *V. cholerae*, shigellae are human pathogens without an animal reservoir but, unlike the vibrios, they are not found in the environment, being spread from person to person by the faecal–oral route and less frequently by contaminated food and water. Shigellae appear to be able to initiate infection from a small infective dose of only 10–100 organisms and therefore spread is easy in situations where sanitation or personal hygiene may be poor. These include refugee camps, nurseries, daycare centres and other residential institutions.

Shigella *diarrhoea is usually watery at first, but later contains mucus and blood.* *Shigella* has a large virulence plasmid that encodes secreted proteins acting on colonic epithelial cells that damage the epithelial lining as well as acting on the host immune response. Shigellae attach to, and invade, the mucosal epithelium of the distal ileum and colon, causing inflammation and ulceration (Fig. 23.16). However, they rarely invade through the gut wall to the bloodstream. *S. dysenteriae* produce a shiga toxin similar to that associated with enterohaemorrhagic *E. coli* (EHEC; see above), which can cause damage to the intestinal epithelium and glomerular endothelial cells, the latter leading to kidney failure (haemolytic–uraemic syndrome, HUS; see *E. coli* section).

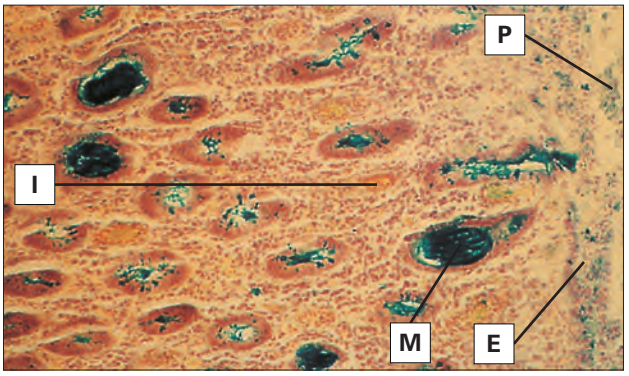


Figure 23.16 Shigellosis. Histology of the colon showing disrupted epithelium covered by pseudomembrane and interstitial infiltration. Mucin glands have discharged their contents and the goblet cells are empty. E, epithelium; I, interstitial infiltration; M, mucin in glands; P, pseudomembrane (colloidal iron stain). (Courtesy of R.H. Gilman.)

The main features of *Shigella* infection are summarized in Table 23.3. Diarrhoea is usually watery initially, but later contains mucus and blood. Lower abdominal cramps can be severe. The disease is usually self-limiting, but dehydration can occur, especially in the young and elderly. Complications can be associated with malnutrition and extraintestinal manifestations can occur.

***Culture and serological typing are helpful in distinguishing* Shigella *from* E. coli.** This is critical for both diagnosis and epidemiological and public health purposes. There are four subgroups, A to D that include *S. dysenteriae* (A), *S. flexneri* (B), *S. boydii* (C) and *S. sonnei* (D).

Antibiotics should be given only for severe* Shigella *diarrhoea. Once again, rehydration is critical and antibiotics, especially those that also decrease intestinal motility, should not be given except in severe cases. Plasmid-mediated resistance is common, and antibiotic susceptibility tests should be performed on *Shigella* isolates if treatment is required.

Education in personal hygiene and proper sewage disposal are important. Infected individuals may continue to excrete shigellae for a few weeks, but longer-term carriage is unusual; therefore, with adequate public health measures and no animal reservoir, the disease is potentially eradicable.

Other bacterial causes of diarrhoeal disease

The pathogens described in the previous sections are the major bacterial causes of diarrhoeal disease. *Salmonella* and *Campylobacter* infections and some types of *E. coli* infections are most often food associated, whereas cholera is more often water borne and shigellosis is usually spread by direct faecal–oral contact.

From a diagnostic perspective (see Ch. 32), although culture, biochemical identification and serological typing are the classical techniques, molecular methods such as mass spectrometry and multiplex PCR panels are being added to the diagnostic armamentarium.

Other bacterial pathogens that cause food-associated infection or food poisoning are described below.

V. parahaemolyticus *and* Yersinia enterocolitica *are food-borne Gram-negative causes of diarrhoea.* *V. parahaemolyticus* is a

halophilic (salt-loving) vibrio found in estuarine, marine and coastal environments and can contaminate seafood and fish. If these foods are consumed uncooked, diarrhoeal disease can result. These bacteria have a number of different virulence factors including adhesins and haemolysins. After binding to the host cell, most strains associated with infection are haemolytic owing to production of a heat-stable cytotoxin and have been shown to invade intestinal cells (in contrast to *V. cholerae*, which is non-invasive and produces cholera toxin, which is not cytotoxic).

The clinical features of infection are summarized in Table 23.3. The methods used for the laboratory diagnosis of *V. parahaemolyticus* infection involve special media for culture. Prevention of infection depends on cooking fish and seafood properly.

Yersinia enterocolitica is a member of the Enterobacteriaceae and is a cause of food-associated infection especially among infants and particularly in the winter months, possibly because the organism can multiply at refrigerator temperatures. *Y. enterocolitica* is a zoonosis and is found in a variety of animal hosts including rodents, rabbits, pigs, sheep, cattle, horses and domestic pets. Transmission to humans from household dogs has been reported. The organism survives and multiplies, albeit more slowly, at low temperatures and has been implicated in outbreaks of infection associated with contaminated milk as well as other foods.

The virulence factors include proteins promoting adhesion and epithelial cell invasion as well as the production of an enterotoxin, but the clinical features of the disease result from invasion of the terminal ileum, necrosis in Peyer's patches and an associated inflammation of the mesenteric lymph nodes (Fig. 23.17). The presentation, with enterocolitis and often mesenteric adenitis, can easily be confused with acute appendicitis, particularly in children. The clinical features are summarized in Table 23.3. As with *V. parahaemolyticus*, an indication of a suspicion of *Yersinia* infection is useful so that the laboratory staff can process the specimen appropriately.

Figure 23.17 *Yersinia enterocolitica* infection of the ileum, showing superficial necrosis of the mucosa and ulceration. (Courtesy of J. Newman.)

Clostridium perfringens *and* Bacillus cereus *are spore-forming Gram-positive rods that cause diarrhoea.* The Gram-negative organisms described in the previous sections invade the intestinal mucosa or produce enterotoxins, which cause diarrhoea. None of these organisms produces spores. Two Gram-positive species are important causes of diarrhoeal disease, particularly in association with spore-contaminated food. These are *Clostridium perfringens* and *Bacillus cereus* and are discussed in the next section.

FOOD POISONING – BACTERIAL TOXIN-ASSOCIATED DIARRHOEA

Toxins elaborated by contaminating bacteria in food before it is consumed include the emetic toxin of *B. cereus*, *Staph. aureus* enterotoxin, *C. botulinum* and *C. perfringens* toxin.

Staphylococcus aureus

Enterotoxigenic strains of *Staph. aureus* are associated with food-borne illness

More than 20 distinct enterotoxin and enterotoxin-like molecules have been reported to be produced by strains of *Staph. aureus*, the classic serotypes are enterotoxins A–E (Table 23.4). All are heat stable and resistant to destruction by enzymes in the stomach and small intestine. Their mechanism of action is incompletely understood; however, similar to the TSST-1 toxin of toxic shock syndrome (see Ch. 27), they generally behave as superantigens (see Ch. 17), binding to major histocompatibility complex (MHC) class II molecules, which results in T-cell stimulation and leads to the production of proinflammatory mediators. Their effect on the central nervous system results in severe vomiting within 3–6 h of consumption. Diarrhoea is not a feature and recovery within 24 h is usual. In addition, the enterotoxins are implicated in autoimmune dysregulation and may be involved in the pathogenesis of inflammatory bowel diseases.

Up to 50% of *Staph. aureus* strains produce enterotoxin and food (especially processed meats) may be contaminated by human carriers; up to 50% of healthy people carry the bacteria on their skin and in their nose. The bacteria grow at room temperature and release toxin. Subsequent heating may kill the organisms, but the toxin is stable and nanogram quantities are sufficient to cause illness. Often there are no viable organisms detectable in the food consumed, but enterotoxin can be detected by a latex agglutination test but immunoassays are more sensitive.

Table 23.4 Staphylococcal enterotoxins

Enterotoxin		
A	Most commonly associated with food poisoning	
B	Associated with staphylococcal enterocolitis (rare)	
C	Rare	Associated with contaminated milk products
D	Second most common	
	Alone or in combination with A	
E	Rare	
TSST-1	Toxic shock syndrome toxin, not food-associated	

Staphylococcus aureus produces at least eight immunologically distinct enterotoxins, the most important of which are listed here. Strains may produce one or more of the toxins simultaneously. Enterotoxin A is by far the most common in food-associated disease.

Botulism

Exotoxins produced by *C. botulinum* cause botulism, which has a mortality rate of about 10%

Botulism is a rare but serious disease caused by the exotoxin of *C. botulinum*. The organism is widespread in the environment, is mesophilic with a minimum and optimum temperature for growth of 12°C and 37°C respectively and spores can be isolated readily from soil samples and from various animals, including fish. There are seven major botulinum neurotoxins, labelled A–G, but only four – A, B, E, and less frequently F – are associated with human disease. While not destroyed by digestive enzymes, the toxins are inactivated after 30 min at 80°C. The toxins are ingested in food (often canned or reheated) or produced in the gut after ingestion of the organism; they are absorbed from the gut into the bloodstream and then reach their site of action: the peripheral nerve synapses. A person need ingest only 30–100 nanograms of neurotoxin to develop botulism. Botulism is characterized by a symmetrical descending flaccid muscle paralysis and starts with the cranial nerves causing blurred vision, swallowing difficulty and slurred speech. Then the respiratory and cardiac muscles are affected if it is not treated quickly. The action of the toxin is to block neurotransmission (see Ch. 17).

Infant botulism is the most common form of botulism

There are three forms of botulism:

1. food-borne botulism
2. infant botulism
3. wound botulism

In food-borne botulism, toxin is elaborated by organisms in food, which is then ingested. Often caused by eating home-canned foods that have undergone inadequate heat processing, the aim is to reach 121°C for 3 minutes. In infant and wound botulism, the organisms are, respectively, ingested or implanted in a wound, and multiply and elaborate toxin in vivo. Infant botulism has been associated with feeding babies honey contaminated with *C. botulinum* spores.

The clinical disease is the same in all three forms and is characterized by flaccid paralysis leading to progressive muscle weakness and respiratory arrest. Intensive supportive treatment is urgently required and complete recovery may take many months. Improvements in supportive care have reduced the mortality from around 70% to approximately 10%, but the disease, although rare, remains life threatening. In addition, since botulinum toxin is one of the most potent biological toxins known, there is concern regarding its potential use as an agent of biowarfare.

Considering botulism in the differential diagnosis is key and then confirming by laboratory diagnosis

Laboratory diagnosis involves demonstrating the presence of toxin in clinical specimens or food or culturing the bacteria. However, a bioassay may need to be used if serum is available, whereby the serum would be injected into mice that have been protected with botulinum antitoxin or left unprotected. Culture of faeces or wound exudate for *C. botulinum* as well as toxin detection by PCR-based assays for toxin sequences and ELISA (see Ch. 32) tests for functional toxin activity.

Polyvalent antitoxin is recommended as an adjunct to intensive supportive therapy for botulism

Since botulinum toxins are antigenic, they can be inactivated and used to produce antitoxin in animals. When botulism is suspected, antitoxin should be promptly administered along with supportive care, which may include mechanical ventilation, due to difficulty in breathing and intravenous and nasogastric nutritional support, due to difficulty in swallowing. Antibiotics are generally used only for treatment of secondary infections.

It is not practical to prevent food becoming contaminated with botulinum spores, so prevention of disease depends on preventing the germination of spores in food by:

- maintaining food at an acid pH
- storing food at <4°C
- inactivating spores by heating at 121°C for 3 minutes before storage
- inactivating toxin by heating for 5 minutes at 80°C

Two Gram-positive species, *Clostridium perfringens* and *Bacillus cereus,* are enterotoxin producers and important causes of diarrhoeal disease, particularly in association with spore-contaminated food. However, much more rarely, *C. perfringens* can also be present in inadequately cooked food, multiply and beta-toxin-producing strains produce an acute necrotizing disease of the small intestine, accompanied by abdominal pain and diarrhoea. The pathogenesis is summarized in Fig. 23.18. This form occurs after the consumption of contaminated meat by people who are unaccustomed to a high-protein diet and do not have sufficient intestinal trypsin to destroy the toxin. It is traditionally associated with the orgiastic pig feasts enjoyed by the natives of New Guinea, but also occurred in people released from prisoner-of-war camps.

The clinical features of the more common enterotoxin type of infection are shown in Table 23.3. *C. perfringens* is an anaerobe and grows readily on routine laboratory media. Enterotoxin production can be demonstrated by a latex agglutination method but more sensitive tests include an ELISA carried out on faecal material and PCR detection.

Antibacterial treatment of *C. perfringens* diarrhoea is rarely required. Prevention depends on thorough reheating of food before serving, or preferably avoiding cooking food too long before consumption.

C. perfringens is also an important cause of wound and soft tissue infections, as described in Chapter 27.

Bacillus cereus is widely distributed in the environment, especially in soil and the spores and vegetative cells contaminate many foods. Food-associated infection takes one of two forms:

- diarrhoea resulting from the production of enterotoxin in the gut
- vomiting due to the ingestion of enterotoxin in food.

Two different toxins are involved in pathogenicity and cause exoenzymes that destroy tissue, as illustrated in Fig. 23.19. In the small intestine, having ingested the spores, the vegetative cells secrete an enterotoxin causing diarrhoea. However, the emetic toxin, which is plasmid encoded, is produced in food products and ingested preformed. The clinical features of the infections are summarized in Table 23.3. *B. cereus* is very serious and can also cause a spectrum of infections

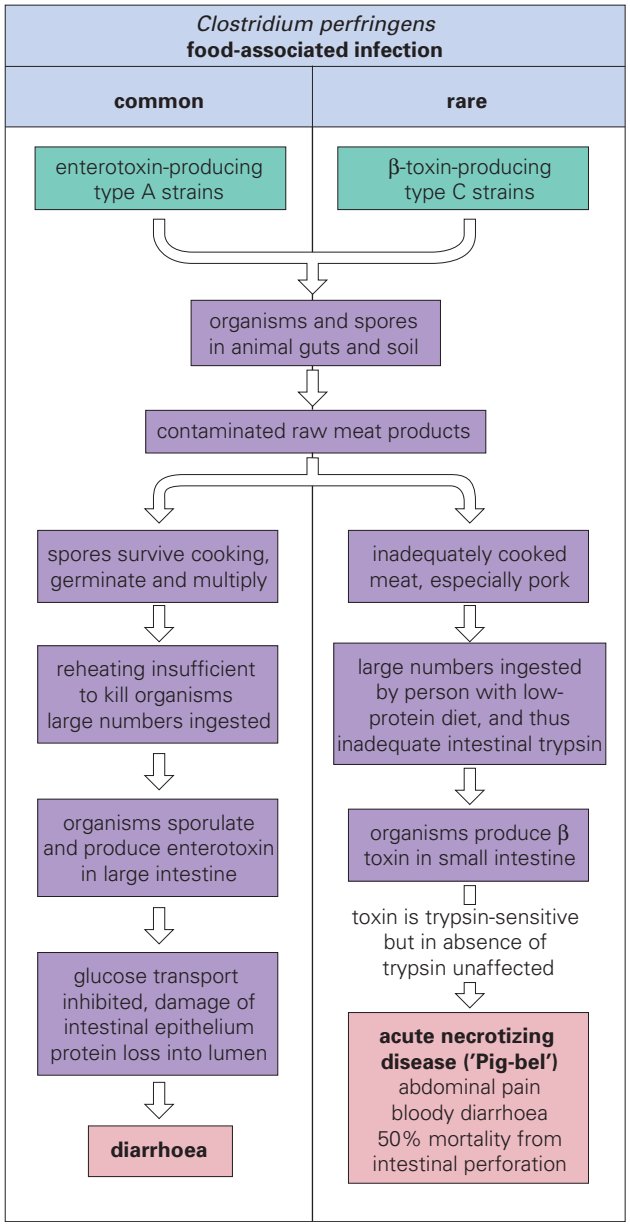


Figure 23.18 *Clostridium perfringens* is linked with two forms of food-associated infection. The common, enterotoxin-mediated infection (left) is usually acquired by eating meat or poultry that has been cooked enough to kill vegetative cells, but not spores. As the food cools, the spores germinate. If reheating before consumption is inadequate (as it often is in mass catering outlets), large numbers of organisms are ingested. The rare form associated with β-toxin-producing strains (right) causes a severe necrotizing disease.

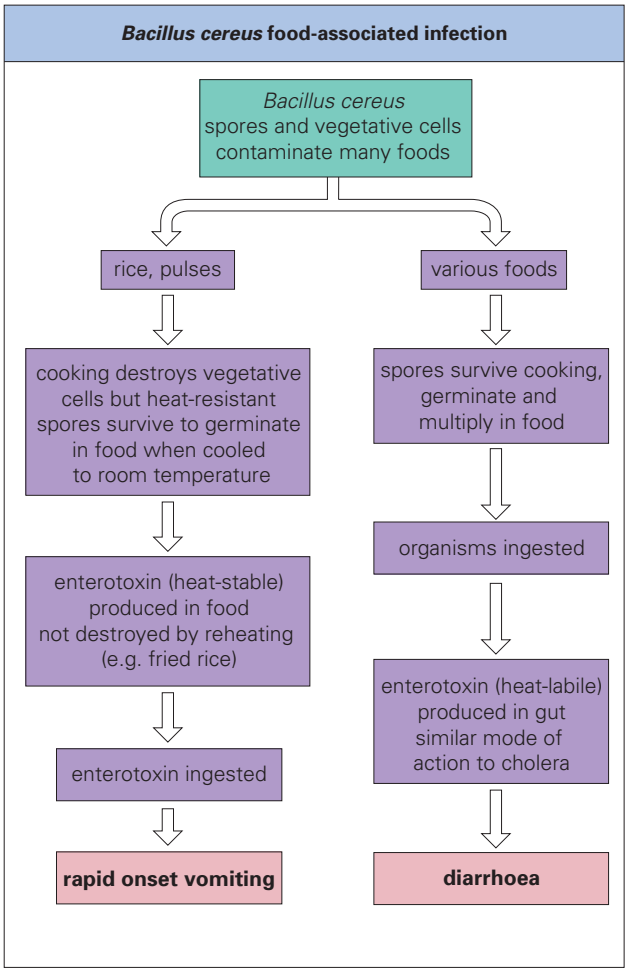


Figure 23.19 *Bacillus cereus* can cause two different forms of food-associated infection. Both involve toxins.

including meningitis, brain abscesses, endophthalmitis and pneumonia. Laboratory confirmation of the diagnosis requires specific media. The emetic type of disease may be difficult to assign to *B. cereus* unless the incriminated food is cultured.

As with *C. perfringens*, prevention of *B. cereus* food-associated infection depends on proper cooking and rapid consumption of food. Specific antibacterial treatment is not indicated in this setting.

Antibiotic-associated diarrhoea – *Clostridium difficile*

Clostridium difficile infection is the most commonly diagnosed bacterial cause of hospital-acquired infectious diarrhoea in resource-rich countries. In the USA, CDC estimated that there were almost 500 000 *C. difficile* infections and 29 000 deaths in 2011. It is the most common cause of healthcare-associated infections in United States hospitals.

Treatment with broad-spectrum antibiotics can be complicated by antibiotic-associated *C. difficile* diarrhoea

All the infections described so far arise from the ingestion of organisms or their toxins. However, diarrhoea can also arise from disruption of the normal gut flora. Even in the early days of antibiotic use, it was recognized that these agents affected the normal flora of the body as well as attacking the pathogens. For example, orally administered tetracycline disrupts the normal gut flora, and patients sometimes become recolonized not with the usual facultative Gram-negative anaerobes but with *Staph. aureus*, causing enterocolitis, or with yeasts such as *Candida*. Soon after clindamycin was introduced for therapeutic use, it was found to be associated with severe diarrhoea in

which the colonic mucosa became covered with a characteristic fibrinous pseudomembrane (pseudomembranous colitis; Fig. 23.20). However, clindamycin is not the cause of the condition; it merely inhibits the normal gut flora and allows *C. difficile* to multiply. This organism is commonly found in the gut of children and to a lesser extent in adults, but can also be acquired from other patients in hospital by cross-infection.

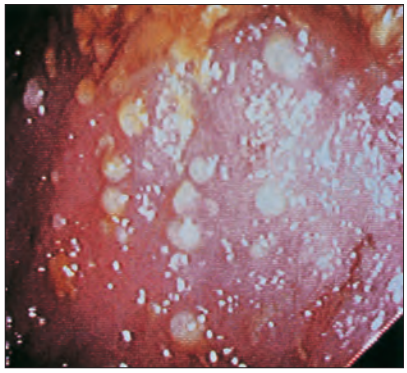


Figure 23.20 Antibiotic-associated colitis due to *Clostridium difficile*. Sigmoidoscopic view showing multiple pseudomembranous lesions. (Courtesy of J. Cunningham.)

C. difficile is a spore former and survives in the environment as it is resistant to heat and acid, for example. The spores contaminate the environment and become vegetative bacteria that can be transmitted between patients on the wards.

In common with other clostridia, *C. difficile* produces exotoxins. Toxin A, an enterotoxin, causes increased intestinal permeability and secretion of fluids and toxin B, a cytotoxin, causes colonic inflammation, haemostasis and tissue necrosis in the colon, resulting in diarrhoea.

Toxin A and toxin B are encoded by the *tcdA* and *tcdB* genes (Fig. 23.21) within a short chromosomal segment carried by pathogenic strains of *C. difficile*, referred to as the pathogenicity locus. Some strains may produce a binary toxin called *C. difficile* transferase (CDT), and its role is not clear as the symptoms are not as severe and the incidence of *C. difficile* infections involving strains that produce only CDT is low.

An emergent epidemic of a *C. difficile* variant strain called *C. difficile* ribotype B1 / NAP1 / 027 has been shown to produce much more toxin A and toxin B. Toxin production is related to spore production, so this is a highly sporulating strain that therefore dominates the environment it inhabits. The increased toxin production causes a number of direct and indirect cytopathic effects causing colonocyte death, the loss of the intestinal barrier function and colitis. This strain detected in the USA, Canada, the UK and other parts of Europe is not

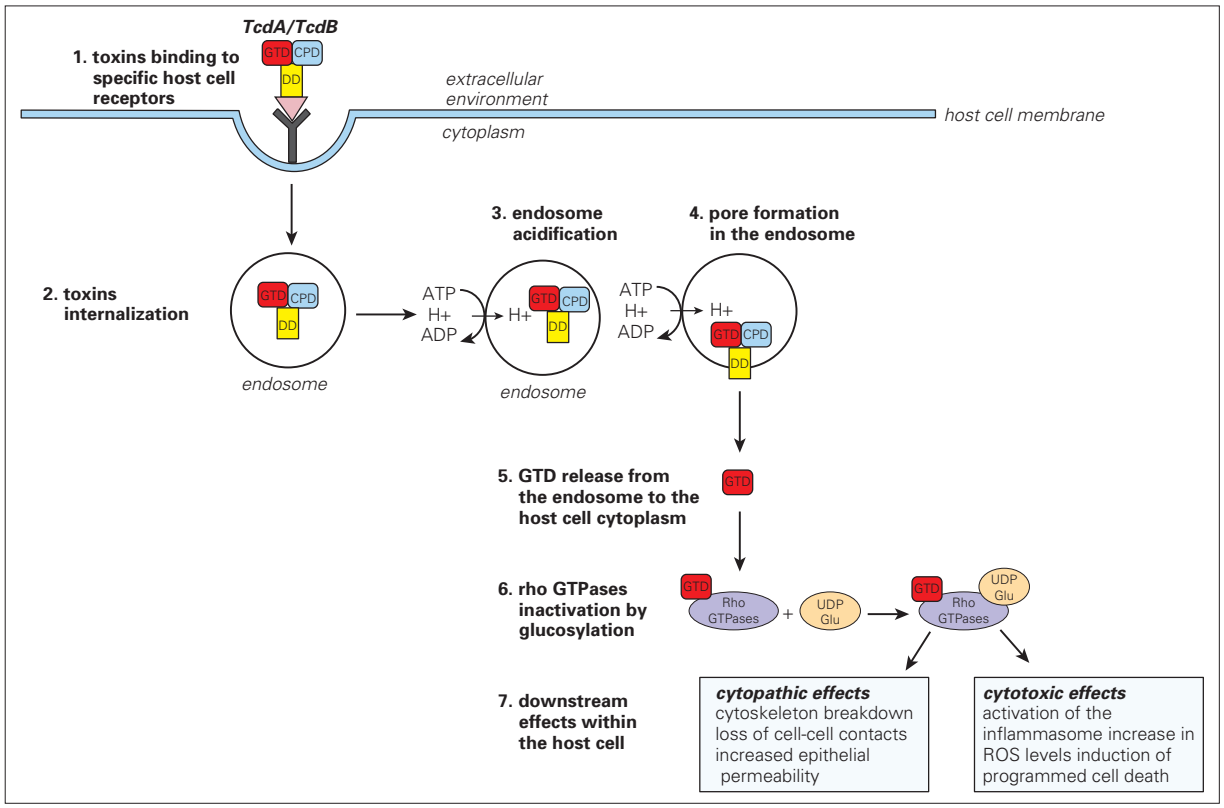


Figure 23.21 Toxins delivery into the host cell cytosol can be divided into seven main steps: (1) toxin binding to the host cell surface receptor, (2) toxins internalization through a receptor-mediated endocytosis, (3) endosome acidification, (4) pore formation, (5) GTD release from the endosome to the host cell cytoplasm, (6) rho GTPases inactivation by glucosylation; and (7) downstream effects within the host cell, i.e. toxins-induced cytopathic and cytotoxic effects. ADP, adenosine diphosphate; ATP, adenosine triphosphate; CPD, cysteine protease domain (cyan); DD, delivery domain (yellow); GTD, *N*-terminal glucosyltransferase domain (red). (Reproduced with permission from Di Bella S., Ascenzi P., Siarakas S et al. *Clostridium difficile* toxins A and B: insights into pathogenic properties and extraintestinal effects. *Toxins* 2016; *8*[5]:134; doi:10.3390/toxins8050134, Fig 2, with permission.)

only highly transmissible but causes more severe disease in individuals in both hospitals and the community. It has been associated with higher case fatality rates, with some infected individuals requiring a colectomy and intensive care unit support, and has also been shown to be more resistant to the fluoroquinolone antibiotics than other strains.

Although initially associated with clindamycin, *C. difficile* diarrhoea has since been shown to follow therapy with many other broad-spectrum antibiotics; hence the term antibiotic-associated diarrhoea or colitis. The infection is often severe and may require treatment with the anti-anaerobic agent metronidazole, or with oral vancomycin. However, the emergence of vancomycin-resistant enterococci, probably originating in the gut flora, has led to the recommendation that oral vancomycin be avoided wherever possible (see Ch. 34).

Hopefully, you are not reading and eating at the same time, as an alternative therapeutic approach, fecal microbiota transplantation is about to be summarized. This is the ingestion of a fecal suspension from a donor in order to reset the diversity of the normal gastrointestinal flora, referred to as the microbiome within the colon. Studies have shown that this is a safe and effective way to treat *C. difficile*-associated diarrhoea. Just in case you are wondering, the routes of administration involve either a nasogastric or nasojejunal tube approach or a rectal tube or colonoscope, but the optimal route is still unclear.

VIRAL CAUSES OF DIARRHOEA

Huge reductions have been seen in diarrhoeal deaths, especially in under-5-year-olds

Non-bacterial gastroenteritis and diarrhoea are usually caused by viruses and infection are seen in all parts of the world, especially in infants and young children (Fig. 23.22). The bacterial and parasitic infections have reduced as a result of improved sanitation and hygiene. Worldwide, deaths in all age groups due to diarrhoea have fallen considerably annually to around 1.3 million in 2013 compared with an estimated 2.6 million in 1990. This is particularly seen in the under-5-year-old age group, where mortality had fallen to just under 600 000 in 2013. However, it is the second most common cause of morbidity in that group.

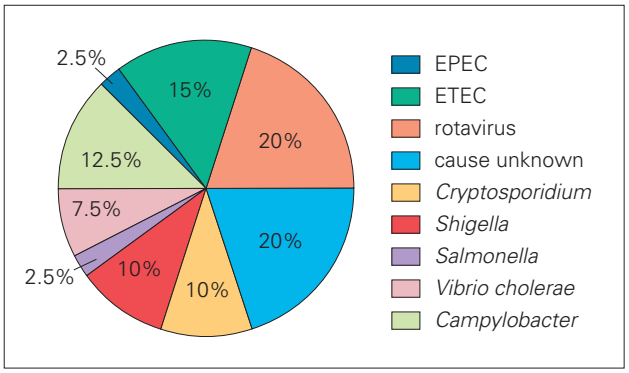


Figure 23.22 Diarrhoeal disease is a major cause of illness and death in children in resource-poor countries. This illustration shows the proportion of infections caused by different pathogens. Note that in as many as 20% of infections a cause is not identified, but many of these are likely to be viral. EPEC, enteropathogenic *E. coli*; ETEC, enterotoxigenic *E. coli*. (Data from WHO.)

Although viruses appear to be the most common causes of gastroenteritis in infants and young children, viral gastroenteritis is not distinguishable clinically from other types of gastroenteritis. The viruses are specific to humans, and infection follows the general rules for faecal–oral transmission. Oral transmission of non-bacterial gastroenteritis was first demonstrated experimentally in 1945, but it was not until 1972 that viral particles were identified in faeces by electron microscopy. It has been difficult or impossible to cultivate most of these viruses in cell culture.

Noroviruses

The most common cause of diarrhoea worldwide, causing nearly 20% of all diarrhoea episodes

Noroviruses, previously known as small round structured viruses (SRSV) or Norwalk-like viruses (NLV) cause 'winter vomiting disease' as well as diarrhoea. They are part of the Caliciviridae family and are 27 nm in diameter, unenveloped, single-stranded RNA viruses. Three of the six genogroups affect humans, namely GI, GII and G IV, and there is much genetic diversity, which is driven by immune selection. Cultivation in vitro has been problematic; co-factors are probably needed and were shown to cause gastroenteritis when fed to adult volunteers. One of the first identified norovirus outbreaks was in a school in Norwalk, Ohio, in 1969. Infection is common in older children and adults. These viruses are highly infectious, spread rapidly and nosocomial infection is common. The incubation period is 12–72 h. In up to 50% of cases there may be chills, headache, myalgia or fever as well as nausea, abdominal pain, vomiting and diarrhoea. Recovery may occur within 24–48 h but may take longer.

Noroviruses bind to cell surface carbohydrates of the ABH histo-blood group antigens and some strains have different binding affinities for different patterns of these antigens. In addition, these antigens are expressed to varying degrees in different individuals, resulting in some people being resistant to infection with specific norovirus strains. Laboratory diagnosis, important in outbreaks and for epidemiological studies, is usually by PCR, electron microscopy or ELISA. Viruses in this group are often implicated in diarrhoea associated with food- or water-borne routes occurring after eating sewage-contaminated shellfish such as cockles or mussels. In particular, noroviruses are a major cause of gastroenteritis in healthcare settings and many outbreaks have been reported in crowded environments such as cruise ships. Noroviruses show a high level of variability, resulting in both limited cross-protection between strains and reduced immunity in the population. In addition, due to this diversity, diagnostic assays have to be modified in order to optimize detection, and vaccine design either has to involve a cross-protective component or the development of a multivalent vaccine.

Virus-like particles are potential vaccines and antibody that blocks their binding to histo-blood group antigens may be a key model as a correlate of protection.

Rotaviruses

These are morphologically characteristic viruses (Fig. 23.23) named after the Latin word *rota* meaning a wheel, with a genome consisting of 11 separate segments of double-stranded RNA. Different rotaviruses infect the young of many mammals, including children, kittens, puppies, calves, foals and piglets,

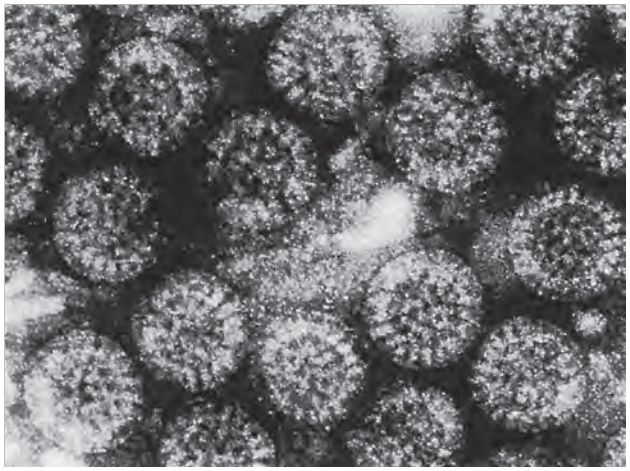


Figure 23.23 Rotavirus. The virus particles (65 nm in diameter) have a well-defined outer margin and capsules radiating from an inner core to give the particle a wheel-like (hence 'rota') appearance. (Courtesy of J.E. Banatvala.)

but it is thought that viruses from one host species occasionally cross-infect another. There are at least two human serotypes.

Replicating rotavirus causes diarrhoea by damaging transport mechanisms in the gut

The incubation period is 1–2 days. After virus replication in intestinal epithelial cells there is an acute onset of vomiting, which is sometimes projectile, and diarrhoea which lasts from 4 to 7 days. The replicating virus damages transport mechanisms in the gut, and loss of water, salt and glucose causes diarrhoea (Fig. 23.24). Infected cells in the intestine are destroyed, resulting in villous atrophy. The villi, long finger-like projections, become flattened, resulting in the loss of both the surface area for absorption and the digestive enzymes, and raised osmotic pressure in the gut lumen causes diarrhoea. There is no inflammation or loss of blood. Exceedingly large numbers of virus particles, 10^{10}–10^{11} / g, appear in the faeces. For unknown reasons, respiratory symptoms such as cough and coryza are quite common. The disease is more severe in infants in resource-poor countries.

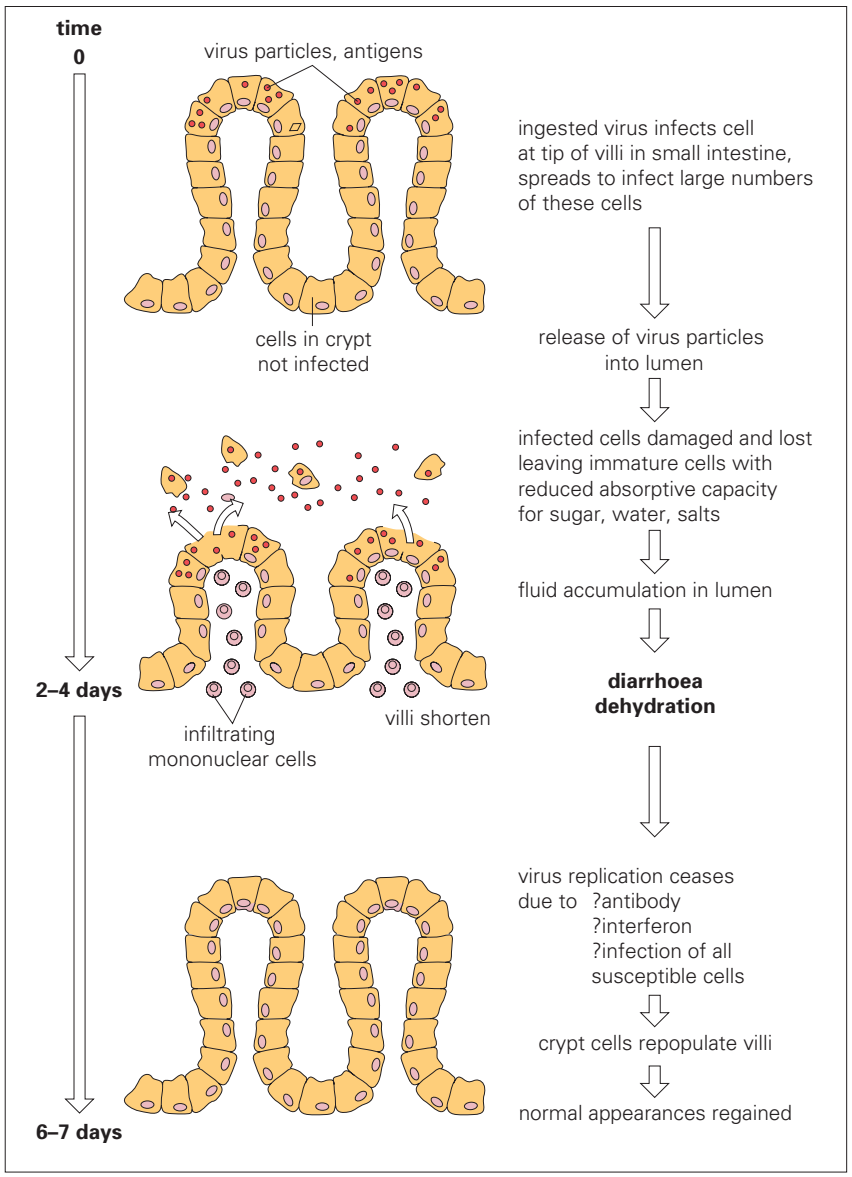


Figure 23.24 The pathogenesis of rotavirus diarrhoea. This may differ with other viral infections of the gastrointestinal tract.

Infection is most common in children under 2 years of age, and has a seasonal pattern, being most frequent in the cooler months of the year in temperate climates. IgA antibodies in colostrum give protection during the first 6 months of life. WHO estimated, as of April 2016, that in 2013 over 200 000 children died of rotavirus infection worldwide compared with 500 000 in 2000. Around 50% of rotavirus-related deaths in 2013 were in the under-5-years age group, in India, Nigeria, Pakistan and the Democratic Republic of the Congo. Outbreaks are sometimes seen in nurseries. Older children are less susceptible to infection, nearly all of them having developed antibodies, but occasional infections occur in adults.

Rotaviruses are well-adapted intestinal infectious agents. As few as ten ingested particles can cause infection, and by generating diarrhoea laden with enormous quantities of infectious particles, together with their stability in the environment, these organisms have ensured their continued transmission and survival.

Rotavirus infection is confirmed by viral RNA or antigen detection

Laboratory diagnosis may not be available in resource-poor countries, but is made by detecting viral RNA or antigen using PCR or ELISA methods, respectively (see Ch. 33). The characteristic 65 nm particles can be seen in faecal samples by electron microscopy. They show cubic symmetry and an outer capsid coat arranged like the spokes of a wheel (Fig. 23.23).

Fluid and salt replacement can be life saving in rotavirus diarrhoea

Dehydration occurs readily in infants, and fluid and salt replacement orally or intravenously can be life saving. There are no antiviral agents available, but a variety of live attenuated oral vaccines have undergone successful trials. In 2006, the US Food and Drug Administration (FDA) announced the approval of a live, oral vaccine for use in preventing rotavirus gastroenteritis in infants. An exciting development has been the introduction of a live, attenuated, orally administered rotavirus vaccine in 86 countries by 2016, around 45% of the world. The first dose should be given between 6 weeks and 14 weeks and 6 days of age as there are insufficient safety data on immunizing older infants.

Other viruses causing diarrhoea in humans include sapoviruses, astroviruses, adenoviruses and coronaviruses

Sapoviruses, also members of the Caliciviridae family, were first detected in an outbreak of diarrhoea in an orphanage in Sapporo, Japan. Astroviruses are 28 nm single-stranded RNA viruses and have characteristic five- or six-pointed star patterns. Most infections occur in childhood and are mild. Both sapoviruses and astroviruses are each thought to account for around 10% of gastroenteritis globally. Adenoviruses are unenveloped, 70–80 nm double-stranded DNA viruses of which types 40 and 41 are associated with around 5% of gastroenteritis. Types 40 and 41 can be grown only in specialized cell culture lines. The role of coronaviruses, human bocavirus and a number of newly identified viral infections in causing gastroenteritis is uncertain.

Although outbreaks of gastroenteritis often have a viral aetiology it may be difficult to be sure about the exact role of a given virus when it is identified in faeces, as there are a number of viruses that replicate in the gastrointestinal tract – enteroviruses, for example, which are not associated with acute diarrhoeal illness.

HELICOBACTER PYLORI AND GASTRIC ULCER DISEASE

Helicobacter pylori is associated with most duodenal and gastric ulcers

It is now well established that the Gram-negative spiral bacterium *H. pylori* is associated with over 90% of duodenal ulcers and 70–80% of gastric ulcers (Fig. 23.25). Marshall and Warren were awarded a Nobel Prize for discovering the bacterium and its role. Marshall bravely ingested an *H. pylori* culture having had a normal endoscopy, developed nausea and vomiting a few days later, and a repeat endoscopy demonstrated gastritis and *H. pylori* was grown from the biopsy, showing cause and effect. *H. pylori* was the first bacterium proven to cause malignancy, gastric cancer, and is the cause of 25% of all infection-associated cancers. The most common presentation is with persistent or recurrent pain in the upper abdomen in the absence of structural evidence of disease.

H. pylori colonizes the host for life, but eradication can be achieved by antibiotics. Persistence is due to the production of urease that breaks down urea to ammonia and CO_2, increasing the pH and providing protection against gastric acid. In addition, *H. pylori* has various surface attributes that help evade the immune response.

H. pylori has a number of virulence factors encoded by *cagA* (cytotoxin-associated gene A, CagA), *vacA* (vacuolating toxin A, VacA), *babA* (sialic-acid-binding adhesin, BabA) and *oipA* (outer inflammatory protein adhesion, OipA). CagA, a cytotoxin, affects cell signalling, reduces cell adhesion and changes the cell phenotype from epithelial to mesenchymal cells, which is associated with carcinogenesis. VacA induces large vacuoles in host cells and forms pore-like structures that result in osmotic swelling. In addition, it causes mitochondrial dysfunction, apoptosis, disrupts the epithelial cell barrier and improves the ability of *H. pylori* to colonize the gastric epithelium. BabA binds to the Lewis b ABO blood group antigen on red blood cells and some epithelial cells. This causes

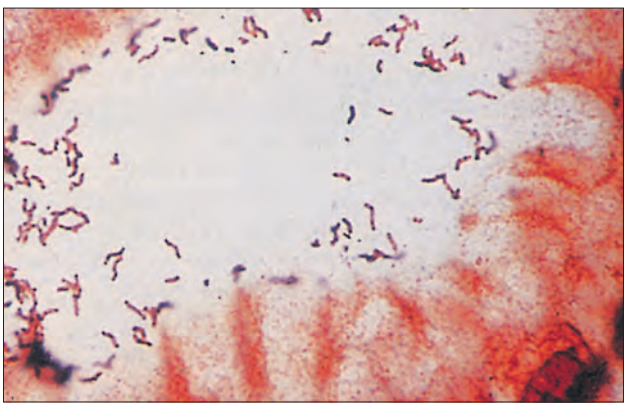


Figure 23.25 *Helicobacter pylori* gastritis, showing numerous spiral-shaped organisms adhering to the mucosal surface (silver stain). (Courtesy of A.M. Geddes.)

ds DNA breaks in host cells and may lead to cancer-associated gene mutations. It may also improve adherence to host cells and OipA is an outer membrane protein that acts as an adhesin and is associated with carcinogenesis.

H. pylori infection is associated with dyspepsia, stomach or upper abdominal pain, due to gastric ulcers, acute and chronic gastritis, and gastric adenocarcinoma and mucosa-associated lymphoid tissue (MALT) lymphoma. In addition, *H. pylori* has been found in other sites of the body and associated with extragastric diseases.

Rapid diagnosis may be made by using either the non-invasive urea breath test or fecal *H. pylori* antigen testing. For the breath test, a person ingests carbon-radiolabelled urea and, as *H. pylori* produces large amounts of urease, it is broken down into ammonia and carbon dioxide. The latter is absorbed into the bloodstream and the radiolabelled carbon is detected in the expired air. Both are more sensitive and specific than ELISA-based serology tests.

Invasive methods involve endoscopy, with a diagnosis made on the basis of histological examination of biopsy specimens. It also allows a direct assessment of inflammation in the stomach. Rapid urease testing can be carried out on gastric biopsy material and this time, when urea is added to the sample, the ammonia produced increases the pH detected in the test device. Biopsies can also be tested by PCR. *H. pylori* can be cultured in the laboratory, but is difficult to grow and is usually used when testing the sensitivity to specific antibiotics.

Eradication of *H. pylori* to promote the remission and healing of ulcers requires combination therapy involving quadruple, triple or sequential drugs. Quadruple therapy involves a proton pump inhibitor (PPI), bismuth salts and two antibiotics such as metronidazole and tetracycline. Triple therapy includes a PPI and two antibiotics, clarithromycin and amoxicillin. Sequential treatment starts with amoxicillin and a PPI for a few days followed by triple therapy (see Ch. 34).

PARASITES AND THE GASTROINTESTINAL TRACT

Many species of protozoan and helminth (worm) parasites live in the gastrointestinal tract, infecting some 3.5 billion people worldwide. Only a few commonly cause serious pathology (Fig. 23.26) and these will form the focus of this part of the chapter.

Transmission of intestinal parasites is maintained by the release of life cycle stages in faeces

The different life cycle stages include cysts, eggs and larvae. In most cases, new infections depend either directly or indirectly on contact with faecally derived material, infection rates therefore reflecting standards of hygiene and levels of sanitation. In general, the stages of protozoan parasites passed in faeces are either already infective or become infective within days. These parasites are therefore usually acquired by swallowing infective stages in faecally contaminated food or water. Worm parasites, with two major exceptions, threadworm (also known as pinworm) and the dwarf tapeworm, produce eggs or larvae that require a period of development outside the host before they become infective. Transmission routes are more complex here:

- Some species are acquired through food or water contaminated with infective eggs or larvae, or are picked up directly via contaminated fingers.

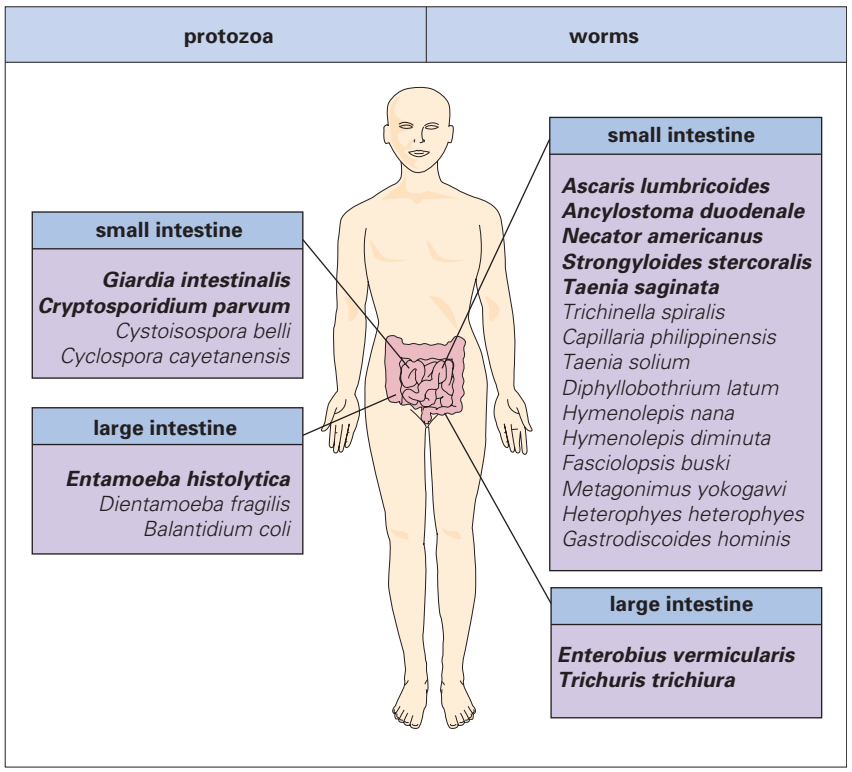


Figure 23.26 Gastrointestinal parasites of humans. The majority of these infections are found in resource-poor countries, but all species may also present in the resource-rich world and some have come to prominence because of their association with AIDS. The most important parasite species are highlighted in bold type.

- Some have larvae that can actively penetrate through the skin, migrating eventually to the intestine.
- Others are acquired by eating animals or animal products containing infective stages.

The symptoms of intestinal infection range from very mild, through acute or chronic diarrhoeal conditions associated with parasite-related inflammation, to life-threatening diseases caused by spread of the parasites into other organs of the body. Most infections fall into the first of these categories.

Protozoan infections

Three species are of particular importance:

- *Entamoeba histolytica*
- *Giardia intestinalis*
- *Cryptosporidium hominis*.

All three can give rise to diarrhoeal illnesses, but the parasites have distinctive morphological features that allow a precise diagnosis to be made fairly easily (Fig. 23.27). Other intestinal protozoa of concern, particularly in immunosuppressed patients, include *Cyclospora cayetanensis*, *Cystoisospora belli* (previously known as *Isospora belli*) and the microsporidia.

Entamoeba histolytica

E. histolytica *infection is particularly common in subtropical and tropical countries.* For many years, it was considered that infections with *E. histolytica* could be asymptomatic or pathogenic, with dysentery a key symptom when the amoebae invaded the mucosa. In fact, rather than a single species behaving differently, the explanation is that two species are involved: *E. histolytica* is invasive and *E. dispar* is non-pathogenic and non-invasive. *E. histolytica* occurs worldwide, but is most often found in subtropical and tropical countries, where the prevalence may exceed 50%. The trophozoite stages of the amoebae live in the large intestine on the mucosal surface. Reproduction of these stages is by simple binary fission, and there is periodic formation of resistant encysted forms, which pass out of the body. These cysts can survive in the external environment (for up to 30 days in water) and act as the infective stages. Infection occurs when food or drink is contaminated either by infected food handlers or as a result of inadequate sanitation. Transmission can also take place as a result of anal sexual activity. The cysts pass intact through the stomach when swallowed and excyst in the small intestine, each giving rise to four progeny. These adhere to the epithelial cells using a combination of adhesins and lectins and damage them via amoebapore-, phospholipase- and protease-induced cytolysis followed by phagocytosis. They invade the mucosa and feed on host tissues including red blood cells, giving rise to amoebic colitis.

E. histolytica *infection may cause mild diarrhoea or severe dysentery.* Infections with *E. dispar* are asymptomatic and this protozoan does not cause illness in humans. In contrast, invasion of the mucosa by *E. histolytica* may produce small localized superficial ulcers or involve the entire colonic mucosa with the formation of deep confluent ulcers (Fig. 23.28). The former causes mild diarrhoea, whereas more severe invasion leads to 'amoebic dysentery', which is characterized by mucus and blood in the stools. Dysenteries of amoebic and bacillary origin can be distinguished by a number of features (Table 23.5).

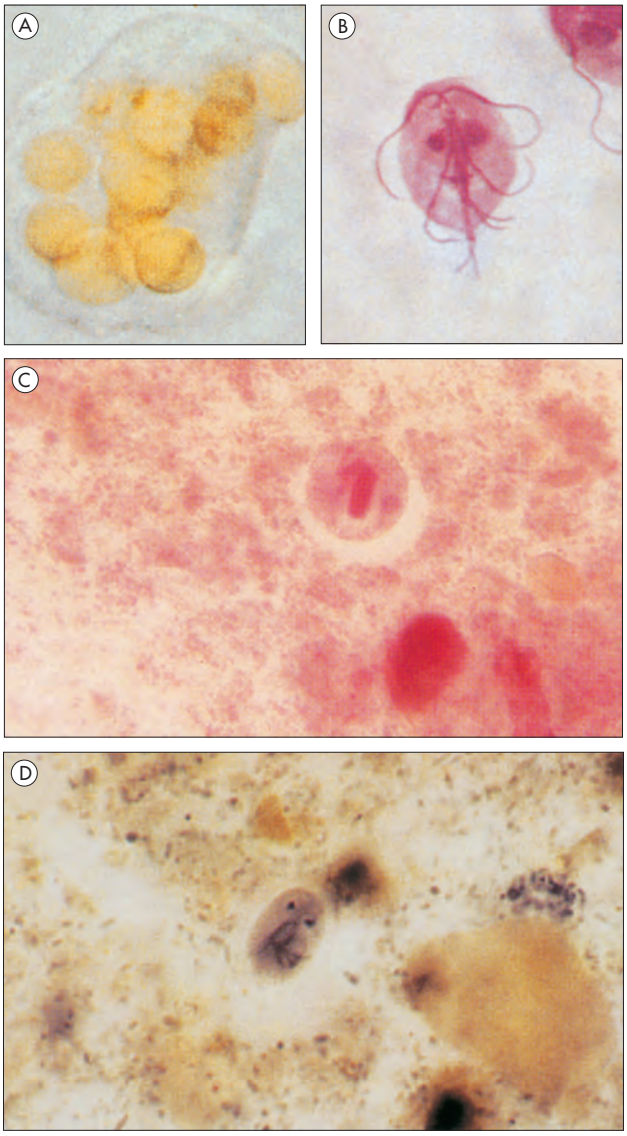


Figure 23.27 Protozoan infections of the gastrointestinal tract. (A) *Entamoeba histolytica*. Trophozoite found in the acute stage of the disease, which often contains ingested red blood cells. (B) *Giardia intestinalis* trophozoite associated with acute infection in humans. (C) Cyst of *E. histolytica*, with only one of the four nuclei visible. The broad chromidial bar is a semicrystalline aggregation of ribosomes (H&E stain). (D) Oval cyst of *G. intestinalis* showing two of the four nuclei (iron haematoxylin stain). ([B] Courtesy of D.K. Banerjee. [D] Courtesy of R. Muller and J.R. Baker.)

Complications include perforation of the intestine, leading to peritonitis, and extraintestinal invasion. Trophozoites can spread via the blood to the liver, with the formation of an abscess, and may secondarily extend to the lung and other organs. Rarely, abscesses spread directly and involve the overlying skin. *E. histolytica* is able to evade the immune response by a variety of methods including immunomodulation, phagocytosis of immune cells and protease-based destruction of soluble immune mediators. Serology (by indirect fluorescent antibody technique [IFAT] or ELISA) is the mainstay of diagnosis for an amoebic liver abscess.

E. histolytica *infection can be diagnosed in asymptomatic patients from the presence of characteristic four-nucleate cysts in the stool.* These cysts may be infrequent in light infections, and repeated stool examination is necessary. Care must be taken to differentiate *E. histolytica* from other non-pathogenic species that might be present (Fig. 23.29). Trophozoites can be found in cases of dysentery (when the stools are loose and wet), but they are fragile and deteriorate rapidly; specimens should therefore be preserved before examination. ELISA tests are available, as is a triage panel assay that can distinguish between *E. histolytica* / *E. dispar*, *Cryptosporidium parvum* and *Giardia intestinalis*. Differentiation of *E. histolytica* from *E. dispar* requires immunological tests or species-specific PCR.

Acute* E. histolytica *infection can be treated with metronidazole or tinidazole. If infection is treated early, recovery is expected and there is some immunity to re-infection. Metronidazole or tinidazole kills amoebic trophozoites in both intestinal and extraintestinal sites of infection and results in rapid clinical improvement, but relapse of the infection may occur unless a second antiamoebic agent is given to eradicate amoebae from the gut lumen. Examples are diloxanide furoate or paromomycin. Prevention of amoebiasis in the community requires the same approaches to hygiene and sanitation as those adopted for bacterial infections of the intestine. A vaccine directed against the Lec A fragment of the Gal / GalNAC lectin, which mediates attachment of *E. histolytica* to the colonic mucosa, is being developed.

Table 23.5 Features of bacillary and amoebic dysentery

	Bacillary	Amoebic
Organism	*Shigella*	*Entamoeba*
Polymorphs and macrophages in stool	Many	Few
Eosinophils and Charcot–Leyden crystals in stool	Few or absent	Often present
Organisms in stool	Many	Few
Blood and mucus in stool	Yes	Yes

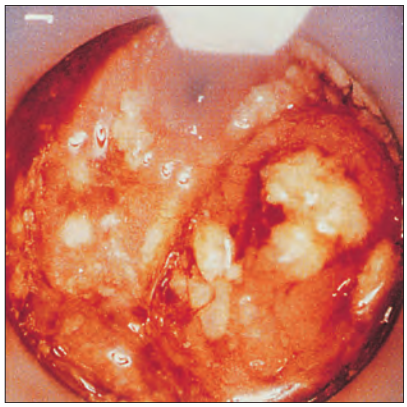


Figure 23.28 Amoebic colitis. Sigmoidoscopic view showing deep ulcers and overlying purulent exudate. (Courtesy of R.H. Gilman.)

Giardia intestinalis

Giardia was the first intestinal microorganism to be observed under a microscope. It was discovered in 1681 by Anton van Leeuwenhoek who used the microscope he had invented to examine specimens of his own stool. It has a global distribution and is a frequent cause of travellers' diarrhoea. *Giardia* is the most commonly diagnosed intestinal parasite in the USA, having been detected in both drinking and recreational water. There is confusion over nomenclature and the species infecting humans is also commonly referred to as *G. lamblia*, and sometimes as *G. duodenalis* (human).

Like* Entamoeba, Giardia *has only two life cycle stages. The two life cycle stages are the flagellate (four pairs of flagella) binucleate trophozoite and the resistant four-nucleate cyst. The trophozoites live in the upper portion of the small intestine, adhering closely to the brush border of the epithelial cells by specialized attachment regions (Fig. 23.30). They divide by binary fission and can occur in such numbers that they cover large areas of the mucosal surface. Cyst formation occurs at regular intervals, each cyst being formed as one trophozoite rounds up and produces a resistant wall. Cysts pass out in the stools and can survive for several weeks under optimum conditions. Infection occurs when the cysts are swallowed, usually as a result of drinking contaminated water. The minimum infective dose is very small: 10–25 cysts.

Epidemics of giardiasis have occurred when public drinking supplies have become contaminated, but smaller outbreaks have been traced to drinking from rivers and streams that have been contaminated by animals. Apart from water-borne transmission, *Giardia* can be passed from person to person, especially within families, with food-borne transmission being rare. *Giardia* may also be transmitted sexually among men who have sex with men. Genotyping has demonstrated that *Giardia* consists of at least seven different assemblages. Assemblages A and B can infect dogs, cats and cattle as well

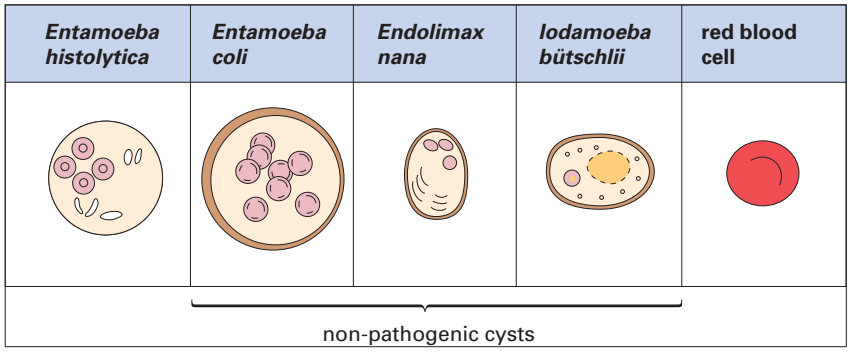


Figure 23.29 Characteristics of cysts (size and number of nuclei) are used to differentiate pathogenic from non-pathogenic protozoa. A red blood cell is shown for comparison.

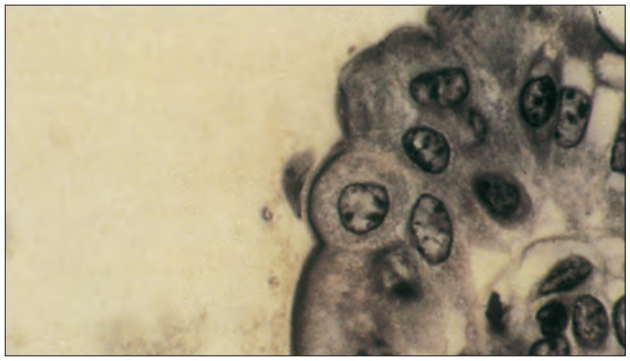


Figure 23.30 Trophozoite of *Giardia intestinalis* attached to the mucosal surface of the small intestine (iron haematoxylin stain). (Courtesy of R. Muller and J.R. Baker.)

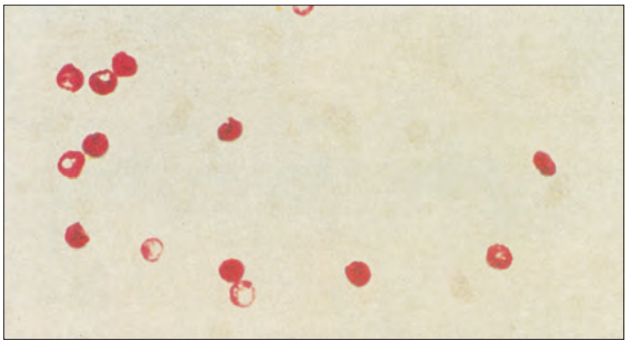


Figure 23.31 *Cryptosporidium* oocysts in faecal specimen. (Courtesy of S. Tzipori.)

as humans, so human infection with these genotypes can be zoonotically acquired.

Mild* Giardia *infections are asymptomatic; more severe infections cause diarrhoea. The diarrhoea may be:

- self-limiting, with 7–10 days being the usual course
- chronic, and develop into a serious condition, particularly in patients with deficient or compromised immunological defences.

It is thought to arise from inflammatory responses triggered by the damaged epithelial cells and from interference with normal absorptive processes. Characteristically, the stools are loose, foul smelling and often fatty.

Diagnosis of* Giardia *infection is based on identifying cysts or trophozoites in the stool. Formalin-ether or formalin ethyl acetate concentration is superior to direct wet film microscopy. Immunofluorescence staining of faecal smears has high specificity. Repeated examination is necessary in light infections. Duodenal intubation or the use of recoverable swallowed capsules and threads, known as the 'string test', may aid in obtaining trophozoites directly from the intestine. Alternatives to microscopic methods are increasingly available, including faecal antigen detecting ELISA tests with good specificity, immunochromatographical tests in cassette form and PCR. Multiplex PCR assays detecting *Giardia, Cryptosporidium* and *E. histolytica* in faecal specimens are now widely available.

Giardia *infection can be treated with a variety of drugs.* The nitroimidazole compounds metronidazole and tinidazole are commonly used. Increasing numbers of nitroimidazole treatment failures have occurred in the last 5 years, however, especially in giardiasis acquired in the Indian subcontinent, and nitazoxanide, albendazole or mepacrine (also known as quinacrine) are alternatives. Community measures for prevention include the usual concerns with hygiene and sanitation, and improved treatment of drinking water supplies (largely filtration and chlorination) where these are suspected as a source. Avoiding drinking from potentially contaminated natural waters is also important.

Cryptosporidium hominis and *Cryptosporidium parvum*

The protozoan genus* Cryptosporidium *is widely distributed in many animals. Awareness of *Cryptosporidium* as an important cause of diarrhoea in humans was established during the early years of the AIDS epidemic, although similar parasites were known to be widely distributed in many animals. Although there are 26 species of *Cryptosporidium*, a very high proportion of human infection is due to *C. hominis*, which is specific to humans, and *C. parvum*, which infects calves but is also capable of causing disease in humans. These two species are responsible for more than 90% of cases of human cryptosporidiosis. The parasite has a complex life cycle, going through both asexual and sexual phases of development in the same host. Transmission requires ingestion of a minimum of 10 of the resistant oocysts (4–5 μm in diameter) in faecally contaminated material (Fig. 23.31). In the small intestine, the oocyst releases infective sporozoites, which invade the epithelial cells, remaining closely associated with the apical plasma membrane such that they are intracellular but extracytoplasmic. Here they form meronts, which produce and release merozoites and these then re-invade further epithelial cells. A second type of meront produces sexual stages known as gamonts. Fertilization occurs, and thick-walled oocysts are released in the faeces. The major risk factors for developing cryptosporidiosis are ingesting contaminated drinking or recreational water, contact with infected people or animals and travel to areas with poor sanitation. Most outbreaks are water borne and in 1993 *Cryptosporidium* caused a massive outbreak of watery diarrhoea affecting 403 000 people in Milwaukee, USA. It was transmitted through the public water supply.

Cryptosporidial diarrhoea ranges from moderate to severe. Symptoms of infection with *Cryptosporidium* range from moderate diarrhoea to more severe profuse diarrhoea that is self-limiting in 15–40 days in immunocompetent individuals, but can become chronic in immunocompromised patients, including those with advanced HIV infection. In individuals with $CD4^+$ T-cell counts <100 / mm^3, diarrhoea is prolonged, may become irreversible in the absence of immune reconstitution and can be life threatening.

Routine faecal wet preparation examinations are inadequate for diagnosing cryptosporidial diarrhoea. Fluorescence microscopy (e.g. with auramine) or modified Ziehl–Neelsen staining can be used to demonstrate oocysts in thin faecal smears. Antigen detection ELISA assays, which are capable of high throughput and immunochromatographic lateral flow cassette tests, are also deployed. Direct immunofluorescence microscopy and PCR both have high specificity and PCR, commonly as a multiplex assay detecting *Cryptosporidium*, *Giardia* and *E. histolytica,* is now widely available.

Antiparasitic treatment for cryptosporidial diarrhoea is suboptimal. Symptomatic therapy is an important part of management. Combination antiretroviral therapy (cART) in individuals with advanced HIV infection suffering from cryptosporidiosis has been reported to improve the diarrhoea symptoms. This may be due to the protease inhibitors used in combination therapy interfering directly with the cryptosporidial proteases involved in the protozoal life cycle. In addition, cART results in lowering the plasma HIV load and promotes immune reconstitution. Paromomycin reduces oocyst output but does not clear infection. Nitazoxanide is effective in HIV-negative patients but is only partially active in those co-infected with HIV. Public health measures are similar to those outlined for controlling giardiasis, although *Cryptosporidium* is more resistant to chlorination. Some water treatment facilities deploy an additional ozonation step to inactivate cryptosporidia.

Cyclospora, *Cystoisospora* and the microsporidia

Cyclospora, like *Cystoisospora belli* and *Cryptosporidium*, is a coccidian parasite, whose life cycle stages take place in epithelial cells of the mucosa. *Cyclospora* and *Cystoisospora* have only been found in humans, unlike other coccidia that are zoonotic.

Cyclospora cayetanensis, named in 1994, is one of the causes of diarrhoea in travellers, but it can also be acquired from contaminated imported food; for example, Guatemalan raspberries were thought to be the cause of five diarrhoeal outbreaks in the USA in the years 1995 to 2000. More recently, in 2015 and 2016, outbreaks of cyclosporiasis occurred in UK travellers having returned from Mexico. Diarrhoea can be prolonged and is severe in immunosuppressed individuals. Trimethoprim-sulphamethoxazole (co-trimoxazole) treatment is effective. Ciprofloxacin is partially effective.

AIDS patients infected with *Cystoisospora belli* may show particularly severe symptoms, with persistent diarrhoea causing weight loss and even death. Treatment is with co-trimoxazole.

Infections with microsporidia, an unusual group, have also become recognized as a cause of diarrhoea in AIDS and other immunosuppressed patients. *Enterocytozoon bieneusi* is the commonest cause, although *Encephalitozoon intestinalis* also occurs. Transmission appears to be direct. Albendazole treatment is effective against *E. intestinalis* but has disappointing activity against *E. bieneusi.* Where possible, immune reconstitution is the mainstay of treatment.

'Minor' intestinal protozoa

The human intestine may harbour a large number of protozoa, many of which appear to be quite harmless. Some have a questionable role in disease: these include *Blastocystis hominis*, *Dientamoeba fragilis* and *Sarcocystis hominis.*

Worm infections

The most important intestinal worms clinically are the nematodes known as the soil-transmitted helminths

Soil-transmitted helminths fall into two distinct groups:

- *Ascaris lumbricoides* (large roundworm) and *Trichuris trichiura* (whipworm), in which infection occurs by swallowing the infective eggs
- *Ancylostoma duodenale* and *Necator americanus* (hookworms) and *Strongyloides stercoralis*, which infect via active skin penetration by infective larvae, which then undertake a systemic migration through the lungs to the intestine.

With the exception of *Trichuris* all the soil-transmitted nematodes inhabit the small bowel.

The pinworm or threadworm *Enterobius vermicularis* is probably the commonest intestinal nematode in resource-rich countries and is the least pathogenic. The females of this species, which live in the large bowel, release infective eggs onto the perianal skin. This causes itching, and transmission usually occurs directly from contaminated fingers, but the eggs are also light enough to be carried in dust.

The soil-transmitted helminths are commonest in warmer resource-poor countries. About one-quarter of the world's population carry these worms, children being the most heavily infected group. Transmission is favoured where there is inadequate disposal of faeces, contamination of water supplies, use of faeces (night-soil) as fertilizer, or low standards of hygiene (see below). Vast numbers of eggs are released in the lifetime of each female worm (tens of thousands by *Trichuris* and *Ancylostoma* and hundreds of thousands by *Ascaris*).

Life cycle and transmission

Female* Ascaris *and* Trichuris *lay thick-shelled eggs in the intestine, which are expelled with faeces and hatch after being swallowed by another host. The thick-shelled eggs of *Ascaris* and *Trichuris* are shown in Fig. 23.32. The eggs require incubation for several days at optimum conditions (warm temperature, high humidity) for the infective larvae to develop. Once this occurs, the eggs remain infective for many weeks or months, depending upon the local microclimate, and *Ascaris* eggs can survive in moist soil for up to 10 years. After being swallowed,

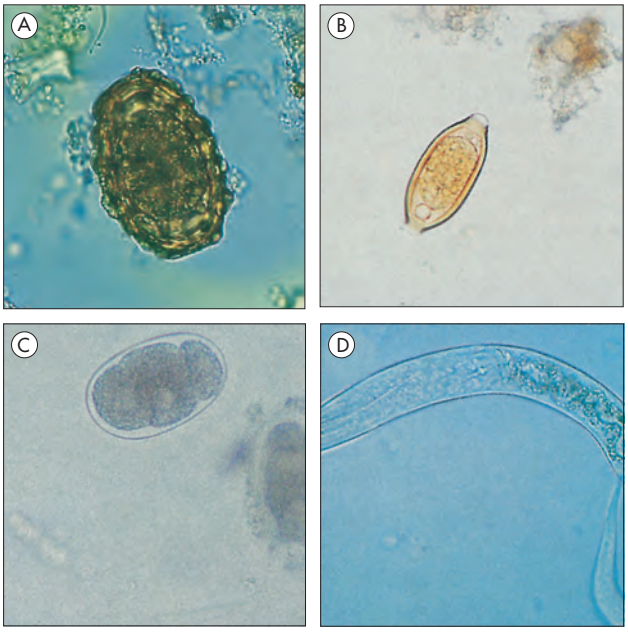


Figure 23.32 Eggs and larvae of intestinal nematodes passed in faeces. (A) Egg of *Ascaris* (fertile). (B) Egg of *Trichuris*. (C) Egg of hookworm. The embryo continues to divide in the faecal sample and may be at the 16- or 32-cell stage by the time the sample is examined. (D) Larva of *Strongyloides stercoralis*. (Courtesy of J.H. Cross.)

the eggs hatch in the intestine, releasing the larvae. Those of *Ascaris* penetrate the bowel wall and are carried in the blood through the liver to the lungs, migrating up the bronchi and trachea before being swallowed and once again reaching the intestine. The adult worms live freely in the gut lumen, feeding on intestinal contents. In contrast, *Trichuris* larvae remain within the large bowel, penetrating into the epithelial cell layer, where they remain as they mature.

Adult female hookworm lay thin-shelled eggs that hatch in the faeces shortly after leaving the host. A hookworm egg is shown in Fig. 23.32C. The larvae of these hookworm (*A. duodenale* and *N. americanus*) feed on bacteria until infective, and then migrate away from the faecal mass. Infection takes place when larvae come into contact with unprotected skin (or additionally, in the case of *Ancylostoma*, are swallowed). They penetrate the skin, migrate via the bloodstream to the lungs, ascend the trachea and are swallowed. Adult worms attach by their enlarged mouths to the intestinal mucosa, ingest a plug of tissue, rupture capillaries and suck blood.

The adult female* Strongyloides *lays eggs that hatch in the intestine. The life cycle of *Strongyloides* is broadly similar to that of hookworm, but shows some important differences. Humans harbour only parthenogenetic females that lay eggs into the mucosa. These eggs hatch in the intestine and the released rhabiditiform larvae usually pass out in the faeces (Fig. 23.32D). Development outside the host can follow the hookworm pattern, with the direct production of skin-penetrating filariform larvae, or may be diverted into the production of a complete free-living generation including adult males and females, which then produce infective larvae. Under certain conditions, and particularly when the host is immunocompromised, *Strongyloides* larvae can develop to the filariform stage and re-invade before they are voided in the faeces. This process of autoinfection can give rise to the severe clinical condition known as disseminated strongyloidiasis, also called hyperinfection, which is often complicated by Gram-negative bacterial septicaemia. All soil-transmitted helminths are relatively long lived (several months to years), but authenticated cases show that *Strongyloides* infections can persist for more than 30 years, presumably through continuous internal autoinfection.

Clinical features

In most individuals, worm infections produce chronic mild intestinal discomfort rather than severe diarrhoea or other conditions. Infections may lead to hypersensitivity responses and can also reduce responses to vaccination. Each parasite has a number of characteristic pathological conditions linked with it.

Large numbers of adult* Ascaris *worms can cause intestinal obstruction. The migration of *Ascaris* larvae through the lungs can cause severe respiratory distress due to pneumonitis; ascariasis is one of the causes of Löffler's syndrome. This stage is often associated with pronounced eosinophilia. Worms in the intestine can cause abdominal pain, nausea and digestive disturbances. In children with a suboptimal nutritional intake, these disturbances can contribute to clinical malnutrition. Large numbers of adult *Ascaris* can cause a physical blockage in the intestine and this may also occur as worms die following antiparasitic chemotherapy. Intestinal worms tend to migrate out of the intestine, often up the bile duct, causing cholangitis or liver abscess. Perforation of the intestinal wall can also occur. Worms have occasionally been reported in unusual locations, including the orbit of the eye and the male urethra. *Ascaris* is highly allergenic and infections often give rise to symptoms of hypersensitivity, which may persist for many years after the infection has been cleared.

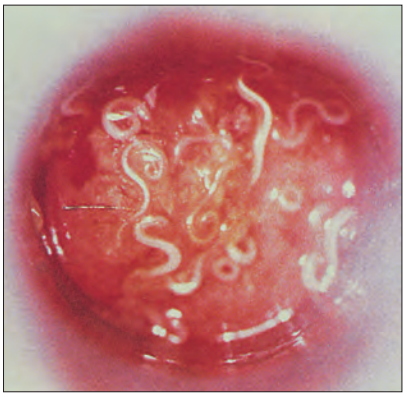


Figure 23.33 Trichuriasis in a healthy, infected, child. Proctoscopic view showing numerous adult *Trichuris trichiura* attached to the intestinal mucosa. (Courtesy of R.H. Gilman.)

Moderate to severe* Trichuris *infection can cause chronic diarrhoea. As with all intestinal worms, children are the members of the community most heavily infected with *Trichuris*. Although previously regarded as of little clinical significance, research has shown that moderate to heavy infections in children can cause a chronic diarrhoea (Fig. 23.33), reflected in impaired nutrition and retarded growth. Occasionally, heavy infections lead to rectal prolapse.

Hookworm disease can result in iron-deficiency anaemia. Migration of hookworm larvae through the skin and lungs can cause dermatitis and pneumonitis, respectively. The blood-feeding activities of the intestinal worms can lead to iron-deficiency anaemia if the diet is inadequate. Heavy infections cause a marked debility and growth retardation.

Strongyloidiasis can be fatal in immunosuppressed people. Heavy intestinal infection with strongyloidiasis causes persistent and profuse diarrhoea with dehydration and electrolyte imbalance. Profound mucosal changes can also lead to a malabsorption syndrome, which is sometimes confused with tropical sprue. People with human lymphotropic virus type 1 (HTLV-1) infection, diseases that suppress immune function such as AIDS and cancer, or who are being treated with immunosuppressive drugs are susceptible to the development of disseminated strongyloidiasis. Invasion of the body by many thousands of autoinfective larvae can be fatal. Gram-negative bacterial septicaemia or meningitis can ensue.

The most common sign of pinworm (threadworm) infection is anal pruritus. Occasionally, this is accompanied by mild diarrhoea. *Enterobius* are sometimes found in the appendix, but their role in appendicitis is controversial. Having emerged onto the perianal skin, adult female worms occasionally enter the vagina in women and produce local irritation.

Laboratory diagnosis

All five of the soil-transmitted species can be diagnosed by finding eggs or larvae in a fresh stool. Although these stages may be detected in direct faecal smears, concentration

Figure 23.34 Filling defect in the small intestine due to the presence of *Ascaris*, seen on a radiograph after a barium meal. (Courtesy of W. Peters.)

techniques are more sensitive and a charcoal culture of faeces is added if *Strongyloides* is suspected. Faecal PCR for *Strongyloides* is now entering service in specialist laboratories. Acute infections with *Ascaris*, hookworms and *Strongyloides* are often accompanied by a marked blood eosinophilia. Although this is not diagnostic, it is a strong indicator of helminth infection. An enzyme immunoassay can be used to detect *Strongyloides* antibody and has approximately 90% sensitivity of detection. However, there is some cross-reactivity with IgG made against other nematode infections and one cannot determine whether the *Strongyloides* infection occurred recently or in the past.

The eggs of* Ascaris, Trichuris *and hookworm are characteristic. These eggs are shown in Fig. 23.32 and are easily recognizable. Identification of the species of hookworm requires charcoal culture of the stool to allow the eggs to hatch and the larvae to mature into the infective third stage. The presence of adult *Ascaris* is sometimes demonstrated incidentally by radiography (Fig. 23.34).

The presence of characteristic rhabditiform larvae in fresh stools is diagnostic of *Strongyloides* infection.

Pinworm infection is diagnosed by finding eggs on perianal skin. Although adult pinworm sometimes appear in the stools, the eggs are seldom seen in fecal concentrates because they are laid directly onto the perianal skin (Fig. 23.35). They can be found by pressing this area with a piece of clear adhesive tape (the 'Scotch tape' test) and examining the tape mounted sticky side down on a microscope slide.

Treatment and prevention

Enterobius is treated with mebendazole, piperazine or pyrantel pamoate; *Ascaris* with mebendazole, albendazole or piperazine; hookworm with mebendazole or albendazole; and *Trichuris* with mebendazole or albendazole. *Strongyloides* requires treatment with ivermectin; thiabendazole is also effective, but is less well tolerated by the patient. At the community level, prevention can be achieved through improved hygiene and sanitation, making sure that faecal material is disposed of properly.

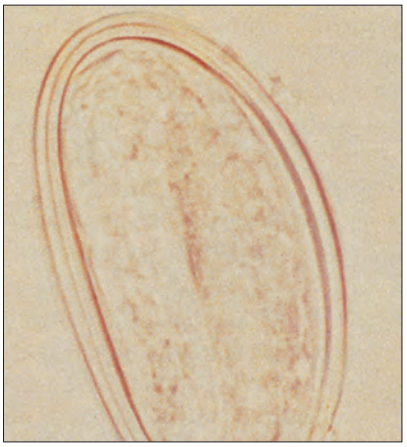


Figure 23.35 Egg of *Enterobius* on perianal skin. (Courtesy of J.H. Cross.)

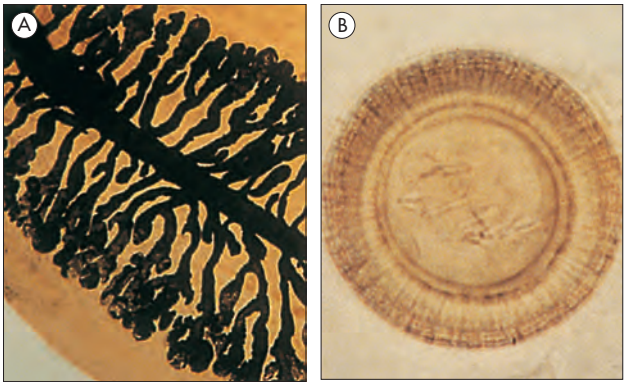


Figure 23.36 *Taenia saginata*. (A) Gravid proglottid stained with India ink to show numerous side branches. (B) Egg containing six-hooked (hexacanth) larva. (Courtesy of R. Muller and J.R. Baker.)

Other intestinal worms

Many other worm species can infect the intestine, but most are uncommon in resource-rich countries. Of the human tapeworms:

- The beef tapeworm *Taenia saginata*, transmitted through infected beef, is the most widely distributed. However, infection is usually asymptomatic, apart from revulsion felt on passing the large segments. Diagnosis involves finding these segments or the characteristic eggs in the stool (Fig. 23.36A, B).
- *Diphyllobothrium latum*, the fish tapeworm, is widely distributed geographically, but infection is restricted to individuals eating raw or undercooked fish carrying the infective larvae. The eggs of this species have a terminal 'lid', known as an operculum and are the diagnostic stage in the stool (Fig. 23.37A).
- *Hymenolepis nana*, the dwarf tapeworm, occurs primarily in children, infection occurring directly by swallowing eggs (Fig. 23.37B). This worm has the ability to initiate autoinfection within the host's intestine, so that a large number of worms can build up, leading to diarrhoea and some abdominal discomfort.

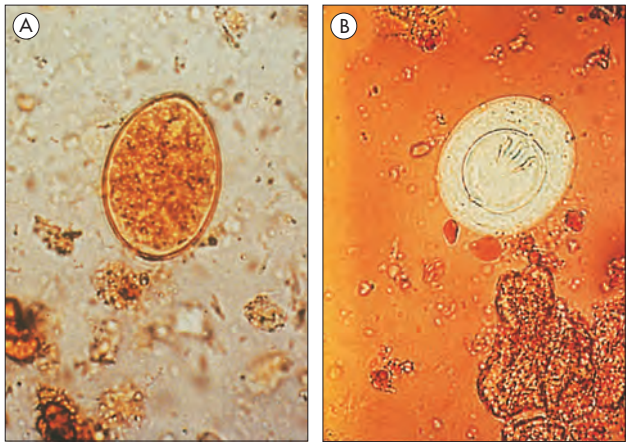


Figure 23.37 Eggs of (A) *Diphyllobothrium latum* and (B) *Hymenolepis nana*. (Courtesy of R. Muller and J.R. Baker.)

All these tapeworms can be treated with praziquantel or niclosamide.

Intestinal symptoms (predominantly diarrhoea and abdominal pain) are associated with infection by the nematode *Trichinella spiralis*, which is better known clinically for the pathology caused by the blood-borne muscle phase (see Chs. 27 and 29). Infection with species of schistosomes situated in mesenteric blood vessels (*Schistosoma japonicum* and *S. mansoni*) can also cause symptoms of intestinal disease. As the eggs pass through the intestinal wall, they cause marked inflammatory responses, granulomatous lesions form and diarrhoea may occur in the early acute phase. Heavy chronic *S. mansoni* infection is associated with inflammatory polyps in the colon, whereas severe involvement of the small bowel is more common with *S. japonicum*.

SYSTEMIC INFECTION INITIATED IN THE GASTROINTESTINAL TRACT

We opened this chapter by noting that infections acquired by the ingestion of pathogens could remain localized in the gastrointestinal tract or could disseminate to other organs and body systems. Important examples of disseminated infection are the enteric fevers and viral hepatitis types A and E. Listeriosis also appears to be acquired via the gastrointestinal tract. For the sake of clarity and convenience, other types of viral hepatitis will also be discussed in this chapter.

Enteric fevers: typhoid and paratyphoid

The term 'enteric fever' was introduced in the last century in an attempt to clarify the distinction between typhus (see Ch. 28) and typhoid. For many years these two diseases had been confused, as the common root of their names suggests (typhus, a fever with delirium; typhoid, resembling typhus), but even before the causative agents were isolated (typhoid caused by *S. typhi* and typhus caused by *Rickettsia* spp.), it was pointed out that it was 'just as impossible to confuse the intestinal lesions of typhoid with the pathological findings of typhus as it was to confuse the eruptions of measles with the pustules of smallpox'. In fact, enteric fevers can be caused by *S. typhi* and three additional *Salmonella* species, but the name 'typhoid' has stuck.

S. typhi and paratyphi types *S. paratyphi A*, *S. schottmuelleri* (previously named *S. paratyphi B*) and *S. hirschfeldii* (previously named *S. paratyphi C*) cause enteric fevers

These species of *Salmonella* are restricted to humans and do not have a reservoir in animals. Therefore, spread of the infection is from person to person, usually through contaminated food or water (Fig. 23.38). After infection, people can carry the organism for months or years, providing a continuing source from which others may become infected. Typhoid Mary, a cook in New York City in the early 1900s, is one such example. She was a long-term carrier who succeeded in initiating at least ten outbreaks of the disease (see Ch. 17, Box 17.1).

The salmonellae multiply within and are transported around the body in macrophages

After ingestion, the salmonellae that survive the antibacterial defences of the stomach and small intestine penetrate the gut mucosa through the Peyer's patches, probably in the jejunum or distal ileum (Fig. 23.39). Once through the mucosal barrier, the bacteria reach the intestinal lymph nodes, where they survive and multiply within macrophages. They are transported in the macrophages to the mesenteric lymph nodes and thence to the thoracic duct and are eventually discharged into the bloodstream. Circulating in the blood, the organisms can seed many organs, most importantly in areas where cells of the reticuloendothelial system are concentrated (i.e. the spleen, bone marrow, liver and Peyer's patches). In the liver, they multiply in Kupffer cells. From the reticuloendothelial system, the bacteria re-invade the blood to reach other organs (e.g. kidney). The gallbladder is infected either from the blood or from the liver via the biliary tract, the bacterium being particularly resistant to bile. As a result, *S. typhi* enters the intestine for a second time in much larger numbers than on the primary encounter and causes a strong inflammatory response in Peyer's patches, leading to ulceration, with the danger of intestinal perforation.

Rose spots on the upper abdomen are characteristic, but absent in up to half of patients with enteric fever

After an incubation period of 10–14 days (range 7–21 days), the disease has an insidious onset with non-specific symptoms of fever and malaise accompanied by aches and respiratory symptoms, and may resemble a flu-like illness. Diarrhoea may be present, but constipation is just as likely. At this stage, the patient often presents with a fever of unknown origin. In the absence of treatment, the fever increases and the patient becomes acutely ill. Rose spots – erythematous maculopapular lesions that blanch on pressure (Fig. 23.40) – are characteristic on the upper abdomen, but may be absent in up to half of patients. They are transient and disappear within hours to days. Without treatment, an uncomplicated infection lasts 4–6 weeks.

Before antibiotics, 12–16% of patients with enteric fever died, usually of complications

The complications can be classified into:

- those secondary to the local gastrointestinal lesions (e.g. haemorrhage and perforation; Fig. 23.41)

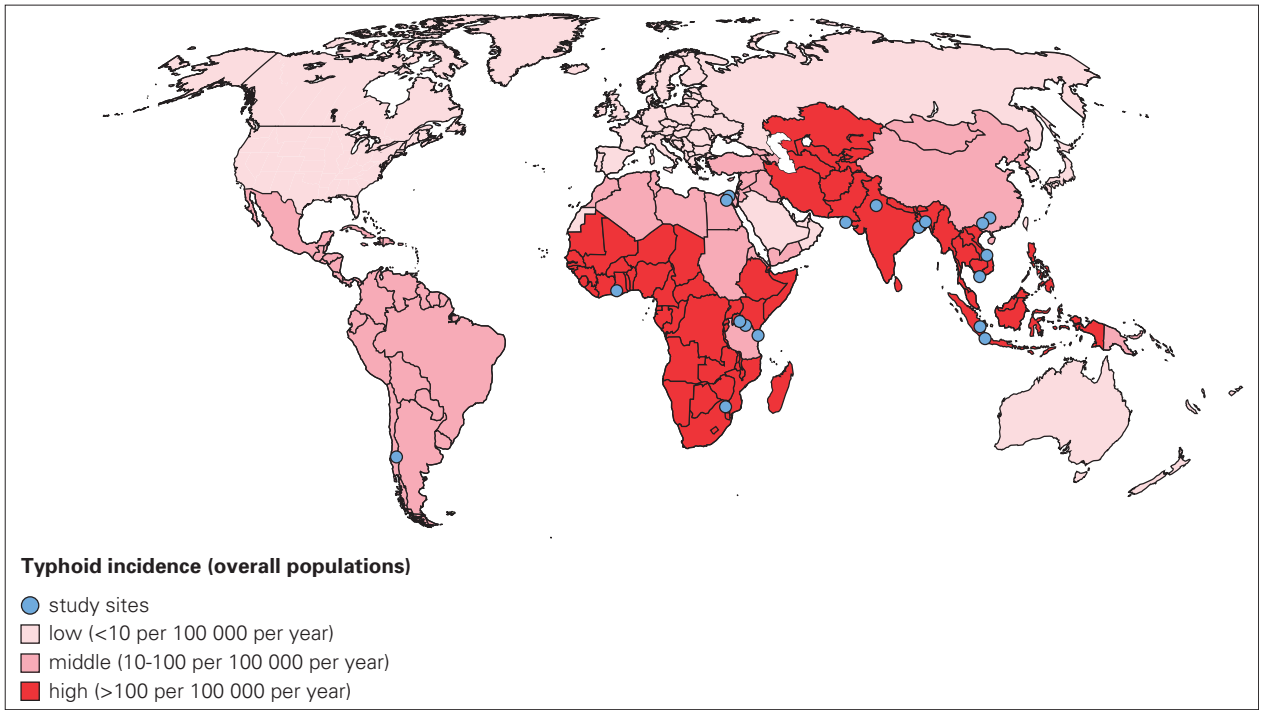


Figure 23.38 Typhoid incidence in low-income and middle-income countries. (Redrawn from Crump J.A., Sjölund-Karlsson M., Gordon M.A., Parry C.M. Epidemiology, clinical presentation, laboratory diagnosis, antimicrobial resistance, and antimicrobial management of invasive *Salmonella* infections. *Clin Micro Rev* 2015; 28[4];901-37. Fig 1, with permission.)

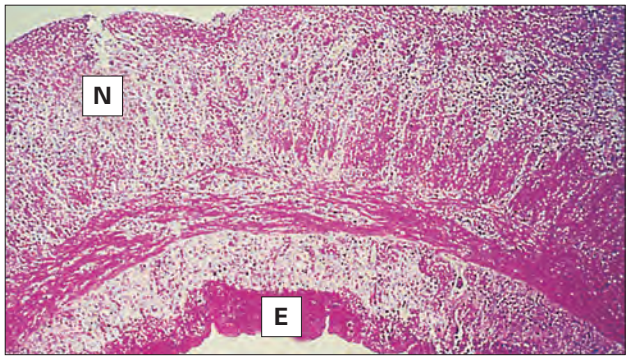


Figure 23.39 Typhoid. Section of ileum showing a typhoid ulcer with a transmural inflammatory reaction, focal areas of necrosis (N) and a fibrinous exudate (E) on the serosal surface (H&E stain). (Courtesy of M.S.R. Hutt.)

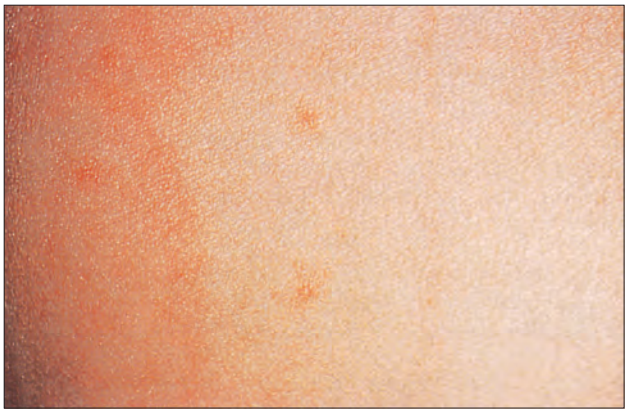


Figure 23.40 Rose spots on the skin in typhoid fever. (Courtesy of W.E. Farrar.)

- those associated with toxaemia (e.g. myocarditis, hepatic and bone marrow damage)
- those secondary to a prolonged serious illness
- those resulting from multiplication of the organisms in other sites, causing meningitis, osteomyelitis or endocarditis.

Before antibiotics became available, 12–16% of patients died, usually of complications occurring in the third or fourth week of the disease. Relapse after an initial recovery was also common.

One to three percent of patients with enteric fever become chronic carriers

Patients usually continue to excrete *S. typhi* in the faeces for several weeks after recovery, and 1–3% become chronic carriers, which is defined as *S. typhi* excretion in faeces or urine for 1 year after infection. Chronic carriage is more common in women, in older patients and in those with underlying disease of the gallbladder (e.g. stones) or urinary bladder (e.g. schistosomiasis).

Diagnosis of enteric fever depends upon isolating *S. typhi* or paratyphi types using selective media

Diagnosis cannot be made on clinical grounds alone, although the presence of rose spots in a febrile patient is highly suggestive. Samples of blood, faeces and urine should be cultured on selective media. An antibody response to infection can be detected by an agglutination test (Widal test), but non-specific cross-reaction with other enterobacteria may also

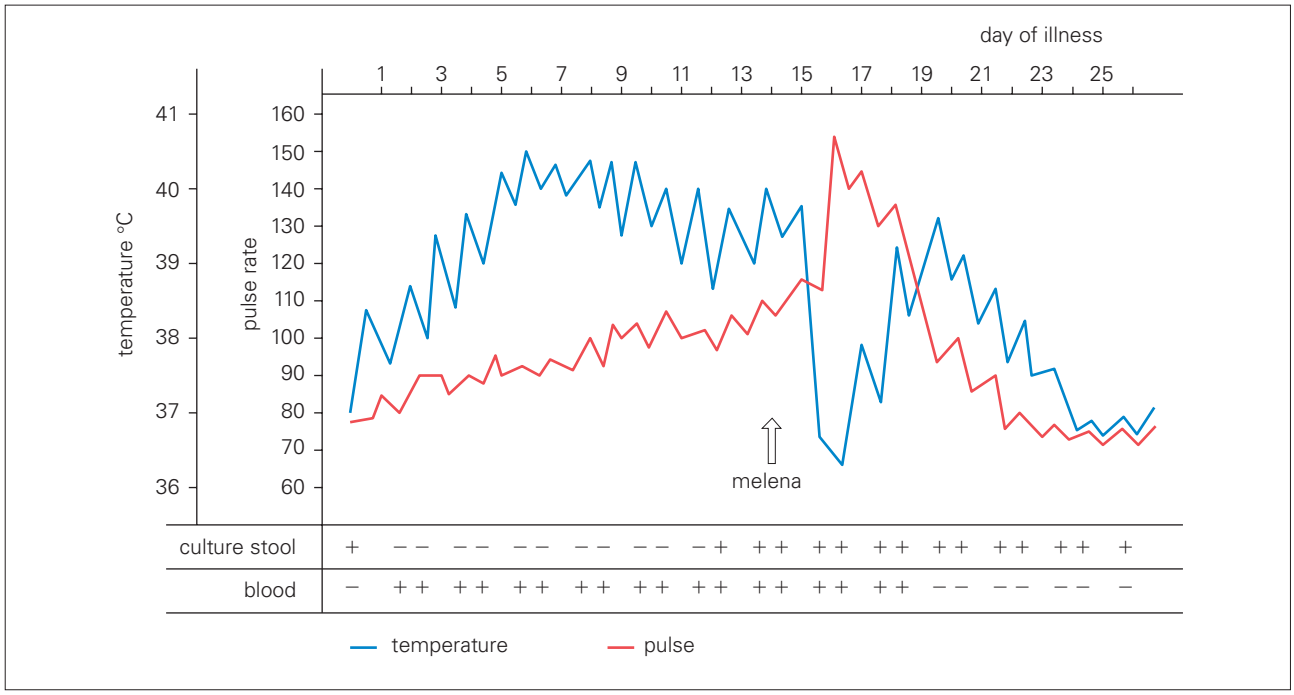


Figure 23.41 The clinical course of typhoid fever. Chart of temperature, pulse rate and bacteriological findings in a patient whose illness was complicated by massive haemorrhage. Melena: dark black, tarry faeces due to upper gastrointestinal blood loss colour due to altered blood. (Courtesy of H.L. DuPont.)

cause an increase in H and O antibody levels. Interpretation of the results is complicated and depends on knowing the normal antibody titres in the population and whether the patient has been vaccinated. A demonstration of a rising titre between acute- and convalescent-phase sera is more useful than examination of a single sample. At best, the results confirm the microbiological diagnosis; at worst they are misleading.

Antibiotic treatment should be started as soon as enteric fever is diagnosed

Ciprofloxacin or ceftriaxone followed by cefixime have been effectively used in antimicrobial chemotherapy, which should continue for at least 1 week after the patient's temperature has returned to normal. Some antibiotics appear active in vitro, but do not achieve a clinical cure, presumably because they do not reach the bacteria in their intracellular location. Isolates of *S. typhi* resistant to a variety of antimicrobial agents have been reported.

Prevention of enteric fever involves public health measures, treating carriers and vaccination

Breaking the chain of spread of infection from person to person depends upon good personal hygiene, adequate sewage disposal and a clean water supply. These conditions exist in the resource-rich world, where outbreaks of enteric fever are rare but still occur.

Typhoid carriers are a public health concern and should be excluded from employment involving food handling. Every effort should be made to eradicate carriage by antibiotic treatment and, if this is unsuccessful, removal of the gallbladder (the most common site of carriage) should be considered.

A single-dose injectable vaccine (Typhim Vi), which contains capsular polysaccharide antigen, and an oral, live-attenuated, vaccine (strain Ty21a) are available and recommended for travellers to resource-poor countries. However, with both vaccines there is complete protection in only 50–80% of recipients.

Listeriosis

Listeria infection is associated with pregnancy and reduced immunity

Listeria monocytogenes is a Gram-positive coccobacillus that is widespread among animals and in the environment. It is a food-borne pathogen, associated particularly with uncooked foods such as pâté, contaminated milk, soft cheeses and coleslaw. Studies of cases involving unpasteurized milk suggest that fewer than one thousand organisms may cause disease, and the ability of the organism to multiply, albeit slowly, at refrigeration temperatures allows an infective dose to accumulate in goods stored in this way. Even then, the population at risk appears primarily to be:

- pregnant women, with the possibility of infection of the baby in the uterus or during birth
- immunocompromised individuals including those with cancer, AIDS, on immunosuppressive drugs
- elderly individuals.

The disease usually presents as meningitis (see Ch. 25).

Viral hepatitis

An alphabetical litany of viruses directly target the liver, from hepatitis A to E

Hepatitis means inflammation and damage to the liver, and has differing aetiologies including non-infectious multisystemic conditions and drug toxicity as well as infectious agents. The latter include viruses and less commonly bacteria (e.g. *Leptospira* spp.), and other microorganisms. There is a broad spectrum of clinical illness ranging from asymptomatic, through

Table 23.6 The main viruses causing hepatitis in humans

Virus	Virus classification	Type of virus	Mode of infection	Incubation period	Other comments
Hepatitis A (HAV)	Hepatovirus	ssRNA	Faecal–oral	2–4 weeks	No carrier state
Hepatitis B (HBV)	Hepadnavirus	dsDNA	Blood-borne, sexual	6 weeks–6 months	Carriage associated with liver cancer
Hepatitis C (HCV)	Flavivirus	ssRNA	Blood-borne	2 months	Carriage associated with liver cancer
Hepatitis D (HDV)	Deltavirus	ssRNA	Blood-borne	2–12 weeks	Needs concurrent hepatitis B virus infection
Hepatitis E (HEV)	Orthohepevirus	ssRNA	Faecal–oral	2–6 weeks	Sporadic infection, large outbreaks in Asia, food-borne, can cause persistent infection in immunosuppressed individuals
Yellow fever	Flavivirus	ssRNA	Mosquito	3–6 days	No person-to-person spread, no carrier state

Other viruses causing hepatitis include Epstein–Barr virus (mild hepatitis in 15% of infected adults and adolescents), cytomegalovirus (CMV), adenovirus and rarely herpes simplex virus, while intrauterine infection with rubella or CMV causes hepatitis in the newborn. ds, double-stranded; ss, single-stranded.

symptomatic with malaise, anorexia, nausea, abdominal pain and jaundice, to acute life-threatening liver failure, which is rare. Jaundice is a clinical term for the yellow tinge to the skin, sclera and mucous membranes. This is a result of liver cell damage which means that the liver cannot transport bilirubin into the bile, causing increased bilirubin levels in the body fluids. More than half of the liver must be damaged or destroyed before liver function fails. Regeneration of liver cells is rapid, but fibrous repair, especially when infection persists, can lead to permanent damage called cirrhosis. Cirrhosis results in a small, shrunken liver with poor function.

At least six different viruses are referred to as hepatitis viruses (Table 23.6), and generally they cannot be distinguished clinically. However, hepatitis A and E viruses are transmitted by the faecal–oral route and generally do not result in a carrier state and both resolve, although chronic HEV infection can occur in immunocompromised individuals. In contrast, hepatitis B, D (delta), and C are transmitted by similar routes involving blood-contaminated equipment, although sexual transmission of hepatitis B is much more common than in hepatitis C, and all can lead to chronic carriage. Some agents have been reported that were thought to be involved in the spectrum of what is referred to as non-A–E hepatitis. However, there is no evidence that the GB, hepatitis G and TT viruses infect the liver directly, the liver being affected as a bystander. Other viruses also cause hepatitis as part of a disease syndrome and are dealt with in other chapters. Dramatic elevations of serum aminotransferase concentration (i.e. alanine aminotransferase, ALT; aspartate aminotransferase, AST) are characteristic of acute viral hepatitis. Specific laboratory tests to make the serological diagnosis of hepatitis A, B, D, C and E virus infections are available, as are PCR tests to detect and quantify the hepatitis B, C and E virus load in those with chronic infections. With the exception of hepatitis A and B there are no licensed vaccines, and specific antiviral treatments with and without immunomodulators are available for hepatitis B, C and E.

Hepatitis A

This infection is caused by hepatitis A virus (HAV), a single-stranded unenveloped RNA virus that has its own genus *Hepatovirus* in the Picornaviridae family. There is only one serotype, and the virus is endemic worldwide.

HAV is transmitted by the faecal–oral route

Virus is excreted in large amounts in faeces (10^8 infectious doses / g) and spreads from person to person by close contact (poor hand hygiene), by intimate contact (anal intercourse) or by contamination of food or water. The incubation period is 3–5 weeks, with a mean of 4 weeks; virus is present in faeces 1–2 weeks before symptoms appear and during the first week (sometimes also the second and third week) of the illness. Person-to-person transmission can lead to outbreaks in places such as schools and camps, and viral contamination of water or food is a common source of infection (Fig. 23.42). In resource-poor countries, up to 90% of children have been infected by 5 years of age, whereas in resource-rich countries up to 20% of young adults have been infected. The latter figure used to be higher but is mostly a result of improved sanitation and less overcrowding.

Clinically, hepatitis A is milder in young children than in older children and adults

After infection, the virus enters the blood from the gastrointestinal tract, where it may replicate. It then infects liver cells, passing into the biliary tract to reach the intestine and appears in faeces (Fig. 23.43). Relatively small amounts of virus enter the blood at this stage. Events during the rather lengthy incubation period are poorly understood, but liver cells are damaged, and this is thought to be due to direct viral action. Common clinical manifestations are fever, anorexia, nausea and vomiting; jaundice is more common in adults. The illness generally has a more sudden onset than hepatitis B. The best laboratory method for the diagnosis of an acute infection is to detect HAV-specific IgM in serum.

Pooled human normal immunoglobulin (HNIG) is no longer the mainstay of protecting contacts, having been replaced by vaccine. HNIG contains antibody to HAV and will prevent or attenuate infection if given as pre- or post-exposure prophylaxis. There is no antiviral therapy, but an effective formaldehyde-inactivated hepatitis A vaccine should be offered to a number of groups at particular risk of

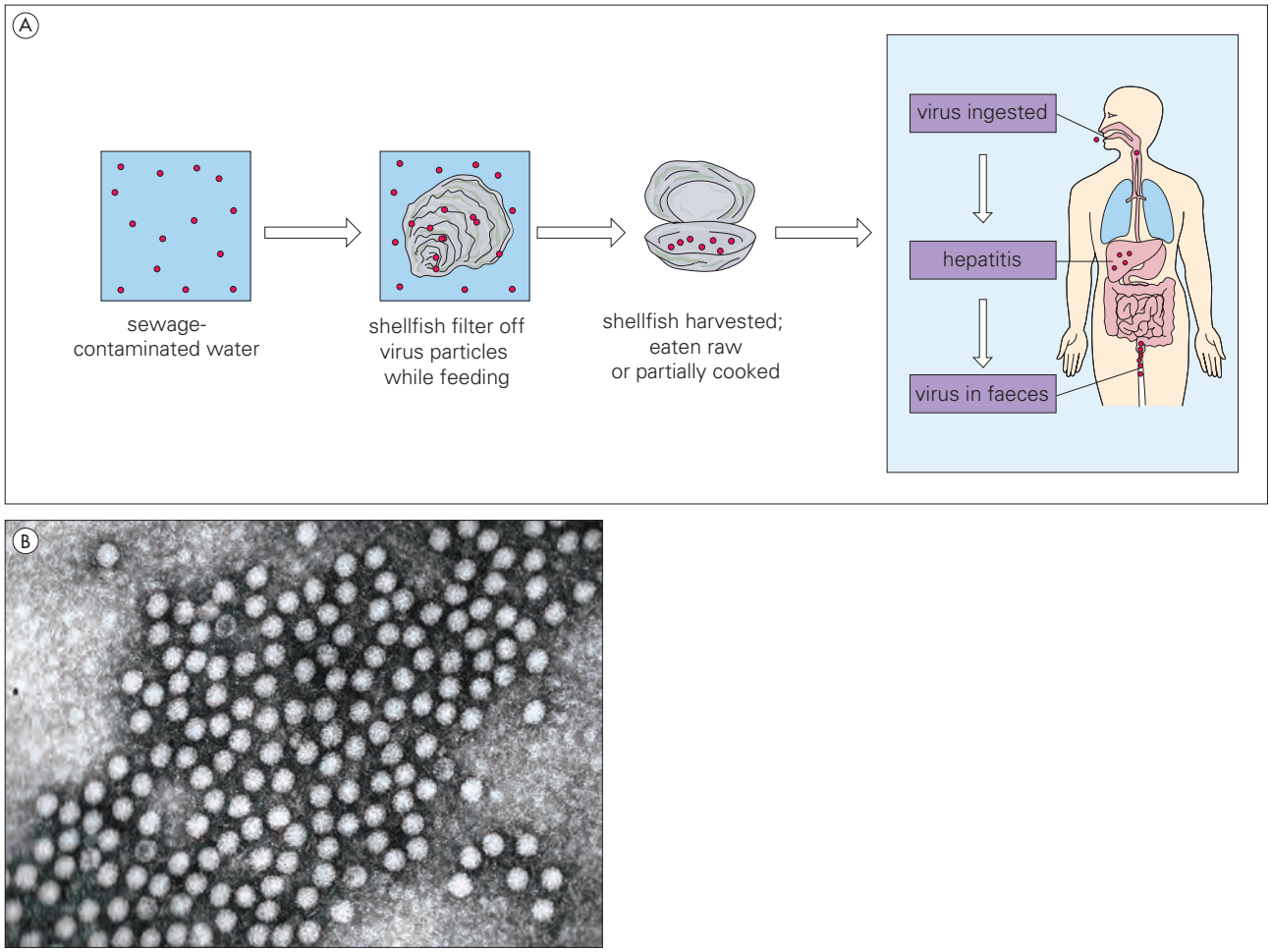


Figure 23.42 (A) Contamination of shellfish by hepatitis A virus (HAV) can lead to human infection. (B) Hepatitis A virus in a faeces sample from a patient with acute HAV infection. Electron micrograph (×170 000). (Courtesy of Professor A.J. Zuckerman, reproduced from *Principles and Practice of Clinical Virology*, 1987. Chichester: John Wiley & Sons, with permission.)

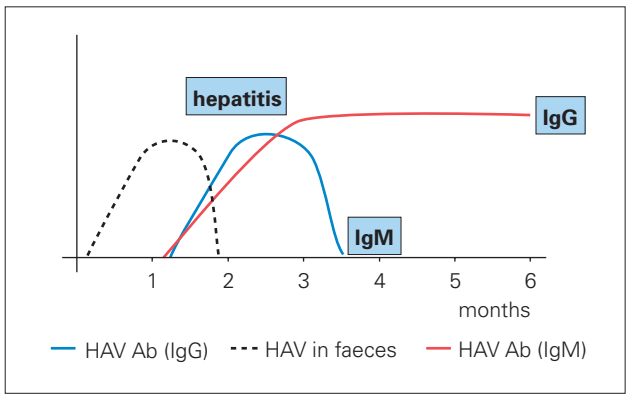


Figure 23.43 The clinical and virological course of hepatitis A virus (HAV). Ab, antibody; Ig, immunoglobulin.

infection. These include travellers to HAV-endemic countries, sewage workers, child daycare centre staff, institutional care workers, men who have sex with men (MSM), injecting drug users, and individuals with chronic liver disease and those with haemophilia. The vaccine is used alone or together with HNIG in certain situations, mostly if the contact is immunosuppressed, in the post-exposure setting providing it can be given to contacts within 14 days of the onset of jaundice in the infected individual. If the exposure was 2–4 weeks previously, HNIG may be offered alone to contacts at risk of severe complications such as those with chronic liver disease.

Hepatitis E

Hepatitis E virus (HEV) spreads by the faecal–oral route

HEV is an unenveloped single-stranded RNA virus which shares similarities with the caliciviruses. It has been classified in the genus *Hepevirus* in the family Hepeviridae, with four genotypes and one serotype.

Genotypes 1 and 2 have been involved in large outbreaks in resource-poor countries, transmitted between humans via the fecal–oral route. Genotypes 3 and 4 infect humans and other animals in both resource-rich and -poor settings and are zoonoses. The virus is excreted in faeces and spreads by the faecal–oral route. It is the major cause of sporadic (up to 60%) as well as epidemic hepatitis in Asia, in the latter due to water-borne routes of transmission. In addition, there are

increasingly identified sporadic infections in resource-rich countries.

HEV has been identified in a variety of animals, especially pigs, rabbits, wild boar, chickens and sika deer, and they constitute a reservoir for infection. Pigs are the major animal reservoir and they are asymptomatic. Zoonotic transmission is mainly due to eating undercooked pork or game meat, although direct contact with infected animals may be important as vets and swine handlers are more likely to have serological evidence of infection compared with the general population. HEV RNA has also been detected in seafood as well as untreated water. Reports of blood-transfusion-transmitted HEV infections in the UK in 2014 resulted in providing HEV RNA-screened negative blood products to transplant recipients as well as advice on cooking pork and pork products properly. HEV RNA had been detected in 1 in 2848 blood donations collected in southeast England and 42% of the recipients who could be followed up had been infected.

The incubation period is 2–6 weeks and the acute infection is usually self-limiting and mild, lasting a few weeks. However, it may be severe in pregnant women, with a high mortality (up to 20% during the third trimester) due to fulminant hepatitis, as well as in immunosuppressed individuals and those with chronic liver disease. An acute HEV infection in immunocompromised patients can result in a chronic hepatitis that can lead to cirrhosis. These patients are monitored by carrying out blood HEV RNA load tests. If detected, the aim is to clear the infection by reducing the immunosuppressive treatment where possible, using the antiviral drug ribavirin or the immunomodulator drug pegylated interferon unless contraindicated.

The diagnosis is made using serological tests to detect HEV IgM and confirmation can be carried out by HEV RNA testing. The 3-D crystal structure of the HEV capsid protein has been determined, which will lead to potential vaccines and antiviral agents. Two recombinant vaccines have undergone successful clinical trials, but in 2015 WHO issued a position paper stating that a recommendation would not be made on introducing the vaccine as there was insufficient information on safety, immunogenicity and efficacy in specific at-risk groups.

Hepatitis B

Hepatitis B virus (HBV) is a hepadna (hepatitis DNA) virus (Box 23.3) containing a partially double-stranded circular DNA genome and three important antigens: HB surface antigen, HB core antigen and HBe antigen (Fig. 23.44; Table 23.7). HBe antigen is a soluble component secreted by the virus core, is expressed on the hepatocyte surface and is targeted by the host immune system. Infection with a given strain of HBV confers resistance to all strains, but antigenic variation occurs. The four classical serological subtypes (*adw, adr, ayw* and *ayr*) have been superseded by the genotypic classification in which eight genotypes A to H have been determined. These can influence the clinical outcome of infection and response to antiviral treatment, and are useful in epidemiological studies.

Box 23.3 ■ Lessons in Microbiology

Hepatitis A

In August 1988, the Florida Department of Health and Rehabilitation Services traced 61 people who had suffered serologically confirmed infection with HAV. These individuals resided in five different states, but 59 of them had eaten raw oysters from the same growing areas in Bay County coastal waters. The oysters had been gathered illegally from outside the approved harvesting areas and were contaminated with HAV. The mean incubation period of the disease was 29 days (range 16–48 days). Probable sources of faecal contamination near the oyster beds included boats with inappropriate sewage disposal systems and discharge from a local sewage treatment plant that contained a high concentration of faecal coliforms.

Hepatitis B

One of the largest outbreaks of hepatitis B virus infections in Europe occurred in London in 1998. A patient went to an alternative medicine clinic and was treated with a technique called autohaemotherapy. This involved mixing a small sample of the patient's blood with saline, then injecting the blood and saline mixture into her buttocks or acupuncture points. She subsequently developed acute hepatitis B and the public health doctors were contacted and an investigation started having identified the practices in the clinic that could have resulted in her becoming jaundiced.

A lookback exercise was carried out involving 352 patients who had attended the clinic between January 1997 and February 1998 and four staff. Evidence of exposure to hepatitis B was found in samples from 57 (16%) of this group. Hepatitis B surface antigen was detected in blood samples collected from a total of 33 patients and staff, 23 of whom had acute hepatitis B. Molecular analysis revealed that 30 (91%) samples had identical nucleotide sequences and were part of a large community outbreak of hepatitis B. Five patients were chronic hepatitis B carriers, one of whom was the likely source of infection, with the vehicle being the contaminated saline in a vial that was used to mix the blood on a number of occasions for the other patients involved in the outbreak.

This demonstrated once again that only single-use vials must be used in healthcare settings, together with the benefits in those countries that offer universal immunization against hepatitis B to their populations.

Hepadnaviruses

Hepadnaviruses are also found in woodchucks, ground squirrels and Peking ducks. In each case, the infection persists in the body, with HBsAg-like particles in the blood and chronic hepatitis and liver cancer as sequelae. These viruses often infect non-hepatic cells. In northeast USA, for instance, 30% of woodchucks carry their own type of hepadnavirus and most develop liver cancer by later life. The virus replicates not only in liver cells, but also in lymphoid cells in the spleen, peripheral blood and thymus and in pancreatic acinar cells and bile duct epithelium.

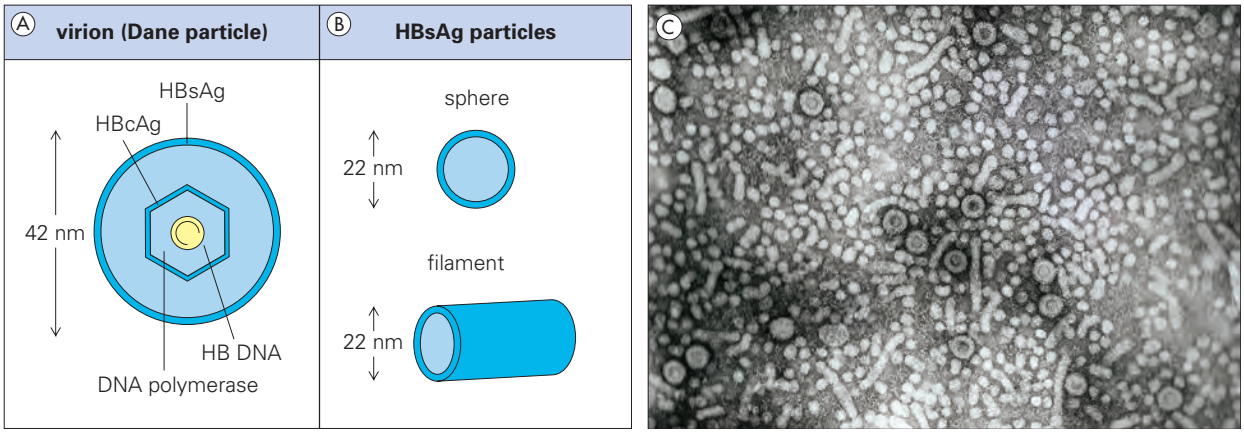


Figure 23.44 During acute infection, and in some carriers there are 10^6–10^7 infectious (Dane) particles/mL of serum (A), and as many as 10^{12} hepatitis B surface antigen (HBsAg) particles/mL (B). (C) Electron micrograph showing Dane particles and HBsAg particles. (Courtesy of Professor A.J. Zuckerman, reproduced with permission from *Principles and Practice of Clinical Virology*, 1987, John Wiley and Sons, Chichester.)

Table 23.7 Characteristics of hepatitis B virus (HBV) antigens (Ag) and antibodies (Ab)

HBsAg	Envelope (surface) antigen of HBV particle also occurs as free particles (spheres and filaments) in blood; indicates infectivity of blood
HBsAb	Antibody to HBsAg; post-hepatitis B vaccine response; appears late after resolved HBV infection (not in carriers)
HBcAb (total)	Antibody to HB core antigen; appears early; includes HB core IgM
HBc IgM	Appears in acute HBV infection; can last for 3 months and is a marker of acute HBV infection if it has resolved; seen in HBeAg-positive carriers with high viral replication; seen in HBeAg HBeAb reversion
HBeAg	Antigen derived from HBV core; indicates high transmissibility
HBeAb	Antibody to the HBV core

HB surface antigen can be found in blood and other body fluids

HBV can be transmitted by various routes including:

- sexual intercourse
- vertically from mother to child: intrauterine, peri- and postnatal infection
- via blood and blood products, blood-contaminated needles and equipment which may be used by injecting drug users
- in association with tattooing, body piercing and acupuncture, again due to reusing needles which may be contaminated by blood.

Transmission has been reported in healthcare settings such as renal units and has been associated with blood-contaminated haemodialysis equipment. This has been reduced dramatically since the introduction of regular HB surface antigen (HBsAg) monitoring of patients and disposable dialysis cartridges. In addition, incidents have been reported involving HBV transmission from hepatitis B carrier healthcare workers (HCWs) to their patients while carrying out exposure-prone procedures, such as cardiothoracic surgery, from intraoperative needlestick injuries resulting in blood-to-blood contact. Hepatitis B immunization and HBsAg screening of HCWs reduces the incidence of these transmission events. Blood and organ donors are also screened for HBsAg and HB core antibody in many countries worldwide, reducing the potential for transmission to recipients.

The number of HBV carriers worldwide is estimated to be over 350 million, and they play a major role in transmission. The HBV carrier prevalence is estimated to be up to 0.5% in north, west and central Europe, North America and Australia, up to 0.7% in east Europe, the Mediterranean littoral, Central and South America, Russia and southwest Asia, and up to 20% in South-East Asia, sub-Saharan Africa and China. In countries where infant and childhood infection is common (possibly because there is a high carrier rate in mothers), overall carrier rates are higher.

HBV is not directly cytopathic for liver cells, and the pathology is largely immune mediated

After entering the body, the virus reaches the blood, then the liver, where the result is inflammation and necrosis. Much of the pathology is immune mediated, as infected liver cells are attacked by virus-specific cytotoxic T cells. The incubation period ranges from 6 weeks to 6 months, the median being 2.5 months.

As liver damage increases, clinical signs of hepatitis appear (Fig. 23.45); the disease is generally more severe than hepatitis A. The immune response slowly becomes effective, virus replication is curtailed, and eventually, although sometimes not for many months, the blood becomes non-infectious.

Certain groups of people are more likely to become carriers of hepatitis B

People with a more vigorous immune response to the infection clear the virus more rapidly, but tend to suffer a more severe illness. However, about 10% of infected adults fail to eliminate the virus from the body, and become carriers.

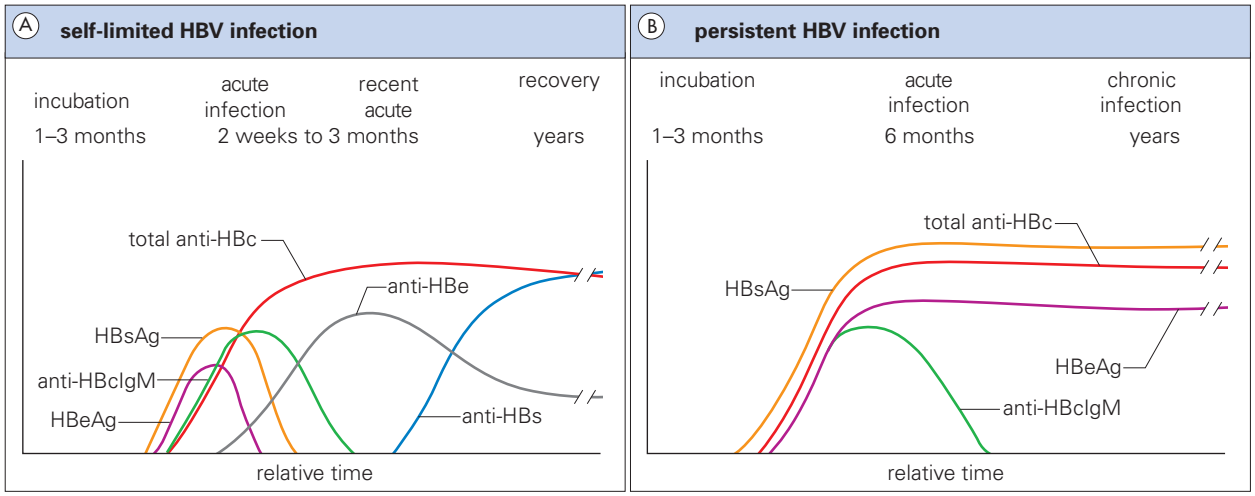


Figure 23.45 (A) Clinical and virological course of hepatitis B virus (HBV) infection, with recovery. (B) Clinical and virological course in a carrier of hepatitis B. (Redrawn from: Farrar, W.E., Wood M.J., Innes, J.A. et al. [1992] *Infectious Diseases*, 2nd edn. London: Mosby International.)

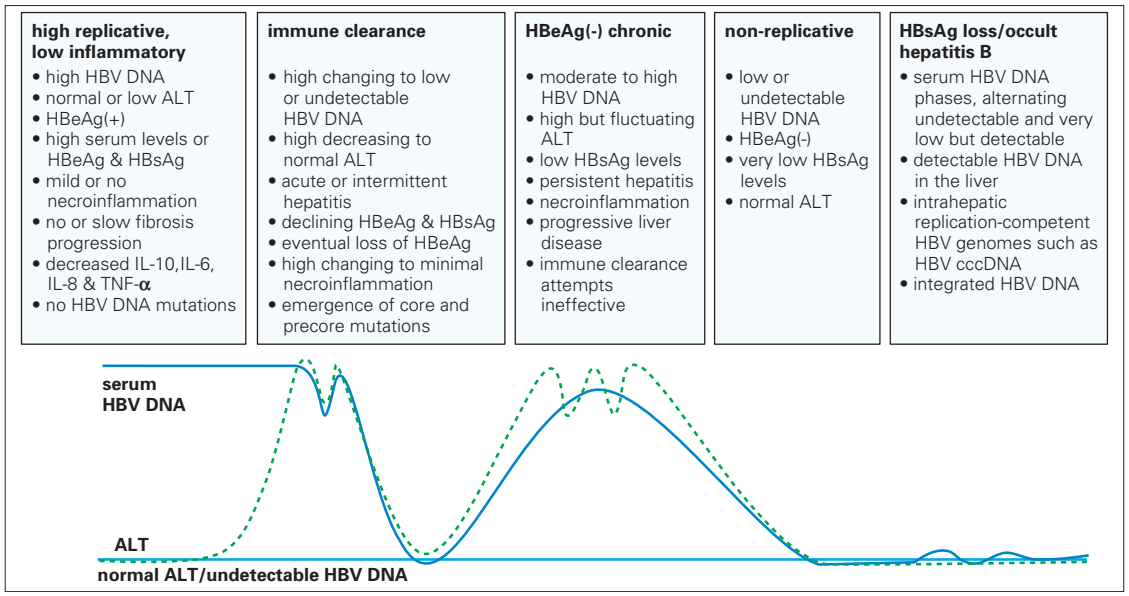


Figure 23.46 Major phases of chronic hepatitis B virus (HBV) infection. The natural history can be divided into five major phases: high-replicative, low-inflammatory; immune clearance; HBeAg(–) chronic hepatitis; non-replicative; and HBsAg loss/occult hepatitis. These phases do not occur in all patients, and transitions between them are dynamic and can be non-consecutive. Ag, antigen; ALT, alternating; IL, interleukin; TNF, tumour necrosis factor. (Redrawn from Ghish R.G., Given B.D., Lai C-L. et al. Chronic hepatitis B; virology, natural history, current management and a glimpse at future opportunities. *Antiviral Research* 2015; 121:47–58, Fig 4.)

The HBV viraemia means the person is infectious, often for life but sometimes spontaneous clearance occurs. Although continuing liver damage can cause chronic hepatitis, the damage may be minimal and the carrier remains in good health. In general, after chronic infection is established there is the high-replicative, low-inflammatory phase, which has replaced what was termed the immune-tolerant phase, and which may last for decades. This is where there is a high HBV DNA load in the blood, no or minimal liver inflammation and mildly deranged liver function tests. The immune clearance stage follows with spikes of acute hepatitis and HBV DNA levels ending with immune control and a stable, low HBV DNA load. These events can cause liver damage leading to fibrosis and cirrhosis (Fig. 23.46). Certain groups of people are more or less likely to become carriers, as follows:

- Immunodeficient patients may have few if any symptoms due to the effect of reducing the host response to the infection, but are more likely to become carriers
- There is a marked age-related effect. For example, in a study carried out in Taiwan, 90–95% of perinatally infected infants became carriers, compared with 23% of those infected at 1–3 years of age and only 3% of those infected as university students
- Gender is another factor, with males being more likely to become carriers.

Complications of hepatitis B are cirrhosis and hepatocellular carcinoma

Complications of hepatitis B include:

- Cirrhosis, as a result of chronic active hepatitis. This is an irreversible form of liver injury which may lead to primary hepatocellular carcinoma.
- Hepatocellular carcinoma is one of the 10 most common cancers worldwide. Hepatitis B carriers are 200 times more likely than non-carriers to develop liver cancer. This is not seen until 20–30 years after the infection. The cancer cells contain multiple integrated copies of HBV DNA (see Ch. 18).

Serological tests are used in the diagnosis of HBV infection

HBsAg appears in the serum during the incubation period in the form of infectious Dane particles, named after David Dane who detected the 42 nm virions by electron microscopy (see Fig. 23.45). The characteristic serological picture in an acute HBV infection includes the detection of HBsAg, HB core IgM and HBe antigen. The HBsAg concentration generally falls and finally disappears during recovery and convalescence. As HBsAg disappears, the HB core IgM level wanes over the next 3 months, HB core total antibody (IgM and IgG) is detected but is almost all IgG by this stage, and HB surface antibody becomes detectable. Therefore, evidence of past infection will give the following serological profile (Table 23.8): HBsAg negative, HB core total antibody positive and HB surface antibody positive. HBV carriage is defined by detecting HBsAg in blood for a period of 6 months after the acute infection. When HBe antigen is detected, there are large amounts of virus in the blood and the carrier is considered to be of high infectivity, and when it disappears HBe antibody may become detectable. HBe antibody-positive carriers are considered to be of low infectivity. However, HBV DNA load is a more useful marker of infectivity as mutations have been detected in the region encoding the e antigen which result in absence of e antigen production yet infectious virus is still assembled. Analagous to the famous Trojan horse, all seems normal but that is not the case. They are known as precore mutant viruses. Therefore, these patients will be HBe antigen negative and HBe antibody positive but could be highly infectious with high HBV DNA loads found in the blood.

The range of antiviral therapy has widened

Reducing the HBV viraemia and improving liver function have been the aims of therapy in order to prevent cirrhosis and liver cancer. A sustained virological response involves the absence of HBsAg and HBV DNA in the blood in individuals having stopped antiviral therapy, with the caveat that HBV DNA is integrated in the hepatocytes and can reactivate owing to immunesenescence and immunosuppression. Seven antiviral drugs are licensed for use and two classes of drugs used to treat hepatitis B virus infections are pegylated interferon and nucleotide / nucleoside analogues (see Ch. 34). Management of HBV carriers has been revolutionized with the advent of oral antiviral therapy, in particular lamivudine (3TC), adefovir, entecavir, emtricitabine and tenofovir. Previously, therapy with interferon α2b, an immunomodulator, was used, but only 30% of selected patients achieved sustained responses. In addition, interferon treatment has significant side effects. However, the better pharmacokinetics of pegylated interferon α2a has improved the results with respect to sustained response after treatment has been discontinued, especially in the e-antigen-positive carriers. Better responses to interferon are seen in females under 50 years old, infected in adulthood with HBV genotype A or B, with a lower HBV DNA load and alanine aminotransferase more than twice the upper limit of normal. Moreover, with the range of antivirals available, courses of treatment are available that depend on a number of factors related to the virus as well as the stage of liver disease. For example, entecavir or tenofovir can be used if lamivudine resistance develops, which it does in 70% of those treated after 5 years. Entecavir and tenofovir are the most effective antivirals in terms of undetectable HBV DNA in blood, improved liver biopsy histology and normal transaminases.

Other direct- and indirect-acting antiviral drugs targeting multiple stages of the viral life cycle and interfering with the host immune function respectively are being investigated.

Hepatitis B infection can be prevented by immunization

The original vaccine was produced in 1981 and consisted of purified HBsAg, prepared from the plasma of carriers, which was chemically treated to kill any contaminating viruses. The current vaccine is genetically engineered HBsAg produced in yeast or mammalian cells. Three injections of vaccine over a 6-month period will lead to a response and protection in over 90% of healthy adults. Immunization is recommended, especially for those who may be exposed to blood or blood products, such as receiving multiple transfusions or dialysis patients, all healthcare workers, sexual contacts of individuals with acute

Table 23.8 Interpretation of hepatitis B virus serological results

	Acute hepatitis B	Hepatitis B carrier	Hepatitis B carrier	Past hepatitis B virus infection[b]	Hepatitis B vaccine response
HBsAg[a]	+	+	+	–	–
HB core antibody (total)	+	+	+	+	–
HB core IgM	+	–	–	–	–
HBe antibody	–	+	–	+	–
HBe antigen	+	–	+	–	–
HB surface antibody	–	–	–	+	+

[a]Always confirm by neutralization if positive.
[b]Or passively acquired antibody having received blood products from someone with a history of past HBV infection.

or chronic hepatitis B, and injecting drug users. One problem is that up to 10% of healthy individuals may not respond to the vaccine, even when re-immunized. This could be due to genetically determined defects in the immune repertoire or because of the induction of immune suppressor cells.

The global prevalence of HBV infections fell after the vaccine was introduced in 1982. The WHO Global hepatitis report of 2017 revealed an estimate that 4.5 million HBV childhood infections were prevented annually. Universal hepatitis B immunization programmes for newborns or babies are conducted in 48 of 53 countries in the WHO European Region, after the UK joined in August 2017 and worldwide in at least 187 countries.

After accidental exposure to infection, hepatitis B immunoglobulin (HBIG) can be used to provide immediate passive protection to unimmunized people. This is prepared from the serum of individuals with high titres of HB surface antibody. It may also be used together with hepatitis B vaccine to prevent transmission to babies born to highly infectious HBV carrier mothers.

Hepatitis C

Hepatitis C virus was the most common cause of transfusion-associated non-A-non-B viral hepatitis

Hepatitis C virus (HCV) was discovered in 1989 as the cause of 90–95% of cases of transfusion-associated non-A-non-B hepatitis. It is an enveloped single-stranded RNA virus in the *Hepacivirus* genus in the Flaviviridae family. The discovery of HCV was a tour de force in molecular virology. The viral RNA was extracted from blood, a complementary DNA (cDNA) clone was made, and viral antigen produced. Serum from individuals with non-A-non-B hepatitis was then tested for the presence of antibody to the viral antigen. The introduction of first-generation HCV antibody-screening tests between 1990 and 1992, and subsequent improvement in sensitivity and specificity of these assays and genome detection methods, has resulted in a massive reduction in transfusion-associated HCV infection. It is estimated that more than 185 million people worldwide are infected with HCV.

HCV transmission routes share similarities with hepatitis B

HCV is present in blood, and transmission routes include blood and blood products, blood-contaminated needles and equipment which may be used by injecting drug users, and in association with tattooing, body piercing and acupuncture, again due to reusing potentially blood-contaminated needles from other clients. Transmission has been reported in healthcare settings such as renal units because of contaminated dialysis equipment and other fomites, including gloves. Although the introduction of regular HCV monitoring of patients and disposable dialysis cartridges has helped in infection control, transmission has also occurred by other routes, probably often involving contaminated gloves worn by HCWs, which may not have been changed between patients. In addition, there have been incidents involving HCV transmission from HCV carrier HCWs carrying out exposure-prone procedures on their patients, such as intraoperative needlestick injuries resulting in blood-to-blood contact during cardiothoracic surgery. Unlike hepatitis B, HCV transmission is uncommon vertically, from mother to infant, and by sexual intercourse. There may be other methods of spread, as the route of transmission is unknown in up to 40% of infected individuals.

The HCV envelope binds to the hepatocyte cell surface membrane allowing viral entry through a number of host cell receptors. It involves a multiple-step entry process that has yet to be fully elucidated. Some of the HCV proteins interfere with the host response and other evasive measures include the high degree of genetic diversity due to the high error rate of RNA replication.

Six major HCV genotypes and more than 100 subtypes have been identified. They have a global distribution, but genotypes 1 and 3 are most common. Around 90% of HCV infections are genotypes 1, 2 and 3 in Europe, whereas in the Americas mostly genotype 1 is seen and the rest are genotype 2. Genotype 4 is found mostly in northeast and central Africa, subtype 4a in Egypt particularly after syringe needles were reused during a schistosomiasis treatment national campaign. Genotype 6 is found mostly in East and South-East Asia. Genotype determination is predictive of antiviral therapy response, genotype 1 being associated with poor response. Viral and host factors affect the disease progression rate, with high HCV load in blood, genotype, and the degree of viral heterogeneity referred to as the quasispecies, being associated with more rapid progression. Viral clearance is associated with both the development and persistence of strong HCV-specific cytotoxic T-cell and helper T-cell responses.

Being infected with one genotype does not protect against the others; therefore multiple infections are possible, making the production of a cross-protective vaccine more difficult.

About 75–85% of HCV-infected individuals develop chronic HCV

The incubation period is 2–4 months, with a mean of 7 weeks. Subclinical infection is the rule, with about 25% of individuals developing jaundice in the acute infection, in contrast to the 90% seen in acute HBV infection. This makes the diagnosis of HCV infection more difficult as many individuals do not know they have been infected. Virus is often detectable in the blood after recovery from the acute illness, and carriers are a source of infection. Up to 2% of apparently healthy individuals in the USA have HCV antibody, and as a result between 2.7 and 3.9 million people have an active infection. About 75–85% of HCV-infected individuals will develop chronic HCV and 10–15% will progress to cirrhosis within the first 20 years, with a resultant 1–4% risk per year of liver cancer in those with established cirrhosis. It is also a leading indication for liver transplantation. The rate of chronic HCV infection depends on the infected individual's age, gender, ethnicity and immune response.

Diagnostic tests for HCV infection involve serological assays to detect HCV antibody or combined HCV antibody and antigen assays, qualitative and quantitative HCV RNA detection methods and genotype analysis. HCV RNA is present in approximately 70% of HCV antibody positive individuals.

Direct-acting antivirals (DAA) have revolutionized HCV treatment in a short time period

The aim of treatment is a sustained virological response (SVR), which means that HCV RNA cannot be detected 6 months after completing a course of treatment (see Ch. 34). It is worth just spending a moment to consider the brief history that rapidly

changed the HCV treatment landscape. Pegylated interferon (IFN)α and ribavirin was the standard of care. Originally, IFNα monotherapy resulted in up to 40% initial response rates, but less than 20% were sustained responses. Treatment with pegylated IFNα, in which polyethylene glycol is attached to interferon extending the half-life and duration of activity, and ribavirin resulted in an SVR in 45% of patients with genotype 1 or 4 infections (48 weeks' treatment) and 80% of those with genotype 2 or 3 (24 weeks' treatment). Combining pegylated interferon and ribavirin with some DAAs improved the SVR rates in the more difficult to treat HCV infections.

Since 2011, after determining the crystal structure of the non-structural (NS) protein domains, numerous DAAs and combinations of these agents were licensed, and combining pegylated interferon and ribavirin with some DAAs improved the SVR rates in the more difficult to treat HCV infections. Targeting the viral NS3 protease and NS5 polymerase, the DAAs can eradicate chronic HCV RNA viraemias within 8 to 24 weeks of starting oral treatment. Pharmaceutical companies have been competing with one another to come up with a more tongue-twisting drug name than previously. The NS3 protease inhibitor drugs started with telaprevir and boceprivir, replaced by simeprevir, asunaprevir and paritaprevir. The NS5 polymerase inhibitors include daclatasvir, elbasvir, ledipasvir, ombitasvir, sofosbuvir and velpatasvir. Different barriers to antiviral resistance are seen, with sofosbuvir having a high barrier to resistance and activity against the genotypic spectrum. These DAAs, singly or combined, have the potential to cure HCV-infected individuals and eradicate infection.

In summary, HCV is another RNA virus that continuously evolves as a quasispecies. As a result, it has the advantage of evading host immune responses, the action of antivirals and makes vaccine development a massive challenge.

Hepatitis D

Hepatitis D virus can multiply only in a cell infected with HBV

This is caused by hepatitis D virus (HDV or delta virus), which has the smallest genome among animal viruses, a circular, single-stranded RNA genome. It is a defective virus, so-named because it can successfully multiply in a cell only when the cell is infected with HBV at the same time. When HDV buds from the surface of a liver cell it acquires an envelope consisting of HBsAg (Fig. 23.47). HDV needs the HBV capsid only to enter hepatocytes and once inside it replicates using host cell RNA polymerases.

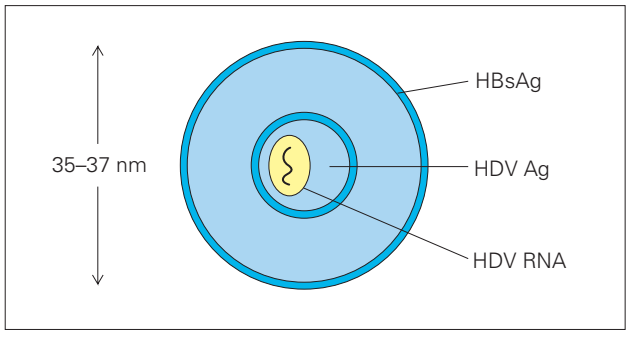


Figure 23.47 Structure of hepatitis D virus (HDV) in serum. Ag, antigen.

Spread of HDV is similar to that of HBV and HBC

Infected blood contains very large amounts of virus (up to 10^{10} infectious doses / mL in experimentally infected chimpanzees) and spread is similar to that of the other parenterally transmitted hepatitis viruses.

HDV infection may occur at the same time as an HBV infection, and the resulting disease is often more severe than with HBV alone. Alternatively, HDV superinfection of an HBV carrier may occur, which may accelerate the course of the chronic hepatitis-B-related liver disease. It is estimated that 15 million people globally have an HDV infection and that 5% of HBV-infected individuals have an HDV co-infection. Over the last two decades, the epidemiology of HDV infection has changed owing to universal hepatitis B immunization. However, high prevalence areas include the Mediterranean, Middle East, Pakistan, central and northern Asia and parts of Africa, South America and the Pacific region.

The diagnosis is made by serological tests for HD antigen ('delta' antigen) or HDV IgM and IgG. HBsAg will also be present. Antiviral treatment is limited by the fact that antivirals used to treat HBV infection have no effect on HDV, although interferon may be effective. However, there are significant relapse rates once treatment is discontinued. Different therapeutic strategies targeting other parts of the HDV life cycle are being investigated.

There is no HDV-specific vaccine, but successful hepatitis B immunization prevents infection with hepatitis D.

Viral hepatitis, the rest of the alphabet

After the discovery of HCV, a small percentage of hepatitis infections known to be transmitted by blood transfusion have yet to be attributed to a virus infection, although hepatitis G virus, referred to as GB virus C, transfusion-transmitted virus or Torque Teno virus (TTV) and SENV 25–28 have been detected in individuals with post-transfusion hepatitis. There are even more human hepatitis viruses waiting to be discovered.

Parasitic infections affecting the liver

Few protozoa affect the liver. Some worms live there as adults and others migrate through the liver to reach other locations.

Inflammatory responses to the eggs of *Schistosoma mansoni* result in severe liver damage

Liver pathology in parasitic infections is most severe in *S. mansoni* infection. Although the worms spend only a relatively short time in the liver before moving to the mesenteric vessels, eggs released by the females can be swept by the bloodstream into the hepatic circulation and be filtered out in the hepatic sinusoids. The inflammatory response to these trapped eggs is the primary cause of the complex changes that result in hepatomegaly, fibrosis and the formation of varices (Fig. 23.48A–D).

Whereas schistosomiasis is widespread in tropical and subtropical regions, other parasitic infections affecting the liver are more restricted in their distribution (e.g. clonorchiasis and alveolar hydatid disease).

In Asia, infections with the human liver fluke *Clonorchis sinensis* are acquired by eating fish infected with the metacercarial stage. Juvenile flukes released in the intestine move up the bile duct and attach to the duct epithelium

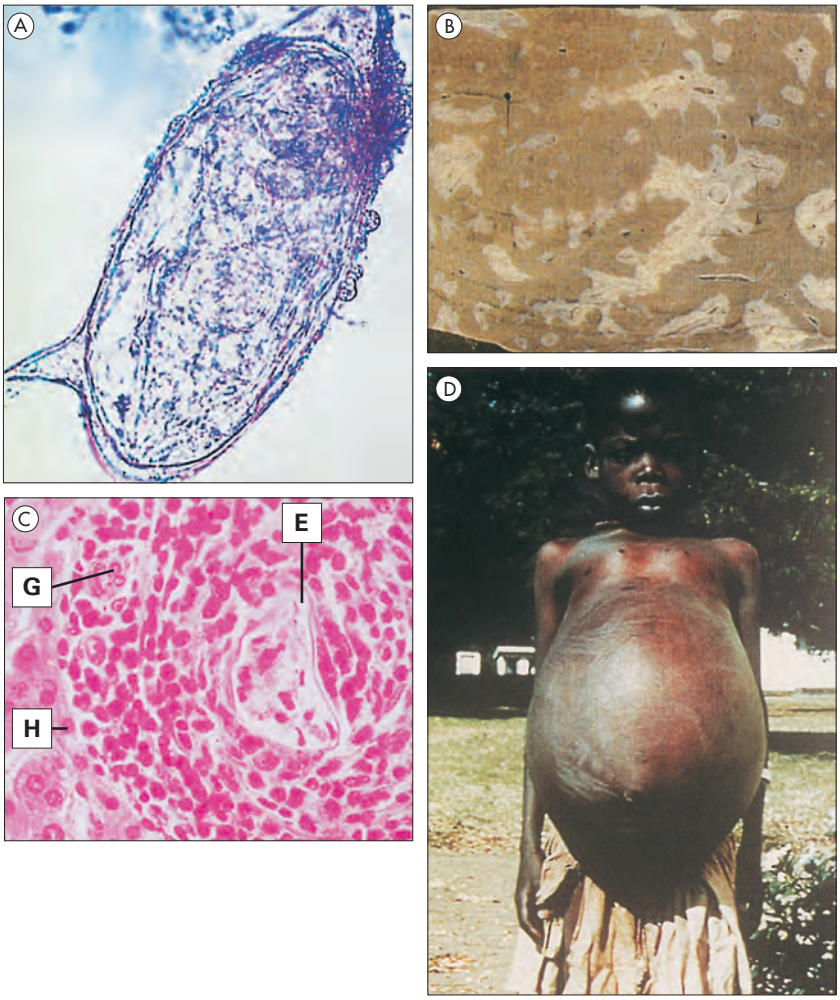


Figure 23.48 The portal fibrosis of *Schistosoma mansoni* is the end result of large numbers of granulomas formed around worm eggs deposited in the liver. In the related *Schistosoma haematobium* infection, a similar process occurs in the wall of the bladder. (A) Egg of *S. mansoni* (×400). (B) Pipe-stem fibrosis in the liver as a result of coalescent calcified granulomas. (C) Cellular reaction around an egg in the liver. E, egg containing miracidium; G, giant cell; H, hepatic cell. (D) Advanced clinical schistosomiasis with massive hepatosplenomegaly and ascites due to portal obstruction. ([A]–[C] Courtesy of R. Muller. [D] Courtesy of G. Webbe.)

where they are able to live for 20 years, feeding on the cells and blood and tissue fluids. In heavy infections, there is a pronounced inflammatory response and proliferation and hyperplasia of the biliary epithelium. Cholangitis, jaundice and liver enlargement are possible consequences, but many people are asymptomatic in the early stages or experience non-specific symptoms. Chronic infection with *C. sinensis* or *Opisthorchis viverrini* is a recognized cause of intrahepatic cholangiocarcinoma and *C. sinensis* is classified as a group 1 biocarcinogen.

A number of animal liver flukes can also establish themselves in humans. These include species of *Opisthorchis* (in Asia and Eastern Europe) and the common liver fluke *Fasciola hepatica*. In general, the symptoms associated with these infections are similar to those described for *C. sinensis*.

The larval stages of the dog tapeworm *Echinococcus granulosus* can develop in humans when the eggs are swallowed. Larvae from the eggs move from the intestine into the portal circulation and develop into large hydatid cysts (cystic echinococcosis) in the liver in around two-thirds of cases, lungs and occasionally other organs. They can be seen on ultrasound or cross-sectional imaging as large cysts. Apart from pressure damage to surrounding tissues, rupture of the cysts leads to secondary spread and may cause anaphylaxis. Treatment strategy is determined by cyst size, site and type. Options include treatment with benzimidazole drugs alone (usually albendazole or mebendazole) for small unilocular cysts, percutaneous aspiration injection and reaspiration (PAIR) plus a benzimidazole drug for larger unilocular cysts, and open operation plus a benzimidazole drug for large cysts with daughter cysts. *E. multilocularis*, acquired from eggs passed by wild carnivores, usually foxes, behaves very differently and develops in the liver not as cysts but as a ramifying mass resembling a carcinoma (alveolar echinococcosis). *E. multilocularis* is treated by radical excision plus benzimidazole therapy. Inoperable cysts require life-long drug therapy. Liver transplantation is sometimes used.

Other parasitic infections associated with liver pathology are malaria, leishmaniasis, ascariasis and extraintestinal amoebiasis, which causes liver abscesses.

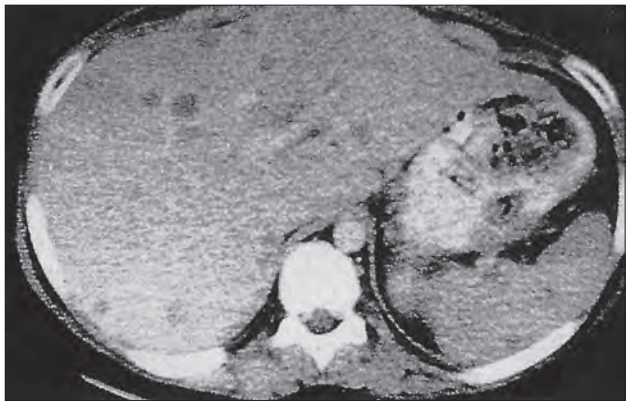


Figure 23.49 Multiple pyogenic liver abscesses due to *Pseudomonas aeruginosa*. (Courtesy of N. Holland.)

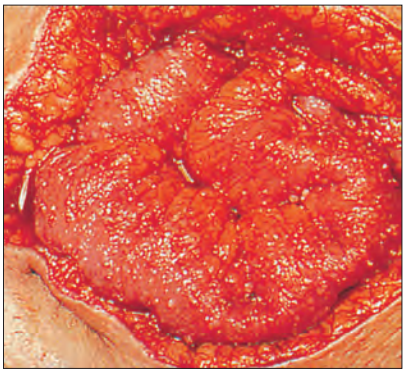


Figure 23.50 Tuberculous peritonitis. Oedematous bowel with multiple lesions on the peritoneal surface. (Courtesy of M. Goldman.)

Liver abscesses

Despite the name, an amoebic liver abscess does not consist of pus

E. histolytica can move from the gastrointestinal tract and cause disease in other sites, including the liver (see above). However, the term amoebic liver abscess is not strictly accurate because the lesion formed in the liver consists of necrotic liver tissue rather than pus. True liver abscesses – walled-off lesions containing organisms and dead or dying polymorphs (pus) – are frequently polymicrobial, containing a mixed flora of aerobic and anaerobic bacteria (Fig. 23.49). Lesions caused by both types of hydatid disease can become secondarily infected with bacteria. The source of infection may be local to the lesion or another body site, but is usually undiagnosed. Antibacterial therapy is required to cover both aerobes and anaerobes.

Biliary tract infections

Infection is a common complication of biliary tract disease

Although infection is not often the primary cause of disease in the biliary tract, it is a common complication. Many patients with gallstones obstructing the biliary system develop infective complications caused by organisms from the normal gastrointestinal flora such as enterobacteria and anaerobes. Local infection can result in cholangitis and subsequent liver abscesses or invade the bloodstream to cause septicaemia and generalized infection. Removing the underlying obstruction in the biliary tree is a prerequisite to successful therapy. Antibacterial therapy is usually broad spectrum, covering both aerobes and anaerobes.

Peritonitis and intra-abdominal sepsis

The peritoneal cavity is normally sterile, but is in constant danger of becoming contaminated by bacteria discharged through perforations in the gut wall arising from trauma (accidental or surgical) or infection. The outcome of peritoneal contamination depends upon the volume of the inoculum (1 mL of gut contents contains many millions of microorganisms) and the ability of the local defences to wall off and destroy the microorganisms.

Peritonitis is generally classified as primary (without apparent source of infection) or secondary (e.g. due to perforated appendicitis, ulcer, colon)

Peritonitis usually begins as an acute inflammation in the abdomen which may progress to the formation of localized intra-abdominal abscesses. In general, the aetiological agents responsible for primary and secondary peritonitis and intraperitoneal abscesses are different. Spontaneous bacterial peritonitis (SBP) is most commonly associated with cirrhosis of the liver. SBP is typically due to Gram-negative enteric bacteria, most commonly *E. coli*. Secondary peritonitis and intra-abdominal abscesses more often involve a mixture of organisms, especially the Gram-negative anaerobe *Bacteroides fragilis*. *Mycobacterium tuberculosis* and *Actinomyces* can also cause intraperitoneal infection (Fig. 23.50). In the absence of appropriate antibiotic therapy, infections are frequently fatal, and even with appropriate treatment the mortality remains at 1–5%. Empiric antibiotic therapy for SBP commonly involves third-generation cephalosporins, such as ceftriaxone (see Ch. 34) with re-evaluation when culture results are available. Initial antimicrobial treatment of secondary peritonitis must especially target the Gram-negative anaerobe *B. fragilis* (e.g. metronidazole) and Gram-negative aerobic pathogens as well as taking steps to eliminate the source of contamination. Mycobacterial infection requires specific antituberculosis therapy (see Ch. 34), while actinomycosis responds well to prolonged treatment with penicillin.

KEY FACTS

- Diarrhoeal disease is a major cause of morbidity and mortality in the resource-poor world. A wide range of diverse pathogens cause infections of the gastrointestinal tract. Diarrhoea, the most common symptom, ranges from mild and self-limiting to severe with consequent dehydration and death.
- Gastrointestinal pathogens are transmitted by the faecal–oral route. They may invade the gut, causing systemic disease (e.g. typhoid), or multiply and produce locally acting toxins and damage only the gastrointestinal tract (e.g. cholera). The number of organisms ingested and their virulence attributes are critical factors in determining whether infection becomes established.
- Microbiological diagnosis is usually impossible without laboratory investigations, but the patient's history, including food and travel history, provides useful pointers.
- The major bacterial causes of diarrhoea are *E. coli*, salmonellae, *Campylobacter*, *V. cholerae* and shigellae. Other less common causes include *C. perfringens*, *B. cereus*, *V. parahaemolyticus* and *Y. enterocolitica*. Food poisoning (i.e. the ingestion of bacterial toxins in food) is caused by *S. aureus* and *C. botulinum*.
- *E. coli* is the major bacterial cause of diarrhoea in resource-poor countries and of traveller's diarrhoea. Distinct groups within the species (ETEC, EHEC, EPEC and EIEC) have different pathogenic mechanisms – some are invasive, others toxigenic.
- Salmonellae and *Campylobacter* are common in resource-rich countries, have large animal reservoirs and spread via the food chain. Both cause disease by multiplication in the gut and the production of locally acting toxins.
- *V. cholerae* and shigellae have no animal reservoirs and the diseases are potentially eradicable. Transmission is prevented by good hygiene, clean drinking water and hygienic disposal of faeces. The pathogenesis of cholera depends upon production of cholera enterotoxin, which acts on the gastrointestinal mucosal cells. In contrast, *Shigella* invades the mucosa, causing ulceration and bloody diarrhoea, symptoms similar to those of amoebic dysentery.
- *H. pylori* is associated with gastritis and duodenal ulcers. Removal of the bacterium by combination treatment with antibiotics and proton pump inhibitors reduces symptoms and encourages healing.
- Disruption of the normal bacterial flora of the gut (usually due to antibiotic treatment) allows organisms normally absent or present in small numbers (e.g. *C. difficile*) to multiply and cause antibiotic-associated diarrhoea.
- Although viruses appear to be the most common causes of gastroenteritis in infants and young children, viral gastroenteritis is not distinguishable clinically from other types of gastroenteritis. The chief culprits are norovirus and rotavirus infections, although rotavirus immunization programmes will reduce the incidence of infections.
- Ingestion of food or water contaminated with *S. typhi* or *paratyphi* types can result in the systemic infection enteric (typhoid) fever. These pathogens invade the gut mucosa and are ingested by, and survive in, macrophages. They are transported via the lymphatics to the bloodstream from whence they seed many organs and give the characteristic multisystem disease. Positive diagnosis depends upon culture of the organism. Specific antibiotic therapy is required and specific prevention is achievable through immunization.
- Hepatitis is usually caused by viruses, especially hepatitis A–E viral infections. Hepatitis A and E are transmitted by the faecal–oral route and the rest by contaminated blood or the sexual route. Infection with HBV and HCV often leads to chronic hepatitis and can result in liver cancer. Antiviral treatments have been developed for both HBV and HCV which are highly effective. In particular, the oral DAAs for treating HCV infection could potentially eliminate this infection with high sustained virological responses. Vaccines can prevent hepatitis A and B virus infections.
- Many protozoa and worms live in the intestine, but relatively few cause severe diarrhoea. Important protozoa are *E. histolytica*, *G. intestinalis* and *Cryptosporidium*, which are acquired by ingestion of infective cysts in faecally contaminated food or water. Important worms are *Ascaris*, *Trichuris, Strongyloides* and hookworm. They have more complex routes of transmission, with the eggs or larvae requiring a development period outside the human host.
- Parasitic infections involving the liver include infections by *Schistosoma mansoni* in the tropics and subtropics, and *Clonorchis sinensis*, the human liver fluke, in Asia. Other parasitic infections with important liver pathology include malaria, leishmaniasis, extraintestinal amoebiasis, echinococcosis (hydatid disease) and ascariasis.
- Infection of the biliary tree is usually secondary to obstruction. The normal intestinal flora causes mixed infections, which may extend to produce liver abscesses and septicaemia.
- Peritonitis and intra-abdominal sepsis follow contamination of the normally sterile abdominal cavity with intestinal pathogens. The presentation is acute, and infection can be fatal. Antibiotic therapy against both aerobic and anaerobic bacteria is essential.

24 Obstetric and perinatal infections

Introduction

During pregnancy, a novel set of tissues potentially susceptible to infection appear, including the fetus, the placenta and the lactating mammary glands. The placenta acts as an effective barrier, protecting the fetus from most circulating microorganisms, and the fetal membranes shield the fetus from microorganisms in the genital tract. Perforation of the amniotic sac, for instance, at a late stage of pregnancy, often results in fetal infection.

During pregnancy, certain infections in the mother can be more severe than usual, including malaria and viral hepatitis, or latent viruses such as herpes simplex virus (HSV) and cytomegalovirus (CMV) can reactivate and infect the fetus, and after delivery the raw uterine tissue is susceptible to streptococcal and other pathogens, causing puerperal sepsis.

The fetus, once infected via the placenta, is highly susceptible, but may survive certain pathogens and develop congenital abnormalities; examples include rubella, CMV, Zika virus (ZIKV), *Toxoplasma gondii* and *Treponema pallidum*. However, not all babies become infected after a maternal primary infection and there is an important distinction between babies being infected and as a result, affected. Bacteria from the vagina, such as group B streptococci, can cause neonatal septicaemia, meningitis and death, and a birth canal infected with *Neisseria gonorrhoeae* or *Chlamydia trachomatis* inoculates the infant to cause neonatal conjunctivitis. Maternal genital HSV infection can cause more serious neonatal disease and is underreported.

Maternal HIV infection, in resource-poor countries or where maternal infection is undiagnosed, can lead to up to 40% of infants infected, about one-third in utero, causing abortion, prematurity and low birth weight and two-thirds perinatally, from maternal viraemia or milk. Hepatitis B carrier mothers can transmit hepatitis B in utero as well as during delivery, and breast milk can be a source of human T-cell lymphotropic virus type 1 (HTLV-1) infection.

Here we describe infections that occur during pregnancy and around the time of birth, and discuss their effects on the mother, the fetus and the neonate.

INFECTIONS OCCURRING IN PREGNANCY

Immune and hormonal changes during pregnancy worsen or reactivate certain infections

The fetus may be considered as an immunologically incompatible implant that must not be rejected by the mother. Reasons for the failure to reject the fetus include:

- the absence or low density of major histocompatibility complex (MHC) antigens on placental cells
- a covering of antigens with blocking antibody
- subtle defects in the maternal immune responses

A severe or generalized immunosuppression in the mother would be undesirable because it would mean potentially disastrous susceptibility to infectious disease. Certain infections, however, are known to be more severe (Table 24.1), and certain persistent infections reactivate during pregnancy. The hormonal changes that accompany pregnancy can also increase susceptibility. The picture is further complicated when there is malnutrition, which in itself impairs host defences by weakening immune responses, decreasing metabolic reserves and interfering with the integrity of epithelial surfaces.

The fetus has poor immune defences

Once the fetus is infected, it is exquisitely susceptible because:

- IgM and IgA antibodies are not produced in significant amounts until the second half of pregnancy
- there is no IgG antibody synthesis
- cell-mediated immune responses are poorly developed or absent, with inadequate production of the necessary cytokines

Indeed, if the fetus were able to generate a vigorous response to maternal antigens, a troublesome graft-versus-host reaction could be unleashed.

Most microorganisms have sufficient destructive activity to kill the fetus once it is infected, leading to spontaneous abortion or stillbirth. Here, our interests focus on the few microorganisms that are capable of subtler, non-lethal effects. They overcome the placental barrier by infecting it so that the

infection then spreads to the fetus. They can then interfere with fetal development, or cause lesions, so that a live but damaged baby is born.

CONGENITAL INFECTIONS

Intrauterine infection may result in death of the fetus or congenital malformations

After primary infection during pregnancy, certain microorganisms enter the blood, establish infection in the placenta, and then invade the fetus. The fetus sometimes dies, leading to abortion, but when the infection is less severe, as in the case of a relatively non-cytopathic virus, or when it is partially controlled by the maternal IgG response, the fetus survives. It may then be born with a congenital infection, often showing malformations or other pathological changes. The infant is generally small and fails to thrive. Virus-specific antibodies may be produced, but often, for instance with CMV, the fetus fails to generate an adequate virus-specific cell-mediated immune response, remaining infected for a long period. Hence, the lesions may progress after birth. It is a striking feature of these infections that they are generally mild or unnoticed by the mother.

Important causes of congenital infections are shown in Table 24.2. Viruses that induce fetal malformations (i.e. act as teratogens) share certain characteristics with other teratogens such as drugs or radiation. The fetus tends to show similar responses (e.g. hepatosplenomegaly, encephalitis, eye lesions, low birth weight) to different infectious agents, and the diagnosis is difficult on purely clinical grounds. Most of these infections – HSV, rubella, CMV and syphilis – can also, at times, kill the fetus. They generally follow primary infection of the mother during pregnancy, so their incidence depends upon the proportion of non-immune females of childbearing age.

Routine antenatal screening for rubella antibody, treponemal antibody (which includes syphilis, yaws, pinta or bejel, which cannot be identified individually by serology), hepatitis B surface antigen and HIV combination antibody and antigen assays are being carried out to differing degrees worldwide. These tests help identify women who are infected with hepatitis B or HIV, infected or have been exposed in the

Table 24.1 The effect of pregnancy on the severity of infectious disease

Infection	Comments
Malaria	? Depressed cell-mediated immunity
Viral hepatitis	The viral load may fluctuate owing to immunomodulation in pregnancy
Influenza	Higher morbidity and mortality
Poliomyelitis	Paralysis more common
Urinary tract infection	Cystitis; pyelonephritis more common; atony of bladder and ureter leads to less-effective flushing, emptying
Candidiasis	Vulvovaginitis
Listeriosis	Influenza-like illness
Coccidioidomycosis	Leading cause of maternal mortality in endemic areas in SW USA and Latin America

Table 24.2 Maternal infections that are transmitted to the fetus

Microorganism	Effects
Rubella virus	Congenital rubella
Cytomegalovirus (CMV)	Congenital CMV, deafness, mental retardation
Human immunodeficiency virus (HIV)	Congenital infection, childhood AIDS; about 1 in 5 infants born to infected mothers in resource-poor countries are infected in utero[a]
Zika virus (ZIKV)	Microcephaly, facial disproportionality, cutis gyrate
Varicella-zoster virus (VZV)	Skin lesions; musculoskeletal, CNS abnormalities when fetus infected before 20 weeks. After infection later in pregnancy, childhood zoster a common sequel[b]
Herpes simplex virus (HSV)	Neonatal HSV infection, often disseminated. Much higher risk when maternal infection primary rather than recurrent, infection in utero is rare
Hepatitis B virus	Congenital hepatitis B, persistent infection[a,c]
Parvovirus B19	After maternal infection 5–10% fetuses lost (abortion, hydrops fetalis)
Zika virus (ZIKV)	Congenital ZIKV syndrome includes microcephaly and other neurological features
Treponema pallidum	Congenital syphilis, classical syndrome
Toxoplasma gondii	Congenital toxoplasmosis
Trypanosoma cruzi	Congenital Chagas disease
Listeria monocytogenes	Congenital listeriosis, pneumonia, septicaemia, meningitis[b]
Mycobacterium leprae	Congenital infection common in mothers with lepromatous leprosy

Congenitally infected babies may be symptomless, especially in cytomegalovirus infection. They are often small, fail to thrive or show detectable abnormalities later in childhood. In all cases, the baby remains infected, often for long periods, and may infect others.
[a]This figure is for resource-poor countries with no intervention (no antiretroviral drugs, no caesarean section, or avoidance of breastfeeding).
[b]Infection also occurs during and immediately after birth.
[c]Protection of newborn by hepatitis B vaccine plus specific immunoglobulin.

past to treponemal infections, the most important of which is syphilis in this setting, or are susceptible to rubella.

Routine screening programmes lead to clinical management issues for both the mother and child. For example, an HIV-positive diagnosis will lead to healthcare staff discussing antiretroviral therapy for the mother and, immediately on birth, the child, planning a vaginal delivery unless a caesarean section is indicated, and advising against breastfeeding to reduce the risk of vertical transmission. In addition, the child will then be followed up for at least 12 months using sensitive tests to determine whether HIV has been transmitted vertically. Diagnosis of chronic hepatitis B virus (HBV) infection will result in determination of the maternal level of infectivity, and the baby subsequently being offered an accelerated course of hepatitis B vaccine alone or, if the mother is highly infectious, vaccine and HBV-specific immunoglobulin to the baby. In addition, there are antiviral drugs for chronic hepatitis B that might be offered, together with long-term follow-up, to the mother. Rubella-susceptible women are offered immunization postnatally, although, in the UK, rubella antibody testing was removed from the antenatal screening programme in 2016. This was because it did not meet the criteria for a screening programme on the basis that the incidence of rubella infection in the UK was so low that it was within the WHO definition of having been eliminated, that rubella infection in pregnancy was very rare, and that the measles, mumps and rubella (MMR) vaccine uptake had improved considerably. This had dropped in previous years, but by 2016 was deemed to be more effective in protecting women against rubella in pregnancy, before becoming pregnant, as the screening test used could potentially be interpreted incorrectly in women with an acute rubella infection.

Women found to have been exposed to treponemal infection in pregnancy are offered antibiotic treatment and the baby is followed up for the first year using serology to identify active infection, as congenital syphilis can result from earlier untreated infection of the mother. In the case of CMV, which is not part of routine antenatal screening in the UK and USA, for example, a primary infection, re-infection or reactivation of the latent virus during pregnancy can lead to fetal infection (see next section).

The likelihood of fetal infection is increased in a primary maternal infection and when the mother has a chronic infection, although there are a number of factors that result in varying risks of fetal infection.

There is no good evidence to suggest that maternal mumps, influenza or poliovirus infection during pregnancy leads to harmful effects in the fetus, but with human parvovirus B19 infection the risk of intrauterine infection is around 15% at 5–16 weeks of pregnancy and 25–70% after 16 weeks gestation, with a risk of fetal death around 9% in the first 20 weeks. The infected fetus develops hydrops fetalis due to severe anaemia, with ascites and hepatosplenomegaly in 3% as the virus infects progenitor erythroid stem cells. Intrauterine exchange blood transfusion is used to manage hydrops fetalis.

In terms of emerging infectious diseases, a Zika virus outbreak in Brazil, which by 2016 had emerged in South, Central and North America and the Caribbean too, resulted in reports of neonatal microcephaly and neurological illness, including Guillain–Barré syndrome.

Congenital rubella

The fetus is particularly susceptible to rubella infection when maternal infection occurs during the first 3 months of pregnancy

At this time, the heart, brain, eyes and ears are being formed and the infecting virus interferes with their development. If the fetus survives, it may show characteristic abnormalities (Fig. 24.1). Not all fetuses are affected despite the risk of

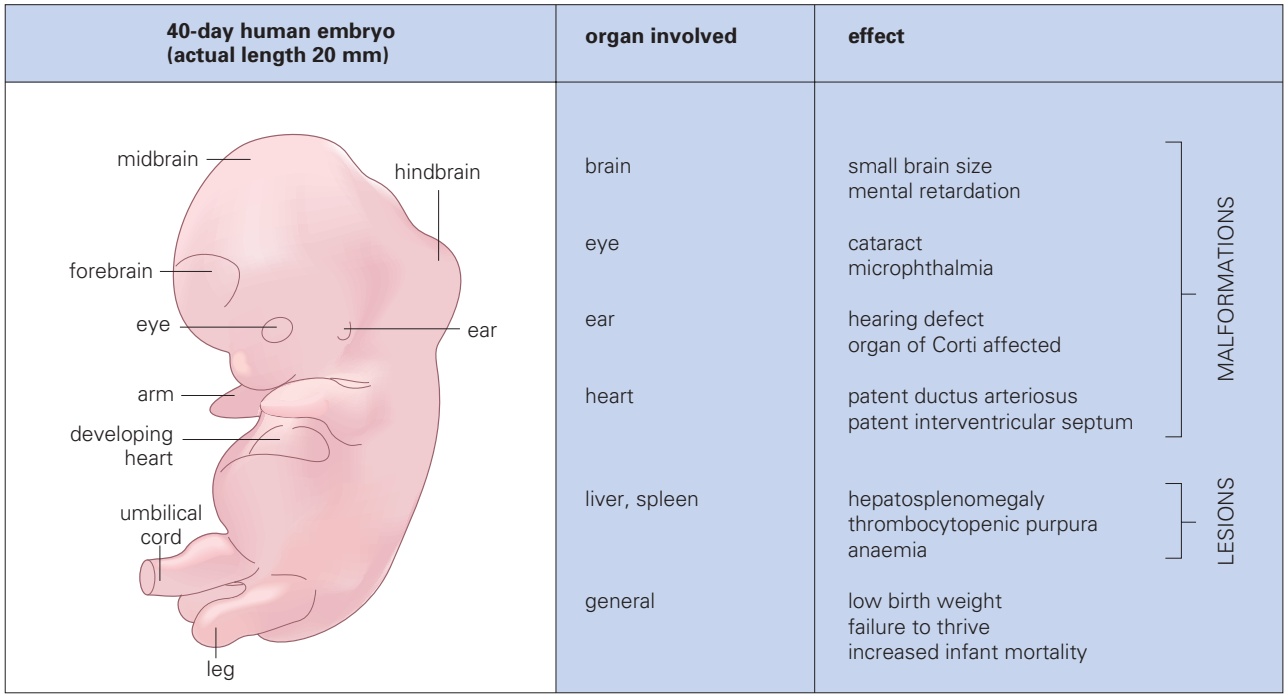


Figure 24.1 Organ involvement and effects in congenital rubella.

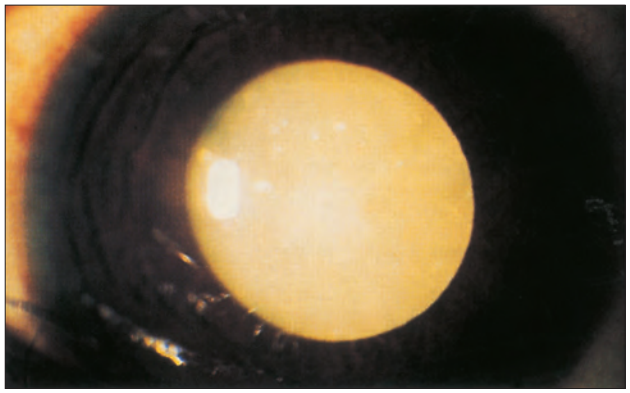


Figure 24.2 Cataract in congenital rubella. (Courtesy of R.J. Marsh and S. Ford.)

intrauterine infection being 90% at under 11 weeks, 55% at 11–16 weeks and 45% at more than 16 weeks. The risk of an adverse fetal outcome was seen in 90% of babies when maternal rubella occurred in the first 11 weeks of pregnancy, 20% when it occurred between 11 and 16 weeks, by 16–20 weeks there was a minimal risk of deafness and no increased risk at more than 20 weeks.

Congenital rubella can affect the eye, heart, brain and ear

Clinical manifestations of congenital rubella include low birth weight and eye (Fig. 24.2) and heart lesions. Effects on the brain and ears may not become detectable until later in childhood, in the form of mental retardation and deafness. Up to 80% of infected infants eventually suffer from deafness. About 25% of congenitally infected children develop insulin-dependent diabetes mellitus later in life (the virus replicates in the pancreas), but rubella is a very uncommon cause of this disease. There is 15% mortality in infants showing signs of infection at birth, often associated with hypogammaglobulinaemia.

Fetal rubella IgM is found in cord and infant blood

Infected fetuses produce their own IgM antibody to rubella virus, which can be detected in cord and infant blood. Maternal IgG antibodies are also present and together with interferons help to control the spread of infection in the fetus. Virus can be isolated from the infant's throat or urine. The infant sheds virus into the throat and urine for several months and can infect susceptible individuals. Rubella virus RNA detection may be carried out in mostly reference centres in order to assist with the diagnosis.

Congenital rubella can be prevented by vaccination

Vaccination with live attenuated rubella virus is given during childhood, usually as the combined MMR vaccine. Pregnancy is a contraindication to vaccination, as it is a live vaccine, and the only safe time during reproductive life is the immediate postpartum period. This is an interesting example of a vaccine that is given to protect an as yet non-existent individual (the future fetus), the infection being only subclinical or mild in the mother. Until effective vaccines became available in the late 1960s (Box 24.1), rubella was an important cause of congenital heart disease, deafness, blindness and mental retardation. The virus continues to circulate in the community and damage fetuses in countries with less-extensive rubella vaccination programmes.

Box 24.1 Lessons in Microbiology

Rubella and the fetus

Dr Norman McAllister Gregg (1892–1966) was ophthalmic surgeon to the Royal Alexandra Hospital for Children in Sydney, and during the Second World War he noticed what he called an 'epidemic' of congenital cataract in infants. He went further and made the astute observation that all the mothers had suffered from rubella during early pregnancy. There were 78 infants with cataract, and 68 of the mothers had a history of rubella in early pregnancy. Many of the infants had heart defects, were small, and two-thirds of them had microphthalmia. He published his findings in 1941, providing the first clear demonstration that an environmental factor could cause congenital malformations. It is a striking feature of the infection that, whereas the fetus suffers cruel malformations, the mother shows little or no signs of illness. We now know that several other viruses, notably CMV, can do this, as well as factors such as thalidomide and folate deficiency. Later studies on rubella revealed that congenitally infected infants also developed deafness and brain defects. Survivors were followed up until 1991 when they were 50, and other abnormalities have been observed, including the development of diabetes by the age of 25 years and certain vascular abnormalities.

It was not until 1962 that the causative virus was isolated and grown in cell culture. A rubella epidemic in the USA in 1964–1965 left in its wake 20 000 infants with the congenital rubella syndrome. By the late 1960s, an effective live virus vaccine was available, and congenital rubella is now seen only when vaccination cover is poor. The fetus is exquisitely vulnerable to rubella during the first trimester of pregnancy. This is the critical stage in embryonic development when key organs (heart, ear, eye, brain) are being formed and, although the virus does no damage to the cells in which it grows, it interferes with mitosis. Interference with programmed mitosis in these major organs causes the malformations, vasculitis playing a part. The fetus is good at repairing damage but it cannot at a later stage compensate for the failure in basic organ development. The antimitotic action of the virus also means that the total number of cells in the body is reduced, and this is why the rubella-infected infants are smaller. The rubella virus remains in infected organs such as the lens and brain for more than 1 year, but eventually there is an adequate cell-mediated immune response and the virus is eliminated.

Congenital CMV infection

Mothers with a poor T-cell proliferative response to CMV antigens are more likely to infect their fetus

After primary maternal infection during pregnancy, about 40% of fetuses are infected, and 5% of these show signs at birth. It is not known whether the fetus is especially vulnerable

at certain stages of pregnancy. The fetus is also infected following CMV reactivation during pregnancy in women with previous CMV exposure, but fetal damage is then uncommon. As many as 1–2% of infants born in the USA are infected, and up to about 10% of these are symptomatic, with up to 1 million infectious doses of virus present per millilitre of urine. However, the incidence of congenital CMV infection is likely to be an underestimate worldwide. In large cohorts of pregnant women studied it has been shown that 2% develop a primary CMV infection, but over 95% were asymptomatic. Of those women with a primary infection in the first trimester, up to 30% of babies may develop central nervous system (CNS) sequelae including sensorineural hearing loss. Although the percentage is much reduced if the maternal infection is later in pregnancy, a degree of CNS damage still occurs. However, the relationship between a first-trimester infection and outcome is much clearer in rubella infections than with CMV. In CMV reactivation or reinfection, partial control of the infection by maternal antibody under these circumstances means that the baby may be infected but not affected, although a small percentage become symptomatic over the next couple of years. The frequency and outcome of congenital CMV infections in reactivation or reinfection in pregnancy is still not well understood. However, the incidence of symptomatic congenital CMV infections has been reported to be similar in pregnant women with primary infections and reactivations or reinfections.

Clinical features of congenital CMV include mental retardation, choroidoretinitis and optic atrophy, hearing defects, hepatosplenomegaly, thrombocytopenic purpura and anaemia (Fig. 24.3). Deafness and mental retardation may not be detectable until later in childhood.

Diagnosis is made by detecting CMV-specific IgM antibodies in infant blood within 3 weeks of delivery, and by detecting and quantifying CMV DNA in the blood or urine during this period. Virus can also be isolated from throat swab or urine samples. Live attenuated vaccines have been investigated (AD169 and Towne strains), and in preliminary studies no-one who became pregnant after vaccination transmitted the virus to the infant.

Antiviral drugs such as ganciclovir and valganciclovir can be considered in managing symptomatic babies with congenital CMV infection.

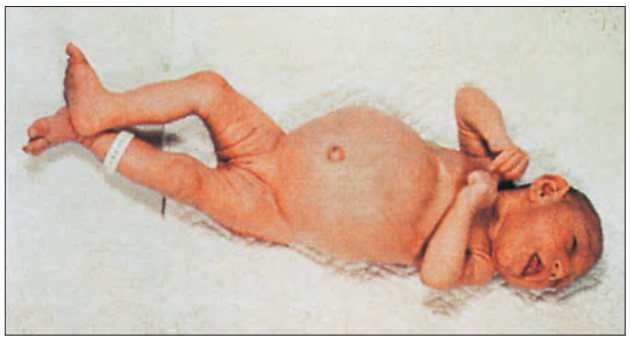


Figure 24.3 Microcephaly with associated severe psychomotor retardation and hepatosplenomegaly in congenital cytomegalovirus infection. (Courtesy of W.E. Farrar.)

Zika virus

Zika virus is a single-stranded virus belonging to the Flaviviridae, the family which includes Dengue virus, West Nile Virus and Yellow Fever virus. Isolated as long ago as 1947 in a monkey in the Zika Forest of Uganda and reported to cause human infection in 1954, it rose to prominence as recently as 2015 with an outbreak in Central and South America, the Caribbean, Oceania and parts of Asia. In 2016 it was declared a public health emergency. Zika virus is transmitted by a variety of *Aedes* mosquitoes, especially *Aedes aegypti*. Approximately 80% of those who become infected are asymptomatic and in those who do develop symptoms the illness is relatively mild in most cases, though it can cause Guillain-Barre syndrome in some instances. However, Zika virus can cross the placenta and maternal infection contracted during pregnancy can result in fetal malformations and congenital microcephaly. An active programme is underway to develop a vaccine against Zika virus but in the meantime, until a vaccine becomes available, prevention of infection depends heavily on mosquito bite prevention. The virus has been detected in semen and in the female genital tract. Sexual transmission of Zika virus has occurred in a small minority of cases and has been reported to occur female to male, or male to female. Females who are pregnant or planning to become pregnant and who might become exposed to the risk of Zika virus infection or whose sexual partner might become exposed, should consult their healthcare provider for individual assessment. Guidance is available from websites of the US Centers for Disease Control; the European Centre for Disease Prevention and Control and the UK National Travel Health Network and Centre.

Congenital syphilis

As a result of routine serological screening for syphilis, a treponemal infection, in antenatal clinics and treatment with penicillin, congenital syphilis is now rare, but is more common in resource-poor countries. Clinical features in the infant include rhinitis (a runny nose), skin and mucosal lesions, hepatosplenomegaly, lymphadenopathy, and abnormalities of bones, teeth and cartilage (saddle-shaped nose). Pregnancy often masks the early signs of syphilis, but the mother will have serological evidence of treponemal infection, and treponemal IgM will be detected in the fetal blood. Vertical transmission most commonly takes place after 4 months of gestation; therefore treatment of the mother before the fourth month of pregnancy should prevent fetal infection.

Congenital toxoplasmosis

Acute asymptomatic infection by *Toxoplasma gondii* during pregnancy can cause fetal malformation

Toxoplasma is a ubiquitous infection worldwide. Depending on the country concerned, approximately 10 to 80% of healthy adults have serological evidence of previous Toxoplasma gondii infection, but the risk factor for congenital toxoplasmosis is primary infection of the mother acquired during pregnancy. The incidence of fetal infection increases from 14% when maternal infection is in the first trimester to 59% when in the third trimester. In contrast, damage to the fetus is more severe the earlier in pregnancy infection is contracted. At birth most infants are asymptomatic and there are often no detectable

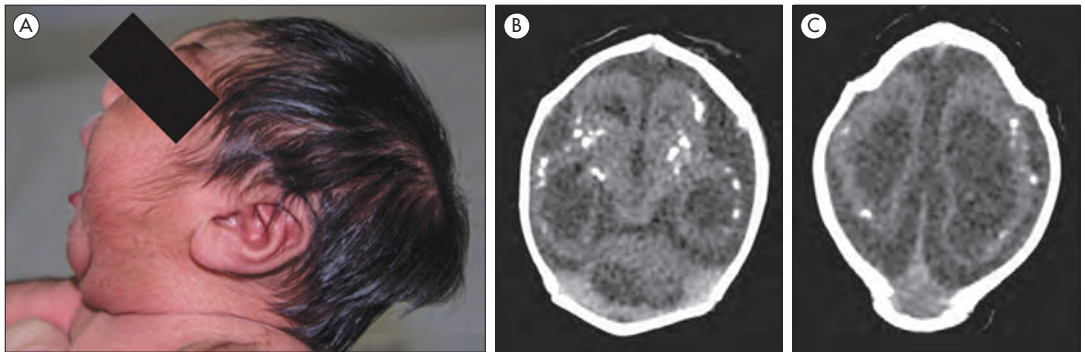


Figure 24.4 (A) Newborn baby with microcephaly with laboratory-confirmed Zika virus. (B, C) Abnormalities detected on computed tomography (CT) scan. The neonate shows phenotypic features previously described during the microcephaly epidemic, including craniofacial disproportion, prominent external occipital protuberance and excessive scalp skin. Radiological features found on brain CT imaging include reduced volume of cortical brain parenchyma, cortical and subcortical calcifications, simplified gyral pattern and ventriculomegaly. (Reproduced with permission from T.V. Barreto de Araújo et al. Association between Zika virus infection and microcephaly in Brazil, January to May, 2016: preliminary report of a case–control study. *Lancet Infect Dis* 2016; 16[12]:1356–1363. ©2016 World Health Organization,)

abnormalities at at that time, but signs (e.g. chorioretinitis) generally appear within a few years.

In severely affected infants the clinical features of congenital toxoplasmosis include convulsions, microcephaly, chorioretinitis, hepatosplenomegaly and jaundice, with later hydrocephaly, mental retardation and defective vision. Some countries undertake serological screening of pregnant women for Toxoplasma-specific antibodies, including IgM. If the serological profile indicates maternal infection acquired in pregnancy, treatment of the mother is commenced with spiramycin to try to prevent transmission to the fetus. Amniotic fluid is tested by Toxoplasma PCR and if it confirms fetal infection, treatment with sulphadiazine plus pyrimethamine plus folinic acid is given instead of spiramycin.

There is no vaccine. Prevention is by avoidance of primary infection which occurs via ingesting cysts from cat faeces or raw or lightly cooked meat during pregnancy.

Congenital Chagas disease

Chagas disease (American Trypanosomiasis) caused by *Trypanosoma cruzi* is transmitted vectorially by Reduviid bugs; orally; congenitally; or via blood transfusion and organ transplantation. As a result of vector control programmes, vertical transmission now accounts for around 20% of new infections. This is concerning, as it permits continuing transmission even in the absence of a competent insect vector. Congenital *T. cruzi* infection is usually asymptomatic, but when illness is present the clinical features include prematurity, low birth weight, anaemia, thrombocytopenia, meningoencephalitis, hepatosplenomegaly and respiratory distress. Congenital infection left untreated progresses to the chronic stage, with a risk of developing cardiac or gastrointestinal complications 20 to 30 years later. Treatment of infected infants in the first year of life with benznidazole or nifurtimox is very effective. In contrast to the situation with adults who commonly experience adverse effects, these drugs are well tolerated in infants. Prevention of congenital Chagas disease depends on serological screening of females born in endemic areas or screening of females whose mother was born in an endemic area. After birth the infant is monitored for the presence of vertical infection by blood film, PCR and serology through the first year of life and treatment offered if there is evidence of *T. cruzi* parasitaemia.

Congenital human immunodeficiency virus (HIV) infection

In resource-poor countries, approximately one-quarter of infants born to mothers with HIV are infected: about one-third of these in utero and the rest perinatally

In 2015, the World Health Organization published data that around 150000 children under 15 years of age had newly diagnosed HIV infection and that, in the same age range, there were around 1.8 million children living with HIV. Clinically, congenital HIV infection manifests as poor weight gain, susceptibility to sepsis, developmental delays, lymphocytic pneumonitis, oral thrush, enlarged lymph nodes, hepatosplenomegaly, diarrhoea and pneumonia, and some infants develop encephalopathy and AIDS by 1 year of age. As most infections take place during late pregnancy or during delivery, transmission rates are reduced by lowering the HIV load by offering antiretroviral drugs during pregnancy, especially during the last trimester or during labour, carrying out an elective caesarean section where indicated (if the HIV load is suppressed, vaginal delivery is recommended) and avoiding breastfeeding.

IgG antibodies present in the neonatal blood sample may be maternal in origin and can persist for at least a year. The mainstay of laboratory diagnosis therefore involves detection of HIV-1 proviral DNA or HIV-1 RNA by polymerase chain reaction (PCR), although these tests may not be positive until several months after birth, in conjunction with testing by HIV antibody and antigen combination assays once maternal antibody has waned.

Congenital and neonatal listeriosis

Maternal exposure to animals or foods infected with *Listeria* can lead to fetal death or malformations

Listeria monocytogenes is a small Gram-positive rod, which is motile and beta-haemolytic. It is distributed worldwide in a great variety of animals including cattle, pigs, rodents

and birds, and the bacteria also occur in plants and in soil. *Listeria* can grow at regular refrigeration temperatures (e.g. 3–4°C). Transmission to humans is by:

- contact with infected animals and their faeces
- consumption of unpasteurized milk or soft cheeses or contaminated vegetables

In the USA, there are about 2000 reported cases of listeriosis each year, about one-third of them in newborn infants. Faecal carriage is uncommon, except in contacts of cases.

L. monocytogenes in the pregnant woman causes a mild influenza-like illness or is asymptomatic, but there is a bacteraemia which leads to infection of the placenta and then the fetus. This may cause abortion, premature delivery, neonatal septicaemia or pneumonia with abscesses or granulomas. The infant can also be infected shortly after birth, for instance from other babies or from hospital staff, and this may lead to a meningitic illness.

L. monocytogenes is isolated from blood cultures, cerebrospinal fluid (CSF) or newborn skin lesions.

Treatment is with amoxicillin, which may need to be combined with gentamicin to achieve a bactericidal effect. There are no vaccines.

Pregnant women should avoid exposure to infected material, but the exact source of infection is generally unknown.

INFECTIONS OCCURRING AROUND THE TIME OF BIRTH

Effects on the fetus and neonate

The routes of infection in the fetus and neonate are shown in Fig. 24.5

Viral infections (e.g. rubella, CMV) are generally less damaging to the fetus when the maternal infection occurs late in pregnancy. Primary infection with varicella-zoster virus (VZV) in the first 20 weeks of pregnancy can lead to limb deformities and other severe lesions in the newborn. HSV infection in this setting is underdiagnosed and can lead to neonatal morbidity and mortality.

Bacterial infections originating from the vagina and perineum late in pregnancy, especially those occurring when the fetal membranes have been ruptured for more than 1–2 days, may result in chorioamnionitis, maternal fever, premature delivery and stillbirth. Infants of low birth weight (<1500 g) tend to be more severely affected. Bacteria involved include:

- group B haemolytic streptococci; 10–30% of pregnant women are colonized in the rectum or vagina
- *E. coli*
- *Klebsiella*
- *Proteus*
- *Bacteroides*
- *Staphylococcus*
- *Mycoplasma hominis*

These infections may also be acquired after delivery to give later-onset disease.

Neonatal septicaemia often progresses to meningitis

Bacterial meningitis (see Table 24.3) is frequently fatal unless treated. Clinical diagnosis is difficult because the infant shows generalized signs such as respiratory distress, poor feeding, diarrhoea and vomiting, but early diagnosis is essential and emergency treatment is required. 'Blind' antibiotic treatment should be started as soon as CSF (Gram stain and culture) and blood samples have been taken.

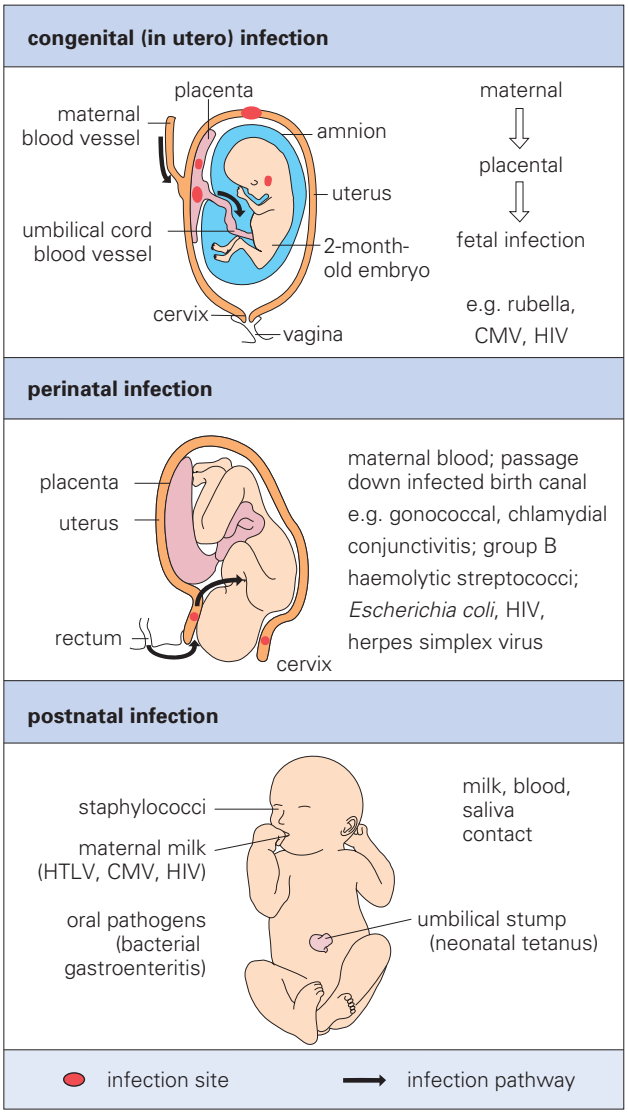


Figure 24.5 Routes of infection in the fetus and neonate. CMV, cytomegalovirus; HIV, human immunodeficiency virus; HTLV, human T-cell lymphotropic virus.

Fetal infection with HSV must be considered in a baby who is acutely ill within a few days or weeks of birth

Fetal infection during labour results from direct contact with the infecting microorganism as the fetus passes down an infected birth canal (Table 24.3). For instance, cutaneous lesions of HSV may develop 1 week after delivery, with generalized infection and severe CNS involvement. Approximately 80% of mothers with primary HSV infection (but only about 10% with recurrent HSV) have cervical lesions and about a third of their infants are infected. Babies less than 4 weeks of age may present with neonatal HSV as acutely ill and 'septic' but classically there are three well-defined clinical presentations:

Table 24.3 Neonatal infections acquired during passage down an infected birth canal

Infectious agent	Site of infection	Phenomenon
Neisseria gonorrhoeae	Conjunctiva	Neonatal conjunctivitis (ophthalmia neonatorum)
Chlamydia trachomatis	Conjunctiva, respiratory tract	Neonatal conjunctivitis (ophthalmia neonatorum), neonatal pneumonia
Herpes simplex virus	Skin, eye, mouth	Neonatal herpetic infection[a]
Genital papillomavirus	Respiratory tract	Laryngeal warts in young children
Group B streptococci,[b] Gram-negative bacilli (*E. coli*, etc.)	Respiratory tract	Septicaemia; death if not treated
Candida albicans	Oral cavity	Neonatal oral thrush

[a]Although preventable by caesarean section, it is often difficult to detect maternal genital infection; infants can be treated prophylactically with aciclovir.
[b]Up to 30% of women carry these bacteria in the vagina or rectum.

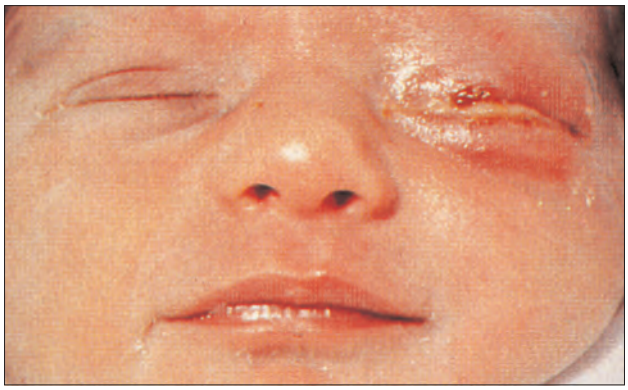


Figure 24.6 Gonococcal ophthalmia neonatorum. Signs appear 2–5 days after birth. The inflammation and oedema are more severe than with *Chlamydia* infection. (Courtesy of J.S. Bingham.)

those with infection affecting the skin, eye and / or mouth (SEM); encephalitis with or without skin involvement; and disseminated disease involving the lungs, liver, central nervous system, adrenal glands and SEM. The diagnosis may be missed as neonatal HSV infection may present without skin lesions in up to 39% of babies. Therefore, there must be a low threshold for considering this diagnosis and intravenous aciclovir therapy must be started as soon as possible. Treatment could be started at the same time as samples are collected for HSV DNA detection that include swabs of the SEM and vesicles, if present, EDTA whole blood samples and CSF. Morbidity and mortality rates are higher in those with encephalitis and disseminated disease.

Gonococci (Fig. 24.6), Chlamydia or staphylococci can infect the eye to cause ophthalmia neonatorum. Infection with group B streptococci generally occurs at this time.

In countries with high hepatitis B carrier rates, maternal blood is a major source of infection during or shortly after birth. More than 90% of infants from carrier mothers become infected and then carry the virus. This is preventable by giving the vaccine plus specific immunoglobulin to the newborn. Hepatitis C, in contrast, is not usually transmitted in this way and the risk is higher if the mother is immunocompromised, as this is likely to lead to a higher maternal viral viraemia.

Human milk may contain rubella virus, CMV, human T-cell lymphotropic virus (HTLV) and HIV. The amount of virus detectable in milk is low and, except in the case of HTLV and HIV, milk is not thought to be an important source of infection. However, it makes sense to pasteurize milk in human milk banks, just as we pasteurize cows' milk.

Effects on the mother

Puerperal sepsis is prevented by aseptic techniques

After delivery (or abortion), a large area of damaged vulnerable uterine tissue is exposed to infection. Puerperal sepsis (childbed fever) was a major cause of maternal death in Europe in the nineteenth century. In 1843, Oliver Wendell Holmes made the unpopular suggestion that it was carried on the hands of doctors, and 4 years later Ignaz Semmelweiss in Vienna showed how it could be prevented if doctors and midwives washed their hands before attending a woman in labour and practised aseptic techniques. This is because:

- group A beta-haemolytic streptococci were the major culprits and came from the nose, throat or skin of hospital attendants
- other possible organisms include anaerobes such as *Clostridium perfringens* or *Bacteroides*, *E. coli* and group B streptococci and originate from the mother's own faecal flora

Puerperal sepsis carried a mortality rate of up to 10% until the 1930s, but, like septic abortion, is now uncommon in resource-rich countries. Predisposing factors include premature rupture of the membranes, instrumentation and retained fragments of membrane or placenta. High vaginal swabs and blood cultures should be taken if there is postnatal pyrexia or an offensive discharge.

Other neonatal infections

Infection may be transmitted to the newborn infant during the first 1–2 weeks after birth, rather than during delivery as follows:

- Group B beta-haemolytic streptococci and Gram-negative bacilli (see above) acquired by cross-infection in the nursery can still cause serious infection at this time, often with meningitis
- Herpes simplex virus from facial cold sores or herpetic whitlows of attending adults
- *Staphylococci* from the noses and fingers of adult carriers may cause staphylococcal conjunctivitis or 'sticky eye', skin

sepsis in the neonate, and sometimes the staphylococcal 'scalded skin' syndrome (Fig. 24.7) due to a specific 'epidermolytic' staphylococcal toxin

During the first 1–2 weeks of life, the nose of the neonate becomes colonized with *Staphylococcus aureus*, which can enter the nipple during feeding to cause a breast abscess. These infections are preventable if hospital staff members pay vigorous attention to hand washing and aseptic techniques.

If hygienic practices are poor, the umbilical stump, especially in resource-poor countries, may be infected with *Clostridium tetani*, usually because instruments used to cut the cord are contaminated with bacterial spores, resulting in neonatal tetanus (Fig. 24.8). It can be prevented by immunization of mothers with tetanus toxoid.

In resource-poor countries, gastroenteritis is an important problem during the neonatal period as well as during infancy.

Diarrhoea leading to water and electrolyte depletion is particularly serious in low-birth-weight infants. Causative agents include strains of *E. coli* and salmonellae rather than rotaviruses. Breastfeeding gives some protection by supplying specific antibodies and other less well-characterized protective factors.

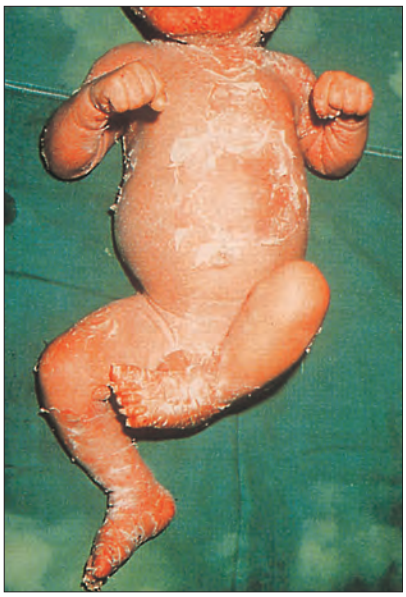


Figure 24.7 Staphylococcal scalded skin syndrome. There are large areas of epidermal loss where bullae have burst. (Courtesy of L. Brown.)

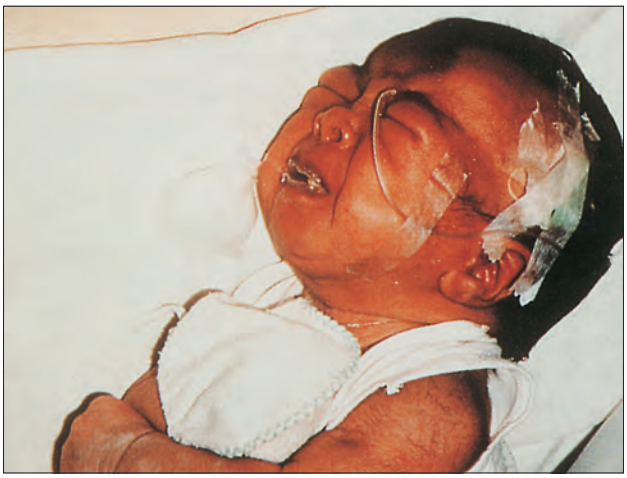


Figure 24.8 Tetanus. Risus sardonicus in a newborn infant. (Courtesy of W.E. Farrar.)

KEY FACTS

- During pregnancy, certain infections (coccidioidomycosis, influenza) can be more severe than usual and there can be reactivation of certain latent infections (HSV, CMV).
- A few infections are able to pass to the fetus via the placenta and cause damage. These infections are generally mild or subclinical in the mother (rubella, CMV, ZIKV, toxoplasmosis, American trypanosomiasis), but this is not always the case (syphilis).
- Once infected, the fetus may die, but if the baby survives it may be born with the infection (HIV, toxoplasmosis), often showing characteristic malformations (rubella, syphilis).
- Infection of the infant during birth or shortly afterwards can cause local disease (conjunctivitis due to gonococci or Chlamydia) or occasionally severe life-threatening illness (*E. coli* meningitis, HSV or group B streptococcal infection).
- Life-threatening bacterial infection of the mother via the postpartum uterus (puerperal sepsis) used to be common but is now rare in resource-rich countries.

25 Central nervous system infections

Introduction

Central nervous system infections are usually blood-borne or infectious agents invading via peripheral nerves

The brain and spinal cord are protected from mechanical pressure or deformation by enclosure in rigid containers, the skull and vertebral column, which also act as barriers to the spread of infection. The blood vessels and nerves that traverse the walls of the skull and vertebral column are the main routes of invasion. However, cellular barriers such as the blood–brain barrier and blood–cerebrospinal fluid (CSF) barrier protect against pathogen invasion. Blood-borne invasion is the most common route of infection, for example, by polioviruses or *Neisseria meningitidis*. Invasion via peripheral nerves is less common; examples include herpes simplex, varicella-zoster and rabies viruses. Local invasion from infected ears or sinuses, local injury or congenital defects such as spina bifida also occurs, whereas invasion from the olfactory tract leading to amoebic meningitis is rare.

Here, we discuss the main routes of central nervous system (CNS) invasion by pathogens and the body's response, followed by a more detailed discussion of the diseases that result. In particular, meningitis, inflammation of the meninges surrounding the brain, encephalitis, inflammation of the white matter substance of the brain, meningoencephalitis and focal CNS syndromes encompass the clinical presentations of these infections.

INVASION OF THE CENTRAL NERVOUS SYSTEM

Natural barriers act to prevent blood-borne invasion

Blood-borne invasion takes place across:

- the blood–brain barrier to cause encephalitis
- the blood–CSF barrier to cause meningitis (Fig. 25.1).

The blood–brain barrier consists of tightly joined endothelial cells surrounded by glial processes, whereas the brain–CSF barrier at the choroid plexus consists of endothelium with fenestrations, and tightly joined choroid plexus epithelial cells. Pathogens can traverse these barriers by:

- growing across, infecting the cells that comprise the barrier
- being passively transported across in intracellular vacuoles
- being carried across by infected white blood cells.

Examples of each route are seen in viral infections. The clinical presentation may be headache and neck stiffness in meningitis, inflammation of the meninges surrounding the brain or a confusional state in encephalitis, inflammation of the white matter substance of the brain, or a mixture of both in a meningoencephalitis. Poliovirus, for instance, invades the CNS across the blood–brain barrier. After the virus gains entry via oral ingestion, a complex stepwise series of events leads to CNS invasion (Fig. 25.2). Poliovirus also invades the meninges after localizing in vascular endothelial cells, and can cross the blood–CSF barrier. Mumps virus behaves in the same way, as do circulating *Haemophilus influenzae*, meningococci and pneumococci. Once infection has reached the meninges and CSF, the brain substance can in turn be invaded if the infection crosses the pia. In poliomyelitis, for instance, a meningitic phase often precedes encephalitis and paralysis.

CNS invasion, however, is a rare event because most microorganisms fail to pass from blood to the CNS across the natural barriers. A large variety of viruses can multiply and cause disease if introduced directly into the brain, but circulating viruses generally fail to invade, and CNS involvement by polio, mumps, rubella or measles viruses is seen in only a very small proportion of infected individuals. Other neurotropic viruses include enteroviruses, herpes simplex virus (HSV), varicella-zoster virus (VZV), cytomegalovirus (CMV), Epstein–Barr virus (EBV), JC (named after John Cunningham), HIV and human T-cell lymphotropic virus (HTLV), Japanese encephalitis virus as well as those that have emerged in recent years including Zika virus and West Nile virus.

Invasion of the CNS via peripheral nerves is a feature of herpes simplex, varicella-zoster and rabies virus infections

HSV and VZV present in skin or mucosal lesions, and travel up axons using the normal retrograde transport mechanisms that can move virus particles (as well as foreign molecules such as tetanus toxin) at a rate of about 200 mm / day, to reach the dorsal root ganglia. Rabies virus, introduced into muscle or subcutaneous tissues by the bite of a rabid animal, infects

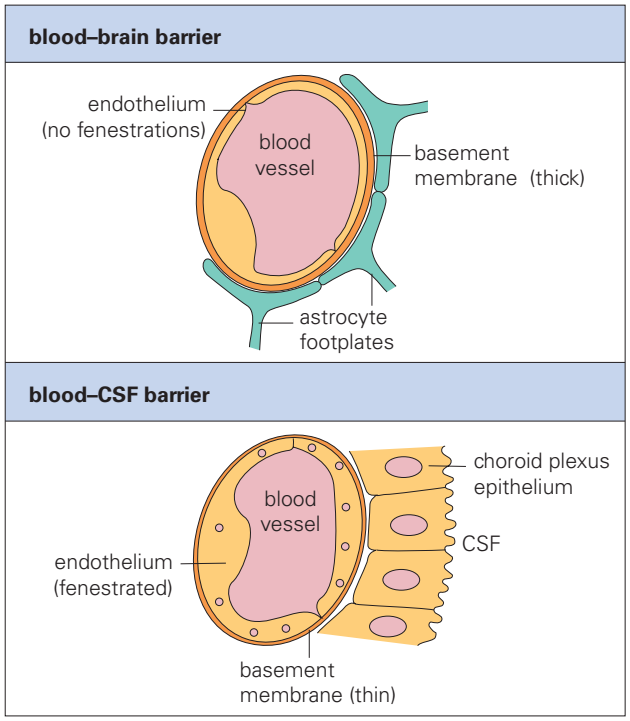


Figure 25.1 Structures of the blood–brain and blood–cerebrospinal fluid (CSF) barriers.

muscle fibres and muscle spindles after the virus binds to the nicotinic acetylcholine receptor. It then enters peripheral nerves and travels to the CNS, to reach glial cells and neurones, where it multiplies.

THE BODY'S RESPONSE TO INVASION

CSF cell counts increase in response to infection

The response to invading viruses is reflected by an increase in lymphocytes, mostly T cells, and monocytes in the CSF (Table 25.1). A slight increase in protein also occurs, the CSF remaining clear. This condition is termed 'aseptic' meningitis. The response to pyogenic bacteria shows a more spectacular and more rapid increase in polymorphonuclear leukocytes and proteins (Fig. 25.3), so that the CSF becomes visibly turbid. This condition is termed 'septic' meningitis. Certain slower growing or less pyogenic microorganisms induce less dramatic changes, such as in tuberculous or listerial meningitis.

The pathological consequences of CNS infection depend upon the microorganism

In the CNS itself, viruses can infect neural cells, sometimes showing a marked preference. Polio and rabies viruses, for instance, invade neurones, whereas JC virus invades oligodendrocytes. The latter are myelin-producing cells of the CNS and the viral infection leads to cell lysis, the axons lose their myelin sheath rendering them dysfunctional and demyelinated lesions appear. Because there is very little extracellular space, spread is mostly direct from cell to cell

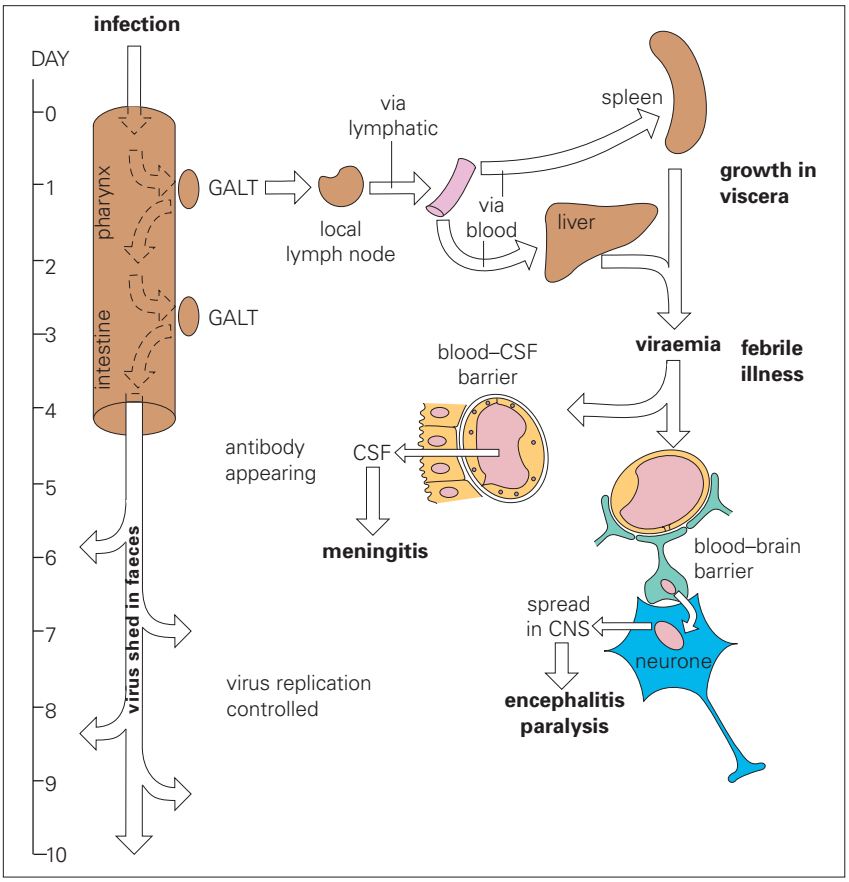


Figure 25.2 The mechanism of central nervous system (CNS) invasion by poliovirus. CSF, cerebrospinal fluid; GALT, gut-associated lymphoid tissue.

Table 25.1 Changes in cerebrospinal fluid (CSF) in response to invading pathogens

	Cells / mL	Protein (mg / dL)	Glucose (mg / dL)	Causes
Normal	0–5	15–45	45–85	
Septic (purulent) meningitis	200–20 000 (mainly neutrophils)	High (>100)	<45 Low	Bacteria, including tuberculosis and leptospira, fingi, amoebae, brain abscess
Aseptic[a] meningitis or meningoencephalitis	100–1000 (mainly mononuclear)	Moderately high (50–100)	Normal	Viruses, tuberculosis, leptospira, fungi, brain abscess, partly treated bacterial meningitis

[a]Aseptic because the CSF is sterile on regular bacteriological culture.

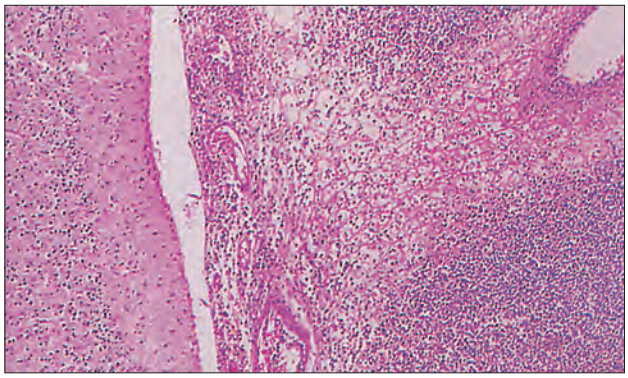


Figure 25.3 Bacterial meningitis. Exudate of acute inflammatory cells in the subarachnoid space (H&E stain). (Courtesy of P. Garen.)

along established nervous pathways. Invading bacteria and protozoa generally induce more dramatic inflammatory events, which limit local spread so that infection is soon localized to form abscesses.

Viruses induce perivascular infiltration of lymphocytes and monocytes, sometimes, as in the case of polio, with direct damage to infected cells. (The pathogenesis of viral encephalomyelitis is shown later, in Fig. 25.7.) Associated immune responses not only to viral, but also often to host CNS components, play a part in postvaccinial encephalitis. Infiltrating B cells produce antibody to the invading microorganism, and T cells react with microbial antigens to release cytokines that attract and activate other T cells and macrophages. The pathological condition evolves over the course of several days and occasionally, when partly controlled by host defences, over the course of years. Subacute sclerosing panencephalitis (SSPE) caused by measles, is an example of this as it is has both a virological, seen by defective, persistent viral replication and an immunological pathogenesis with high titres of neutralizing antibodies in the serum and CSF against viral structural proteins. The immune response is ineffective in clearing measles virus in the CNS. Bacteria cause more rapidly evolving pathological changes, with local responses to bacterial antigens and toxins playing an important part.

In all cases, a degree of inflammation and oedema that would be trivial in striated muscle, skin or liver may be life threatening when it occurs in the vulnerable 'closed box' containing the leptomeninges, brain and spinal cord. It may be several weeks after clinical recovery before cellular infiltrations are removed and histological appearances are restored to normal.

CNS invasion only rarely assists in the transmission of infection

From the point of view of a parasitic microorganism that needs to be transmitted to a fresh host, invasion of the CNS is generally foolish because it damages the host. The only occasions on which it makes sense are:

- when dorsal root ganglion neurones are invaded as an essential step in establishing latency (HSV and VZV); this gives a mechanism for reactivation and further episodes of shedding from mucosal or skin lesions.
- in the case of rabies (see below), where CNS invasion in the animal host is necessary for two reasons. First, it enables the virus to spread from the CNS down peripheral nerves to the salivary glands, from where transmission takes place. Second, invasion of the limbic system of the brain causes a change in behaviour of the infected animal so that it becomes less retiring, more aggressive and more likely to bite, thus transmitting the infection. Invasion of the limbic system can be regarded as a fiendish strategy on the part of rabies virus to promote its own transmission and survival.

MENINGITIS

Bacterial meningitis

Acute bacterial meningitis is a life-threatening infection, needing urgent specific treatment

Bacterial meningitis is more severe, but less common, than viral meningitis and may be caused by a variety of agents (Table 25.2). Prior to the 1990s, *Haemophilus influenzae* type b (Hib) was responsible for most cases of bacterial meningitis. However, the introduction of the Hib vaccine into childhood immunization regimens has lowered overall Hib incidence in favour of *Neisseria meningitidis* and *Streptococcus pneumoniae*, which are now responsible for most bacterial meningitis. These three pathogens have several virulence factors in common (Table 25.3), including possession of a polysaccharide capsule (Table 25.4).

Meningococcal meningitis

Neisseria meningitidis *is carried by about 20% of the population, but higher rates are seen in epidemics.* *Neisseria meningitidis* is a Gram-negative diplococcus which closely resembles *N. gonorrhoeae* in structure, but with an additional polysaccharide capsule that is antigenic and by which the serotype of *N. meningitidis* can be recognized. The bacteria are carried asymptomatically in the population, up to 20%

Table 25.2 The important causative agents of non-viral meningitis, their treatment and prevention

Pathogen	Treatment[a]	Prevention
Neisseria meningitidis	Ceftriaxone (or chloramphenicol)	Ciprofloxacin prophylaxis for close contacts; polysaccharide vaccine
Haemophilus influenzae	Ceftriaxone or cefotaxime (or chloramphenicol)	Polysaccharide vaccine against type b (Hib)
Streptococcus pneumoniae	Ceftriaxone (or chloramphenicol)	Prompt treatment of otitis media and respiratory infections; polyvalent (23 serotypes) polysaccharide vaccine
Escherichia coli (and other coliforms), group B streptococci	Gentamicin + cefotaxime or ceftriaxone (or chloramphenicol)[b]	No vaccines available
Listeria monocytogenes	Amoxicillin + gentamicin	No vaccines available
Mycobacterium tuberculosis	Isoniazid and rifampin and pyrazinamide ± streptomycin	BCG vaccination; isoniazid prophylaxis for contacts recommended in USA
Cryptococcus neoformans	Amphotericin B and flucytosine	No vaccines available

[a]Treatment should be initiated immediately and the susceptibility of the infecting isolate confirmed in the laboratory.
[b]If isolate is shown to be susceptible (10–20% of isolates are resistant because they produce a plasmid coded beta lactamase).

Table 25.3 Virulence factors in bacterial meningitis

Virulence factor	Bacterial pathogen		
	Neisseria meningitidis	***Haemophilus influenzae***	***Streptococcus pneumoniae***
Capsule	+	+	+
IgA protease	+	+	+
Pili	+	+	–
Endotoxin	+	+	–
Outer membrane proteins	+	+	–

Table 25.4 Polysaccharide capsules are important virulence factors in the pathogenesis of bacterial meningitis

Pathogen	Capsule	Important type	Vaccine
Neisseria meningitidis	Polysaccharide	A, B, C, Y, W-135	A, C, Y, W quadrivalent vaccine B vaccine
Haemophilus influenzae	Polysaccharide	B	Hib vaccine for <1 year olds
Streptococcus pneumoniae	Polysaccharide	Many	Pneumovax: 23-valent most common types
Group B streptococcus	Polysaccharide rich in sialic acid	(Ia, Ib, II) III in neonatal meningitis	In development
Escherichia coli		KI in meningitis	—

depending on geographical location, and are attached by their pili to the epithelial cells in the nasopharynx. Invasion of the blood and meninges is a rare and poorly understood event. The known virulence factors are summarized in Table 25.3. People possessing specific complement-dependent bacterial antibodies to capsular antigens are protected against invasion. Those with C5–C9 complement deficiencies show increased susceptibility to bacteraemia (as they do to *N. gonorrhoeae* bacteraemia; see Ch. 22). Those most often infected include young children who have lost the antibodies passively acquired from their mother and adolescents who have not previously encountered the infecting serotype and therefore have no type-specific immunity.

Person-to-person spread takes place by droplet infection and is facilitated by other respiratory infections, often viral, that cause increased respiratory secretions. Thus, conditions of overcrowding and confinement such as prisons, military barracks and college dormitories contribute to the frequency of infection in populations. During outbreaks of meningococcal meningitis, which most frequently occur in late winter and early spring, the carrier rate may reach 60–80%. Specific serotypes associated with infection exhibit some geographical variation. However, serotypes B, W, Y and C, in that order, tend to predominate in more resource-rich countries, whereas serotypes A and W-135 are more common in less-developed regions. Available vaccines target serotypes A, B, C, Y and

W-135 (Table 25.4). The UK was the first country to introduce the meningitis C conjugate vaccine. It has been part of routine childhood immunization since November 1999 and resulted in a 96% fall in incidence of meningitis C infections.

Group B meningococcal disease is diagnosed in more than 50% of meningitis cases, particularly in infants and toddlers. A national meningitis B immunization programme for infants was introduced in the UK in September 2015. In addition, as meningitis W infections were increasing, a meningitis ACWY conjugate vaccine was introduced as part of the national immunization programme in England in 2015 for school leavers.

Clinical features of meningococcal meningitis include a haemorrhagic skin rash. After an incubation period of 1–3 days, the onset of meningococcal meningitis is sudden with a sore throat, headache, drowsiness and signs of meningitis which include fever, irritability, neck stiffness and photophobia. There is often a haemorrhagic skin rash with petechiae, reflecting the associated septicaemia (Fig. 25.4). In about 35% of patients, this septicaemia is fulminating, with complications due to disseminated intravascular coagulation, endotoxaemia and shock, and renal failure. In the most severe cases there is an acute Addisonian crisis, with bleeding into the brain and adrenal glands referred to as Waterhouse–Friedrichsen syndrome. Mortality from meningococcal meningitis reaches 100% if untreated, but remains around 10% even if treated.

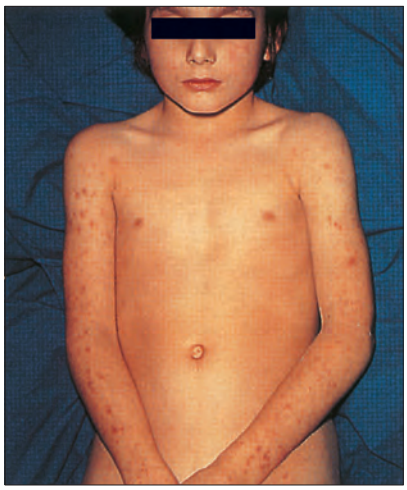


Figure 25.4 Meningococcal septicaemia showing a mixed petechial and maculopapular rash on the extremities and exterior surfaces. (Courtesy of W.E. Farrar.)

In addition, serious sequelae such as permanent hearing loss may occur in some survivors (Table 25.5).

A diagnosis of acute meningitis is usually suspected on clinical examination. Laboratory identification of the bacterial cause of acute meningitis is essential so that appropriate antibiotic therapy can be given and prophylaxis of contacts initiated. Preliminary microscopy results involving white cell counts and Gram staining for bacteria should be available within an hour of receipt of the CSF sample in the laboratory. The CSF/serum glucose ratio is also useful as bacteria breakdown glucose and so a low CSF sugar compared with serum glucose indicates a bacterial infection in the CSF (Table 25.1). Results of culture of CSF and blood should follow after 24 h. Molecular diagnosis of meningococcal infection can also be carried out and may be of clinical assistance as early treatment saves lives, but makes culture of viable organisms from specimens more difficult.

Serology is not helpful in the diagnosis because the infection is too acute for an antibody response to be detectable. Bacterial meningitis is a medical emergency and antibiotic therapy, such as ceftriaxone or chloramphenicol if the patient is allergic to penicillin, must be given immediately if the diagnosis is suspected (see Table 25.2) and is the treatment of choice if the diagnosis is confirmed.

Close contacts in the family, referred to as 'kissing contacts', should be given single-dose ciprofloxacin. Note that penicillin is not used for prophylaxis because it does not eliminate nasopharyngeal carriage of meningococci. Rifampicin used to be recommended but it is associated with rapid induction of resistance, has to be taken for a longer time period and interacts with oral contraceptives.

Haemophilus meningitis

Type b* H. influenzae *causes meningitis in infants and young children. *H. influenzae* is a Gram-negative coccobacillus. 'Haemophilus' means 'blood-loving', and the name 'influenzae' was given because it was originally thought to be the cause of influenza, but is now known to be a common secondary invader in the lower respiratory tract. There are six types (a–f) of *H. influenzae*, distinguishable serologically by their capsular polysaccharides:

- Unencapsulated strains are common and are present in the throat of most healthy people.
- The capsulated type b, a common inhabitant of the respiratory tract of infants and young children (where it may cause infection: see Ch. 19), very occasionally invades the blood and reaches the meninges.

Maternal antibody protects the infant up to 3–4 months of age, but as it wanes there is a 'window of susceptibility' until the

Table 25.5 Clinical features of bacterial meningitis

Pathogen	Host (patient)	Important clinical features	Mortality[a]	Sequelae[a,b]
Neisseria meningitidis	Children and adolescents	Acute onset (6–24 h); skin rash	7–10	<1
Haemophilus influenzae	Children <5 years of age	Onset often less acute; (1–2 days)	5	9
Streptococcus pneumoniae	All ages, but especially children <2 years of age and elderly	Acute onset may follow pneumonia and/or septicaemia in elderly	20–30	15–20

[a]As percentage of treated cases.
[b]Major central nervous system deficit; in addition, up to 10% of patients develop deafness.

child produces his / her own antibody. Anticapsular antibodies are good opsonins which allow the bacteria to be phagocytosed and killed, but children do not generally produce them until 2–3 years of age, possibly because these antibodies are T cell independent. In addition to the capsule, *H. influenzae* has several other virulence factors, as shown in Table 25.3.

Acute* H. influenzae *meningitis is commonly complicated by severe neurological sequelae. The incubation period of *H. influenzae* meningitis is 5–6 days, and the onset is often more insidious than that of meningococcal or pneumococcal meningitis (see Table 25.5). The condition is less frequently fatal but, as with meningococcal infection, serious sequelae such as hearing loss, delayed language development, and mental retardation and seizures may occur.

General diagnostic features are the same as for meningococcal meningitis, as explained above. It is important to note that the organisms may be difficult to see in Gram-stained smears of CSF, particularly if they are present in small numbers. Ceftriaxone treatment is recommended.

H. influenzae *type b (Hib) vaccine is effective for children from 2 months of age.* General features of treatment are referred to above under meningococcal meningitis; details are summarized in Table 25.2. An effective Hib vaccine, suitable for children 2 months of age and upwards, is available. Rifampicin prophylaxis is recommended for close contacts of patients with invasive Hib disease.

Pneumococcal meningitis

Streptococcus pneumoniae *is a common cause of bacterial meningitis, particularly in children and the elderly.* *Strep. pneumoniae* was first isolated more than 100 years ago but relatively little is known about its virulence attributes apart from its polysaccharide capsule (Tables 25.3, 25.4), and the pneumococcus remains a major cause of morbidity and mortality. (Pneumococcal respiratory tract infections are reviewed in Ch. 20.)

Strep. pneumoniae is a capsulate Gram-positive coccus carried in the throats of many healthy individuals. Invasion of the blood and meninges is a rare event, but is more common in the very young (<2 years of age), in the elderly, in those with sickle cell disease, in debilitated or splenectomized patients and following head trauma. Susceptibility to infection is associated with low levels of antibodies to capsular polysaccharide antigens: antibody opsonizes the organism and promotes phagocytosis, thereby protecting the host from invasion. However, this protection is type specific and there are more than 85 different capsular types of *Strep. pneumoniae*.

The clinical features of pneumococcal meningitis are generally worse than with *N. meningitidis* and *H. influenzae* and are summarized in Table 25.5. The general diagnostic features are the same as for meningococcal meningitis described above.

Treatment and prevention of pneumococcal meningitis are summarized in Table 25.2. Since penicillin-resistant pneumococci have been observed worldwide, attention must be paid to the antibiotic susceptibility of the infecting strain, and empirical chemotherapy usually involves a combination of vancomycin and either cefotaxime or ceftriaxone.

An effective pneumococcal conjugate vaccine (PCV) containing polysaccharide from 13 common capsular types conjugated to protein is available and is recommended for all babies and children younger than 2 years old (i.e. to be given with other recommended childhood vaccines) and for 2–64 year olds at high risk (e.g. sickle cell disease, HIV infection, chronic illness or weakened immune systems) for serious pneumococcal infection. The older 23-valent polysaccharide vaccine (PPV) remains available for all adults 65 years of age or older and individuals from 2 to 64 years of age at high risk as above. This is because children under 2 years old have poor antibody responses to PPV.

Listeria monocytogenes meningitis

Listeria monocytogenes *causes meningitis in immunocompromised adults.* *Listeria monocytogenes* is a Gram-positive coccobacillus and an important cause of meningitis in immunocompromised adults. It also causes intrauterine infections and infections of the newborn, as summarized in Chapter 24. *L. monocytogenes* is less susceptible than *Strep. pneumoniae* to penicillin and the recommended treatment is a combination of ceftriaxone and amoxicillin.

Neonatal meningitis

In general, neonates, especially those with low birth weight, are at increased risk for meningitis because of their immature immunological status. This is illustrated by problems with, for example, humoral and cellular immunity, phagocytic capability and inefficient alternative complement pathway. This is especially true as a result of medical advances that have contributed to the increased survival of preterm infants.

Although mortality rates due to neonatal meningitis in resource-rich countries are declining, the problem is still serious. Neonatal meningitis can be caused by a wide range of bacteria, but the most frequent are group B haemolytic streptococci (GBS) and *E. coli* (Table 25.6; see also Ch. 24). This may occur by routes such as nosocomial infection. However, the infant may also be infected from the mother. For example, with women vaginally colonized by GBS, the infant may swallow maternal secretions such as infected amniotic fluid during delivery.

Neonatal meningitis often leads to permanent neurological sequelae such as cerebral or cranial nerve palsy, epilepsy, mental retardation or hydrocephalus. This is partly because the clinical diagnosis of meningitis in the neonate is difficult, perhaps with no more specific signs than fever, poor feeding, vomiting, respiratory distress or diarrhoea. In addition, due to the possible range of aetiological agents, 'blind' antibiotic therapy in the absence of susceptibility tests may not be optimal, and adequate penetration of the antibiotic into the CSF is also an issue. Antibiotic treatment includes benzylpeniciilin and gentamicin.

Tuberculous meningitis

Patients with tuberculous meningitis always have a focus of infection elsewhere, but approximately 25% may have no clinical or historic evidence of such an infection. In >50% of cases, meningitis is associated with acute miliary tuberculosis (Fig. 25.5). In areas with a high prevalence of tuberculosis, meningitis tends to be most commonly seen in children from 0 to 4 years of age. However, in areas where tuberculosis is less frequent, most meningitis cases are in adults.

Tuberculous meningitis usually presents with a gradual onset over a few weeks. There is a gradual onset of generalized

Table 25.6 Group B streptococci are a major cause of neonatal meningitis

Group B streptococci (*Streptococcus agalactiae*) are normal inhabitants of the female genital tract and may be acquired by the neonate		
	At or soon after birth	**In the nursery**
	Early onset disease	***Late onset disease***
Age	<7 days	1 week–3 months
Risk factors	Heavily colonized mother lacking specific antibody Premature rupture of membranes Preterm delivery Prolonged labour, obstetric complications	Lack of maternal antibody Exposure to cross-infection from heavily colonized babies Poor hygiene in nursery
Type of disease	Generalized infection including bacteraemia, pneumonia and meningitis	Predominantly meningitis
Type of group B streptococcus	All serotypes but meningitis mostly due to type III	90% type III
Outcome	Approximately 60% fatal; serious sequelae in many survivors	Approximately 20% fatal
Treatment	Take blood and CSF for culture	Treat on suspicion
	Treat on suspicion	Take blood and CSF for culture
	Gentamicin and benzylpenicillin	Gentamicin and benzylpenicillin
Prevention	Antibiotic treatment does not reliably abolish carriage in mother; not recommended	Good hygiene practices in nursery
	'Blind' treatment of sick baby who has risk factors Future: ? immunize antibody-negative females of child-bearing age	Do not allow mothers to handle other babies

CSF, cerebrospinal fluid.

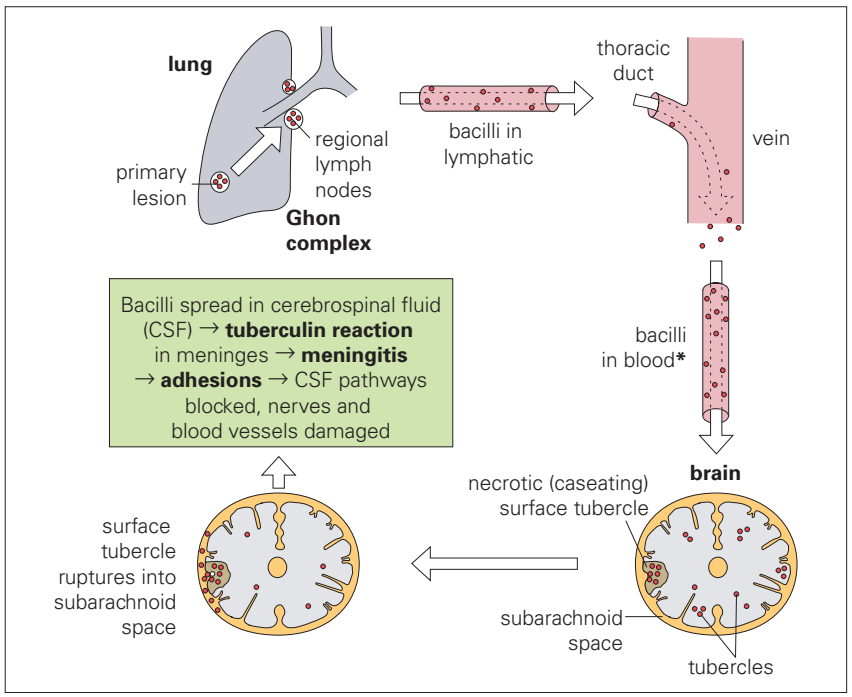


Figure 25.5 The association between acute miliary tuberculosis and meningitis. (* Leads to miliary tuberculosis [Latin: *milium*, millet seed – each tubercle resembles a millet seed]. Miliary tuberculosis also occurs in the lungs and elsewhere.)

illness beginning with malaise, apathy and anorexia and proceeding within a few weeks to photophobia, neck stiffness and impairment of consciousness. Occasionally, the onset is much more rapid and may be mistaken for a subarachnoid haemorrhage. The variability of presentation means that the clinician needs to maintain an awareness of possible tuberculous meningitis to make the diagnosis. A delay in making the diagnosis and in starting appropriate antimicrobial therapy (Table 25.2) results in serious complications and sequelae.

Spinal tuberculosis is uncommon now except in resource-poor countries; bacteria in the vertebrae destroy

the intervertebral disks to form epidural abscesses. These compress the spinal cord and lead to paraplegia.

Fungal meningitis

Cryptococcus neoformans and *Coccidioides immitis* can invade the blood from a primary site of infection in the lungs and then travel to the brain to cause meningitis. *Cryptococcus* has a marked tropism for the CNS and is the major cause of fungal meningitis. *C. neoformans* occurs as two varieties, each with two serotypes.

Cryptococcus neoformans meningitis is seen in patients with depressed cell-mediated immunity

It therefore occurs in individuals with AIDS and other immunosuppressive conditions. The onset is usually slow, over days or weeks. The capsulate yeasts can be seen in Indian-ink-stained preparations of CSF (Fig. 25.6) and can be cultured. Antigen detection is also a useful diagnostic tool and evidence of a decline in antigen and an increase in antibody levels in the CSF can be used as a measure of successful therapy. Treatment with the antifungal drugs amphotericin B and flucytosine in combination is recommended, the former penetrating poorly into CSF.

Coccidioides immitis infection is common in particular geographical locations

These locations are notably southwest USA, Mexico and South America. CNS infection occurs in <1% of infected individuals, but is fatal unless treated. It may be part of the generalized disease or may represent the only extrapulmonary site. The organisms are rarely visible in the CSF, and cultures are positive in <50% of cases, but the diagnosis can be made by demonstrating complement-fixing antibodies in the serum. Treatment with amphotericin B or fluconazole is recommended.

Protozoal meningitis

The thermophilic free-living amoeba *Naegleria fowleri* lives in warm fresh water, especially in the sludge at the bottom of lakes and swimming pools, where it feeds on bacteria. If inhaled, *N. fowleri* can reach the meninges via the olfactory tract and cribriform plate. Primary amoebic meningoencephalitis caused by *Naegleria* affects healthy individuals with no obvious defect in immunity. The disease has a rapid onset, and the mortality rate is very high.

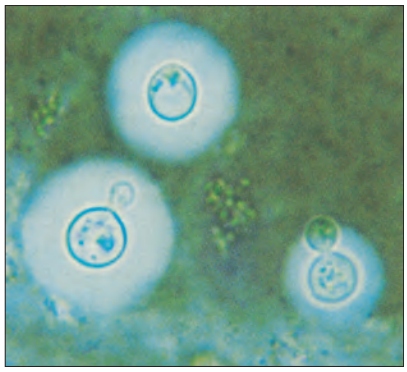


Figure 25.6 *Cryptococcus neoformans* in India ink-stained preparation of cerebrospinal fluid sediment. (Courtesy of A.E. Prevost.)

Acanthamoeba spp. are widespread in the environment. They more commonly affect those who are already unwell or immunocompromised and are thought to enter via the skin or the respiratory tract. *Acanthamoeba* causes a chronic progressive condition (granulomatous amoebic encephalitis) which is almost always fatal.

Balamuthia mandrillaris is found in soil or stagnant water as vegetative trophozoites and dormant cysts. Humans become infected by inhalation of cysts or direct contamination of skin. Several months may elapse between the appearance of cutaneous lesions and invasion of the CNS. Cases have been reported in patients with a variety of underlying medical conditions but also in immunocompetent individuals. Infection of the CNS produces granulomatous encephalitis with raised protein, lymphocytic CSF and normal or low CSF glucose, but in the presence of severely compromised cellular immunity there may be little granuloma formation. Death occurs in days to weeks.

Under the microscope, *N. fowleri* appears as slowly motile amoebae on careful examination of a fresh wet sample of CSF. *Acanthamoeba* spp. are rarely seen in the CSF but can be visualized in brain biopsies. They also grow well in cultures prepared from tissue biopsies. PCR is available in specialist centres and serology is sometimes available. Diagnosis of *Balamuthia* infection is by histopathology of biopsy samples and PCR on brain tissue or CSF. Serology is available in only a few specialist centres. Treatment is not fully satisfactory. Amphotericin B, with miconazole and rifampin, has been used for *Naegleria*; a variety of drugs have been used for *Acanthamoeba*. Mortality rates for *Balamuthia* encephalitis are reportedly greater than 90%. Combination therapy is given for all three forms of amoebic encephalitis. The choice of agents is based on case reports and the inclusion of miltefosine in the regimen is reported to be associated with a greater chance of survival in *Balamuthia* and *Acanthamoeba* cases.

Viral meningitis

Viral meningitis is the most common type of meningitis

It is a milder disease than bacterial meningitis, with headache, fever and photophobia, but less neck stiffness. The CSF is clear in the absence of bacteria and the cells are mainly lymphocytes, although polymorphonuclear leukocytes may be present in the early stages (Table 25.1). Before the advent of molecular-based methods of detection, viruses were isolated from the CSF in <50% of cases.

There are five groups of human enteroviruses which include the echoviruses, coxsackie group A and B viruses, and the three types of polioviruses. The non-polio enteroviruses are the most common causes of seasonal aseptic meningitis, usually from late spring to autumn. In contrast to bacterial meningitis, viral meningitis usually has a benign course, and complete recovery is the rule.

ENCEPHALITIS

Encephalitis is usually caused by viruses, but there are many cases where the infectious aetiology is not identified

The pathogenesis of viral encephalitis is shown in Fig. 25.7. The estimated incidence of encephalitis in a study in

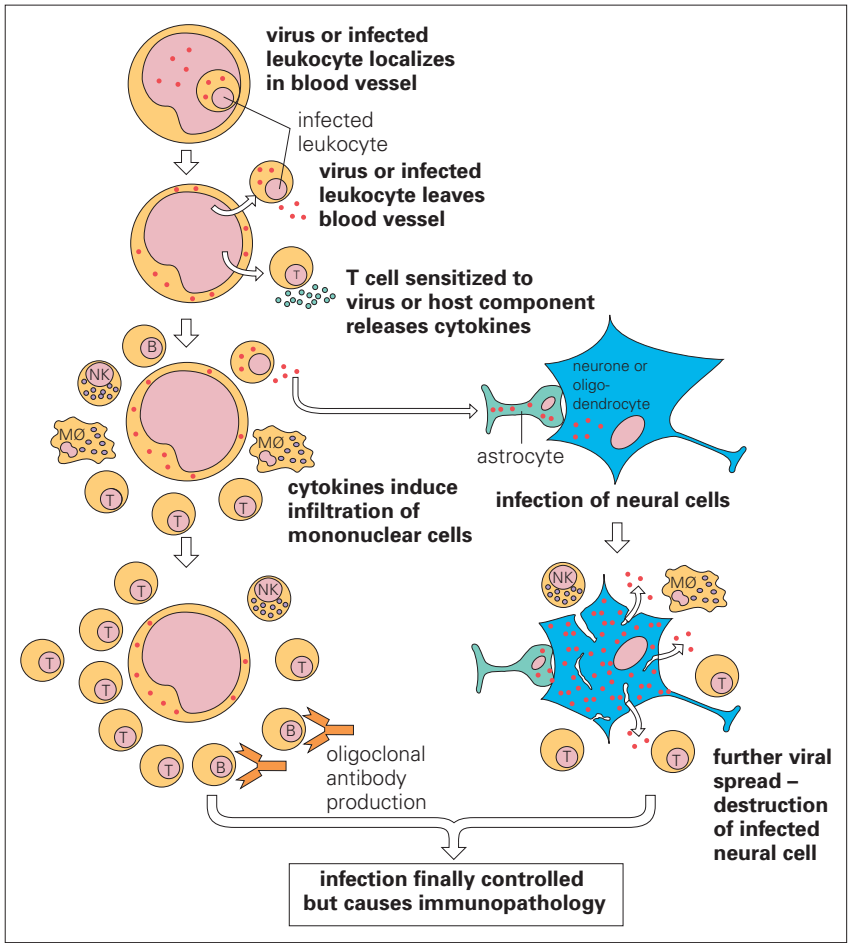


Figure 25.7 The pathogenesis of viral encephalomyelitis. Mφ, macrophage; NK, natural killer cell.

the USA between 1998 and 2010 reported around 20 000 encephalitis-associated hospital admissions per year, with a 6% mortality rate and substantial morbidity including physical, cognitive and behavioural difficulties. It is thought that the annual costs of illness caused by encephalitis to the United States health service amounts to around US$630 million.

Characteristically, there are signs of cerebral dysfunction, as the substance of the brain is affected, unlike meningitis where the lining of the brain is inflamed. Someone with an encephalitic illness will present with abnormal behaviour, confusion, seizures and altered consciousness, often with nausea, vomiting and fever.

Up to 85% of individuals diagnosed with encephalitis globally are of unknown aetiology. Emerging viruses that can cause encephalitis include the Nipah virus, bat lyssaviruses, and avian influenza A H5N1 virus infections. Immune-mediated forms of encephalitis, including voltage-gated potassium channel and *N*-methyl-D-aspartate (NMDA) receptor antibody-associated encephalitis, must be considered in the differential diagnosis as they have a presentation similar to that of infectious causes. Steroids can be used to treat the immune-mediated encephalitides, including acute disseminated encephalomyelitis (ADEM).

Antiviral drugs such as aciclovir are used to treat herpes simplex and varicella-zoster virus encephalitis. Preventative measures include measles, mumps and rubella immunization.

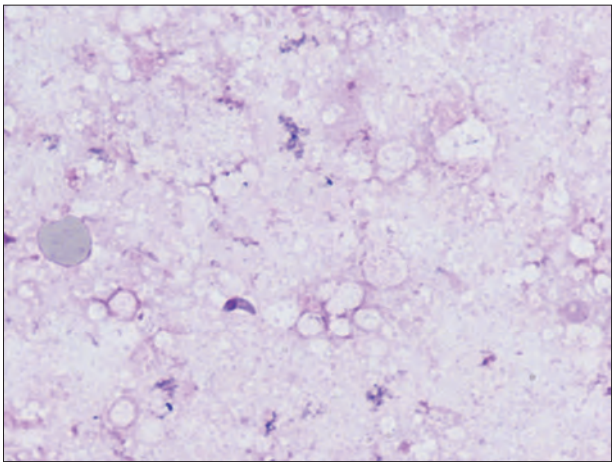


Figure 25.8 *Toxoplasma* tachyzoite in a brain biopsy smear. (Courtesy of Peter Chiodini.)

Toxoplasma gondii and *C. neoformans* can also cause life-threatening encephalitis or meningoencephalitis (Fig. 25.8). This is particularly likely in those with defective cell-mediated immunity, and cerebral malaria as a complication of *Plasmodium falciparum* infection is frequently fatal. Encephalitis may occur in Lyme disease (*Borrelia burgdorferi*) and Legionnaires'

disease (*Legionella pneumophila*), but the relative importance of bacterial invasion, bacterial toxins and immunopathology is unknown.

HSV encephalitis (HSE) is the most common form of severe sporadic acute focal encephalitis and early aciclovir treatment is critical

It is thought that the incidence of HSE in the USA is about 1 / 250 000 to 500 000 population per year. A distinction is made between HSV infections of the CNS during the neonatal period and those in older children and adults. Neonates may acquire a primary and disseminated infection with diffuse encephalitis after vaginal delivery from a mother shedding HSV-2 in the genital tract. Most HSE seen in older children and adults is due to HSV-1, of which most are due to virus reactivation in the trigeminal ganglia, the infection then passing back to the temporal lobe of the brain, and the minority are due to a primary infection. About 30% of HSE is seen in people <20 years old, and 50% in the over-50 age range.

Herpetic skin or mucosal lesions may be present. The diagnosis is indicated by finding temporal lobe enhancement using CT and MRI scans of the head (Fig. 25.9). HSV DNA detection is carried out on a CSF sample using PCR. An electroencephalogram (EEG) may also be helpful. The 70% mortality rate in untreated patients is greatly reduced by early and prolonged treatment with intravenous aciclovir. The 21-day treatment course is important, as relapse can occur.

Other herpesviruses less commonly cause encephalitis

With VZV, encephalitis generally occurs as a sequel to reactivation, and with cytomegalovirus during either primary infection in utero or reactivation as a complication of immunosuppression (e.g. in allogeneic bone marrow transplant recipients). Human herpes virus 6 (HHV-6) encephalitis has also been reported in immunosuppressed patients. Finally, B virus is a Cercopithecine herpesvirus of macaque monkeys that does not really affect the animal but can cause severe and fatal encephalitis in humans who are bitten or scratched by an infected monkey. The wound should be cleaned immediately and antiviral prophylaxis is recommended.

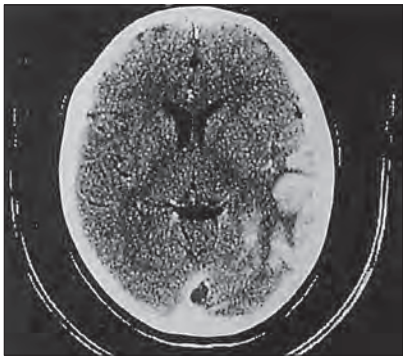


Figure 25.9 Herpes simplex encephalitis. Computerized tomographic head scan showing enhancement of gyral structures in the left temporal lobe and associated cerebral oedema. (Courtesy of J. Curé.)

Enteroviral infections

Enterovirus 71-associated hand, foot and mouth epidemic resulted in a high rate of neurological complications

Other enteroviruses such as coxsackieviruses and echoviruses occasionally cause meningoencephalitis. However, in 1998, there was a large outbreak of enterovirus 71 (EV71) hand, foot and mouth (HFMD) infection in Taiwan, in which most of the 405 patients were children under 5 years of age, with a mortality rate of 19%. The most severely affected children had brainstem involvement, and many were left with permanent neurological sequelae. Treatment is supportive and there is no vaccine. Since then, EV71, having been identified in Guangdong province in the People's Republic of China and having caused epidemics in south China, then caused several epidemics reported in the middle or north of China. Overall, by August 2016, nearly 1 800 000 HFMD infections had been reported nationwide, including 172 deaths.

Poliovirus used to be a common cause of encephalitis

In the great 1916 polio epidemic in New York City, 9000 cases of paralysis were reported, nearly all in children <5 years of age. CNS disease occurs in <1% of those infected. After an initial 1–4 days of fever, sore throat and malaise, meningeal signs and symptoms appear, followed by involvement of motor neurones and paralysis (see Fig. 25.2).

There are successful vaccines; the structure (Fig. 25.10) and replication of the virus are better understood, and efforts to eradicate the disease by 2002 had driven the incidence of polio to its lowest point in history. The disease is completely preventable by vaccination (see Ch. 32) and has been disappearing in resource-rich countries since vaccination programmes were first carried out in the 1950s (Fig. 25.11).

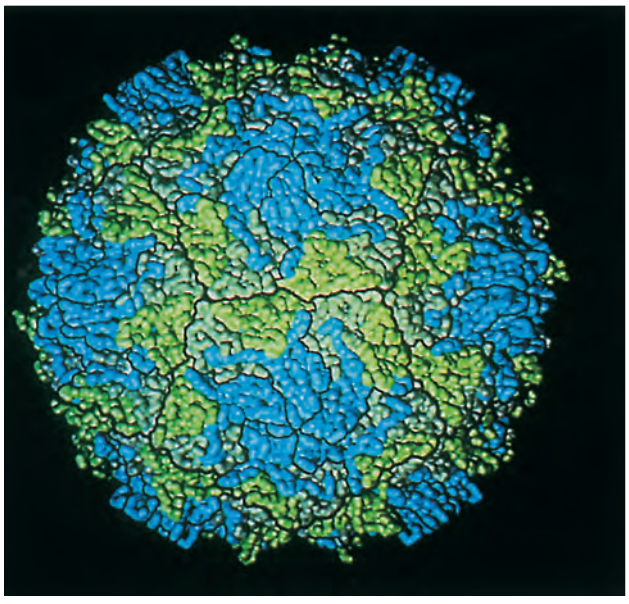


Figure 25.10 Computer graphic model of the surface of a poliovirus based on X-ray diffraction studies. The capsid protein subunits visible on the surface of the virus particle are viral protein 1 (VP1) in blue, VP2 in green and VP3 in grey. (Courtesy of A.J. Olson, Research Institute of Scripps Clinic, La Jolla, California.)

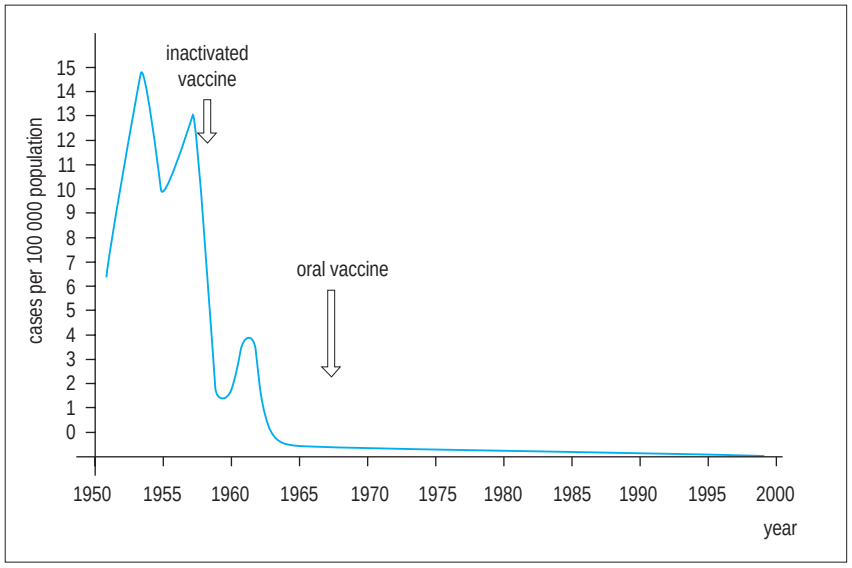


Figure 25.11 The incidence of paralytic poliomyelitis in the USA from 1951 to 2000.

The Global Polio Eradication Initiative reduced the number of polio-endemic countries, and the fall in poliovirus transmission in these countries was due to a new bivalent oral polio vaccine and new ways of delivering the vaccine. All of the South-East Asia region of WHO was certified as polio free in 2014, encompassing 11 countries from India to Indonesia. By 2016, 80% of the global population lived in regions where polio had been eradicated. However, endemic transmission was still occurring in Pakistan, Nigeria and Afghanistan.

There are three serological (antigenic) types of poliovirus, with little cross-reaction between them, so that antibody to each type is necessary for protection. At least 75% of paralytic cases are due to type 1 polioviruses.

Paramyxoviral infections

Mumps virus is a common cause of mild encephalitis

Asymptomatic CNS invasion may be common because there are increased numbers of cells in the CSF in about 50% of patients with parotitis. However, meningitis and encephalitis are often seen without parotitis.

Nipah virus encephalitis, a zoonotic paramyxovirus infection

In 1998, an outbreak of encephalitis with a high mortality rate was reported among pig farm workers in Malaysia. In total, there were 105 deaths among 265 patients with Nipah virus encephalitis. Some patients have respiratory symptoms early on in the infection. At first attributed to Japanese encephalitis, the clinical, epidemiological and virological characteristics showed that the virus was a paramyxovirus which was transmitted to humans by close contact with infected pigs, probably by aerosol. The outbreak was ended by culling more than 1 million infected or exposed pigs in the local and surrounding regions in Malaysia. The island flying fox, *Pteropus hypomelanus*, a fruit bat, is the natural reservoir for the virus and the virus can be found in the urine and saliva of infected bats. The pigs were infected having eaten food contaminated by fruit bat secretions. Human-to-human transmission can also play an important role in Nipah virus transmission. Further outbreaks were reported in 2001 in Bangladesh and India, where there were reports of human-to-human transmission in hospital settings.

Rabies encephalitis

More than 55 000 people die of rabies worldwide each year

- Rabies occurs in more than 150 countries and territories.
- Wound cleansing and immunization within a few hours after contact with a suspect rabid animal can prevent the onset of rabies and death.
- Every year, more than 15 million people worldwide receive a post-exposure preventive regimen to avert the disease – this is estimated to prevent 327 000 rabies deaths annually.

The causative agent of rabies is a rhabdovirus, a bullet-shaped single-stranded RNA virus. The *Lyssavirus* genus sits within the Rhabdoviridae family and there are seven genotypes: genotype 1 occurs worldwide and is the classic rabies virus, genotypes 2, 3 and 4 are the African Lagos, Mokola and Duvenhage bat viruses, respectively, genotypes 5 and 6 are the European bat Lyssaviruses (EBLV) 1 and 2, respectively, and genotype 7 is the Australian bat Lyssavirus.

The virus is excreted in the saliva of infected dogs, foxes, jackals, wolves, skunks, raccoons and vampire and other bats, and transmission to humans follows a bite or salivary contamination of other types of skin abrasions or wounds. The infection is eventually fatal, although the course of the disease varies considerably between species. If an apparently healthy dog is still healthy 15 days after biting a human, rabies is extremely unlikely. However, the virus may be excreted in the dog's saliva before the animal shows any clinical signs of disease.

The virus can infect all warm-blooded animals. Rabies from vampire bats causes more than 1 million deaths per year in cattle in Central and South America. Dogs are the source of more than 99% of human rabies deaths and are involved in most of the 55 000 cases of human rabies that occur in the world each year. In all the mainland masses, the infection

maintains itself in non-human mammalian hosts; islands such as Australia, Great Britain, Japan, Hawaii, most of the Caribbean islands and also Scandinavia, are free of rabies because of strict controls over the importation of animals such as dogs and cats, although this is changing. Since the development of the Channel Tunnel linking the UK and the rest of Europe, a number of rabies infections occurred associated with contact with bats. Thirty different bat species have been identified in Europe, a number of which carry EBLV 1 or 2. They are distinct from rabies genotype 1 infections in foxes, dogs and other terrestrial animals. In the USA, the incidence of human rabies has been falling since the 1940s and 1950s, when most cases followed exposure to infected dogs. Since then, the source has more often been non-domesticated animals such as skunks, raccoons and bats, or exposure to dogs in other countries.

Raccoon rabies spread slowly northwards from Florida in the 1950s, and in the 1980s caused an explosive epidemic in Virginia, Maryland and the District of Columbia. This outbreak was due to the importation of raccoons from infected areas for sporting purposes.

The incubation period in humans is generally 4–13 weeks, although it may occasionally be as long as 6 months, possibly due to a delay in virus entry into peripheral nerves. The virus travels up peripheral nerves and, in general, the further the bite is from the CNS, the longer is the incubation period. For instance, a bite on the foot leads to a longer incubation period than does a bite on the face.

While the virus is travelling up the axons of motor or sensory neurones, there is no detectable antibody or cell-mediated immune response, possibly because antigen remains sequestered in infected muscle cells. Hence, passive immunization using rabies-specific immunoglobulin may be given during the incubation period.

Once in the brain, the virus spreads from cell to cell until a large proportion of neurones are infected, but there is little cytopathic effect, even when viewed by electron microscopy, and almost no cellular infiltration. The striking symptoms of this disease are largely due to dysfunction rather than to visible damage to infected cells. The change in behaviour of infected animals results from virus invasion of the limbic system.

Clinical features of rabies include muscle spasms, convulsions and hydrophobia

After developing a sore throat, headache, fever and discomfort at the site of the bite, the patient becomes excited, with muscle spasms and convulsions. Involvement of the muscles of swallowing when attempting to drink water gave the old name for rabies – hydrophobia – as the symptoms are sometimes precipitated by the mere sight of water.

Once rabies has developed it is fatal, death occurring following cardiac or respiratory arrest. Paralysis is often a major feature of the disease.

Rabies can be diagnosed by detecting viral antigen or RNA

Laboratory diagnosis can be made by the detection of viral antigen by immunofluorescence or using PCR to detect rabies viral RNA in skin biopsies, corneal impression smears or brain biopsy. Characteristic intracytoplasmic inclusions called Negri bodies are seen in neurones (Fig. 25.12). There is no treatment

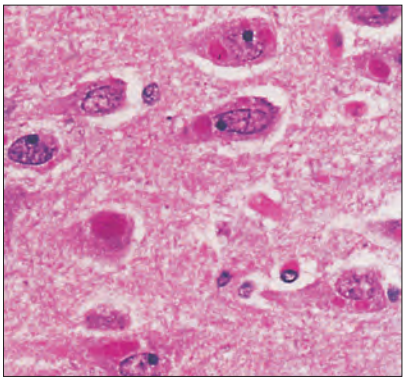


Figure 25.12 Multiple cytoplasmic Negri bodies in pyramidal neurones of the hippocampus in rabies. (Courtesy of P. Garen.)

except supportive care. Five individuals have survived having received immunoprophylaxis before symptom onset. There was a report in 2005 of a 15-year-old girl who was bitten by a bat and developed rabies, had not received rabies immunoprophylaxis and was put into a medically induced coma in order to rest and protect the brain from injury and to allow the immune response to mature. The coma was induced using specific receptor antagonists and agonists that reduced brain metabolism, autonomic reactivity and excitotoxicity. Antivirals were given too and she survived. This was referred to as the Milwaukee protocol.

Many countries (e.g. France) have developed vaccination programmes for domestic dogs and, in Canada and elsewhere, wild foxes have been vaccinated by dropping food baited with live virus vaccine from the air. For the rabies-free countries, constant vigilance at borders and strict quarantine regulations are necessary to prevent the introduction of infected animals. In 1886, there were 36 human rabies deaths in England, 11 of them in London. As recently as 1906, rabies was still endemic in England, and there were deaths due to rabies in the deer in Hampton Court Park, London.

After exposure to a possibly infected animal, immediate preventive action should be taken

This action includes:

- prompt cleaning of the wound (alcoholic iodine, debridement)
- confirmation of whether or not the animal is rabid (clinical observation of suspected dogs)
- administration of human rabies immunoglobulin (RIG) to ensure prompt passive immunization; RIG is infiltrated intramuscularly around the wound site
- active immunization with killed diploid cell-derived rabies virus (see Ch. 35). The chances of preventing the disease are greater when vaccination is started as early as possible after infection. Vaccine and RIG must never be administered at the same anatomical site.

Togavirus meningitis and encephalitis

Numerous arthropod-borne togaviruses can cause meningitis or encephalitis

These togaviruses sometimes cause outbreaks of infection. In different parts of the world, different mammals, birds or even reptiles act as reservoirs and there are a variety of arthropod

(mosquito and tick) vectors. Usually, <1% of humans infected develop neurological disease (see Ch. 28). There may be a febrile illness, but asymptomatic infection is common. In California, for instance, western equine encephalomyelitis (WEE) virus and St Louis encephalitis (SLE) virus are prevalent and are transmitted by the mosquito *Culex tarsalis*; a WEE vaccine is available, but only for horses.

Flavivirus infections

Japanese encephalitis virus infection is an important cause of encephalitis in South East Asia and mostly affects children

Japanese encephalitis virus, a flavivirus infection related to dengue, yellow fever and West Nile viruses is, you may have guessed, prevalent in South-East Asia. The majority of reported infections, around 70000 per year, are in China and India. It is transmitted by *Culex* mosquitoes with pigs and wading birds as intermediate hosts. Many adults in endemic countries have been infected in childhood and are immune. Infection can result in a mortality of more than 30%. Inactivated and live attenuated vaccines have been developed, with immunization programmes in endemic countries.

West Nile virus infection swept rapidly through the USA after the initial reports

In 1999, a dramatic epidemic of viral encephalitis was reported in New York City, leading to 62 patients with encephalitis, seven of whom died. Meningoencephalitis was rare in the younger age groups and more common in those over 50 years of age. Originally thought to be due to SLE, once again the clinical, epidemiological and virological characteristics resulted in the correct identification of West Nile virus (WNV) infection, as there had been an epidemic of deaths among wild and other birds which are the avian reservoir of SLE, but are not usually killed by the virus. How the virus had been introduced into North America, having been circulating in Israel and Tunisia, was unclear. Israeli domestic geese had developed fatal WNV infections in 1997–1998 and human WNV infections occurred in August 1999 in both countries. *Infected Culex* species mosquitoes transmit WNV to birds, some of which develop high levels of viraemia and other mosquitoes bite them and become infected, helping cycle WNV. In New York City, an outbreak of infection in birds with a high mortality rate had been reported but not linked with the cluster of human infections. Extensive pathological assessments were carried out and genome sequencing demonstrated that the WNV strains were homologous. To illustrate the remarkable speed of WNV spread in North America, there were 21 human infections in 10 counties in the northeast in 2000; 66 in 38 counties in 10 states by 2001 and by 2003 there were over 4000 human infections with nearly 2500 cases of meningoencephalitis and 284 deaths. This was the largest outbreak of WNV meningoencephalitis ever recorded, with severe central nervous system disease seen in older people and milder, feverish illness in younger people. Bird death and mosquito pool surveillance was important to track events from a public health perspective. Transmission was also reported in four organ transplant recipients who had received organs from one donor with West Nile viraemia antemortem, as well as by blood transfusion.

West Nile virus belongs to the Japanese encephalitis serogroup of flaviviruses that includes SLE; it had not been seen in the western hemisphere but was well recognized in Africa and the Middle East. West Nile virus is primarily an infection of birds and culicine mosquitoes, with humans and horses acting as incidental hosts. Since 1999, the virus has been successfully dispersed by migratory birds and has spread through most of the USA. By 2017, WNV infection infection in humans had been reported by the European Centre for Disease Prevention and Control in a host of European countries including Bulgaria, Italy, Hungary, Romania and Austria.

The diagnosis can be made by detecting West Nile viral RNA or an IgM response in serum and / or CSF samples. Treatment is supportive, there is no vaccine, and prevention includes mosquito control programmes.

HIV meningitis and encephalitis

HIV can cause subacute encephalitis, often with dementia

HIV often invades the CNS shortly after initial infection, resulting in an increase in cells in the CSF and a mild meningitic illness. At a later stage, and quite independently of the disease picture that results from immune deficiency, a subacute encephalitis may develop, often with dementia. Early in the HIV epidemic, a number of opportunistic CNS infections were detected, AIDS-defining diagnoses, and were a result of advanced HIV infection and immunosuppression. These included *Toxoplasma gondii*, *Cryptococcus neoformans*, CMV and JC virus infections. JC virus, a polyomavirus, occasionally invades oligodendrocytes in immunodeficient people, particularly in AIDS, and eventually gives rise to progressive multifocal leukoencephalopathy (PML). In HIV-related dementia, the brain is shrunken, with enlarged ventricles and vacuolation of myelin tracts. HIV mainly infects macrophages and microglia in the CNS and the virus may also enter astrocytes, although this is controversial. It may be that HIV directly and indirectly affects neuronal activity by altering neuronal pathways and is involved in local inflammation.

Combined antiretroviral therapy has reduced the incidence of HIV-associated dementia but neurological effects of HIV are still seen as the population gets older and are reported as HIV-associated neurocognitive disorders (HAND). HAND is correlated with long-term CNS inflammation and neurotoxicity.

Viral myelopathy

A number of viral infections can cause inflammation of the spinal cord, a myelitis. Acute myelitis may result in symmetrical symptoms if it transverses the spinal cord. These will include motor weakness and sensory loss, for example. The symptoms will be asymmetrical if only part of the spinal cord is involved. When the anterior horn cells of the cord are affected by polio, coxsackie, enterovirus 71 and West Nile virus infection, the symptoms are motor and result in acute flaccid paralysis. A number of herpesviruses including HSV, CMV, EBV and VZV have been associated with myelitis. Post-infectious causes have also been reported.

Chronic myelopathy can be caused by HTLV-1 infection and patients present with tropical spastic paraparesis (TSP), also called HAM (HTLV-1-associated myelopathy). HIV-1 infection is also part of the differential diagnosis.

Guillain–Barré syndrome – an inflammatory demyelinating condition of the peripheral nervous system

Some 2–4 weeks before Guillain–Barré syndrome (GBS) develops, there is usually a history of an upper respiratory tract or other infection, leading to a rapidly evolving ascending muscle weakness with little sensory loss. This can be severe and patients can deteriorate rapidly, requiring mechanical ventilation to support their breathing.

GBS has been associated with a variety of infections, as well as with immunization with non-infectious material. The viral infections include EBV, CMV, HIV, West Nile and Zika virus and, of the bacteria, *Campylobacter jejuni* may be seen in one-third of patients, with *Mycoplasma pneumoniae* and *Borrelia burgdorferi* as rare associations.

In 1976, most adults in the USA were given inactivated influenza virus vaccine, which resulted in a small but highly significant number of cases of GBS.

Treatment is general supportive care, and plasma exchange, which directly removes immune complexes, cytokines, autoantibodies and other inflammatory mediators that may be involved in the pathogenesis, as well as intravenous immunoglobulin have been shown to be effective.

Post-infectious encephalitis

Encephalitis following viral infection or vaccination possibly has an autoimmune basis

Encephalitis very occasionally occurs 1–2 weeks after an apparently normal measles virus infection, and even less commonly after varicella. It is also seen after *Mycoplasma* infection and various influenza-like illnesses. The infectious agent is generally not recoverable from the CNS, and the perivascular infiltration, sometimes with demyelination, suggests an autoimmune pathogenesis. A similar condition occurs after administration of brain-derived inactivated rabies vaccine, which is now obsolete, and after other immunizations with non-infectious materials. The clinical picture resembles experimental allergic encephalitis, and is probably due to autoimmune responses triggered by the infection or by the injected material.

In addition, rubella or measles virus invades the CNS, but virus growth is slow, often incomplete and partially controlled by host defences; clinical disease appears after an incubation period of up to 10 years. For instance:

- In otherwise uncomplicated measles, CNS invasion can take place and eventually result in SSPE.
- Rubella very occasionally causes a similar disease to SSPE but more commonly, like CMV, it invades the brain of the fetus, interfering with development to cause mental retardation.

NEUROLOGICAL DISEASES OF POSSIBLE VIRAL AETIOLOGY

It has often been suggested that certain neurological diseases of unknown origin, including multiple sclerosis, amyotrophic lateral sclerosis, Parkinson's disease, schizophrenia and dementia, have a viral origin. Although so far there is no definitive evidence for this, it is possible that viruses and other infectious agents may, at times, trigger dangerous autoimmune-type responses in the CNS.

SPONGIFORM ENCEPHALOPATHIES CAUSED BY SCRAPIE-TYPE AGENTS

Scrapie-type agents are closely associated with host-coded prion protein

Scrapie-type agents infect a variety of mammals, including humans, and are transmissible to laboratory rodents or primates. They show a number of remarkable biological characteristics; their molecular biology is now well described and experiments in laboratory mice have revealed much about their interaction with host tissues (see Ch. 8). Disease is characterized by the appearance of a spongiform appearance of nervous tissues, caused by vacuolation and plaque formation. Infections in animals seem to have originated from sheep and goats with scrapie (see Fig. 8.4), which has been present in Europe for 200–300 years. Affected animals itch and scrape themselves against posts for relief.

CNS DISEASE CAUSED BY PARASITES

The CNS is an important target in toxoplasmosis

Although congenitally acquired infection with *Toxoplasma gondii* is initially generalized, it may become localized in the CNS. Damage to the eye is the most common consequence (see Ch. 26), but the brain may also be affected, resulting in hydrocephalus and intracerebral calcification. In the days before the advent of combined antiretroviral therapy, cerebral toxoplasmosis due to reactivation of infection from dormant tissue cysts was an important cause of death in AIDS patients, with encephalitis and toxoplasma abscess due to necrosis as contributory causes.

Cerebral malaria is a major killer

The life cycle of *Plasmodium falciparum* shows an unusual feature in that red blood cells containing the asexual stages (asexual stages are in humans; sexual stages are in mosquitoes, see Ch. 28) adhere to the walls of capillaries. When this occurs in the brain, cerebral malaria may result and is an important cause of mortality in African children. Fever is followed by a variety of symptoms, including convulsions and coma, which lead rapidly to death if not treated. Artemisinin combination therapy has replaced quinine as the treatment of choice as it has a clear survival advantage in serious and complicated malaria. Coma is reversible, mostly without residual neurological deficit, when treatment is successful.

Toxocara infection can result in granuloma formation in the brain and retina

The cat and dog roundworms *Toxocara cati* and *Toxocara canis* infect humans, usually children, when *Toxocara* eggs derived from kitten or puppy faeces are ingested. After ingestion by humans, the eggs hatch and the larvae migrate from the gut to the liver, lung, eye, brain, kidney and muscles. However, as humans are dead-end hosts for these parasites, they cannot reach full maturity. Granulomas form around the larvae, which in the brain may cause convulsions and eosinophilic meningoencephalitis. In the eye it can present as a tumour-like mass in the peripheral retina or as a posterior pole granuloma. Granulomas can cause retinal detachment, and blindness can ensue if the macula is involved. Peripheral blood eosinophilia is rarely seen in ocular toxocariasis.

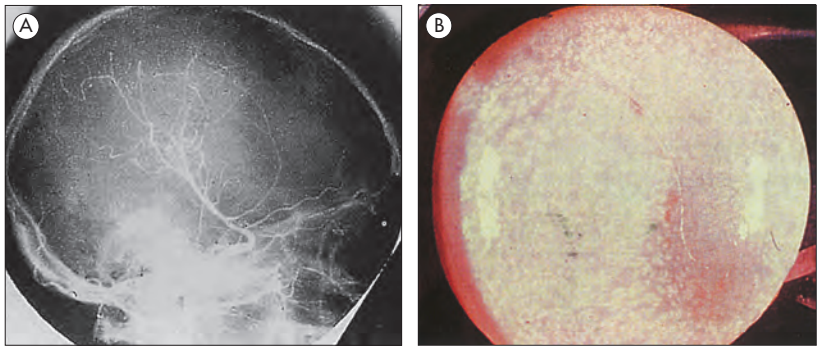


Figure 25.13 Echinococcosis. (A) Cerebral angiography showing displacement of vessels by a large frontal mass. (B) Cyst removed from patient in (A). (Courtesy of H. Whitwell.)

Serum can be tested for antibodies to *Toxocara* excretory–secretory antigen by enzyme-linked immunosorbent assay (ELISA), confirmed by Western blot, but may give false-negative results in ocular toxocariasis. Antibody detection in ocular vitreous fluid samples is more sensitive. The disease is prevented by deworming puppies and kittens, and by reducing the contamination of children's play areas by dog excreta. Albendazole under corticosteroid cover can be given for neurotoxocariasis. Anthelmintic therapy is not always given in ocular toxocariasis. Corticosteroids and appropriate ophthalmic surgery are the mainstays of therapy.

Cystic hydatid disease is characterized by cyst formation, potentially in any organ but most commonly in the liver

Cystic echinococcosis (cystic hydatid disease) is caused by the tapeworm *Echinococcus granulosus*, which has a worldwide distribution, especially in sheep-rearing areas. When humans ingest eggs from infected dogs, the embryos emerge and migrate through the gut to the portal blood vessels. From there, they are carried mainly to the liver where they subsequently develop into hydatid cysts. These may occur in any organ but are found especially in the liver and, less commonly, in the lungs, brain and kidney. Disease is caused by local pressure from the cyst, and sometimes hypersensitivity reactions to hydatid antigens. Neurological symptoms include nausea and vomiting, seizures and altered mental status.

Hydatid disease is diagnosed by detecting serum antibody to hydatid antigens and, specifically for CNS involvement, by CT or preferably MRI scanning, to demonstrate the presence of cysts (Fig. 25.13). Hydatid cysts in the CNS require surgical removal, with special care to avoid cyst rupture, plus adjunctive therapy with albendazole.

The disease is prevented by interrupting the natural dog–sheep, dog–goat, or other carnivore–herbivore transmission cycle.

Cysticercosis is characterized by cyst formation in the brain and eye

Cysticercosis results from infection with the larval stage of *Taenia solium*, the pork tapeworm. Eggs present in human faeces infect pigs, which develop cysts in their muscles ('measly pork') and are a source of further human infection if the meat is eaten raw or undercooked. Humans ingest eggs in material contaminated with human faeces, often from another person rather than from a tapeworm infection of their own, which explains why vegetarians can contract cysticercosis. After passing through the gut wall, larvae released from the eggs

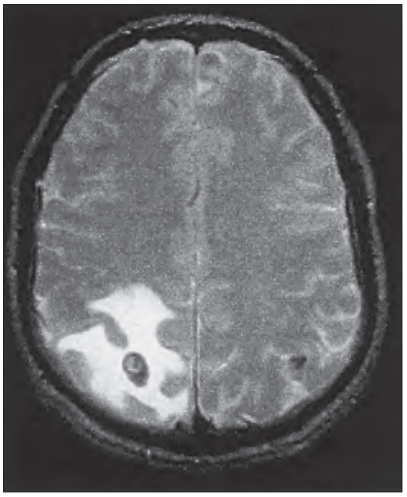


Figure 25.14 Cerebral cysticercosis. Magnetic resonance imaging head scan showing a cyst containing a developing larva. (Courtesy of J. Curé.)

are carried in the circulation, usually to skeletal muscle, but also and more importantly to the brain (Fig. 25.14) or eye, where they develop into cysts known as cysticerci. These may cause no symptoms at first if few in number, or may cause convulsions or, if very heavily infected, cysticercotic encephalopathy. Diagnosis is by detecting specific antibody in serum or CSF using a Western blot (interestingly, there is a higher positivity rate in serum than in CSF) and visualizing cysts, preferably by MRI scan. Treatment is with albendazole plus praziquantel under corticosteroid cover, which is superior to albendazole plus corticosteroids for parenchymal neurocysticercosis.

Sleeping sickness is a trypanosomal infection that is being better controlled

There are two forms of human African vector-borne trypanosomiasis or sleeping sickness, with most infections due to the protozoan *Trypanosoma brucei gambiense*. They are transmitted to people in mostly rural sub-Saharan Africa after they have been bitten by the tsetse fly, the flies having acquired their infection from trypanosome-carrying humans or animals.

A person can be infected and asymptomatic for a long time and only present at an advanced disease stage with CNS signs. Initially, the trypanosomes replicate in the bloodstream, lymphatic and subcutaneous tissue and symptoms include fever, headache and arthralgia. Then they can cross the blood–brain

barrier resulting in a meningoencephalitis, the hallmark of which is sleepiness, confusion and behavioural change.

Fewer infections are due to *Trypanosoma brucei rhodesiense* which runs a shorter, more aggressive clinical course.

Diagnosis is made by microscopy carried out by skilled, experienced staff and treatment is complex

In order to detect trypanosomal parasites in body fluid or tissue by microscopy, for which concentration of samples is often needed. Lymph node aspirates and CSF must be examined too.

Treatment involves knowing which trypanosome is involved and the disease stage. The various drugs include pentamidine, suramin, melarsoprol, eflornithine and nifurtimox.

There were huge epidemics in Africa in the twentieth century but sustained tsetse fly control efforts, supported by case finding in the case of T. b. gambiense, have been effective. In 2009, under 10 000 cases were reported for the first time in 50 years, and in 2015 there were just under 3000 cases.

BRAIN ABSCESSES

Brain abscesses are usually associated with predisposing factors

Since the development of antibiotics, brain abscesses have become rare and usually follow surgery or trauma, chronic osteomyelitis of neighbouring bone, septic embolism or chronic cerebral anoxia. They are also seen in children with congenital cyanotic heart disease in whom the lungs fail to filter off circulating bacteria. Acute abscesses are caused by various bacteria, generally of oropharyngeal origin, including anaerobes. There is usually a mixed bacterial flora. Chronic abscesses may be due to *Mycobacterium tuberculosis* (referred to as tuberculomas; Fig. 25.15) or *C. neoformans*. In immunosuppressed patients, opportunistic infection may occur with fungi and protozoan aetiological agents.

Brain abscesses are diagnosed clinically and by CT and MRI brain scans. If an abscess is suspected, lumbar puncture is contraindicated but, if performed, generally shows raised CSF cells and proteins (see Table 25.1). Treatment is by surgical drainage if the abscess is well encapsulated, and antibiotics should be given for at least 1 month. Other infections that may manifest as chronic meningitis or brain abscess are summarized in Table 25.7.

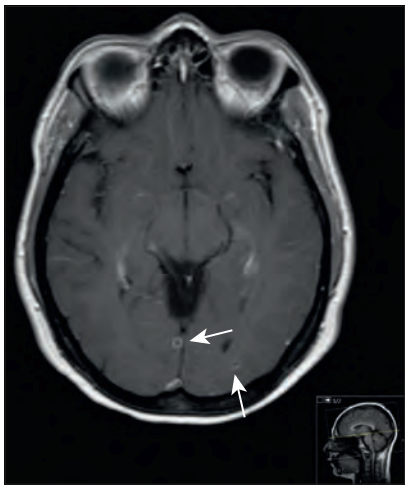


Figure 25.15 Magnetic resonance imaging head scan with arrows pointing at two tuberculomas. (Courtesy of Dr G. Bain, London North West Healthcare NHS Trust.)

TETANUS AND BOTULISM

Several bacteria release toxins that act on the nervous system, but do not themselves invade the CNS. In the case of *Clostridium tetani* and *Clostridium botulinum*, the major clinical impact is neurological.

Tetanus

Cl. tetani toxin is carried to the CNS in peripheral nerve axons

Tetanus spores are widespread in soil and originate from the faeces of domestic animals. The spores enter a wound, and if necrotic tissue or the presence of a foreign body permits local and anaerobic growth of bacteria, the toxin tetanospasmin (see Ch. 18) is produced. All strains of *Cl. tetani* produce the same toxin. The wound can be anything from a small gardener's scratch or cut to that seen in a large automobile or battlefield injury. However, in as many as 20% of cases, there is no history of injury. Infection of the umbilical stump can cause neonatal tetanus, which killed an estimated 34 000 newborns in 2015 worldwide (compared with the WHO estimate of around 790 000 in 1989), especially in resource-poor countries.

The toxin is carried in peripheral nerve axons and probably in the blood to the CNS, where it binds to neurones and blocks the release of inhibitory mediators in spinal synapses, causing overactivity of motor neurones. It can also pass up sympathetic nerve axons and lead to overactivity of the sympathetic nervous system.

Clinical features of tetanus include muscle rigidity and spasms

After a period of 3–21 days, but sometimes longer, there are exaggerated reflexes, muscle rigidity and uncontrolled

Table 25.7 Infections causing chronic meningitis or brain abscess

Bacterial	
Tuberculosis	*Mycobacterium tuberculosis*
Syphilis	*Treponema pallidum*
Brucellosis	*Brucella abortus*
Lyme disease	*Borrelia burgdorferi*
Nocardiosis[a]	*Nocardia asteroides*
Actinomycosis[a]	*Actinomyces fumigatus*
Fungal	
Cryptococcosis	*Cryptococcus neoformans*
Coccidioidomycosis	*Coccidioides immitis*
Histoplasmosis	*Histoplasma capsulatum*
Candidiasis	*Candida albicans*
Blastomycosis[a]	*Blastomyces dermatitidis*
Parasitic	
Toxoplasmosis[a]	*Toxoplasma gondii*
Cysticercosis	*Taenia solium*

[a]Disease manifests as brain abscess.

muscle spasms. Lockjaw (trismus) is due to contraction of jaw muscles. Dysphagia, *risus sardonicus* (a sneering appearance), neck stiffness and opisthotonos (especially in neonatal tetanus) are also seen. Muscle spasms may lead to injury and eventually there is respiratory failure. Tachycardia and sweating can result from effects on the sympathetic nervous system. Mortality is up to 50%, depending on the severity and quality of treatment.

The diagnosis is clinical. Organisms are rarely isolated from the wound, and only a small number of bacteria are needed to form enough toxin to cause disease.

Human antitetanus immunoglobulin should be given as soon as tetanus is suspected clinically

The wound should be excised if necessary and penicillin given to inhibit bacterial replication. Muscle relaxants are used and, if necessary, respiratory support in an intensive care unit.

Immunization with toxoid prevents tetanus, the effects of the vaccine lasting for 10 years after the last dose. Thus, tetanus represents a vaccine-preventable disease that is unique in not being communicable but, instead, acquired from the environment as a result of exposure to *Cl. tetani* spores. Wounds should be cleaned, necrotic tissue and foreign bodies removed, and a tetanus toxoid booster given. Those with badly contaminated wounds should also be given tetanus immunoglobulin and penicillin.

In resource-poor countries, routine immunization of women with tetanus toxoid and improved hygienic birth practices are having a significant impact in reducing the rates of neonatal tetanus.

Botulism

Spores of *Cl. botulinum* are widespread in soil and contaminate vegetables, meat and fish. When foods are canned or preserved without adequate sterilization (often at home), contaminating spores survive and can germinate in the anaerobic environment, leading to the formation of toxin.

Cl. botulinum toxin blocks acetylcholine release from peripheral nerves

Preformed botulinus toxin is ingested, then absorbed from the gut into the blood. It acts on peripheral nerve synapses by blocking the release of acetylcholine. It is therefore a type of food poisoning that affects the motor and autonomic nervous systems. Sometimes spores contaminate a wound and the toxin is then absorbed from this site. If the organism is ingested by infants, in the honey smeared on pacifiers, for instance, it can multiply in the gut and produce the toxin, causing infant botulism.

Clinical features of botulism include weakness and paralysis

After an incubation period of 2–72 h, there is descending weakness and paralysis, with dysphagia, diplopia, vomiting, vertigo and respiratory muscle failure. There is no abdominal pain, diarrhoea or fever. Infants develop generalized weakness ('floppy babies'), but usually recover.

Botulism is treated with antibodies and respiratory support

A diagnosis of botulism is mainly clinical. The toxin can be demonstrated in contaminated food and occasionally in the patient's serum.

Since the specific *Cl. botulinum* strain(s) responsible are normally unknown, trivalent antitoxin (for type A, B and E toxins) must be given promptly together with respiratory support. The mortality is <20%, depending upon the success of the respiratory support.

Prevention is by avoiding imperfectly sterilized canned or preserved food. Contaminated cans are often swollen due to the release of gas by clostridial enzymes. Home-preserved foods are often incriminated, but fruit, with its acidic pH, usually prevents the development of the spores. The toxin is heat labile and is destroyed by adequate cooking, for example, boiling for 10 min. The spores can, however, survive boiling for 3–5 h.

KEY FACTS

- Microbial invasion of the CNS is uncommon, owing to the presence of the blood–brain and blood–CSF barriers, which limit the spread of infection.
- Once infectious agents have traversed these barriers, they generally cause neurological disease by involving the meninges (meningitis) or the brain substance (encephalitis).
- Viral aetiology of meningitis is most common, followed by bacterial meningitis, with cerebral abscesses and viral encephalitis as rarities. The spinal cord (in myelitis) or peripheral nerves (in neuritis) are occasionally affected.
- Disease results from interference with the function of infected nerve cells (e.g. rabies), from direct damage to infected nerve cells (e.g. poliomyelitis), or from the inflammatory sequel to CNS invasion (e.g. bacterial meningitis, viral encephalitis).
- Herpes simplex encephalitis is a critical diagnosis to consider as aciclovir therapy must be given as soon as possible.
- Autoimmune causes of encephalitis may present with a fever, seemingly infectious in origin, but there is a good response to steroids and so a diagnosis must be made quickly.
- Because the anatomically defined compartments of the nervous system are adjacent or interconnected, more than one of them can be involved in a given infectious disease.
- CNS disease is sometimes seen in the helminth infections toxocariasis, hydatid disease and cysticercosis.
- CNS disease can also result when bacterial neurotoxins reach the CNS either from extraneural sites of growth (tetanus) or from contaminated food (botulism).

26 Infections of the eye

Introduction

Because the outer surface of the eye is exposed to the external world, it is easily accessible to infective organisms. The conjunctiva is particularly susceptible. Not only is it a vulnerable epithelial surface, it is covered by the eyelids, which create a warm, moist, enclosed environment in which contaminating organisms can quickly establish and set up a focus of infection. The eyelids and tears protect the external surfaces of the eye, both mechanically and biologically; any interference with their function increases the chance of a pathogen becoming established.

Eyelid infections are generally due to *Staphylococcus aureus*, *Streptococcus pneumoniae* or *Haemophilus influenza*, with involvement of the lid margins causing blepharitis, and eyelid glands or follicles causing styes or hordeolums.

The conjunctiva can be invaded by other routes, such as the blood or nervous system. The deeper tissues of the eye can also be invaded from within, particularly by protozoan and worm parasites. Differentiating between the different causes of conjunctivitis on the basis of clinical signs and symptoms can be difficult.

CONJUNCTIVITIS

A wide variety of viruses and bacteria can cause conjunctivitis or pinkeye (Table 26.1). Conjunctivitis can start in one eye and then progress to the other. The eye will be red, irritated and there will be a lot of tear fluid. A sticky discharge is likely to be secondary to a bacterial infection. Some infections are common in children and resolve quickly; others are potentially more serious. Keratoconjunctivitis from adenovirus, herpes simplex virus or varicella-zoster virus infection can result in severe damage. An acute haemorrhagic conjunctivitis is highly contagious and outbreaks have been reported around the world. It presents as a pink eye, fast-onset eye pain with tear formation and light sensitivity or photophobia. It can follow infection with enterovirus 70 or coxsackievirus A24.

Chlamydial infections

Different serotypes of *Chlamydia trachomatis* cause inclusion conjunctivitis and trachoma

To establish infection on the conjunctiva, microorganisms must avoid being rinsed and wiped away in tears. The best way of achieving this is to have a specific mechanism of attachment to conjunctival cells. *Chlamydia*, for example, has surface molecules that bind specifically to receptors on host cells. This is one of the reasons that, of all the organisms infecting the conjunctiva (Table 26.1), they are among the most successful. There are eight different serotypes of *C. trachomatis* responsible for inclusion conjunctivitis (D–K) (Fig. 26.1) and another four serotypes responsible for trachoma (A, B, Ba and C), which, globally, is the most important eye infection in the world.

Two million people worldwide are visually impaired because of trachoma

Over 200 million people in 42 countries are affected by trachoma. Of these, about 2 million have some degree of visual impairment and the disease accounts for 1–2% of the world's blindness. Trachoma is endemic in resource-poor countries (Fig. 26.2) where prevalence rates in preschool children can reach 60–90%. Trachoma was known in ancient Egypt 4000 years ago, and tweezers to remove in-turned eyelashes have been found in royal tombs. Transmission of *C. trachomatis* is by contact, for example, by contaminated flies, fingers and towels.

Trachoma itself is the result of chronic repeated infections (Fig. 26.3), which are especially prevalent when there is poor access to water, preventing regular washing of the hands and face. Under these circumstances, chlamydial infection is frequently spread from one conjunctiva to another and this can be referred to as 'ocular promiscuity', comparable with the spread of genital secretions in non-specific urethritis (see Ch. 22). Some chlamydial serotypes can infect the urogenital tract (see Ch. 22) as well as the conjunctiva, and the conjunctiva or lungs of a newborn infant may become infected after passage down an infected birth canal (see Ch. 24) requiring systemic treatment with erythromycin.

Chlamydial infections are treated with antibiotic and prevented by face washing

Nucleic acid amplification tests (NAATs; e.g. PCR) are the most accurate for laboratory diagnosis of chlamydial infections (see Chs. 22 and 32) although trachoma is most often diagnosed in endemic areas based on clinical symptoms as well as microscopic analysis of conjunctival fluid or scrapings.

Table 26.1 Examples of microbial infections of the conjunctiva

Organism	Comments
Adenovirus	Very common, especially types 8 and 19
Measles virus	Infection of conjunctiva via blood
Herpes simplex virus	Virus reactivating in ophthalmic division of trigeminal ganglia causes corneal lesion (dendritic ulcer)
Varicella-zoster virus	May involve conjunctiva
Enterovirus 70, coxsackievirus A24	Acute haemorrhagic conjunctivitis
Zika virus	May cause infections including conjunctivitis and uveitis
Chlamydia trachomatis Types A–C Types D–K	 Cause of trachoma and commonly blindness Cause of inclusion conjunctivitis; infection via fingers, or in newborn via birth canal
Neisseria gonorrhoeae	Infection of newborn via birth canal
Staphylococcus aureus *Streptococcus pneumoniae* *Haemophilus influenza*	Cause eyelid infection (styes) and 'sticky eye' in neonates

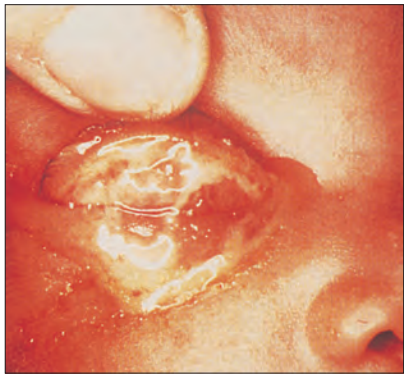


Figure 26.1 Chlamydial conjunctivitis is the most common form of neonatal conjunctivitis. (Courtesy of G. Ridgway.)

Treatment is with topical or oral antibiotics (e.g. azithromycin, doxycycline, etc.). Because infection and reinfection are facilitated by overcrowding, shortage of water and abundant fly populations, the disease can be prevented by improvements in standards of hygiene. In many areas with high rates of endemic trachoma, disease leading to blindness has been sharply reduced or eliminated by socioeconomic development and specific intervention steps (e.g. face washing). This has led the World Health Organization to establish an international alliance for the global elimination of blinding trachoma by the year 2020.

In spite of many decades of research, there are still no vaccines for chlamydial infections. This is partly because immunopathology itself makes a major contribution to the disease, and vaccine-induced immune responses could be harmful.

Other conjunctival infections

In resource-rich countries, conjunctivitis is caused by a variety of bacteria

Several bacteria (especially *H. influenzae, Staph. aureus,* and *Strep. pneumoniae*) can cause conjunctivitis (Fig. 26.4).

Infection by *Neisseria gonorrhoeae* is a hazard of birth through an infected birth canal, and can result in a severe purulent condition. It is seen on the first or second day of life (ophthalmia neonatorum) and requires urgent treatment with ceftriaxone (penicillin resistance is widespread). *Staph. aureus* also produces infections in newborns as well as in adults. The eyes of infants may be invaded by this organism if the organism is transferred from the child's own body or from an infected adult. Conjunctivitis caused by *H. influenzae* has decreased in areas with available vaccine but can continue to be a problem for children with non-typeable (non-encapsulated) strains or in resource-poor areas.

Direct infection of the eye may be associated with wearing contact lenses

Excessive wearing of contact lenses can lead to a reduction in the effectiveness of the eye's defence mechanisms, allowing pathogens to become established, but more likely hazards are the use of contaminated eye drops or cleaning solutions and the insertion of contaminated lenses. A number of bacteria can be transmitted directly in this way. Species of the free-living amoeba *Acanthamoeba* can multiply in some unchanged lens cleaning fluids (although newer products are more effective at killing) and be transferred when the lens is inserted, causing corneal ulceration. Diagnosis is by microscopy and culture of corneal scrapings. NAATs are becoming more readily available.

Conjunctival infection may be transmitted by the blood or nervous system

Several organisms invade the superficial tissues of the eye after transport through the blood or, in the case of herpes simplex virus (HSV), by movement along the trigeminal nerve. Reactivation of this virus can result in the development of a keratitis with the formation of dendritic ulcers (Fig. 26.5). The keratitis can lead to corneal scarring with new blood vessel formation (neovascularization) resulting in loss of sight. Antiviral drugs such as aciclovir and famciclovir, combined with steroid treatment, may be effective. However, if uncontrolled, corneal transplantation may be necessary.

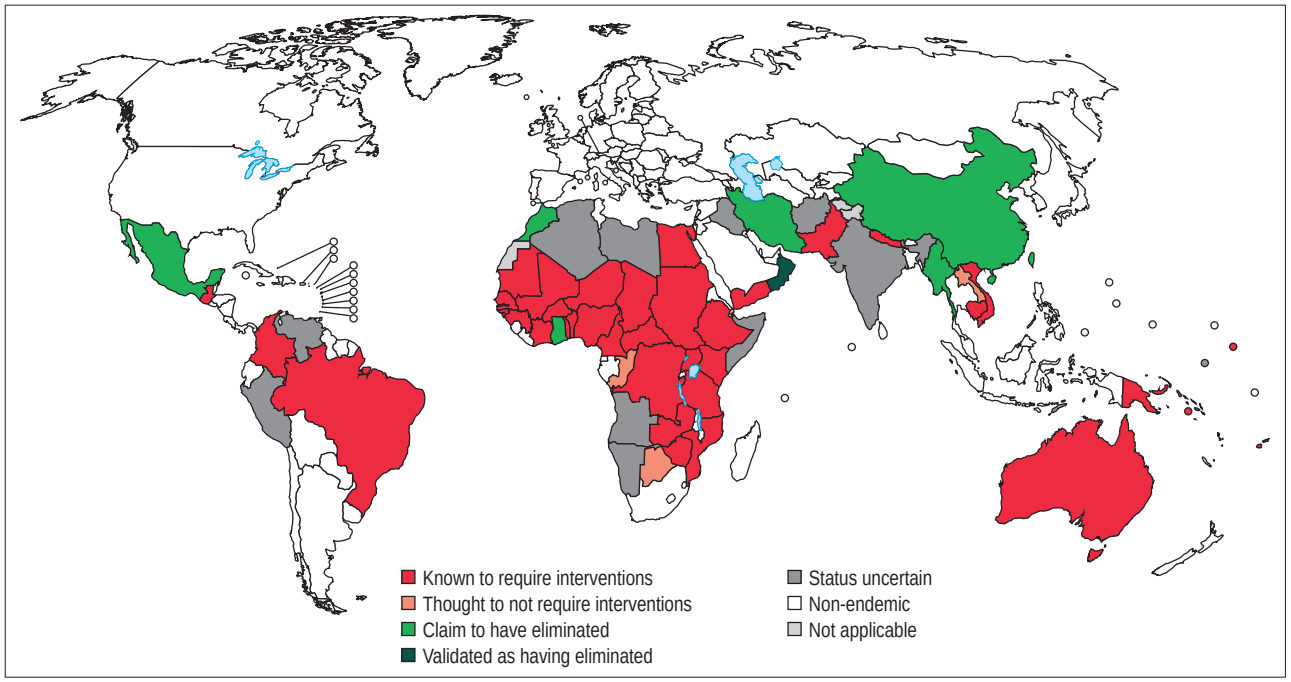


Figure 26.2 Global trachoma incidence, 2016. (Adapted from http://gamapserver.who.int/mapLibrary/Files/Maps/Trachoma_2016.png,)

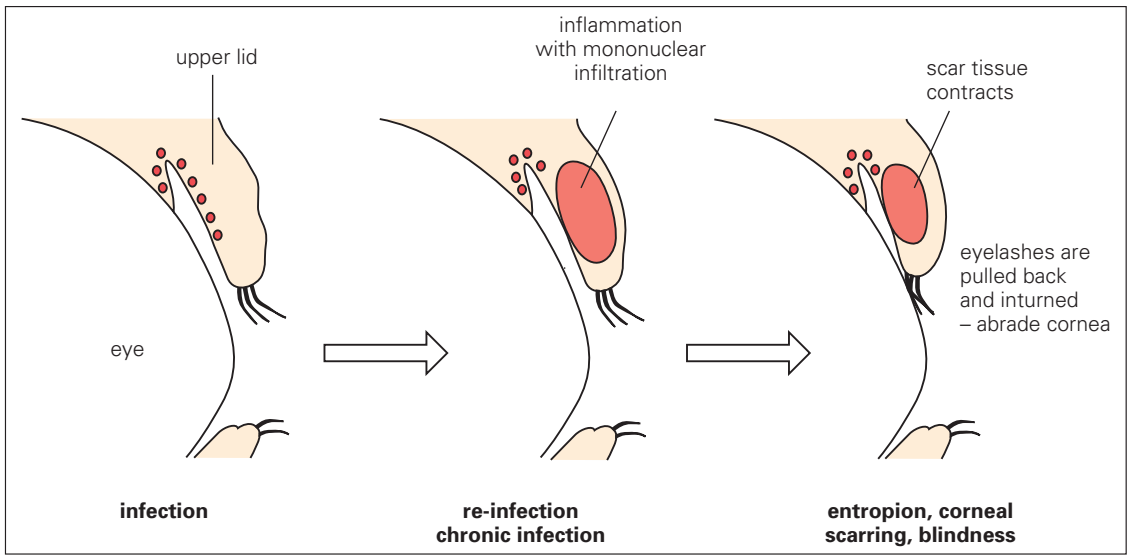


Figure 26.3 Steps in *Chlamydia trachomatis* pathogenesis leading to blindness.

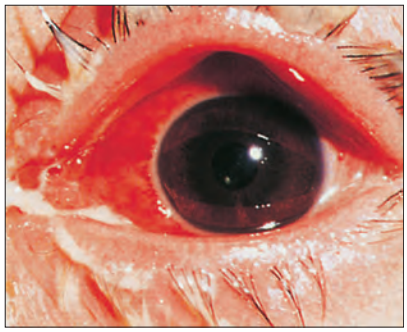


Figure 26.4 Purulent discharge in bacterial conjunctivitis is often associated with infections by *Streptococcus pneumoniae, Haemophilus influenzae* or *Staphylococcus aureus*. (Courtesy of M. Tapert.)

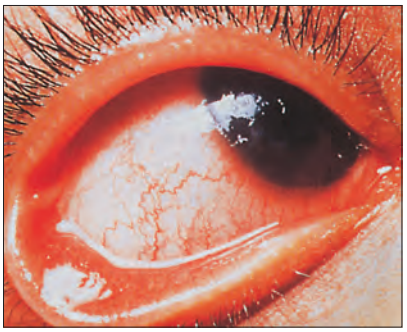


Figure 26.5 Herpes simplex virus (HSV) keratitis. Dendritic ulcers, seen here on the cornea, are common in recurrent HSV infections. (Courtesy of M.J. Wood.)

Varicella-zoster virus may cause conjunctivitis associated with chickenpox or as a secondary infection. Overall, viral conjunctivitis is most commonly caused by adenovirus infections. Many years ago, due to the strong occupational association, shipyard eye was the name given to adenoviral conjunctivitis seen in shipbuilders and other workers exposed to the risk of eye injuries that could then result in an adenovirus infection. These viruses also cause pharyngoconjunctival fever, which includes, as one might expect, pharyngitis, fever and an acute follicular conjunctivitis that clears within a few weeks.

INFECTION OF THE DEEPER LAYERS OF THE EYE

The spectrum of organisms causing disease in the deeper layers of the eye is wider than that associated with the conjunctiva (Table 26.2).

Entry into the deeper layers occurs by many routes

Trauma to the eye may result in the opportunistic establishment of a *Pseudomonas aeruginosa* infection, giving rise to serious inner eye infection. This organism may also be introduced via contaminated eye drops. Congenital syphilis produces a retinopathy with quiescent lesions, and keratitis may appear in later life. Secondary syphilis is also associated with ocular inflammation.

Rubella virus and cytomegalovirus (CMV) may invade the fetal eye in utero, the former causing cataracts and microphthalmia, the latter a severe chorioretinitis. CMV can also cause chorioretinitis in AIDS patients, although highly active antiretroviral therapy has resulted in a reduction of eye disease (see Fig. 22.26E). Ocular complications have been reported in patients with West Nile virus infection.

Toxoplasmosis

Toxoplasma gondii infection can cause retinochoroiditis leading to blindness

Infection with this protozoan is widespread in adults and children (see Ch. 5), and is normally acquired by swallowing oocysts released by infected cats (the definitive host) or by eating meat containing tissue cysts. Women who become infected in pregnancy may transmit the infection to the fetus, as tachyzoites can cross the placenta. Tissue cysts can form in the retina of the fetus and undergo continuous proliferation, producing progressive lesions particularly when levels of immunity are low. These lesions may also involve the choroid (Fig. 26.6) and lead ultimately to blindness. One or both eyes may be affected.

Infection is not serious unless:

- acquired in utero, when the organism invades all tissues, especially the central nervous system (CNS)
- acquired (or reactivated) under immunosuppression.

Damage to the eye occurs in both congenital and postnatally acquired toxoplasmosis and may present at any age. Ocular toxoplasmosis may present years after the initial infection, whether congenital or acquired postnatally, and can be more serious in the elderly population.

Parasitic worm infections

Toxocara canis larvae cause an intense inflammatory response and can lead to retinal detachment

Larval tapeworms (e.g. the hydatid cyst stage of *Echinococcus granulosus* transmitted by eggs passed from infected dogs) occasionally enter the eye, with growth of the cysts causing severe mechanical damage. The larval form (cysticercus) of *Taenia solium* (the pork tapeworm) is acquired when humans ingest eggs of this tapeworm. Cysticerci develop mainly in skeletal muscle, but can invade the nervous system or the eye.

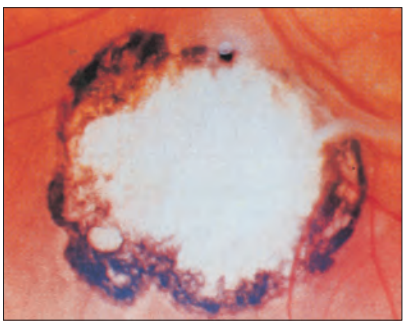


Figure 26.6 Congenital toxoplasmosis. Fundal photograph showing the scar of healed chorioretinitis. (Courtesy of M.J. Wood.)

Table 26.2 Examples of infections of the deep layers of the eye

Organism	Disease	Route of infection
Rubella virus	Cataracts, microphthalmia	Infection in utero
Cytomegalovirus	Chorioretinitis	Infection in utero; may occur in AIDS and other immunocompromised individuals
Pseudomonas aeruginosa	Serious inner eye infection	After trauma; foreign bodies in eye; eye operations; bacteria can contaminate eye drops
Toxoplasma gondii (toxoplasmosis)	Chorioretinitis	Infection in utero
Echinococcus granulosus (hydatid disease)	Distortion of the eye by growth of larval tapeworm in hydatid cyst	Transmission by eggs passed by dogs
Toxocara canis (ocular toxocariasis)	Chorioretinitis, posterior pole granuloma, blindness	Transmission by eggs passed by dogs
Onchocerca volvulus (river blindness)	Sclerosing keratitis, chorioretinitis	Larvae transmitted by blood-feeding *Simulium* flies

Ocular cysticerci are diagnosed by direct vision (e.g. seen as a translucent vesicle in the vitreous), ultrasonography (e.g. as a subretinal subchoroidal cyst), cross-sectional imaging and serology. Treatment of intraocular cysticercosis is by surgical removal. Antiparasitic drugs are not given, to avoid causing an inflammatory reaction. Corticosteroids are given if uveitis is present. Invasion by migratory larvae of the nematode *Toxocara canis* (commonly called dog roundworm) is more common. This parasite occurs naturally in the intestines of dogs, releasing thick-shelled resistant eggs into the environment. The eggs can hatch if swallowed by humans, the larvae initiating, but failing to complete, their customary migration through the tissues. In the canine host, migration results in the worms re-entering the intestine where they mature. In humans, larvae can enter almost any organ, especially the liver and often the CNS or eye (Fig. 26.7), triggering an intense eosinophilic inflammatory response. In the eye, *Toxocara* larvae may lead to posterior uveitis, localized retinal granuloma, traction bands and retinal detachment. The misdiagnosis of retinal granuloma as retinoblastoma has led to enucleation. Serology on vitreous samples is preferable to serum samples in diagnosing ocular toxocariasis. Anthelmintic treatment is not routinely given as it may lead to worsening of inflammation; corticosteroids are used to suppress the inflammatory response. Laser photocoagulation and cryoretinopexy have been used to destroy ocular granulomas.

Onchocerca volvulus infection causes 'river blindness' and is transmitted by *Simulium* flies

Onchocerca volvulus infection is transmitted by biting *Simulium* flies, which take up microfilariae larvae from the skin of infected hosts and reintroduce the larvae after they have further developed to become infective, at a future feed. Adult worms live in subcutaneous nodules, and are comparatively harmless. The microfilariae, released by the females in enormous numbers, induce intense inflammatory reactions in the skin (see Ch. 27). The larvae migrate through the subcutaneous tissue, and invasion of the eye (resulting in 'river blindness') is particularly common in regions of Africa, Yemen, as well as Central America.

The inflammatory responses in the eye cause a number of pathological changes, which may affect both the anterior and posterior chambers (Fig. 26.8). These include:

- punctate and sclerosing keratitis
- iridocyclitis
- chorioretinitis
- optic atrophy.

The disease is called river blindness because the *Simulium* flies develop in fast flowing rivers, and people living near these sites are most affected. In the past, blindness rates have reached 50% of the adult population in endemic areas, but vector control and especially ivermectin treatment are important in reducing the incidence of new infections. Unfortunately, the blindness is irreversible.

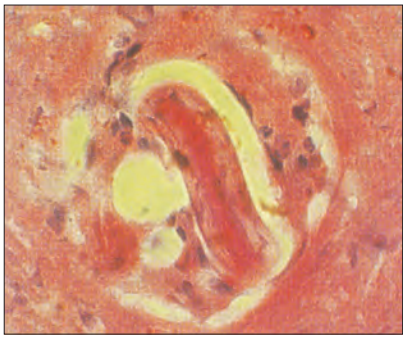


Figure 26.7 *Toxocara*. Granuloma in the posterior pole of an infected eye. The larval nematode is clearly visible in the centre of the granuloma. (Courtesy of D. Spalton.)

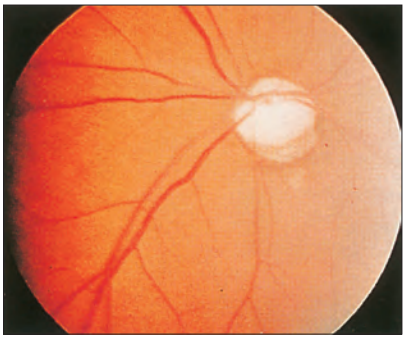


Figure 26.8 Onchocerciasis. Sclerosis of the choroidal vessels caused by invading microfilaria of *Onchocerca volvulus*. (Courtesy of J. Anderson.)

KEY FACTS

- The external surfaces of the eye are vulnerable to infection. They are protected by the eyelids and by factors such as lysozyme in tears.
- It can be difficult to make a diagnosis regarding the aetiology of conjunctivitis on clinical signs and symptoms alone.
- The consequences of eye infection are always potentially serious given that sight is dependent upon the presence of an intact transparent cornea.
- Pathogens infecting the conjunctiva have specific attachment mechanisms.
- Inflammatory responses, though 'designed' to limit invasion and repair damage, can irreversibly damage conjunctival and corneal surfaces.
- Relatively few organisms invade the retina, and those that do are potentially sight threatening.
- Some of the most serious infection-related diseases of the eye involve invasion by protozoan or helminth parasites. The diagnosis then often follows rather than precedes the development of visual impairment.

27 Infections of the skin, soft tissue, muscle and associated systems

Introduction

Healthy intact skin protects underlying tissues and provides excellent defence against invading pathogens

The microbial load of normal skin is kept in check by various factors, as shown in Box 27.1. Alterations in these factors (e.g. prolonged exposure to moisture) upset the ecological balance of the commensal flora, and predispose to infection.

A small number of pathogens cause diseases of muscle, joints or the haemopoietic system. Invasion of these sites is generally from the blood, but the reason for localization to particular tissues is often obscure. Circulating pathogens tend to localize in growing or damaged bones (acute osteomyelitis) and in damaged joints, but we do not know why coxsackieviruses or *Trichinella spiralis* invade muscle. On the other hand, some viruses infect a given target cell, and plasmodia invade erythrocytes because they have specific attachment sites for these cells.

Box 27.1 Factors Controlling the Skin's Microbial Load

- The limited amount of moisture present
- Acid pH of normal skin
- Surface temperature <optimum for many pathogens
- Salty sweat
- Excreted chemicals such as sebum, fatty acids and urea
- Competition between different species of the normal flora.

The number of bacteria on the skin varies from a few hundred/cm^2 on the arid surfaces of the forearm and back, to tens of thousands/cm^2 on the moist areas, such as the axilla and groin. This normal microbiota plays an important role in preventing 'foreign' organisms from colonizing the skin, but it too needs to be kept in check.

Infections of the skin

In addition to being a structural barrier, the skin is colonized by an array of organisms which forms its normal flora. The relatively arid areas of the forearm and back are colonized with fewer organisms, predominantly Gram-positive bacteria and yeasts. In the moister areas, such as the groin and the armpit, the organisms are more numerous and more varied and include Gram-negative bacteria. The normal microbiota of the skin and other body sites plays an important role in defending the surface from 'foreign invaders'.

An appreciation of the structure of the skin helps in understanding the different sorts of infection to which the skin and its underlying tissues are prone (Fig. 27.1). If organisms breach the stratum corneum the host defences are mobilized, the epidermal Langerhans cells elaborate cytokines, neutrophils are attracted to the site of invasion, and complement is activated via the alternative pathway.

Microbial disease of the skin may result from any of three lines of attack

These lines of attack are:

- breach of intact skin, allowing infection from the outside
- skin manifestations of systemic infections, which may arise as a result of blood-borne spread from the infected focus to the skin or by direct extension (e.g. draining sinuses from actinomycotic lesions, or necrotizing anaerobic infection from intra-abdominal sepsis)
- toxin-mediated skin damage due to production of a microbial toxin at another site in the body (e.g. scarlet fever, toxic shock syndrome).

The sequence of events in the pathogenesis of mucocutaneous lesions caused by bacterial, fungal and viral infections is outlined in Fig. 27.2. Breaches in the skin range from microscopic to major trauma, which may be accidental (e.g. lacerations or burns) or intentional (e.g. surgery). Hospitalized patients are liable to other skin breaches (e.g. pressure sores and intravenous catheter insertions), which may become infected. Infections in compromised individuals such as patients with burns are discussed in Chapter 31. Here, we will consider primary infections of the skin and underlying soft tissues, together with mucocutaneous lesions resulting from certain systemic viral infections. Examples of systemic bacterial and fungal infections that cause mucocutaneous lesions are summarized in Table 27.1.

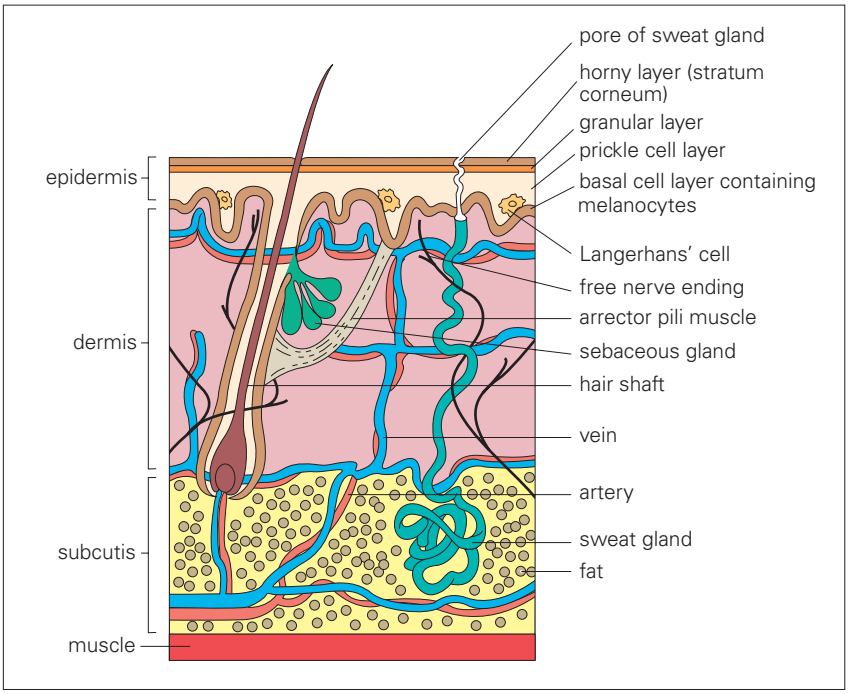


Figure 27.1 Infection of the skin and soft tissue can be related to the anatomy of the skin. Pathogens usually enter the lower layers of the epidermis and dermis only after the skin surface has been damaged.

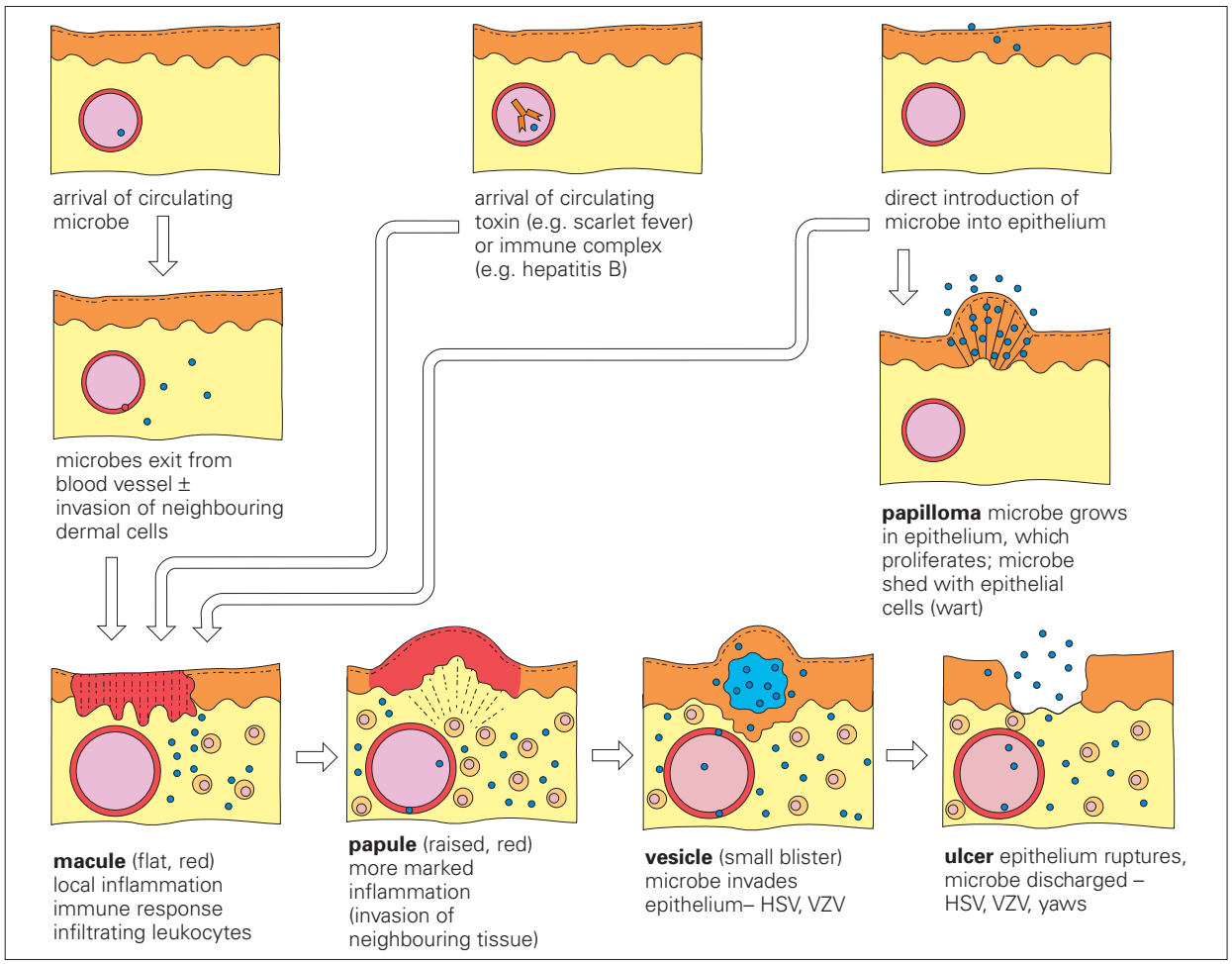


Figure 27.2 The pathogenesis of mucocutaneous lesions. In different infections, the starting point (arrival of pathogen or toxin or immune complex) and the final picture (e.g. maculopapular rash, vesicle) will be different. HSV, herpes simplex virus; VZV, varicella-zoster virus.

Table 27.1 Skin manifestations of systemic infections caused by bacteria and fungi

Organism	Disease	Skin manifestation
Salmonella typhi, Salmonella schottmuelleri	Enteric fever	'Rose spots' containing bacteria
Neisseria meningitidis	Septicaemia, meningitis	Petechial or maculopapular lesions containing bacteria
Pseudomonas aeruginosa	Septicaemia	Ecthyma gangrenosum, skin lesion pathognomonic if infected by this organism
Treponema pallidum *Treponema pertenue*	Syphilis Yaws	Disseminated infectious rash seen in secondary stage of disease after infection
Rickettsia prowazekii *Rickettsia typhi* *Rickettsia rickettsii*	Typhus Spotted fever	Macular or haemorrhagic rash
Streptococcus pyogenes	Scarlet fever	Erythematous rash caused by erythrogenic toxin
Staphylococcus aureus	Toxic shock syndrome	Rash and desquamation due to toxin
Blastomyces dermatitidis	Blastomycosis	Papule or pustule develops into granuloma lesions containing organisms
Cryptococcus neoformans	Cryptococcosis	Papule or pustule, usually on face or neck

Skin lesions are often associated with systemic infection with particular bacteria and fungi. The lesions may provide useful diagnostic aids. Sometimes they are a site from which organisms are shed.

Table 27.2 Direct entry into skin of bacteria and fungi

Structure involved	Infection	Common cause
Keratinized epithelium	Ringworm	Dermatophyte fungi (*Trichophyton, Epidermophyton* and *Microsporum*)
Epidermis	Impetigo	*Streptococcus pyogenes* and / or *Staphylococcus aureus*
Dermis	Erysipelas	*Strep. pyogenes*
Hair follicles	Folliculitis Boils (furuncles) Carbuncles	*Staph. aureus*
Subcutaneous fat	Cellulitis	*Strep. pyogenes*
Fascia	Necrotizing fasciitis	Anaerobes and microaerophiles, usually mixed infections
Muscle	Myonecrosis gangrene	*Clostridium perfringens* (and other clostridia)

Direct introduction of bacteria or fungi into the skin is the most common route of skin infection. Infections range from mild, often chronic, conditions such as ringworm to acute and life-threatening fasciitis and gangrene. Relatively few species are involved in the common infections.

BACTERIAL INFECTIONS OF SKIN, SOFT TISSUE AND MUSCLE

These can be classified on an anatomic basis

The classification depends upon the layers of skin and soft tissue involved, although some infections may involve several components of the soft tissues:

- *Abscess formation*. Boils and carbuncles are the result of infection and inflammation of the hair follicles in the skin (folliculitis).
- *Spreading infections*. Impetigo is limited to the epidermis and presents as a bullous, crusted or pustular eruption of the skin. Erysipelas involves the blocking of dermal lymphatics and presents as a well-defined, spreading erythematous inflammation, generally on the face, legs or feet, and often accompanied by pain and fever. If the focus of infection is in the subcutaneous fat, cellulitis, a diffuse form of acute inflammation is the usual presentation.
- *Necrotizing infections*. Fasciitis describes the inflammatory response to infection of the soft tissue below the dermis. Infection spreads, often with alarming rapidity, along the fascial planes causing disruption of the blood supply. Gangrene or myonecrosis may follow infection associated with ischaemia of the muscle layer. Gas resulting from the fermentative metabolism of anaerobic organisms may be palpable in the tissues (gas gangrene).

The common causative organisms are shown in Table 27.2. Note that the same pathogen (e.g. *Streptococcus pyogenes*) can cause different infections in different layers of the skin and soft tissue.

Staphylococcal skin infections

Staphylococcus aureus is the most common cause of skin infections and provokes an intense inflammatory response

Staph. aureus causes minor skin infections such as boils or abscesses as well as more serious postoperative wound infection. Infection may be acquired by 'self-inoculation' from a carrier site (e.g. the nose) or acquired by contact with an exogenous source, usually another person. People who are

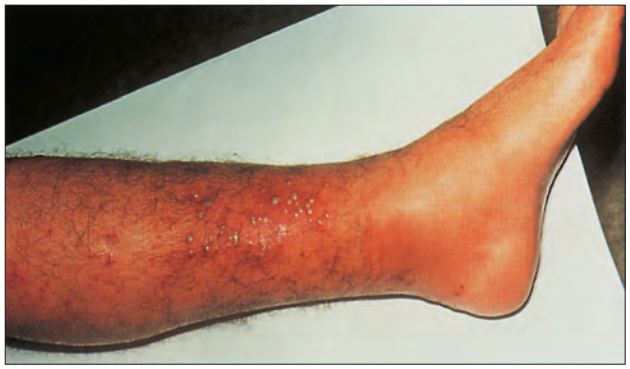


Figure 27.3 Folliculitis. A superficial infection is shown here localized in the hair follicles on the leg. The boils contain creamy-yellow pus and masses of bacteria. *Staphylococcus aureus* is the most common cause. (Courtesy of A. du Vivier.)

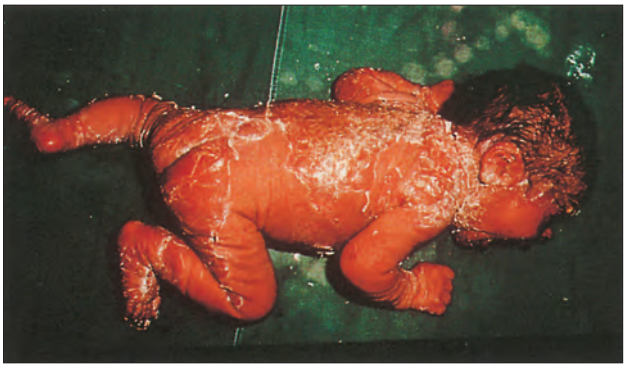


Figure 27.4 Scalded skin syndrome results from infection of the skin with strains of *Staphylococcus aureus* producing a specific toxin, which destroys the intercellular connections in the skin, resulting in large areas of desquamation. The appearance may be confused with a burn. (Courtesy of A. du Vivier.)

nasal carriers of virulent *Staph. aureus* may suffer from recurrent boils, but an inoculum of about 100 000 organisms is thought to be required in the absence of a wound or foreign body. *Staph. aureus* can also cause serious skin disease due to toxin production (scalded skin syndrome, toxic shock syndrome; see below). In addition, skin and soft tissue infections caused by community-associated, methicillin-resistant *Staph. aureus* strains (CA-MRSA) are of increasing incidence and concern (see Ch. 37).

A boil begins within 2–4 days of inoculation, as a superficial infection in and around a hair follicle (folliculitis; Fig. 27.3). In this site, the organisms are relatively protected from the host defences, multiply rapidly and spread locally. This provokes an intense inflammatory response with an influx of neutrophils. Fibrin is deposited, and the site is walled off. Abscesses typically contain abundant yellow creamy pus formed by the massive number of organisms and necrotic white cells. They continue to expand slowly, eventually erode the overlying skin, 'come to a head' and drain. Drainage inwards can result in seeding of the staphylococci to underlying body sites to cause serious infections such as peritonitis, empyema or meningitis.

Staph. aureus infections are often diagnosed clinically and treatment includes drainage and antibiotics

Staph. aureus is the most common cause of boils, and diagnosis is made on clinical grounds. Isolation and further characterization of the infecting staphylococcus in hospital patients and staff are important in the investigation of hospital infections (see Ch. 37).

Treatment involves drainage and this is usually sufficient for minor lesions, but antibiotics may be given in addition when the infection is severe and the patient has a fever. Most *Staph. aureus* are beta-lactamase producers, but methicillin-susceptible *Staph. aureus* (MSSA) can be treated with enzyme-stable penicillins such as nafcillin. Isolates resistant to these compounds (i.e. methicillin-resistant *Staph. aureus* [MRSA]; see Ch. 34) may be treated with vancomycin, linezolid, quinopristin-dalfoprisin, or daptomycin. Treatment with these agents does not necessarily eradicate carriage of the staphylococci.

Recurrent infections may be treated in nasal carriers of *Staph. aureus* with nasal creams containing antibiotics. For example, mupirocin has been used successfully for carriers of methicillin-resistant staphylococci (see Ch. 37). Good skin care and personal hygiene should be encouraged.

Staphylococcal scalded skin syndrome is caused by toxin-producing *Staph. aureus*

This condition, also known as 'Ritter's disease' in infants and 'Lyell's disease' or 'toxic epidermal necrolysis' in older children, occurs sporadically and in outbreaks. It is caused by strains of *Staph. aureus* producing a toxin known as 'exfoliatin' or 'scalded skin syndrome toxin'. The initial skin lesion may be minor, but the toxin causes destruction of the intercellular connections and separation of the top layer of the epidermis. Large blisters are formed, containing clear fluid, and within 1 or 2 days, the overlying areas of skin are lost (Fig. 27.4), leaving normal skin underneath. The baby is irritable and uncomfortable, but rarely severely ill. However, treatment should take into account the risk of increased loss of fluid from the damaged surface, and fluid replacement may be needed. As mentioned above, antimicrobial chemotherapy would employ beta-lactamase stable penicillins (e.g. nafcillin) against MSSA, whereas vancomycin, linezolid, quinopristin-dalfoprisin, or daptomycin would be used for MRSA.

Toxic shock syndrome is caused by toxic shock syndrome toxin-producing *Staph. aureus*

This systemic infection came to prominence through its association with tampon use by healthy women, but it is not confined to women and can occur as a result of *Staph. aureus* infection at non-genital sites (e.g. a wound). Toxic shock syndrome (TSS) involves multiple organ systems and is characterized by fever, hypotension and a diffuse macular erythematous rash followed by desquamation of the skin, particularly on the soles and palms (Fig. 27.5). TSS is caused by exotoxins of *Staph. aureus*, most commonly TSST1, which behaves as a superantigen (stimulating T-cell proliferation and cytokine release; see Ch. 17). While the prevalence of TSS in the USA is low (estimated at <200 cases / year) >90% of adults carry antibodies to TSST1. Treatment of TSS includes steps to open the infected site (e.g. drainage), fluid replacement and antistaphylococcal chemotherapy.

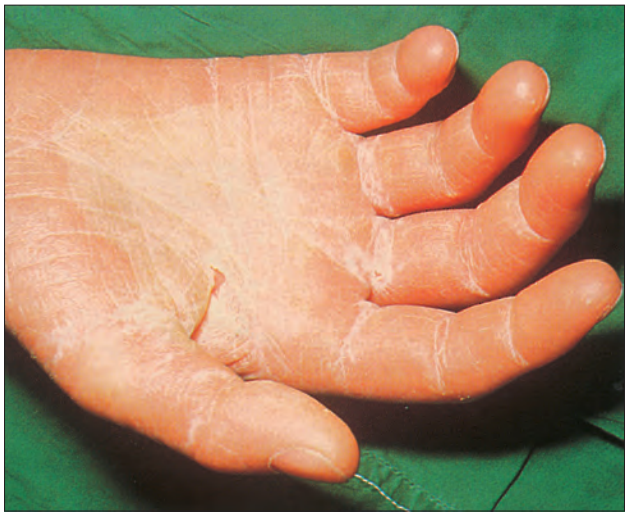


Figure 27.5 Toxic shock syndrome results from systemic infection with *Staphylococcus aureus*, but has skin manifestations in the form of desquamation, particularly of the palm and soles. (Courtesy of M.J. Wood.)

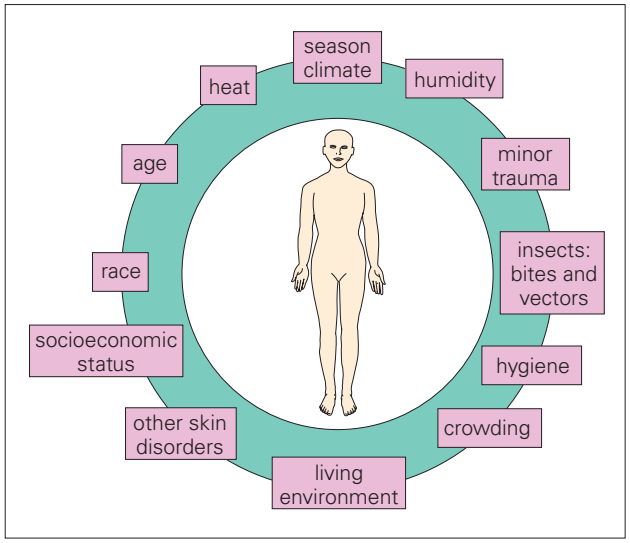


Figure 27.6 Various factors are involved in the development of streptococcal skin infections. Particular M types of *Streptococcus pyogenes* have a predilection for skin, but various factors predispose the host (usually a child) to infection. Mixed infections with *Staphylococcus aureus* are also common.

Streptococcal skin infections

Streptococcal skin infections are caused by *Strep. pyogenes* (group A streptococci)

Streptococcal impetigo develops independently of streptococcal upper respiratory tract infection, and although up to 35% of patients carry the same strain in their nose or throat, colonization may well occur after the skin has become infected. The organisms are acquired through contact with other people with infected skin lesions and may first colonize and multiply on normal skin before invasion through minor breaks in the epithelium and the development of lesions. The various risk factors involved in the development of streptococcal impetigo are shown in Fig. 27.6. *Strep. pyogenes*

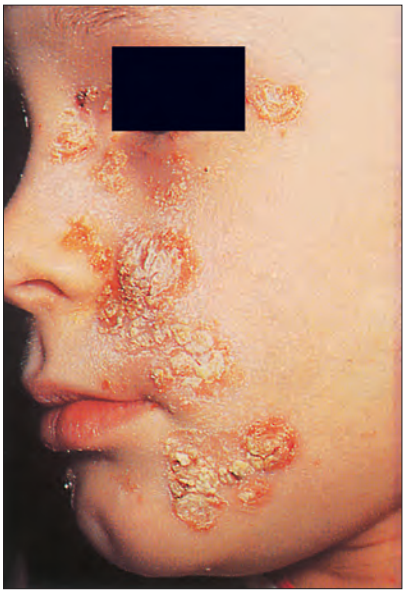


Figure 27.7 Impetigo is a condition limited to the epidermis, with typically yellow, crusted lesions. It is commonly caused by *Streptococcus pyogenes* either alone or together with *Staphylococcus aureus*. (Courtesy of M.J. Wood.)

may also cause erysipelas, an acute deeper infection in the dermis. About 5% of patients with erysipelas go on to develop bacteraemia which carries a high mortality if untreated. As discussed previously, impetigo may also be caused by *Staph. aureus* and occasionally presents in more extreme bullous form (i.e. bullous impetigo) as blisters resembling localized scalded skin syndrome (see above).

Strep. pyogenes possesses certain surface proteins (M and T) which are antigenic. The species can be subdivided (typed) on the basis of these antigens, and it has been recognized that certain M and T types are associated with skin infection (and these differ from the types associated with sore throats). T proteins play no known role in virulence, and their function is unknown. M proteins are important virulence factors because they inhibit opsonization and confer on the bacterium resistance to phagocytosis. A variety of additional factors contribute to the virulence of the organism, such as lipoteichoic acid (LTA; a component of the Gram-positive cell wall) and F protein, which facilitate binding to epithelial cells.

Clinical features of streptococcal skin infections are typically acute

They develop within 24–48 h of skin invasion and trigger a marked inflammatory response as the host attempts to localize the infection (Figs 27.7 and 27.8). *Strep. pyogenes* elaborates a number of toxic products and enzymes, such as hyaluronidase, which help the organism to spread in tissue. Lymphatic involvement is common, resulting in lymphadenitis and lymphangitis.

Lysogenic strains of *Strep. pyogenes* produce pyrogenic exotoxins (SPE; formally called erythrogenic toxins). As with TSST1 in *Staph. aureus* (discussed previously), these toxins are superantigens with a potent influence on the immune system. The toxins (e.g. SPEA, B, and C) also act on skin blood vessels to cause the diffuse erythematous rash of scarlet fever, which

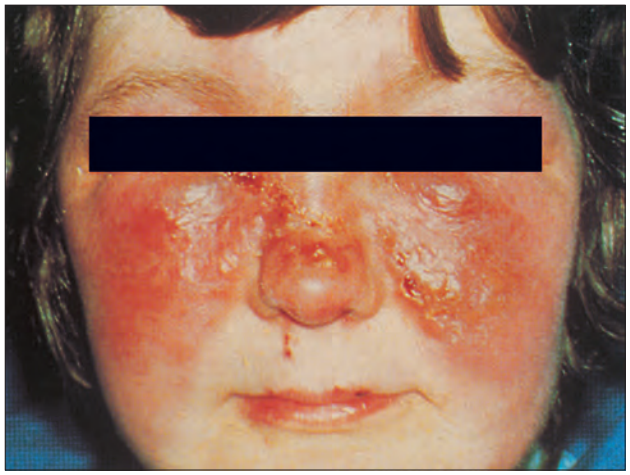


Figure 27.8 Erysipelas. Infection with *Streptococcus pyogenes* involves the dermal lymphatics and gives rise to a clearly demarcated area of erythema and induration. When the face is involved, there is often a typical 'butterfly-wing' rash, as shown here. (Courtesy of M.J. Wood.)

may occur with streptococcal pharyngitis. *Strep. pyogenes* may also cause a form of toxic shock syndrome which has been especially associated with the production of the SPEA.

M protein is a major virulence factor in *Strep. pyogenes* with over 100 types, some of which (e.g. M49) are specifically associated with diseases such as acute glomerulonephritis

Acute glomerulonephritis (AGN) occurs more often after skin infections than after infections of the throat (see Ch. 19). It is characterized by the deposition of immune complexes on the basement membrane of the glomerulus but the precise role of the streptococcus in the causation is still unclear (see Ch. 18); 10–15% of individuals infected with a nephritogenic strain will develop AGN about 2–3 weeks after the primary infection. Most people recover completely, and recurrence after a subsequent streptococcal infection is rare. Rheumatic fever (see Ch. 19) very rarely follows skin infections with *Strep. pyogenes*.

Streptococcal skin infections are usually diagnosed clinically and treated with penicillin

Gram stains of pus from vesicles in impetigo show Gram-positive cocci, and culture reveals *Strep. pyogenes* sometimes mixed with *Staph. aureus* (Fig. 27.9). In erysipelas, skin cultures are often negative, although culture of fluid from the advancing edge of the lesion may be successful.

Depending on the cause of infection and antibiotic susceptibility, dicloxacillin is a commonly used drug, although erythromycin, newer macrolides, or an oral cephalosporin may be used for penicillin-allergic patients. However, the prevalence of resistance (e.g. to erythromycin) in streptococci is increasing, and these drugs are not effective in mixed infections with *Staph. aureus*. Severe infections may require hospitalization.

Impetigo is prevented by improving the host factors associated with acquisition of the disease, as illustrated in Fig. 27.7. Since AGN rarely recurs on subsequent streptococcal infection, long-term prophylaxis with penicillin is not indicated (in contrast to the long-term prophylaxis following rheumatic fever; see Ch. 19).

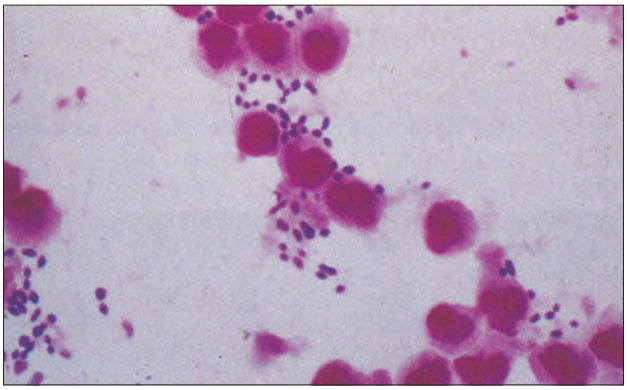


Figure 27.9 Gram-positive cocci in pus.

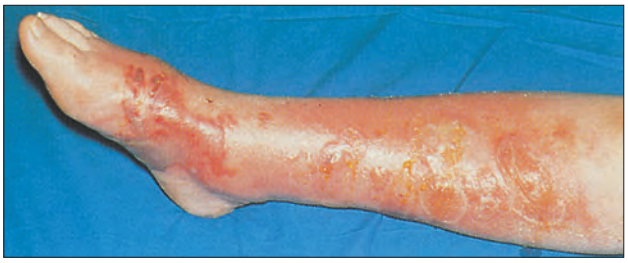


Figure 27.10 When the focus of infection is in the subdermal fat, cellulitis – a severe and rapidly progressive infection – is the typical presentation. Large blisters and scabs may also be present on the skin surface. (Courtesy of M.J. Wood.)

Cellulitis and gangrene

Cellulitis is an acute spreading infection of the skin that involves subcutaneous tissues

Cellulitis extends deeper than erysipelas and usually originates either from superficial skin lesions such as boils or ulcers or following trauma. It is rarely blood-borne, but conversely it may lead to bacterial invasion of the bloodstream. Infection develops within a few hours or days of trauma and quickly produces a hot red swollen lesion (Fig. 27.10). Regional lymph nodes are enlarged and the patient suffers malaise, chills and fever.

The great majority of cases of cellulitis are caused by *Strep. pyogenes* and *Staph. aureus*. Occasionally, in patients who have had particular environmental exposure, other organisms may be implicated. For example, *Erysipelothrix rhusiopathiae* is associated with cellulitis in butchers and fishmongers, while *Vibrio vulnificus* and *Vibrio alginolyticus* may complicate traumatic wounds acquired in saltwater environments.

The pathogen causing cellulitis is isolated in only 25–35% of cases, and initial therapy should cover streptococci and staphylococci. Attempts can be made to confirm the clinical diagnosis by culture of:

- aspirates from the advancing edge of the cellulitis
- the site of trauma (if present)
- skin biopsies
- blood.

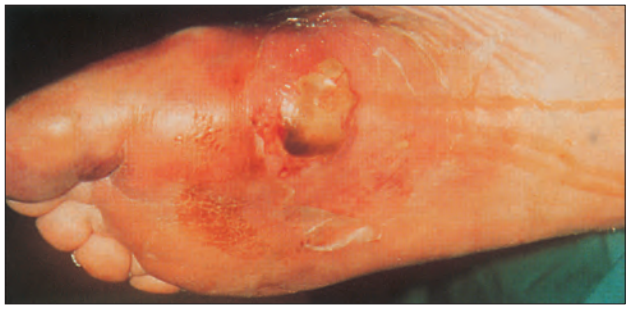


Figure 27.11 Severe progressive cellulitis of the foot. Such cellulitis is usually caused by anaerobic bacteria or a mixture of aerobes and anaerobes and is a particular problem in diabetic patients with peripheral vascular and neuropathic damage. (Courtesy of J.D. Ward.)

Treatment should be initiated on the basis of the clinical diagnosis because of the potential for rapid progression of the disease.

Anaerobic cellulitis may develop in areas of traumatized or devitalized tissue

Such damaged tissue is associated with surgical or traumatic wounds or is found in ischaemic extremities. Diabetic patients are particularly prone to anaerobic cellulitis of their feet (Fig. 27.11). The causative organisms depend upon the circumstances of the trauma: infections in the lower parts of the body are most often caused by organisms from the faecal flora whereas wounds from human bites are infected with oral organisms. Foul-smelling discharge, marked swelling and gas in the tissues are characteristic of anaerobic cellulitis, and a mixture of organisms is usually cultured from the wound. Treatment needs to be aggressive to halt the spread of infection, and both antibiotics and surgical debridement are required. Osteomyelitis (see below) is a common sequela.

Synergistic bacterial gangrene is a relentlessly destructive infection

This rare infection is caused by a mixture of organisms, typically *microaerophilic streptococci* and *Staph. aureus*. The gangrene most commonly follows surgery in the groin or genital area, starting at the site of a drain or suture. Cellulitis develops in the surrounding skin and extends rapidly (within hours), leaving a black necrotic centre. The condition is often fatal, and treatment requires radical excision of the necrotic area and systemic antibiotic therapy.

Necrotizing fasciitis, myonecrosis and gangrene

Necrotizing fasciitis is a frequently fatal mixed infection caused by anaerobes and facultative anaerobes

Although apparently resembling synergistic bacterial gangrene, necrotizing fasciitis is a much more acute and highly toxic infection, causing widespread necrosis and undermining of the surrounding tissues, such that the underlying destruction is more widespread than the skin lesion (Fig. 27.12). Necrotizing fasciitis has been most prominently linked by the popular media with *Strep. pyogenes*, where it has been frequently termed 'flesh-eating bacteria'. However, the infection may be caused by a variety of other organisms, especially including MRSA. Patients with necrotizing fasciitis deteriorate rapidly and frequently die. Radical excision of all necrotic fascia is an essential part of therapy, along with antibiotics given both locally to the wound and systemically.

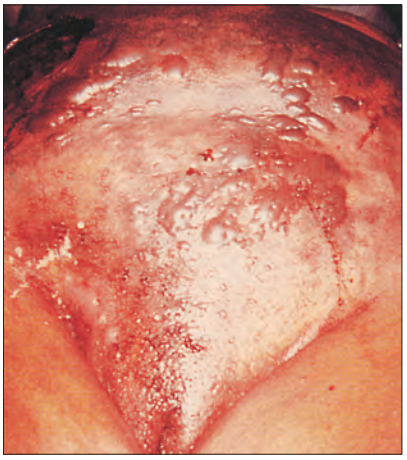


Figure 27.12 Necrotizing fasciitis of the abdominal wall. In patients such as this, infection can be seen rapidly spreading from its origin and causing deep and widespread necrosis. Complete debridement and intensive antimicrobial therapy is required, but the condition is often fatal. (Courtesy of W.M. Rambo.)

Traumatic or surgical wounds can become infected with *Clostridium* species

Clostridium tetani gains access to the tissues through trauma to the skin, but the disease it produces is entirely due to the production of a powerful exotoxin (see Ch. 18).

Gas gangrene or *clostridial myonecrosis* can be caused by several species of *clostridia*, but *Clostridium perfringens* is the most common. The organism and its spores are found in the soil and in human and animal faeces, and can therefore gain access to traumatized tissues by contamination from these sources. Infection develops in areas of the body with poor blood supply (anaerobic), and the buttocks and perineum are common sites, particularly in patients with ischaemic vascular disease or peripheral arteriosclerosis. The organisms multiply in the subcutaneous tissues, producing gas and an anaerobic cellulitis, but a characteristic feature of clostridial infection is that the organisms invade deeper into the muscle, where they cause necrosis and produce bubbles of gas, which can be felt in the tissue and sometimes seen in the wound (Fig. 27.13). The infection proceeds very rapidly and causes acute pain. Much of the damage is due to the production by *Cl. perfringens* of a lecithinase (also known as alpha toxin), which hydrolyses the lipids in cell membranes, resulting in cell lysis and death. The presence of dead and dying tissue further compromises the blood supply, and the organisms multiply and produce more toxin and more damage. Other extracellular enzymes may also play a role in helping the clostridia to spread. If the toxin escapes from the affected area and enters the bloodstream, there is massive haemolysis, renal failure and death.

Amputation may be necessary to prevent further spread of clostridial infection

Because of the rapid progression and fatal outcome of this type of clostridial infection, gangrenous areas require immediate

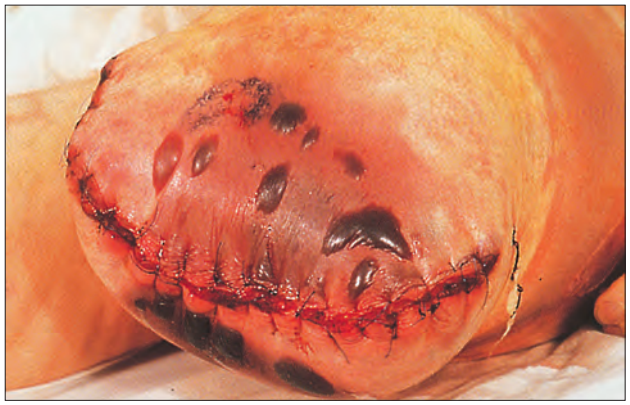


Figure 27.13 Gas gangrene caused by *Clostridium perfringens*. Organisms from the fecal flora may contaminate a wound and grow and multiply in poorly perfused (anaerobic) tissue. Infection spreads rapidly, and gas can be felt in the tissue and seen on radiographs. (Courtesy of J. Newman.)

surgery to excise all the affected tissue, and amputation may be necessary. Although some reports suggest that anti-alpha toxin may help if given early enough, antitoxin treatment is not generally viewed as effective, while treatment in a hyperbaric oxygen chamber, where available, may be helpful (i.e. oxygenation of tissue) in some cases.

Antibiotics are adjuncts to, not replacements for, surgical debridement.

Prevention of infection is of foremost importance. Wounds should be cleansed and debrided early to remove dead and poorly perfused tissue, which the anaerobes favour. Prophylactic antibiotics should be given preoperatively to patients having elective surgery of body sites liable to contamination with faecal flora.

Propionibacterium acnes and acne

P. acnes go hand in hand with the hormonal changes of puberty which result in acne

An increased responsiveness to androgenic hormones leads to increased sebum production plus increased keratinization and desquamation in pilosebaceous ducts. Blockage of ducts turns them into sacs in which *P. acnes* and other members of the normal flora (e.g. micrococci, yeasts, staphylococci) multiply. *P. acnes* acts on sebum to form fatty acids and peptides which, together with enzymes and other substances released from bacteria and polymorphs, cause the inflammation (Fig. 27.14). Comedones are greasy plugs composed of a mixture of keratin, sebum and bacteria and capped by a layer of melanin (blackheads in popular terminology) (Fig. 27.15).

Treatment of acne includes long-term administration of oral antibiotics

The antibiotics used to treat acne are usually one of the tetracyclines. Other treatments include skin care, keratolytics and, in severe cases, synthetic vitamin A derivatives such as isotretinoin. Orally administered antibiotics reduce the surface numbers of *P. acnes* with a concomitant lowering of the free fatty acids, which act as skin irritants, which result from the activity of bacterial enzymes on sebum. Acne can be a problem for teenagers, but often disappears in older age groups as the sebaceous follicles become less active.

Figure 27.14 Typical lesions of acne. 'blackheads' are seen when plugs of keratin block the pilosebaceous duct. (Courtesy of A. du Vivier.)

Other Gram-positive rods related to *P. acnes*, such as corynebacteria and brevibacteria, can also cause skin infections.

MYCOBACTERIAL DISEASES OF THE SKIN

Leprosy

Leprosy is decreasing in incidence but still remains a concern

Leprosy has been recognized since biblical times, but in the past, the word was a generic term applied to several different diseases and also implying 'moral uncleanliness'. Leprosy is thought to have spread to Europe in the sixth century, and by the thirteenth century there were some two hundred leper hospitals in England. Over the centuries that followed, leprosy declined in incidence, and by the fifteenth century was no longer endemic in England; in contrast tuberculosis was on the increase. Now leprosy is rare in the UK and USA and the WHO estimates that the number of new cases worldwide has decreased to approximately 200 000 cases detected in 2015. However, the disease still represents a problem in South-East Asia, Africa and the Americas.

Leprosy is caused by *Mycobacterium leprae*

Mycobacterium leprae was discovered in 1873 by G.A. Hansen, who identified it as the first bacterial agent capable of causing human disease. Leprosy (Hansen's disease) appears to be confined to humans. *M. leprae* is found in nine-banded armadillos, chimpanzees and mangabey monkeys; however, epidemiological studies have not demonstrated a significant link between this carriage and human disease. Transmission of infection is directly related to overcrowding and poor hygiene and occurs by direct contact and aerosol inhalation. Relatively few organisms are shed from skin lesions, but nasal secretions of patients with lepromatous leprosy are laden with *M. leprae*. Arthropod vectors may play a role in transmission. Leprosy is not highly contagious, and prolonged exposure to an infected source is necessary; it seems that children living under the same roof as an open case of leprosy are most at risk. Ironically, because the lesions of leprosy are more obvious, patients were in the past excluded from the community and gathered in leper colonies, whereas tuberculosis is much more contagious, but people with tuberculosis were not shunned.

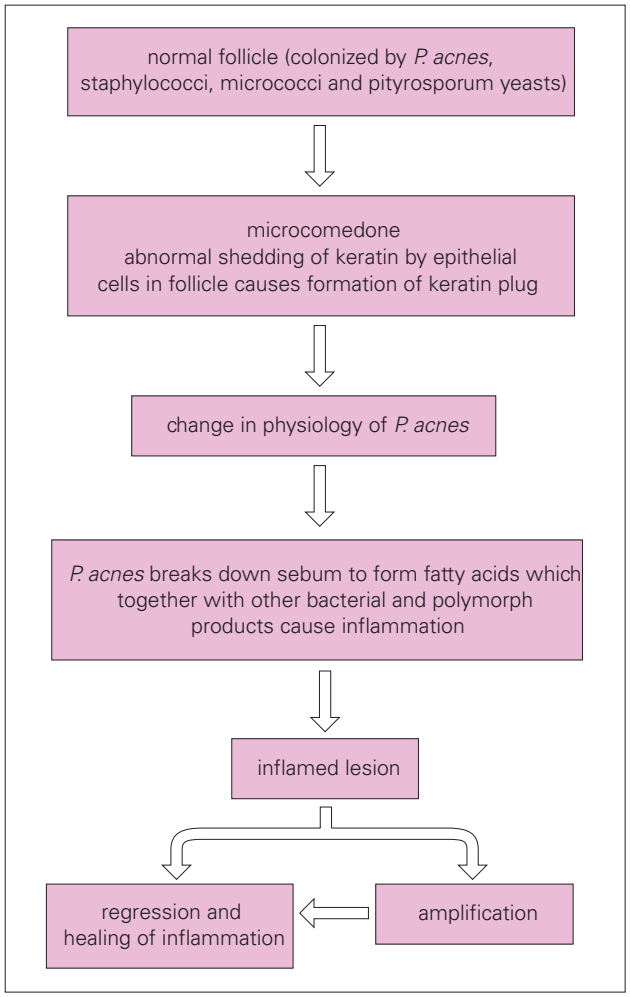


Figure 27.15 The proposed mechanism of the pathogenesis of acne. Hormonal changes in the host initiate the formation of comedones from normal follicles and thereby change the environment of *Propionibacterium acnes* and its physiological properties. *P. acnes* is also known to be an immunostimulator.

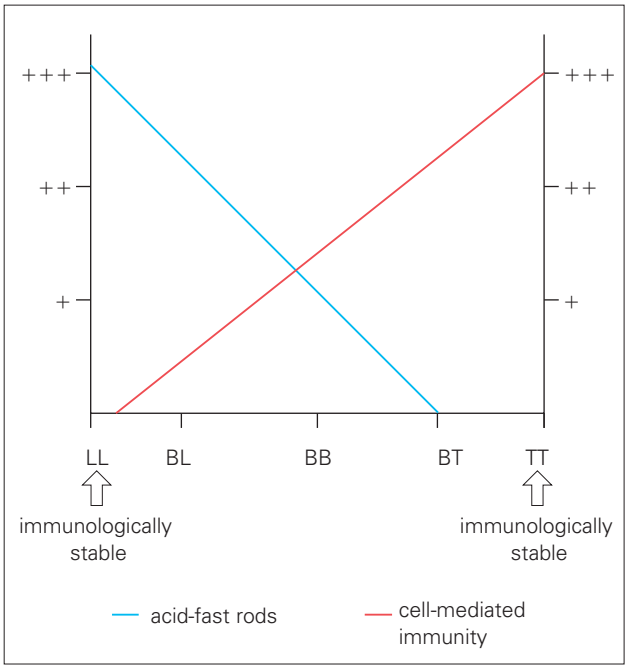


Figure 27.16 Immunological responses in leprosy. In tuberculoid leprosy (TT) the patient is capable of mounting an effective cell-mediated immune (CMI) response, which makes it possible for macrophages to destroy the organisms and contain the infection. At the other extreme, in lepromatous leprosy (LL) the patient is incapable of producing a CMI response and the organisms multiply unhindered. These patients have many acid-fast rods in their skin and nasal secretions, and are much more infectious than TT patients. Borderline lepromatous (BL), borderline borderline (BB), and borderline tuberculoid (BT) responses are found between these extremes.

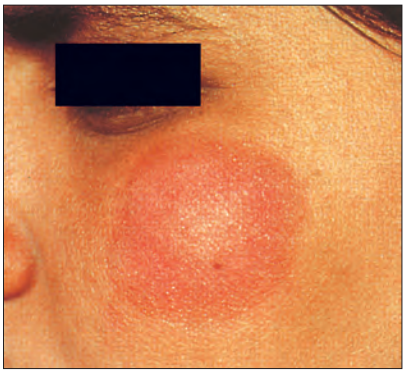


Figure 27.17 Tuberculoid leprosy – a characteristic dry blotchy lesion on the face, but the diagnosis needs to be confirmed by microscopic examination of skin biopsy (see Fig. 27.20). (Courtesy of the Institute of Dermatology.)

The clinical features of leprosy depend upon the cell-mediated immune response to *M. leprae*

M. leprae cannot be grown in artificial culture media, and little is known about its mechanism of pathogenicity. Two animal models have been used: infection in the armadillo and in the footpads of mice. The organism grows better at temperatures below 37°C, hence its concentration in the skin and superficial nerves, and it grows extremely slowly; in the mouse footpad the generation time is 11–13 days. Likewise in humans, the incubation period may be many years.

M. leprae grows intracellularly, typically within skin histiocytes and endothelial cells and the Schwann cells of peripheral nerves. The immune response is all important in deciding the type of disease.

M. leprae shares many pathobiological features with *M. tuberculosis*, but the clinical manifestations of the diseases are quite different. After an incubation period of several years, the onset of leprosy is gradual and the spectrum of disease activity is very broad depending upon the presence or absence of a cell-mediated immune (CMI) response to *M. leprae* (Fig. 27.16). At one end of the spectrum is tuberculoid leprosy (TT), characterized by blotchy red lesions with anaesthetic areas on the face, trunk and extremities (Fig. 27.17). There is palpable thickening of the peripheral nerves because the organisms multiply in the nerve sheaths. The local anaesthesia renders the patient prone to repeated trauma and secondary bacterial infection. This disease state is equivalent to secondary tuberculosis (see Ch. 20), with a vigorous CMI response leading to phagocytic destruction of bacteria, and exaggerated allergic responses. TT carries a better prognosis than lepromatous

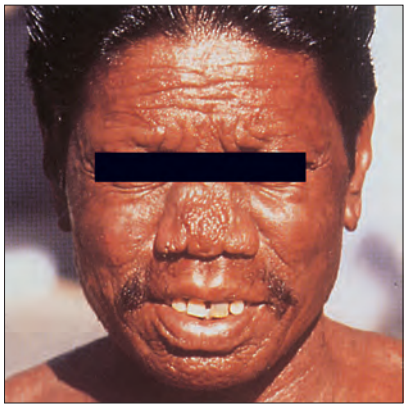


Figure 27.18 Extensive skin involvement in lepromatous leprosy results in a characteristic leonine appearance. (Courtesy of D.A. Lewis.)

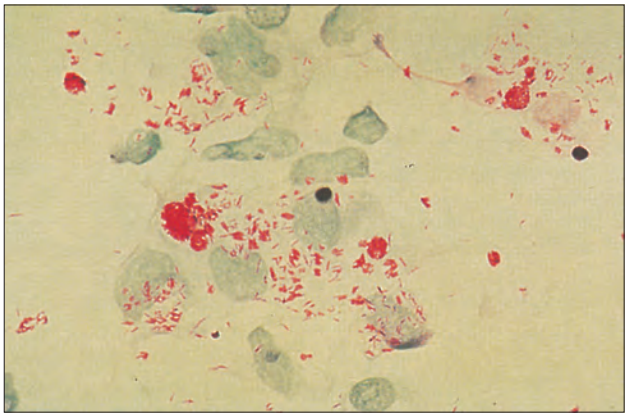


Figure 27.19 In lepromatous leprosy, the nasal mucosa is packed with *Mycobacterium leprae*, seen here in an acid-fast stain (Ziehl–Neelsen) of nasal scrapings. (Courtesy of I. Farrell.)

leprosy (LL) and in some patients is self-limiting, but in others may progress across the spectrum towards LL.

In LL, there is extensive skin involvement with large numbers of bacteria in affected areas. As the disease progresses there is loss of eyebrows, thickening and enlargement of the nostrils, ears and cheeks, resulting in the typical leonine (lion-like) facial appearance (Fig. 27.18). There is progressive destruction of the nasal septum, and the nasal mucosa is loaded with organisms (Fig. 27.19). This form of the disease is equivalent to miliary tuberculosis (see Ch. 20) with a weak CMI response and many extracellular organisms visible in the lesions. The gross deformities characteristic of late disease result primarily from infectious destruction of the nasomaxillary facial structures, and secondarily from pathological changes in the peripheral nerves predisposing to repeated trauma of the hands and feet and subsequent superinfection with other organisms.

Whether a patient develops TT or LL may in part be genetically determined. Patients with intermediate forms of the disease may progress to either extreme.

M. leprae are seen as acid-fast rods in nasal scrapings and lesion biopsies

Alertness to the possibility of leprosy when confronted with a patient with dermatological, neurological or multisystem complaints is of fundamental importance. Although the majority of cases are in people who are not native to Europe or the USA, the diagnosis should also be considered in those who have worked in endemic areas.

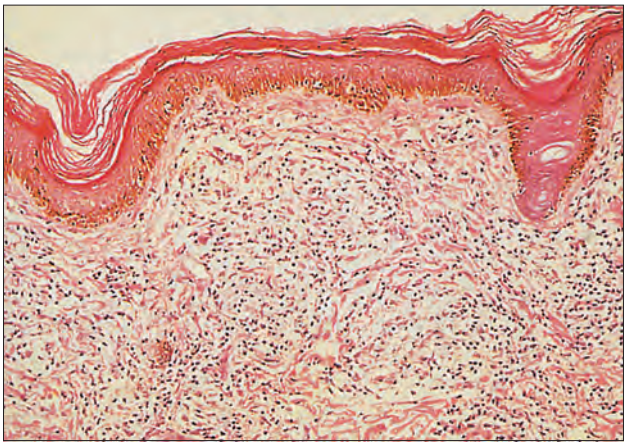


Figure 27.20 In tuberculoid leprosy, the organisms are much sparser but characteristic granulomas form in the dermis, as shown in this histological preparation. (Courtesy of C.J. Edwards.)

Nasal scrapings and biopsies of skin lesions can be stained by Ziehl–Neelsen or auramine stain to demonstrate acid-fast rods. In LL these are numerous, but in TT few if any organisms are seen, but the appearance of granulomas is sufficiently typical to allow the diagnosis to be made (Fig. 27.20). Remember that, in contrast to *M. tuberculosis*, the organism cannot be grown in vitro.

Treatment

Leprosy is treated with dapsone given as part of a multidrug regimen to avoid resistance

If the disease is diagnosed early and treatment initiated promptly the patient has a much better prognosis. Dapsone (see Ch. 34) had long been the mainstay of therapy, but multidrug therapy is now used because of dapsone resistance:

- For LL, triple therapy with dapsone, rifampin and clofazimine is commonly given for 1–2 years and may be longer or until all skin scrapings and biopsies are negative for acid-fast rods.
- For TT, a combination of dapsone and rifampin for 6 months is recommended, the rationale being that in this form of disease there are many fewer organisms and therefore less chance of emergence of resistant mutants.

As a result of multidrug therapy, which is reasonably cheap, well tolerated and effects a complete cure, steady progress is being made towards the elimination of leprosy as a public health problem.

Destruction of the organisms by effective antimicrobial therapy may result in an inflammatory response, erythema nodosum leprosum, which may be severe and, occasionally, fatal. Treatment with corticosteroids may be indicated.

Vaccination with bacille Calmette–Guérin (BCG) has been used in countries with high incidence where potential protection outweighs negative factors such as a positive skin test. Vaccination is not useful for immunocompromised individuals.

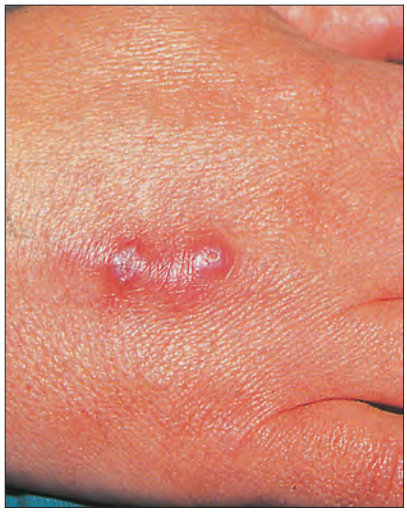


Figure 27.21 Fish-tank granuloma caused by *Mycobacterium marinum* infection of a lesion acquired while cleaning out a fish tank. (Courtesy of M.J. Wood.)

Other mycobacterial skin infections

Mycobacterium marinum, *M. ulcerans* and *M. tuberculosis* also cause skin lesions

Mycobacterium marinum and *M. ulcerans* are two slow-growing mycobacterial species that prefer cooler temperatures and cause skin lesions. As its name suggests, *M. marinum* is associated with water and marine organisms. Human infections follow trauma, often minor such as a graze acquired while climbing out of a swimming pool or while cleaning out an aquarium, which becomes contaminated with mycobacteria from the wet environment. After an incubation period of 2–8 weeks, initial lesions appear as small papules, which enlarge and suppurate and may ulcerate. Histologically, the lesions are granulomas and hence the name 'swimming pool granuloma' or 'fish-tank granuloma' (Fig. 27.21). Sometimes the nodules follow the course of the draining lymphatic and produce an appearance that may be mistaken for sporotrichosis (see below).

M. ulcerans causes chronic, relatively painless cutaneous ulcers known as 'Buruli ulcers'. This disease is seen in Africa and Australia, but is rarely elsewhere.

Tuberculosis of the skin is exceedingly uncommon. Infection can occur by direct implantation of *M. tuberculosis* during trauma to the skin (lupus vulgaris) or may extend to the skin from an infected lymph node (scrofuloderma).

FUNGAL INFECTIONS OF THE SKIN

Fungal infections may be confined to the very outermost layers of the skin and hair shafts or penetrate into the keratinized layers of the epidermis, nails and hair (the superficial and cutaneous mycoses); others develop in the dermal layers (subcutaneous mycoses). In addition, some systemic fungal infections acquired by the airborne route have skin manifestations (see Table 27.1).

Superficial and cutaneous mycoses

These are some of the most common infections in humans. Superficial infections of the skin and hair (pityriasis versicolor, tinea nigra, black and white piedras) mainly cause cosmetic problems; cutaneous infections (ringworm, tineas) caused by the dermatophyte fungi are more significant. The important causative agents are the superficial basidiomycete yeast *Malassezia furfur* and the cutaneous ascomycete dermatophytes *Epidermophyton*, *Trichophyton* and *Microsporum*.

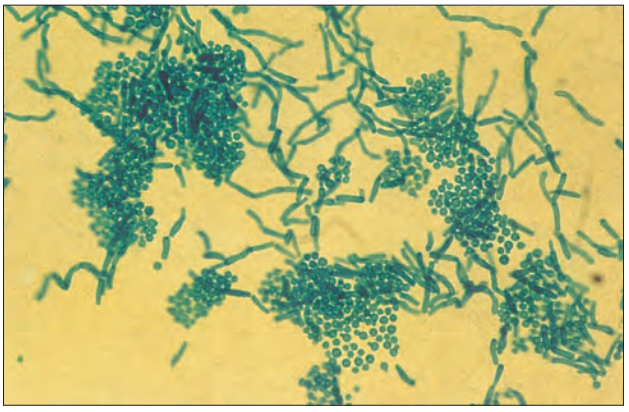


Figure 27.22 Infected skin scales stained to show the thick-walled yeast forms of *Malassezia furfur* and the short angular hyphae. (Courtesy of Y. Clayton and G. Midgley.)

Pityriasis versicolor

M. furfur is the cause of pityriasis or tinea versicolor

The yeast *M. (Pityrosporum) furfur* is a common skin inhabitant. The change from commensalism to pathogenicity appears to be associated with the phase change from yeast to hyphal forms of the fungus, but the stimulus for this is unknown. As part of their adaptation to living in the skin, *Malassezia* secrete acid sphingomyelinases and aspartate proteases and as they cannot synthesize fatty acids, they secrete lipases and phospholipases C, which release fatty acids from host lipids. Infections are usually confined to the trunk or proximal parts of the limbs and are associated with hypo- or hyperpigmented macules that coalesce to form scaling plaques. The lesions are not usually itchy and in some patients, they resolve spontaneously.

Malassezia yeasts are also involved in the pathogenesis of seborrhoeic dermatitis and dandruff, but the relative roles of the host's immune system, enzymatic activity of *Malassezia* or secondary metabolites in promoting it have yet to be clearly defined.

Diagnosis of pityriasis versicolor can be confirmed by direct microscopy of scrapings

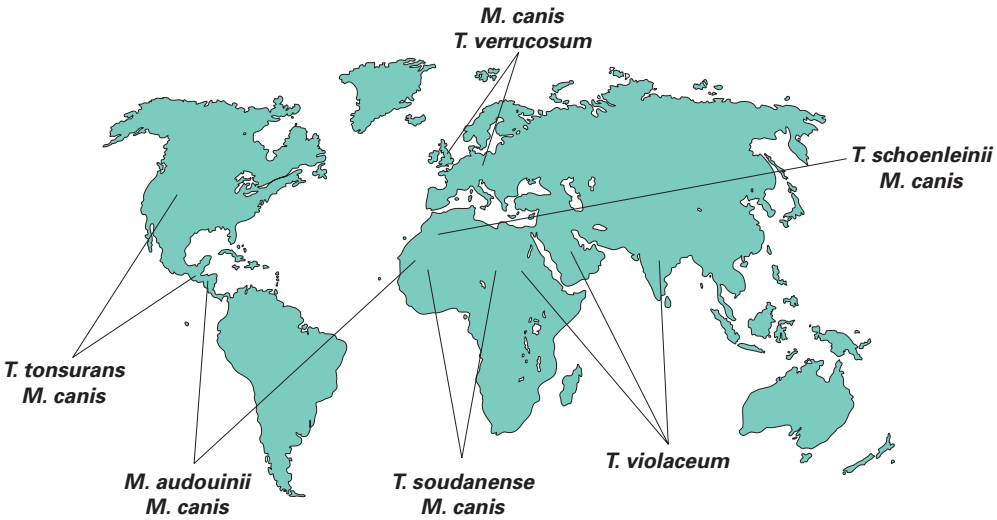
Direct microscopy of scrapings shows characteristic round yeast forms (Fig. 27.22), and treatment with a topical azole antifungal (see below) or with selenium sulphide (2.5%) lotion is appropriate.

Cutaneous dermatophytes

Dermatophyte infections are acquired from many sources and are spread by arthrospores

Species of dermatophytes are described as anthropophilic, zoophilic or geophilic depending upon their primary source (human, animal or soil). The species concerned differ in their geographical distribution, in their predilection for different body sites and in the degree of host response elicited in

geographic distribution of anthropophilic and zoophilic fungi causing tinea capitis



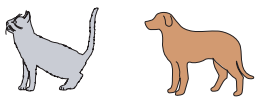
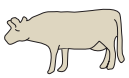
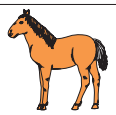
anthropophilic dermatophytes	zoophilic dermatophytes	
	dermatophyte species	**natural host**
Microsporum audouinii	*Microsporum canis*	
Trichophyton mentagrophytes var. *mentagrophytes*		
T. rubrum	*M. gallinae*	
T. tonsurans	*Trichophyton verrucosum*	
T. violaceum		
T. soudanense	*T. equinum*	
T. schoenleinii	*T. mentagrophytes* var. *mentagrophytes*	
T. concentricum		
Epidermophyton floccosum	*T. erinacei*	

Figure 27.23 Three genera of dermatophytes are important causes of disease: *Microsporum*, *Trichophyton* and *Epidermophyton*. Within each genus there are anthropophilic, zoophilic and geophilic species. The natural host and therefore distribution of anthropophilic species varies. *Microsporum gypseum* is the geophilic species of importance.

humans. The source of an infection determines its route of transmission to humans and, to some extent, its distribution in human populations (Fig. 27.23), although population movements are changing established patterns. For example, for a time migration from Latin America replaced *Microsporum audouinii* by *Trichophyton tonsurans* as the common cause of tinea capitis in the USA, but the latter (which responds poorly to treatment) is now again predominant.

The anthropophilic species are the most common causes of dermatophyte infections. In temperate countries, *Trichophyton verrucosum* from cattle, *T. mentagrophytes* from rodents, and *Microsporum canis* from cats and dogs, are the most common zoophilic causes of human infection. Geophilic species such as *Microsporum gypseum* are uncommon causes of human disease, but are seen in people who have appropriate exposure, such as gardeners and agricultural workers. Zoophilic and geophilic

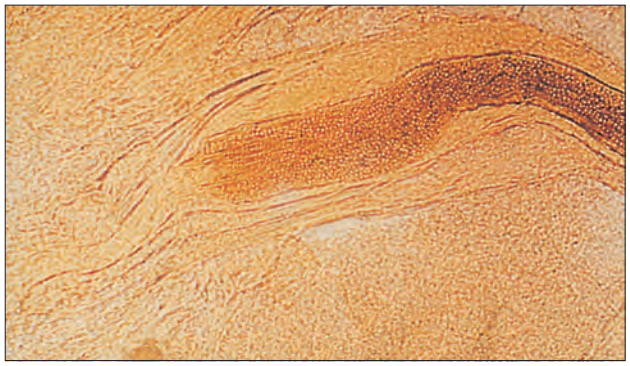


Figure 27.24 Arthrospores of *Trichophyton tonsurans* in an infected hair shaft. These thick-walled spores are the form in which infection is spread. They can survive in the environment for weeks or months before infecting a new host. (Courtesy of A.E. Prevost.)

species tend to cause a greater inflammatory response than anthropophilic species.

Infections are spread by contact with arthrospores, the thick-walled vegetative cells formed by dermatophyte hyphae (Fig. 27.24), which can survive for months. In anthropophilic and zoophilic species, these are shed from the primary host in skin scales and hair.

Dermatophytes invade skin, hair and nails

The dermatophytes are keratin-loving organisms and invade the keratinized structures of the body (i.e. skin, hair and nails). Dermatophytes have septate hyphae and form arthrospores which adhere to keratinocytes, germinate and invade. In adapting to life in the skin, they produce proteases to break down keratin, Lys M proteins to evade recognition by the host and kinases and pseudokinases to modulate host cell metabolism. The Latin word *tinea* (meaning a maggot or grub) or 'ringworm' is used for these infections because they were originally thought to be caused by a worm-like parasite. Thus, tinea capitis affects the hair and skin of the scalp, tinea corporis the body, tinea cruris the crotch, tinea manuum the hands, tinea unguium the nails and tinea pedis the feet (Fig. 27.25).

The typical lesion is an annular or serpentine scaling patch with a raised margin. The main symptom is itching, but this is variable in degree. The skin is often dry and scaly and sometimes cracks (e.g. between the toes in tinea pedis), while infections of hair cause hair loss (Fig. 27.26). The degree of associated inflammation varies with the infecting species, usually being greater with zoophilic than with anthropophilic species. Individuals also differ in their susceptibility to infection, but the factors determining these differences are not clearly understood. Similarly, dermatophyte species differ in their ability to elicit an immune response; some, such as *Trichophyton rubrum*, cause chronic or relapsing conditions, whereas other species induce long-term resistance to re-infection. In some patients, circulating fungal antigens give rise to immunologically mediated hypersensitivity phenomena in the skin (e.g. erythema or vesicles) known as dermatophytid reactions. When the skin becomes cracked and macerated as a result of infection, it is liable to superinfection with other organisms such as Gram-negative bacteria in moist sites.

Very rarely, dermatophytes invade the subcutaneous tissues via the lymphatics, causing granulomas, lymphoedema and

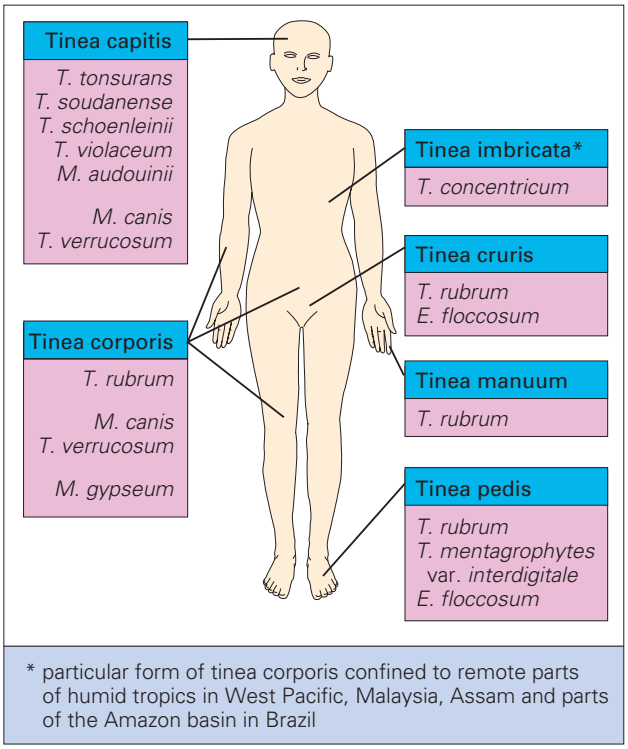


Figure 27.25 Tinea (or ringworm) is the disease of skin, hair and nails caused by dermatophyte fungi. Different species have predilections for different body sites. *E.*, *Epidermophyton*; *M.*, *Microsporum*; *T.*, *Trichophyton*.

draining sinuses. Further extension to sites such as the liver and brain may be fatal.

Most dermatophyte species fluoresce under ultraviolet light

This feature can be used as a diagnostic aid, particularly for tinea capitis, in the clinic. Laboratory diagnosis depends upon culture of scrapings or clippings from lesions on Sabouraud agar or other agars to which inhibitory agents (antibiotics / cycloheximide) have been added to provide some selectivity (Fig. 27.27). Dermatophytes infecting hair show a characteristic distribution, which may be helpful for identification:

- Some, such as most *Microsporum* species, form arthrospores on the outside of the hair shaft (ectothrix infections).
- The majority of *Trichophyton* infections form arthrospores within the hair shaft (endothrix infection, Fig. 27.28).

Confirmation of identity is useful for determining the source of infection and depends upon the colonial and microscopic characteristics of the fungi cultured on Sabouraud agar (Fig. 27.29). Growth may take up to 3 weeks, so more rapid molecular methods are also deployed and matrix-assisted laser desorption ionization-time-of-flight mass spectrometry (MALDI-TOF MS) is coming into use.

Dermatophyte infections are treated topically if possible

A range of agents is available for topical treatment (see Ch. 34), both antifungals (e.g. miconazole) and keratolytic

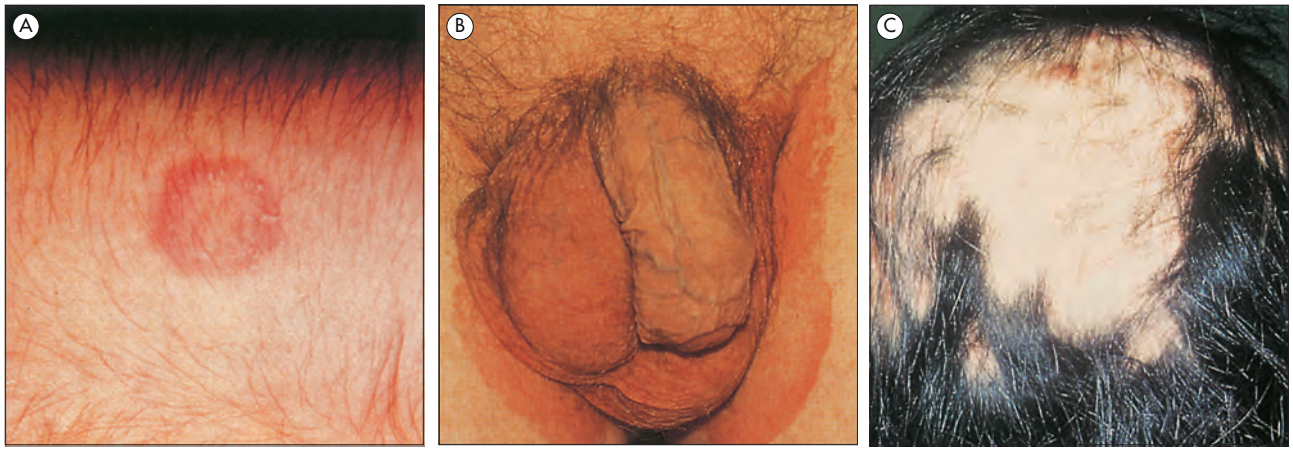


Figure 27.26 (A) Classic annular lesion of tinea corporis, caused here by infection with a *Microsporum* species. (B) Tinea cruris or 'jock itch' is a scaly rash on the thighs; the scrotum is usually spared. (C) Tinea capitis is characterized by scaling on the scalp and hair loss. Some dermatophytes fluoresce under ultraviolet light, and this can be an aid to diagnosis. ([A] Courtesy of A.E. Prevost. [B] Courtesy of M.J. Wood. [C] Courtesy of M.H. Winterborn.)

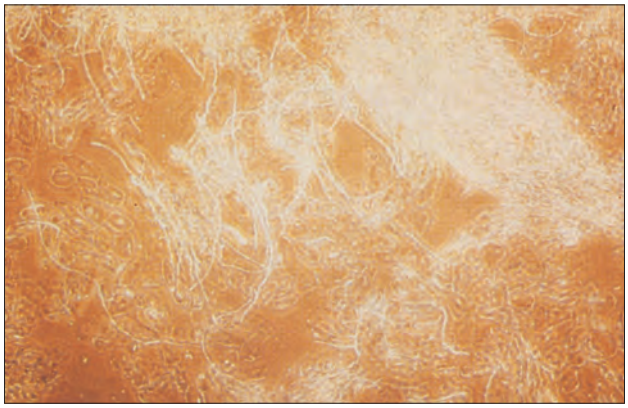


Figure 27.27 Dermatophyte infection. Samples of skin, hair and nails need to be 'cleared' by treatment with potassium hydroxide before examining under the microscope for the presence of fungal hyphae. (Courtesy of R.Y. Cartwright.)

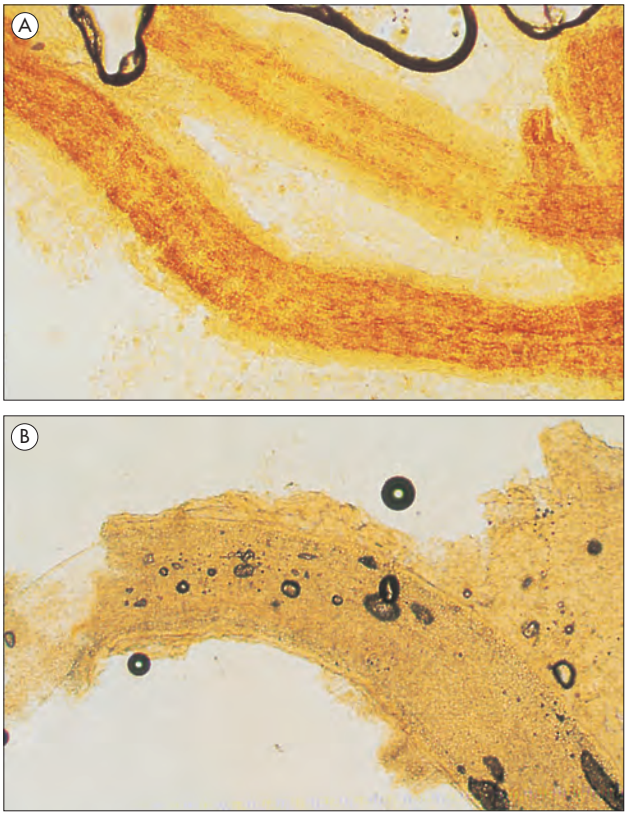


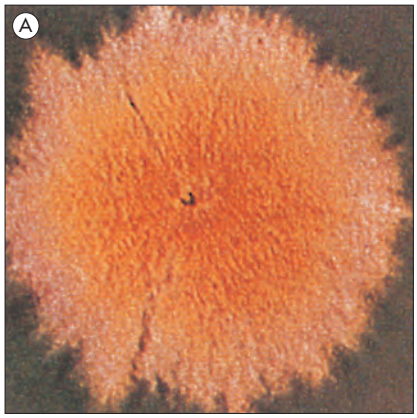
Figure 27.28 Dermatophytes may form arthrospores within the hair shafts (endothrix infection) as shown in (A) and less commonly outside the shaft (ectothrix infection) as shown in (B). (Courtesy of Y. Clayton and G. Midgley.)

agents such as Whitfield's ointment (a mixture of salicylic and benzoic acids). In order to prevent relapse, treatment should be continued for 1 to 2 weeks after resolution of clinical signs. Systemic therapy with oral antifungal drugs is required for scalp infection and is more effective than topical agents for nail infections. Terbinafine or itraconazole are now used in preference to griseofulvin. These newer agents may give a cure rate of 70–80% for nail infections.

Candida and the skin

Candida requires moisture for growth

The relative dryness of most areas of skin limits the growth of fungi such as *Candida* that require moisture. *Candida* is found in low numbers on healthy intact skin, but rapidly colonizes damaged skin and intertriginous sites (opposed skin sites which are often moist and become chafed, Fig. 27.30). *Candida* also colonizes the oral and vaginal mucosa and overgrowth may result in disease in these sites (thrush, see Ch. 22). However, a substantial lowering of host resistance (e.g. neutropenia leading to invasion via the gastrointestinal tract) is necessary for *Candida* to invade deeper subcutaneous tissue, and disseminated candidiasis does not often originate from skin infection unless there is disruption to the skin barrier, for example presence of a central venous catheter.



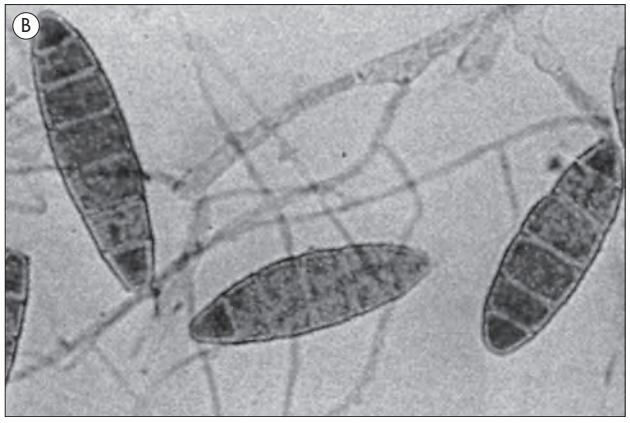


Figure 27.29 (A) Macroscopic growth (colony) and (B) microscopic preparation showing the macroconidia of *Microsporum gypseum*.

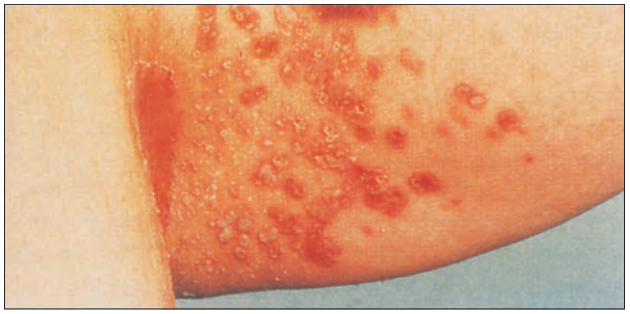


Figure 27.30 *Candida* infection of the skin. Here, infection has occurred between two apposing skin surfaces, which provide a suitably moist environment for this yeast to multiply. (Courtesy of A. du Vivier and St Mary's Hospital.)

Subcutaneous mycoses

Subcutaneous fungal infections can be caused by a number of different species

Lesions usually develop at sites of trauma (a thorn, a bite) where the fungus becomes implanted. With the exception of sporotrichosis, subcutaneous fungal infections are rare, but similar diseases can be caused by certain bacteria such as *Actinomyces* and atypical mycobacteria, and therefore it is important to establish the aetiology in order to select optimal therapy. The fungi involved are difficult to eradicate with antifungal agents, and surgical intervention, in the form of excision or even amputation, is often required.

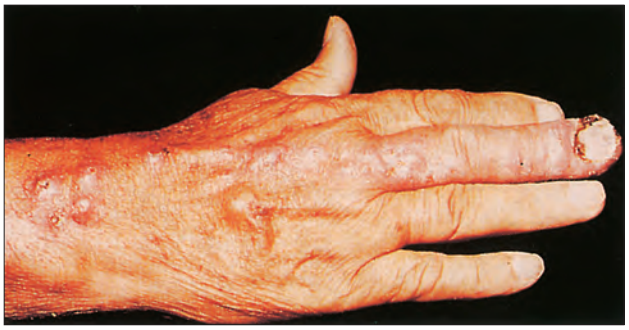


Figure 27.31 *Sporotrichosis* spreading up the draining lymphatics of the hand following a primary infection in the nailbed of the third finger. (Courtesy of T.F. Sellers, Jr.)

Sporotrichosis is a nodular condition caused by *Sporothrix schenckii*

Sporothrix schenckii is a saprophytic dimorphic fungus that is widespread in nature in soil, on rose and berberis bushes, tree bark and sphagnum moss. Infection is acquired through trauma (e.g. a thorn) and is an occupational hazard for people such as farmers, gardeners and florists. Clinical presentation depends on host immune status, size and depth of inoculum, and pathogenicity and thermal tolerance of the infecting strain. Commonly, a small papule or subcutaneous nodule develops at the site of trauma 1 week to 6 months after inoculation, and infection spreads, producing a series of secondary nodules along the lymphatics that drain the site (Fig. 27.31). Diagnosis is made by culture of drained or aspirated material onto Sabouraud agar. Molecular diagnosis is more rapid and can be helpful in culture-negative cases. Serological tests, for example IgG ELISA, are available. Azole drugs are highly effective and, itraconazole has replaced treatment with oral potassium iodide.

Disseminated disease can occur following cutaneous or pulmonary infection with *S. schenckii*. It is more common in compromised patients such as those with underlying carcinoma or sarcoidosis, but many cases occur in people in whom no underlying disease is recognized. Treatment with amphotericin B is indicated for induction therapy, followed by itraconazole. Long-term maintenance therapy may be needed where it is not possible to reverse underlying immunosuppression.

Other species causing subcutaneous infections include *Cladosporium* and *Phialophora* (chromoblastomycosis).

Mycetoma

Mycetoma is a chronic progressive subcutaneous infection which gives rise to tumour-like swellings complicated by the development of sinuses discharging grains. It most commonly involves the foot (hence Madura foot) but it can also affect the hand or other parts of the body. The lesion develops after trauma which introduces the infecting organism into subcutaneous tissue so is more common in farmers and the poor who may have no footwear. Its true global distribution and prevalence are not established. Most cases have been reported from Mexico, Sudan and India. As a result of global migration patterns, clinicians in regions considered non-endemic for mycetoma are now seeing imported cases for the first time.

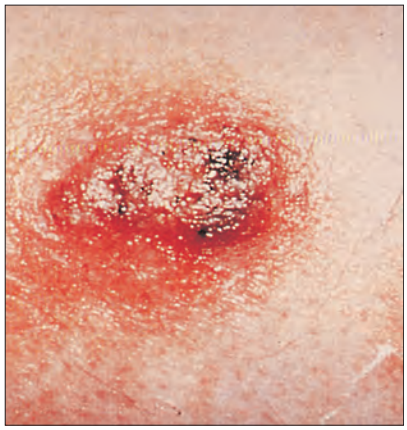


Figure 27.32 Typical skin lesion of blastomycosis. Infection is acquired by the respiratory route, and the primary site of infection is the lung. However, in chronic blastomycosis the skin is the most common extrapulmonary site of infection. (Courtesy of K.A. Riley.)

There are two types of mycetoma:

- eumycetoma, caused by fungi, of which *Madurella mycetomatis* is the commonest causative agent
- actinomycetoma, caused by bacteria, commonly *Nocardia brasiliensis* and *Streptomyces somaliensis*.

Biopsy for histopathology, bacterial and fungal culture is required to establish the diagnosis and identify the infecting organism. PCR is valuable in identifying the organism responsible, but culture is essential for antimicrobial susceptibility testing.

Actinomycetoma responds well to antibiotic therapy, for example co-trimoxazole. Eumycetoma requires surgery in addition to prolonged antifungal therapy, often with itraconazole and where possible, wide local excision should be performed. Repeat operation may be necessary to deal with recurrent disease and amputation may be required in advanced cases.

Systemic fungal infections with skin manifestations include blastomycosis, coccidioidomycosis and cryptococcosis

Skin lesions occur in 40–80% of cases of blastomycosis, a disease endemic in Central and North America and Africa and caused by the dimorphic fungus *Blastomyces dermatitidis*. Infection is acquired by aspiration of the fungal spores and spreads from the primary site in the lung. Blastomycosis can be a systemic disease in apparently immunologically normal hosts (Fig. 27.32). It also causes disease in horses and dogs.

Other systemic fungal infections that may have skin manifestations are those caused by *Coccidioides immitis* and *Cryptococcus neoformans*.

PARASITIC INFECTIONS OF THE SKIN

The skin is a major route of entry for parasites, which may:

- penetrate directly (e.g. schistosomes, hookworm)
- be injected by blood-feeding vectors.

Many of these parasites leave the skin almost immediately as they progress through their life cycle, but some remain there and others, for example animal parasites unable to complete their life cycle in humans, may become trapped. A few parasites actually exit from the body through the skin (e.g., release of Guinea worm larvae). Pathological responses to parasites associated with the skin range from mild to disablingly severe. Some species causing severe conditions are described briefly below.

Leishmaniasis may be cutaneous or mucosal (formerly termed mucocutaneous)

Two major disease complexes caused by the protozoan *Leishmania* affect the skin and both are transmitted by the bite of sandfly vectors:

- The cutaneous leishmaniases, which occur in both the Old World (Asia, Africa, Southern Europe) and New World (Central and South America), include conditions ranging from localized self-healing ulcers to non-curing, disseminated lesions similar to leprosy in appearance.
- In the New World, mucosal leishmaniasis occurs when the parasite in the skin invades mucosal surfaces (nose, mouth), giving rise to chronic disfiguring conditions. Leishmaniasis is discussed in detail in Chapter 28.

Schistosome infection can cause a dermatitis

Transmission of schistosomal infection to humans is achieved via active skin penetration by larvae (cercariae) released into fresh water by the snail intermediate host (see Ch. 28). This stage of infection can give rise to a dermatitis known as swimmers' itch. It may also be produced by the cercariae of bird schistosomes and is relatively common where natural water used for recreation is populated with aquatic birds. It is a frequent problem in lakes in North America. Topical anti-inflammatory treatments are effective therapy. Occasionally, topical 1% hydrocortisone ointment is required.

Cutaneous larval migrans is characterized by itchy inflammatory hookworm larvae trails

Human hookworm (the nematodes *Ancylostoma* and *Necator*) invade the body through the skin, the infective larvae burrowing into the dermis, then migrating via the blood to eventually reach the intestine. Invasion may cause dermatitis (known as ground itch) and this becomes more severe upon repeated infection. Humans, however, can also be invaded by the larvae of the cat and dog species of *Ancylostoma*. Infection is acquired when exposed skin comes into contact with soil that has been contaminated by animals carrying the adult worms in their intestines. Eggs in the faeces hatch to produce the infective larvae, which remain viable for prolonged periods. As the human host is foreign for these species, the larvae fail to escape from the dermis after invasion, and may live for some time, migrating parallel to the skin and leaving intensely itchy sinuous inflammatory trails (creeping eruption), which are easily visible at the surface (Fig. 27.33). Treatment is with topical thiabendazole paste or oral ivermectin.

Onchocerciasis is characterized by hypersensitivity responses to larval antigens

Onchocerciasis is also known as river blindness. The adult stages of *Onchocerca volvulus* live for many years in subcutaneous nodules. Female worms release live microfilariae, which migrate away from the nodules, remaining largely in the dermal layers. They can invade the eye, causing

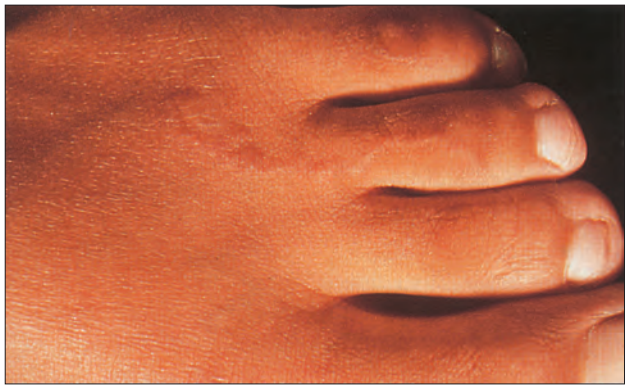


Figure 27.33 Cutaneous larval migrans (creeping eruption), showing the raised inflammatory track left by the invading hookworm larvae. (Courtesy of A. du Vivier.)

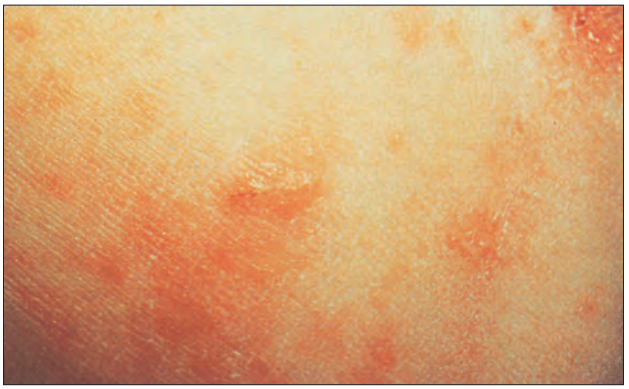


Figure 27.34 A characteristic cutaneous burrow in scabies. (Courtesy of M.J. Wood.)

river blindness (see Ch. 26). The slow build-up of parasite numbers and the development of a hypersensitivity response to the antigens released by living and dying larvae give rise to inflammatory skin conditions. In the early stages, these appear as erythematous papular rashes accompanied by intense itching. Later, there is skin thickening, elasticity is lost, and excessive wrinkling occurs and depigmentation is also common. The microfilariae can be killed by ivermectin treatment, but the skin changes, once advanced, are irreversible. Dermal inflammatory condition and secondary bacterial infection are not uncommon during infection with lymphatic filarial nematodes.

Arthropod infections

Some flies, mainly in the tropics and subtropics, have larvae that develop within the skin

Myiasis is a condition associated with invasion of the body by the larvae (maggots) of dipteran flies such as *Dermatobia*. Several species of fly have a cycle in which the larvae feed and grow in the skin of a mammal, just below the surface, escaping before or after pupation to continue their life cycle and lead ultimately to the release of adult forms. Female flies lay eggs or larvae directly onto the skin, and larvae may then invade wounds or natural orifices. The activities and feeding of the larvae cause intense painful reactions, and large lesions may develop. A number of these species have been found in humans, and infections have been recorded in many countries, although primarily in tropical and subtropical regions. Treatment involves removal of the larvae, alleviation of symptoms and prevention of secondary bacterial infection.

There is a revival of interest in using maggots of non-myiasis species to remove necrotic tissue from wounds, their secretions also preventing bacterial contamination.

Certain ticks, lice and mites live on blood or tissue fluids from humans

Some feed non-selectively on humans, the normal hosts being animals; other species are human specific. The feeding processes and the inevitable release of saliva, give rise to skin irritation, which becomes more intense as the body responds immunologically to the proteins present in the saliva. Prolonged feeding, as practiced by ticks, may leave painful lesions in the skin, which can become secondarily infected. Species such as lice and scabies mites, which spend the greater part or the whole of their lives on the human body, can cause severe skin conditions when populations accumulate. These conditions arise from:

- the activity of the arthropods themselves
- their production of excreta
- the oozing of blood and tissue fluids from the feeding sites
- the host's inflammatory reaction.

Pediculosis – infection with head and body lice of the genus *Pediculus* – can, when severe, give rise to encrusting inflammatory masses in which fungal infections may establish. Good personal hygiene prevents infestation; use of insecticidal creams, lotions, shampoos and powders containing permethrin helps to clear the insects directly.

The scabies mite has a more intimate contact with the human host than lice, living its whole life in burrows within the skin. The female lays eggs into these burrows, and so the area of infection can spread to cover large areas of the body from the original site, which is usually on the hands or wrists (Fig. 27.34; see Ch. 22). Infection causes a characteristic rash with itching, and secondary infections may follow scratching. Very heavy infections may develop in immunocompromised individuals or in people who are unable to care adequately for themselves. Under these conditions, there is extensive thickening and crusting of the skin (Norwegian scabies). Treatment with permethrin or malathion is recommended; benzyl benzoate can also be used on unbroken skin but is less effective. Oral ivermectin may be required in addition to topical therapy for Norwegian scabies.

MUCOCUTANEOUS MANIFESTATIONS OF VIRAL INFECTIONS

Rashes can be divided into

- vesicular (blistering) eruptions
- maculopapular (flat, papules) and erythematous (red)

and also into:

- where the virus is restricted to the body surface at the site of initial infection
- where the virus spreads systemically through the body (Table 27.3).

The skin rash has a characteristic distribution in many infectious diseases, but with the exception of zoster, which

Table 27.3 Mucocutaneous manifestations of viruses

Virus	Lesion	Virus shedding from lesion
No systemic spread		
Papillomavirus (wart)	Common wart; plantar wart; genital wart	+
Molluscum contagiosum (poxvirus)	Fleshy papule	+
Orf (poxvirus from sheep, goats)	Papulovesicular	+
Systemic spread		
Herpes simplex virus Varicella-zoster virus	Vesicular (neural spread and latency)	+
Coxsackievirus A (9, 16, 23)	Vesicular, in mouth (herpangina)	+
Coxsackievirus A16 Enterovirus 71	Vesicular (hand, foot and mouth disease)	+
Parvovirus B19	Facial maculopapular (erythema infectiosum)	–
Human herpesvirus 6	Exanthem subitum (roseola infantum)	–
Measles virus	Maculopapular skin rash	–
Rubella virus Echoviruses	Maculopapular not distinguishable clinically	–
Dengue and other arthropod transmitted viruses	Maculopapular	–

The pathogenesis of these diseases is illustrated in Fig. 27.2. Papillomas and vesicular lesions are generally sites of virus shedding. The distribution as well as the nature of the lesion can be important in diagnosis (e.g. varicella), but many maculopapular rashes are clinically indistinguishable.

involves the dermatome of the skin innervated by the affected nerve / dorsal root ganglion, the reason for this is unknown.

Rashes are particular features of human infection and are rare in animals. This is because human skin is naked and is a turbulent, highly reactive tissue in which immune and inflammatory events are clearly visible. Rashes cause discomfort and may be painful but they may be very helpful for the clinician who needs to make a diagnosis. The veterinarian is less privileged because the skin of most other mammals is largely covered with fur, and skin lesions generally involve hairless areas such as udders, scrotums, ears, prepuces, teats, noses or paws, which have the human properties of thickness, sensitivity and vascular reactivity.

Papillomavirus infection

Over 120 different types of papillomavirus can infect humans and are species specific

Papillomaviruses are 55 nm in diameter, icosahedral, double-stranded DNA viruses and cause skin papillomas (warts). The 70 different types that can infect humans show <50% cross-hybridization of DNA, although not all types are common. Human papillomaviruses (HPV) are species specific and distinct from animal papillomaviruses. They are highly adapted to human skin and mucosa and are ancient associates of our species; therefore, for most of the time they cause little or no disease. They show some adaptation to definite sites on the body:

- At least 40 types, including HPV 6, 11, 16 and 18, can infect the anogenital tract and other mucosal areas and are sexually transmitted.
- HPV 1 and 4 tend to cause plantar warts.
- HPV 2, 3 and 10 cause warts on the knees and fingers.

Papillomaviruses are generally transmitted by direct contact, but they are stable and can also be spread indirectly. For instance, plantar warts can be acquired from contaminated floors or from the non-slip surfaces at the edges of swimming pools, and in a given individual, warts can be spread from one site to another by shaving as they are autoinoculated.

Papillomavirus infects cells in the basal layers of skin or mucosa and are tissue tropic

After entering the body via surface abrasions, the virus infects cells in the basal layers of the skin or mucosa (see Fig. 27.2). There is no spread to deeper tissues. Virus replication is slow and is critically dependent on the differentiation of host cells. Viral DNA is present in basal cells, but viral antigen and infectious virus are produced only when the cells begin to become squamified and keratinized as they approach the surface. The infected cells are stimulated to divide and finally, 1–6 months after initial infection, the mass of infected cells protrudes from the body surface to form a visible papilloma or wart (Fig. 27.35). There is marked proliferation of prickle cells, and vacuolated cells are present in the more superficial layers. Warts can be:

- filiform with finger-like projections
- flat topped
- flat because they grow inwards due to external pressure (plantar warts)
- a cauliflower-like protuberance (e.g. genital warts)
- a flat area of dysplasia on the cervix.

Immune responses eventually bring virus replication under control and, several months after infection, the wart regresses. Antibodies are demonstrable, but CMI responses are more important in recovery. Viral DNA remains in a latent state in the basal cell layer, infecting an occasional stem cell, and is therefore retained within the layer as epidermal cells differentiate and are shed from the surface. When patients are subsequently immunocompromised (e.g. post-transplant)

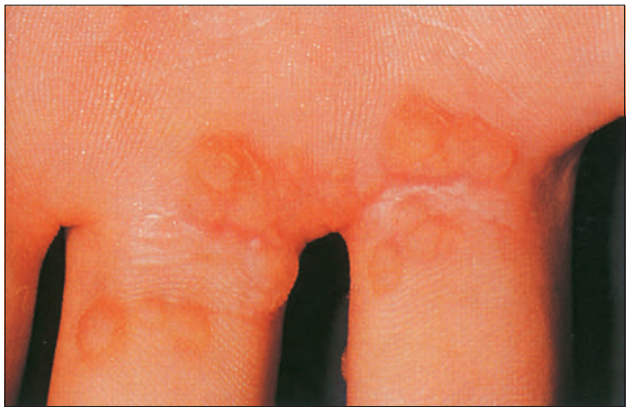


Figure 27.35 Common warts (papillomas) on the hand. (Courtesy of M.J. Wood.)

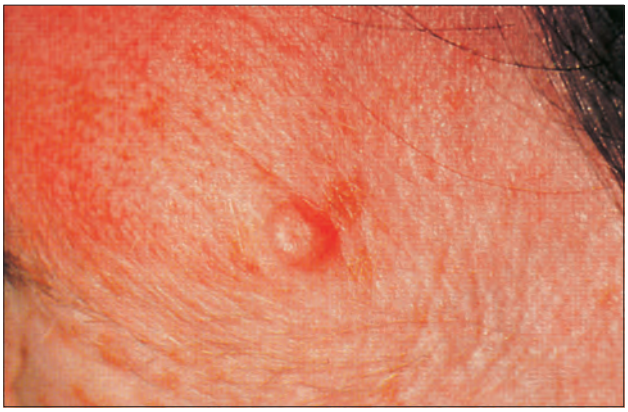


Figure 27.36 Single umbilicated lesion in *Molluscum contagiosum*. (Courtesy of M.J. Wood.)

crops of warts may result from reactivation of latent virus in the skin.

Papillomavirus infections are associated with cancer of the cervix, vulva, penis, rectum, head, and neck

Human papillomavirus infections are associated with nearly 4% of all cancers. The association between genital warts and cancer of the cervix, vulva, penis and rectum is referred to in Chapter 18. Infection with specific genital HPVs causes invasive cervical cancer. There is a rare autosomal recessive disease, epidermodysplasia verruciformis, characterized by multiple warts containing many different HPV types which are not normally seen causing skin warts and immunological defects. Warts may undergo malignant change (squamous cell carcinomas) in nearly 30% of these patients, usually in sun-exposed sites.

Diagnosis of papillomavirus infection is clinical and there are many treatments

Wart viruses cannot be cultivated in the laboratory, and serological tests are mainly of epidemiological, rather than diagnostic, use. HPV DNA detection methods can be used to examine samples not only for the HPV type but also to quantify the viral load.

Many treatments have been used for warts, some of them doubtless seeming effective because skin warts eventually disappear without treatment. Treatments of skin warts include the application of karyolytic agents such as salicylic acid and destruction of wart tissue by cryotherapy, freezing with dry ice (solid carbon dioxide) or with liquid nitrogen. The latter is the most commonly used and most effective treatment. Genital intraepithelial lesions, especially cervical, can lead to malignant disease, and treatment to eliminate the infection may involve laser therapy, loop excision, and surgery. Immunomodulating and antiviral agents such as imiquimod and topical cidofovir, respectively, have been used in certain clinical settings.

Molluscum contagiosum is an umbilicated lesion caused by a poxvirus

The poxvirus infects epidermal cells to form a fleshy lesion, often with an umbilicated centre (Fig. 27.36). It only infects humans and is spread by contact, or in the case of genital lesions, by sexual intercourse. There are two antigenically distinct types. Poxvirus particles can be seen by electron microscopy (see Ch. 3).

Orf is a papulovesicular lesion caused by a poxvirus

Orf (contagious pustular dermatitis) is an uncommon infection of the epidermis and is acquired by direct contact with infected sheep or goats. There is a papulovesicular lesion, generally on the hands, which may ulcerate. It is a clinical diagnosis and can be confirmed by electron microscopy.

Herpes simplex virus infection

Herpes simplex virus infections are ubiquitous

Herpes simplex virus (HSV) is a medium-sized (120 nm) double-stranded DNA virus of the herpesvirus group. Two types, HSV-1 and HSV-2, are distinguishable antigenically. It is a ubiquitous infection in early childhood. They cause a wide variety of clinical syndromes, the basic lesion being an intraepithelial vesicle, from which the virus is shed.

Infection is usually transmitted from the saliva or cold sores of other individuals and frequently by kissing and sexual intercourse.

Clinical features of HSV infection include painful vesicles and a latency state

After infection, the virus replicates in cells in the oral mucosa and forms virus-rich vesicles which ulcerate and become coated with a whitish-grey slough (Fig. 27.37).

During the primary infection, virus particles enter sensory nerve endings supplying the affected area of the skin and are transported to the dorsal root ganglion, where they initiate a latent infection (see Ch. 17). The lesion resolves as antibody and CMI responses develop. The latent virus remains in the sensory ganglion for life, and under certain circumstances such as local trauma, can reactivate and spread down sensory nerves to cause cold sores at the site of the original infection (Fig. 27.38).

Primary infection can occur in various sites of the body and may be a result of inadvertent autoinoculation and include:

- in and around the mouth, lips and nose, causing painful, recurrent ulcers
- the eye, to cause conjunctivitis and keratitis, often with vesicles on the eyelids (see Ch. 26)

- the finger, to cause herpetic whitlow
- other skin sites following direct contact with infected individuals where there is rubbing or trauma, for instance in rugby football ('scrum pox') or in wrestlers ('herpes gladiatorum')

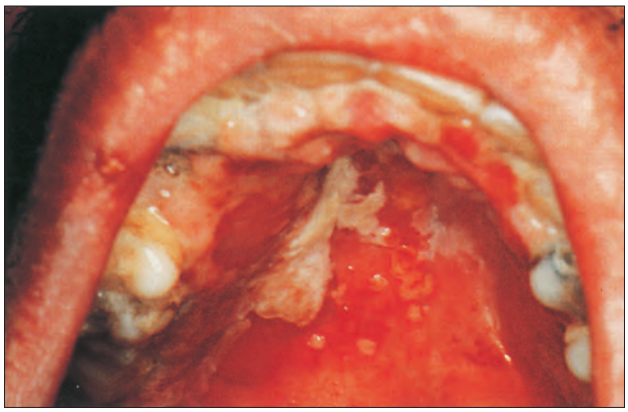


Figure 27.37 Primary herpes simplex virus infection. There are shallow ulcers with white exudate on the palate and gums. (Courtesy of J.A. Innes.)

- the genital tract (see Ch. 22). Although HSV-2 arose as a sexually transmitted variant of HSV-1, the sites infected by the two types are now less clearly distinct.

Serious complications associated with HSV infection include:

- herpetic infection of eczematous skin areas leading to severe disease in young children, eczema herpeticum (Fig. 27.39)
- acute necrotizing encephalitis following either primary infection or reactivation (see Ch. 25)
- neonatal infection acquired from the genital tract of the mother (see Ch. 24)
- primary or reactivating HSV infection in immunocompromised individuals, causing very severe disease (see Ch. 31).

HSV reactivation is provoked by a variety of factors

In healthy individuals, HSV reactivation is provoked by:

- certain febrile illnesses (e.g. common cold, pneumonia)
- direct sunlight
- stress
- trauma
- menstruation
- immunocompromise

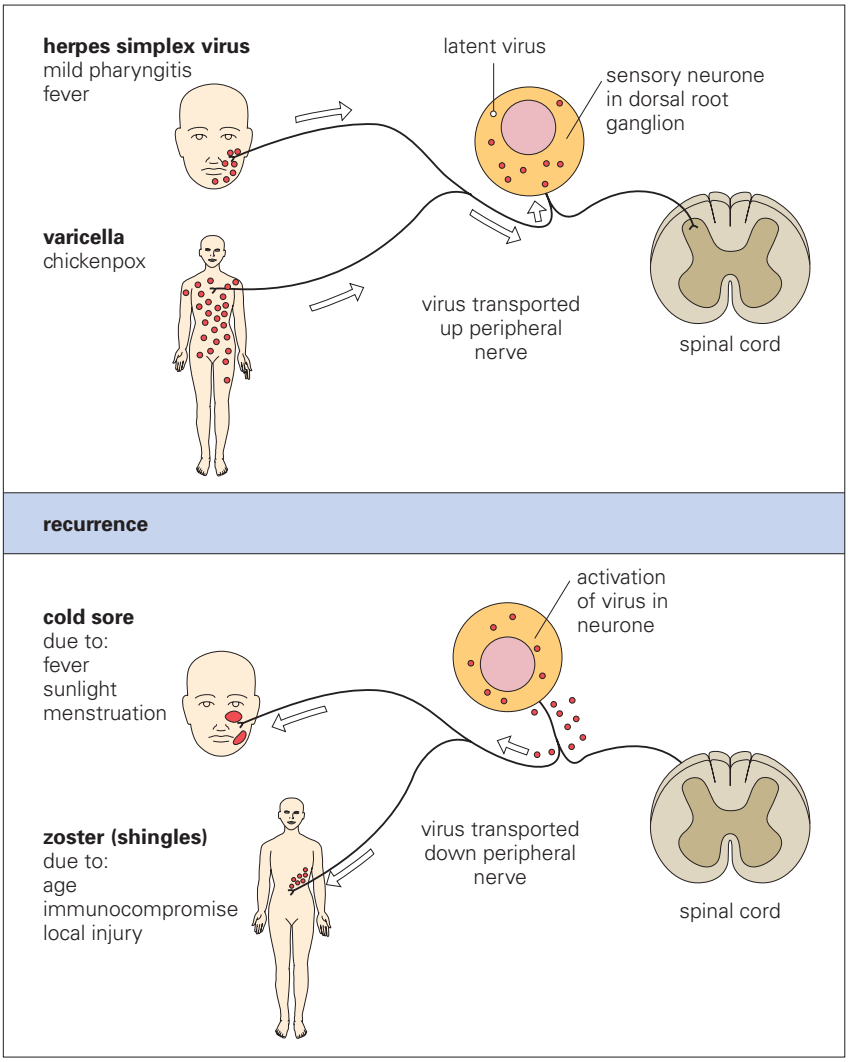


Figure 27.38 Pathogenesis of cold sores and zoster. In both herpes simplex virus and varicella-zoster virus infections, the virus in mucocutaneous nerve endings travels up the axon to reach the dorsal root ganglion where it becomes latent. Recurrences are due to reactivation of the virus within the dorsal root ganglion to become infectious followed by passage of virus down the axon to mucocutaneous site(s) and local spread and replication to form clinical lesion(s).

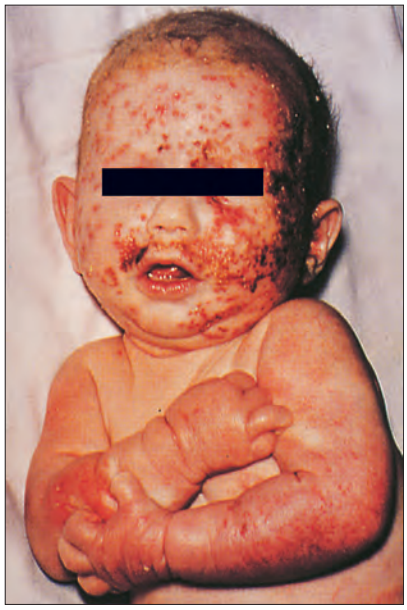


Figure 27.39 Eczema herpeticum due to herpes simplex virus infection in an infant. (Courtesy of M.J. Wood.)

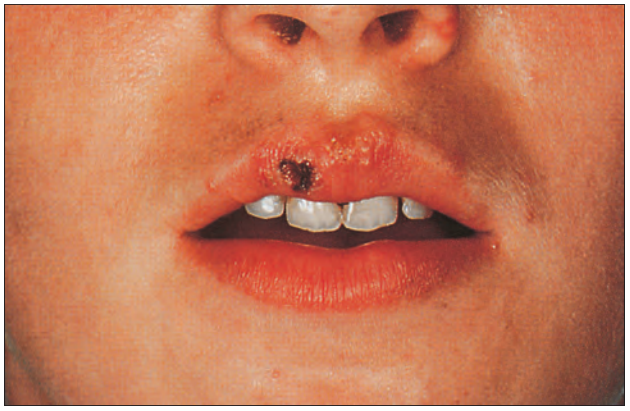


Figure 27.40 Recurrent herpes simplex virus vesicles on the mucocutaneous margin of the lip. (Courtesy of A. du Vivier.)

Reactivation may be very severe in immunocompromised patients (see Ch. 31).

A sensory prodrome in the affected area may include feeling pins and needles, pain, burning, and itching which precedes the appearance of the lesion and is due to virus activity in sensory neurones. The lesion, a so-called 'cold sore', generally occurs around the mucocutaneous junctions in the nose or mouth (Fig. 27.40). Less commonly, when the ophthalmic branch of the trigeminal ganglion is involved, the lesion is a dendritic ulcer of the cornea. Large amounts of virus are shed in the cold sore, which scabs over and heals over the course of about 1 week. Occasionally, the sensory prodrome occurs without proceeding to a cold sore (see also varicella-zoster virus recurrence below).

HSV DNA can be detected in vesicle fluid and infection is treated with aciclovir

HSV DNA may be detected by PCR in vesicular fluid collected from lesions at affected sites. The majority of samples sent to laboratories are from genital lesions, but as HSV can infect various sites, cerebrospinal fluid, skin, eye and mucous membrane swab samples are part of the routine diagnostic laboratory workload. HSV causes a distinct cytopathic effect when grown in cell culture lines such as human embryo lung. However, highly sensitive and specific molecular-based techniques have improved the diagnosis of HSV infection by detecting HSV types 1 and 2 DNA in a variety of samples and have replaced virus culture in many laboratories around the world.

Aciclovir revolutionized the treatment of HSV infection (see Ch. 34) and can be used either systemically or topically, although the antiviral drug penetration is better when given systemically. It is relatively non-toxic and acts specifically in virus-infected cells (see Ch 34). Recurrent HSV can be very disabling and aciclovir prophylaxis may be successful using a lower doses of aciclovir given twice daily for 6 to 12 months, at which time treatment can be stopped and the frequency of recurrent infection reassessed.

Other antiviral treatment options include valaciclovir and famciclovir. Aciclovir must be given intravenously when treating severe HSV infections such as herpes simplex encephalitis or disseminated HSV infection in immunocompromised individuals. Alternative antivirals such as ganciclovir, foscarnet or cidofovir may be used when antiviral resistance is being considered.

Varicella-zoster virus infection

Varicella-zoster virus (VZV) infections are highly infectious and cause chickenpox (varicella) and zoster (shingles)

VZV is a medium-sized (100–200 nm in diameter) double-stranded DNA virus of the herpesvirus group and is morphologically indistinguishable from HSV. There is only one serological type. The virus grows more slowly than HSV and is not released from the infected cell. Infection is by inhalation of droplets from respiratory secretions and saliva, or by direct contact from skin lesions. Primary infection with VZV causes varicella (chickenpox). Immunity develops and prevents re-infection (a second attack of varicella), but the virus persists in the body, and later in life, after reactivation, causes zoster (shingles). Nearly all individuals in resource-rich countries are infected during childhood, but there are many areas of the world where the incidence of chickenpox in children is low, e.g. Africa and the Caribbean islands.

Varicella is characterized by crops of vesicles that develop into pustules and then scab over

After primary infection, the virus passes across surface epithelium in the respiratory tract to infect mononuclear cells, and is then carried to lymphoid tissues. There are no symptoms and no detectable lesions at the site of entry into the body. The virus slowly replicates in lymphoreticular tissues for about 1 week, and then enters the blood in association with mononuclear cells and is seeded out to epithelial sites. These are mainly the respiratory tract and the skin, but also include the mouth, often the conjunctiva, and probably also the alimentary and urogenital tracts. In the skin, for unknown reasons, the trunk, face and scalp are especially involved. At these epithelial sites, the virus exits from small blood

vessels, infecting subepithelial and finally epithelial cells. Multinucleated giant cells with intranuclear inclusions are present in the lesions. In the oropharynx and respiratory tract, the virus reaches the surface and is shed to the exterior to infect other individuals about 2 weeks after initial infection. In the skin, it takes a day or two longer, and it is at this stage, when the characteristic varicella vesicles appear in a centripetal distribution, that a clinical diagnosis can be made (Fig. 27.41). The mean incubation period is 14 days (range 10–23 days).

The patient remains well until a day or two before the rash, when there may be slight fever and malaise, but the illness is usually mild and often unnoticed. The vesicles appear first on the trunk, then on the face and scalp, and less commonly, on the arms and legs. They often come in 'crops' over the course of several days and all stages of lesions occur simultaneously, then develop into pustules, break down, and scab over. The lesions are deeper than with HSV, and scarring is more common. Lesions in the mouth may be painful.

Varicella is usually more severe and more likely to cause complications in adults

The skin lesions of varicella can become infected with staphylococci or streptococci to produce secondary impetigo, but varicella in a child is characteristically a mild illness. The main complications are:

- interstitial pneumonia, especially in adults who smoke; secondary bacterial pneumonia can also occur
- CNS involvement, which may consist of a lymphocytic meningitis or an encephalomyelitis (see Ch. 25).

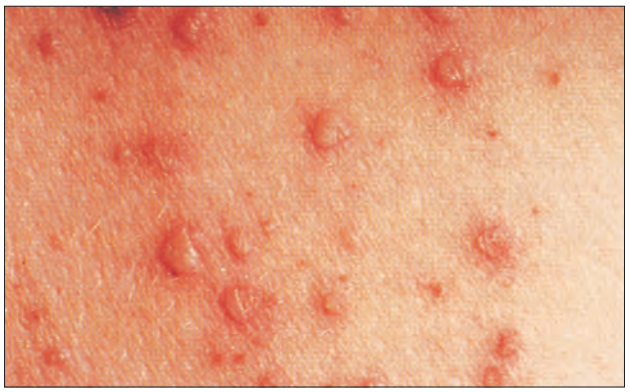


Figure 27.41 Early rash in varicella (chickenpox), with macules, papules and vesicles. (Courtesy of M.J. Wood.)

Thrombocytopenia can occur, but it is usually symptomless. Varicella can be a life-threatening disease in any immunocompromised patients.

After a primary infection during pregnancy, the virus may infect the fetus (see Ch. 24). Congenital varicella syndrome is seen in up to 1–2% if maternal infection occurs in the first or second trimester. Clinical features include skin scarring, hypoplastic limbs, and other stigmata involve the eyes and brain. When the mother is infected a few days before or after delivery, the infant is exposed without the protection of maternal antibody and can suffer a serious disease. Passive immunization with varicella-zoster immune globulin (VZIG) may prevent or attenuate the infection in the infant.

Zoster results from reactivation of latent VZV

During primary infection, VZV in mucocutaneous lesions enters sensory nerve endings and establishes latent infection in the dorsal root ganglia (see Fig. 27.38). Later in life, reactivation can occur to cause zoster in the dermatome, the area of skin supplied by that nerve, at the site of the reactivation. Thoracic dermatomes are most commonly affected because these are the most common sites for the original varicella lesions. Zoster is usually unilateral, unless the individual is immunocompromised, because the reactivation is a localized event in a single dorsal root ganglion. Zoster therefore originates from inside the body and is not directly acquired from either varicella or zoster in other individuals. During reactivation in sensory neurones (see Fig. 27.38), there is paraesthesia and pain. Pain may be severe and precedes the development of the erythematous rash in which virus-rich vesicles appear (Fig. 27.42) by several days. It takes a few days for the virus to travel down peripheral nerves and multiply in the skin. Fever and malaise may accompany the rash. Sometimes, the immune response controls the reactivating virus before skin lesions have had time to form, and in this case, the sensory phenomena occur without the skin eruption. This is referred to as zoster sine herpete, for the classical scholars amongst the readers.

Conditions that predispose to zoster include:

- increasing age – although zoster is very occasionally seen in childhood, its incidence increases with increasing age, rising from 3 / 1000 per year in 50- to 59-year-olds to 10 / 1000 per year in 80- to 89-year-olds
- immunocompromise due to leukaemia, lymphoma, AIDS, solid organ transplantation and drug-induced immunosuppression
- trauma or tumours affecting the brain or spinal cord.

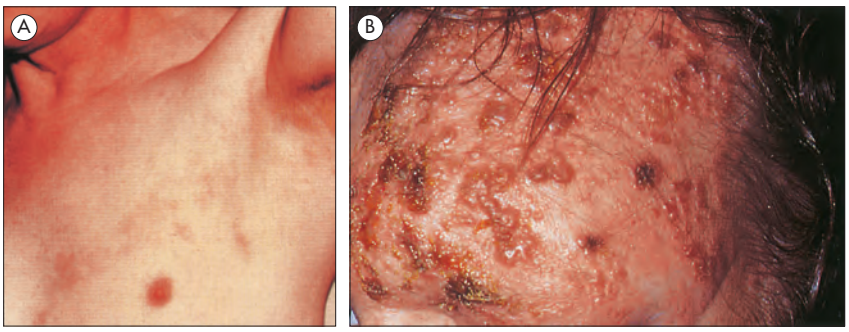


Figure 27.42 Zoster rash. (A) A band of faint erythema, an early sign of shingles, along an intercostal nerve. (B) Rash affecting the ophthalmic division of the trigeminal nerve. (Courtesy of M.J. Wood.)

The skin areas affected by zoster reflect the distribution of the original varicella rash, as might be expected from its pathogenesis (see Fig. 27.38). Hence, the trunk is most commonly involved. Ophthalmic zoster involving the upper eyelid, forehead and scalp is a particularly unpleasant manifestation, which can be sight threatening and is highly infectious as there is an opportunity for a lot of VZV to be shed into the air.

Postherpetic neuralgia is a common complication of zoster

In the healthy host, postherpetic neuralgia (also known as zoster-associated pain, ZAP) is common, especially in the elderly. The pain, which can be severe early in the illness, continues for up to several months after the lesions have resolved. It is difficult to treat, although antiviral agents reduce the incidence, duration and severity of ZAP if started as soon as possible after zoster occurs.

Zoster may be severe in immunocompromised patients. A few days after the localized eruption, the virus, with inadequate control by cell-mediated immunity, spreads via the blood to produce skin and visceral lesions throughout the body. Haemorrhagic complications and pneumonia may occur.

Laboratory diagnosis of VZV

It is a clinical diagnosis that can be assisted by carrying out molecular tests to detect VZV DNA in swabs from the vesicles. Alternative tests less widely used include immunofluorescence tests on skin lesion scrapings using VZV-specific monoclonal antibodies or by isolating VZV in cell culture, although the cytopathic effect may take a couple of weeks. Herpesvirus particles can be seen by electron microscopy in vesicle fluid, but are indistinguishable from other herpesviruses, and in particular HSV, which also causes vesicular lesions. Past infection is determined by detecting VZV IgG by enzyme-linked immunosorbent assay (ELISA) or other methods. A VZV IgM result may be helpful if the skin lesions have healed and the diagnosis needs to be made for clinical reasons.

Treatment of varicella and zoster infection

Higher dose aciclovir is given to treat VZV infections, compared with HSV infections, but this drug, or the more readily bioavailable valaciclovir or famciclovir, can be used orally to treat varicella and zoster. Treating chickenpox is not often considered as it is thought of as a mild infection that causes little discomfort, mostly by those who cannot remember having had chickenpox. However, varicella can cause complications in adolescents and adults, and antiviral treatment should be offered, especially as new lesion formation, viral shedding and symptoms will be reduced. Severe infections must be treated with intravenous aciclovir, especially in high-risk groups. Varicella-zoster immunoglobulin (VZIG) contains a high titre of VZV IgG, pooled from blood donors with a past history of chickenpox. VZIG is used to prevent or attenuate varicella in susceptible individuals at risk of complications (e.g. immunocompromised patients) after exposure, but must be given within 7–10 days of exposure to the source. Varicella skin lesions may be treated with calamine lotion to relieve itching and to prevent scratching and secondary infection.

A live attenuated chickenpox vaccine is licensed in a number of countries, and universal childhood immunization was started in the USA in 1995. In addition, there is a shingles (herpes zoster) vaccine used since 2006 in the United States and reduces the risk in the target age group, 60 years old and over, of both developing shingles and ZAP by just over 50% and nearly 70%, respectively. Other countries have different age ranges for those eligible in their national shingles vaccination programmes.

Rashes caused by enteroviruses

Coxsackieviruses and echoviruses cause a variety of exanthems (skin rashes)

Enteroviruses are positive-sense, single-stranded RNA viruses that are in the Picornaviridae family. There are 71 human enterovirus serotypes and the genus has 12 species, enterovirus A–H and J within which are the coxsackieviruses, echoviruses, enteroviruses, rhinoviruses and polioviruses. Sometimes, such infections are accompanied by an enanthem (lesions on internal epithelial surfaces such as the oral cavity). These infections are generally seen in young children, are not usually distinguishable on clinical examination, and are not severe. These viruses are also responsible for illnesses affecting the CNS (see Ch. 25), the upper respiratory tract (see Ch. 19), and striated and heart muscle (see below). In addition, enterovirus infections have been increasingly associated with the development of type 1 diabetes mellitus.

The lesions are usually vesicular and occur mostly on the buccal mucosa and the tongue. Most children complain of a sore mouth or tongue and there is slight fever. When vesicular lesions are also seen on the skin, principally on the hands and feet, the condition is called 'hand, foot and mouth disease' (Fig. 27.43). The virus is present in the lesions, and coxsackievirus A16 and enterovirus 71 are the most common causes.

Maculopapular rashes resembling rubella and often occurring in the summer are common manifestations of some coxsackie A and echovirus infections.

Rashes caused by human parvovirus B19

Parvovirus B19 causes slapped cheek syndrome

Viruses are small anyway (!), but parvoviruses, as Latin scholars might know, are very small (22 nm in diameter) single-stranded DNA viruses. Parvovirus B19 was identified by

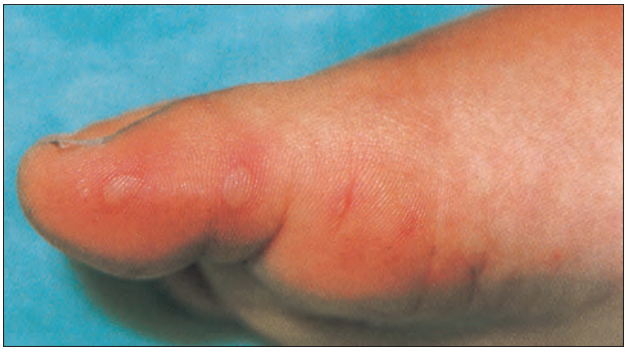


Figure 27.43 Vesicular lesions on the foot in hand, foot and mouth disease. (Courtesy of M.J. Wood.)

chance in 1974 when serum sample number 19 in panel B gave some odd results when tested in hepatitis B surface antigen assays. At that time, electron microscopy was the catch-all method for detecting viruses and particles that looked like animal parvoviruses were seen. Parvovirus B19 is the only member of the Parvoviridae family known to cause human disease and is tropic to erythroid progenitor cells and binds to the P antigen cellular receptor. It causes a febrile illness in children with a characteristic maculopapular rash on the face ('slapped cheek syndrome'). The condition is referred to as 'erythema infectiosum' and sometimes 'fifth disease', it being the fifth of the six common exanthematous infections recognized by nineteenth-century physicians.

Symptomless parvovirus B19 infection is common and spreads by respiratory droplets

Nearly 50% of the population has had parvovirus B19 in the past. The virus grows in haemopoietic cells in the bone marrow, and although this normally causes no more than a temporary and barely detectable fall in haemoglobin levels, it can lead to serious consequences in those with chronic anaemia. In children with sickle cell anaemia, for instance, the effect on erythropoiesis may cause an aplastic crisis. The virus can also cause arthralgia when it infects adults. Laboratory diagnosis is made by testing sera for parvovirus B19-specific IgM. Molecular tests may be used to detect B19 DNA in fetal blood when hydrops fetalis is suspected, after the mother has either had a parvovirus infection or an ultrasound examination of the baby has shown hydrops. Parvovirus B19 cannot be isolated in cell culture.

Rashes caused by human herpesviruses-6 and -7

Human herpesvirus-6 (HHV-6) is present in the saliva of over 85% of adults and causes roseola infantum

Human herpesviruses-6 and -7 were discovered in 1986 and 1990, respectively, the preceding five HHVs being HSV-1, HSV-2, VZV, CMV and EBV. Both infections are ubiquitous globally and occur in most of the population in the first 2 years of life. HHV-6 replicates in T and B cells and also in the oropharynx, from where it is shed into saliva. The virus persists in the body after initial infection. There are two HHV-6 variants, namely HHV-6A and -6B. Their tissue distribution differs in that HHV-6B can be detected in blood, saliva and in brain tissue whereas 6A occurs more often in the lungs and skin.

HHV-6B is the cause of exanthem subitum (also called *roseola infantum*), a very common acute febrile illness in infants and young children. After an incubation period of about 2 weeks, children develop a high fever which lasts for 3–5 days. The disease is mild, and within 2 days of the fever subsiding a maculopapular rash is seen (Fig. 27.44). HHV-6B is also associated with about 30% of febrile fits in children and HHV-6 encephalitis has been reported in bone marrow transplant recipients.

The diagnosis is difficult as HHV-6 DNA may be integrated into human cell chromosomes, so detecting HHV-6 DNA may not be diagnostic of an active infection. This means that the HHV-6 DNA load needs to be quantified in the peripheral blood as well as the sample from the body compartment affected, in order to make a diagnosis of HHV-6 infection.

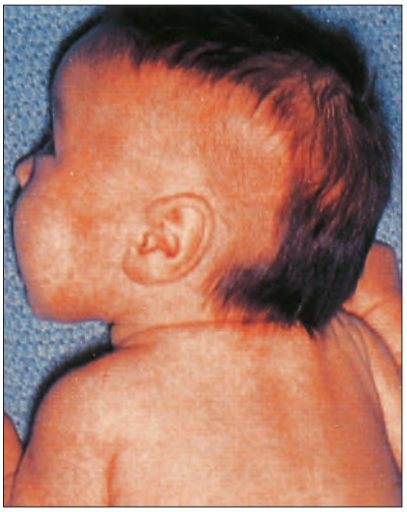


Figure 27.44 Maculopapular rash in roseola infantum. (Courtesy of M.J. Wood.)

Human herpesvirus-7 (HHV-7) is acquired slightly later in early childhood

HHV-7 has been isolated from CD4-positive T cells. Infection occurs later than HHV-6 during infancy and early childhood. Persistence in the saliva and exanthem subitum has been reported with HHV-7.

Human herpesvirus-8 (HHV-8) is associated with all forms of Kaposi's sarcoma skin lesions

After a number of epidemiological reports, it had been thought that a transmissible agent was involved in the development of Kaposi's sarcoma (KS), a skin malignancy more common in some Mediterranean areas and parts of Africa in addition to AIDS-associated KS. A number of molecular technology developments allowed the identification of KS-associated herpesvirus (KSHV), also known as HHV-8, in 1994 in the endothelial cells of KS lesions. It is not a ubiquitous infection and has also been associated with two other rare malignancies, primary effusion lymphoma and multicentric Castleman's disease. Transmission is mostly via saliva, and interactions between defective cellular immune responses, the endothelial system and KSHV result in the pathogenesis of KS.

It is a clinical diagnosis. The incidence of AIDS-associated KS has dropped since the advent of combined antiretroviral therapy. In addition, retrospective studies have shown that ganciclovir and foscarnet treatment resulted in a reduction of KS lesions.

SMALLPOX VIRUS INFECTION

Smallpox (variola) was a major scourge of humankind for at least 3000 years. It was caused by a poxvirus and spread from person to person by contact with skin lesions and via the respiratory tract. The disease was severe, with a generalized rash (Fig. 27.45), and was fatal in up to 40% of cases, depending upon the strain of virus.

Global smallpox eradication was officially certified in December 1979

During the first part of the twentieth century, smallpox was largely eradicated from Oceania, North America and Europe

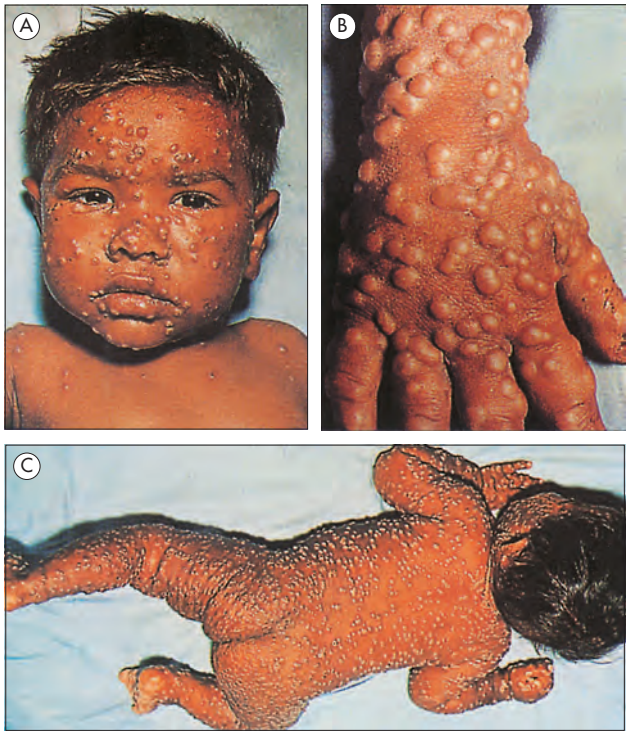


Figure 27.45 Smallpox. (A–C) These pictures were used as smallpox recognition cards by the World Health Organization during its smallpox eradication campaign. After upper respiratory tract infection, the virus reached the skin, where it replicated to cause a widespread vesiculopustular rash, with later scarring, especially on the face. The fatality rate was up to 40%, depending on the age of the host and the strain of the virus. (Courtesy of the World Health Organization.)

by widespread vaccination, as originally developed by Edward Jenner (see Ch. 35), using a live attenuated strain of virus (vaccinia virus), together with strict controls at frontiers. In 1967, the World Health Organization (WHO) started a campaign to eradicate smallpox from the world, focusing on South America, Africa, India and Indonesia, making use of vaccination, surveillance and containment of cases. Despite such daunting difficulties as cultural barriers, warfare and transport to remote areas, the campaign was successful. Occasional cases had continued to occur in the USA until the 1940s, and in 1974, there were 218 000 cases worldwide, mostly in Asia, but the last case was recorded in Somalia in October 1977. The total cost to WHO was about US$150 million.

Global eradication of smallpox was possible for a variety of reasons

These reasons are as follows:

- There were no subclinical infections, so cases could be readily identified.
- The virus was eliminated from the body on recovery, with no carriers.
- Humans were the only host (no animal reservoir).
- An effective vaccine was available.

For a few years there were concerns about monkeypox, a simian disease caused by a similar virus and acquired by contact with infected monkeys in Africa. It is, however, poorly transmitted from human to human. However, over 80 people were believed to have contracted monkeypox in the USA in 2003. This was thought to be due to contact with infected prairie dogs. Concerns about the use of smallpox by bioterrorists have resulted in countries making contingency plans for the potential threat, which include the stockpiling of smallpox vaccine.

MEASLES VIRUS INFECTION

Measles has several special features, as follows:

- Nearly all infected individuals become unwell and develop disease. This is in contrast to most other viral infections, in which a significant proportion of individuals undergo an asymptomatic or subclinical infection.
- The disease is so characteristic that a clinical diagnosis can nearly always be made without the need for laboratory help. We can recognize measles as described 1000 years ago by the Arabian physician, Rhazes.
- There is only one antigenic type of measles virus.
- After infection, there is complete resistance to re-infection, which is probably life-long. Second attacks are almost unknown.
- Measles is highly infectious, and nearly all susceptible children contract the disease on exposure. Measles was regarded as a routine inescapable part of childhood, and more than 99% of individuals were infected until immunization programmes were developed.
- There is a striking contrast between measles in well-nourished children with good access to medical care (i.e. in resource-rich countries) and measles under conditions of malnutrition or starvation or with poor medical services (i.e. resource-poor countries, Table 27.4).

Aetiology and transmission

Measles outbreaks occur every few years in unvaccinated populations

The basic virology of this paramyxovirus is described in Chapter 3 and it is transmitted by respiratory droplets. Although the virus is soon inactivated as it dries on surfaces, it is more stable in droplets suspended in the air. In unvaccinated populations, outbreaks tend to occur every few years when the number of susceptible children reaches a high enough level. There were a number of measles outbreaks in Europe in 2011, some of which were large, including more than 7000 cases in France that resulted in further immunization campaigns in a number of countries.

Clinical features of measles include respiratory symptoms, Koplik's spots and a rash

The inhaled virus enters the body in the upper or lower respiratory tract or conjunctiva and spreads to subepithelial and local lymphatic tissues, without causing detectable lesions or symptoms. During the next few days, there is a primary viraemia and the virus, which is highly lymphotropic, slowly spreads and multiplies in lymphoid tissues elsewhere in the body, including the spleen and the respiratory tract. There is then a secondary viraemia around 5 days after the initial infection and the virus disseminates to a variety of epithelial sites including the skin, kidney, and bladder. Clinical signs soon appear in the respiratory tract where there are only one or two layers of epithelial cells to traverse. The patient

Table 27.4 The clinical impact of measles depends upon the condition of the host

Site	Well-nourished child Good medical care	Malnourished child Poor medical care
Lung	Mild respiratory illness	Life-threatening pneumonia
Ear	Otitis media quite common	Otitis media more common, more severe
Oral mucosa	Koplik's spots	Severe ulcerating lesions
Conjunctiva	Conjunctivitis	Severe corneal lesions, secondary bacterial infection, blindness may result
Skin	Maculopapular rash	Haemorrhagic rashes may occur ('black measles')
Intestinal tract	No lesions	Diarrhoea – exacerbates malnutrition, halts growth, impairs recovery
Overall impact	Serious disease in a small proportion of those infected	Major cause of death in childhood (estimated 1 million deaths/year worldwide)

Measles is a much more serious disease in malnourished children with poor access to medical care. The same epithelial surfaces are infected more extensively and with more serious sequelae.

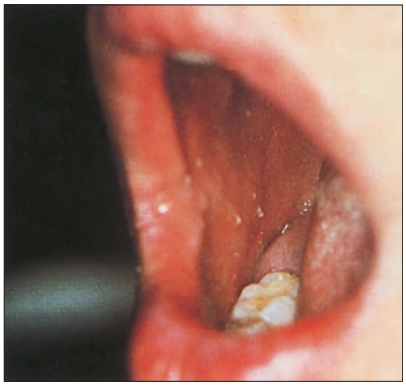


Figure 27.46 Koplik's spots seen as minute white dots on the inflamed buccal mucosa of a patient with measles. (Courtesy of M.J. Wood.)

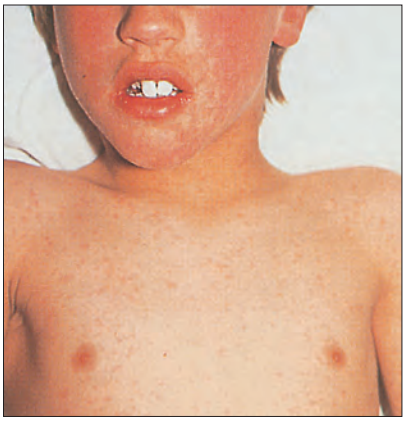


Figure 27.47 Maculopapular rash on the face and trunk of a patient with measles. (Courtesy of M.J. Wood.)

is well until 9–10 days after infection and then develops an acute respiratory illness with a runny nose, fever and cough. Conjunctivitis is also a feature, and as a result of the large amounts of virus being shed in respiratory secretions, the patient is highly infectious. The diagnosis may be suspected during this prodromal illness, especially after known exposure to measles. It takes a day or two longer for the foci of infection at mucosal and skin surfaces to cause lesions. Koplik's spots, pathognomonic of measles, appear inside the cheek (Fig. 27.46), and shortly afterwards at around 12 days after infection, the maculopapular rash (Fig. 27.47) is seen, first on the face, then spreading down the body to the extremities.

Measles rash results from a cell-mediated immune response

Antibodies are formed, but a cell-mediated immune (CMI) response is needed to control the virus in the lungs and elsewhere. Without it, the virus continues to multiply and gives rise to giant cell pneumonia (see Ch. 20). The CMI response is also responsible for the skin lesions, which are not seen in patients with serious defects in this type of immunity. Children with agammaglobulinaemia, on the other hand, have a normal course of disease, develop normal immunity, and can be protected by vaccination. In uncomplicated cases, recovery is rapid.

During measles, as in a variety of other acute infections, there are temporary defects in immune responses to unrelated antigens. For instance, at about the time the rash appears, individuals who are known to be tuberculin-positive give negative skin test responses to tuberculin. This returns to normal in about 1 month. During the 'virgin-soil' epidemic, when measles reappeared after a long absence in Southern Greenland, in 1953, and adults as well as children were infected, there was increased mortality in those previously infected with tuberculosis.

Complications of measles are particularly likely among children in resource-poor countries

Complications of measles, due to the loss of memory B and T cells and a resulting generalized immune suppression, include:

- opportunistic bacterial superinfections, which are quite common, especially otitis media and pneumonia, as a result of virus damage to respiratory surfaces
- a primary measles virus pneumonia (giant cell pneumonia), which is seen in patients with serious CMI response defects
- post-infectious encephalitis, which occurs in about 1 in 1000 patients (see Ch. 25)
- very rarely, subacute sclerosing panencephalitis (SSPE). This develops 1–10 years after apparent recovery from acute infection.

Table 27.5 Clinical consequences of rubella virus invasion of different body tissues

Site	Result	Comment
Respiratory tract	Virus shedding but symptoms minimal (mild sore throat, coryza, cough)	Patient infectious 5 days before to 3 days after symptoms
Skin	Rash	Often fleeting, atypical; immunopathology involved (Ag-Ab complexes)
Lymph nodes	Lymphadenopathy	More common in posterior triangle of neck or behind ear
Joints	Mild arthralgia, arthritis	Immunopathology involved (circulating immune complexes)
Placenta/fetus	Placentitis, fetal damage	Congenital rubella

Children in countries where there is poor medical care and malnutrition develop a more serious disease (see Table 27.4), especially during famine. This is attributable to:

- poor local mucosal defences, which can be improved by vitamin A administration
- impaired immune defences due to protein–calorie malnutrition, with the added impact of measles virus-induced immunosuppression
- poor medical services, with less ready availability of antibiotics to control secondary infection
- high levels of bacterial contamination of the environment
- exposure to a larger virus dose – a possible factor if others with severe measles shed larger amounts of virus from the respiratory tract.

Diagnosis, treatment and prevention

Measles is usually diagnosed clinically; ribavirin can be used as antiviral treatment if clinically indicated and there is a safe and effective vaccine

Although the clinical diagnosis should be clear, the rash is similar to a number of other viral exanthems which affect the same age group. Koplik's spots and conjunctivitis help with the definitive diagnosis. In addition, with the success of the vaccine, the incidence of measles infection fell and it was less likely that healthcare workers would see children with measles in resource-rich countries. However, after the controversial publication of a study in 1998 in which measles, mumps and rubella (MMR) vaccine was linked with autism, which was never confirmed and all the subsequent epidemiological investigations showed there was no link, there was both a reduction in immunization rates and increase in measles and mumps infections around the world. Since then, MMR vaccine rates have risen with a corresponding reduction in infection.

Detecting measles virus RNA or carrying out a measles-specific IgM assay is helpful in confirming the diagnosis either on blood or saliva samples. Virus isolation in cell culture is rarely necessary. Complicated measles infection can be treated with ribavirin.

A live attenuated vaccine has been available since 1963. It is effective, safe and long lasting, and is combined with mumps and rubella vaccines (MMR vaccine; see Ch. 35). Before a vaccine became available, measles killed 7–8 million children each year worldwide. By 1996, this was reduced to 1 million, and the WHO/UNICEF initiatives included applying the mass immunization programmes used in the Americas and Europe to resource-poor countries.

RUBELLA VIRUS INFECTION

Rubella virus infection causes a multisystem infection, but its main impact is on the fetus

There is only one serotype of this single-stranded RNA togavirus, and its principal impact is on the fetus (see Ch. 24). It is transmitted by droplet infection, and is less contagious than measles, but more so than mumps.

After entering the body via the upper respiratory tract, the virus replicates for a period in local lymphoid tissues, followed by spread to the spleen and to lymph nodes elsewhere in the body. One week after infection further multiplication in these tissues leads to viraemia and localization of virus in the respiratory tract and skin, and sometimes the placenta, joints and kidney. The clinical consequences of infection in various tissues of the body are shown in Table 27.5.

After an incubation period of 14–21 days, there is a mild disease, with fever, malaise and an irregular maculopapular rash lasting 3 days. Enlarged lymph nodes are often evident behind the ear, but the infection is commonly subclinical.

Rubella is diagnosed in the laboratory; there is no treatment, but there is a vaccine

Clinical diagnosis of rubella is sometimes possible but must be confirmed in the laboratory. Laboratory diagnosis is made by demonstrating rubella-specific IgM antibodies or detecting rubella virus RNA (see Ch. 33). Virus isolation from the throat is rarely indicated – virus isolation requires specialized cell lines, and indirect methods are needed to demonstrate its growth. Viral RNA can be detected in samples from different sites.

There is no antiviral treatment. A live attenuated rubella vaccine that is safe and effective is given by injection, generally in combination with measles and mumps vaccines (MMR vaccine). Prevention of congenital rubella is referred to in Chapter 24.

OTHER MACULOPAPULAR RASHES ASSOCIATED WITH TRAVEL-RELATED INFECTIONS

The maculopapular rashes seen in certain arthropod-borne virus infections (e.g. dengue) and in zoonotic virus infections (e.g. Marburg disease) are referred to in Chapters 28 and 29. A maculopapular rash may be rarely seen in the prodromal stage of hepatitis B virus infection and is immune complex mediated.

OTHER INFECTIONS PRODUCING SKIN LESIONS

Other bacterial, fungal and rickettsial infections produce a variety of rashes or other skin lesions

Most of these are referred to elsewhere in this book, and they are listed in Table 27.1. Rashes in rickettsial infections are often striking, as in the case of Rocky Mountain spotted fever or typhus (see Ch. 28). Most rickettsia invade vascular endothelial cells and are shed into the blood to infect blood-sucking arthropod vectors. Invasion of vascular endothelial cells in the skin provides the basis for the skin rash but is not a source of direct shedding to the exterior.

KAWASAKI SYNDROME

Kawasaki syndrome is an acute vasculitis and is probably caused by superantigen toxins

The Kawasaki syndrome is a childhood illness that occurs in genetically susceptible hosts with dysregulated T-cell activation after exposure to infectious triggers. Patients, who are generally under 4 years of age, develop fever, conjunctivitis and a rash. There is dryness and redness of the lips and red palms and soles with some oedema, desquamation of fingertips, often arthralgia and myocarditis, which gives a case mortality of about 2%. The basic pathology is an acute multisystemic vasculitis, and 20% of untreated patients develop coronary artery aneurysms. The disease is more common in those of Asian ethnicity, but occurs worldwide. There is no clear evidence for person-to-person transmission and the disease is endemic with seasonal fluctuations and outbreaks. It is thought to be of infectious origin, and the mechanism of immune activation may be due to either an antigen or superantigen such as the toxins (see Ch. 17) of *Staph. aureus* or *Strep. pyogenes*. A superantigen is a group of proteins that can stimulate many T cells by attaching to part of the T-cell receptor in association with the MHC class II molecules without needing antigen processing.

Treatment with intravenous immunoglobulin and aspirin reduces the incidence of coronary artery damage and prevents the aneurysms if given early enough.

VIRAL INFECTIONS OF MUSCLE

Viral myositis, myocarditis and pericarditis

Some viruses, particularly coxsackievirus B, cause myocarditis and myalgia

A cytotoxic effect is seen in animal models after viral attachment to the cellular receptors found in cardiac myocytes and macrophages. Group B, and to a lesser extent group A, coxsackieviruses and certain enteroviruses are the main viral causes of acute myocarditis and pericarditis. There is a slight male predominance in myocarditis and both myocarditis and pericarditis can be mistaken for myocardial infarction, yet the prognosis is good and complete recovery is the rule. There is also evidence for persistent infection linked with chronic myocarditis and chronic dilated cardiomyopathy. The most common cause of viral myocarditis in infants is the coxsackievirus B group, and it may be rapid in onset and fatal. These infections are transmitted by the faecal–oral route and occasionally from pharyngeal secretions. Ingested coxsackieviruses spread from the pharynx or gastrointestinal mucosa to the lymphatics and then to the blood. Invasion of striated muscles, heart or pericardium takes place across small blood vessels and results in acute inflammation. In the heart and pericardium, this gives rise to dyspnoea, pain in the chest, and sometimes mimics a myocardial infarction. Coxsackievirus may be isolated from throat swabs, faecal specimens or occasionally pericardial fluid but enterovirus RNA detection methods and then typing positive samples are more widely used, for example in-situ hybridization on endomyocardial biopsy tissue. Mumps and influenza are less common causes of myocarditis or pericarditis. Rubella (see Ch. 24) can cause myocarditis and associated congenital lesions in the fetus.

Group B coxsackieviruses also cause pleurodynia or epidemic myalgia. This condition is sometimes called 'Bornholm disease', after the Danish island where there was an extensive outbreak in 1930. There is pain and inflammation involving intercostal or abdominal muscles.

Influenza (especially influenza B in children) can cause pain and tenderness in muscles, but it is not known whether this is associated with viral invasion of muscle. Myalgias are also seen in dengue and in rickettsial and other febrile infections and are probably caused by circulating cytokines.

Laboratory diagnosis in these settings can be difficult, as molecular-based methods, serology and virus isolation can give only circumstantial evidence for association between that virus infection and a specific organ. Direct detection methods in affected tissue may be more helpful.

The antiviral drug pleconaril has been used to treat enterovirus infections. However, it is no longer in production. There are no specific vaccines for coxsackievirus infections. The mainstay of treatment involves medical management of acute heart failure. This can involve mechanical circulatory support and extracorporeal membrane oxygenation (ECMO) in some clinical situations.

Postviral fatigue syndrome

It has been difficult to establish postviral fatigue syndrome as a clinical entity

The postviral fatigue syndrome or chronic fatigue syndrome is sometimes referred to as myalgic encephalomyelitis, but this is inappropriate because there is no evidence for CNS pathology. It consists of:

- chronic and severe muscle weakness, lasting at least 6 months, often as a sequel to an acute febrile illness
- severe tiredness
- less regularly associated symptoms such as depression, headache and anxiety.

It is more reliably identified when the first two symptoms appear in a previously healthy individual with no history of psychosomatic illness. Several viruses have been suggested as causes. There have been repeated claims for the role of coxsackie B viruses, based on antibody tests and on the detection of a virus-specific protein in the serum of patients, but these results have not been widely confirmed, and the picture remains unclear. A small proportion of cases appear to be due to chronic infection with Epstein–Barr virus (EBV). Occasional reports have associated the condition with HHV-6 as well as other viruses. It has also been suggested that it is due to 'allergic reactions' triggered by virus infections.

In 2009, a gammaretrovirus called xenotropic murine leukaemia virus (XMRV) was detected in peripheral blood mononuclear cells from nearly 67% of people with chronic fatigue syndrome compared with 4% of healthy controls. However, the association was not confirmed in other studies and it was a salutary lesson about sensitive methods of detection as well as good laboratory practice as it was shown that the genomic sequences detected were actually part of the enzymes used in the PCR process.

PARASITIC INFECTIONS OF MUSCLE

Relatively few protozoan or helminth parasites invade muscle tissues and cause serious disease. Three of the more common are described here to illustrate the variety of organisms and the range of pathology.

Trypanosoma cruzi infection

Trypanosoma cruzi is a protozoan and causes Chagas disease

Chagas disease is also known as American trypanosomiasis (see Ch. 28). The disease is restricted to Mexico, Central and South America, where up to 15 million people are infected. It is a zoonosis, and *Trypanosoma cruzi* has been isolated from more than 150 species of mammal. The parasite is carried by blood-sucking reduviid bugs, which deposit infective trypomastigote stages on to the skin as they defecate while feeding. If these are rubbed into mucous membranes or wounds, the parasites penetrate cells, transform into amastigotes and multiply. The infected cells then burst, liberating trypomastigotes, and a local lesion is formed. The parasite is dispersed around the body to reinvade other cells. Major sites of infection include the CNS, intestinal myenteric plexus, reticuloendothelial system and cardiac muscle.

Chagas disease is complicated by cardiac conduction disorders, ventricular aneurysm formation or heart failure many years later

Chagas disease may be asymptomatic from the outset or occur as an acute febrile phase, with intense inflammatory changes, followed by a chronic phase that may produce no apparent damage (indeterminate phase), or progress to cause damage 20–30 years later. In the chronic phase, there is gradual tissue destruction with autoimmune damage playing a part. 95% of those with clinical features of chronic Chagas disease have cardiac manifestations and 5% have either megaoesophagus or megacolon. The parasite invades the myofibrils of the heart (see Fig. 28.15) causing myocarditis, and muscle fibrils and Purkinje fibres may be replaced by fibrous tissue. As a result of the conduction defects this causes, the heart enlarges, there are cardiac arrhythmias, and heart failure can occur.

Benznidazole or nifurtimox are used to treat the acute phase and some cases in the indeterminate or chronic phase. These drugs are available from the World Health Organization. At the time of writing, no vaccine is available, and prevention of infection is the most important measure.

Taenia solium infection

The larval stages of *Taenia solium* invade body tissues

Tapeworms are intestinal parasites, but the larval stages of several species may invade deeper tissues. The most important of these are:

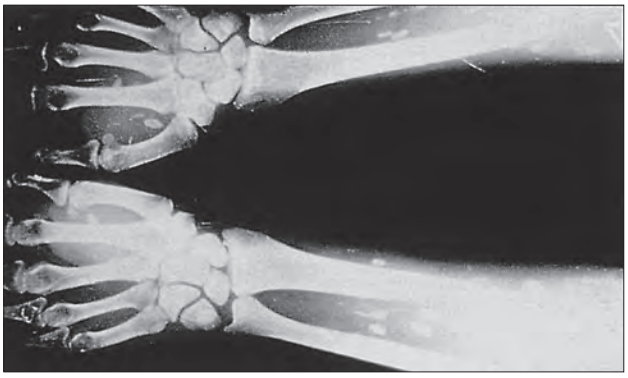


Figure 27.48 Radiograph showing numerous calcified cysts of *Taenia solium* in the forearms. (Courtesy of R. Muller and J.R. Baker.)

- *Echinococcus granulosus*, which causes cystic echinococcosis or cystic hydatid disease; see Chs. 25 and 29.
- the pork tapeworm *Taenia solium*.

Humans acquire *T. solium* infection by eating undercooked infected pig meat in which the cysticercus larvae are found as small, bladder-like structures in the muscle tissue. These larvae are digested out in the intestine and mature into the adult tapeworm, which may reach a length of several metres. *T. solium* eggs released in human faeces and ingested by a pig hatch in the pig intestine and release larvae which cross the intestinal wall and are carried via the blood stream to muscle. *T. solium* is unusual in that its eggs can hatch directly in the human intestine and behave in the same way as they would do in a pig. In areas of poor sanitation, this may result from accidental swallowing of water or food contaminated with eggs. If hatching occurs, larvae can invade and form cysticerci in human muscle or, much more seriously, in the CNS. Cysts in muscle eventually become calcified and can be seen on radiography (Fig. 27.48). Muscle infection is not serious, being largely asymptomatic. Infections are common in many parts of the world, particularly South and Central America and Asia. Avoidance of undercooked pork products is the safest precaution against developing pork tapeworm, whereas good sanitation and good personal hygiene practice are required to avoid ingesting eggs and thus developing cysticercosis.

Trichinella infection

The larvae of *Trichinella* invade striated muscle

This nematode has many unique features. It is able to infect almost any warm-blooded animal, and has a life cycle in which a complete generation (infective stage to infective stage) develops within the body of a single host. Humans can be infected by a variety of *Trichinella* species, *T. spiralis* being the most common. Transmission depends upon the ingestion of muscle tissue containing viable infective larvae. As far as humans are concerned, the commonest route of transmission is through infected pig meat, but many other meat sources have been known to transmit infection (e.g. bear, boar, horse). Infections occur worldwide. When infected undercooked meat is eaten, the larvae are digested out in the small intestine and develop rapidly into the adult worms. These live in the mucosa, each female releasing about one thousand newborn larvae directly into the intestinal tissues, from where they are

carried in blood or lymph around the body. Eventually, the larvae penetrate striated muscles and mature into the infective stage, transforming muscle cells into a parasite-sustaining nurse cell (see Fig. 29.11).

Light infections are asymptomatic, but the migration and penetration of the larvae is associated with inflammatory reactions, which can be severe and life threatening when a person is heavily infected. Various symptoms are associated with this phase, of which fever, muscle pains, weakness and eosinophilia are characteristic. Myocarditis may also occur, although the parasite does not develop in the heart.

Diagnosis on clinical criteria is usually made after the parasites have invaded the muscles and treatment then is difficult. Albendazole or mebendazole are used to kill adult females in the intestine and prevent production of more larvae. Adjunctive corticosteroids are given in severely symptomatic cases to treat myositis.

Sarcocystis

Sarcocystis is a rare muscle parasite

The cyst stages of *Sarcocystis*, a protozoan related to *Toxoplasma*, are occasionally reported in human muscles. Outbreaks of myalgia and myositis due *Sarcocystis nesbitti* occurred in 2011 to 2012 in visitors to South-East Asia.

JOINT AND BONE INFECTIONS

Joints and bones will be considered separately for convenience, but joint lesions often spread to involve neighbouring bone, and vice versa (e.g. in tuberculosis).

Reactive arthritis, arthralgia and septic arthritis

Arthralgia and arthritis occur in a variety of infections and are often immunologically mediated

Examples of such infections are outlined in Table 27.6. Joints can become infected by the haematogenous route or directly following trauma or surgery, but in many cases the condition is immunologically mediated rather than due to microbial invasion of the joint. The pathogen responsible is at a distant site in the body and causes a 'reactive arthritis'. Reactive arthritis and arthralgia occur after certain enteric bacterial infections, and the arthralgia in rubella and hepatitis B infections is of similar origin. In this type of arthritis, more than one joint is usually affected.

Ankylosing spondylitis is associated with *Klebsiella* infection, and it has been suggested that the antigenic similarity between *Klebsiella* and HLA B27 antigens provokes a cross-reactive immune response that causes the disease. So far, there is no evidence that rheumatoid arthritis is caused by either viruses or other microbes.

Circulating bacteria sometimes localize in joints, especially following trauma

Such bacterial localization can then cause a suppurative (septic) arthritis. Generally, a single joint is involved. Joints are very susceptible, particularly if they are already damaged, for instance by rheumatoid arthritis, or if a prosthesis has been inserted. Knees are most commonly affected, followed by hips, ankles (see Fig. 22.5B) and elbows. Signs include a fever, joint pain, limitation of movement and swelling,

Table 27.6 Arthralgia and arthritis in infectious diseases

Infectious agent	Comments
Viral arthritis	
Hepatitis B	Occurs in prodromal period; due to circulating immune complexes
Rubella	Especially in young women, often follows live virus vaccine
Mumps	Unusual; mostly in men
Ross River and other togaviruses	Mosquito-transmitted infections in Australia (Ross River) and Africa
Parvovirus	May follow adult infection
Reactive arthritis	
Campylobacter, Yersinia, salmonellae, shigellae, *Chlamydia trachomatis* (Reiter's syndrome[a])	'Post-infectious' arthritis, HLA B27-associated, no bacterial invasion of joint, immune mediated
Septic arthritis	
Staphylococcus aureus	Commonest cause of suppurative arthritis
Streptococci (Group A and B)	Common in adults and children
Haemophilus influenza	Occurrence in children has decreased with *H. influenzae* vaccine
Neisseria gonorrhoeae	May affect multiple joints
Mycobacterium tuberculosis	Often with bone lesions, especially weight-bearing joints and bones
Borrelia burgdorferi	Arthritis a late feature of Lyme disease
Gram-negative bacilli	Neonates, the elderly, patients with immune deficiency disorders
Sporothrix schenckii	Fungal infection of joints; increased risk with HIV infection

[a]Urethritis, arthritis, uveitis, mucocutaneous lesions; complicates a small percentage of cases of chlamydial urethritis.

and usually a joint effusion. Bacteria can be isolated from the joint fluid or seen in the centrifuged deposit, and the commonest organism is *Staph. aureus*. Sometimes the source of the circulating bacteria is obvious (e.g. a septic skin lesion), but often no source is apparent.

Osteomyelitis

Bone can become infected by adjacent infection or haematogenously

As with joints, infection of bones can be by the direct route (e.g. from a nearby focus of infection, after fractures, after orthopaedic surgery) or from circulating pathogens. The commonest cause of haematogenous osteomyelitis is *Staph. aureus*, but when infection is from a neighbouring site it is generally mixed, with Gram-negative rods and occasionally anaerobes also present. There seems to be no equivalent to reactive arthritis, in which inflammation is due to infection at a distant site.

Acute osteomyelitis typically involves the growing end of a long bone, where sprouting capillary loops adjacent to epiphyseal growth plates promote the localization of circulating bacteria. It therefore tends to be a disease of children and adolescents, and may follow non-penetrating injury to the bone.

Osteomyelitis results in a painful tender bone lesion and a general febrile illness.

Osteomyelitis is treated with antibiotics and sometimes surgery

The infection is diagnosed from blood cultures taken before the start of antimicrobial therapy or, if there is an open lesion, from a bone biopsy. Periosteal reaction and bone loss may be visible radiologically (Fig. 27.49). Treatment is begun on a 'most likely' basis (e.g. nafcillin for MSSA, see above) as soon as microbiological samples have been taken.

Osteomyelitis can become chronic, especially when there are necrotic bone fragments to act as a continued source of infection. Surgical intervention for debridement and drainage, as well as prolonged courses of antibiotics, may be necessary.

Tuberculosis may affect the spine, the hip, the knee and the bones of the hands and feet, and in resource-rich countries is particularly seen in immigrants from the Indian subcontinent. Constitutional disturbances are often absent, but the site is generally painful and pressure from a tuberculous abscess in the spine can cause paraplegia.

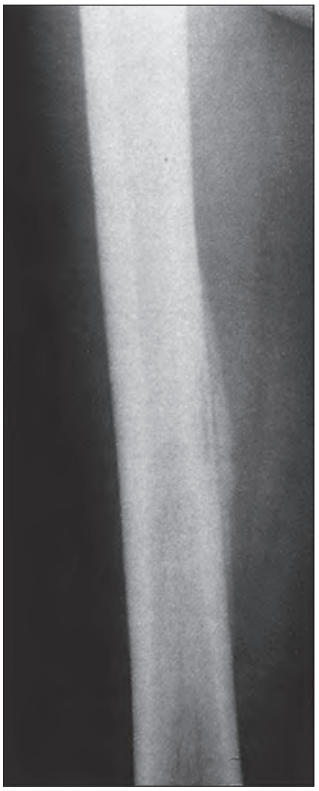


Figure 27.49 Acute staphylococcal osteomyelitis in the femur of a 24-year-old woman. There is a well-defined periosteal reaction in relation to the midshaft of the femur and an underlying lucency. (Courtesy of A.M. Davies.)

INFECTIONS OF THE HAEMOPOIETIC SYSTEM

Many infectious agents cause changes in circulating blood cells

Examples of such agents include:

- *Bordetella pertussis*, which causes lymphocytosis
- EBV and cytomegalovirus infections, which cause mononucleosis
- *Plasmodium* spp., which cause anaemia and thrombocytopenia.

A smaller number of infectious agents act directly on cells in the bone marrow (human parvovirus) or cause malignant transformation of lymphocytes, for example, human T-cell lymphotropic virus (HTLV) type 1. The range of possibilities is summarized in Table 27.7. HTLV-1 and HTLV-2 are mentioned earlier (see Ch. 24) but are described more fully below.

Human T-lymphotropic virus type 1 infection

HTLV-1 is mainly transmitted by maternal milk

Human T-lymphotropic virus type 1 was first isolated in 1980 from a patient with adult T-cell leukaemia (ATLL). Infection is widespread, especially in certain islands in the West Indies and Japan, where 5–15% of the population are infected, and also in South America and parts of Africa. Transmission is primarily via maternal milk, less effectively via sexual intercourse, and by blood-contaminated equipment in injecting drug users.

HTLV-1 infects T cells and up to 5% of those infected develop T-cell leukaemia

HTLV-1 infects T cells and persists. The tax gene product, a transcriptional activator protein, stimulates transcription of host genes controlling production of interleukin 2 (IL-2), IL-2 receptor and other molecules, thus affecting cell replication. Infected T cells proliferate, and if in addition there are certain chromosomal abnormalities, malignant transformation takes place.

Clinically, the patient develops a mild febrile disease with lymphadenopathy. The skin is often involved, with nodule and plaque formation, and pleural effusion or aseptic meningitis can occur. There is also increased susceptibility to opportunist infections such as *Pneumocystis jirovecii* and *Strongyloides stercoralis*. Depressed delayed hypersensitivity responses to

Table 27.7 Examples of pathogens affecting blood cells or haemopoiesis

Pathogen	Disease	Effect	Mechanism
Plasmodium spp.	Malaria	Anaemia	Replication in erythrocytes
Babesia spp.	Babesiosis (tick-borne)	Anaemia	Replication in erythrocytes
Bartonella bacilliformis	Oroya fever[a] (rare, sandfly transmitted, occurs in Peru)	Anaemia	Replication in erythrocytes
Ehrlichia spp. (Rickettsiae)	Human ehrlichiosis (tick transmitted in Southern USA and Japan)	Leukopenia, thrombocytopenia	Replication in leukocytes
Human parvovirus B19	Erythema infectiosum	Temporary fall in haemoglobin levels Aplastic crisis (individual with chronic anaemia)	Replication in erythropoietic cells
Colorado tick fever virus	Colorado tick fever	No effect on survival of infected erythrocytes	Replication in erythropoietic cells
Human T-cell lymphotropic virus (HTLV-1)	T-cell leukaemia/lymphoma	Malignant transformation of infected T cells	Replication in T cells
HIV	AIDS	Immunosuppression	Infection of CD4-positive T cells
Epstein–Barr virus (EBV)	Infectious mononucleosis	Thrombocytopenia, anaemia	Autoantibody to platelets, erythrocytes
Cytomegalovirus (CMV)	Congenital CMV complication of adult infection	Anaemia, thrombocytopenia	Infection of, or autoantibody to, erythrocytes, platelets

[a]A cutaneous form (Verrugas) also occurs; in 1885 a Peruvian medical student, Daniel Carrion, demonstrated the common bacterial origin by inoculating himself with infected blood from the cutaneous form of the disease and developing Oroya fever.

tuberculin are associated. Polymyositis has been described. Up to 5% of infected individuals eventually develop T-cell leukaemia, which has a high and rapid mortality rate, and a similar proportion progress to 'tropical' spastic paraparesis (TSP), also known as HTLV-associated myelopathy (HAM), in which there is primary demyelination (see Ch. 25). Neural cells do not appear to be infected, and it is not known how the virus causes a neurological disease.

Detection of HTLV-1- and HTLV-2-specific antibody is based on serological methods with type differentiation by immunoblot. Antiretroviral agents other than protease inhibitors have been shown to inhibit viral replication and may be used as part of the management of individuals with ATLL or TSP. Other treatments have been examined with limited success. Allogeneic bone marrow transplantation has been carried out with some success, with some survivors in those with a graft versus ATLL effect 3 years post-transplant. HTLV infection can be transmitted by HTLV antibody-positive individuals and they should not donate blood or organs. HTLV antibody screening of blood donors is now included in many countries.

HTLV-2 infection

HTLV-2 was first isolated in 1982 from a patient with T-cell hairy leukaemia, although it is not the usual cause of this condition. HTLV-2 is closely related to HTLV-1, is transmitted by similar routes and has been reported in injecting drug users and native Amerindian tribes in North, Central and South America. It has been associated with a number of neurological conditions including occasional reports of myelopathy.

KEY FACTS

- The intact skin is an invaluable barrier that defends the body against invasion.
- A wide range of organisms is associated with skin infection and disease.
- Bacteria, fungi and viruses usually gain access through breaches of the barrier caused by trauma.
- Some parasites initiate their own penetration into the skin (hookworm, schistosomes).
- Other pathogens are introduced into the skin by arthropod vectors.
- Once in the skin, pathogens cause local infections or disseminate through the body to distant sites.
- Pathogens may be acquired by other routes, disseminate in the body and then localize in the skin or cause toxic or immunopathological manifestations in the skin.
- Superficial infections of the skin are among the commonest human infections (boils, impetigo, warts, acne, ringworm).
- Invasion of pathogens deeper into dermal and subdermal tissues may produce severe infections that can be rapidly fatal, as in gangrene, or slow but progressive deformation and destruction, as in leprosy.
- Infections of muscle usually arise from invasion from the outside, whereas infections of joints are more often blood-borne.
- Bone infections may arise either by local spread from an infected joint or as a result of haematogenous seeding.
- Bone marrow cells or leukocytes may be invaded by viruses that interfere with haemopoiesis (parvovirus), cause malignant transformation (HTLV-1), or interfere with the immune system (EBV, HIV).

28 Vector-borne infections

Introduction

A number of important human diseases, caused by organisms ranging from viruses to worms, are transmitted by blood-feeding arthropods. These vectors inject the organisms into humans as they take a blood meal. Two classes of arthropods make the major contribution to disease transmission: the six-legged insects and the eight-legged ticks and mites. Arthropod-transmitted infections are commonest in warmer countries, but occur worldwide. Of these, malaria is undoubtedly the most important. This chapter will also cover schistosomiasis, a major tropical disease that is often described as vector transmitted, but the aquatic snail 'vectors' are more accurately referred to as intermediate hosts.

Transmission of disease by vectors

In sparsely populated areas, transmission by insects is an effective means of spread

Disease transmission by insects has major implications for the host, the vector and the parasite. To consider the parasite first, it requires the organism to be present in the right place (in the blood) and at the right time (some insects, for example, bite only at night). Blood is an inhospitable environment and this may require quite subtle evasion mechanisms for parasite survival. In addition, the conditions found in the vector are likely to be very different from those in the human host and the parasite may have to make a remarkably complex transition in a short time. With the larger protozoal and helminth parasites, this transition often involves clearly visible changes in appearance and is responsible for much of the complicated nomenclature of parasite life cycles. As some insect vectors have lifespans hardly longer than those of their parasites, there is considerable wastage due to death of the vector before the parasite has matured to the infective stage for humans. A difference of a few days in a mosquito's lifespan can make an enormous difference to the effectiveness of malaria transmission and indeed this simple factor is believed to underlie much of the difference between the African pattern of endemic infection and the Indian pattern of sporadic epidemics. However, what may be lost from wastage is more than compensated for by the increased distances over which spread of the parasite can occur.

Vector transmission of disease means that the disease may be controlled by controlling the vector and is, for instance, a major reason why malaria is not endemic in many European countries, where it used to be common.

A potential advantage of this type of transmission for the host is that it is sometimes possible to immunize specifically against the stages infective to humans or those responsible for infecting the vector of the parasite. Again, malaria can serve as an example – vaccines against the sporozoites, gametocytes and gametes having been clearly shown to block transmission in animal models. Once transmission is blocked, there is a mathematically calculable possibility that the disease will die out. A vaccine against sporozoites has shown promising activity in protecting African children from *Plasmodium falciparum* malaria and in 2017–2020 is being rolled out in pilot projects in sub-Saharan Africa.

ARBOVIRUS INFECTIONS

Arboviruses are arthropod-borne viruses

A wide range of about 500 different viruses is transmitted by arthropods such as ticks, mosquitoes and sandflies. These arboviruses multiply in the arthropod vector and for each virus there is a natural cycle involving vertebrates (various birds or mammals) and arthropods. The virus enters the arthropod when the latter takes a blood meal from the infected vertebrate and passes through the gut wall to reach the salivary gland, where replication takes place. Once this has occurred, 1–2 weeks after ingesting the virus, the arthropod becomes infectious and can transmit the virus to another vertebrate during a blood meal. Certain arboviruses that infect ticks are also transmitted directly from adult tick to egg (transovarial transmission), so that future generations of ticks are infected without the need for a vertebrate host.

Only a small number of arboviruses are important causes of human disease

Arboviruses tend to replicate in vascular endothelium, the central nervous system (CNS), skin and muscle and are therefore multisystem infections. They are generally named after the clinical disease (e.g. yellow fever) or the place where they were first discovered (e.g. Rift Valley fever, Japanese encephalitis). A few (e.g. Ross River virus in Australia and the Pacific and Chikungunya virus in Africa and Asia) cause arthritis.

The human stage of the virus cycle may be essential (e.g. urban yellow fever, dengue), there being no other vertebrate host, or it may be 'accidental' from the virus's point of view, with humans acting as 'dead-end' hosts who do not form a

necessary part of the natural cycle (e.g. equine encephalitides, West Nile virus).

Yellow fever

Yellow fever virus is transmitted by mosquitoes and is restricted to Africa, Central and South America and the Caribbean

Yellow fever virus is an RNA virus of the family Flaviviridae. It was taken by the early slave traders to the Americas where the first recorded case was in Yucatan in 1640. The virus is transmitted by two different cycles:

- from human to human by the mosquito *Aedes aegypti*, which is well adapted to breeding around human habitations; the infection can be maintained in this way as 'urban' yellow fever
- from infected monkeys to humans by mosquitoes such as *Haemagogus*. This is 'jungle' yellow fever and is seen in Africa and South America.

Yellow fever is not transmitted directly from human to human by day-to-day contact, but transmission from ill patients to healthcare workers has been reported, notably after needlestick injury.

Clinical features of yellow fever may be mild, but in 10–20% of cases classic yellow fever with liver damage occurs, which can prove fatal

The virus enters dermal tissues or blood vessels at the site of a mosquito bite and spreads through the body. The liver is the most affected organ, but the kidneys, spleen, lymph nodes and heart are also damaged. Studies in Africa estimated that the ratio of inapparent to apparent infection was 7–12:1. When symptoms occur, after an incubation period of 3–6 days, there is a sudden onset of fever, nausea, vomiting, headache and muscular aches. Although mild cases occur, severe infection results in hepatocellular jaundice, renal failure, including acute tubular necrosis and shock. Coagulation defects (due to reduced synthesis and increased consumption of clotting factors) cause haemorrhage into the gastrointestinal tract (manifesting as haematemesis and melena) and elsewhere. The case fatality rate is approximately 20% in Africa and 40–60% in South America.

Clinical diagnosis is unreliable; there is no specific treatment, but there is a vaccine

The virus can be isolated or detected by reverse transcriptase polymerase chain reaction (PCR) from blood during the acute stage. A postmortem histopathological diagnosis can be made from the severe mid-zonal changes and acidophilic inclusion bodies (Councilman bodies) seen in the liver. Virus-specific immunoglobulin M (IgM) antibodies are detectable after a week but there is cross-reactivity with other flaviviruses, a particular problem in endemic areas.

The best prevention is to give the live attenuated 17D yellow fever vaccine to those who may be exposed. Vaccination is necessary for entry into and travel through endemic areas. Protection is long lasting and in 2016 the International Health Regulations (IHR) stipulated that the period of protection provided by the vaccine changed from 10 years to the lifetime of the person vaccinated. The IHR permit a state to require a valid certificate of vaccination from a traveller from an endemic area to another country where the right mosquitoes are present but the disease does not occur (e.g. from tropical Africa to India). Vaccines based on recombinant DNA technology have been developed. As with all arthropod-borne infections, control of arthropod vectors (insecticides, attention to breeding sites) and reduced exposure (insect repellents, mosquito nets) are also important.

Dengue fever

Dengue virus is transmitted by mosquitoes and occurs in SE Asia, the Pacific area, India, South and Central America

Dengue is one of the most rapidly re-emerging arbovirus diseases, with more than 50 million infections each year. Dengue virus is a flavivirus with four antigenic subtypes, all of which now circulate in Asia, Africa and the Americas. The mosquito *A. aegypti* is the principal human vector. The virus also circulates in monkeys and can be transmitted by mosquitoes to cause 'jungle' dengue in humans, a disease analogous to jungle yellow fever.

Dengue fever may be complicated by dengue haemorrhagic fever / dengue shock syndrome

Dengue virus replicates in dendritic cells, peripheral blood monocytes, liver parenchymal cells and macrophages in lymph nodes, liver and spleen. After an incubation period of 4–8 days, there is malaise, fever, headache, arthralgia, nausea, vomiting and sometimes a maculopapular or erythematous rash. Recovery may be followed by prolonged fatigue and / or depression.

Dengue haemorrhagic fever / dengue shock syndrome (DHF / DSS) is a particularly severe form of the disease. In the past, mortality rates were high, but with prompt access to expert hospital care a fatality rate of below 1% can be achieved. The pathogenesis of this syndrome is shown in Fig. 28.1. After an earlier attack of dengue, antibodies are formed that are specific for that serotype. On subsequent infection with a different serotype, the antibodies bind to the virus and not only fail to neutralize it (as might be expected for a different subtype), but actually enhance its ability to infect monocytes. The Fc portion of the virus-bound immunoglobulin (Ig) molecule attaches to Fc receptors on monocytes and entry into the cell by this route increases the efficiency of infection. Infection of increased numbers of monocytes results in an increased release of cytokines into the circulation (see Ch. 18) and this leads to vascular damage, shock and haemorrhage, especially into the gastrointestinal tract and skin. Similar 'enhancing' antibodies are formed in many other virus infections, but it is only in dengue haemorrhagic fever that they are known to play a pathogenic role. A number of other factors can influence the course of dengue infection, including age, female gender, several HLA class I alleles and dengue virus strain virulence.

There is no antiviral therapy for dengue fever. Treatment is supportive. The World Health Organization (WHO) has published a revised dengue case classification based on the presence or absence of warning signs in order to improve patient care (see Bibliography).

It has taken many years to develop a dengue vaccine. It was essential that it be tetravalent, to avoid the danger that

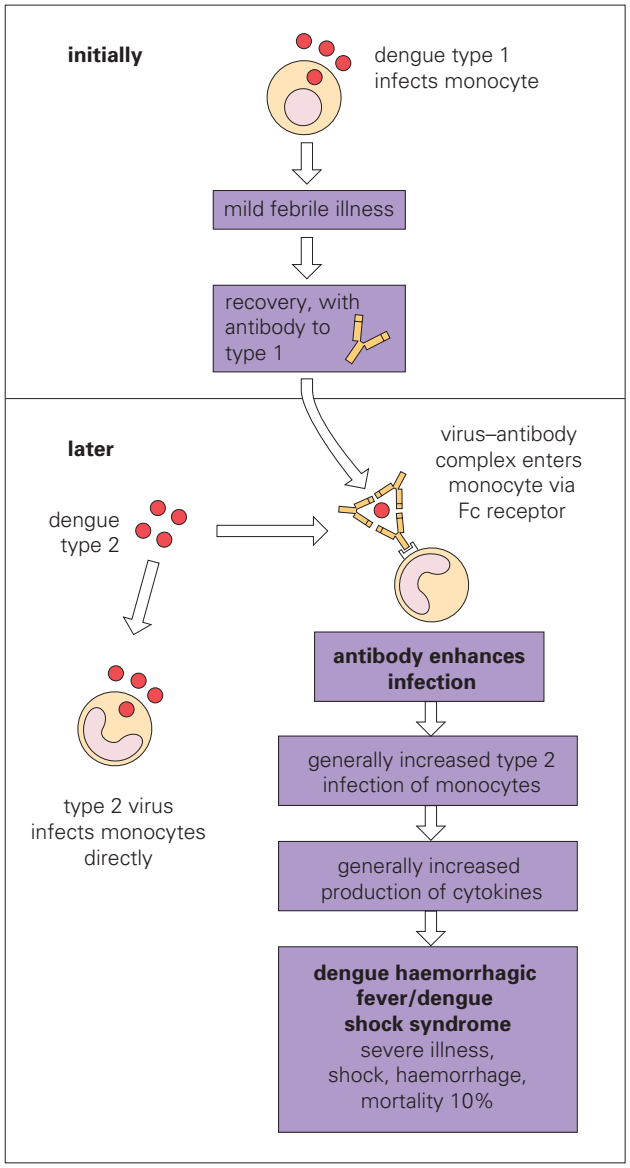


Figure 28.1 The pathogenesis of dengue haemorrhagic fever/dengue shock syndrome. There are four serotypes of dengue virus. Types 1 and 2 are illustrated as examples. Antibody to type 1 binds to type 2 without preventing infection with type 2.

a vaccine could induce the type of antibody associated with DHF / DSS. The first product was licensed in December 2015 and is a live attenuated tetravalent vaccine. WHO recommends that its introduction should be considered only in locations where there is a high burden of disease. There is no current recommendation for vaccination of travellers.

Chikungunya virus infection (CHIKV)

Chikungunya is an arbovirus of the family Togaviridae transmitted mainly by *Aedes aegypti*. The disease is present mostly in Africa and Asia but there was in an outbreak in Italy in 2007 and a major outbreak in the Americas in 2015. The illness is similar to dengue but polyarthritis is very common and retro-orbital pain rare in CHIKV.

Zika virus

Zika virus, a member of the Flaviviridae, was discovered in 1947 in a rhesus macaque in the Zika Forest of Uganda and was recognized as a cause of human disease in 1953. It remained an uncommon cause of human illness and thus has not appeared in *Mims' Medical Microbiology* until this, the 6th edition, after rising to prominence from 2013 in the Pacific and from 2015 in the Americas. Thus a little-known arbovirus long thought to result in only mild illness has become a major threat to humans. Possible reasons for its spread through Latin America include changes in climate and in land use, poverty and movement of people. In February 2016, WHO declared that the spread of the Zika virus was an emergency of public health concern.

The virus is transmitted by the mosquito vectors *Aedes aegypti* or *A. albopictus*. Infection acquired during pregnancy can lead to vertical infection of the fetus with resulting microcephaly or other congenital abnormality. Infection can also be transmitted by sexual intercourse or via blood transfusion.

Symptoms and signs of Zika virus infection include mild fever, a maculopapular rash, arthralgia, myalgia and conjunctivitis. Guillain–Barré syndrome can ensue. Congenital Zika syndrome includes microcephaly (see Fig. 29.2A, B), a variety of ocular abnormalities, craniofacial disproportion, spasticity and seizures.

Zika virus diagnosis is by reverse transcription-polymerase chain reaction (RT-PCR) for viral RNA on serum, saliva or urine and by antibody detection (IgM and IgG) after the first week of symptoms. Flavivirus antibodies show significant cross-reactivity and care is required in test interpretation to differentiate Zika virus infection from dengue.

There is no vaccine currently available, but Zika virus strains are strongly conserved at the nucleotide level, raising the possibility of a vaccine to protect against all strains.

Arbovirus encephalitis

The encephalitic arboviruses only occasionally cause encephalitis

Six of the ten encephalitis arboviruses listed in Table 28.1 cause disease in the USA and, although most infections are subclinical or mild, fatal encephalitis can occur. The viruses replicate in the CNS, but a cell-mediated immune response to infection makes a major contribution to the encephalitis. Vaccines against Western equine encephalitis (WEE), Eastern equine encephalitis (EEE) and Venezuelan equine encephalitis (VEE), each of which may cause disease in horses, have been used for laboratory workers. A Japanese encephalitis vaccine is also available and is used in the UK for at-risk travellers. Laboratory diagnosis is carried out in special centres, occasionally by virus isolation, but more commonly by demonstrating a rise in specific antibody.

Prior to the mid 1990s, West Nile virus (WNV), a flavivirus transmitted from infected birds by *Culex* mosquitoes and for which humans are considered to be dead-end hosts, was not considered a major public health problem, but viral changes then resulted in cases with severe neurological disease. The virus, which had not previously been reported from the Western hemisphere, was recorded in New York in 1999 and since then has spread widely in the USA, Canada, Mexico and the Caribbean. 20–40% of humans infected with WNV develop

Table 28.1 Arboviruses causing encephalitis

Virus and disease	Geographical distribution	Vector for human infection	Vertebrate reservoir	Severity of infection
Eastern equine encephalitis (alphavirus)	USA (Atlantic Gulf states)	*Aedes* spp. mosquitoes	Wild birds, horses (dead-end hosts)	50% case fatality
Western equine encephalitis (alphavirus)	USA (west of Mississippi)	*Culex* spp. mosquitoes	Wild birds, horses (dead-end hosts)	Up to 2% case fatality
West Nile encephalitis (flavivirus)	Africa, Europe, Central Asia, USA	*Culex* spp. mosquitoes	Birds	Up to 5% case fatality
St Louis encephalitis (flavivirus)	USA (Southern, Central and Western States)	*Culex* spp. mosquitoes	Wild birds	10% case fatality
California encephalitis (bunyavirus)	USA (Northern and Central States)	*Aedes* spp. mosquitoes	Small mammals	Fatalities rare
Japanese encephalitis (flavivirus)	Far East, South-East Asia	*Culex* spp. mosquitoes	Birds, pigs	Greater than 10% case fatality
Murray valley encephalitis (flavivirus)	Australia	*Culex* spp. mosquitoes	Birds	Up to 70% case fatality
Tick-borne encephalitis (flavivirus)	Eastern Europe	Tick	Mammals, birds	Up to 10% case fatality (variable)
Venezuelan encephalitis (alphavirus)	Southern USA, Central and South America	Mosquito	Rodents	70% case fatality (cases rare)
Powassan (flavivirus)	USA, Canada	Tick	Rodents	Cases rare

The great majority of infections are either subclinical or associated with non-specific febrile illness (e.g. 70% case fatality in encephalitis due to Venezuelan encephalitis virus, but only 3% develop encephalitis).

symptoms. In 2006, the CDC reported a total of more than 1500 human cases in the USA and more than 150 blood donors with the virus. By 2010, it was reported to have caused more than 25000 cases, 12000 of whom had severe neurological disease, with more than 1100 deaths. Quite how the virus crossed the Atlantic is unknown, though it has been suggested that it was probably imported in a live bird. Most clinical episodes present as a mild flu-like illness. West Nile neuroinvasive disease can be divided into three different syndromes: meningitis, encephalitis and acute flaccid paralysis. Cerebrospinal fluid (CSF) cell count and protein are elevated. Specific diagnosis can be made by virus isolation from serum, detection of viral RNA in blood, urine and CSF, and antibody detection in serum (and CSF where appropriate). Vaccines are available for horses and are under development for humans.

Arboviruses and haemorrhagic fevers

Arboviruses are major causes of fever in endemic areas of the world

Arbovirus infections are often subclinical or mild, but occasionally there is a severe haemorrhagic illness. Some of the best known of these infections are listed in Table 28.2. Laboratory diagnosis by isolation of virus, detection of viral genome or demonstration of a rise in antibody is possible in special centres.

INFECTIONS CAUSED BY RICKETTSIAE

The rickettsiae are a group of intracellular, arthropod-transmitted Gram-negative aerobic rods (see Ch. 2 and Appendix). Previously the group included, among others, the genera *Rickettsia*, *Bartonella*, *Coxiella*, *Ehrlichia* and *Orientia*. Genomic-based analysis has resulted in a complete reclassification of the group and only the genera *Rickettsia* and *Orientia* remain in the family Rickettsiaceae. *Bartonella*, *Coxiella* and *Ehrlichia* have been transferred to other families and are not discussed further in this chapter. The rickettsiae are obligate intracellular parasites, carried in arthropod or animal reservoirs (Fig. 28.2). Person-to-person transmission does not occur.

The rickettsiae are small bacteria and infections tend to be persistent or become latent

Howard T. Ricketts identified 'Rocky Mountain spotted fever' in 1906 and showed that the infection was transmitted transovarially in ticks. Rickettsiae probably arose as parasites of blood-sucking or other arthropods in which they were maintained by vertical transmission, transfer to the arthropod's vertebrate host being initially 'accidental' and not necessary for rickettsial survival. The infected arthropod does not appear to be adversely affected. *Rickettsia prowazekii* is perhaps a more recent parasite of the human body louse, because the louse dies 1–3 weeks after infection. As with most arthropod-borne infections, transmission from person to person does not occur.

Typical clinical symptoms of rickettsial infection are fever, headache and rash

Rickettsiae multiply in the vascular endothelium to cause vasculitis in skin, CNS and liver, and hence are multisystem infections (Table 28.3). In spite of immune responses, there is a tendency for rickettsial infections to persist in the body for long periods or become latent.

The typical clinical features are fever, headache and rash. A history suggesting contact with rickettsial vectors or reservoir

Table 28.2 Arboviruses causing fevers and haemorrhagic disease

Viruses	Disease reservoir	Geographical distribution	Vector	Animal
Yellow fever (alphavirus)	Fever, hepatitis	Africa, Central and South America	Mosquito *Aedes* spp.	Nil (monkeys for jungle type)
Dengue (4 serotypes) (flavivirus)	Fever, rash (haemorrhagic shock syndrome)	India, South-East Asia, Pacific, South America, Caribbean	Mosquito	Nil
Kyasanur forest (flavivirus)	Haemorrhagic fever	India	Tick	Monkeys, rodents
Ross river (alphavirus)	Fever, arthralgia, arthritis	Australia, Pacific Islands	Mosquito	Birds
Rift Valley fever (bunyavirus)	Fever, sometimes haemorrhage	Africa	Mosquito	Sheep, cattle, camels
Sandfly fever (bunyavirus)	Fever (mild disease)	Asia, South America, Mediterranean	Sandfly	Gerbils
Congo-Crimean haemorrhagic fever (bunyavirus)	Fever, haemorrhage	Asia, Africa	Tick	Rodents
Colorado tick fever (reovirus)	Fever, myalgia	USA (Rocky Mountains)	Tick	Rodents
La Crosse (bunyavirus)	Fever	USA	Mosquito	Rodents

There are many other less important arboviruses. For example, there are almost 200 in the bunyavirus family, most of which are arthropod borne, with about 40 occasionally causing human disease.

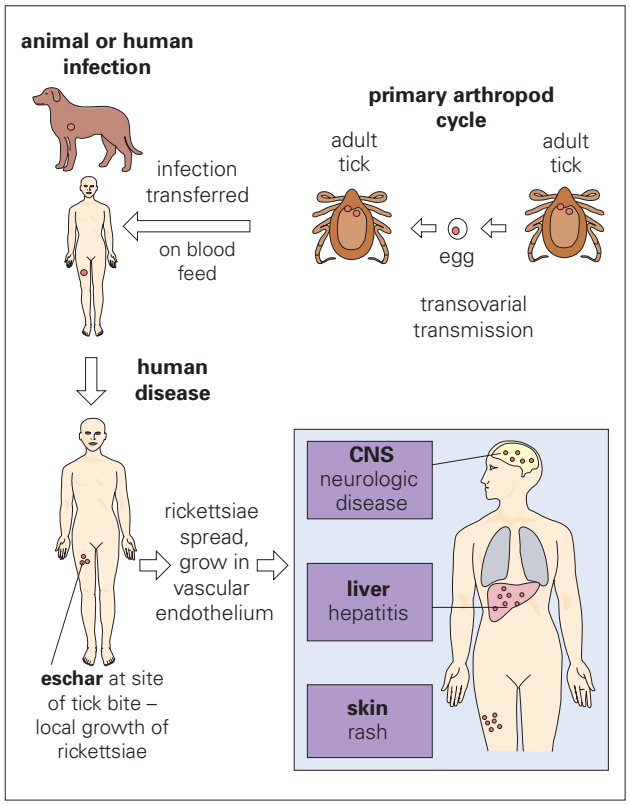


Figure 28.2 Typical events in rickettsial infection. There is no direct person-to-person spread. Typhus is unusual because the infected arthropod transmits from person to person, eventually dies and there is no eschar. CNS, central nervous system.

animals may suggest a diagnosis (e.g. camping, working, engaging in military activities in endemic areas).

Laboratory diagnosis is based on serological tests

Microimmunofluorescence methods are the most common serological approach, and demonstration of a fourfold or greater rise in titre is considered to be a positive result. Western blot analysis is used in reference laboratories. Seroconversion occurs 7–15 days after the onset of illness, but can take as long as 28 days. Infected patients also make antibody to the rickettsiae that cross-react with the O antigen polysaccharide of various strains of *Proteus vulgaris*, as detected by agglutination in the Weil–Felix test. Although the phenomenon is of interest, the Weil–Felix test is not of great value, however, because of false-positive and false-negative results. Earlier diagnosis can often be made by fluorescent antibody staining of skin biopsy material. PCR tests are deployed on skin biopsies or swabs from eschars, but a negative PCR does not exclude rickettsial infection. Isolation of rickettsiae is difficult and dangerous and laboratory infections have occurred.

All rickettsiae are susceptible to tetracyclines

Prevention is based on reducing exposure to the vector (e.g. ticks). A killed *R. prowazekii* vaccine has been used in the past by the military, but at present there is no commercially available anti-*Rickettsia* vaccine.

Rocky Mountain spotted fever

Rocky Mountain spotted fever is transmitted by dog ticks and has a mortality of 10% or more

The rickettsiae causing this disease are carried by the dog tick (*Dermacentor variabilis*) or by wood ticks (*D. andersoni*) and are transmitted vertically from adult tick to egg. Human infection occurs in the warm months of the year as ticks become active. Children are most commonly infected, but their disease is milder.

The rickettsiae multiply in the skin at the site of the tick bite, then spread to blood and infect vascular endothelium in the lung, spleen, brain and skin. After an incubation period of about 1 week, there is onset of fever, severe headache, myalgia, and often respiratory symptoms. A generalized maculopapular rash develops 2–4 days after the onset of fever, becoming petechial or purpuric in 50–60% of cases (Fig. 28.3). Splenomegaly may occur and neurological involvement is

Table 28.3 The principal rickettsial diseases in humans

Organism	Disease	Arthropod vector	Vertebrate reservoir	Clinical severity	Geographical distribution
Spotted fevers[a]					
Rickettsia rickettsii	Rocky Mountain spotted fever	Tick[b]	Dogs, rodents	++	Rocky Mountain states, eastern USA
R. akari	Rickettsial pox	Mite[b]	Mice	–	Asia, Far East, Africa, USA
R. conorii	Mediterranean spotted fever	Tick	Dogs	+	Mediterranean
Typhus					
R. prowazekii	Epidemic typhus	Louse	Human[c]	++	Africa, South America
R. typhi	Endemic typhus	Flea	Rodents	–	Worldwide
Orientia tsutsugamushi	Scrub typhus	Mite[b]	Rodents	++	Far East

[a]Other rickettsiae cause similar tick-borne fevers in Africa, India, Australia.
[b]Vertically transmitted in arthropod.
[c]Non-human vertebrates are possibly also involved.

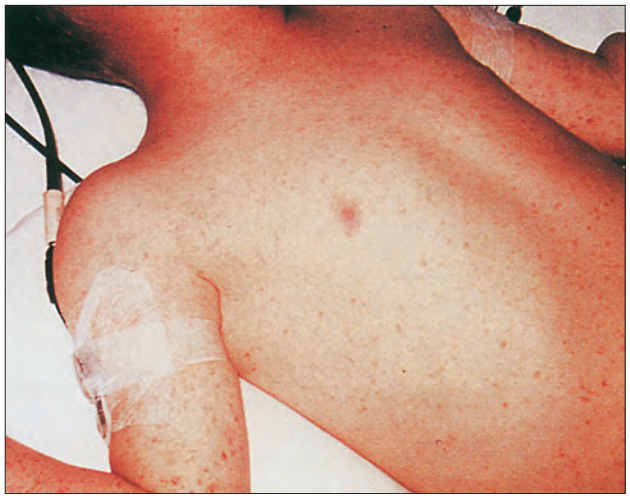


Figure 28.3 Generalized maculopapular rash with petechiae in Rocky Mountain spotted fever. (Courtesy of T.F. Sellers Jr.)

frequent, with later onset of clotting defects (disseminated intravascular coagulation), shock and death. Fatal cases are usually those with a delayed diagnosis. Peak mortality is seen in 40- to 60-year-olds.

Mediterranean spotted fever

Mediterranean spotted fever is transmitted by dog ticks

Mediterranean spotted fever is caused by *Rickettsia conorii*, carried by the dog tick *Rhipicephalus sanguineus*. Human infection, which occurs mainly in the summer, is known in all Mediterranean countries and can occur in urban as well as rural areas. After an incubation period of about 1 week, 50% of cases develop fever, headache and myalgia, then 2–4 days later a maculopapular rash, especially on the palms and soles. Approximately 50–75% of cases show an eschar.

African tick-bite fever

Eight pathogenic rickettsial species are currently recognized in Africa. *R. africae* is found mainly in urban areas and *R. conorii* in semi-rural and rural areas. African tick-bite fever is regularly seen in travellers returning from Africa to the temperate zone.

Rickettsialpox

Rickettsialpox is a mild infection

About 5 days after the bite of a rodent-associated mite (*Liponyssoides sanguineus*) infected with *R. akari*, a local eschar develops with fever and headache occurring approximately 1 week later. After a few more days, a generalized papulovesicular rash appears. The disease is, however, self-limiting and usually settles in 14–21 days.

Epidemic typhus

Epidemic typhus is transmitted by the human body louse

Epidemic typhus is transmitted from person to person by *Pediculus humanus*. The rickettsiae (*R. prowazekii*) multiply in the gut epithelium of the louse and are excreted in faeces during the act of biting. The rickettsiae enter the skin when the bite is scratched. The disease cannot maintain itself unless enough people are infested with lice. Epidemic typhus is therefore classically associated with poverty and war, when clothes and bodies are washed less frequently. There were 30 million cases in Eastern Europe and the Soviet Union from 1918 to 1922. The disease is seen in Africa, Central and South America and sporadically (as a sylvatic form) in the USA. As there is no direct person-to-person spread, outbreaks can be terminated by delousing campaigns.

Untreated epidemic typhus has a mortality as high as 60%

Rickettsiae proliferate at the site of the bite and then spread in the blood to infect vascular endothelium in skin, heart, CNS, muscle and kidney. About 10–14 days after the louse bite (there is no eschar) the infected person develops fever, headache and flu-like symptoms. A generalized maculopapular rash appears 5–9 days later in 20–40% of cases. Neurological involvement occurs in 80% and sometimes there is severe meningoencephalitis with delirium and coma. In untreated

cases, mortality can range from 20% in healthy individuals to as high as 60% in elderly or compromised patients, owing to peripheral vascular collapse or secondary bacterial pneumonia. Well-treated cases have a mortality rate of approximately 4%.

Convalescence may take months. In some individuals, the rickettsiae are not eliminated from the body on clinical recovery and remain in the lymph nodes. As much as 50 years later, the infection can reactivate to cause Brill–Zinsser disease and the patient once again acts as a source of infection for any lice that may be present.

Endemic (murine) typhus

Endemic typhus is caused by *R. typhi* and is transmitted to humans by the rat flea. The disease is similar to epidemic typhus, but is less severe and can present as a non-specific febrile illness.

Scrub typhus

Scrub typhus is a severe illness caused by *Orientia tsutsugamushi* and is transmitted to humans by larval trombiculid mites. It occurs throughout Asia where it is a common cause of fever in rural areas. The rickettsiae are maintained in the mites by transovarial transfer and are transmitted to humans or rodents during feeding. There is fever, headache and an eschar, then a macular rash appears after 5–8 days of illness. Pneumonitis, meningitis, disseminated intravascular coagulation and circulatory collapse may ensue. Immunochromatographic rapid diagnostic tests are available and should improve diagnosis in the field. Treatment is with doxycycline or azithromycin and must be given early. The human immune response to *O. tsutsugamushi* is unable to produce sterile, long-lasting, cross-protective immunity. As a result, attempts to develop a vaccine have so far proven unsuccessful, but work is underway to identify candidate antigens recognized by T cells.

BORRELIA INFECTIONS

Relapsing fever

The epidemic form of relapsing fever is caused by *Borrelia recurrentis*, which is transmitted by human body lice

Borrelia recurrentis is a Gram-negative spirochete consisting of an irregular spiral, 10–30 µm long and is highly flexible, moving by rotation and twisting.

Epidemics of relapsing fever (Fig. 28.4) are due to transmission of infection by the human body louse. Bacteria multiply in the louse and when louse bites are rubbed the lice are crushed and the bacteria are introduced into the bite wound. *B. recurrentis* can also penetrate intact mucosa and skin. Lice are essential for person-to-person transmission of louse-borne relapsing fever. As with other louse-borne infections (e.g. typhus), spread of the disease in humans is favoured when people rarely wash and when clothes are not changed (e.g. in wars, natural disasters). The last great epidemic in North Africa and Europe during the Second World War caused 50000 deaths and it remains an infection of public health concern in northern and eastern Africa.

The endemic form of relapsing fever in humans is transmitted by tick bites

Infections with other species of *Borrelia* are endemic in rodents in many parts of the world, including western USA, and are transmitted by soft ticks of the genus *Ornithodoros*. In the tick, the bacteria are transmitted transovarially from generation to generation, which, together with their ability to survive for up to 10 years, helps maintain the endemic cycle of this form of relapsing fever.

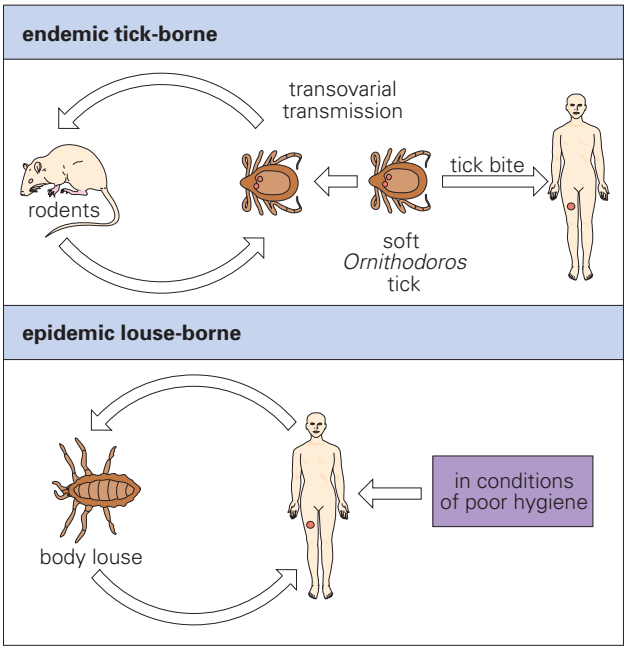


Figure 28.4 Transmission in relapsing fever.

Relapsing fever is characterized by repeated febrile episodes due to antigenic variation in the spirochetes

The bacteria multiply locally and enter the blood. After an incubation period of 3–10 days, there is a sudden onset of illness with chills and fever, lasting for 3–5 days (Fig. 28.5). The afebrile period lasts about a week before there is a second attack of fever, which is followed by another afebrile period. Generally, there are 3–10 such episodes, of diminishing severity. More serious illness can occur if there is extensive growth of the bacteria in the spleen, liver and kidneys.

Agglutinating and lytic antibodies are formed against the infecting bacteria, which are cleared from the blood. Under the 'pressure' of this immune response, a new antigenic type emerges and is free to multiply and cause a fresh febrile episode.

Antigenic variation involves switching of variable proteins on the bacterial surface. The *Borrelia* species have arrays of genes (variable large proteins [Vlp] and variable small proteins [Vsp]) that are altered and activated by gene conversion involving plasmids carrying collections of these genes. The result is that a single cloned bacterium can give rise spontaneously to approximately 30 serotypes and switching occurs at a rate of 1:1000 to 1:10000 per cell generation. Similar phenomena are seen in trypanosomes. Direct person-to-person transmission does not occur. Mortality with endemic (tick-borne) relapsing fever is <5%, but may be up to 40% in untreated epidemic (louse-borne) relapsing fever (4% if treated).

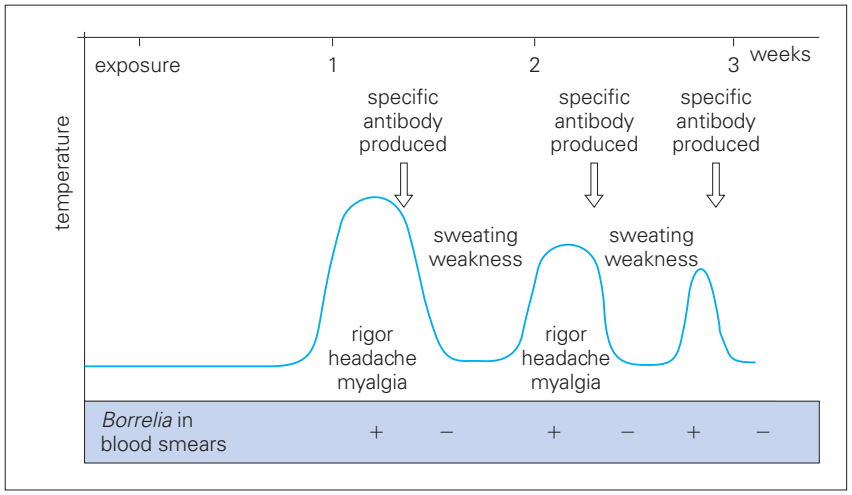


Figure 28.5 Course of events in relapsing fever.

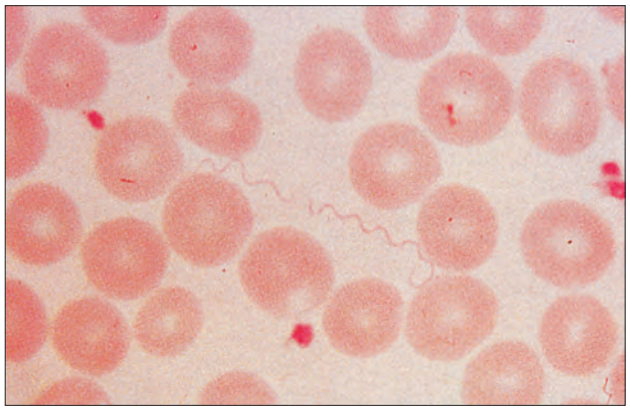


Figure 28.6 Tightly coiled helical spirochetes of *Borrelia recurrentis* in the blood of a patient with relapsing fever. (Courtesy of T.F. Sellers.)

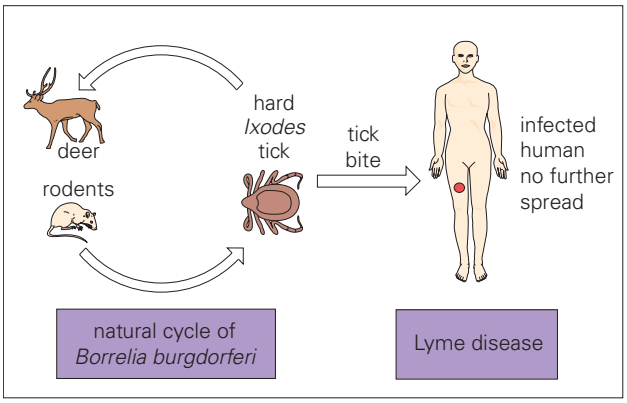


Figure 28.7 Transmission of Lyme disease.

Relapsing fever is diagnosed in the laboratory and treated with tetracycline

The bacteria can be cultivated in the laboratory and in approximately 70% of cases can be seen in Giemsa-stained smears of blood taken during the febrile period (Fig. 28.6). ELISA or immunofluorescence assays are able to detect specific antibody after a week of infection. PCR and molecular typing are available in reference laboratories.

Tetracycline is used in treatment and to prevent relapses. A Jarisch–Herxheimer reaction, with worsening of symptoms, high fever, rigors and hypotension, occurs in the first few hours after the start of treatment in 50–75% of cases. The best preventative measure against louse-borne relapsing fever is good personal hygiene, good sanitation and control of the louse vector. For tick-borne relapsing fever a key measure is avoidance of the vector.

Lyme disease

Lyme disease is caused by *Borrelia* spp. and is transmitted by *Ixodes* ticks

Lyme disease (or Lyme borreliosis) occurs in Europe, the USA and most continents of the world and is named after the town in Connecticut, USA where the first cases were recognized in 1975. About 30 000 new cases of Lyme diseases are estimated to occur each year in the United States. Lyme disease is caused by *Borrelia burgdorferi* in the USA, and in Europe by *B. garinii* and *B. afzelii*, with *B. burgdorferi* less common. The natural cycle of infection takes place in small mammals, in which it is transmitted by hard ticks of the genus *Ixodes* (Fig. 28.7). Human infection follows the bite of an infected tick (most commonly the nymph). In Europe and the USA, infection is commoner in summer months when recreational exposure to infected ticks is more likely. Person-to-person transmission does not occur.

Erythema migrans is a characteristic feature of Lyme disease

The bacteria multiply locally and, after an incubation period of about 1 week, fever, headache, myalgia, lymphadenopathy and a characteristic lesion at the site of the tick bite develop. The skin lesion is called erythema migrans (Fig. 28.8), its name describing its main features. It begins as a macule and enlarges over the next few weeks, remaining red and flat, but with the centre clearing, until it is several centimetres in diameter. In 50% of patients, fresh transient lesions appear on the skin elsewhere in the body. Immunological findings include circulating immune complexes and sometimes elevated serum IgM levels and cryoglobulins that contain IgM. *Borrelia*

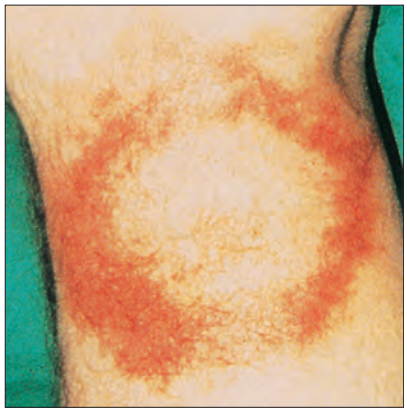


Figure 28.8 Rash of erythema chronicum migrans on the leg in Lyme disease. (Courtesy of E. Sahn.)

is capable of evading the human immune response and mechanisms include antigenic variation and the ability to evade complement-mediated killing.

Lyme disease commonly causes additional disease 1 week to 2 years after the initial illness

In 75% of untreated patients, in spite of antibody and T-cell responses to the *Borrelia*, there are additional later manifestations of disease. These are seen from 1 week to >2 years after the onset of illness. The initial manifestations to appear are neurological (meningitis, encephalitis, peripheral neuropathy) and cardiological (heart block, myocarditis). The subsequent manifestations are arthralgia and arthritis, which may persist for months or years. Immune complexes are found in affected joints. These late manifestations are immunological in origin and are probably due to antigenic cross-reactivity between *Borrelia* and host tissues. The *Borrelia* themselves are rarely detectable at this stage.

Lyme disease is diagnosed serologically and treated with antibiotics

Borrelia can be cultured (in BSK or MKP medium) from early-stage cutaneous tissues but culture has low sensitivity (40–60% in erythema migrans) and culture may take several weeks. Thus, Lyme disease is primarily diagnosed on clinical presentation and known exposure. When indicated, serological tests such as enzyme-linked immunosorbent assay (ELISA) are useful, with Western blot confirmation of all positive and equivocal results. Specific IgM antibodies are detected 3–6 weeks after infection and IgG antibodies at a later stage. PCR diagnosis has been disappointing except for Lyme arthritis, in which synovial fluid PCR is positive in 70–85% of cases.

Doxycycline or amoxicillin is effective in treatment of early disease. Late disease, especially with neurological complications, may require more aggressive therapy, for example, with intravenous ceftriaxone for up to 28 days.

Prevention of Lyme disease is by avoidance of tick bites. A vaccine is available for use in dogs (which can become naturally infected) but there is no Lyme vaccine currently licensed for humans. A Lyme vaccine based on recombinant outer surface protein A (Osp A) was marketed in North America for human use from 1998 to 2002, but concerns were expressed that it might induce arthritis, uptake was poor and it was voluntarily withdrawn from the market. Current Lyme research is exploring the option of a vaccine to block both tick feeding and *Borrelia* transmission.

PROTOZOAL INFECTIONS

Malaria

Malaria is initiated by the bite of an infected female anopheline mosquito

Between 2000 and 2015 the global incidence rate for malaria is estimated to have decreased by 41%. But it remains a formidable opponent, with approximately 200 million cases per year worldwide. Malaria is restricted to areas where the anopheline mosquitoes can breed – that is, the tropics between 60°N and 40°S (except areas higher than about 2000 m). Ninety percent of malaria cases occur in the WHO African region; 7% in the WHO South-East Asia region; and 2% in the WHO Eastern Mediterranean region. Despite the advances made, drug and insecticide resistance present major challenges to malaria elimination and there are around 420 000 malaria deaths globally each year. Increased international travel means malaria is regularly seen as an imported disease in non-malarious countries and, unless the possibility of malaria is constantly borne in mind, the diagnosis may be delayed or missed altogether, with fatal results. Malaria can also be transmitted by blood transfusion, needlestick accidents or from mother to fetus or neonate.

The life cycle of the malaria parasite comprises three stages

Five species of *Plasmodium* cause malaria in humans, of which *P. falciparum* and *P. knowlesi* are the most virulent (Table 28.4). All have similar life cycles, which are the most complex of any human infection, comprising three quite distinct stages and characterized by alternating extracellular and intracellular forms (Figs 28.9 and 28.10).

Invasion of red cells requires at least two separate receptor–ligand interactions; the lack of one red cell surface receptor, the Duffy antigen, explains the absence of *P. vivax* from most West Africans as there is a high prevalence of Duffy-negative people in the region. However, *P. vivax* infection of Duffy-negative individuals has now been confirmed, indicating that this malaria parasite is capable of using other receptors to invade erythrocytes and suggesting it is evolving rapidly. Other genetic traits that contribute to resistance to malaria include haemoglobin S (sickle cell), beta-thalassaemia, and glucose-6-phosphate dehydrogenase (G6PD) deficiency.

Clinical features of malaria include a fluctuating fever and drenching sweats

Symptoms range from fever to fatal cerebral disease or multiorgan failure and are associated exclusively with the asexual blood stage (see Fig. 28.9). The clinical picture depends upon the age and immune status of the patient, as well as the species of parasite. The most characteristic feature is fever, which follows rupture of erythrocytic schizonts and is mainly due to the induction of cytokines such as interleukin 1 (IL-1) and tumour necrosis factor (TNF). The synchronous cycle in red cells means that the different species of malaria give characteristic patterns of fever, with either a 48-h (tertian: days 1 and 3), 72-h (quartan: days 1 and 4) or, rarely, 24-h

Table 28.4 Human malaria parasites

Species	*Plasmodium falciparum*	*P. vivax*	*P. malariae*	*P. ovale*	*P. knowlesi*
Major distribution	West, East and Central Africa, Middle East, Far East, South America	India, North and East Africa, South America, Far East	Tropical Africa, India, Far East	Tropical Africa	Asia-Pacific region
Common name	Malignant tertian	Benign tertian	Quartan	Ovale tertian	
Duration of liver stage (incubation period)	6–14 days	12–17 days (with relapses up to 3 years)	13–40 days (with recrudescence up to 20 years)	9–18 days (with rare relapses)	9–12 days
Duration of asexual blood cycle (fever cycle)	48 h	48 h	72 h	50 h	24 h
Major complications	Cerebral malaria; anaemia; hypoglycaemia; jaundice; pulmonary oedema; shock		Nephrotic syndrome		Respiratory distress; renal failure

The most important and life-threatening complications occur with *P. falciparum*, hence its old name 'malignant tertian malaria'.

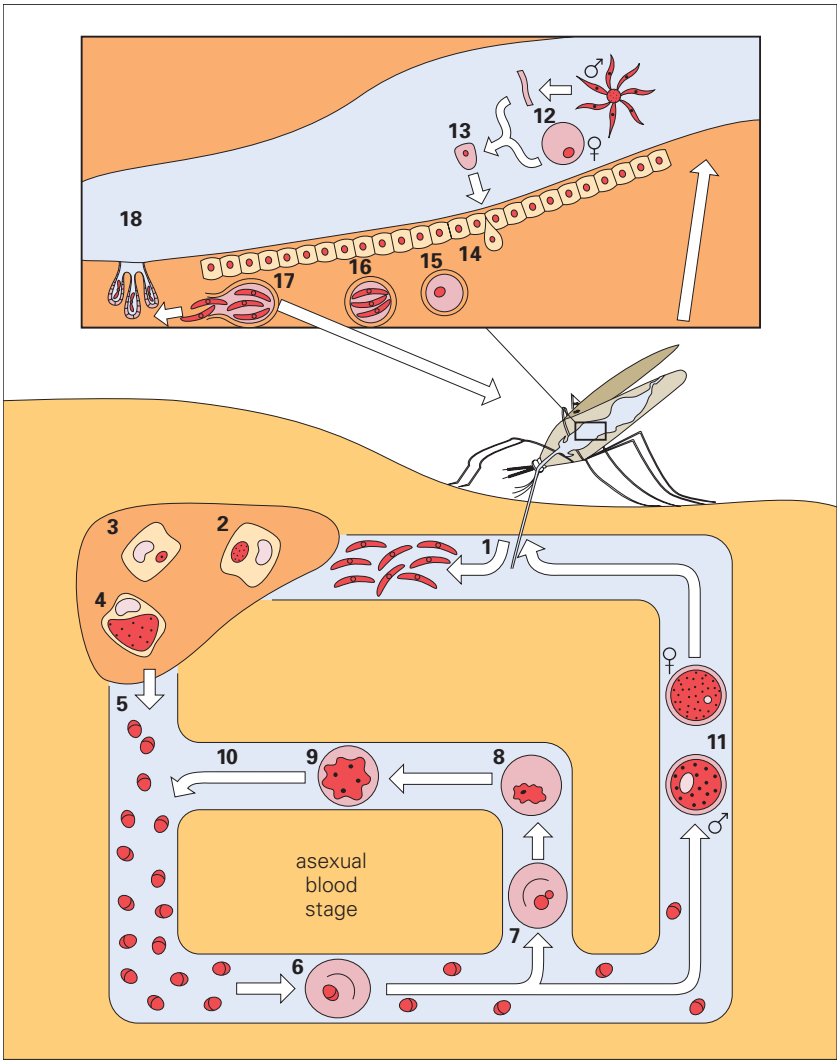


Figure 28.9 The life cycle of malaria in human and mosquito. In the symptomless pre-erythrocytic stage, sporozoites from the saliva of an infected *Anopheles* mosquito are injected into the human bloodstream when the mosquito bites (1). They then enter the parenchymal cells of the liver (2), where they mature in approximately 2 weeks into pre-erythrocytic (tissue) schizonts (4), finally rupturing to produce 10 000–40 000 merozoites (5). These circulate in the blood for a few minutes before entering the red blood cells (6) to initiate the asexual blood stage. For *P. vivax* and *P. ovale* only, some parasites, however, remain within the liver to lie dormant as hypnozoites (3), which are the cause of relapses. Once in the red blood cells, the merozoites mature into the ring form (7), trophozoite (8) and schizont (9), which complete the cycle by maturing to release merozoites back into the circulation (10). This cycle may last for months or even years. Some merozoites, however, go on to initiate the sexual stage, maturing within the red blood cells to form male and female gametocytes (11), which can be taken up by the *Anopheles* mosquito on feeding. On entering the gut of the insect, the male gametocyte exflagellates (12) to form male microgametes, which fertilize the female gamete to form the zygote (13). This then invades the gut mucosa (14), where it develops into an oocyst (15). This develops to produce thousands of sporozoites (16), which are released (17) and migrate to the salivary glands of the insect (18), whence the cycle begins again.

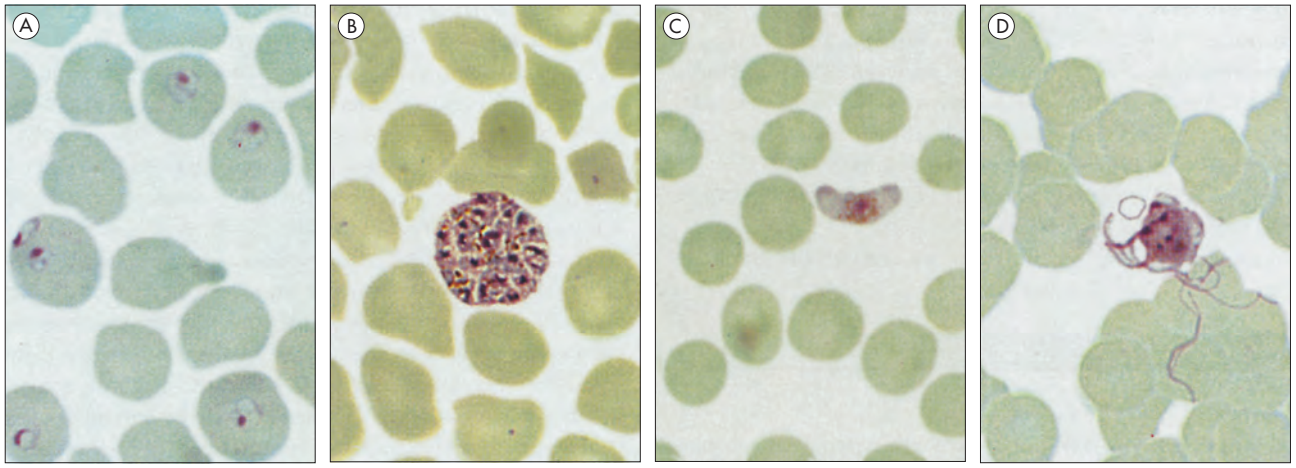


Figure 28.10 Different stages of the malaria parasites. (A) *Plasmodium falciparum* ring forms in red blood cells. (B) *Plasmodium vivax* erythrocytic schizont. (C) *P. falciparum* female gametocyte. (D) *P. vivax* male gametocytes exflagellating to form microgametes 20–25 μm long.

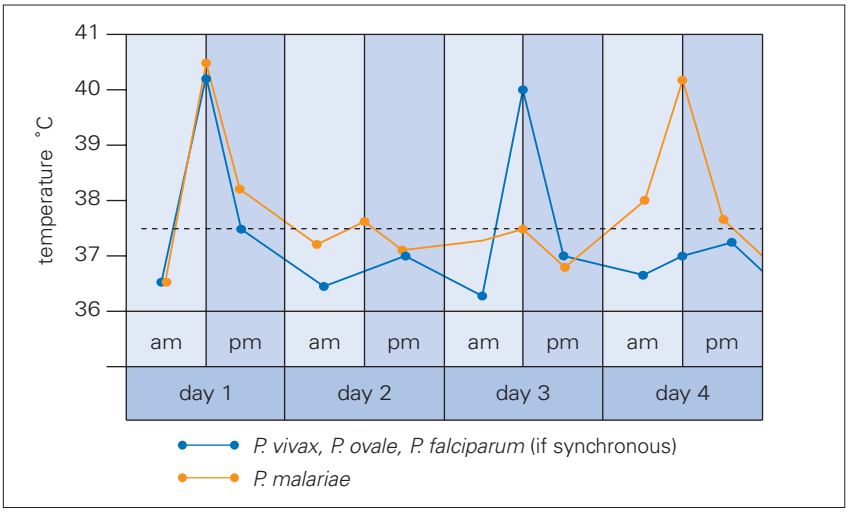


Figure 28.11 Malaria fever charts showing cyclical fluctuations in temperature. The peaks coincide with the maturation and rupture of the intraerythrocytic schizonts, occurring every 48 h (*Plasmodium falciparum*, *P. vivax* and *P. ovale*) or every 72 h (*P. malariae*), when the cycles are synchronized.

(quotidian: daily) periodicity (Fig. 28.11). However, this classical pattern of fever is seldom seen in clinical practice, where a chaotic fever pattern is common. Furthermore, it is possible to be afebrile yet obviously very unwell with malaria. A typical paroxysm starts with a feeling of intense cold with shivering, followed by a hot, dry stage and finally a period of drenching sweats. Headache, muscle pains and vomiting are common. The symptoms of malaria closely resemble those of influenza, which is a common misdiagnosis. Jaundice may be present and may lead to an erroneous diagnosis of viral hepatitis. Fever may be the only physical sign in early malaria, but later enlargement of the spleen and liver is common and anaemia is almost invariable.

P. vivax produces a chronic debilitating febrile illness resulting in significant morbidity and mortality in endemic areas. Indeed, there are proven cases of *P. vivax* infection that fulfill the case definition for severe malaria, manifesting as severe anaemia and respiratory distress. In the absence of re-infection, *P. ovale* and *P. malariae* malarias are normally self-limiting though debilitating infections. *P. malariae* may persist in the blood at a low level for decades and recrudesce to cause symptoms from time to time. Relapses (defined as hypnozoite-induced) may occur with *P. vivax* and *P. ovale* months or even 1–2 years after the initial malarial illness.

P. falciparum malaria is frequently fatal during the first 2 weeks because of a variety of complications (see Table 28.4). In hyperendemic areas, complicated falciparum malaria is most common in children aged between 6 months and 5 years, and in pregnant, particularly primigravid, women. However, it can occur at any age in non-immune individuals (e.g. tourists). The most dangerous complication is cerebral malaria, with convulsions and diminished level of consciousness progressing to coma. Possible causes include binding (sequestration) of parasitized red cells in cerebral capillaries, endothelial dysfunction, increased permeability of the blood–brain barrier, dysregulation of coagulation pathways, and excessive induction of proinflammatory cytokines such as TNF. If successfully treated, there is usually little or no impairment of cerebral function, although neurological and psychiatric sequelae may occur in 5–10% of childhood cases.

Also common is severe anaemia, which is due partly to red cell destruction and partly to dyserythropoiesis in the bone marrow. Of the other complications, hypoglycaemia and lactic acidosis are thought to be important contributors

to mortality. Acute renal failure due to acute tubular necrosis is an important complication of falciparum malaria, and nephrotic syndrome may occur with *P. malariae* (quartan malarial nephropathy).

In children, the main components of severe malaria are cerebral malaria, severe malarial anaemia and metabolic acidosis. In adults, multiorgan failure is seen, with metabolic acidosis, acute renal failure, jaundice and respiratory failure, with or without cerebral malaria.

Malaria has an immunosuppressive effect and interacts with HIV infection

The strong epidemiological correlation between malaria and endemic Burkitt's lymphoma probably reflects reduced T-cell cytotoxicity against Epstein–Barr virus (EBV)-infected cells. Malaria may also interfere with the efficacy of vaccines against common viral or bacterial infections.

In pregnant females, HIV-1 infection is associated with more peripheral blood malaria, more placental malaria, higher parasite densities, more fever, and increased risk of adverse birth outcomes. In semi-immune non-pregnant adults, HIV-1 infection is associated with higher rates of malaria infection and higher rates of clinical disease. In non-immune, non-pregnant adults, HIV-1 infection is associated with higher rates of severe malaria and death. HIV-infected patients have a higher rate of malaria treatment failure.

Immunity to malaria develops gradually and seems to need repeated boosting

Immunity to malaria develops in stages and, in endemic areas, children who survive early attacks become resistant to severe disease by about 5 years. Parasite levels fall progressively until adulthood, when they are low or absent most of the time. However, 1 year spent away from exposure is sufficient for much of this immunity to wane, albeit incompletely (i.e. repeated boosting is needed to maintain it). The actual mechanisms are still being worked out, but involve both antibody- and cell-mediated immunity (Fig. 28.12).

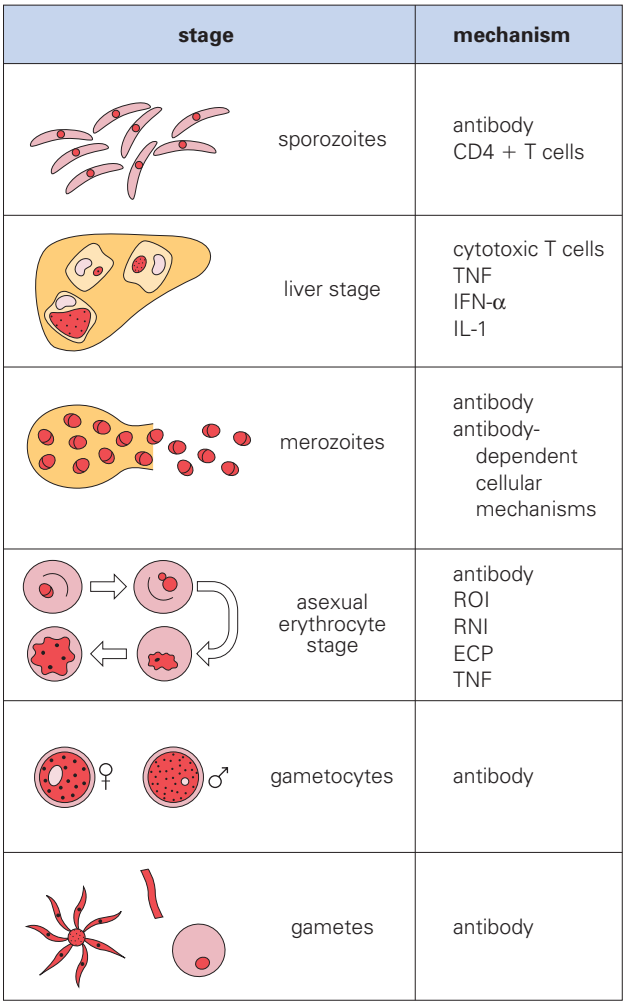


Figure 28.12 Immunity to malaria. The principal mechanisms thought to be responsible for immunity at each stage of the cycle. CD, cluster of differentiation; ECP, eosinophil cationic proteins; IFN, interferon; IL, interleukin; RNI, reactive nitrogen intermediates; ROI, reactive oxygen intermediates; TNF, tumour necrosis factor.

Malaria is diagnosed by finding parasitized red cells in thin and thick blood films

Lateral-flow devices (dipsticks) to detect malarial antigen are also widely used and can be performed without the need for a laboratory. Molecular assays to detect malarial DNA or RNA are much more sensitive and are available in reference laboratories.

In the case of *P. falciparum* infection, later (schizont) stages may be sequestered in deep tissues, so parasites may be deceptively scarce in, or even absent from, the peripheral blood. A single negative blood film does not exclude malaria of any species. Further samples should be taken 12–24, and 48 h later. A severe febrile illness, especially with anaemia, splenomegaly or cerebral signs, with a negative blood film, in a patient who conceivably could have malaria may therefore need to be treated as malaria, while healthcare workers continue to look for other diagnoses and seek expert help. However, the presence of parasites in the blood of an ill patient from an endemic area does not mean that malaria is the cause of the illness, so other causes of fever should still be borne in mind while they receive treatment for malaria. For example, they might have lobar pneumonia and coincidental malarial parasitaemia, as low-grade parasitaemia may be asymptomatic in those with partial malarial immunity.

Where available, intravenous artesunate (in combination with other antimalarials to avoid the development of drug resistance) is the drug of choice for severe malaria. Intravenous quinine is used if artesunate cannot be obtained without delay.

Uncomplicated falciparum malaria is treated with oral artemisinin combination therapy. Malaria due to *P. vivax*, *P. ovale*, *P. knowlesi* or *P. malariae* is treated with oral artemisinin combination therapy or oral chloroquine. Severe or complicated malaria due to any of these species is treated as for severe falciparum malaria. Primaquine (contraindicated in G6PD deficiency) is used to kill hypnozoites of *P. vivax* or *P. ovale* in the liver and thus prevent relapses of these infections.

In endemic areas, the most important method of prevention is the use of long-lasting insectide-impregnated bed nets. Indoor residual spraying with insecticides has an important effect in rapidly reducing malaria transmission when at least 80% of houses in a given area is sprayed. Development of malaria vaccines is discussed in Chapter 35.

Trypanosomiasis

Three species of the protozoan *Trypanosoma* cause human disease

Trypanosoma brucei gambiense and *T. b. rhodesiense* cause human African trypanosomiasis or sleeping sickness, and *T. cruzi* causes South American trypanosomiasis or Chagas disease. The diseases differ markedly in:

- the insect vector
- the localization of the parasite
- the effects on the immune system.

Human African trypanosomiasis

Human African trypanosomiasis is transmitted by the tsetse fly and restricted to equatorial Africa

The vector of human African trypanosomiasis (HAT) is the tsetse fly *Glossina*, and there is a reservoir of *T. b. rhodesiense* infection in several domestic and wild animals (e.g. cattle, pigs, deer). In humans, *T. brucei* remains extracellular, first in the tissues near the insect bite and then in the blood, where it divides rapidly and continuously.

Clinical features of HAT include lymphadenopathy and sleeping sickness

Following an infected bite, a swollen chancre develops at the site (*T. b. rhodesiense* only), with widespread lymph node enlargement. Posterior cervical lymphadenopathy (Winterbottom's sign; Fig. 28.13A) is typical of *T. b. gambiense*. The parasite establishes in the blood and multiplies rapidly, with fever, splenomegaly and, often, signs of myocardial involvement. The CNS may become involved (more acutely in the East African *T. b. rhodesiense* than the West African *T. b. gambiense*), with the gradual development of headache, psychological changes, weight loss and finally coma (sleeping sickness; Fig. 28.13B) and death. Unlike malaria, parasitologically cured trypanosomiasis can leave the patient with severe residual neurological and mental disability.

T. brucei evades host defences by varying the antigens in its glycoprotein coat

T. brucei survives freely in the blood because of its remarkable degree of antigenic variation, based on switching between some 900 different genes for the surface glycoprotein coat, expressing one at a time. A high concentration of IgM is found in the blood, and later in the CSF, and this is manufactured by the plasma cells (Mott cells), which are a feature of the lymphocytic infiltrate seen as perivascular cuffing around blood vessels in the brain (Fig. 28.14).

HAT is diagnosed by demonstrating parasites microscopically in blood, lymph nodes (by puncture) or in late cases in the CSF. Detection of antitrypanosomal antibody is used to screen populations for *T. b. gambiense*, with further parasitological examination of those who are seropositive. Immunochromatographic tests have been developed for field use and molecular diagnostics are available in reference laboratories.

East African trypanosomiasis is treated with suramin intravenously for the haemolymphatic stage, followed by melarsoprol intravenously (very toxic) if the CNS is involved. West African trypanosomiasis is treated with pentamidine intravenously or intramuscularly for the haemolymphatic stage. CNS involvement is treated with nifurtimox orally plus eflornithine intravenously (NECT). A new nitroimidazole compound, oral fexinidazole, is in clinical trial as an alternative to NECT.

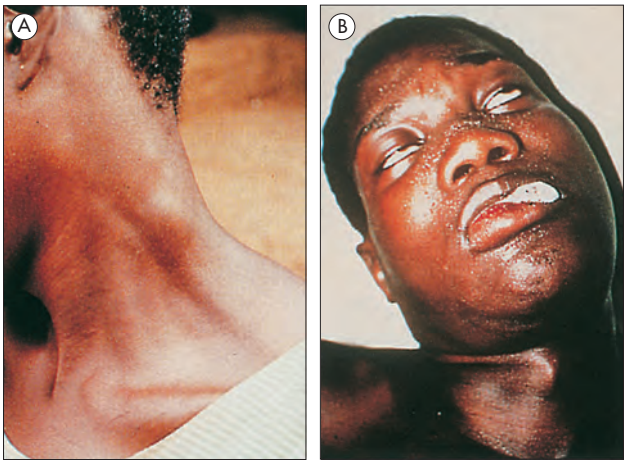


Figure 28.13 African trypanosomiasis. (A) Enlargement of the lymph nodes in the neck (Winterbottom's sign). (B) Coma (sleeping sickness) due to generalized encephalitis. ([A] Courtesy of P.G. Janssens. [B] Courtesy of M.E. Krampitz and P. de Raadt.)

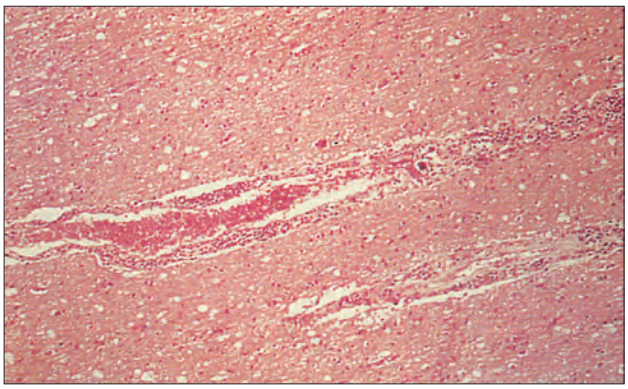


Figure 28.14 Lymphocytic infiltration around a blood vessel in the brain in *Trypanosoma brucei* infection (H&E stain). (Courtesy of R. Muller and J.R. Baker.)

Pentamidine prophylaxis is no longer deployed

Some 97% of HAT cases are due to *T. b. gambiense*. Its control is based on case finding and treatment, supported by vector control. Bed nets are ineffective, as the flies feed during daylight hours.

Chagas disease

T. cruzi is transmitted by the reduviid ('kissing') bug

T. cruzi is transmitted by reduviid (kissing) bugs, which readily inhabit poor housing, so Chagas disease is characteristically a disease of the rural poor. Almost all species of mammal can act as reservoirs of infection. The parasite invades host cells, notably macrophages and cardiac muscle cells.

Vectorial transmission occurs in parts of North, Central and South America. Oral transmission via food or drink contaminated by reduviid bugs also occurs in endemic areas.

Vertical transmission from mother to fetus and transmission by blood or organ donation also take place.

Due to migration from rural to urban settings, many people with Chagas disease now live in the large cities of Latin America and, as a result of international migration, in the United States and parts of Europe.

Chagas disease has serious long-term effects, which include fatal heart disease

A nodular lesion (chagoma) or oedematous swelling of the eyelid (Romaña's sign) may develop at the site of infection, with a transient febrile illness that may rarely lead to death by heart failure. Following invasion of host cells, the disease pursues an extremely slow and chronic course. Approximately 70% of infected individuals remain in the indeterminate phase of the disease and do not develop complications. In cases where the disease does progress, the major complications, which can take years to appear, involve the heart and the intestinal tract. The major cause of death is myocarditis, with progressive weakening and dilatation of the ventricles due to destruction of cardiac muscle as a result of parasite persistence and the host's inflammatory response (Fig. 28.15). Cardiac aneurysm and heart block are particularly serious features. Dilation of the intestinal tract is due to similar processes in nerve cells, and the organs become incapable of proper peristalsis; megaoesophagus and megacolon are the two commonest manifestations.

Chronic Chagas disease is usually diagnosed serologically

In the acute phase, parasites may be seen in a blood film, but the chronic disease is usually diagnosed by serology, plus PCR on peripheral blood where available. *T. cruzi* parasites can also be detected by xenodiagnosis. Clean reduviid bugs are fed on the patient and their rectal contents examined 1–2 months later, or homogenized and injected into mice, in which even a single trypanosome will produce a patent infection. The use of PCR on bug faeces instead of microscopy increases the sensitivity of xenodiagnosis.

Antiparasitic therapy of Chagas disease is with oral benznidazole or oral nifurtimox. Children respond better to antitrypanosomal drugs than do adults. In recent years, there has been a re-evaluation of the role of drug therapy in chronically infected adults such that most practitioners now consider them for antiparasitic drug therapy. Both benznidazole and nifurtimox commonly produce side effects, so patients need to be followed carefully during treatment.

Prevention is achieved by improved housing and living standards, vector control plus active case finding and treatment. However, vector control by insecticides is difficult as some triatomine bugs can adapt to different habitats and re-invade houses after spraying. *Trypanosoma cruzi* is adept at evading the immune response – a major challenge to vaccine development. However, a therapeutic vaccine is under development as immunotherapy for those with chronic or indeterminate Chagas disease.

Leishmaniasis

Leishmania parasites are transmitted by sandflies and cause New World and Old World leishmaniasis

Several species of *Leishmania* parasites cause disease in both the New World and the Old World (Table 28.5). In the latter areas especially, dogs can act as an important reservoir of infection. All are transmitted by sandflies.

Leishmania is an intracellular parasite and inhabits macrophages

Leishmania evades the killing mechanisms of macrophages (Fig. 28.16) unless they are strongly activated, for example by

Table 28.5 *Leishmania* species – their distribution and clinical syndromes

Species	Distribution	Diseases
L. donovani *L. infantum*	Africa, India, Mediterranean	Visceral leishmaniasis
L. chagasi	South America	
L. major *L. tropica*	Africa, India, Mediterranean	Cutaneous leishmaniasis
L. aethiopica	Africa	
L. Mexicana	Mexico and Central America	
L. braziliensis	South America	

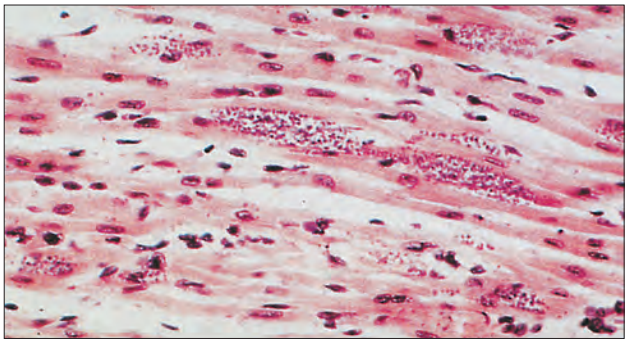


Figure 28.15 Amastigote forms of *Trypanosoma cruzi* in cardiac muscle in Chagas disease (H&E stain). (Courtesy of H. Tubbs.)

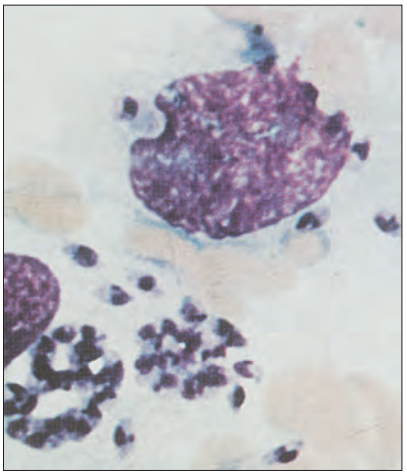


Figure 28.16 *Leishmania* within macrophages in aspirate from a lesion of New World leishmaniasis. (Courtesy of M.J. Wood.)

interferon gamma (IFNγ). The two principal sites of parasite growth are:

- the spleen, liver and bone marrow (visceral leishmaniasis)
- the skin (cutaneous leishmaniasis).

Untreated visceral leishmaniasis ('kala-azar') is fatal in 80–90% of cases

Visceral leishmaniasis, or kala-azar, usually develops slowly, with fever and weight loss, followed months or years later by hepatomegaly and, especially, splenomegaly. With appropriate treatment, only those who are very ill at diagnosis die. Skin lesions known as post-kala-azar dermal leishmaniasis (PKDL) may appear following treatment. They contain *Leishmania* amastigotes and constitute a reservoir of infection that can infect biting sandflies.

Cutaneous leishmaniasis is characterized by plaques, nodules or ulcers

Classic cutaneous leishmaniasis progresses insidiously, from a small papule at the site of infection to a large ulcer. This may eventually heal with considerable scarring (Fig. 28.17), leaving the patient relatively immune to reinfection. Old World leishmanial lesions are known as Oriental sores (also 'Baghdad boil' and 'Delhi sore') and New World leishmaniasis as espundia (mucosal leishmaniasis due to *Leishmania (Viannia) braziliensis*) and chiclero ulcer (*Leishmania mexicana* infection of the pinna).

Immunodeficient patients may suffer more severe leishmaniasis

In immunodeficient patients, widespread chronic skin lesions can occur – diffuse cutaneous leishmaniasis – analogous to lepromatous leprosy. Visceral leishmaniasis (VL) is a major complication of HIV infection not only in the tropics but also around the Mediterranean, though it is now easier to manage with antileishmanial drugs since the advent of highly active antiretroviral therapy (HAART). Immunocompromised patients with VL have lower cure rates and higher relapse rates. A newly emerging risk factor for more severe leishmaniasis is monoclonal antibody therapy directed against tumour necrosis factor alpha (TNFα). More cases are therefore likely to be seen, as such biologics are increasingly being used to treat a variety of medical conditions.

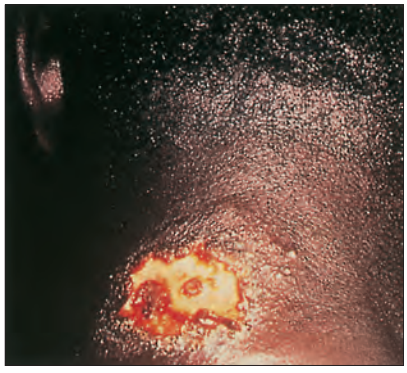


Figure 28.17 Cutaneous lesion on the neck in *Leishmania braziliensis* infection. (Courtesy of P.J. Cooper.)

Leishmaniasis is diagnosed by demonstrating the organism microscopically and is treated with antimonials

Demonstration of the organism by microscopy of splenic aspirate or biopsies of bone marrow or skin lesions (depending upon the clinical picture) is definitive proof of leishmaniasis. PCR is more sensitive than microscopy and culture.

Detection of antileishmanial antibody by the *Leishmania* direct agglutination test and rK39 rapid test is valuable in the diagnosis of visceral leishmaniasis.

Where available, PCR is now the method of choice for the detection and species identification of *Leishmania* in skin biopsies.

The precise choice of agent depends on the infecting species but, in principle, cutaneous leishmaniasis is treated by local injection of the edge of the ulcer with sodium stibogluconate (an antimonial). Intravenous sodium stibogluconate is used to treat multiple or potentially disfiguring lesions. Oral miltefosine is an alternative. The agent of choice for the treatment of visceral leishmaniasis is intravenous liposomal amphotericin B. Intravenous sodium stibogluconate is an alternative, though there is now significant antimony-resistant visceral leishmaniasis in parts of India.

Impregnated bed nets are effective against the sandfly vector and a *Leishmania infantum* vaccine is available for use in dogs.

A variety of vaccines against the cutaneous disease are under development for human use, including those composed of sandfly salivary proteins with or without *Leishmania* antigens.

HELMINTH INFECTIONS

Schistosomiasis

Schistosomiasis is transmitted through a snail vector

All digenean flukes must pass through a mollusc intermediate host in order to complete their larval development. However, schistosomes are the only group in which larvae penetrate directly into the final host after release from the snail.

The life cycle of schistosomes is illustrated in Fig. 28.18. Infected freshwater snails, which are always aquatic, release fork-tailed larvae into the surrounding water. These penetrate the host's skin, enter the dermis and pass via the blood, through the lungs to the liver, where they mature and form permanent male and female pairs before relocating to their final site:

- the veins surrounding the bladder for *Schistosoma haematobium*
- the mesenteric veins around the colon for *S. japonicum* and *S. mansoni*.

The life cycle is completed when eggs laid by the female worms move across the walls of the bladder or bowel and leave the body.

Clinical features of schistosomiasis result from allergic responses to the different life cycle stages

The stages of skin penetration, migration and egg production are each associated with pathological changes, collectively affecting many body systems. Penetration can cause a dermatitis, which becomes more severe on repeated re-infection. The developmental stages are associated with

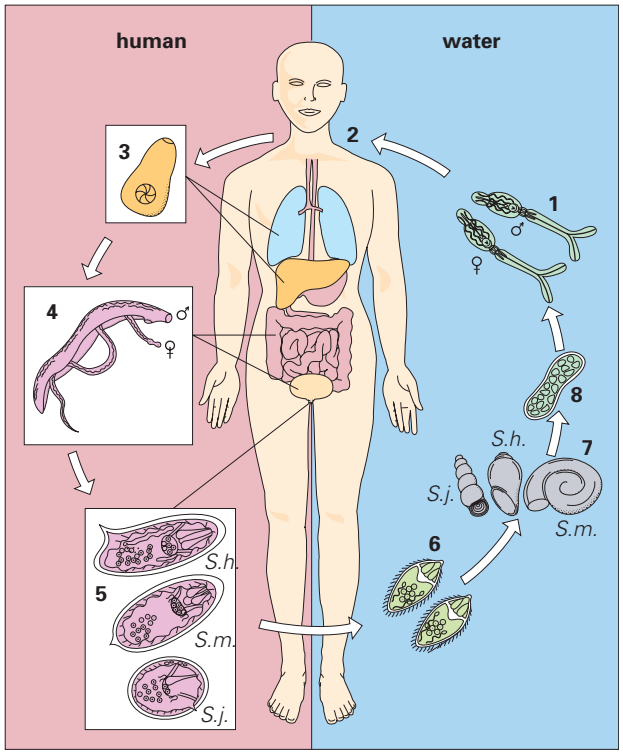


Figure 28.18 Life cycle of schistosomes. Free-swimming cercariae in water (1) penetrate unprotected skin. (2) During penetration, they lose their tails to become schistosomulae. (3) These migrate through the bloodstream via the lungs and liver to the veins of the bladder (*Schistosoma haematobium, S.h.*) or bowel (*S. mansoni, S.m.*; *S. japonicum, S.j.*), where they mature (4), to produce characteristic eggs (5) within 6–12 weeks. The eggs then penetrate the bladder or colon, to be passed in the urine or the faeces (6). Eggs passed into fresh water release miracidia, which penetrate snail intermediate hosts (7) where they mature into sporocysts (8). These release cercariae (1) into the water to complete the cycle.

the onset of allergic symptoms (e.g. fever, eosinophilia, lymphadenopathy, spleno- and hepatomegaly, diarrhoea), but the most severe pathology arises following the onset of egg laying. The body becomes hypersensitive to antigens released by the eggs as they pass through tissues to the outside world, or become trapped in other organs after being swept away in the bloodstream.

- In urinary schistosomiasis caused by *S. haematobium*, movement of eggs through the bladder wall causes haemorrhage. With time, the bladder wall becomes inflamed. Infiltrated polyps develop and malignant changes may follow; nephrosis may also occur (see Ch. 21).
- Release of the eggs of *S. japonicum* and *S. mansoni* similarly causes intestinal haemorrhage and inflammation.

A more serious consequence of these infections results from the inflammatory responses to eggs that become trapped in other organs of the body, primarily the liver, but also the lung and CNS. These consequences do not develop in all patients, but if they do then severe disease may ensue (see Ch. 23). Formation of granulomas by delayed hypersensitivity reactions around eggs in the presinusoidal capillaries interferes with blood flow and, together with extensive periportal fibrosis (Symmers' pipestem fibrosis), which occurs in about 10% of those infected with *S. mansoni*, leads to portal hypertension. As a consequence, there is hepatosplenomegaly, collateral connections form between the hepatic vessels and fragile oesophageal varices develop. The collateral circulation can lead to eggs being washed into the capillary bed of the lungs.

Schistosomiasis is diagnosed by microscopy and treated with praziquantel

Diagnosis of schistosomiasis is made by visualization of eggs on microscopy of stool or urine samples. Serum antibody detection is helpful in non-endemic areas, especially in travellers. Antigen detection assays and molecular diagnostics are in use in some centres.

Treatment of individuals with praziquantel removes the worms, but does not kill the eggs, which die naturally in about 2 months. In advanced cases, the pathology is irreversible. Three candidate schistosome vaccines based on the Sm-14, Sm-TSP-2 and Sm-p80 antigens are expected to enter safety and efficacy clinical trials in humans over the coming years.

Control of infection at a population level is achieved by breaking the transmission cycle, through avoidance of infected water and improvement in sanitation. Mass drug administration (MDA) programmes aim to reduce morbidity but can also reduce transmission. On the way to eradication it is hoped to move from MDA to selective treatment, but that will require more sensitive diagnostics suitable for field use.

Filariasis

Filarial nematodes depend upon blood-feeding arthropod vectors for transmission

The filarial nematodes parasitize the deeper tissues of the body (see Ch. 6). The most important species can be divided into those located in the lymphatics (*Brugia*, *Wuchereria*) and those in subcutaneous tissues (*Onchocerca*). A number of less harmful species also occur. In all species, the female worms release live larvae (microfilaria), which are picked up by the vector from the blood (lymphatic species) or skin (*Onchocerca*). Both groups can cause severe inflammatory responses, reflected in a variety of pathological responses in the skin and lymph nodes, but each is associated with additional and characteristic pathology. (Descriptions of the diseases caused by *Onchocerca* are given in Chs. 26 and 27.)

Lymphatic filariasis caused by *Brugia* and *Wuchereria* is transmitted by mosquitoes

The mosquitoes introduce the infective larvae into the skin as they feed. These larvae migrate to the lymphatics and develop slowly into long thin adult worms (females 80–100 mm×0.25 mm), found in the lymph nodes and lymphatics of the limbs (usually lower) and groin. Infections become patent after about 8–12 months, when sheathed microfilariae appear in the blood. Infected individuals may show few clinical signs or have acute manifestations such as fever, rashes, eosinophilia, lymphangitis, lymphadenitis (Fig. 28.19) and orchitis. Initial damage to the lymphatics is vessel dilatation in response to mediators released by the adult worms. Gradual impairment of lymphatic contractility follows. The lymphatic valves become incompetent, resulting in lymphatic stasis. Later, chronic obstructive changes, caused by repeated episodes of lymphangitis, may block lymphatics,

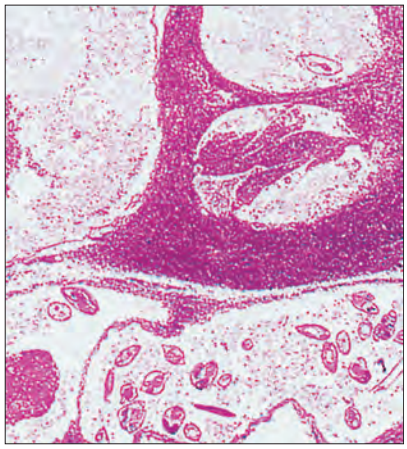


Figure 28.19 Lymph node containing adult *Wuchereria*, showing dilated lymphatics and tissue reaction in the vessel walls. (Courtesy of R. Muller and J.R. Baker.)

Figure 28.20 Elephantiasis of the leg, caused by *Brugia malayi*. (Courtesy of A.E. Bianco.)

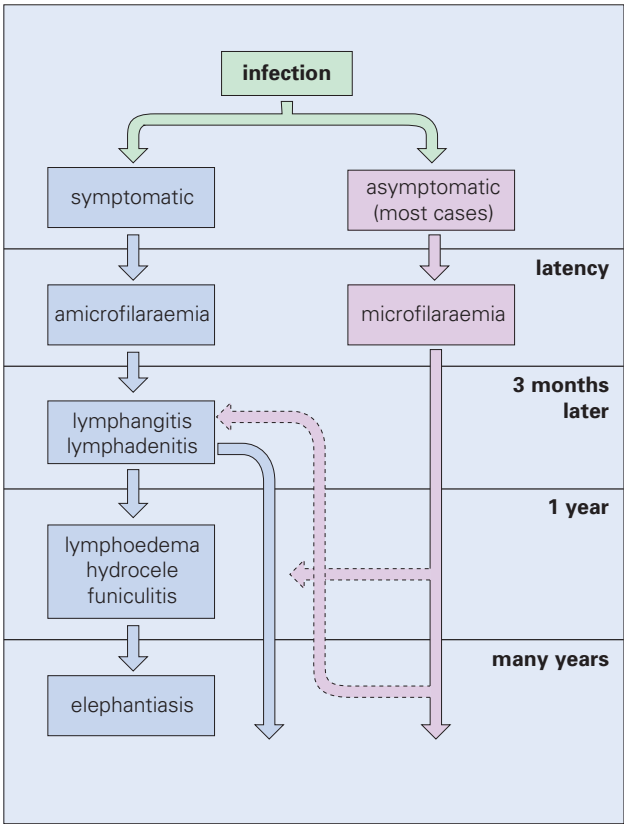


Figure 28.21 Course of lymphocytic filariasis in symptomatic cases. (Redrawn from: Muller R., Baker J.R. *Medical Parasitology*. London: Gower Medical Publishing, 1990.)

leading to hydrocele and to the gross enlargement of breasts, scrotum and limbs – the latter condition being known as elephantiasis (Fig. 28.20). Secondary bacterial infection of the skin (e.g. with streptococci) is a major factor in the development and progression of filarial adenolymphangitis.

A feature of filarial infections in endemic regions is that not everyone exposed develops symptomatic infections. Many, although microfilaraemic, remain asymptomatic, and relatively few show gross pathology (Fig. 28.21). Some individuals develop pulmonary symptoms known as 'tropical pulmonary eosinophilia' (see Ch. 20).

Few drugs are really satisfactory for treating filariasis

Diethylcarbamazine (DEC), which primarily kills microfilariae, is no longer used for the treatment of onchocerciasis as it produces a violent allergic response when microfilariae are killed. A single low dose of DEC is, however, used in the Mazzotti test to diagnose onchocerciasis in patients whose skin snips are negative for microfilariae. Onchocerciasis is treated with ivermectin plus doxycycline.

DEC is still used to treat lymphatic filariasis, in combination with doxycycline, which kills the *Wohlbachia* symbionts in the adult worm. Albendazole plus either DEC or ivermectin is used in MDA programmes to eliminate lymphatic filariasis.

It is difficult to prevent transmission of filariasis and MDA should be supported by vector control and prevention of biting.

KEY FACTS

- Many important infections (arboviruses, rickettsiae, *Borrelia*, protozoa, helminths) are transmitted by vectors – insects, ticks or snails.
- Some infections are chronic (Lyme disease, leishmaniasis, schistosomiasis) or can be lethal (malaria, viral encephalitis).
- Often they are restricted to tropical countries because of the distribution of the vector. Climate change may alter this distribution and therefore the pattern of diseases transmitted.
- Strong immune responses are mounted, often leading to immunopathological complications. Treatment is usually by chemotherapy.
- Vector control is difficult, but can lead to disease eradication.
- With very few exceptions (yellow fever), vaccines are not available for this group of diseases.

29 Multisystem zoonoses

Introduction

Some multisystem infections in humans are animal diseases (i.e. zoonoses)
In these infections, a non-human vertebrate host is the reservoir of infection and humans are involved only incidentally. The human infection follows contact with or ingestion of infective material passed by an infected host, but infection of a human is not essential for the pathogen's life cycle, or for its maintenance in nature. One striking feature of zoonotic infections, and of the arthropod-borne infections described in Chapter 28, is that few are transmitted effectively between humans, who thus represent dead-end-hosts for the infecting organism. However, the largest Ebola virus disease outbreak to date, between 2013 and 2016, demonstrated that there is the potential to do so and how important it is to control and prevent these infections.

Sometimes, the zoonotic origin of these infections is less clear. For example, tularaemia can be acquired either by direct contact with the reservoir host or from an arthropod vector, and is included in this chapter. Plague is included because it is transmitted from infected rats via the rat flea, although it is also transmissible directly from human to human.

Other zoonoses are dealt with in their relevant chapters (e.g. toxoplasmosis in Chs. 24–26, rabies in Ch. 25, salmonellosis in Ch. 23).

ARENAVIRUS INFECTIONS

Arenaviruses are transmitted to humans in rodent excreta

Many zoonoses are caused by enveloped single-stranded RNA viruses with a genome consisting of two RNA segments called arenaviruses. On electron microscopy (Fig. 29.1) these pleomorphic virus particles with a diameter of 50–300 nm can be seen to contain ribosomes that have a sand-like granular appearance, giving rise to the name *arena* (Latin: arena, sand). Arenaviruses are carried by various species of rodent in which they cause a harmless lifelong infection with continuous excretion of virus in urine and faeces of apparently healthy infected animals. Humans may become infected via direct contact with infected rodents, inhalation of infectious excreta, working in agricultural environments or trekking in areas where the rodents exist, and may develop severe and often lethal disease involving extensive haemorrhaging and multiorgan involvement. A selection of arenaviruses and the diseases they cause are included in Table 29.1. Since 2007, nine new arenaviruses have been identified, some as a result of recombination events within one segment. They are divided into the Old and New World groups, of which the Old World viruses, Lassa fever and lymphocytic choriomeningitis virus (LCMV) are associated with the most common human infections involving this family. The distribution of the host is concordant with the distribution of the virus. LCMV is the only arenavirus with a worldwide distribution, the rest being seen in Africa or the New World. Of the New World Tacaribe serocomplex viruses, serious illness is associated with the Junin and Machupo viruses that cause Argentine and Bolivian haemorrhagic fevers, respectively. LCMV can cause acute central nervous system disease. As with most zoonoses, infection is not transmitted, or is transmitted with low efficiency, from human to human. However, healthcare workers have been infected by direct contact with blood or secretions from patients infected with Lassa fever virus, but this can be prevented by using barrier nursing techniques.

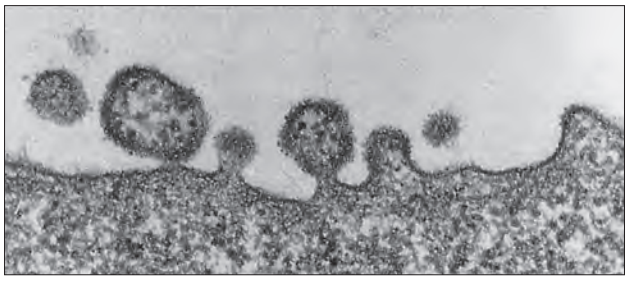


Figure 29.1 Electron micrograph of lymphocytic choriomeningitis virus budding from the surface of an infected cell. The sand-like granules in the virus particles are characteristic of arenaviruses. (Courtesy of K. Mannweiler and F. Lehmann-Grübe.)

Arenavirus infection is diagnosed by viral genome detection, serology or virus isolation

Diagnosis by testing for viral genome or specific antibodies, or by isolating viruses, can be carried out in special centres.

Prevention of infection by reducing exposure to the virus concerned was dramatically illustrated when rodent trapping terminated outbreaks of Bolivian haemorrhagic fever (Box 29.1, Fig. 29.2). Treatment with the antiviral agent ribavirin has been successful if used early in Lassa fever infection.

Table 29.1 Viral fevers and haemorrhagic diseases acquired from vertebrates or from unknown sources

Virus	Virus group	Disease	Animal of origin	Lethality	Geographical distribution
Lymphocytic choriomeningitis (LCM)	Arenavirus	LCM	Mouse, hamster	–	Worldwide
Lassa fever	Arenavirus	Lassa fever	African bush rat (*Mastomys natalensis*)	+	West Africa
Machupo	Arenavirus	Bolivian haemorrhagic fever	Bush mouse (*Calomys callosus*)	+	NE Bolivia
Junin	Arenavirus	Argentinian haemorrhagic fever	*Calomys* spp., mice	+	Argentina
Hantaan	Bunyavirus	Haemorrhagic fever Fever with renal syndrome (Korean haemorrhagic fever) Severe pulmonary syndrome	Mice, rats	+	Far East, Scandinavia, E. Europe SW USA
Marburg	Filovirus	Marburg disease	Fruit bats	+ +	Africa (lab. infections in Marburg, Germany)
Ebola	Filovirus	Ebola disease	Fruit bats	+ +	Africa (Sudan, Zaire, Sierra Leone, Guinea, Liberia)

Box 29.1 ■ Lessons in Microbiology

Bolivian haemorrhagic fever: a lesson in ecology

In 1962, there was an outbreak of a severe and often lethal infectious disease in the small town of San Joachim, Bolivia. Patients developed fever, myalgia and an enanthem (internal rash), followed by capillary leakage, haemorrhage, shock and a neurological illness. This disease was termed 'Bolivian haemorrhagic fever' and had a mortality rate of 15%. Extensive investigations failed to incriminate an arthropod vector, but the evidence pointed to a role for mice in the epidemic. Acting on this possibility, hundreds of mouse-traps were airlifted to the beleaguered town, and it was soon shown that trapping mice had a dramatic effect on the incidence of the disease. The epidemic was completely halted. Quite separately, a virus was isolated from the tissues of a trapped local bush mouse (*Calomys callosus*). The virus was shown to cause a harmless lifelong infection in this animal, with continued excretion of virus in urine and faeces. The virus (given the name 'Machupo') was an arenavirus, a group that includes lymphocytic choriomeningitis (LCM) virus (infecting mice and hamsters) and Lassa fever virus (infecting an African bush rat). These viruses cause a harmless, persistent infection in the natural rodent host, but an often severe disease in humans exposed to infected animals.

This outbreak of Bolivian haemorrhagic fever provided an important lesson in ecology. Because of the high incidence of malaria in the San Joachim area, extensive DDT spraying had been carried out to control mosquitoes. As a result, geckos (small lizards that eat insects) accumulated DDT in their tissues and the local cats that preyed on geckos began to die with lethal concentrations of DDT in their livers. The shortage of cats, in turn, allowed the bush mice to invade human dwellings. The close vicinity of infected mice to humans and human food led to the epidemic (Fig. 29.2).

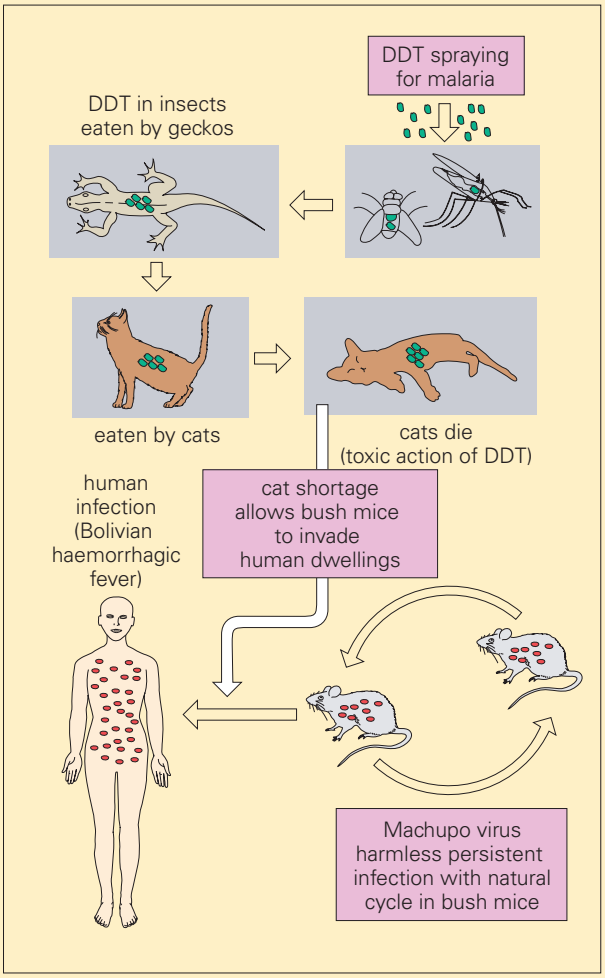


Figure 29.2 Bolivian haemorrhagic fever – a lesson in ecology. DDT, dichlorodiphenyltrichloroethane. (Courtesy of the late Dr Davis Ellis, London School of Hygiene & Tropical Medicine.)

Post-exposure prophylaxis with oral ribavirin has been used. There are no World Health Organization-approved vaccines against arenaviruses. However, a live attenuated Junin virus vaccine was licensed in 2006 for use only in Argentina.

Lassa fever virus is an arenavirus that occurs naturally in bush rats in parts of West Africa

Infection arising from human exposure to infected rats, *Mastomys natalensis*, or their urine results in a febrile disease, which is generally not very severe. Viral entry into host cells is directed by a fusion glycoprotein sited in the viral outer lipid envelope. The cellular receptor for Lassa fever and certain other arenaviruses is α-dystroglycan, a membrane protein found in the mast cells, which anchors the cytoskeleton and the extracellular matrix. There are about 300 000 cases with 5000 deaths / year, and Lassa fever is the commonest febrile illness in hospitals in parts of Sierra Leone. Transfer of virus from hospital patient to healthcare worker via blood or tissue fluids can result in a more severe illness with high mortality. This involves haemorrhage, capillary damage, haemoconcentration and collapse, and was seen when the disease was first recognized in Americans in the village of Lassa in 1969. However, person-to-person transmission via droplet spread is thought to be rare. The usual incubation period is 5–10 days.

Outbreaks have been reported in Central Africa, Liberia, Nigeria and Sierra Leone. An outbreak in Sierra Leone, from January 1996 to April 1997, involved 823 cases with a mortality rate of 19%. The incubation period would allow an infected individual to carry the disease anywhere in the world and, indeed, there have been cases imported into Europe and the USA. Therefore, Lassa fever must be considered in travellers from these endemic areas with fevers of unknown origin.

Lymphocytic choriomeningitis virus occurs worldwide

Lymphocytic choriomeningitis (LCM) has caused sporadic infection in people living in mouse-infested dwellings, and has been reported in children possessing apparently healthy, but infected hamsters. There is generally a non-specific febrile illness, but occasionally aseptic lymphocytic meningitis occurs, with recovery.

HAEMORRHAGIC FEVER WITH RENAL SYNDROME (HFRS)

The Hantaan and Seoul viruses infect rodents and cause HFRS in Asia

The Hantaan and Seoul viruses are bunyaviruses that causes a harmless persistent infection in various species of mice and rats. They differ from other bunyaviruses as the latter are transmitted by arthropod vectors. After exposure to the urine of infected animals, there is a febrile illness, often with hypotension, haemorrhage and a renal syndrome. Many American soldiers suffered severe infections in Korea, and a milder disease is seen in Eastern Europe and Scandinavia. Related viruses are present in mice and rats in the USA, and outbreaks in the southwestern USA caused 26 deaths with severe pulmonary disease. The latter is called hantavirus cardiopulmonary syndrome and has been reported in the Americas as a result of Sin Nombre virus infection. In Europe, Puumala virus causes a mild form of HFRS known as nephropathia epidemica. Laboratory diagnosis is by molecular and serological methods detecting viral RNA or specific IgM or IgG antibody, respectively.

EBOLA AND MARBURG HAEMORRHAGIC FEVERS

Fruit bats are the reservoir for Ebola and Marburg viruses

Ebola and Marburg haemorrhagic fevers occur in central and east Africa, are infections caused by Ebola virus (EBOV) and Marburg virus, members of the family Filoviridae, and are long filamentous single-stranded RNA viruses (Fig. 29.3A, B). There are five Ebola viruses (EBOV) in the *Ebolavirus* genus, Zaire virus, Sudan virus, Tai Forest virus and Bundibugyo virus that can infect humans and primates. Reston virus is also a member, but does not cause disease in humans. Ebola and Marburg virus-infected individuals can develop fever, haemorrhage, rash and disseminated intravascular coagulation (see Ch. 18). The reservoir of origin and natural cycle of maintenance for Marburg virus was not known until Marburg virus RNA was detected in cave-dwelling fruit bats after a small outbreak of Marburg haemorrhagic fever was seen in some miners in Uganda in 2007. A fruit bat reservoir was also found for the Zaire Ebola virus.

Marburg virus infection was first recognized in 1967 in Marburg, Germany, after exposure of laboratory workers to infected African green monkeys from Uganda. However, these monkeys are not the natural hosts. Mortality was about 20% and, as with Ebola virus infection, it was noted that the virus could be detected in semen for months after clinical recovery; one patient transmitted the infection to his wife by this route.

Ebola virus disease (EVD) – gradual evolution of outbreaks to an unprecedented epidemic in West Africa from 2013 to 2016

Outbreaks of a similar disease to Marburg virus infections occurred in 1976 in southern Sudan and in the region of the Ebola River in Zaire (now Democratic Republic of the Congo). Overall, there were 602 people with Ebola virus disease (EVD) and 397 deaths. Person-to-person transmission took place in local hospitals via contaminated syringes and needles, burial preparations and sexual contact.

The virus enters through mucous membranes or abraded skin. Infection does not occur through aerosol transmission. In 1989, monkeys infected with EBOV were inadvertently imported into the USA from the Philippines. A number of the monkeys died but, although at least four people were infected, none developed disease.

A large epidemic was seen in Kikwit, Zaire, in 1995, with 315 cases and 244 deaths. Gabon had three epidemics between 1994 and 1997. EVD appeared in northern Uganda in 2000 and caused large outbreaks with high mortality rates in Congo-Brazzaville in 2003, also killing many gorillas and chimpanzees, and then in Angola between 2004 and 2005.

The largest and longest epidemic of EVD occurred between December 2013 and April 2016 in West Africa, in Guinea, Liberia and Sierra Leone. Overall, 28 616 people had been seen with suspected, probable and confirmed EVD, with 11 310 deaths, although the true figures were likely to have been greater. Due to local travel and EBOV infected healthcare

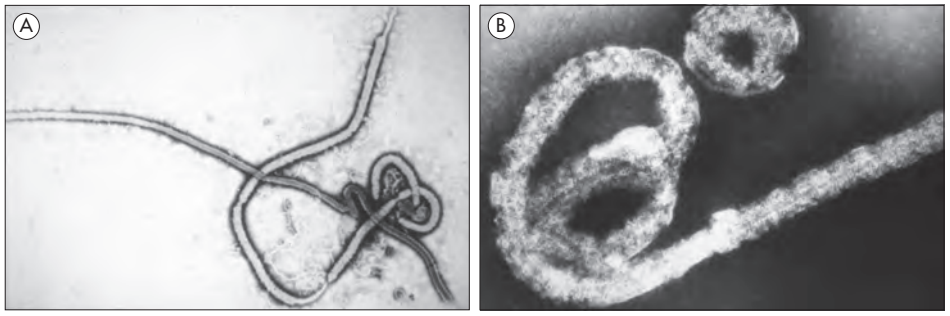


Figure 29.3 (A, B) Electron micrographs of Ebola Zaire virus. (Courtesy of the late Dr David Ellis, London School of Hygiene and Tropical Medicine.)

workers returning home, there were 36 people with EVD reported in Nigeria, Senegal and Mali and the United States, Great Britain, Spain and Italy, respectively.

It was thought to have started in Guinea, where a 2-year-old boy had died within 2 days of falling ill with the Zaire EBOV strain (Fig. 29.3B) possibly having been in contact with a bat. Subsequently, direct contact with blood or body fluids of EBOV-infected symptomatic individuals was the main route of transmission. The outbreaks in West African countries varied in size owing to the time period of the growth rate of the epidemic, as well as the population size. In addition, a proportion of EBOV-infected individuals were 'superspreaders', who infected the majority of people who made up the next generation of infected individuals.

Interventions that reduced the transmission rate

The effective control measures included finding symptomatic people, contact tracing, isolation of patients and contacts, admission of patients to specific Ebola treatment centres where clinical supportive care could be given, ensuring good infection control practices and providing safe burials. In the absence of antiviral treatments, management of symptomatic people that was critical involved relatively simple measures such as carrying out blood tests to measure electrolyte imbalances and intravenous rehydration. The development of a vaccine and rapid diagnostic tests in biocontainment field laboratories helped too, in terms of protection and faster EVD detection, respectively.

The epidemic peaked in September 2014, after 10 months and further EBOV infections were seen for another 18 months, with the end determined by the passing of two incubation periods, 42 days, from the last reported EBOV-infected person. It was known that transmission could occur in the absence of a viraemia as viral RNA could be detected in semen, breast milk, eye fluid and CSF. Reactivation from sanctuary sites could also result in a viraemia leading to transmission. Little had been known about potential clinical sequelae whilst recovering from EVD, but a clinic in Sierra Leone reported that 76% of survivors had arthralgia, 18% uveitis and 24% hearing loss. Neurological symptoms have also been reported in EVD survivors.

Rapid development of diagnostic tests, antiviral agents and vaccines

A major global effort by the international community led to almost 40 field laboratories being built in West Africa, with biocontainment facilities and the equipment to carry out extraction of nucleic acid from various sample types and real-time reverse transcriptase PCR assays to detect EBOV RNA.

In those 27 people who were medically evacuated for care in their countries of origin in Europe and the United States, including those diagnosed with EVD in those countries having been infected with EBOV in West Africa, careful monitoring, intravenous rehydration, correcting electrolyte imbalances and critical care management were all critical in the near 82% survival figure.

Experimental treatments were also used including immunotherapies such as convalescent plasma, monoclonal antibodies (ZMapp, ZMab or MIL77) and antiviral agents such as brincidofovir and favipravir. It was impossible to determine whether they had an effect as there were small numbers of patients and no controls (see Box 29.2).

Infection control strategies to manage travellers from EBOV-affected countries

From an infection control perspective, screening algorithms were prepared across the world for managing people travelling from the countries in West Africa where there were EBOV outbreaks. The key questions involved whether the traveller had had a fever in the previous 24 hours and had developed symptoms within 21 days of leaving an EBOV-affected country. Screening at airports and other ports of entry into countries involved measuring the person's temperature and asking about symptoms, preparing isolation rooms in emergency departments in hospitals for travellers or contacts with symptoms, making arrangements in laboratories to both test samples and send samples for EBOV RNA as well as malaria, the latter being the most common diagnosis. In addition, ambulance and hospital staff were trained on what protective equipment to wear when in contact with people with potential EBOV infection and to know which infectious disease containment facilities they were to be admitted to for management if found to have EVD.

A number of vaccines were rapidly developed, starting with virus inactivation and moving on rapidly to DNA vaccines, recombinant viral vector vaccines and recombinant and subunit proteins. All involved expressing the EBOV glycoprotein, which is involved in attachment and virus–cell membrane fusion and is a target for neutralizing antibodies. Two vaccines that underwent clinical investigation were a replication-competent vesicular stomatitis virus-based vaccine expressing the glycoprotein of the Zaire strain of EBOV (ZEBOV) and a monovalent, replication-deficient, chimpanzee adenovirus type 3 vector-based ZEBOV vaccine.

Box 29.2 ■ Lessons in Microbiology

Although there have been a number of Ebola virus disease (EVD) outbreaks in Africa, complications in survivors had not been recognized. A number of healthcare workers were repatriated to their countries having volunteered to assist in managing patients with EVD in the epidemic in West Africa and subsequently developed symptoms associated with Ebola virus (EBOV) infection. One nurse became symptomatic having returned to Great Britain. She was transferred to a specialist infectious disease high containment unit and received intravenous fluid and electrolyte replacement, an antiviral agent called brincidofovir and convalescent plasma that had been collected from another survivor. However, she developed respiratory failure and needed mechanical ventilation, high-volume diarrhoea, erythroderma and mucositis. The initial high plasma EBOV RNA load, with an RT-PCR crossing threshold (CT) value of 25, had become raised at a value of 13 by day 6 of admission. This fell after two doses of ZMAb, an experimental monoclonal antibody raised against an EBOV glycoprotein, had been given and plasma EBOV RNA was not detectable by day 25.

She was then discharged from hospital, but 3 weeks later developed thyrotoxicosis, an overactive thyroid gland, due to thyroiditis. Plasma EBOV RNA was not detected in blood, a test carried out after she had developed joint pains and some ankle joint effusions. However, 9 months after discharge, she had a fever, severe headache and meningism. A diagnosis of EBOV relapse was made when a lumbar puncture was carried out and EBOV RNA was detected at a CT of 24 in the CSF and 31 in the plasma. No other pathogens were detected in the CSF sample. She subsequently developed meningoencephalitis, had two tonic–clonic seizures and given another monoclonal antibody drug, MIL 77, that had to be discontinued. An experimental nucleoside analogue, GS-5734, that had successfully treated EBOV infected non-human primates was then given, together with a steroid, dexamethasone and she improved slowly and was again discharged after nearly 2 months.

The central nervous system was the most likely site of EBOV relapse, probably after viral dissemination during the acute infection and persistence in this immunologically privileged sanctuary site. EBOV sequence analysis showed that the virus had not changed since the initial infection.

Careful monitoring of EVD survivors is critical, together with continuing research and development of effective antiviral therapies.

Box 29.3 ■ Lessons in Microbiology

Molecular epidemiology, phylogenetic analyses, high-throughput sequencing and bioinformatics have revolutionized the approach to investigating outbreaks of infections, determining the origin, evolution and spread.

High-throughput sequencing of Ebola virus genomes in the 2013–2016 Ebola virus epidemic in West Africa allowed rapid real-time molecular epidemiological investigation of transmission chains that resulted in improved outbreak responses.

Analysis of the genome sequences answered the key question as to whether one cross-species transmission event involving humans or had a number of zoonotic events from a widespread animal EBOV reservoir occurred leading to the epidemic. It was likely it was the former as the EBOV genomes sequenced at the start of the epidemic were genetically similar. Molecular clock analyses showed that all recorded human EVD outbreaks shared a common ancestor around 1975, close to the first described outbreak in southern Sudan and in the region of the Ebola River in 1976.

Genomic data were used to assist with infection control and public health policies during the epidemic. Phylogeographic approaches were used to determine how EBOV spread through the communities, allowing direct intervention to be employed in transmission hotspots. Phylogenetic analyses shed light on individual transmission events, showing that sexual transmission had occurred and that multiple reoccurrences happened during the epidemic and how they were related to transmission events involving survivors, together with the human 'super-spreaders'.

Ecological niche modelling models have been used to predict where one might expect to find these filovirus infections. Interestingly, Ebola mapped to the broadleaf tropical rainforest and humid areas in equatorial Central Africa and parts of West Africa (although Angola did not fit this model). Marburg, however, mapped to the opposite, drier, more open areas away from the equator. In these models, bats were thought to be the potential reservoir hosts. Subsequently, tropical rain forest fruit bats were identified as the Ebola virus reservoir. Developments in high-throughput next generation sequencing and large-scale sequence data sets and bioinformatics analyses allowed molecular epidemiological investigations of EBOV transmission chains that could assist outbreak management (see Box 29.3).

There is no treatment, post-exposure prophylaxis or vaccine prevention option for Marburg virus infections.

CRIMEAN–CONGO HAEMORRHAGIC FEVER, A TICK-BORNE VIRUS

Crimean–Congo haemorrhagic fever (CCHF), a severe haemorrhagic fever, with shock and disseminated intravascular coagulation, was described clinically during a large outbreak in the Crimea, part of the former Soviet Union, in 1944. The CCHF virus of the Bunyaviridae family, *Nairovirus* genus, was identified in 1967 and has a wide geographic range, including Africa, Asia, Central and Eastern Europe and the Middle East. It is transmitted by the bite of ixodid ticks (both reservoir and vector), by contact with infected animals or person to person by exposure to infected body fluids including blood. A number of nosocomial outbreaks have been reported around the world. Although mortality rates of up to 80% have been reported, supportive management and the use of ribavirin have been shown to be effective.

Q FEVER

Coxiella burnetii is the rickettsial cause of Q fever

The disease Q fever was first recognized in Australia in 1935, but the cause was unknown for several years – hence Q ('query') fever. The causative rickettsia, *Coxiella burnetii*, differs from other rickettsiae (see Ch. 28) in the following ways:

- It is not transmitted to humans by arthropods.
- It is relatively resistant to desiccation, heat and sunlight, and is therefore stable enough to be acquired from infected material by inhalation.
- Its main site of action is the lung rather than vascular endothelium elsewhere in the body, so that there is usually no rash.

C. burnetii is transmitted to humans by inhalation

C. burnetii can infect many species of wild and domestic animals. In many countries (e.g. USA) infection of livestock is quite common, but there are few human cases (132 reported in 2008 in the USA). Large seasonal Q fever outbreaks occurred in the Netherlands between 2007 and 2009. Infected dairy goat farms were the source of infection. More than 3500 human infections were notified over that time period. The southern part of the Netherlands was most affected, with >12% of the population found to have *C. burnetii* antibodies. People who come into contact with infected animals, especially their placentas (e.g. veterinarians, farmers, abattoir workers) are at risk from aerosolized organisms. Unpasteurized milk, tissue fluids and dust from infected stock can also transmit the disease.

After inhalation, the microbe multiplies in the terminal airways of the lung, and about 3 weeks later the patient develops fever, severe headache, and often respiratory symptoms and an atypical pneumonia. The rickettsia can also spread to the liver, commonly causing hepatitis. Recovery is usually complete in 2 weeks, but the disease can become chronic. The heart is then sometimes involved (endocarditis), with thrombocytopenia and purpura in some patients, and this condition is fatal if untreated.

Q fever is diagnosed serologically and treated with antibiotics

Polymerase chain reaction (PCR) can be used to determine whether a patient has Q fever; however, the sensitivity of this approach decreases after the first week of illness. *C. burnetti* cannot be detected in blood cultures and cannot be isolated by culture except in specialized laboratories. Thus, serological diagnosis is important. A fourfold or greater rise in complement fixing antibody titre is significant. There are two antigenic forms of the rickettsial lipopolysaccharide (LPS): phase 1 and phase 2. Increased antibody to phase 2 compared with phase 1 is seen in acute Q fever, while the reverse (higher antibody titres to phase 1 than phase 2) is seen in chronic disease. Definitive serological confirmation of acute Q fever is demonstrated by a fourfold increase in antibody titres measured by indirect immunofluorescence assay (IFA). The Weil–Felix test (see Ch. 28) is not used.

Acute infection is treated with oral tetracyclines; chronic infections may require drug combinations such as rifampin and doxycycline or trimethoprim-sulphamethoxazole. A killed vaccine is available for those at risk. The rickettsiae are destroyed when milk is pasteurized.

ANTHRAX

Anthrax is caused by *Bacillus anthracis* and is primarily a disease of herbivores

Most members of the genus *Bacillus* are harmless saprophytes, present in soil, water, air and vegetation. *Bacillus cereus* is a cause of food poisoning, but *B. anthracis* is the principal pathogen. It is a large, aerobic and non-motile Gram-positive rod and is unique in having an antiphagocytic capsule made of D-glutamic acid. It forms spores which survive for years in soil.

Anthrax is a disease of herbivores such as sheep, goats, cattle and horses, and bacilli are excreted in faeces, urine and saliva. Humans are relatively resistant, infection occurring following direct contact with infected animals, or by contact with spores present in animal products. The spores can enter the body via the skin and mucous membranes or, less commonly, via the respiratory tract. In resource-rich countries, where animal infection is now uncommon, human infection is rare and when present has been due to exposure to contaminated imported goods such as hides, skin, wool, goat hair and bristles, bones and bonemeal in fertilizers. Spores have also been used in bioterrorism.

Anthrax is characterized by a black eschar, and the disease can be fatal if untreated

B. anthracis spores germinate in tissues at the site of entry. The bacteria then multiply and produce the anthrax toxin, which consists of a protective antigen, an oedema factor (an adenylate cyclase) and a lethal factor; all of them plasmid-coded. Toxic activity requires the protective antigen and at least one of the other two. Host defences are inhibited by the antiphagocytic capsule surrounding the bacillus (see Ch. 15).

The skin is the usual site of entry. As the toxic material accumulates, there is oedema and congestion, and a papule develops within 12–36 h. The papule ulcerates, the centre becoming black and necrotic to form an eschar or 'malignant pustule' (although there is no pus) which is painless and is often surrounded by a ring of vesicles (Fig. 29.4). The bacilli spread to the lymphatics and in about 10% of cases reach the blood to cause septicaemia. Continued multiplication and production of the toxin causes generalized toxic effects, oedema and death.

When the spores are inhaled and enter alveolar macrophages, bacterial growth in the lung leads to pulmonary oedema and mediastinal haemorrhage, with spread to the blood and subsequent death. Pulmonary anthrax is now very rare in most resource-rich countries, where it was referred to as 'woolsorter's disease'.

Cutaneous anthrax is diagnosed by culture and treated with ciprofloxacin

Films from skin lesions show Gram-positive bacilli, but diagnosis can be confirmed and the organism distinguished from non-pathogenic bacilli by culture on blood agar or by PCR assay. Antibodies to toxin antigens indicate presence of the bacillus.

Cutaneous anthrax is successfully treated by ciprofloxacin. Cutaneous anthrax is fatal in 20% of cases when untreated. Systemic anthrax is treated with combination antimicrobial therapy plus antitoxin.

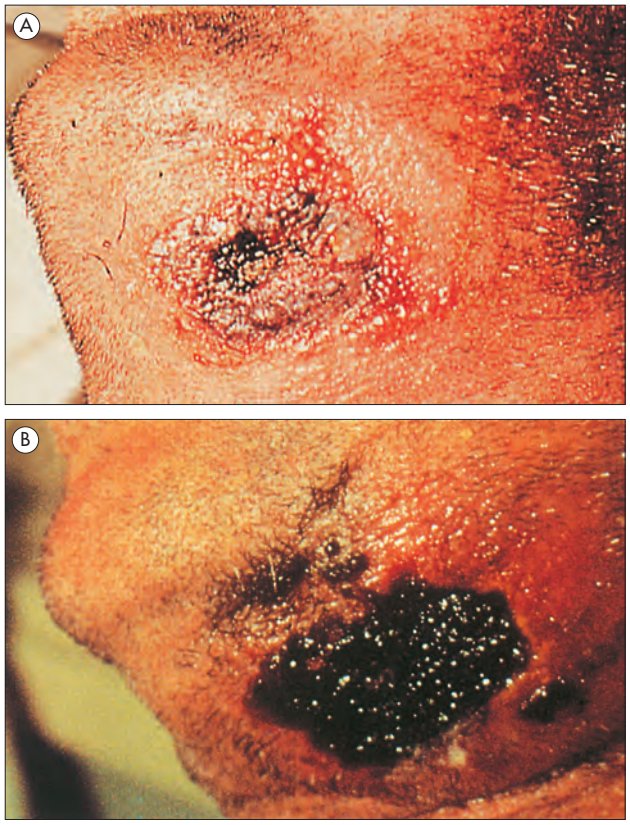


Figure 29.4 Anthrax. (A) Characteristic black eschar surrounded by a ring of vesiculation. (B) Some 8 days later the eschar has enlarged to cover the previously vesicular area, and the surrounding oedema has diminished. (Courtesy of F.J. Nye.)

Anthrax, as a natural infection, is now mainly confined to resource-poor countries. Vaccines are available. Bioterrorism is an important threat.

The disease is largely confined to resource-poor countries (parts of Asia, Africa, Middle East).

Animals can be protected by vaccination with live avirulent bacteria. Infected animals are isolated, killed and buried or cremated without autopsy. A vaccine consisting of purified protective antigen is available for humans at high risk. Human infection is reduced by rigidly controlled disinfection of imported animal products such as hides, hair and wool.

Anthrax is one of the three bacteria categorized by CDC as high-priority bioterrorism threats. Spores sent by mail infected 22 people in the USA in 2001 and this generated renewed interest in antimicrobial post-exposure control (e.g. a fluoroquinolone or doxycycline).

PLAGUE

The plague is caused by *Yersinia pestis*, which infects rodents and is spread from them by fleas to humans

Yersinia pestis is a small Gram-negative rod with a surrounding antiphagocytic capsule that is associated with virulence. The sylvatic reservoirs are rodents such as rats, squirrels, gerbils and field mice, in which the infection is generally mild, the bacteria being spread between animals and to humans by fleas (Fig. 29.5). Infections in urban rats have been the most important sources of plague in humans, and the disease has at times decimated populations and influenced the course of history. In the fourteenth century, about 25% of the population of Europe died in plague epidemics (Box 29.4). Early in the twentieth century, the disease arrived in North America and is at present endemic in wild rodents in western USA. Plague in humans is now extremely rare in Europe and uncommon in the USA.

The rat flea (*Xenopsylla cheopis*) carries infection from rat to rat and from rat to human. *Y. pestis* causes blood to clot in the gut of the flea, multiplies profusely in the clot and eventually blocks the gut lumen, so that the flea regurgitates infected material as it attempts to feed. As infected rats sicken, their fleas leave and may bite humans, thus transmitting 'bubonic' plague. This disease is not generally transmitted from person to person. However, when there is extensive replication of bacteria in the lung, with bronchopneumonia and large numbers of bacteria in the sputum, the infection can spread from person to person by droplets, causing 'pneumonic' plague, with extremely rapid onset.

Rodent infection is endemic in India, SE Asia, central and southern Africa, South America, Mexico and the western states of the USA. Sporadic plague continues to occur in these parts of the world, for instance, in over an 8-week period in 2010, 31 cases of plague were reported in Peru leading to three deaths in a province containing important export harbours. In 2009, a plague outbreak in a farming area of northwestern China resulted in three deaths.

Clinical features of plague include buboes, pneumonia and a high death rate

The infecting bacteria multiply at the site of entry in the skin, and spread via the lymphatics to local and regional lymph nodes. They produce a number of virulence factors, including an antiphagocytic capsular antigen (fraction 1, coded by a plasmid), endotoxin and various other protein toxins. Lymph nodes in the axilla or groin become very tender and enlarge to form 'buboes' with haemorrhagic inflammation 2–6 days after the flea bite. The patient develops fever. In mild forms, the infection is arrested at this stage, but spread to the blood often occurs, with septicaemia, haemorrhagic illness and multisystem involvement (spleen, liver, lungs, CNS).

Common complications are disseminated intravascular coagulation, pneumonia and meningitis. The death rate is about 50% in untreated bubonic plague, and nearly 100% in pneumonic plague. On recovery, there is solid immunity, and bacteria are eliminated from the body.

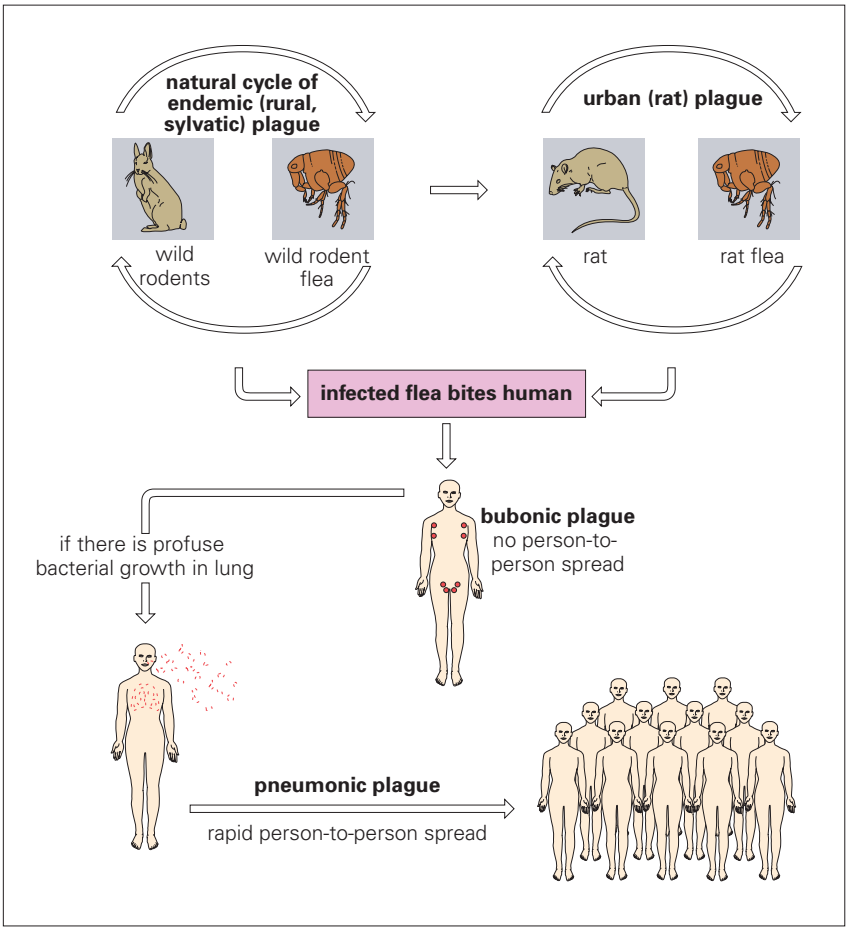
Plague is diagnosed microscopically and treated with antibiotics

Organisms can be recovered in fluid aspirated from lymph nodes, or from sputum in pneumonic plague and stained with Giemsa, Gram or fluorescent antibody (the staining is bipolar); they can also be cultivated. Streptomycin is the standard treatment; doxycycline or ciprofloxacin are also used.

Plague has been prevented by the following measures:

- classically, by quarantine measures in ports and on ships
- by rodent control, especially of rats at the site of entry of ships and aircraft into plague-free countries

Figure 29.5 The epidemiology of plague.



- by strict isolation of patients with plague
- by chemoprophylaxis (doxycycline) during an epidemic or visit to an affected area
- by vaccination of military personnel and of certain workers in endemic areas.

An older vaccine formulation consisting of formalin-killed bacteria has been replaced by efforts to develop a more effective (recombinant) formulation.

YERSINIA ENTEROCOLITICA INFECTION

Yersinia enterocolitica is a cause of diarrhoeal disease (see Ch. 23) and is mentioned here because it has a reservoir in rodents, rabbits, pigs and other livestock.

TULARAEMIA

Tularaemia is caused by *Francisella tularensis* and is spread by arthropods from infected animals

Tularaemia is caused by the small Gram-negative rod *Francisella tularensis*, first isolated from rodents in Tulare County, California, in 1912 and later shown by Edward Francis to cause human disease. It is present in rodents and in a wide variety of other wild animals in many countries in the northern hemisphere, including the USA (especially Arkansas and Missouri), Russia, Scandinavia and Spain, and can occur in contaminated water. The variety found in North America causes a more severe disease than that found in Europe and Asia. In the infected animal, it causes a plague-like disease and is spread via ticks, mites, lice and biting flies. In *Dermacentor* ticks, the bacteria are transmitted vertically by infected female ticks to her offspring via the ovum. Human infection is sporadic, the normal means of infection being contact with the carcass of an infected animal (e.g. skinning of hares, rabbits, muskrats) or the bite of an arthropod vector. There is no spread from person to person.

Clinical features of tularaemia include painful swollen lymph nodes

F. tularensis parasitizes the reticuloendothelial system and lives intracellularly in macrophages, inhibiting phagosome–lysosome fusion. It spreads at the site of entry, aided by an antiphagocytic capsule, and after 3–5 days forms a skin ulcer. There is a febrile illness, and lymphatic spread results in swollen painful regional lymph nodes. Blood invasion and involvement of lungs, gastrointestinal tract and liver is not uncommon, with the formation of granulomatous nodules around infected reticuloendothelial cells. There may be a rash. Mortality in untreated patients is 5–15%. The conjunctiva or oral mucosa can be infected via contaminated fingers, resulting in ocular or oral manifestations. Infection by inhalation is less common and gives a febrile illness with respiratory symptoms.

Box 29.4 ■ Lessons in Microbiology

The Black Death in fourteenth-century England

For thousands of years, *Yersinia pestis* has been endemic in rodents in the Far East, with occasional epidemic spread into Europe and elsewhere. In January 1348, three galleys laden with spices from the East brought the plague to the port of Genoa, Italy. The disease, for reasons that are not clear, became known as 'The Black Death' and soon spread to the rest of Europe, arriving in London in December 1348. To the medieval mind, the speed and violence with which the illness passed from person to person (in the pneumonic form in the winter) was its most terrifying feature. The bubonic form was also important, especially in the warmer summer months, there being at least one family of black rats per household and three fleas to a rat.

The disease was attributed to earthquakes, to the movement of the planets, to a Jewish or Arab plot (350 massacres of Jews took place during the Black Death in Europe), and most commonly to God's punishment for human wickedness. One could become infected without touching a plague victim, and to many it seemed that there was something – a miasma or a poison – in the air. Physicians wore strange masks, and infected houses were labelled and boarded up, together with the inhabitants. But it was impossible to isolate all those who were sick. Rich and poor perished.

The population of England was about 4 million, and over a period of 2.5 years, approximately 35% (more than 1 million) died. The clergy, for unknown reasons, suffered an even greater mortality of nearly 50%. Altogether in Europe, at least 25 million people died. The Black Death was a major human disaster, with lasting effects on economic and social structure. There were a further five, less severe, outbreaks in England in the fourteenth century. The epidemic in 1665, the year before the Great Fire of London, was graphically described by Daniel Defoe (who was only 5 years old at the time) in his *Journal of a Plague Year in London*. The last pandemic arose in China and reached Hong Kong in 1894, where Yersin and (independently) Kitasato described the causative bacillus.

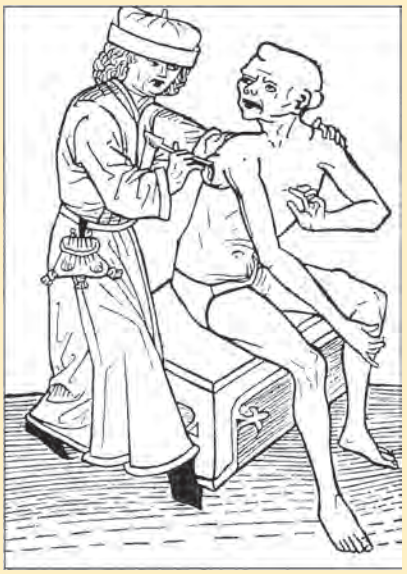


Figure 29.6 Fifteenth-century German woodcut showing incision of a bubo. (Courtesy of the World Health Organization.)

Tularaemia is diagnosed clinically and serologically. Streptomycin is the drug of choice, although other antimicrobials have been used (doxycycline and gentamicin).

Infected tissues can be examined by fluorescent antibody staining, but isolation of bacteria is not often attempted, because of the high risk of laboratory infection. Antibody tests are more commonly used in diagnosis.

Streptomycin is an effective treatment. A live attenuated bacterial vaccine is available for people with an occupational risk (e.g. fur trappers) but has issues of toxicity and incomplete protection, prompting efforts to develop a more effective preparation. Handling animals with gloves, particularly when skinning or eviscerating, gives protection, and contact with ticks should be avoided.

PASTEURELLA MULTOCIDA INFECTION

Pasteurella multocida is part of the normal flora of cats and dogs and is transmitted to humans by an animal bite or scratch

Pasteurella multocida is an encapsulated Gram-negative rod and is distributed worldwide. A number of capsular types exist. It is part of the normal oral flora in cats, dogs and other domestic and wild animals, in which it can also cause pneumonia and septicaemia. It is transmitted to humans by animal bites (especially cat bites) or scratches.

P. multocida infection causes cellulitis, is diagnosed by microscopy and treated with amoxicillin / clavulanate

Local multiplication of bacteria leads within a day or two to cellulitis and lymphadenitis; other types of bacteria including anaerobes are often present in the lesion. Infection can become systemic in patients with compromised immune systems. Virulence factors include endotoxin and the capsule.

P. multocida can be cultivated and identified in material from the wound.

Amoxicillin / clavulanate is an effective treatment, and has also been used in prophylaxis after cat or dog bites. Bite wounds should be cleansed and debrided.

LEPTOSPIROSIS

Leptospirosis is caused by the spirochete _Leptospira interrogans_, which infects mammals such as rats

Leptospires are tightly coiled spirochetes 5–15 µm long. They show active rotational movement and have two flagella, originating at each end but located within the cell as in *Borrelia*. Their delicate outline is best seen by dark field microscopy

Table 29.2 Disease caused by the three main serogroups of the *Leptospira interrogans* complex

Leptospiral serogroups	Animal host	Geographical distribution	Clinical features
Canicola	Dog	Worldwide	Influenza-like illness ('canicola fever', '7-days fever') is the commonest; can progress to aseptic meningitis, liver and kidney damage (Weil's disease)
Icterohaemorrhagiae	Rat	Worldwide	
Hebdomadis	Mice, voles, rats, cattle	Japan, Europe	

There are 19 different serogroups of this organism, other serogroups including Seroja (pigs) and Pomona (swine and cattle in USA and Europe). Among the serogroups, there are 172 different serotypes.

because they are not very well stained by dyes. There are many species, each with several serotypes. The *biflexa* complex is free-living, the *interrogans* complex is pathogenic. The ends of *L. interrogans* are bent into a question-mark shape, hence the specific name. This species infects many domestic and wild mammals in various parts of the world (Table 29.2), dogs and rats being important sources of infection. Infected animals develop a chronic kidney infection with excretion of large numbers of bacteria in urine. The spirochetes are soon killed on drying, heating and exposure to detergents or disinfectants, but they remain viable for several weeks in stagnant alkaline water or wet soil. Humans are infected by ingestion of, or exposure to, contaminated water or food. The bacteria, aided by their motility, enter through breaks in skin or mucosae, so infection can be acquired by swimming, working or playing in contaminated water. Therefore, miners, farmers, sewage workers, and water sports enthusiasts are especially at risk. There are about 50 cases / year in England and Wales, and about 100 / year are reported in the USA. Bacteria are excreted in human urine, but person-to-person transmission is rare. Immunity is serotype specific.

Clinical features of leptospirosis include kidney and liver failure

The bacteria reach the blood and, after an incubation period of 1–2 weeks, cause a febrile, influenza-like illness. In about 90% of cases, this resolves uneventfully, but multiplication can cause:

- hepatitis, jaundice and haemorrhage in the liver
- uraemia and bacteriuria in the kidney
- aseptic meningitis and conjunctival or scleral haemorrhage in the cerebrospinal fluid (CSF) and the aqueous humor (Fig. 29.7).

The main clinical signs result from damage to the endothelia of blood vessels, the clinical picture depending to some extent upon the particular type of leptospire involved. Weil's disease, the severe form with haemorrhagic complications and kidney and liver failure, occurs in only 5–10% of patients with leptospirosis.

Leptospirosis is diagnosed mainly by serological tests and treated with antibiotics

There is often a history of exposure. Bacteria can be isolated from blood, CSF and urine, and a rise in agglutinating serotype-specific antibody can be demonstrated.

Penicillin and doxycycline have been valuable in treatment when given within a day or two of the onset of illness, and doxycycline will prevent disease in those exposed to infection.

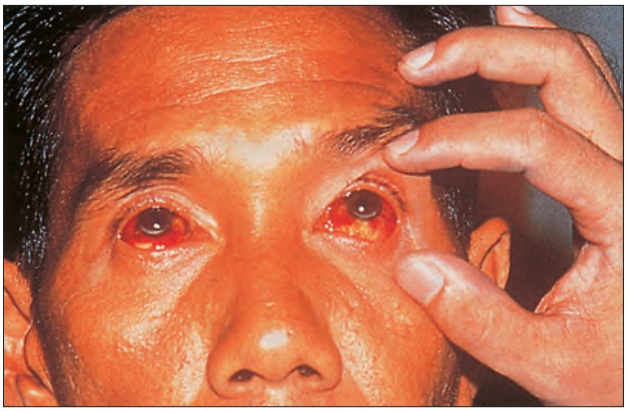


Figure 29.7 Conjunctival haemorrhages in a jaundiced patient with leptospirosis. (Courtesy of D. Lewis.)

Measures for prevention include:

- rodent control
- protective clothing
- prophylactic penicillin after cuts and abrasions in those at risk.

RAT-BITE FEVER

Rat-bite fever is caused by bacteria transmitted to humans by a rodent bite

This uncommon but worldwide condition is caused by one of two species: *Spirillum minus*, a Gram-negative spiral-shaped organism (spirillar fever), or *Streptobacillus moniliformis*, a Gram-negative filamentous bacillus (streptobacillary fever). These bacteria are found in the oropharyngeal flora of 50% of healthy wild and laboratory rats and also in other rodents. Transmission to humans is by biting.

Clinical features of rat-bite fever can include endocarditis and pneumonia

After an incubation period of 7–10 days there is an onset of fever, headache and myalgia. Bacteria multiply at the site of the bite, and in the case of *S. moniliformis*, cause an inflamed local lesion. Spread of infection to lymph nodes and the blood leads to lymphadenopathy, rash and arthralgia. Fever may be recurrent if untreated.

Complications include endocarditis and pneumonia, and there is a mortality of up to 10% in untreated patients.

Rat-bite fever is diagnosed by microscopy or culture and is treated with antibiotics

S. moniliformis can be cultured from the wound site, lymph nodes and blood, but *Spirillum minus* cannot be cultivated and must be demonstrated in tissues by dark field microscopy.

Penicillin and streptomycin are effective treatments. Measures for prevention include:

- rodent control
- prevention of rat bites in laboratory workers.

BRUCELLOSIS

Brucellosis occurs worldwide and is caused by *Brucella* species

Brucellae are small Gram-negative non-motile coccobacilli, adapted to intracellular replication. Four 'species' cause disease in humans: *Brucella abortus, B. melitensis, B. suis, B. canis,* but, on the basis of DNA homology, these are all variants of *B. melitensis*. The first three share common A and M antigens (*B. abortus* primarily A and *B. melitensis* primarily M); *B. canis* is distinct.

Brucellae are primarily animal pathogens, infecting humans after contact with infected animals or their products (Fig. 29.8):

- *B. abortus* infects cows worldwide, but has been eliminated from several resource-rich countries. It causes mild disease in humans.
- *B. melitensis* infects goats and sheep and is common in Malta and other Mediterranean countries, Mexico and South America. It causes more severe disease in humans.
- *B. suis* infects pigs in the USA, South America, and SE Asia. It causes severe disease with destructive lesions in humans.
- *B. canis* infects dogs and is an uncommon cause of mild disease in humans.
- In cows and goats, brucellae localize in the placenta, causing contagious abortion, and also in mammary glands, from where they are shed for long periods in milk. They are present in uterine discharges, faeces and urine.

Human brucellosis (undulant fever, Malta fever) occurs when the bacteria enter the body via abrasions in the skin, via the alimentary tract or, most commonly, via the respiratory tract. Infection is therefore more common in farmers, veterinarians and abattoir workers. Unpasteurized cows' milk (UK, USA), goats' milk or cheese (Mediterranean countries) are less frequent sources of infection. There is no spread from person to person. Infection is common worldwide, but incidence is low in the resource-rich world.

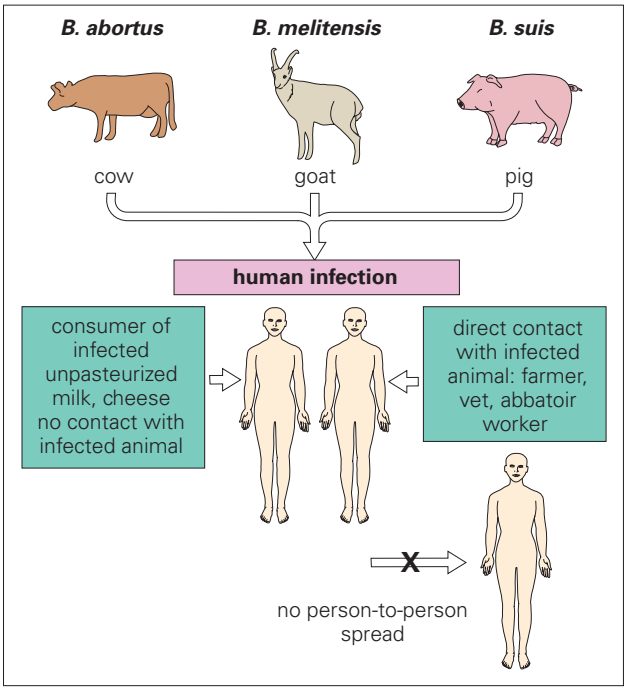


Figure 29.8 Transmission of brucellosis. Human infection follows contact with infected animals or consumption of infected animal products.

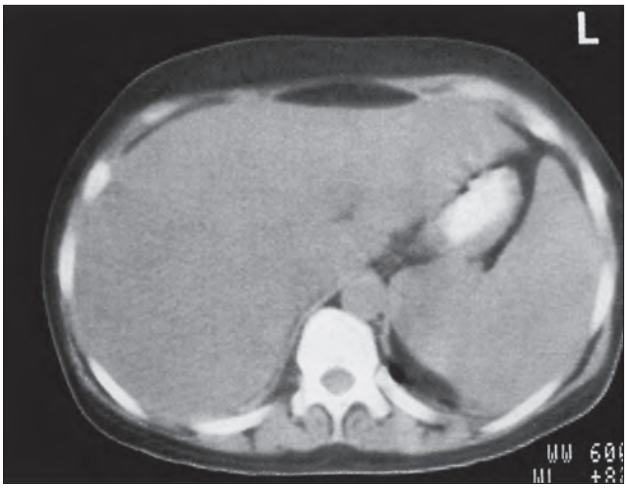


Figure 29.9 Computerized tomographic (CT) scan showing hepatosplenomegaly in *Brucella melitensis* infection. (Courtesy of H. Tubbs.)

Clinical features of brucellosis are immune mediated and include an undulant fever and chronicity

The infecting bacteria pass from the site of entry into local and regional lymph nodes, the thoracic duct and thus the blood (septicaemic phase). Reticuloendothelial cells are infected (liver, spleen, bone marrow, lymphoid tissues) and here the bacteria can survive for prolonged periods. The result is an inflammatory (granulomatous) reaction with epithelioid and giant cells, central necrosis and peripheral fibrosis.

Quite commonly, the infection is subclinical. The symptoms of acute brucellosis begin after an incubation period of 2–6 weeks with a gradual onset of malaise, fever, drenching sweats, aching and weakness. A rising and falling (undulant) fever is seen in a minority of patients. Enlarged lymph nodes and spleen may be detected and hepatitis can occur (Fig. 29.9). The bone marrow lesions may progress to osteomyelitis, and cholecystitis, endocarditis and meningitis are occasionally seen. Abortion occurs in infected cows, sows and goats, but not in humans, who lack the sugar compound erythritol, which stimulates bacterial growth in the placenta.

The patient generally recovers after a few weeks or months, but a chronic stage (more than 1 year's illness) can develop with tiredness, aches and pains, anxiety, depression and

occasional fever. Relapses and remissions may occur. Brucellae cannot be isolated at this stage, and chronic brucellosis is often a difficult diagnosis. Agglutinin titres are generally high, but antibodies are less relevant than cell-mediated immunity for this intracellular parasite.

Brucellosis is diagnosed by culture and by serological tests and treated with antibiotics

Brucellae can be isolated in some cases from blood cultures (or from bone marrow or lymph nodes), and urine culture may be successful. This takes up to 4 weeks. IgM antibodies are present in acute brucellosis, IgG and IgA in chronic brucellosis. A rising titre suggests a current infection.

Brucellosis is typically susceptible to tetracycline and streptomycin; co-trimoxazole is also used. Because of the intracellular location of the bacteria, brucellosis is typically treated with combination therapy (e.g., doxycycline plus streptomycin) for a minimum of 6 weeks.

Brucellae in milk are destroyed by pasteurization. In the USA and UK, brucellosis has gradually declined (about 100 cases / year now reported in the USA) following eradication and control programmes. Protective clothing and goggles may be used by those in close contact with infected animals (farmers, veterinarians, abattoir workers). There is no satisfactory vaccine available for humans. Indeed, veterinarians may develop illness when accidentally infected with the live RB51 animal vaccine.

HELMINTH INFECTIONS

Few helminth infections are true multisystem diseases

It is a somewhat arbitrary decision to include a particular helminth infection in a chapter on multisystem zoonotic infections. Many of the worm parasites that can be acquired from animals have stages that invade a number of the body systems. Others are primarily located in a particular organ, but cause pathological changes that can be widespread in their effects. Conversely, although stages of certain worms may be widely distributed in the body, their pathological effects are most commonly associated with a particular organ.

For example:

- The larvae of the pork tapeworm *Taenia solium*, which cause the disease cysticercosis, develop in a variety of tissues, including muscle. However, the most serious pathology is caused by larvae found in the CNS. Accordingly, this infection is discussed in Ch. 25.
- After infection with eggs of the dog nematode *Toxocara canis*, larvae migrate through the body, causing visceral larval migrans or ocular larva migrans. Again, the most serious effects are associated with larvae in the CNS (see Ch. 25) and the eye (see Ch. 26).

However, three helminths can be considered as genuinely multisystem in their effects. These are:

- the tapeworm *Echinococcus granulosus*
- the nematode *Trichinella spiralis*
- the nematode *Strongyloides stercoralis*.

Echinococcus

Echinococcus adults are tiny tapeworms in the small intestine of dogs or foxes, and their larvae cause hydatid disease in humans. They cause two major types of echinococcosis, both of which result in significant human morbidity.

Echinococcus granulosus (cystic echinococcosis; cystic hydatid disease)

The adults of this species live as tiny (3–5-mm long) tapeworms in the intestine of the dog. Eggs laid by the worm are passed in faeces, surviving for long periods. If swallowed (by sheep or accidentally by humans), the eggs hatch, releasing larvae which then penetrate the small intestinal mucosa to enter a blood vessel. Larvae then lodge in the tissues, most commonly in the liver, with the lung next most common, but any organ can potentially be affected. They then grow slowly into large, thick-walled, fluid-filled hydatid cysts. The resulting symptoms and signs are largely due to the mechanical pressure exerted by the cysts (Fig. 29.10) but patients may also present with fever due to cyst leakage or to secondary bacterial infection.

Cystic hydatid disease is diagnosed by ultrasonography, CT or MRI scans and serological tests assist diagnosis, but sensitivity and specificity of serology are variable. Finding hooklets and protoscoleces in aspirated cyst fluid provides confirmation, but suspected hydatid cysts in the lung must never be aspirated. Treatment is according to the WHO CE ultrasound classification (see Bibliography). Depending on cyst type, therapy is with albendazole, plus praziquantel in some cases, with or without PAIR (**P**uncture, **A**spiration, **I**njection, and **R**easpiration) or open surgery. Dead cysts do not require treatment. Special care must be taken during aspiration or surgical removal to prevent leakage of fluid from the cysts. Not only may this trigger anaphylactic responses in sensitized individuals, but also the numerous larvae present in the fluid (produced by asexual division) can cause local recurrence or metastatic infection in other sites.

Echinococcus multilocularis (alveolar echinococcosis; alveolar hydatid disease)

Echinococcus multilocularis results in the formation of a multilocular mass lesion consisting of hundreds of small vesicles. The parasite generally occurs as a fox–rodent cycle in China, Northern Europe, Siberia and parts of North America, and human infections occur via ingestion of eggs spread by contamination with fox faeces. Its pathogenesis

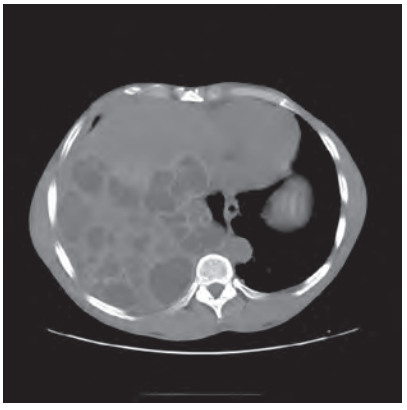


Figure 29.10 CT scan showing extensive cystic hydatid disease of the liver. (Courtesy of P. Chiodini.)

and clinical features are significantly different from those of cystic echinococcosis and the macroscopic appearance of alveolar echinococcosis is similar to that of a hepatic carcinoma. Almost all cases occur in the liver where the parasite leads to obstructive jaundice and weight loss. Metastasis to the lung and brain may occur. Treatment of hepatic disease is with radical excision plus albendazole. Inoperable cases require life-long albendazole therapy. Liver transplantation is sometimes needed.

Trichinella

Trichinella spiralis is transmitted in undercooked pork and causes the disease trichinosis

The genus *Trichinella* consists of eight different species and the genus is capable of infecting almost any warm-blooded animal. Its natural cycle involves predators (e.g. bears, seals) and their prey, or scavengers and the carrion they feed on, but a domestic cycle has become established in pigs and rats.

Humans are infected by eating undercooked meat (pork, horse or wild game animal) containing the encysted infected larval stages. These larvae mature rapidly into adults in the small intestine, their invasion of the mucosa causing acute enteritis.

The clinical features of trichinosis are mainly immunopathological in origin

Female worms release live larvae into the mucosa, which invade the blood vessels and become distributed around the body. The larvae attempt to invade the cells of many organs (including the heart and CNS), although they can mature only in striated muscles, where they form the characteristic cysts (Fig. 29.11). Severity depends on the number of larvae originally ingested by the patient and there is a wide spectrum of pathological signs, such as fever, joint and muscle pains, eosinophilia, periorbital oedema, myositis, petechial haemorrhage; encephalitis and myocarditis may also occur. These signs are mainly caused by hypersensitivity and inflammatory responses.

Trichinosis is diagnosed by microscopy and serologically and treated with anthelmintics and anti-inflammatories

Diagnosis of trichinosis is by muscle biopsy and demonstration of specific antibody by ELISA or IFAT. Molecular methods are required for species identification. Treatment is with benzimidazoles, which kill the adult worms and thus prevent further release of larvae. Benzimidazoles do not kill larvae encysted in the muscles, so they need to be given as early as possible in the course of the infection. Systemic corticosteroid therapy is administered in moderate to severe cases and non-steroidal anti-inflammatory agents are given in mild cases.

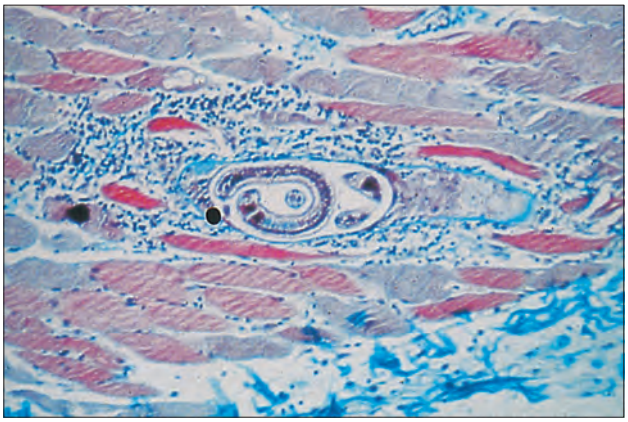


Figure 29.11 Inflammatory reaction around a nurse cell containing a coiled larva of *Trichinella spiralis*. Trichrome stain. (Courtesy of I.G. Kagan.)

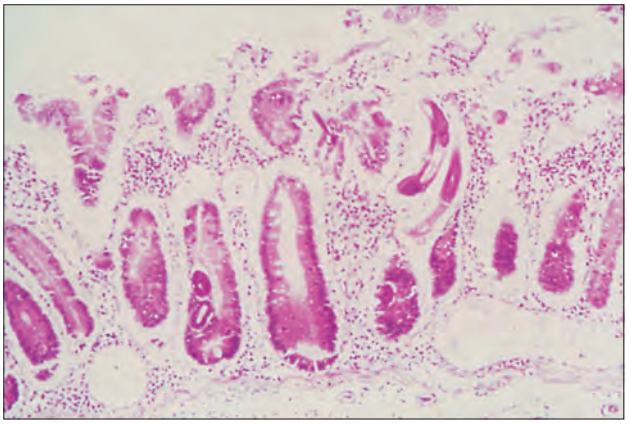


Figure 29.12 *Strongyloides stercoralis*. Adults and larvae in the mucosa of small intestine, showing disruption of the villous surface.

Strongyloides

Strongyloides infections are generally passed between humans, but can develop in animal hosts including dogs

Strongyloides infection is acquired by the penetration of infective larvae through the skin. The larvae migrate to the lung, enter the alveoli, pass up the bronchi and trachea, and are then swallowed. Only females develop in the human host. They reproduce parthenogenetically and lay strings of eggs into the intestinal mucosa (Fig. 29.12). The eggs hatch within the intestine to release larvae that pass out with the faeces and require warm, moist soil to become infective. The geographic distribution of strongyloidiasis is similar to that of hookworm (tropical areas and in rural southern states of the USA).

Infections are most often passed between humans, but two species can also develop in animal hosts including dogs (*S. stercoralis*) and African primates (*S. fuelleborni*). Faecal larval stages may develop directly into the infective stage whilst still in the intestine and penetrate the mucosa or perianal skin to re-infect the host – the process of autoinfection.

Strongyloides infections are usually asymptomatic, but can cause disseminated disease in patients with immunodeficiency states or malnutrition

Many infected individuals are asymptomatic, though abdominal pain, vomiting or diarrhoea may occur. However, in immunodeficiency due to corticosteroid therapy,

immunosuppression for transplantation, advanced malignancy, human T-cell lymphotropic virus (HTLV) infection and malnutrition, autoinfection can lead to hyperinfection or disseminated strongyloidiasis, the larvae invading almost all organs and causing severe and sometimes fatal pathology. Infected patients may show vomiting, abdominal pain, diarrhoea with malabsorption and dehydration, paralytic ileus and pneumonitis. Eosinophilia is often absent in *Strongyloides* hyperinfection. Disseminated strongyloidiasis can arise long after initial infection. It has been firmly established that infections can persist for many years (>30), being maintained by low-level autoinfection, then disseminate once the patient's immune defences are reduced. HTLV-1 antibody testing should be advised in this setting, as there is a correlation between *Strongyloides* and HTLV-1 infection. Furthermore, any patient for whom immunosuppression is planned should be asked about their residence and travel history. If they have been potentially exposed to *Strongyloides*, they should be screened for it, ideally before immunosuppression is commenced.

Strongyloides infection is diagnosed by microscopy and *Strongyloides* culture of faeces to detect larvae. They are often scarce in asymptomatic infections, but are very readily seen in hyperinfection as the parasite load is so high in that condition. Faecal PCR is used in some centres but is not yet widely deployed. Serology for IgG antibody to *Strongyloides* is helpful in migrants from endemic areas, but less sensitive in travellers. It may be negative in hyperinfestation.

Treatment of *Strongyloides* infection is with ivermectin. Thiabendazole is also effective but much less well tolerated by the patients. Albendazole is inferior to both.

KEY FACTS

- The multisystem infections described in this chapter are zoonoses, being maintained naturally in a reservoir of non-human vertebrates.
- Humans are infected incidentally, generally from rodents (arenaviruses, hantaviruses, plague, tularaemia, leptospirosis), bats (filoviruses, rabies virus) or from domestic animals (brucellosis, leptospirosis, trichinosis).
- There is generally no transmission from person to person, except for plague and Ebola virus infections.
- The nature and the extent of human–animal contact are determining factors.
- Some of these infections are highly virulent.
- When the reservoir host is common in crowded human communities (e.g. plague), disease epidemics have been major events in history.
- Most of these infections are now less frequent in resource-rich countries (e.g. anthrax, brucellosis, hydatid disease), but remain as frequent causes of disease in other parts of the world and thus may present in migrants from those regions.
- Anthrax is seen as a major bioterrorism threat.
- There are satisfactory antimicrobial agents for most of the non-viral infections, but effective vaccines are generally not available.
- The Ebola virus epidemic between 2013 and 2016 demonstrated the potential for transmission worldwide and how planning and preparedness is critical in terms of prevention, infection control and management.

30 Fever of unknown origin

Introduction

Fever is an abnormal increase in body temperature and may be continuous or intermittent

The homeostatic mechanisms of the body maintain a constant body temperature with daily fluctuations (circadian temperature rhythm) not exceeding ±1–1.5°C. Although 37°C (98.6°F) is taken as 'normal', individuals vary in their body temperature; in some it may be as low as 36°C, in others as high as 38°C. Fever is defined as an abnormal increase in body temperature – an oral temperature higher than 37.6°C (100.4°F) or a rectal temperature higher than 38°C (101°F) – and may be continuous or intermittent:

- In continuous fever the body temperature is elevated over the whole 24-h period and swings less than 1°C; this is characteristic of, for example, typhoid and typhus fever.
- In an intermittent fever the temperature is above normal throughout the 24-h period, but swings more than 1°C during that time. A swinging fever is typical of pyogenic infections, abscesses and tuberculosis.

Fever may be produced in response to:

- exogenous pyrogen such as endotoxin in Gram-negative cell walls
- endogenous pyrogen such as interleukin 1 (IL-1) released from phagocytic cells.

It is thought that fever may be a protective response by the host (Fig. 30.1).

DEFINITIONS OF FEVER OF UNKNOWN ORIGIN

Fever is a common complaint of patients presenting to a doctor. The cause is usually immediately apparent or is discovered within a few days, or the temperature settles spontaneously. However, if the patient's fever is >38.3°C (101°F) on several occasions and continues for more than 3 weeks despite 1 week of intensive evaluation, a provisional diagnosis of 'fever of unknown origin' (FUO) is made based on the classic definition of FUO. However, increased awareness of FUO causes and an increasing number of patients with serious underlying diseases successfully kept alive by modern medicine has led to additional categorization of FUO with regard to particular patient risk groups (Table 30.1).

CAUSES OF FUO

Infection is the most common cause of FUO

For centuries, fever has been recognized as a characteristic sign of infection and, historically, infection has been the most common cause of FUO, especially in children. However, there are important non-infectious causes of fever, most notably:

- malignancies
- collagen–vascular diseases.

These non-infectious causes need to be differentiated from infections during the investigation of a patient with a FUO. Despite intense and prolonged investigations, the cause of fever may remain undiagnosed in a significant number of patients. However, in the absence of significant weight loss or indication of severe underlying disease, the outcome, though potentially long term, is generally positive. The reported incidence of different FUO aetiologies has varied over time, owing in part to patient demographics and advances in medical diagnostics. One must also consider that patients may have a factitious fever (produced artificially by the patient, e.g. in Munchausen syndrome).

Infective causes of classical FUO

The most common infective causes of classic FUO are shown in Table 30.2. These can be divided into two main groups:

- infections such as tuberculosis and typhoid fever, caused by specific pathogens
- infections such as biliary tract infections and abscesses, which can be caused by a variety of different pathogens.

Most of these infections are described in detail elsewhere in this book. Bacterial endocarditis, which was long associated with FUO but now more easily diagnosed, is discussed below.

Significant infection may be present in the absence of fever in some groups of patients, notably:

- seriously ill neonates
- the elderly
- patients with uraemia
- patients receiving corticosteroids
- those taking antipyretic drugs continuously.

In these people, other signs and symptoms of infection have to be sought. This chapter deals only with patients whose presenting complaint is fever.

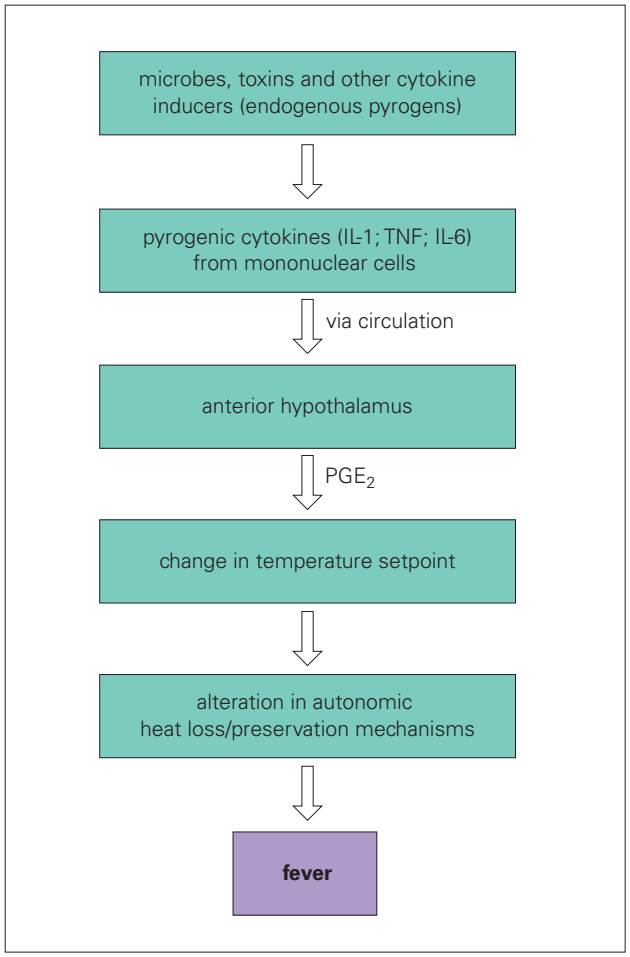


Figure 30.1 Mechanisms of fever. Fever may be induced either by exogenous pyrogens such as pathogens or their toxins or by endogenous pyrogens released in the host, and may have a protective effect. IL, interleukin; PG, prostaglandin; TNF, tumour necrosis factor.

INVESTIGATION OF CLASSIC FUO

Steps in the investigative procedure

Because of the many possible infectious and non-infectious causes of FUO, it is clearly not practical to attempt specific investigations for each at the outset. However, guidelines for the minimum diagnostic evaluation necessary to categorize a presenting case as FUO have remained consistent over the years, an example of which is shown in Box 30.1. In addition, the diagnostic pathway can be divided into a series of stages, each stage attempting to focus the investigation on the likely (e.g. infective) causes (Table 30.2).

Stage 1 comprises careful history taking, physical examination and screening tests

Careful history taking is essential and should include questions about travel, occupation, hobbies, exposure to animals and known infectious hazards, antibiotic therapy within the previous 2 months, substance misuse and other habits. Some of the infections listed in Table 30.2 are zoonoses (e.g. leptospirosis, spotted fevers), whereas others are vector-borne (e.g. malaria, trypanosomiasis) and / or of limited geographic distribution (e.g. histoplasmosis), hence the importance of a travel history.

In the light of the history and the differential diagnosis, a complete physical examination of the patient with FUO is essential, in particular:

- the skin, eyes, lymph nodes and abdomen should be examined
- the heart should be auscultated.

It is also important to confirm that the patient does have a fever. In some series, as many as 25% of patients whose presenting complaint was an FUO may not have a fever, but rather a naturally exaggerated circadian temperature rhythm. The possibility of a factitious fever must also be considered.

Routine investigations such as chest radiography and blood tests should be performed at this stage.

Table 30.1 Definitions of fever of unknown origin (FUO)

Definition	Symptoms	Diagnosis
Classical FUO	Fever (>38.3°C) on several occasions and more than 3 weeks' duration	Uncertain despite appropriate investigations after at least three outpatient visits or 3 days in hospital, including at least 2 days' incubation of microbiological cultures
Nosocomial (healthcare-associated) FUO	Fever (>38.3°C) on several occasions in a healthcare setting; infection not present or incubating on admission	Uncertain after 3 days despite appropriate investigations, including at least 2 days' incubation of microbiological cultures
Neutropenic FUO	Fever (>38.3°C) on several occasions; neutrophil count <500/mm^3 in peripheral blood, or expected to fall below that number within 1–2 days	Uncertain after 3 days despite appropriate investigations, including at least 2 days' incubation of microbiological cultures
HIV-associated FUO	Fever (>38.3°C) on several occasions; fever of more than 4 weeks' duration as an outpatient or more than 3 days' duration as an inpatient; confirmed positive HIV serology	Uncertain after 3 days despite appropriate investigations, including at least 2 days' incubation of microbiological cultures

The classic definition of FUO requires that the fever is of 3 or more weeks' duration, but infections in compromised patients frequently progress rapidly because of inadequate host defences. Consequently, the pace of the investigations needs to be rapid if appropriate therapy is to be initiated.

Table 30.2 Representative infective causes of fever of unknown origin (FUO)

Infection	Usual cause
Bacterial	
Tuberculosis	*Mycobacterium tuberculosis*
Enteric fevers	*Salmonella typhi*
Osteomyelitic	*Staphylococcus aureus* (also *Haemophilus influenzae* in young children, *Salmonella* in patients with sickle-cell disease)
Endocarditis	Oral streptococci, *Staph. aureus*, coagulase-negative staphylococci
Brucellosis	*Brucella abortus*, *B. melitensis* and *B. suis*
Abscesses (esp. intra-abdominal)	Mixed anaerobes and facultative anaerobes from gut microbiota
Biliary system infections	Gram-negative facultative anaerobes, e.g. *E. coli*
Urinary tract infections	Gram-negative facultative anaerobes, e.g. *E. coli*
Lyme disease	*Borrelia burgdorferi*
Relapsing fever	*Borrelia recurrentis*
Leptospirosis	*Leptospira interrogans* serovar icterohaemorrhagiae
Rat bite fever	*Streptobacillus moniliformis*, *Spirillum minus*
Typhus	*Rickettsia prowazekii*
Spotted fever	*Rickettsia rickettsii*, *R. conorii*
Psittacosis	*Chlamydophila psittaci*
Q fever	*Coxiella burnetii*
Parasitic	
Malaria	*Plasmodium* species
Trypanosomiasis	*Trypanosoma brucei gambiense*
Amoebic abscesses	*Entamoeba histolytica*
Toxoplasmosis	*Toxoplasma gondii*
Fungal	
Candidiasis	*Candida albicans*
Cryptococcosis	*Cryptococcus neoformans*
Histoplasmosis	*Histoplasma capsulatum*
Viral	
AIDS	HIV
Infectious mononucleosis	Epstein–Barr virus, cytomegalovirus
Hepatitis	Hepatitis viruses

A wide range of infections can present as FUO. Some, such as brucellosis, are zoonoses, and many are vector-borne. Therefore the patient must have had appropriate exposure to contract these infections. For example, there are approximately 2000 cases of malaria annually in both the UK and the USA, the overwhelming majority of which are contracted outside the country. A travel history is therefore very important.

Stage 2 involves reviewing the history, repeating the physical examination, specific diagnostic tests and non-invasive investigations

A review of the patient's history, particularly after discussion with colleagues and perhaps carried out by a second physician, is valuable to check for omissions such as exposure to particular risk factors in the recent or more distant past. The physical examination should also be repeated because rashes and other signs of infection can be transient.

Clues to the diagnosis elicited by careful history taking should direct specific investigations. As the most common cause of unexplained fever is infection, collection and careful examination of appropriate specimens are essential. Skin tests may also be appropriate at this stage. The most important specimens include:

- blood for culture
- blood for examination of antibodies. A sample of serum collected when the patient presents should also be stored

Box 30.1 Example of Minimum Diagnostic Evaluation Necessary to Categorize a Case as Classical Fever of Unknown Origin

- Comprehensive history (including travel history, risk for venereal diseases, hobbies, contact with pet animals and birds, etc.)
- Comprehensive physical examination (including temporal arteries, rectal digital examination, etc.)
- Routine blood tests (complete blood count including differential, ESR or CRP, electrolytes, renal and hepatic tests, creatine phosphokinase and lactate dehydrogenase)
- Microscopic urinalysis
- Cultures of blood, urine (and other normally sterile compartments if clinically indicated, e.g. joints, pleura, cerebrospinal fluid)
- Chest radiograph
- Abdominal (including pelvic) ultrasonography
- Antinuclear and antineutrophilic cytoplasmic antibodies, rheumatoid factor
- Tuberculin skin test
- Serological tests directed by local epidemiological data
- Further evaluation directed by abnormalities detected by above test, e.g. HIV antibodies depending on detailed history
- CMV-IgM and EBV serology in case of abnormal differential WBC count
- Abdominal or chest helical CT scan
- Echocardiography in case of cardiac murmur

CMV, cytomegalovirus; CRP, C-reactive protein; CT, computed tomography; EBV, Epstein–Barr virus; ESR, erythrocyte sedimentation rate; Ig, immunoglobulin; WBC, white blood cell.
(Adapted from Knockaeert, D.C., Vanderschuern, S., Blockmans, D. [2003] Fever of unknown origin in adults: 40 years on. From the Department of General Internal Medicine, Gasthuisberg University Hospital, Leuven, Belgium. *J Intern Med* 253:263–275.)

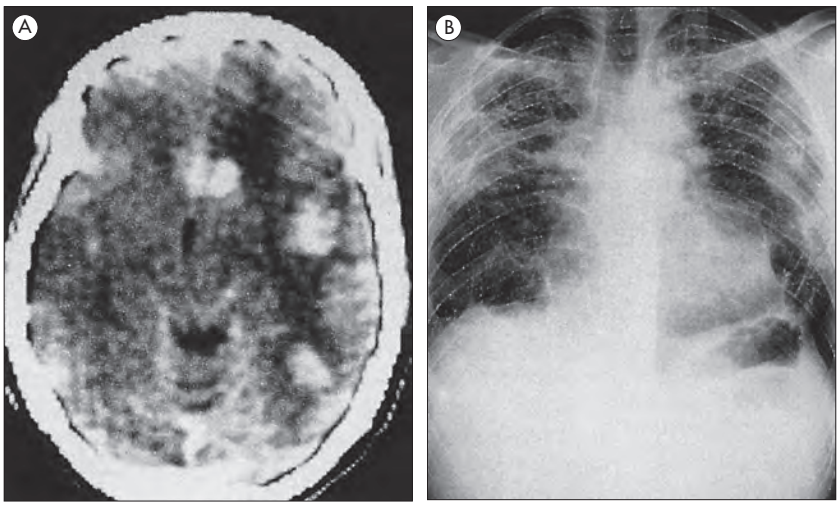


Figure 30.2 Computerized tomography (CT) scans help in the demonstration of abscesses. The patient in (A) has a tuberculoma of the brain, but the CT appearance is not sufficiently characteristic to distinguish this from a pyogenic abscess or a meningioma. The chest radiograph in (B) shows a patient with sarcoidosis. The differential diagnosis between infective and non-infective causes of granulomas is important, and can be difficult in the early stages of the investigation. ([A] Courtesy of J. Ambrose. [B] Courtesy of M. Turner-Warwick.)

for comparison with later samples to detect rising antibody titres even if the patient is some weeks into the infection. Serological tests are helpful, particularly in the diagnosis of cytomegalovirus (CMV) and Epstein–Barr virus (EBV) infection, toxoplasmosis, psittacosis and rickettsial infections. Positive results in syphilis serology should be viewed with caution as other infections can cause biological false positives (see Ch. 22)

- direct examination of blood to diagnose malaria, trypanosomiasis and relapsing fever.

Repeated sampling of blood, urine and other body fluids may be required, and the laboratory should be alerted to search for unusual and fastidious organisms (e.g. nutritionally variant streptococci as a cause of endocarditis; see below). If possible, serial cultures should be collected before antimicrobial therapy is commenced.

Technical advances in diagnostic imaging techniques have provided the physician with a wide range of non-invasive investigative methods (e.g. ultrasound, computed tomography [CT] scan, magnetic resonance imaging [MRI], etc.). Some radiological procedures such as chest radiographs are routine in the work-up of patients with FUO (Fig. 30.2), while others such as gallium or technetium scans may be applied depending on the likely diagnosis (Fig. 30.3).

Stage 3 comprises invasive tests

Biopsy of liver and bone marrow should always be considered in the investigation of classic cases of FUO, but other tissues such as skin, lymph nodes and kidney may also be sampled. It is undesirable or impossible to repeat biopsies, and therefore it is important to organize the laboratory examination of material carefully to maximize the information obtained.

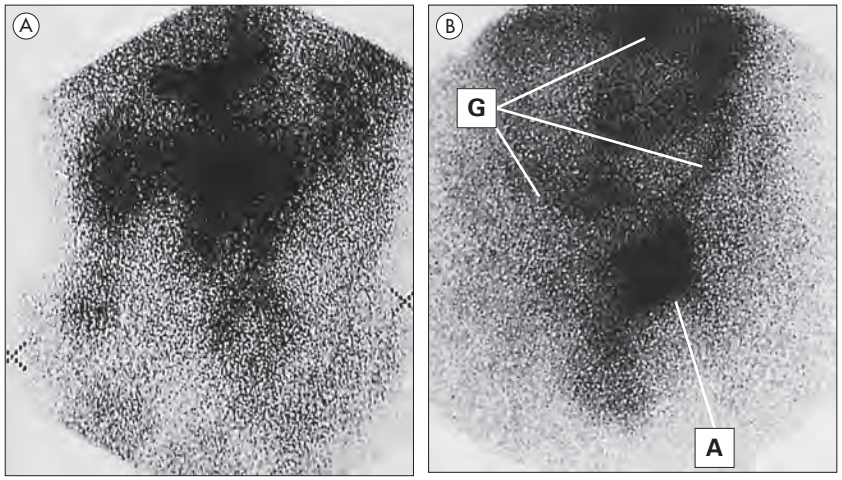


Figure 30.3 Gallium concentrates in many inflammatory and neoplastic tissues and is a useful non-invasive technique in the investigation of a patient with fever of unknown origin. (A) Retroperitoneal lymphadenopathy of Hodgkin's disease highlighted by a gallium scan. (B) Intra-abdominal abscess shown by a gallium scan. A, abscess; G, gallium in colon. ([A] Courtesy of H Tubbs. [B] Courtesy of W.E. Farrar.)

Stage 4 involves therapeutic trials

Trials of corticosteroids (e.g. prednisone, dexamethasone) or prostaglandin inhibitors (e.g. aspirin, indometacin) may be indicated if a non-infectious cause has been essentially eliminated. There are few indications for empirical antimicrobial or cytotoxic chemotherapy in the management of classic FUO. However, a trial of antituberculosis drugs may be advocated in patients with a history of tuberculosis in the absence of supporting microbiological evidence. Infections can progress very rapidly in people who are neutropenic or have AIDS, and 'blind' therapy is warranted (see below).

TREATMENT OF FUO

The investigation and management of a patient with FUO requires persistence and an informed and open mind in order to reach the correct diagnosis. As the range of infective causes of FUO is enormous, the correct diagnosis is an essential prelude to the choice of appropriate treatment. As soon as the cause has been identified, specific therapy, if available, should be given.

FUO IN SPECIFIC PATIENT GROUPS

The main difference between FUO in these groups and classic FUO is the time course

As mentioned above, an increasing number of people are surviving with severe underlying disease that predisposes them to infection or are receiving treatment, such as cytotoxic drugs, that compromises their defences against infection. These groups of patients are discussed in more detail in Chapter 31, but are included here because, in addition to classic FUO, other classifications of FUO (see Table 30.1) define:

- nosocomial FUO
- neutropenic FUO
- HIV-associated FUO.

Classically, an FUO may exist for weeks or months before a diagnosis is made, whereas for healthcare-associated (nosocomial) FUO and in neutropenic patients the time course is hours to days. The more common infective causes of FUO in these groups are shown in Table 30.3.

Investigation should proceed as noted above, but with the particular emphasis depending upon the patient. In hospital patients the emphasis will depend upon:

- the type of operative procedures performed; fever is a common complaint in patients who have received transplants and may indicate graft-versus-host disease rather than infection
- the presence of foreign bodies, especially intravascular devices
- drug therapy, as drug fevers are a common non-infective cause of FUO
- the underlying disease and stage of chemotherapy in neutropenic patients
- the presence of known risk factors such as intravenous drug misuse, travel and contact with infected individuals in patients with HIV. Although the major opportunist infections in people with AIDS are well described (see Ch. 31), common infections can present atypically and new infections continue to emerge.

INFECTIVE ENDOCARDITIS

Although now more easily diagnosed than in the past, infective endocarditis is an uncommon disease that historically has often presented as an FUO which is fatal if untreated. The infection involves the endothelial lining of the heart, usually including the heart valves. It may occur as an acute, rapidly progressive disease or in a subacute form. The majority of these patients have a pre-existing heart defect, either congenital or acquired (e.g. as a result of rheumatic fever), or a prosthetic heart valve in situ. However, the patient may be unaware of any defect before the infection.

Almost any organism can cause endocarditis, but native valves are usually infected by oral streptococci and staphylococci

Infection of native valves is most commonly caused by species of oral streptococci viridans group (such as *Streptococcus sanguinis*, *Strep. oralis* and *Strep. mitis*), *Staphylococcus aureus,* and coagulase-negative staphylococci (Table 30.4). Intravenous drug misusers have the added complication of infection due to organisms they inject into themselves. Coagulase-negative

Table 30.3 Representative infective causes of fever of unknown origin (FUO) in specific patient groups

Category of FUO	Infection	Usual cause
Nosocomial	Vascular line related	Staphylococci
	Other device related	Staphylococci, *Candida*
	Transfusion related	Cytomegalovirus
	Cholecystitis and pancreatitis	Gram-negative rods
	Pneumonia (related to assisted ventilation)	Gram-negative rods, including *Pseudomonas*
	Postoperative abscesses, e.g. intra-abdominal	Gram-negative rods and anaerobes
	Post-gastric surgery	Systemic candidiasis
Neutropenic	Vascular line related	Staphylococci
	Oral infection	*Candida*, herpes simplex virus
	Pneumonia	Gram-negative rods, *Candida*, *Aspergillus*, CMV
	Soft tissue, e.g. perianal abscesses	Mixed aerobes and anaerobes
HIV-associated	Respiratory tract	Cytomegalovirus, *Pneumocystis*, *Mycobacterium tuberculosis*, *M. avium-intracellulare*
	Central nervous system	*Toxoplasma*
	Gastrointestinal tract	*Salmonella*, *Campylobacter*, *Shigella*
	Genital tract or disseminated	*Treponema pallidum*, *Neisseria gonorrhoeae*

Patients with healthcare-associated FUO are most likely to be infected with 'healthcare-associated pathogens', either from their own microbiota or from the healthcare environment. This also applies to neutropenic patients if they are inpatients, but some are treated as outpatients and may therefore be exposed to a wider range of pathogens. People with AIDS commonly become infected with opportunistic pathogens, though an increasing range of organisms is now implicated. It is important to take a detailed history, as latent infections can become florid as the patient's immune status deteriorates. CMV, cytomegalovirus.

Table 30.4 Causative agents of endocarditis in different groups of patients (in general order of decreasing importance)

Patient group	Major aetiological agents of infective endocarditis
Native valve	Oral streptococci and enterococci *Staphylococcus aureus* Coagulase-negative staphylococci Gram-negative (enteric) rods Fungi (mainly *Candida*)
Intravenous drug misuser	*Staph. aureus* Oral streptococci and enterococci Gram-negative (enteric) rods Fungi (mainly *Candida*) Coagulase-negative staphylococci
Prosthetic valve (early)	Coagulase-negative staphylococci *Staph. aureus* Gram-negative (enteric) rods Oral streptococci and enterococci Fungi (mainly *Candida*)
Prosthetic valve (late)	Oral streptococci and enterococci Coagulase-negative staphylococci *Staph. aureus* Gram-negative (enteric) rods Fungi (mainly *Candida*)

Although almost any organism can cause endocarditis, the majority of cases are caused by a relatively small range of species. The relative importance of these species varies depending upon whether the patient has his/her own heart valves or a prosthetic valve.

staphylococci are common causes of early prosthetic valve endocarditis and are probably acquired at the time of surgery. The species causing late infections – more than 3 months after cardiac surgery – are somewhat more like those causing native valve endocarditis (Fig. 30.4).

Endocarditis is an endogenous infection acquired when organisms entering the bloodstream establish themselves on the heart valves. Therefore, any bacteraemia can potentially result in endocarditis. Most commonly, streptococci from the oral flora enter the bloodstream, for example, during dental procedures or vigorous teeth cleaning or flossing, and adhere to damaged heart valves. It is thought that fibrin–platelet vegetations are present on damaged valves before the organisms implant, and that adherence is probably associated with the ability of the organisms to produce dextran as well as adhesins and fibronectin-binding proteins. Having attached themselves to the heart valve, the organisms multiply and attract further fibrin and platelet deposition. In this position, they are protected from the host defences, and vegetations can grow to several centimetres in size. This is probably quite a slow process and correspondingly the time period between the initial bacteraemia and the onset of symptoms averages around 5 weeks.

A patient with infective endocarditis almost always has a fever and a heart murmur

The signs and symptoms of infective endocarditis are very varied, but relate essentially to four ongoing processes:

- the infectious process on the valve and local intracardiac complications
- septic embolization to virtually any organ

- bacteraemia, often with metastatic foci of infection
- circulating immune complexes and other factors.

The patient almost always has a fever and a heart murmur and may also complain of non-specific symptoms such as anorexia, weight loss, malaise, chills, nausea, vomiting and night sweats, symptoms that are common to many of the causes of FUO listed in Table 30.2. Peripheral manifestations may also be evident in the form of splinter haemorrhages and Osler's nodes (Fig. 30.5). Microscopic haematuria resulting from immune complex deposition in the kidney is characteristic (see Ch. 18).

Blood culture is the most important test for diagnosing infective endocarditis

Microbiological and cardiological investigations are of critical importance. The blood culture is the single most important laboratory test. Ideally, three separate samples of blood should be collected within a 24-h period and before antimicrobial therapy is administered. Isolation of the causative organism is essential so that antibiotic susceptibility tests can be performed and optimum therapy prescribed. Nutritionally variant strains of oral streptococci are known to cause infective endocarditis. These may fail to grow in blood culture media unless pyridoxal is added. Alternatively, they grow as satellite colonies around *Staph. aureus* colonies on blood agar.

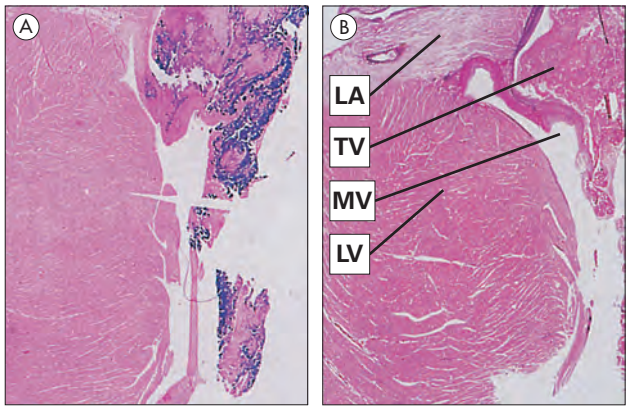


Figure 30.4 Bacteria circulating in the bloodstream adhere to, and establish themselves on, the heart valves. Multiplication of the pathogens is associated with destruction of valve tissue and the formation of vegetations, which interfere with, and may severely compromise, the normal function of the valve. These histological sections show the virtual destruction of the leaflet at the mitral valve by staphylococci. (A) Gram stain. (B) Eosin–Van Gieson stain. LA, left atrium; LV, left ventricle; MV, remnant of mitral valve; TV, thrombotic vegetation. (Courtesy of R.H. Anderson.)

The mortality of infective endocarditis is approximately 20–50% despite treatment with antibiotics

In the past, most organisms causing infective endocarditis have been susceptible to a range of antimicrobials. However, antibiotic resistance has become an increasing issue (see Ch. 34). Even with appropriate treatment, complete eradication takes weeks to achieve, and relapse is common. This is probably due to factors such as:

- relative inaccessibility of the organisms within the vegetations both to antibiotics and to host defences
- the organism's high population density and relatively slow rate of multiplication.

Before the advent of antibiotics infective endocarditis had a mortality of 100%. Even today, despite appropriate antimicrobial chemotherapy and, depending on individual circumstances, the mortality remains at approximately 20–50%.

The antibiotic treatment regimen for infective endocarditis depends upon the susceptibility of the infecting organism

For prosthetic valve endocarditis with penicillin-susceptible streptococci, high-dose penicillin is the treatment of choice. Patients with a good history of penicillin allergy can be treated with ceftriaxone or vancomycin. MIC (minimum inhibitory concentration) and MBC (minimum bactericidal concentration) tests (see Ch. 34) should be performed to detect organisms that are less susceptible or tolerant to penicillin (inhibited, but not killed; e.g. MBC=32×MIC). Organisms less susceptible to penicillin and enterococci, which are always more resistant to penicillin, are treated with a combination of a beta-lactam antibiotic and an aminoglycoside. Combinations such as this act synergistically against streptococci and enterococci (see Ch. 34). However, vancomycin-resistant enterococci (VRE; usually *E. faecium*) pose a therapeutic challenge and require drugs such as linezolid or daptomycin.

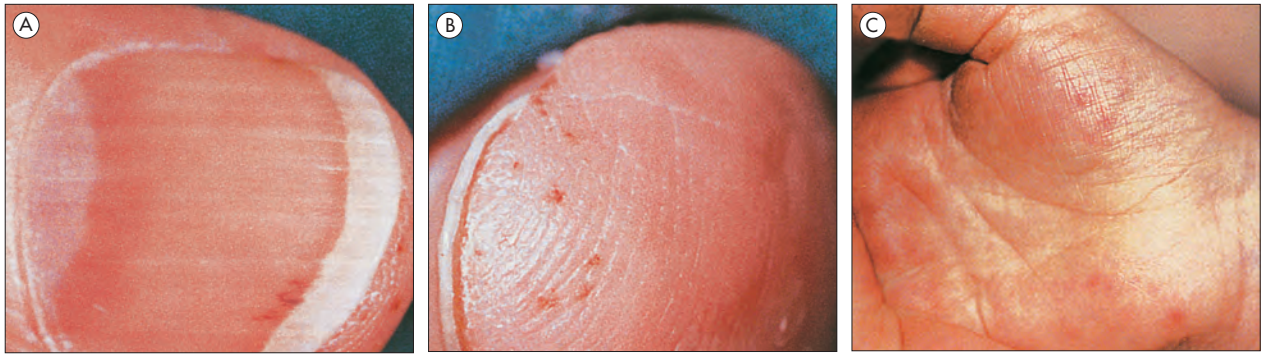


Figure 30.5 Outward signs of endocarditis may be helpful in suggesting the diagnosis. These result from the host's response to infection in the form of immune-complex-mediated vasculitis, focal platelet aggregation and vascular permeability. (A and B, different views.) Splinter haemorrhages in the nailbed and petechial lesions in the skin. (C) Osler's nodes. These are tender nodular lesions that tend to affect the palms and fingertips. (Courtesy of H. Tubbs.)

Staphylococcal endocarditis, particularly in prosthetic valve endocarditis when the organisms may be healthcare associated and consequently often resistant to many antibiotics, often presents a more difficult therapeutic challenge. The increasing incidence of methicillin-resistant staphylococci requires a combination approach (vancomycin plus rifampin and gentamicin). A number of sources exist for detailed treatment regimens including the American Heart Association and the British Society for Antimicrobial Chemotherapy.

People with heart defects need prophylactic antibiotics during invasive procedures

People with known heart defects should be given prophylactic antibiotics to protect them during dental surgery and any other invasive procedure that is likely to cause a transient bacteraemia.

Most people with an FUO have a treatable disease presenting in an unusual manner

The clinical investigation needs to be individualized, but this chapter outlines the essential stages in the investigation of patients and draws attention to the important infective causes of FUO.

Although classically a patient with FUO presents with a long history (weeks or months of fever), patients also present with fevers that are not immediately diagnosed by routine laboratory investigations. Definitions of FUO have also been proposed for these groups (nosocomial, neutropenic and HIV associated). The list of pathogens causing fever in these patients is growing.

The clinician's aim in the investigation of every patient with FUO should be to discover the cause (i.e. to change a FUO to a fever of known origin) and to initiate appropriate treatment.

KEY FACTS

- Fever is the body's response to exogenous and endogenous pyrogens. It is a common symptom and may have a protective effect.
- The term fever of unknown origin (FUO) is used when the cause of fever is not obvious, has classically exceeded 3 weeks' duration, and is not revealed by routine clinical and laboratory investigations.
- Increased numbers of immunocompromised patients have prompted the definition of FUO groups other than classical (i.e. nosocomial, neutropenic and HIV-associated FUO).
- Among the causes of FUO, infection is the most common, but neoplasms and autoimmune diseases are also significant. Cases of FUO often remain undiagnosed.
- The list of infective causes is long; therefore the first stage of investigation (i.e. the patient's history and results of physical examination and screening tests) is a critical pointer to subsequent specific diagnostic tests.
- Therapeutic trials may be indicated if a diagnosis has not been achieved, but may confuse the results of further tests.
- The correct diagnosis is paramount to direct appropriate specific therapy.
- Infective endocarditis is now an uncommon, but classic, example of an FUO. It is usually caused by Gram-positive cocci, the species depending upon the patient's underlying predisposition, and is fatal unless treated.

31 Infections in the compromised host

Introduction

The human body has a complex system of protective mechanisms to prevent infection. This involves both the adaptive (cellular and humoral) immune system and the innate defence system (e.g. skin, mucous membranes). (These have been described in detail in Chapters 10–12.) So far, we have concentrated on the common and serious infections occurring in people whose protective mechanisms are largely intact. In these circumstances, the interactions between host and microorganism are such that the microorganism has to use all its guile to survive and invade the host, and the healthy host is able to combat such an invasion. The focus of this chapter involves the infections that arise when the host defences are compromised, resulting in the host–microorganism equation being weighted heavily in favour of the microorganism.

THE COMPROMISED HOST

Compromised hosts are individuals with one or more defects in their body's natural defences against microbial invaders. Consequently they are much more liable to suffer from severe and life-threatening infections. Modern medicine has effective methods for treating many types of cancers, is improving organ transplantation techniques and has developed technology that enables people with otherwise fatal diseases to lead prolonged and productive lives. A consequence of these achievements, however, is an increasing number of compromised people prone to infection. In addition, viral infections including human immunodeficiency virus (HIV) and human T-cell lymphotropic virus (HTLV) result in a compromised immune system referred to as acquired immune deficiency syndrome (AIDS) (see Ch. 22) and adult T-cell leukaemia / lymphoma (ATLL), respectively.

The host can be compromised in many different ways

Compromise can take a variety of forms, falling into two main groups:

- defects, accidental or intentional, in the body's innate defence mechanisms
- deficiencies in the adaptive immune response.

These disorders of the immune system can be further subclassified as 'primary' or 'secondary' (Table 31.1):

- Primary immunodeficiency is inherited or occurs by exposure in utero to environmental factors or by other unknown mechanisms. It is rare, and varies in severity depending upon the type of defect.
- Secondary or acquired immunodeficiency is due to an underlying disease state (Table 31.2) or occurs as a result of treatment for a disease.

Primary defects of innate immunity include congenital defects in phagocytic cells or complement synthesis

Congenital defects in phagocytic cells confer susceptibility to infection, and of these perhaps the best known is chronic granulomatous disease (Fig. 31.1), in which an inherited failure to synthesize cytochrome b-245 leads to a failure to produce reactive oxygen intermediates during phagocytosis. As a result, the neutrophils cannot kill invading pathogens.

The central role of complement in the innate defence mechanisms is undisputed, and inability to generate classical C3 convertase (see Ch. 11) through congenital defects in the synthesis of the early components, particularly C4 and C2, is associated with a high frequency of extracellular infections.

Secondary defects of innate defences include disruption of the body's mechanical barriers

A variety of factors can disrupt the mechanical non-specific barriers to infection. For example, burns, traumatic injury and major surgery destroy the continuity of the skin and may leave poorly vascularized tissue near the body surface, providing a relatively defenceless site for pathogens to colonize and invade. In health, the mucosal barriers of the respiratory and alimentary tract are vital to prevent infection. Damage sustained, for example, through endoscopy, surgery or radiotherapy, provides easy access for infecting organisms. Devices such as intravascular and urinary catheters, or procedures such as lumbar puncture or bone marrow aspiration, allow organisms to bypass the normal defences and enter normally sterile parts of the body. Foreign bodies such as prostheses (e.g. hip joints or heart valves) and cerebrospinal fluid (CSF) shunts alter the local non-specific host responses and provide surfaces that pathogens can colonize more readily than the natural equivalents.

The adage 'obstruction leads to infection' is a valuable reminder that the defences of many body systems work partly through the clearance of undesirable materials, for example by urine flow, ciliary action in the respiratory tract, and peristalsis in the gut. Interference with these mechanisms as a result of pathological obstruction, central nervous system dysfunction or surgical intervention tends to result in infection.

Table 31.1 Factors that make a host compromised

Factors affecting innate systems	
Primary	Complement deficiencies, phagocyte cell deficiencies
Secondary	Burns, trauma, major surgery, catheterization, foreign bodies (e.g. shunts, prostheses), obstruction
Factors affecting adaptive systems	
Primary	T-cell defects, B-cell deficiencies, severe combined immunodeficiency
Secondary	Malnutrition, infectious diseases, neoplasia, irradiation, chemotherapy, splenectomy

Table 31.2 Infections that cause immunosuppression

Viral	Bacterial
Measles	*Mycobacterium tuberculosis*
Mumps	*Mycobacterium leprae*
Congenital rubella Epstein–Barr virus Cytomegalovirus HIV-1, HIV-2 HTLV-1	*Brucella* spp.

HIV, human immunodeficiency virus; HTLV, human T-cell lymphotropic virus.

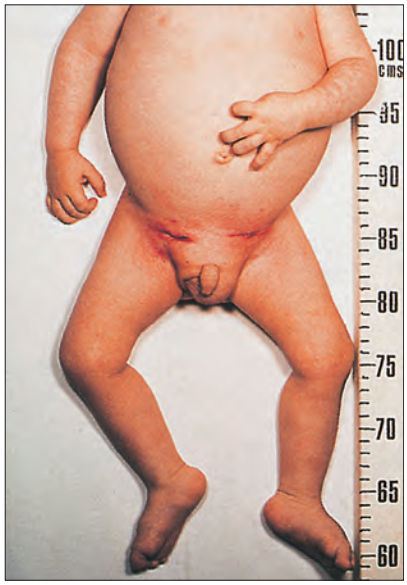


Figure 31.1 Bilateral draining lymph nodes in an 18-month-old boy with chronic granulomatous disease. Abscesses caused by *Staphylococcus aureus* had developed in both groins and had to be surgically drained. (Courtesy of A.R. Hayward.)

Primary adaptive immunodeficiency results from defects in the primary differentiation environment or in cell differentiation

The major congenital abnormalities arising in the adaptive immune system are depicted in Fig. 31.2. A defect in the stromal microenvironment in which lymphocytes differentiate may lead to failure to produce B cells (Bruton-type agammaglobulinaemia) or T cells (DiGeorge syndrome).

Differentiation pathways themselves may also be affected. For example, a non-functional recombinase enzyme will prevent the recombination of gene fragments that form the B-cell antibody or the T-cell receptor variable regions for antigen recognition, with a resulting severe combined immunodeficiency (SCID).

The most common form of congenital antibody deficiency – common variable immunodeficiency – is characterized by recurrent pyogenic infections and is probably heterogeneous in mechanism. Although the number of immature B cells in the marrow tends to be normal, the peripheral B cells are either low in number or in some cases absent. Where present, they are unable to differentiate into plasma cells in some cases or to secrete antibody in others.

Transient hypogammaglobulinaemia of infancy, characterized by recurrent respiratory infections, is associated with a low serum IgG concentration, which often normalizes abruptly by 3–4 years of age (Fig. 31.3).

Immunoglobulin deficiency occurs naturally in human infants as the maternal serum IgG concentration decays. It is a serious problem in very premature babies as, depending on the gestational age, maternal IgG may not have crossed the placental barrier.

Causes of secondary adaptive immunodeficiency include malnutrition, infections, neoplasia, splenectomy and certain medical treatments

Worldwide, malnutrition is common and the most important cause of acquired immunodeficiency. The major form, protein–energy malnutrition (PEM) presents as a wide range of disorders, with kwashiorkor and marasmus at the two poles. It results in:

- drastic effects on the structure of the lymphoid organs (Fig. 31.4)
- gross reductions in the synthesis of complement components
- sluggish chemotactic responses of phagocytes
- lowered concentrations of secretory and mucosal IgA
- reduced affinity of IgG
- in particular, a serious deficit in circulating T-cell numbers (Fig. 31.5), leading to inadequate cell-mediated responses

Infections themselves are often immunosuppressive (see Table 31.2), and none is more so than HIV infection, which gives rise to AIDS (see Ch. 22). Neoplasia of the lymphoid system frequently induces a state of reduced immunoreactivity, and splenectomy results in impaired humoral responses.

Treatment of disease can also cause immunosuppression. For example:

- Cytotoxic agents such as cyclophosphamide and azathioprine cause leukopenia or deranged T- and B-cell function.
- Corticosteroids reduce the number of circulating lymphocytes, monocytes and eosinophils and suppress leukocyte accumulation at sites of inflammation.
- Radiotherapy adversely affects the proliferation of lymphoid cells.

Therefore a patient receiving treatment for neoplastic disease will be immunocompromised as a result of both the disease and the treatment.

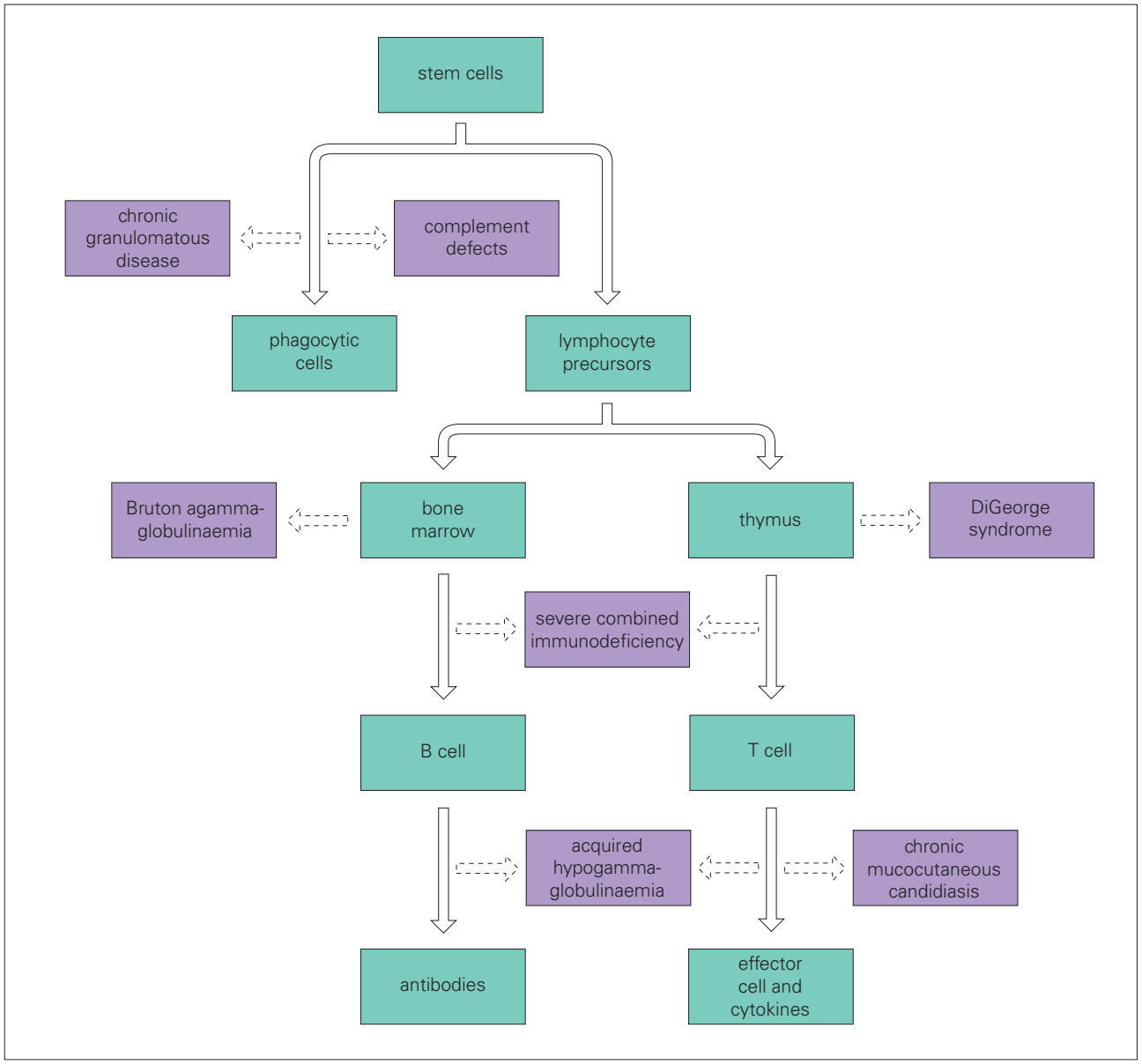


Figure 31.2 The major primary cellular immunodeficiencies. The deficiency states (shown in purple boxes) derive either from defects in the primary differentiation environment (bone marrow or thymus) or during cell differentiation (shown as dashed arrows derived from the differentiation state indicated).

It is important to recognize immunodeficiencies and to understand which procedures are likely to compromise the natural defences of a patient. Due to improvements in medical technology, many immune defects, particularly immunosuppression resulting from radiotherapy or cytotoxic drugs, are transient, and patients who survive the period of immunosuppression have a good chance of a complete recovery.

Pathogens that infect the compromised host

Immunocompromised people can become infected with any pathogen able to infect immunocompetent individuals as well as those opportunist pathogens that do not cause disease in a healthy person. They may be lethal when the host defences are lowered. Different types of defect predispose to infection with different pathogens depending upon the critical mechanisms operating in the defence against each microorganism (Fig. 31.6). Here, we will concentrate mainly on the opportunist infections and refer to other chapters for information about other pathogens.

INFECTIONS OF THE HOST WITH DEFICIENT INNATE IMMUNITY DUE TO PHYSICAL FACTORS

Burn wound infections

Burns damage the body's mechanical barriers, neutrophil function and immune responses

Burn wounds are sterile immediately after the burn is inflicted, but inevitably become colonized within hours with a mixed bacterial flora. Burn injuries cause direct damage

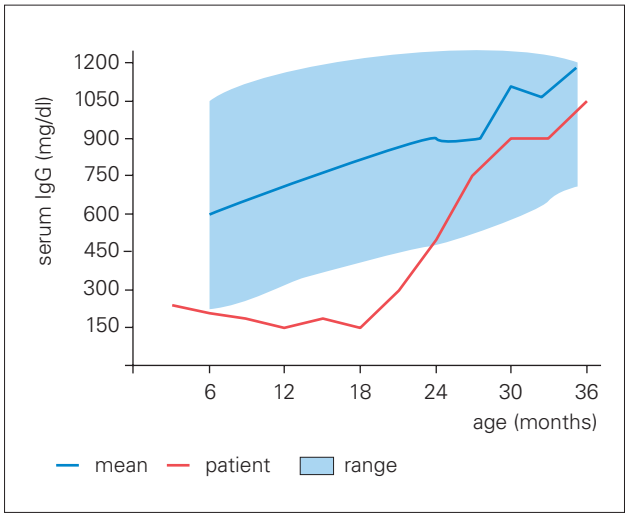


Figure 31.3 Serum immunoglobulin concentrations in a boy with transient hypogammaglobulinaemia compared with the range of normal controls. The patient developed mild paralytic polio when immunized at 4 months of age with live attenuated (Sabin) vaccine.

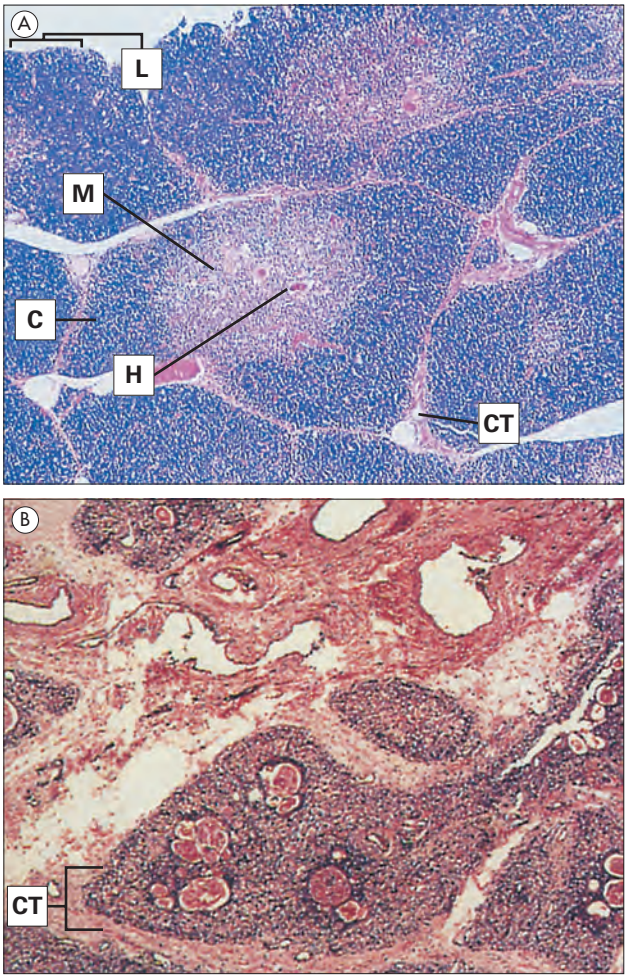


Figure 31.4 Thymic histology in normal children and children with protein–energy malnutrition (PEM). (A) Normal thymus showing a cortex and medullary zones. (B) Acute involution in PEM characterized by lobular atrophy, loss of distinction between cortex and medulla, depletion of lymphocytes and enlarged Hassall's corpuscles. C, cortex; CT, connective tissue; H, Hassall's corpuscle; L, lobule; M, medulla. (Courtesy of R.K. Chandra.)

to the mechanical barriers of the body and abnormalities in neutrophil function and immune responses. In addition, there is a major physiological derangement with loss of fluids and electrolytes. The burn provides a highly nutritious surface for organisms to colonize, and the incidence of serious infection varies with the size and depth of the burn and the age of the patient. Topical antimicrobial therapy should prevent infection of burns of <30% of the total body area, but larger burns are always colonized. Non-invasive infection is confined to the eschar, which is the non-viable skin debris on the surface of deep burns. It is characterized by rapid separation of the eschar from the underlying tissue and a heavy exudate of purulent material from the burn wound. The systemic symptoms are usually relatively mild. However, organisms can invade from heavily colonized burn eschars into viable tissue beneath and rapidly destroy the tissue, converting partial-thickness burns into full-skin-thickness destruction. From here, it is a small step to invasion of the lymphatics and thence to the bloodstream or direct invasion of blood vessels, and to septicaemia. Septicaemia in patients with burns is often polymicrobial.

The major pathogens in burns are aerobic and facultatively anaerobic bacteria and fungi

The most important pathogens in burn wounds are:

- *Pseudomonas aeruginosa* and other Gram-negative rods
- *Staphylococcus aureus*
- *Streptococcus pyogenes*
- other streptococci
- enterococci.

Candida spp. and *Aspergillus* together account for about 5% of infections. Anaerobes are rare in burn wound infections. Herpesvirus infections have been reported and are most likely due to reactivation at a damaged skin site.

P. aeruginosa is a devastating Gram-negative pathogen of burned patients

P. aeruginosa is an opportunist Gram-negative rod that has a long and infamous association with burn infections. It grows well in the moist environment of a burn wound, producing a foul, green-pigmented discharge and necrosis. Invasion is common, and the characteristic skin lesions (ecthyma gangrenosum) that are pathognomonic of *P. aeruginosa* septicaemia may appear on non-burned areas (see Fig. 31.6). Host factors predisposing to infection include:

- abnormalities in the antibacterial activities of neutrophils
- deficiencies in serum opsonins.

Added to these are the virulence factors of the organism, which include the production of elastase, protease and exotoxin. This combination makes *P. aeruginosa* the most devastating Gram-negative pathogen of burned patients. Treatment is difficult because of the organism's innate resistance to many antibacterial agents. A combination of aminoglycoside, usually gentamicin or tobramycin, with one of the beta-lactams

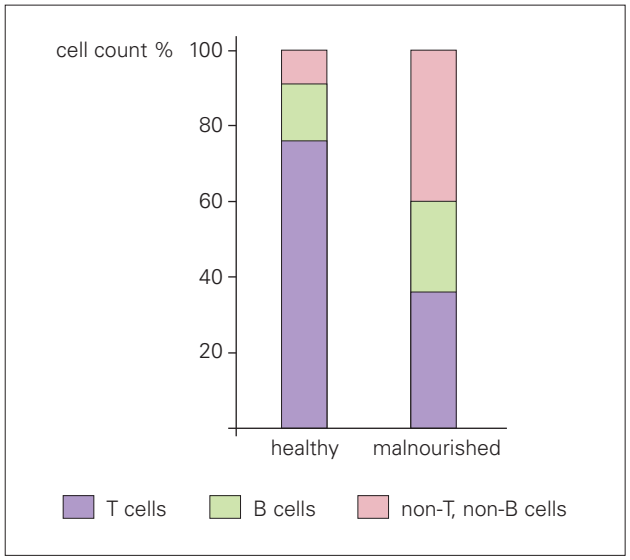


Figure 31.5 The proportion of T cells is decreased in malnourished patients compared with healthy controls. B-cell counts are usually unaltered, and lymphocytes lacking T- and B-cell markers are increased.

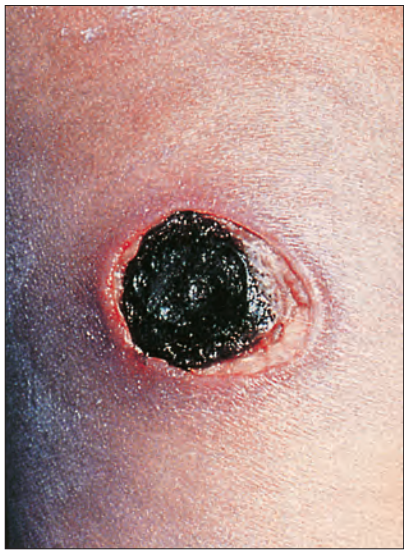


Figure 31.6 Ecthyma gangrenosum in a child with *Pseudomonas* septicaemia associated with immunodeficiency. (Courtesy of H. Tubbs.)

such as ceftazidime or imipenem is usually favoured, but several units have reported strains resistant to these agents.

It is virtually impossible to prevent colonization. Prevention of infection depends largely on inhibiting the multiplication of organisms colonizing the burn by applying topical agents such as silver nitrate.

Staph. aureus is the foremost pathogen of burn wounds

The most important predisposing factor to *Staph. aureus* infection in burns patients appears to be an abnormality of the antibacterial function of neutrophils. Infections follow a more insidious course than streptococcal infections (see below), and it may be several days before the full-blown infection is apparent. The organism is capable of destroying granulation tissue, invading and causing septicaemia. *Staph. aureus* infections of skin are discussed in detail in Chapter 27. Treatment with antistaphylococcal agents such as flucloxacillin or a glycopeptide if methicillin-resistant *Staph. aureus* is isolated should be administered if there is evidence of invasive infection. Every effort should be made to prevent the spread of staphylococci from patient to patient. Although transmissible by both airborne and contact routes, the contact route is by far the more important.

The high transmissibility of *Strep. pyogenes* makes it the scourge of burns wards

Strep. pyogenes (group A strep) infections of skin and soft tissue are discussed in some detail in Chapter 27. *Strep. pyogenes* was the most common cause of burn wound infection in the pre-antibiotic era and is still to be feared in burns wards. The infection usually occurs within the first few days of injury and is characterized by a rapid deterioration in the state of the burn wound and invasion of neighbouring healthy tissue. The patient may become severely toxic and will die within hours unless treated appropriately. *Strep. pyogenes* rarely infects healthy granulation tissue, but freshly grafted wounds may become infected, resulting in destruction of the graft. Every effort should be made to prevent spread. Penicillin is the drug of choice for treatment, and erythromycin or vancomycin can be used for penicillin-allergic patients.

Beta-haemolytic streptococci of other Lancefield groups (notably groups C and G) and enterococci are also important pathogens of burn wounds.

Traumatic injury and surgical wound infections

Both accidental and intentional trauma destroy the integrity of the body surface and leave it liable to infection. Accidental injury may result in pathogens being introduced deep into the wound. The species involved will depend upon the nature of the wound, as discussed in Chapter 27.

Staph. aureus is the most important cause of surgical wound infection

Staph. aureus surgical wound infection (see Ch. 37) may be acquired during surgery or postoperatively and may originate from the patient or from another patient or staff member. The wound is less well defended than normal tissue; it may have a damaged blood supply and there may be foreign bodies such as sutures. Classic studies of wound infections have shown that far fewer staphylococci are needed to initiate infection around a suture than in normal healthy skin. Wound infections can be severe and the organisms can invade the bloodstream, with consequent seeding of other sites such as the heart valves, causing endocarditis (see Ch. 30) or bones, causing osteomyelitis (see Ch. 27), thereby further compromising the patient.

Catheter-associated infection of the urinary tract is common

Urinary catheters disrupt the normal host defences of the urinary tract and allow organisms easy access to the bladder. Such catheter-associated infection of the urinary tract is especially common if catheters are left in place for >48 h (see Ch. 21). The organisms involved are usually Gram-negative rods from the patient's own faecal or periurethral flora, but cross-infection also occurs (see Ch. 37).

Staphylococci are the most common cause of intravenous and peritoneal dialysis catheter infections

Intravenous and peritoneal dialysis catheters breach the integrity of the skin barrier and allow organisms from the skin flora of the patient or hands of the carer easy access to deeper sites. Staphylococci are the most common cause of infection, but coryneforms, Gram-negative rods and *Candida* are also implicated.

Coagulase-negative staphylococci, particularly *Staph. epidermidis*, account for more than 50% of the infections. These opportunists are members of the normal skin flora and for many years were considered to be harmless. However, they have a particular propensity for colonizing plastic and can therefore seed sites adjacent to plastic devices and cause invasive infections. Their ability to produce an adhesive slime material and grow as biofilms on plastic surfaces is important. They are sticky bacterial clusters attached to the surface and embedded in an extracellular matrix. Protection is provided against antibiotics and host defence by the mechanical barrier, slowing down cell processes such as protein synthesis and replication as well as being made up of 'persister' cells that are less susceptible to antibiotics. There are a number of virulence factors that promote biofilm production and these include proteins, adhesins and polysaccharides. Infections are characteristically more insidious in onset than those caused by the more virulent *Staph. aureus*, and recognition is hampered by the difficulty in distinguishing the infecting strain from the normal flora. Treatment is also difficult because many *Staph. epidermidis* strains carry multiple antibiotic resistances, and agents such as a glycopeptide (vancomycin or teicoplanin) and rifampicin may be required (see Ch. 34). Whenever possible, the plastic device should be removed.

Infections of plastic devices in situ

The technical developments in plastics and other synthetic materials have enabled many advances in medicine and surgery, but in the process have produced further ways of introducing infectious agents. *Staph. epidermidis* is an important cause of infection of cardiac pacemakers, vascular grafts and CSF shunts.

Staph. epidermidis is the most common cause of prosthetic valve and joint infections

Patients with prosthetic heart valves or prosthetic joints are compromised by:

- the surgery to implant the prosthesis
- the continued presence of a foreign body.

Staph. epidermidis is again the most common pathogen, gaining access either during surgery or from a subsequent bacteraemia originating from, for example, an intravascular line infection. Endocarditis associated with prosthetic heart valves is discussed in Chapter 30.

The most common complication of joint replacement is loosening of the prosthesis, while infection is the second most common complication and is much more likely to lead to permanent failure of the procedure. The difficulties of treatment have been outlined above, but there is understandably great reluctance to remove a prosthetic device, even though it is sometimes the only way to eradicate an infection.

Infections due to compromised clearance mechanisms

Stasis predisposes to infection, and in health the body functions to prevent stasis. In the respiratory tract, damage to the ciliary escalator predisposes the lungs to invasion, particularly in patients with cystic fibrosis who are infected with *Staph. aureus* and *Haemophilus influenzae* and later with *P. aeruginosa* (see Ch. 20).

Obstruction and interruption of normal urine flow allows Gram-negative organisms from the periurethral flora to ascend the urethra and to establish themselves in the bladder. Septicaemia is an important complication of urinary tract infection superimposed on obstruction.

INFECTIONS ASSOCIATED WITH SECONDARY ADAPTIVE IMMUNODEFICIENCY

The underlying immunodeficiency state determines the nature and severity of any associated infection, and in some cases infection is the presenting clinical feature in a patient with an immunological deficit. However, septicaemia and related infectious complications of immunodeficiency are most commonly encountered in patients hospitalized for chemotherapy for malignant diseases or organ transplantation. In these patients, infection continues to be a major cause of morbidity and mortality (Table 31.3). Increasingly, these infections are iatrogenic and caused by opportunist pathogens acquired in hospital.

Haematological malignancy and bone marrow transplant infections

A lack of circulating neutrophils following bone marrow failure predisposes to infection

Susceptibility to infection of patients with haematological malignancies is primarily due to the lack of circulating neutrophils that inevitably follows bone marrow dysfunction that is either due to the disease or the treatment. Septicaemia may be the presenting feature, but is much more common when the patient has received cytotoxic chemotherapy to induce a remission of the disease. Neutropenia, defined as a count of 0.5×10^9 neutrophils / L, may persist for a few days to several weeks. Similarly, prolonged periods of neutropenia occur after bone marrow transplantation until engraftment has taken place.

The length of time over which the patient is neutropenic influences the nature of any associated infection and the frequency with which it occurs. For example, fungal infections are much more common in patients who are neutropenic for more than 21 days. Although Gram-negative rods such as *Escherichia coli* and *P. aeruginosa* from the bowel flora have in the past been the most common cause of septicaemia in neutropenic patients, Gram-positive organisms such as staphylococci, streptococci and enterococci are also important. *Staph. epidermidis* septicaemia associated with intravascular catheters (see above) is common. Infections caused by fungi are also increasing, partly because more patients are surviving the early neutropenic period with the aid of modern antibacterial agents and granulocyte transfusions. Cytomegalovirus infections may reactivate in the more intensive type of bone marrow transplantation known as allogeneic transplants, where the recipient

Table 31.3 Examples of opportunistic pathogens in immunocompromised hosts

Bacteria
Gram-positive
Staphylococcus aureus
Coagulase-negative staphylococci
Streptococci
Listeria spp.
Nocardia asteroides
Mycobacterium tuberculosis
Mycobacterium avium-intracellulare
Gram-negative
Enterobacteriaceae
Pseudomonas aeruginosa
Legionella spp.
Bacteroides spp.
Fungi
Candida spp.
Aspergillus spp.
Cryptococcus neoformans
Histoplasma capsulatum
Pneumocystis jirovecii[a]
Parasites
Toxoplasma gondii
Strongyloides stercoralis
Viruses
Herpesviruses, e.g. HSV, CMV, VZV, EBV, HHV-6, HHV-7, HHV-8
Hepatitis B
Hepatitis C
Polyomaviruses, e.g. BKV, JCV
Adenoviruses
HIV

[a]Formerly *P. carinii*.
BKV, BK virus; JCV, JC virus; CMV, cytomegalovirus; EBV, Epstein–Barr virus; HIV, human immunodeficiency virus; HHV, human herpesvirus; HSV, herpes simplex virus; VZV, varicella-zoster virus.

receives bone marrow from a matched donor, compared with autologous transplants where the patient receives their own stem cells having received cytotoxic chemotherapy, for example. CMV reactivation is often associated with the development of graft-versus-host disease. In addition, adenovirus, Epstein–Barr virus (EBV) and BK virus (BKV) infections may be seen, especially in allogeneic bone marrow transplant recipients. Aciclovir prophylaxis is effective at preventing other latent herpesvirus infections from reactivating, such as HSV and VZV.

Solid organ transplant infections

Most infections occur within 3–4 months of transplantation

Suppression of a patient's cell-mediated immunity is necessary to prevent rejection of an engrafted organ, and the immunomodulatory regimens used will suppress humoral immunity to some extent as well. In addition, high doses of corticosteroids to suppress inflammatory responses are required. The combination of these factors results in a severely compromised host, and those that have an effect on infection in recipients of solid organ transplants include:

- the underlying medical condition of the patient
- the patient's previous immune status
- the type of organ transplant
- the immunosuppressive regimen
- the exposure of the patient to pathogens.

The organisms that cause the most common and most severe infections are shown in Table 31.3. Some of the viral infections are latent and reactivate when cell-mediated surveillance is suppressed.

From 3–4 months after transplantation, the risk of infection is reduced, but remains for as long as the patient is immunosuppressed.

HIV infection leading to AIDS

The clinical definition of AIDS includes the presence of one or more opportunistic infections

Individuals with AIDS are often infected concomitantly with multiple pathogens, which they fail to eradicate despite prolonged, appropriate and aggressive antimicrobial chemotherapy. Most of the pathogens involved are intracellular pathogens that require an intact cell-mediated immune response for effective defence. As the HIV-infected individual progresses to AIDS (see Ch. 22), organisms that are usually controlled by cell-mediated immunity are able to reactivate to cause disseminated infections not seen in the immunologically intact individual. Improved immune surveillance as a result of combined antiretroviral therapy has reduced the incidence of infections that are the hallmark of AIDS including *Candida*, Kaposi's sarcoma and other opportunist pathogens described in more detail below.

Many of the pathogens that cause infections in the immunocompromised host (Table 31.3) are described elsewhere in this book.

OTHER IMPORTANT OPPORTUNIST PATHOGENS

Fungi

Candida is the most common fungal pathogen in compromised patients

This yeast is an opportunist pathogen in a variety of patients and in various body sites. It is the cause of:

- vaginal and oral thrush (see Ch. 22)
- skin infections (see Ch. 27)
- endocarditis, particularly in injecting drug users (see Ch. 30).

Candida manifests itself in different ways depending on the nature of the underlying compromise:

- *Chronic mucocutaneous candidiasis*. This is rare and is a persistent but non-invasive infection of mucous membranes,

hair, skin and nails in patients, often children, with a specific T-cell defect rendering them anergic to *Candida* (Fig. 31.7). It may require repeated or long-term treatment with azole antifungal drugs. Diminished sensitivity to these agents may occur after repeated use.

- *Oropharyngeal and oesophageal candidiasis*. This is seen in a variety of compromised patients, including HIV-infected individuals (Fig. 31.8) and people with ill-fitting dentures, diabetes mellitus or on antibiotics or corticosteroids. Oropharyngeal candidiasis generally responds to treatment with antifungal mouthwashes (nystatin or an azole compound). Those individuals who do not respond can be treated with fluconazole. Oesophageal candidiasis requires systemic therapy.
- *Gastrointestinal candidiasis*. This is seen in patients who have undergone major gastric or abdominal surgery and in those with neoplastic disease. The organism can pass through the intestinal wall and spread from a gastrointestinal focus. Antemortem diagnosis is difficult, and as many as 25% of patients do not have any symptoms in the early stages of disease. If there is dissemination from the gut, blood cultures may become positive and *Candida* antigens may be detectable in the serum. A high index of suspicion is required to initiate antifungal therapy early in these patients, but disseminated disease is often fatal.
- *Disseminated candidiasis*. This is probably acquired via the gastrointestinal tract, but also arises from intravascular catheter-related infections. Patients with lymphoma and leukaemia are most at risk. Blood-borne spread to almost any organ can occur. Infections of the eye (endophthalmitis; Fig. 31.9) and the skin (nodular skin lesions; see Ch. 27) are important because they provide diagnostic clues, and without these the non-specific symptoms of fever and septic shock make early diagnosis difficult. Immunocompromised patients are often given antifungal therapy 'blindly' if they have a fever and fail to respond to broad-spectrum antibacterial agents.

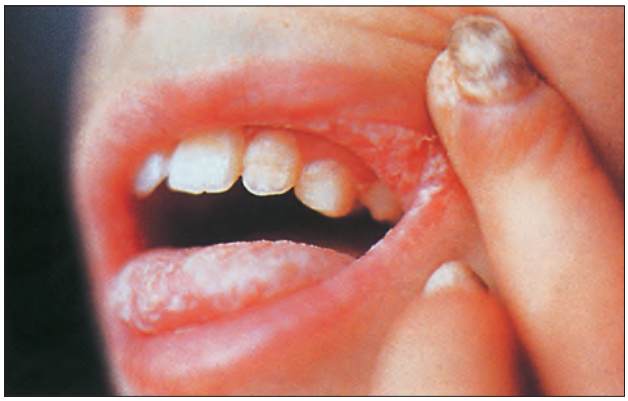


Figure 31.7 Chronic mucocutaneous candidiasis in a child with impaired T-cell response to antigens. (Courtesy of M.J. Wood.)

Cryptococcus neoformans infection is most common in people with impaired cell-mediated immunity

C. neoformans is an opportunistic yeast with a worldwide distribution. It can cause infection in the immunocompetent host, but infection is seen more frequently in people with impaired cell-mediated immunity. The onset of disease may be slow and usually results in lung infection or meningoencephalitis; occasionally other sites such as skin, bone and joints are involved (see Ch. 27).

C. neoformans can be demonstrated in the CSF and is characterized by its large polysaccharide capsule (see Fig. 25.6). Rapid identification can be made by antigen detection in a latex agglutination test using specific antibody-coated latex particles. Treatment involves a combination of amphotericin and flucytosine (see Ch. 34) and can be monitored by detecting a fall in CSF antigen concentration. The prognosis depends largely upon the patient's underlying disease; in the severely immunocompromised, mortality is approximately 50%. In patients with AIDS it is almost impossible to eradicate the organism even with intensive treatment. Fluconazole can be given as post-treatment prophylaxis.

Disseminated *Histoplasma capsulatum* infection may occur years after exposure in immunocompromised patients

This is a highly infectious fungus that causes an acute but benign pulmonary infection in healthy people, but can produce chronic progressive disseminated disease in the compromised host. The organism is endemic only in tropical parts of the world and notably in the so-called 'histoplasmosis belt' of the central USA, particularly in the Ohio and Mississippi river valleys. The natural habitat of the organism is the soil. It is transmitted by the airborne route and the fungal spores are

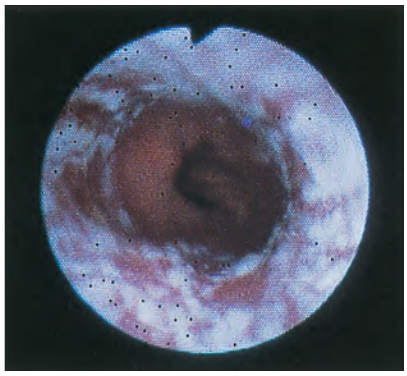


Figure 31.8 *Candida* oesophagitis. Endoscopic view showing extensive areas of whitish exudate. (Courtesy of I. Chesner.)

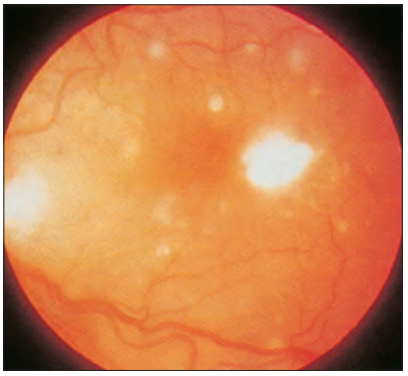


Figure 31.9 *Candida* endophthalmitis. Fundal photograph showing areas of white exudate. (Courtesy of A.M. Geddes.)

deposited in the alveoli, from where the fungus spreads via the lymphatics to the regional lymph nodes. As disseminated disease may occur many years after the initial exposure in immunocompromised patients it may present in patients who have long since left endemic areas. The infection may occur in HIV-infected individuals who have visited such regions.

Cultures of blood, bone marrow, sputum and CSF may yield *Histoplasma*, but biopsy and histological examination of bone marrow, liver or lymph nodes is often required to make the diagnosis (Fig. 31.10). Approximately 50% of cases of progressive disease in the immunocompromised are successfully treated with amphotericin. Itraconazole can be given for post-treatment prophylaxis.

African histoplasmosis, caused by *Histoplasma duboisii*, is found in Equatorial Africa. Patients may present with localized cutaneous or disseminated disease.

Invasive aspergillosis has a very high mortality rate in the compromised patient

The role of *Aspergillus* spp. in diseases of the lung has been outlined in Chapter 20, but this fungus is now increasingly reported as a cause of invasive disease in compromised patients, usually in profoundly neutropenic patients or those receiving high-dose corticosteroids (Fig. 31.11). Like *Histoplasma*, aspergilli are found in soil, but have a worldwide distribution. Infection is spread by the airborne route, and the lung is the site of invasion in almost every case. Dissemination to other sites, particularly the central nervous system (Fig. 31.12) and heart, occurs in about 25% of compromised individuals with lung infection. Diagnosis involves microscopy, culture, antigen detection and polymerase chain reaction (PCR) on bronchoalveolar lavage specimens. Lung biopsy may be required to make a tissue diagnosis.

Invasive aspergillosis has a high fatality rate in the compromised patient. Prophylactic antifungal agents such as caspofungin, posaconazole and voriconazole, early diagnosis and institution of treatment using an intravenous lipid formulation of amphotericin B known as liposomal amphotericin B complexes or AmBisome (see Ch. 34), together with a reduction in corticosteroid and cytotoxic therapy wherever possible, appear to improve the prognosis. Outbreaks of hospital-acquired infection have been reported (see Ch. 37), especially in relation to recent building work.

Pneumocystis jirovecii (formerly *P. carinii*) causes symptomatic disease only in people with deficient cellular immunity

P. jirovecii is an atypical fungus which appears to be widespread; a large proportion of the population has antibodies to the organism, but it only causes symptomatic disease in people whose cellular immune mechanisms are deficient. There is therefore a high incidence of *P. jirovecii* pneumonia in patients receiving immunosuppressive therapy to prevent transplant rejection and in individuals with HIV. It is very rare to find *Pneumocystis* infection in any other site in the body, but the reason for this is unknown.

Diagnosis is not easy and requires a high index of suspicion. The symptoms are non-specific and can mimic a variety of other infectious and non-infectious respiratory diseases. In addition, unlike the other fungi described above, the organism cannot be isolated in expectorated sputum using conventional culture methods, and invasive techniques such as bronchoalveolar lavage are required. In samples obtained by

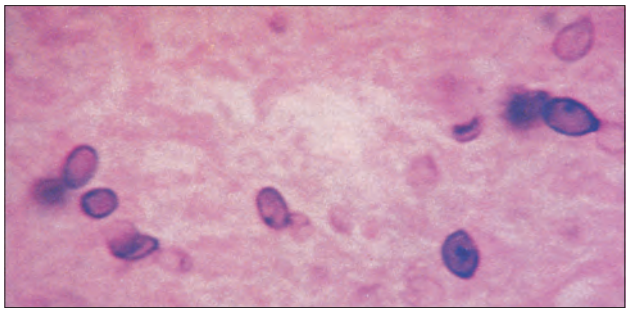


Figure 31.10 Histological section of the lung showing yeast forms of *Histoplasma capsulatum* (methenamine silver stain). (Courtesy of T.F. Sellers, Jr.)

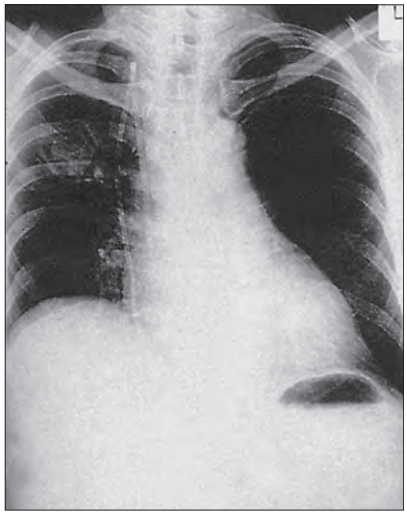


Figure 31.11 Chest radiograph showing invasive aspergillosis in the right lung of a patient with acute myeloblastic leukaemia. (Courtesy of C. Kibbler.)

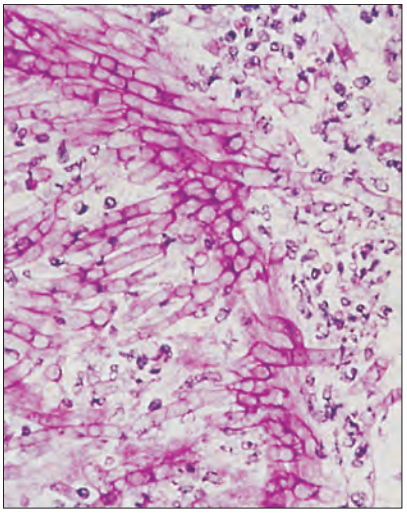


Figure 31.12 Numerous septate hyphae invading a blood vessel wall in cerebral aspergillosis (periodic acid-Schiff stain). (Courtesy of W.E. Farrar.)

these techniques, the organism can be demonstrated by silver or immunofluorescent stains (Fig. 31.13). DNA amplification by PCR improves the sensitivity of the diagnostic tests.

Treatment is with high dose co-trimoxazole (trimethoprim-sulfamethoxazole). Pentamidine is an alternative (see Ch. 34). Adjunctive corticosteroid therapy is given in moderate to severe infections in HIV co-infected individuals. Co-trimoxazole is used prophylactically.

Bacteria

Nocardia asteroides is an uncommon opportunist pathogen with a worldwide distribution

The family Actinomycetes, relatives of the mycobacteria but resembling fungi in that they form branching filaments, contain two pathogenic genera: *Actinomyces* and *Nocardia*. *N. asteroides* infections have been reported in immunocompromised, especially in renal transplant, patients. The lung is usually the primary site (Fig. 31.14), but infection can spread to the skin, kidney or central nervous system. As with *Aspergillus*, hospital outbreaks of nocardiosis have been described.

Nocardia can be isolated on routine laboratory media, but is often slow to grow and is consequently easily overgrown by commensal flora. Therefore the laboratory staff should be informed if nocardiosis is suspected clinically, so that appropriate media are inoculated. The organism is a Gram-negative branching rod and weakly acid fast (Fig. 31.15).

Sulphonamides or co-trimoxazole are the drugs of choice, but treatment can be difficult and various other regimens involving aminoglycosides or imipenem have been described.

Mycobacterium avium-intracellulare disease is often a terminal event in AIDS

Although mycobacterial infections are well documented in immunosuppressed patients, the association between AIDS and mycobacteria includes disseminated infection with *Mycobacterium tuberculosis* and *M. avium-intracellulare* (*M. avium* complex, or MAC). These organisms can be isolated from blood cultures from patients with AIDS. *M. tuberculosis* has been described in detail in Chapter 20. *M. avium-intracellulare* belongs to the so-called 'atypical' mycobacteria or mycobacteria other than tuberculosis (MOTT). It resembles *M. tuberculosis* in that it is slow growing, but it is resistant to the conventional antituberculosis drugs. Multidrug therapy with macrolides

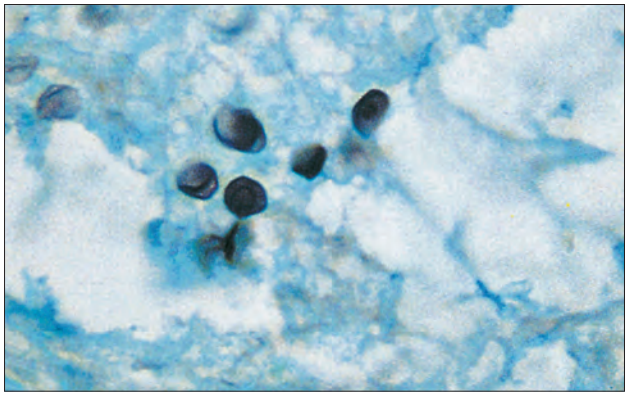


Figure 31.13 Darkly staining cysts of *Pneumocystis jirovecii* in an open lung biopsy from an AIDS patient with pneumonia (Grocott silver stain). (Courtesy of M. Turner-Warwick.)

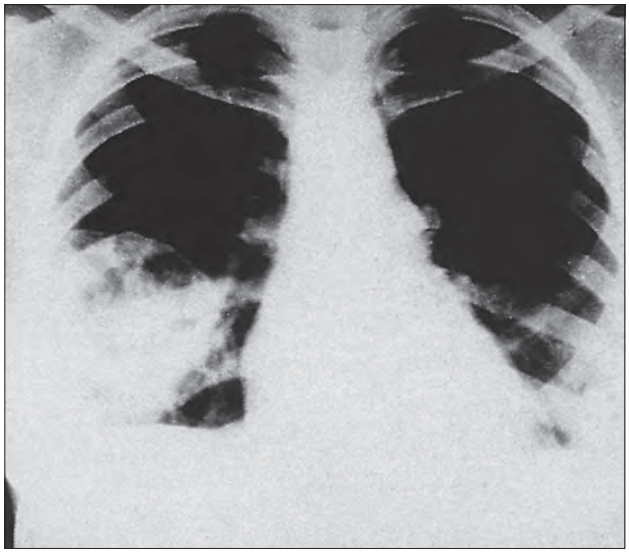


Figure 31.14 Pulmonary nocardiosis. Chest radiograph showing a large rounded lesion in the right lower zone with multiple cavities. (Courtesy of T.F. Sellers, Jr.)

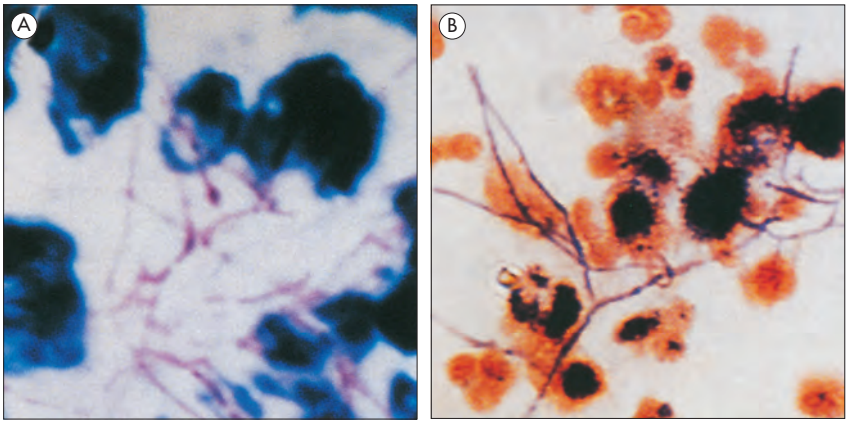


Figure 31.15 *Nocardia asteroides* in sputum. (A) Acid-fast stain. (B) Gram's stain. ([A] Courtesy of T.F. Sellers, Jr. [B] Courtesy of H.P. Holley.)

such as azithromycin or clarithromycin from clarithromycin plus ethambutol (and rifabutin may be considered too) have been recommended.

Protozoa and helminths

Cryptosporidium and *Cystoisospora belli* infections cause severe diarrhoea in AIDS

Cryptosporidium (Fig. 31.16) is a protozoan parasite that causes human disease, and is well known to veterinarians as an animal pathogen. It causes significant but self-limiting diarrhoea in healthy people with an intact immune system (see Ch. 23), but severe and chronic diarrhoea in severely immunocompromised people, e.g. with advanced HIV-infection. Combined active antiretroviral therapy (HAART) in individuals with AIDS infected with *Cryptosporidium* has been reported to improve the diarrhoea symptoms. Paromomycin reduces oocyst output but does not clear infection. Nitazoxanide is effective in HIV-negative patients but is only partially active in those co-infected with HIV. *Cystoisospora belli* (Fig. 31.17) is another protozoan parasite very similar to *Cryptosporidium* and also produces severe diarrhoea in people with AIDS. Unlike *Cryptosporidium*, however, it responds to co-trimoxazole.

Cyclospora cayetanensis, also related to *Cryptosporidium*, likewise produces prolonged and severe diarrhoea in immunosuppressed individuals. Co-trimoxazole treatment is effective. Ciprofloxacin is partially effective.

Infections with microsporidia also cause diarrhoea in people with AIDS and other immunosuppressed patients. *Enterocytozoon bieneusi* is the most common cause, although *Encephalitozoon intestinalis* also occurs. Albendazole treatment is effective against *E. intestinalis* but has disappointing activity against *E. bieneusi*. Where feasible, immune reconstitution is the mainstay of treatment.

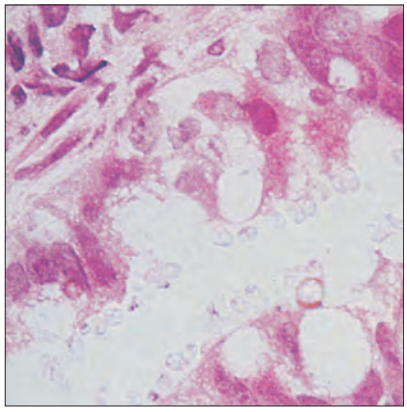


Figure 31.16 Numerous organisms in the brush border of the intestine in cryptosporidiosis. (Courtesy of J. Newman.)

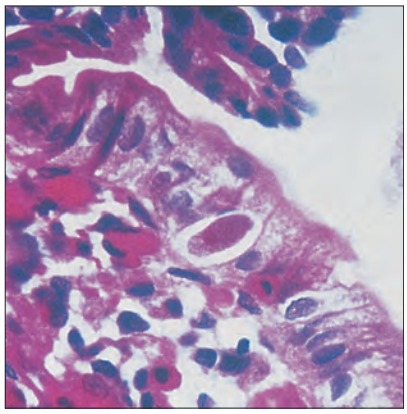


Figure 31.17 Human coccidiosis, with a single *Isospora belli* organism within an epithelial cell and a chronic inflammatory reaction in the lamina propria. (Courtesy of G.N. Griffin.)

Immunosuppression may lead to reactivation of dormant *Strongyloides stercoralis*

Strongyloides stercoralis is a parasitic roundworm that remains dormant for years following initial infection, but may be reactivated by immunosuppression, e.g. with steroid therapy, to produce massive autoinfection. Human T-cell lymphotropic virus type 1 (HTLV-1) infection is associated with disseminated strongyloidiasis due to the modified immune response to this enteric helminth. The lungs, liver and brain are the most common organs affected. Although rare in the UK and most of the USA, *Strongyloides* should be borne in mind in patients who have lived in endemic areas such as the tropics and southern USA, even if this was many years before their immunosuppression.

Viruses

Certain virus infections are both more common and more severe in compromised patients, and regular surveillance is critical

The virus infections that are more common or more severe in the compromised patient (see Table 31.3) have been described in detail elsewhere in this book. Many of these represent reactivation of latent infections. Pre-transplantation baseline serology is carried out to determine both the donor and recipient status for a number of virus infections, including HIV, HTLV hepatitis B and C, CMV, EBV and herpes simplex virus (HSV).

Suppression of specific virus infections using antiviral agents is part of the management of the recipient in conjunction with regular virological surveillance post-transplantation using viral genome detection methods that have generally superseded antigen detection.

As part of a pre-emptive treatment strategy, blood samples are collected for early detection of viraemia or antigenaemia, which precedes disease. For example, transplant donors and recipients are screened for CMV IgG. CMV causes a broad spectrum of clinical disease in this setting, including pneumonitis, oesophagitis, colitis, hepatitis and encephalitis. If there is a transplant mismatch (i.e. the donor is CMV IgG positive and the recipient CMV IgG negative), the infection may be acquired from the donor organ or bone marrow. If possible, transplant centres try to avoid this situation, as the risk of a primary CMV infection in the first month post-transplantation is extremely high, as is the morbidity and mortality. In this case, CMV DNA monitoring is carried out on blood samples on a regular basis post-transplantation to detect early infection and start antiviral therapy as soon as possible. Some centres offer antiviral therapy in the immediate post-transplant period in this clinical setting to delay the onset of infection to a time when the recipient is less immunosuppressed. CMV IgG positive recipients are at

risk of reactivation or re-infection and will also be monitored regularly post-transplantation. A primary CMV infection is usually detected around 4 weeks, compared with reactivation at around 6–8 weeks, post-transplantation respectively.

Antiviral prophylaxis for HSV reactivation that may occur in the immediate post-transplantation period is often given to bone marrow transplant recipients for prolonged periods post-transplantation. Aciclovir is given at a low dose and is effective in preventing HSV and varicella-zoster virus (VZV) reactivation. Virus surveillance is therefore not carried out, but if a breakthrough infection occurs it is important to collect material from the lesions for virus isolation or genome sequence analysis to determine the antiviral susceptibility. Herpetic lesions can be persistent and involve the lips, oesophagus and other parts of the gastrointestinal tract, and may cause a pneumonitis, hepatitis or encephalitis.

Herpes zoster, a reactivation of VZV infection, may occur within a few months post-transplantation, affecting the skin dermatome supplied by the involved nerve. Sometimes the distribution may be multidermatomal and dissemination can occur to other sites.

HHV-6 and HHV-7 infection, re-infection or reactivation has been reported in transplant recipients, in particular with neurological conditions including encephalitis. HHV-8 has been associated with the development of Kaposi's sarcoma (KS) in individuals with AIDS as well as classic and endemic KS in HIV-uninfected individuals. If a recipient or donor has had a hepatitis B virus infection previously, antiviral prophylaxis with lamivudine, tenofovir or entecavir is also given to prevent reactivation. HBsAg and HBV DNA monitoring is also carried out on blood samples.

EBV infection can lead to tumour development

EBV infection has been associated with the development of Hodgkin's disease, non-Hodgkin's lymphomas in individuals with HIV infection, post-transplantation lymphoproliferative disease and smooth muscle tumours in immunosuppressed children. EBV has a broad spectrum of clinical syndromes ranging from infectious mononucleosis to malignancies which include EBV associated post-transplant lymphoproliferative disorder (PTLD), containing clonal chromosomal abnormalities with a high mortality rate, especially with the monoclonal tumours. The risk factors recognized for PTLD development in solid organ transplant recipients include post-transplantation primary EBV infection, mismatched donor and recipient CMV status, CMV disease, and intensity and type of immunosuppressive therapy. With respect to EBV infection, EBV-susceptible recipients have a 10–76-fold higher risk of PTLD compared with recipients with previous EBV exposure.

As the two peaks of primary EBV infection are in children and adolescents, the incidence of PTLD is higher in paediatric transplant recipients. In addition, without an effective cytotoxic T-cell response owing to post-transplant immunosuppression to prevent graft rejection, the EBV-infected B lymphocytes may proliferate in an uncontrolled fashion. This results in B-cell hyperplasia with CD20-positive lymphocytes that ranges from polyclonal and benign to development of a monoclonal or oligoclonal B-cell lymphoma. The prevalence of PTLD in paediatric liver transplant recipients ranges from 4% to 14%, depending on the immunosuppressive regimen. Retrospective studies have shown that up to 50% of paediatric transplant recipients with primary EBV infections are at risk of developing PTLD. The infection may be acquired in the community or, in the transplant setting, from the donor organ or blood products. The natural history of EBV infection and pathophysiology of post-transplant EBV-driven lymphoproliferation is not well understood.

Diagnostic criteria for EBV-associated PTLD have been developed. However, in the absence of randomized, placebo-controlled trials, there is little information on the efficacy of specific treatment protocols. The treatment of PTLD includes reducing immunosuppression to allow a better host response to control the infection, although there is a risk of rejecting the graft, using rituximab, an anti-CD20 monoclonal antibody that targets the B cells with the EBV receptor, and chemotherapy. Treatment of post-transplant lymphomas by adoptive transfer of EBV-specific cytotoxic T lymphocytes has been reported.

Respiratory virus infections

Immunocompromised patients, especially transplant recipients, are at increased risk of pneumonia and death if they develop respiratory tract infections with viruses such as respiratory syncytial virus (RSV), influenza, parainfluenza and adenoviruses. Preventive measures include influenza immunization, prophylaxis with palivizumab, an RSV-specific monoclonal antibody that is used in specific clinical settings, and early diagnosis of an upper respiratory tract infection using sensitive tests such as viral genome detection. There are some specific antiviral treatments that include oseltamivir for influenza and ribavirin for RSV infections.

Adenovirus infection has a high mortality rate

Primary and reactivated adenovirus infections can result in disseminated disease in immunocompromised hosts, in particular paediatric and adult bone marrow transplant recipients. Hepatitis and pneumonia are most frequently reported. Again, adenovirus surveillance is often carried out in centres by collecting blood samples post-transplantation, which are tested for adenovirus DNA in order to detect early viraemia. Where adenovirus viraemia is detected, management options include reducing immunosuppression and treating with an antiviral agent such as ribavirin or cidofovir. However, there are few reports of successful outcomes in patients with disseminated infections.

Hepatitis B and C infection in transplant recipients

Hepatitis B virus (HBV) infection has an immunopathological pathogenesis, with jaundice occurring after cytotoxic T cells have lysed the hepatitis B surface-antigen-bearing hepatocytes. The virus is integrated in the hepatocytes after acute hepatitis B. Bone marrow transplant recipients with evidence of previous, not current, hepatitis B infection are likely to suffer a hepatitis B reactivation post-transplantation. They will be asymptomatic as they are immunosuppressed and will not mount a cytotoxic T-cell response until they have engrafted. It is at this stage they will become symptomatic, develop jaundice and the morbidity and mortality can be high. Antiviral prophylaxis with antiviral agents such as lamivudine, tenofovir or entecavir is given to prevent reactivation, together with HBV DNA monitoring. Antiviral treatment will be given pre- and post-transplant

if a transplant recipient has a current HBV infection (i.e. is hepatitis B surface antigen positive).

Hepatitis C overtook hepatitis B as the main viral cause of cirrhosis leading to liver transplantation when antiviral treatment options became available. By 2017, HCV infected individuals with advanced liver disease could be treated with direct acting antivirals and the potential for delisting liver transplant candidates was being reported.

HCV infection is also associated with veno-occlusive disease in bone marrow transplant recipients. Venous congestion occurs in the liver owing to a non-specific vasculitis and results in liver necrosis. Multiorgan failure can be precipitated because of increased capillary permeability throughout the body.

Polyomaviruses can cause haemorrhagic cystitis and progressive multifocal leukoencephalopathy

BK or JC viruses are polyomaviruses acquired via the respiratory tract that lie latent in the kidney, and may be detected in the urine of bone marrow transplant recipients (see Ch. 21). BK viruria is associated with haemorrhagic cystitis.

JC virus can reactivate and disseminate to cause central nervous system infections such as progressive multifocal leukoencephalopathy (PML) in individuals with AIDS. However, since the advent of HAART resulting in higher CD4 counts and suppressed HIV load, PML is seen less often.

KEY FACTS

- A compromised person is one whose normal defences against infection are defective. Immunodeficiencies may involve the innate or adaptive immune systems and may be primary or secondary.
- Compromised patients can be infected with any of the pathogens capable of infecting immunocompetent individuals. In addition, they suffer many infections caused by opportunist pathogens. The type of infection is related to the nature of the compromise.
- Effective antimicrobial therapy is often difficult to achieve in the absence of a functional immune response, even when the pathogen is susceptible to the drug in vitro.
- Important bacterial opportunists include *P. aeruginosa*, especially in neutropenic patients and those with major burns, and *Staph. epidermidis* in patients with plastic devices in situ. In AIDS, the predominant bacterial opportunists are intracellular pathogens benefiting from the lack of cell-mediated immunity.
- Neutropenia following cytotoxic therapy and in advanced HIV infection (AIDS) predisposes to fungal infections (e.g. *Candida*, *Aspergillus* and *Cryptococcus*) especially when the patient has received previous antibacterial therapy.
- Viral infections are more common and severe in immunodeficient patients than in immunocompetent patients, particularly reactivation of latent infections (e.g. HSV, CMV, JCV).

32 Diagnosis of infection and assessment of host defence mechanisms

Introduction

Good quality specimens are needed for reliable microbiological diagnoses

The precise identification of the causative organism in infection has become increasingly important now that therapeutic intervention is possible. The ability to achieve this depends upon a positive interaction between the clinician and the microbiologist; the clinician must be aware of the complexity of the tests and the time required to achieve a result. In turn, the microbiologist must appreciate the nature of the patient's condition and be able to assist the clinician in interpreting the laboratory report. A fundamental step in any diagnosis is the choice of an appropriate specimen, which ultimately depends upon an understanding of the pathogenesis of infections.

Microbiology differs from other clinical laboratory disciplines in the amount of interpretative input required. When a specimen is received, decisions are made regarding the appropriate processing pathway, and when the result is received, it must be interpreted in relation to the specimen and the patient.

AIMS OF THE CLINICAL MICROBIOLOGY LABORATORY

The aims of the microbiology laboratory are:

- to provide accurate information about the presence or absence of microorganisms in a specimen that may be involved in a patient's disease process
- where relevant, to provide information on the antimicrobial susceptibility of the microorganisms isolated.

Identification is achieved by detecting the microorganism or its products or the patient's immune response

Laboratory tests are carried out:

- to detect microorganisms or their products in specimens collected from the patient
- to detect evidence of the patient's immune response (production of antibodies) to infection.

While there are different protocols for different specimens (e.g. urine, faeces, genital tract, blood, etc.), the tests fall into three main categories:

1. *Identification of microorganisms by isolation and culture*. Microorganisms may grow in artificial media or, in the case of viruses, in cell cultures. In some instances, quantification is important (e.g. more than 10^5 bacteria / mL of urine is indicative of infection whereas lower numbers are not; see Ch. 21). Once an organism has been isolated in culture, its susceptibility to antimicrobial agents can be determined.
2. *Identification of a specific microbial gene or product*. Non-cultural techniques that do not depend upon the growth and multiplication of microorganisms to detect microorganisms have the potential to yield more rapid results. These techniques include the detection of structural components of the cell (e.g. cell wall antigens) and extracellular products (e.g. toxins). Alternatively, molecular approaches are increasingly available such as the detection of specific gene sequences in clinical specimens using DNA probes or the polymerase chain reaction (PCR; see below). They are potentially applicable to all microorganisms, but actual antimicrobial susceptibilities cannot be determined without culture (although the presence of resistance genes may be detectable molecularly).
3. *Detection of specific antibodies to a pathogen*. This is especially important when the pathogen cannot be cultivated in laboratory media (e.g. *Treponema pallidum*, many viruses) or when culture would be particularly hazardous to laboratory staff (e.g. culture of *Francisella tularensis*, the cause of tularaemia, or the fungus *Coccidioides immitis*). Detection of IgM and / or IgG antibodies in a single serum collected during the acute phase of illness can be helpful in diagnosis of, for example, rubella by specific IgM, hepatitis A by IgM and hepatitis B by HepB surface antigen, or in rare diseases such as Lassa fever. The classic diagnostic method is by detection of a rise (fourfold or greater) in antibody titre between 'paired' sera, collected in the acute phase of an infection (5–7 days after onset of symptoms) and in convalescence (e.g. after 3–4 weeks). Such tests therefore tend to result in a delayed or retrospective diagnosis and are therefore of limited help for clinical management.

SPECIMEN PROCESSING

Specimen handling and interpretation of results is based upon a knowledge of normal microbiota and contaminants

Specimens intended for cultivation of microorganisms can be divided into two types:

- those from sites that are normally sterile
- those from sites that usually have commensal microorganisms (Box 32.1; see also Ch. 9).

A thorough knowledge of the microorganisms normally isolated from specimens from non-sterile sites, and the common contaminants of specimens collected from sterile sites, is important to ensure that specimens are properly handled and the results are correctly interpreted. Some specimens from sites that are normally considered sterile (e.g. bladder urine, sputum from the lower respiratory tract) are usually collected after passage through orifices that have a normal flora, which may contaminate the specimens. This needs to be considered when interpreting the culture results of these specimens.

In an ideal world, each specimen arriving in the laboratory would be considered in turn together with the information provided about the patient on the request form so that the microbiologist could assess the pathogens likely to be present and devise an 'individualized' processing plan. However, in reality, this approach is not practical because of constraints on time and money. Thus, specimens tend to be processed by type (e.g. urine, blood, faeces) and the microbiologist looks for easily cultivated pathogens known to be associated with each sample type. However, if the laboratory is provided with suitable information, such as a statement of possible aetiology, more fastidious or unusual pathogens can be sought and relevant antibiotic susceptibilities assessed. To obtain a test result that correctly identifies the infection, it is important to collect an appropriate specimen, to use the appropriate transport conditions and to deliver specimens rapidly to the laboratory. These conditions all affect the accuracy of the laboratory report, and therefore its value to the clinician and ultimately to the patient. Key points to remember about specimen collection are summarized in Box 32.2.

Routine culture takes at least 18 h to produce a result

Time is a key factor because the conventional methods of microbiological diagnosis depend upon growth and identification of the pathogen. Reliable results of routine culture cannot be achieved in <18 h and may take much longer (e.g. several weeks) for a minority of pathogens such as the mycobacteria, which grow very slowly. Antibiotic susceptibility tests involve a further incubation period. Thus, specimen processing can be categorized according to the time required to achieve a result and the method – cultural or non-cultural. An alternative, more immediate, route to the diagnosis of an infection is an immunological one, relying on the detection of an antibody response to the putative pathogen in the patient's blood, or a molecular one such as PCR and nucleic acid probes (see below).

CULTIVATION (CULTURE) OF MICROORGANISMS

Bacteria and fungi can be cultured on solid nutrient or liquid media

While cultures can be made in liquid media (broth), it is not possible to tell whether there is more than one species

Box 32.1 Sampling Sites, the Normal Microbiome and Interpretation of Results

Body sites that are normally sterile

- Blood and bone marrow
- Cerebrospinal fluid
- Serous fluids
- Tissues
- Lower respiratory tract
- Bladder

Body sites that have a normal commensal organisms

- Mouth, nose and upper respiratory tract
- Skin
- Gastrointestinal tract
- Female genital tract
- Urethra

Some sites in the body are sterile in health so that growth of any organism is indicative of infection provided that the specimen has been properly collected and transported, and examined in the laboratory without delay. The significance of isolates from sites that have a commensal flora depends upon the identity of the isolate and the quantity, as well as the immune status of the patient.

Box 32.2 Important Steps in Specimen Collection and Delivery to the Laboratory

- Take the appropriate specimen, e.g. blood and cerebrospinal fluid in suspected meningitis.
- Collect the specimen at the appropriate time, during the acute phase of the disease, e.g. malarial films, virus isolation, viral genome detection, IgM detection.
- If possible, collect specimen before patient receives antimicrobials.
- Collect enough material and an adequate number of samples, e.g. enough blood/serum for more than one set of blood cultures.
- Avoid contamination: from normal flora, e.g. midstream urine from non-sterile equipment.
- Use the correct containers and appropriate transport media.
- Label specimens properly.
- Complete request form with enough clinical information and a statement of possible aetiology.
- Inform the laboratory if special tests are required.
- Transport specimens rapidly to the laboratory.

Responsibility does not end with collection of the specimen and requesting tests. Good communication with the microbiologist is essential.

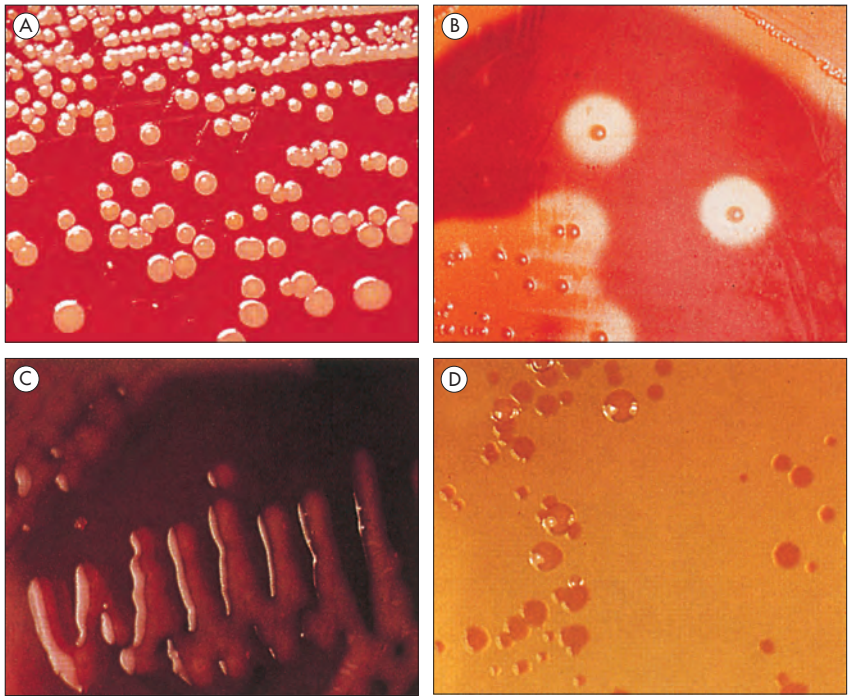


Figure 32.1 Bacterial colonies. A bacterial cell implanted on a solid nutrient medium will multiply to produce a colony containing millions of cells. Different species produce characteristically different colonies, and this feature can be used as a preliminary clue to the identity of the organism. (A) Golden colonies of *Staphylococcus aureus*. (B) Additional features such as the ability to lyse red blood cells can be demonstrated by culturing bacteria on blood-containing media. Here, beta haemolysis (complete haemolysis) is produced by *Streptococcus pyogenes* on blood agar. (C) Culture media can be made selective by including agents that are inhibitory to some species. For example, MacConkey agar contains bile salts so only those organisms tolerant to bile will grow. In addition, it contains lactose and a pH indicator. Species that ferment lactose change the indicator to bright pink. (D) Non-lactose-fermenting species, such as *Salmonella* and *Shigella*, form yellowish colonies.

present. Therefore, solid media are more useful in diagnostic microbiology. Bacteria and fungi grow on the surface of solid nutrient media (agar-based) to produce colonies composed of thousands of cells derived from a single cell implanted on the surface. Colonies of different species often have characteristic appearances, which can give a clue to their likely identity (Fig. 32.1).

Different species of bacteria and fungi have different growth requirements

It is possible to grow the majority of species of bacteria and fungi of medical importance in artificial media in the laboratory, but there is no one universal culture medium that will support the growth of them all, and there are still some species that can be grown only in experimental animals (e.g. *Mycobacterium leprae* and *Treponema pallidum*). Some bacteria that cannot be cultivated on artificial media (e.g. *Chlamydia* and *Rickettsia*) can be grown in cell cultures (see below).

Many culture media are designed not only to support the growth of the desired organisms, but also to inhibit the growth of others (i.e. they are 'selective media').

Specimens collected from body sites that have a normal commensal microbiota will contain a mixture of organisms from which the pathogen has to be recognized. Specimens are 'plated out' on a carefully chosen range of nutrient and selective media to produce single colonies to insure a pure culture. These are subcultured to fresh media for identification and antibiotic susceptibility tests (see below), a procedure which can take 48 h or longer by conventional (non-molecular) approaches.

Parasites such as *Leishmania*, *Trypanosoma* and *Trichomonas* can be cultivated in liquid media to allow small numbers present in the original specimen (e.g. blood or vaginal secretions) to multiply and thus become easier to detect by microscopic examination. Parasites do not form colonies on solid media in the same way as bacteria and fungi.

Growth of viruses, *Chlamydia* and *Rickettsia* requires cell or tissue cultures

This is because these organisms are incapable of a free-living existence. Cell cultures used are human or animal cells adapted to growth in vitro that can be stored at –80°C until required. The specimen is introduced into the cell culture medium where growth / replication ultimately allows detection. Cell culture techniques are specialized, labour intensive and take time to produce an observed result (>1 week for cytomegalovirus). Therefore, alternative methods such as antigen and antibody detection (see below) and PCR-based approaches are important for diagnosis.

IDENTIFICATION OF MICROORGANISMS GROWN IN CULTURE

Bacteria are identified by simple characteristics and biochemical properties

A preliminary identification of many of the bacteria of medical importance has traditionally been made on the basis of the following few simple characteristics of the cells (Fig. 32.2):

- Gram reaction
- cell morphology (e.g. rod or coccus) and arrangement (e.g. pairs or chains)
- ability to grow under aerobic or anaerobic conditions
- growth requirements (simple or fastidious).

Further identification is made on the basis of biochemical properties such as:

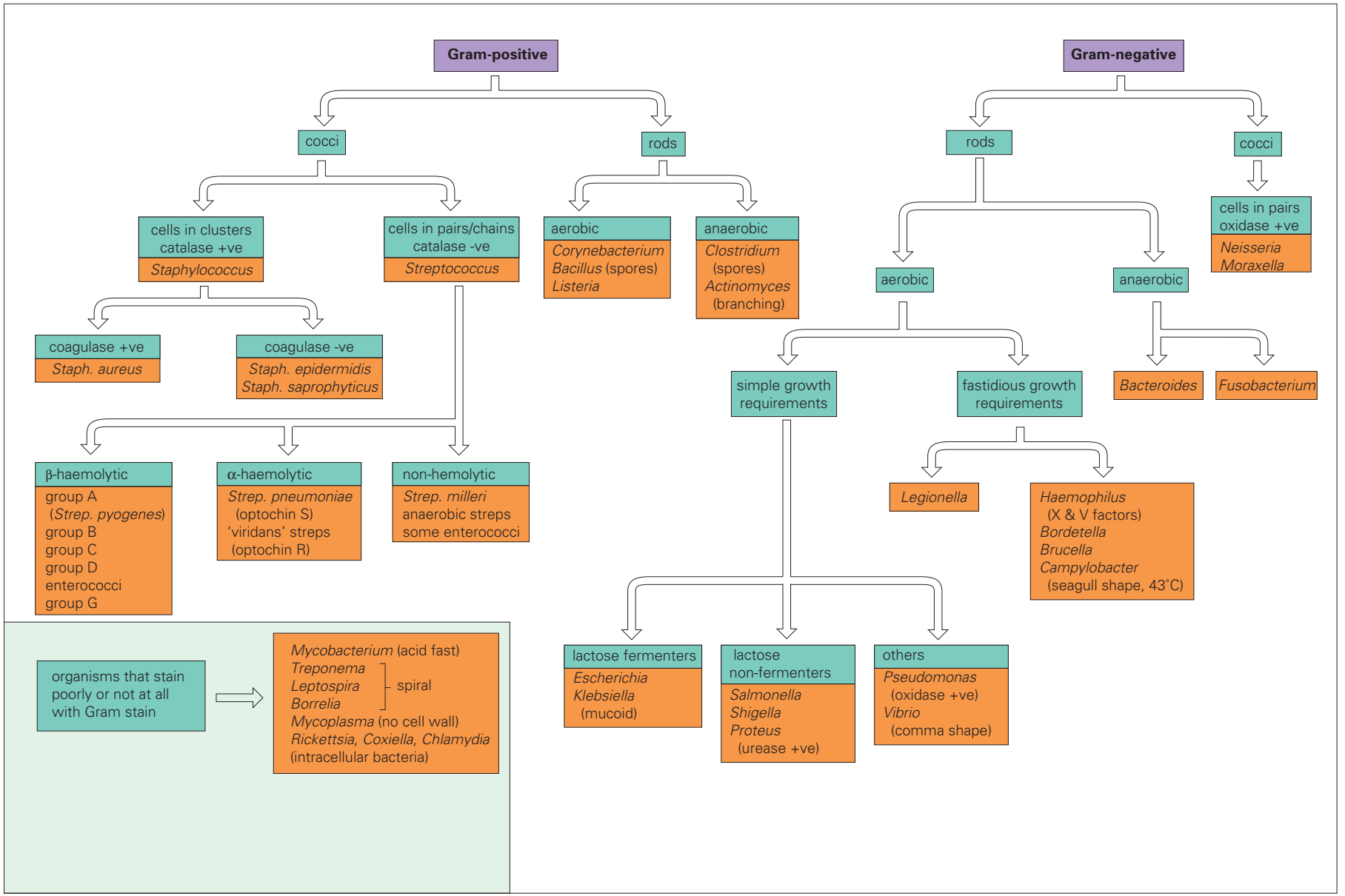


Figure 32.2 Identifying bacteria. The preliminary investigation of the bacteria of medical importance has traditionally been made on the basis of a few key characteristics (see text). Further identification may then be made on the basis of biochemical and serological tests.

- ability to produce enzymes that can be detected by simple tests
- ability to metabolize sugars oxidatively or fermentatively (aerobically or anaerobically)
- ability to use a range of substrates for growth (e.g. glucose, lactose, sucrose).

While these tests were historically done individually (e.g. in broth media containing the specifically required reagents), they are now commonly performed using commercial kits or automated systems which have the potential to give a rapid (e.g. 2–4 h) indication of pathogen identity based on biochemical profiles.

Some species are identified on the basis of their antigens by reacting cell suspensions with specific antisera.

Antibiotic susceptibility can be accurately determined only after the bacteria have been isolated in a pure culture

A variety of methods are available for antimicrobial susceptibility testing, including broth microdilution and automated instrument approaches. However, the most widely employed method assesses antibiotic susceptibility by applying filter paper disks, which contain different antibiotics, onto a lawn of the test organism which has been seeded onto an agar plate (i.e. disk diffusion). During overnight incubation, the organisms grow and multiply and the antibiotics diffuse out from the disks and inhibit growth around the disk. Therefore, after isolation of bacteria from a specimen, a further incubation period (overnight for disk diffusion testing) is required before antibiotic susceptibility results are available. Methods for antibiotic susceptibility tests are described in more detail in Chapter 34.

Fungi are identified by their colonial characteristics and cell morphology

Fungi are identified from colonies or pure cultures largely on the basis of colonial characteristics (e.g. colour) and the morphology of the individual cells viewed under the microscope (Fig. 32.3). Biochemical tests can be used for detailed identification of yeasts of medical importance. In general, fungi grow more slowly than bacteria, and final identification may take weeks.

Protozoa and helminths are identified by direct examination although newer molecular methods are also available

Many protozoa and parasites can be identified by direct examination of specimens without resorting to culture, and therefore the results can be obtained on the day of receipt of the specimen in the laboratory:

- Protozoa are traditionally identified on the basis of their morphological characteristics – different stages of the life cycle may be visible in different specimens from the same patient and at different stages in the disease (Fig. 32.4).
- Helminths are commonly identified by the macroscopic appearance of the worm (e.g. *Ascaris* or *Enterobius*) or by microscopic examination of specimens (e.g. faeces or urine) for eggs of, for example, schistosomes (see Ch. 23).

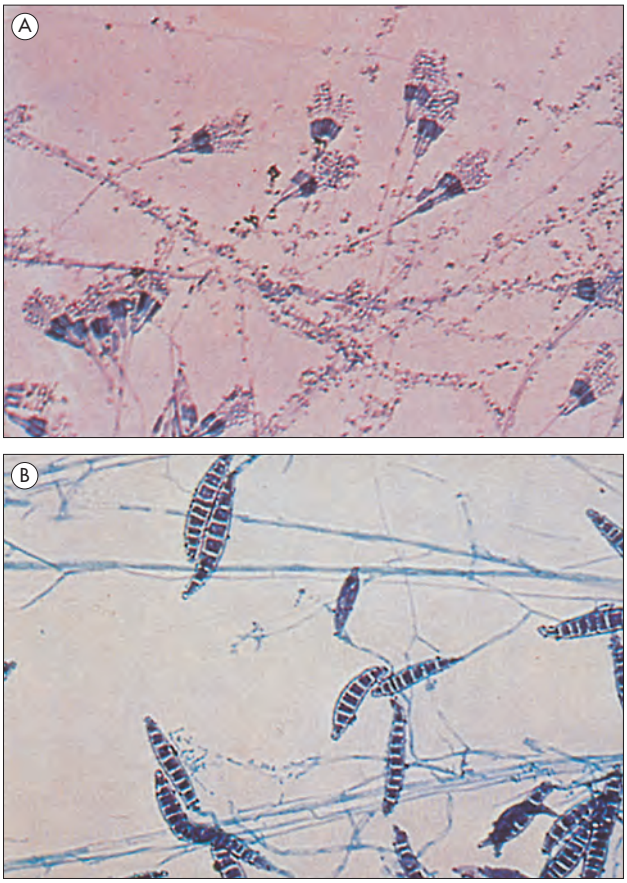


Figure 32.3 Fungi under the microscope. Fungi can be grown on agar culture media in the same way as bacteria, but most species grow much more slowly than bacteria and it may take weeks for a colony to form. Colonial characteristics (such as colour) are helpful in the identification of fungi, but confirmation depends upon microscopic examination of the hyphae and sporing structures. (A) Penicillium in a wet preparation showing the conidiophores and free conidia. (B) Macroconidia of *Microsporum canis* stained with lactophenol cotton blue.

A variety of molecular approaches have improved diagnosis over traditional methods.

Viruses are usually identified using serological and nucleic-acid-based tests

A number of viruses may now be identified by nucleic-acid-based tests (e.g. PCR; see below), as well as detection of viral antigens and the presence of specific antibodies in the patient's serum (see below).

Mass spectrometry heralds a novel diagnostic era

One of the most promising approaches to the identification of bacteria and fungi involves the use of mass spectrometry or, more specifically, matrix-assisted laser desorption–ionization time-of-flight mass spectrometry (MALDI-TOF). MALDI-TOF is being increasingly employed for the identification of microbial pathogens through analysis of their predominant mass spectral protein fingerprints, which can then be compared with established databases of known organisms.

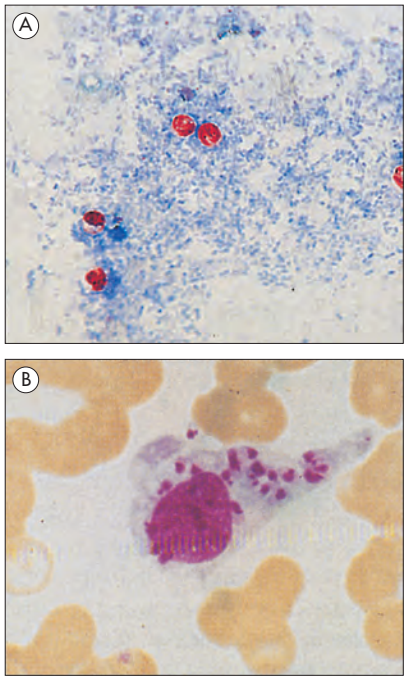


Figure 32.4 Although some parasites can be cultivated in the laboratory, identification is usually based on microscopic appearances in the specimen. (A) Acid-fast stain of *Cryptosporidium* in faeces. Like mycobacteria, this organism is able to retain the pink carbol fuchsin stain when challenged with acid alcohol. (B) *Leishmania donovani* (Donovan bodies) in a stained preparation from a specimen of bone marrow.

NON-CULTURAL TECHNIQUES FOR THE LABORATORY DIAGNOSIS OF INFECTION

Non-cultural techniques do not require microorganism multiplication before detection

Although medical microbiology has long been synonymous with the cultivation of microorganisms from patients' specimens, these techniques are labour intensive and slow to produce results (days rather than hours) because replication of organisms is a necessary, but rate-limiting, step. In addition, some microorganisms cannot be cultured in artificial media, and viable organisms may be difficult to recover from specimens of patients who have received antimicrobial therapy. Non-cultural techniques do not require multiplication of the microorganism before its detection. Techniques such as microscopy, detection of microbial antigens in specimens, DNA probes, and amplification of DNA by PCR may provide a rapid answer (e.g. minutes to hours).

Microscopy

Microscopy is an important first step in the examination of specimens

Microscopy plays a fundamental role in microbiology. Although microorganisms show a wide range in size (see Ch. 1) they are too small to be seen individually by the naked eye, and therefore a microscope is an essential tool in microbiology. The light microscope magnifies objects and therefore improves the resolving power of the naked eye from about 100 000 nm (0.1 mm) to 200 nm; although not routinely used in the clinical microbiology laboratory, the electron microscope can improve this to 0.1 to 1.0 nm.

Light microscopy

Bright field microscopy is used to examine specimens and cultures as wet or stained preparations

Wet preparations are used to demonstrate:

- blood cells and pathogens in fluid specimens such as urine, faeces or cerebrospinal fluid (CSF)
- cysts, eggs and parasites in faeces
- fungi in skin
- protozoa in blood and tissues.

Living organisms can be examined to detect motility.

Dyes are used to stain cells so that they can be seen more easily. Stains are usually applied to dried material that has been fixed (by heat or alcohol) onto the microscope slide. Samples from specimens themselves, or pure cultures, can be stained. The slide can then be viewed in the light microscope with an oil immersion lens, which improves the resolving power of the microscope.

The most important differential staining technique in bacteriology is the Gram stain

Differential staining procedures exploit the fact that cells with different properties stain differently and thus can be distinguished. Based on their reaction to Gram stain (Fig. 32.5), bacteria are divided into two broad groups:

- Gram positive (stain purple)
- Gram negative (stain pink).

This difference is related to differences in the structure of the cell walls of the two groups (see Ch. 2).

Acid-fast stains are used to detect mycobacteria

Some organisms, particularly mycobacteria, which have waxy cell walls, do not readily take up the Gram stain. To demonstrate their presence, special staining techniques are used which rely on the ability of such organisms to retain the stain in the presence of 'decolourizing' agents such as acid and alcohol. The Ziehl–Neelsen stain (see Fig. 20.24) is a classic differential staining procedure that uses heat to drive the fuchsin stain into the cells; mycobacteria stained with fuchsin withstand decolourization with acid and alcohol and are therefore known as 'acid-' and 'alcohol-fast' (typically abbreviated AFB; acid-fast bacteria), whereas other bacteria lose the stain after acid and alcohol treatment. Alternatively, many laboratories use a fluorescent auramine-rhodamine stain, which has a strong affinity for the waxy cell wall of mycobacteria, to demonstrate these organisms by fluorescence microscopy (Fig. 32.6).

Other staining techniques can be used to demonstrate particular features of cells

Examples of such features to aid identification include stains to detect bacterial spores, polymetaphosphate storage (volutin) granules in *Corynebacterium* spp. (dark spots in blue-green cells using Albert stain), and lipid storage granules in *Bacillus* spp. stained with Sudan black (black lipid against red cells)

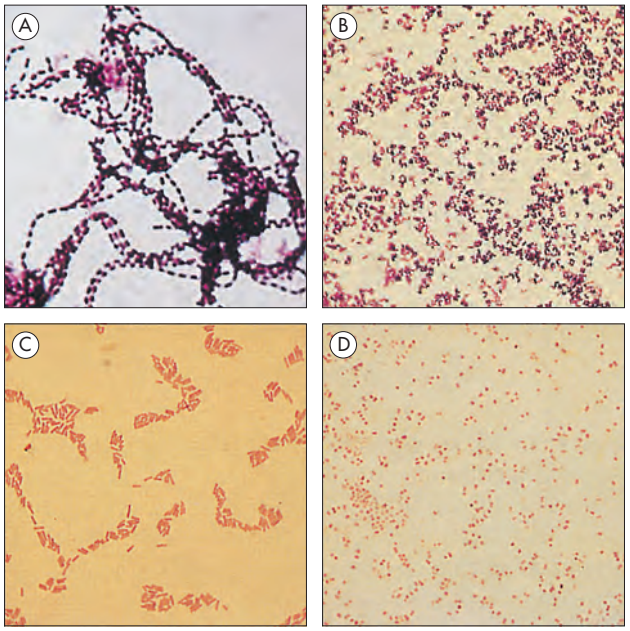


Figure 32.5 The Gram stain is the most important stain for studying bacteria. The combination of the violet dye (crystal violet) and iodine (acting as a mordant) binds to the cell wall. Gram-positive cells retain the stain when challenged with acetone and remain purple. Gram-negative cells lose the purple stain and appear colourless until stained with a pink counterstain (neutral red or safranin). Examination of Gram-stained films also allows the shape of the cells to be noted. Some examples are shown: (A) Gram-positive cocci in chains (*streptococci*); (B) Gram-positive rods (*Listeria*); (C) Gram-negative rods (*E. coli*); (D) Gram-negative cocci (*Neisseria*).

Dark field (dark ground) microscopy is useful for observing motility and thin cells such as spirochetes

The light microscope may be adapted by modifying the condenser so that the object appears brightly lit against a dark background. Living organisms can be examined by dark field microscopy and thus motility can be observed. The method is also used for visualizing very thin cells such as spirochetes because the light reflected from the surface of the cells makes them appear larger and therefore more easily visible than when examined by bright field microscopy (Fig. 32.7).

Phase contrast microscopy increases the contrast of an image

This technique enhances the very small differences in refractive index and density between living cells and the fluid in which they are suspended and therefore produces an image with a higher degree of contrast than that achieved by bright field microscopy.

Fluorescence microscopy is used for substances that are either naturally fluorescent or have been stained with fluorescent dyes

If light of one wavelength shines on a fluorescent object, it emits light of a different wavelength. Some biological substances are naturally fluorescent; others can be stained with fluorescent dyes and viewed in a microscope with an ultraviolet light source instead of white light (see Fig. 32.6).

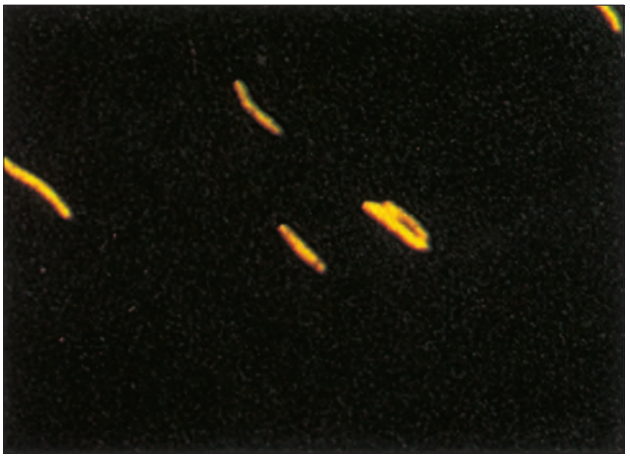


Figure 32.6 Fluorochrome stain of *Mycobacterium tuberculosis* with a mixture of auramine O and rhodamine B. Mycobacteria appear fluorescent under ultraviolet light. (Courtesy of D.K. Banerjee.)

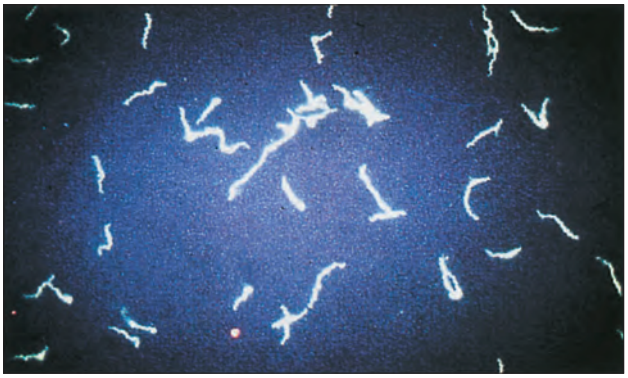


Figure 32.7 Spirochetes visualized by dark ground microscopy. Spirochetes and leptospires are much thinner than most bacterial cells (approximately 0.1 μm in diameter compared with 1 μm for *E. coli*), but they appear larger when viewed by dark ground illumination.

Fluorescence microscopy is widely used in microbiology and immunology and has been developed to detect microbial antigens in specimens and tissues by 'staining' with specific antibodies tagged with fluorescent dyes (immunofluorescence). The method can be made more sensitive or can be adapted to the detection of antibody by labelling a second antibody in an indirect test (Fig. 32.8).

Electron microscopy

Although not routinely used in the clinical laboratory, electron microscopy provides the ultimate in microbe visualization and can aid in microbe identification

The electron microscope uses a beam of electrons instead of light, and magnets are used to focus the beam instead of the lenses used in a light microscope. The whole system is operated under a high vacuum. Electron beams penetrate poorly, and a single microbial cell is too thick to be viewed directly. To overcome this, the specimen is fixed and mounted in plastic and cut into thin sections, which are examined individually. Electron-dense stains such as osmium tetroxide, uranyl acetate or glutaraldehyde are applied to the specimen to improve contrast. The electrons pass through the section

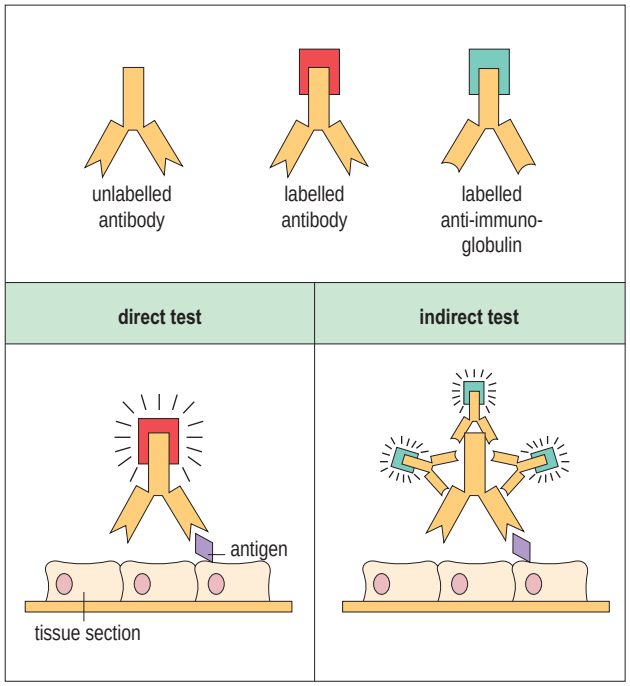


Figure 32.8 The fluorescent antibody test for detection and identification of microbial (or tissue) antigens or antibodies directed against them. In the direct test, antibody labelled with a fluorescent dye is applied to a tissue section bearing the antigen, unbound antibody is washed away, and the bound antibody showing the presence and location of the antigen is visualized by fluorescence microscopy. In the indirect test, antigen is revealed by successive treatments with unlabelled antigen-specific antibody and then fluorescent-labelled anti-immunoglobulin, which amplifies the signal (thus if the first antibody is human, the labelled antibody will be an anti-human Ig).

and produce an image on a fluorescent screen. Alternatively, electrons interact with the specimen at an angle to produce a view in three dimensions (scanning electron microscopy). In either case, images are photographed and enlarged so that the original specimen is magnified many thousand-fold.

Detection of microbial antigens in specimens

Detection of specific microbial antigens can be a more rapid method for detecting the presence of an organism than attempting to grow and identify the microbe. The methods include:

- those that detect antigens by their interaction with specific antibodies
- those that detect microbial toxins.

They are summarized in Box 32.3. Detection of microbial genes using DNA probes and PCR is discussed later in this chapter.

Specific antibody coated onto latex particles will react with the organism or its product, resulting in visible clumping

For example, common causative agents of bacterial meningitis (e.g. *Streptococcus pneumoniae* and *Haemophilus influenzae*) can be detected in CSF by mixing the specimen with specific antibody coated onto latex particles. If the antigen (i.e. the organism or its product) is present, the particles will clump together (Fig. 32.9). These tests give results within minutes of receipt of the specimen and are an especially useful diagnostic when the patient has received antibiotics and organisms may appear morphologically unidentifiable in the CSF and fail to grow in culture.

Immunoassay can be used to measure antigen concentration

Usually, an antibody is adsorbed for convenience to a solid phase and the amount of bound antigen is assessed using a second antibody labelled with an enzyme which acts on a substance to produce a colour or luminescence (Fig. 32.10) or a fluorescent probe.

- The test employing an enzyme label is referred to as an enzyme-linked immunosorbent assay (ELISA).
- The use of chemiluminescent or time-resolved fluorescent labels gives assays of very high sensitivity.

Box 32.3 Non-Cultural Techniques for Detection of Microbial Products

Non-specific techniques for detection of microbial products

Fatty acid end-products of metabolism of anaerobes can be detected in fluid specimens (e.g. pus, blood) by gas liquid chromatography.

Antigen detection

Detection of soluble carbohydrate antigens by agglutination of antibody-coated latex particles or red blood cells (see Fig. 32.9) e.g.:

- *Streptococcus pneumoniae* capsule in CSF and urine
- *Haemophilus influenzae* type b capsule in CSF and urine
- *Cryptococcus neoformans* capsule in CSF and urine
- *Strep. pyogenes* group antigen in throat swabs.

Detection of particular antigens by binding to antibodies labelled with:

- Enzymes (see Fig. 32.10), e.g. ELISA for hepatitis B, rotavirus
- Fluorescent molecules (see Fig. 32.8)

Toxin detection

Detection of exotoxins has historically involved tissue culture or injection into animals. Endotoxin from cell walls of Gram-negative bacteria has been historically detected by clotting of amoebocyte extracts of the horseshoe (*Limulus*) crab (*Limulus* lysate assay) but also by colorimetric and turbidimetric assays.

Identification of specific microbial products can be a more rapid method for detecting microorganisms than isolation and culture. The available techniques vary in their specificity. Toxins may be detected either by virtue of their antigenic properties or by demonstrating their action. Molecular methods such as PCR are used to assess the potential of microorganisms to produce specific microbial products (e.g. toxins) by detecting the presence of their respective genes.

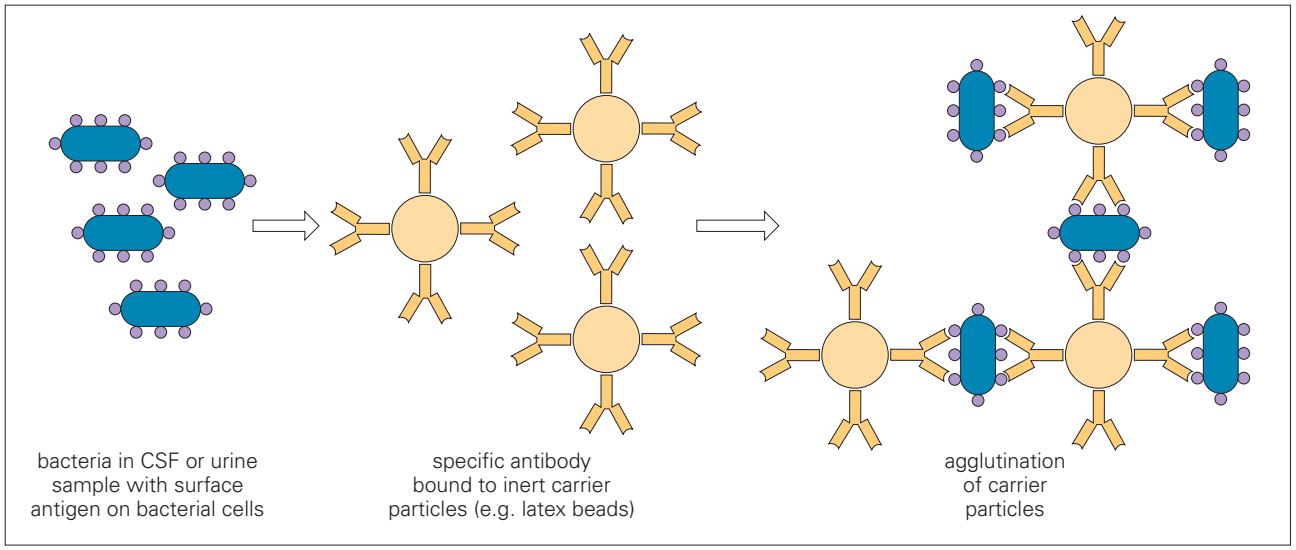


Figure 32.9 When a specimen of cerebrospinal fluid (CSF) containing bacteria (e.g. *Haemophilus influenzae*) is mixed with a suspension of latex particles coated with specific antibody (e.g. *H. influenzae* anticapsular antibodies), the interaction between antigen and antibody causes an immediate agglutination of particles, which is visible to the naked eye.

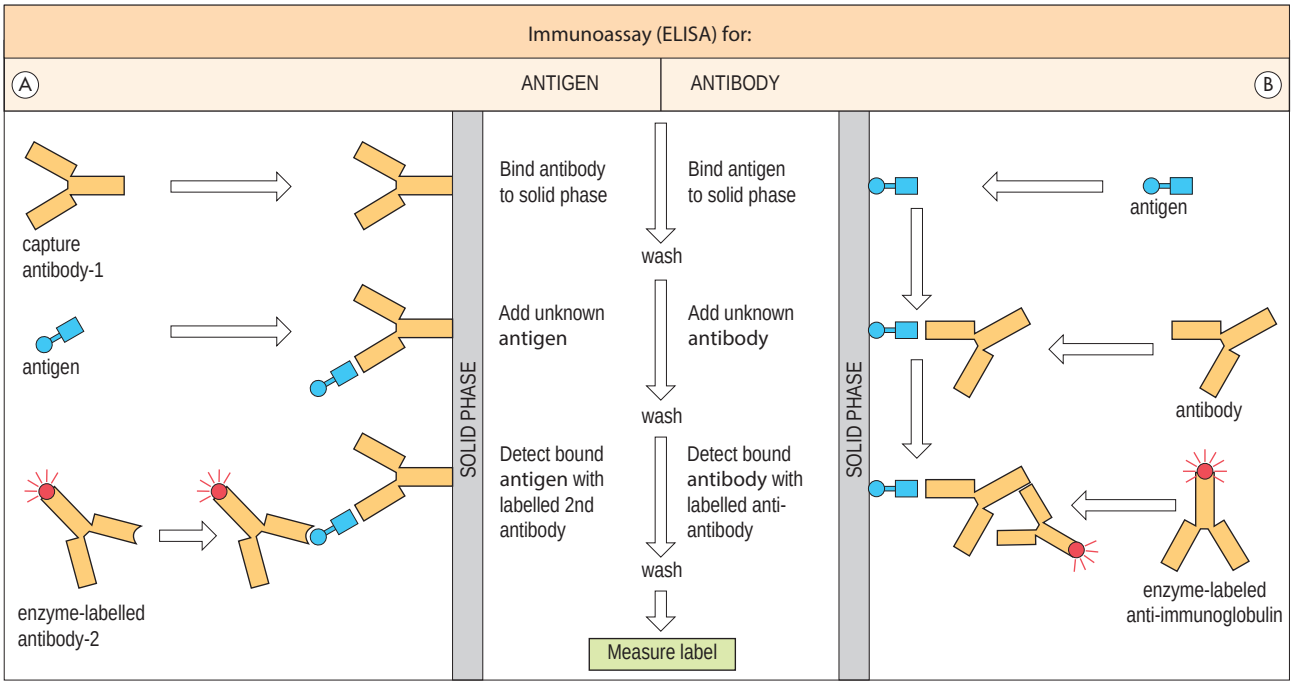


Figure 32.10 Immunoassay on a solid phase (enzyme-linked immunosorbent assay, ELISA). (A) The test antigen is added to solid phase antibody-1 and the occupancy measured by adding an enzyme-labelled second antibody and reading bound enzyme (e.g. peroxidase or alkaline phosphatase) through a colorimetric or luminometric reaction. In some cases, particularly with small antigens, unoccupied sites can be detected by adding a standard amount of labelled antigen. (B) Antibody to be tested is added to solid phase antigen and is detected by addition of an enzyme-labelled anti-immunoglobulin. (Compare with the indirect test in Fig. 32.8, which uses a fluorescent anti-immunoglobulin to detect bound antibody. Similarly, the label in the above assays can be a fluorescent probe rather than an enzyme.)

With modern techniques, multiple assays can be performed on single samples (see below).

Monoclonal antibodies can distinguish between species and between strains of the same species on the basis of antigenic differences

Hybridomas produced by the fusion of 'immortal' B-cell tumours and individual normal antibody-producing cells provide a copious source of monoclonal antibodies, all with identical specificities for their relevant antigen. These monoclonal antibodies can be used as diagnostic tools. In direct ELISA (see above), enzyme-conjugated monoclonal antibodies are frequently employed to detect antigens in specimens from patients. Rotaviruses, HIV, hepatitis B virus, herpesvirus and respiratory syncytial virus (RSV) can all be detected directly with monoclonal antibodies in ELISAs. *Chlamydia trachomatis*

infection can be diagnosed within a few hours by a direct fluorescent antibody test employing a monoclonal antibody labelled with fluorescein (see Fig. 32.8; Ch. 22).

Detection of microbes by probing for their genes

Organisms can be identified using nucleic acids probes that match specific gene sequences

A gene probe is a nucleic acid molecule which, when in a single-stranded state and labeled, can be used to detect a complementary sequence by hybridizing to it. The nucleic acid probe is labelled with a fluorescent dye and hybridized to the extracted microbial nucleic acid that has been denatured (to make single-stranded) and immobilized onto a nitrocellulose membrane. The labelled probe can be visualized by chemiluminescent methods, depending on the label used. Such 'blotting' techniques are time consuming and prone to contamination in routine use and have now been superseded by polymerase chain reaction (PCR) methods.

PCR can be used to amplify a specific DNA sequence to produce millions of copies within a few hours

PCR has the capability to rapidly detect a single gene target – that is, a single organism in the sample being analysed (Fig. 32.11) – within 1–3 hours, depending on the type of technology used. It is particularly useful for diagnostic work with pathogens (e.g. viruses) that are difficult to culture. Earlier methods required the PCR products to be analysed on agarose gels and, for diagnostic certainty, some form of nucleic acid probe technique to unequivocally identify the target. This added a considerable amount of time to the analysis.

For diagnostic purposes, traditional PCR has been largely replaced by real-time PCR

Real-time PCR uses the same basic reagents and techniques as the original PCR method, but with the addition of fluorescently labelled sequence-specific probes. The TaqMan is one of the most widely used types of probe, because it is relatively easy to design and it demonstrates inherently low levels of background fluorescence (Fig. 32.12A). The nucleotide probe sequence has two fluorescent molecules attached: at the 5′ end a reporter dye, and at the 3′ end a quencher molecule. When the probe is intact no signal is detected owing to reporter–quencher proximity; any fluorescence given off by the reporter is immediately absorbed, a feature contributing to the low background of the reaction. During the PCR the polymerase removes the 5′ nucleotides to which the reporter dye is attached. The reporter is now free to move away from the quencher and its fluorescence can be detected. This 5′–3′ exonuclease activity is an inherent proof-reading ability of the enzyme, removing unexpected double-stranded regions of DNA and restricting the probe to register fluorescence only when the PCR is working. The result is the ability to monitor the amplification process in real time (hence the name). The amount of fluorescence detected during the

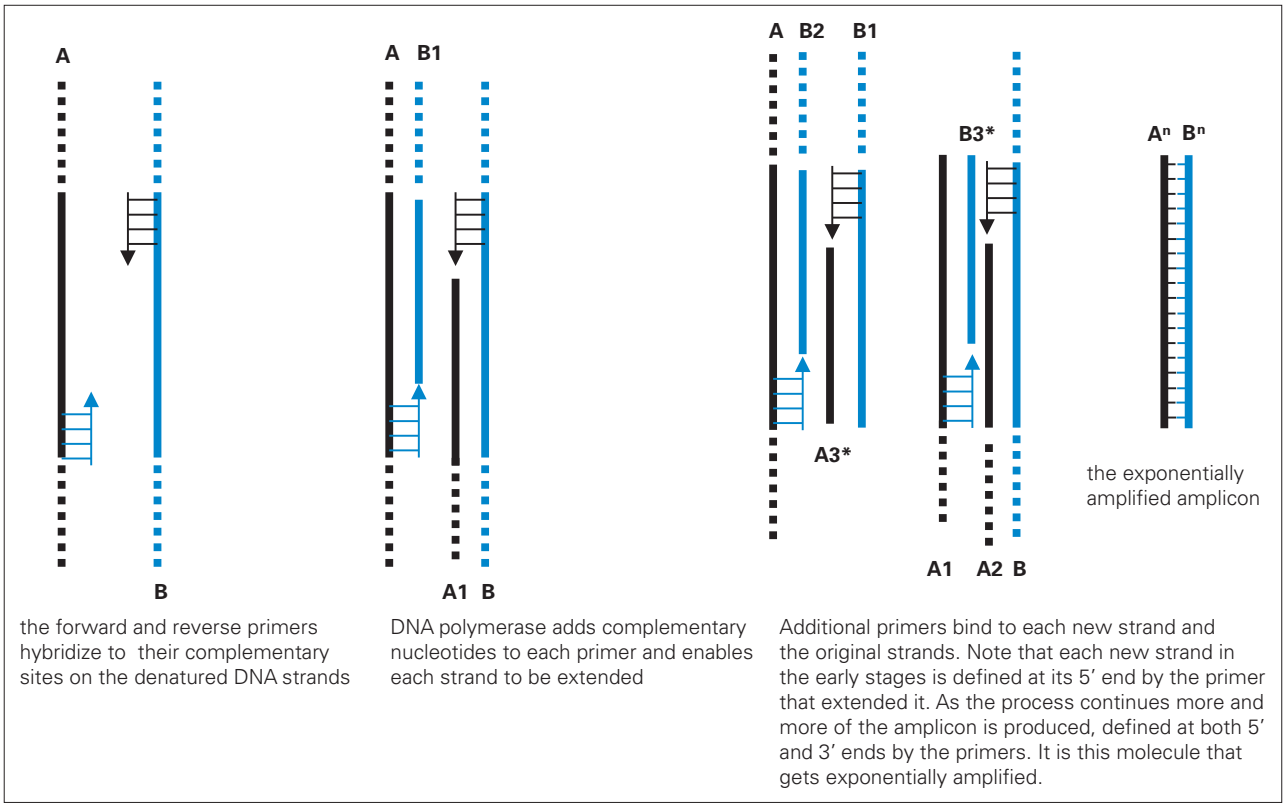


Figure 32.11 Conventional polymerase chain reaction. Short oligonucleotide (ca. 20 bases of DNA) hybridize to complementary sequences on each DNA strand to be amplified. The strands are separated by denaturation enabling the primers to bind, which are extended by the thermostable polymerase adding complementary nucleotides by repeating the thermal cycling rounds of denaturation, annealing and extension 30–60 times. The original strands to be amplified are shown in the figure as A and B with subsequent amplified copies numbered. After rounds of amplification the desired fragment of DNA (amplicon) is copied exponentially.

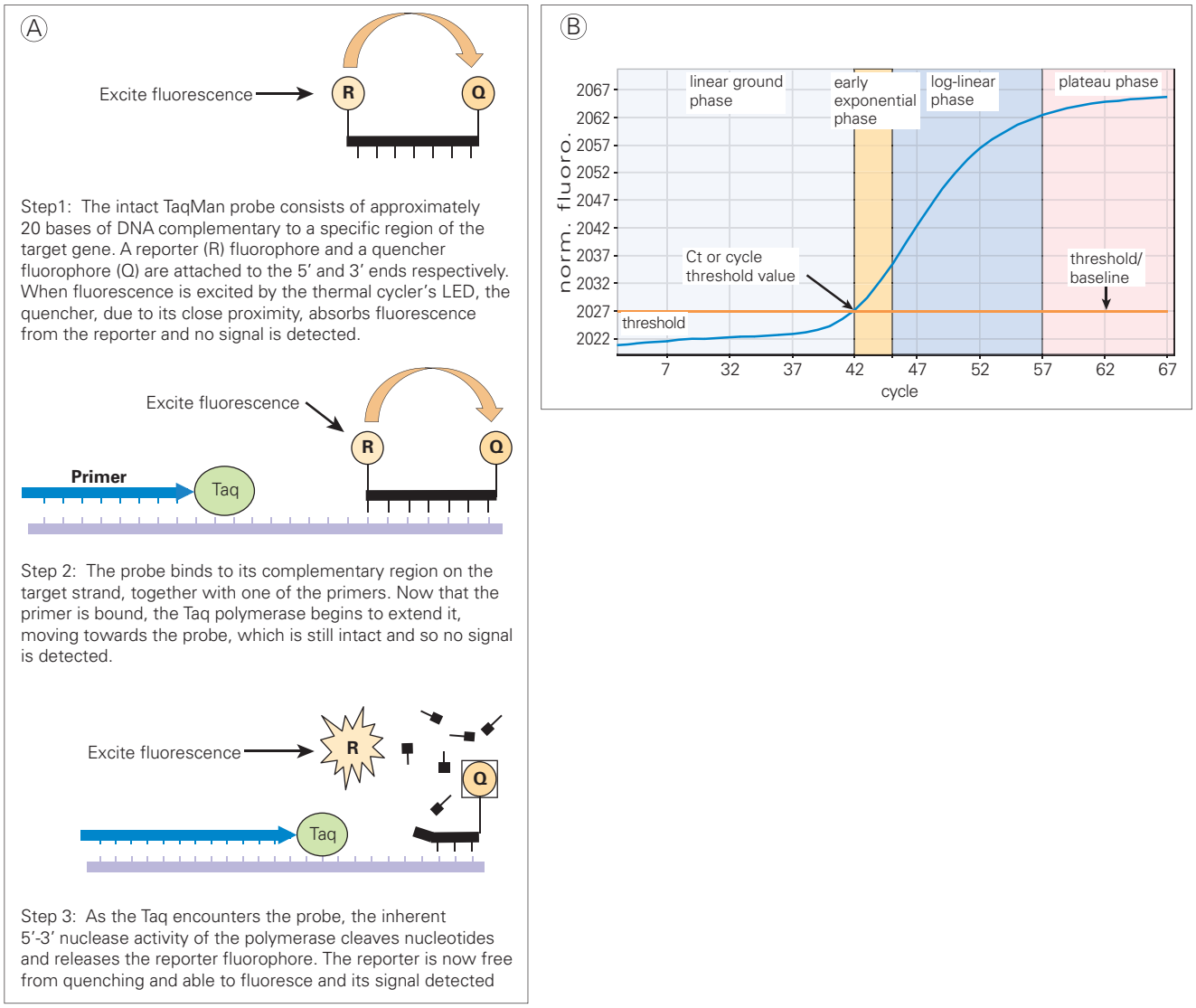


Figure 32.12 Real-time PCR. (A) The steps involved in real-time detection of PCR products by TaqMan probes. (B) A typical S-shaped real-time PCR amplification curve. The number of cycles in the reaction is shown on the *X*-axis and the levels of fluorescence, derived from the TaqMan probe, representing the accumulating amplicon is shown on the *Y*-axis. The threshold or baseline value can be set by the user. The *C*t value represents the PCR cycle number at which the exponential phase of amplification begins.

reaction is directly proportional to the amount of amplicon produced (Fig. 32.12B). By including a set of prequantified DNA standards, co-amplified during the reaction, the copy number of nucleic acid in the original sample can be estimated. Because there is no need for post-PCR analysis, the reaction tubes do not need to be opened, which reduces the potential for contamination and provides results in as little as 1 hour. If the pathogen's nucleic acid is in the form of RNA, it must first be converted into complementary DNA (cDNA) before it can be amplified. This is achieved in an enzymatic step using a reverse transcriptase prior to the PCR (RT-PCR).

More than one pathogen can be detected in a single reaction – multiplexing

The PCR can be multiplexed by adding primers and probes for more than one pathogen, further reducing costs and time to diagnosis. Multiplexing is an extremely useful approach to diagnostic PCR, which apart from its economic benefits, allows tests to be grouped into disease syndromes, such as respiratory or sexually acquired infections, making the requesting and diagnostic procedure much more efficient. The syndromic approach can also provide a diagnosis for multiple infections and pathogens not originally requested by the clinician. For example analysis of a swab from the buttock region of a patient attending a sexual health clinic, with a likely diagnosis of HSV, could reveal herpes zoster (shingles), not considered as part of the original differential diagnosis. Current technical limitations to this approach relate to the number of available fluorescent dyes currently detecting up to four targets while a typical viral respiratory panel can consist of up to 12 pathogens. Approaches to higher levels of multiplexing are currently in development.

Advances in molecular diagnoses for infectious diseases: sequencing-based techniques

Dideoxy chain terminator sequencing

This method was developed in the 1970s by Fredrick Sanger and colleagues (Sanger sequencing) and although newer techniques continue to be developed, it is still the cornerstone of routine sequencing technology. The reaction is similar to PCR; a DNA polymerase is used to make copies of the nucleic acid to be sequenced. However, in addition to the four standard deoxynucleotide triphosphate bases (dNTPs), four dideoxynucleotide (ddNTPs) base analogues are also used (Fig. 32.13A and B). The ddNTPs lack the 3′–OH group on ribose of the dNTPs, necessary for extending the DNA molecule. When one of these molecules is incorporated into the growing chain, extension is terminated, hence the name. The four ddNTPs are each labelled with different fluorescent dyes emitting light at different wavelengths. The fragments are separated according to their lengths by electrophoresis. A laser excites the different fluorescent dyes, the resulting different wavelengths emitted are detected, and the sequence of the original nucleic acid is read as a series of fluorescent peaks.

Second-generation 'sequencing by synthesis'

The need for greater speed in generating data and reducing costs has led to significant improvements in sequencing methods. One of the most widely used (the so-called next or second generation) is sequencing by synthesis. The basic methodology is similar to dideoxy sequencing but there are differences in how the reactions are carried out. The method employs a flow cell, which simplifies the addition of new reagents and the removal of previous reactants at each cycle of the process. A library of the microbe's DNA is created by randomly cutting the genome into short (50–80 bases) fragments either enzymatically or mechanically and attaching (ligating) adapters of known DNA sequence to both ends of the fragments. The adapters are complementary to primers already attached to the surfaces of the flow cell channels. As illustrated in Fig. 32.14, the fragments are denatured and the single-stranded products bound to the complementary primers. The library is amplified for sequencing, resulting in the formation of double-stranded bridge structures which are denatured to form templates for further amplification, eventually producing millions of cluster copies in the flow cell channels. The design of the fluorescence detection allows

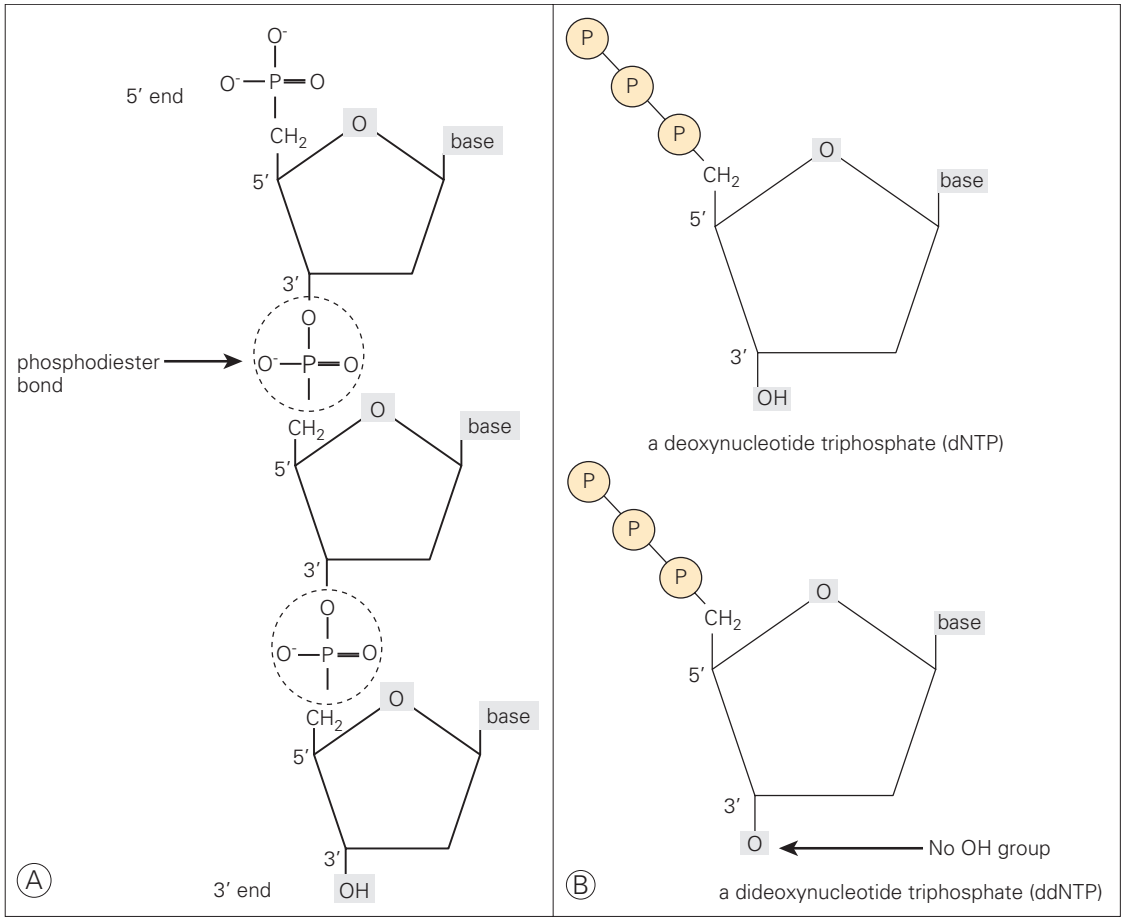


Figure 32.13 Dideoxy chain terminator sequencing. (A) Polymerization occurs in a 5′ to 3′ direction via the formation of phosphodiester bonds. (B) Dideoxynucleotides lack reactive –OH on 3′ carbon. The 5′–C can form a phosphodiester bond with the previous nucleotide in the chain, but the 3′–C cannot form a bond with the incoming dNTP (no OH group). Addition of a ddNTP during DNA replication stops chain elongation.

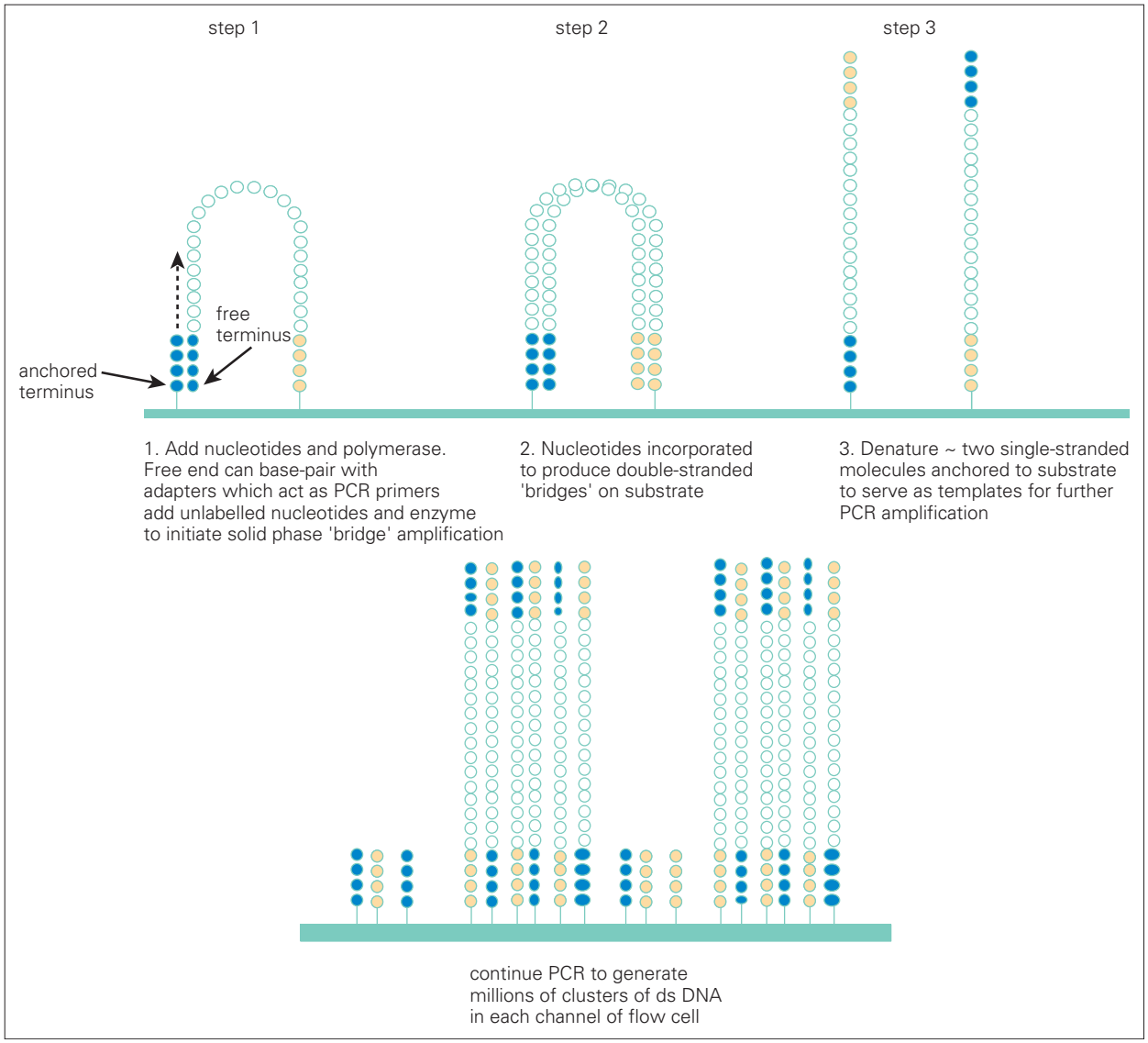


Figure 32.14 The steps involved in second-generation sequencing by synthesis. PCR, polymerase chain reaction.

sequences to be read from each cluster in each of the channels simultaneously in such a way that a whole bacterial genome can be read in a single run in less than one day. This technology requires large amounts of computing power to assemble the sequence fragments as they overlap to produce the final contiguous sequence, often by comparison with a known reference sequence (see Ch. 37).

Single-molecule sequencing – the third generation

Further developments promise even more rapid and cost-effective data acquisition. These methods focus on sequencing a single molecule and do not need PCR amplification of the pathogen's genome. One of the most promising of these techniques is based on the movement of the molecule to be sequenced through a protein nanopore. Each base has its own individual 'electronic signature' and disturbs the current flowing across the pore in a specific manner, allowing each base to be uniquely identified as it passes through the pore. This approach is capable of producing longer stretches of sequence more amenable to assembly and analysis.

Amplification-based techniques and point of care (POC) tests

By definition a POC test requires a specimen to be tested at or near the patient with the results available instantly or within a very short time to provide an immediate diagnosis and enable rapid and appropriate treatment. As noted earlier, traditional culture-based microbiological methods can take at least 24 hours for a result. In a molecular laboratory, the test itself can be completed in 1–2 hours allowing rapid decisions about the management of the patient, with many of the bottlenecks removed because the patient is next to the testing device. This dictates that the equipment used should be small and portable, such that it could be used conveniently in a consulting room or at the bedside. This is particularly important in infectious disease management, where empirical

treatment can lead to inappropriate antibiotic use and the development of microbial resistance. In addition, infection control issues involving isolating infectious patients can be made quickly. Despite considerable interest in POC testing, there are difficulties related to the need for extracting the sample's nucleic acid and POC analysis by PCR which have been addressed by some manufacturers using cassette-based or microfluidic approaches. However, such instrumentation commonly tests only one sample at a time compared with the multiplex capabilities of current real-time applications. In some instances the size of the equipment may require it to be sited in laboratories and these have been termed 'near-patient tests' (e.g. a near-POC respiratory panel with 17 viral targets plus *Bordetella pertussis, Chlamydophila pneumoniae* and *Mycoplasma pneumoniae*). More advanced systems are in development to reduce both equipment size and reaction times using microfluidics and nanotechnology where analytes, in very small volumes, interact rapidly to changes in temperatures, speeding up the PCR. For example, newly developed approaches to single-molecule sequencing employ highly portable devices that may be as small as the size of a USB flash drive.

Personalized molecular medicine and infectious disease

Personalized medicine is not new; in fact clinicians have been attempting to tailor treatment to individual patients needs for centuries. The difference now is the amount of data made available by dramatic technological advances that are providing clearer insights into the molecular basis of disease. The original concept of personalized medicine relied on interrogating the patient's genetic information to determine the effectiveness of a particular therapeutic regimen. This typically involved the management of genetic disease or chronic disorders such as the use of molecular cancer biomarkers in disease screening to predict treatment efficacy or toxicity in an individual. Employing a combination of molecular techniques and infectious disease diagnosis to achieve personalized medicine is an increasing reality owing to improvements in PCR, sequencing, and the range of clinically useful biomarkers (e.g. immune response, host susceptibility to infection, hypersensitivity to antimicrobial drug treatment). Personalized medicine, although often not considered as such, has been a key driver for the management of HIV / AIDS since the early 2000s. Sequencing data has been used to guide antiviral treatment based on viral subtype and sequence comparison with known antiretroviral resistance mutations. In the absence of genotyping, inappropriate therapy may be initiated, viral suppression is unlikely, and antiretroviral resistance may develop. The development of diagnostic tests in parallel with targeted therapeutics has led to the concept of theranostics. Linking a drug identified through personalized medicine with a companion diagnostic test helps to ensure that the patient will benefit from the drug and determine its long-term usefulness by monitoring the therapy in real time. Other benefits from this approach potentially relate to drug development and comprehensive genomic data applied in selecting candidates for clinical trials, which will hopefully reduce the outcomes of potentially harmful side effects and the time taken to demonstrate safety and efficacy.

ANTIBODY DETECTION METHODS FOR THE DIAGNOSIS OF INFECTION

Serological tests (the study of antigen–antibody interactions) are used:

- to diagnose infections
- to identify microorganisms (see above)
- to type blood for blood banks and tissues for transplantation.

Diagnoses based on detecting antibodies in patients' sera are retrospective

The major disadvantage of a diagnosis based on the detection of antibodies in a patient's serum is that it is retrospective, as 2–4 weeks must elapse before IgG antibodies produced in response to the infection are detectable. What is more, a positive result indicates only that the patient has come into contact with the infection at some time in the past. However, IgM antibodies are detected earlier in the infection (7–10 days) and are usually indicative of active, as opposed to past, infection. It may also help to show that the patient has 'seroconverted' by demonstrating a fourfold or greater rise in antibody titre between sera collected in the acute and the convalescent phases of the disease.

Common serological tests used in the laboratory to diagnose infection

Solid-phase immunoassays can be used to estimate antibody in a given sample

These assays have been described previously (see Fig. 32.10). The amount of antibody binding to the solid-phase antigen is a measure of the antibody content of the original sample, and can be detected by adding a second antibody conjugated with a fluorochrome or an enzyme (e.g. phosphatase or peroxidase) that produces a colour or luminescent reaction with a given substrate.

Modern techniques permit the simultaneous assay of several analytes in the same sample. For example, multiplexed arrays of beads core labelled with different fluorochromes and coated with antibodies to cytokines are being increasing used.

ASSESSMENT OF HOST DEFENCE SYSTEMS

Blood samples may be checked for complement components

The complement system is a complex set of blood proteins that work to respond to infection (e.g. inflammation and immune response). Tests for specific complement proteins in the blood provide an indication of the robustness of host immune defence systems.

Phagocytic activity is a key element of proper immune function

The ability of neutrophils to become phagocytic and to concurrently release reactive oxygen (i.e. oxygen burst) has traditionally been assayed by the nitroblue tetrazolium (NBT) test. When yellow NBT dye is added to blood, it forms complexes with heparin or fibrinogen in the sample. These complexes are then phagocytosed by neutrophils that have been activated by the addition of exogenous endotoxin. The dye complex is taken into the stimulated neutrophils and substitutes for oxygen by acting as a substrate for the reduction

process, forming blue, insoluble formazan (Fig. 32.15). More recently, flow cytometry (see below) has been employed to assess oxidative burst using the dye dihydrorhodamine 123 (i.e. the DHR test).

Lymphocytes

The development of T-effector cells to an antigen can often be revealed by intradermal challenge with that antigen. Such an intradermal challenge usually gives rise to erythema and induration, peaking at around 48 h (Fig. 32.16). This time course has led to the reaction being described as 'delayed-type hypersensitivity', and is the basis of the Mantoux skin test for tuberculosis (see Ch. 20).

Overall responsiveness of the T-cell population can be probed by flow cytometry using cells which have incorporated specific modified nucleosides. These cells attract and form a bond with a dye label. By adding specific antibodies, flow cytometry can detect responsive T-cell populations by their fluorescent signal. The technique can also provide information regarding overall T-cell health (e.g. apoptosis). The fluorescence-activated cell sorter (FACS) separates subpopulations delineated by their cytofluorimetric parameters measuring multiple fluorescent labels simultaneously, allowing both the surface phenotype of the cells and its function to be assessed (Fig. 32.17).

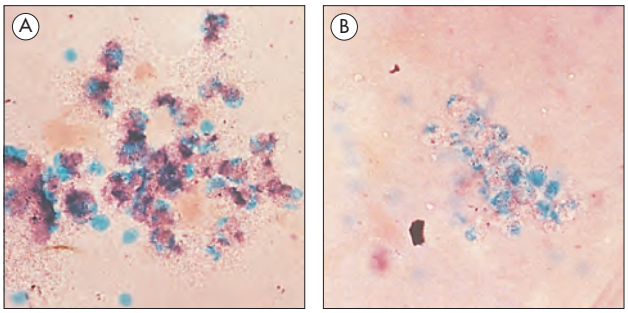


Figure 32.15 Nitroblue tetrazolium (NBT) test. In normal polymorphs and monocytes, reactive oxygen intermediates (ROIs) are activated by phagocytosis, and yellow NBT is converted to purple-blue formazan (A). Patients with chronic granulomatous disease (CGD) cannot form ROIs and so the dye stays yellow (B). (Courtesy of A.R. Hayward.)

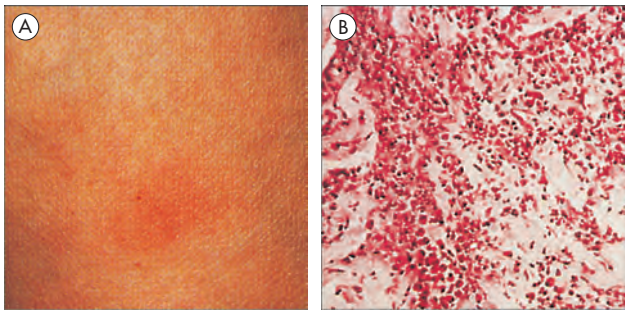


Figure 32.16 Tuberculin-type delayed sensitivity. The dermal response to antigens of leprosy bacillus in a sensitive subject (the Fernandez reaction) is characterized by (A) red induration maximal at 48–72 h and (B) dense infiltration of the injection site with lymphocytes and macrophages. (H&E, ×80.)

Individual cells secreting antibodies or cytokines can be counted by the ELISPOT technique or by flow cytometry

The lymphocytes are incubated on a membrane impregnated with antigen, for antibody detection or anticytokine monoclonal antibody to detect cytokines (Fig. 32.18). The secreted product is identified by conventional ELISA-type readout.

An alternative approach utilizes inhibitors of cytokine export (e.g. metabolic poisons such as brefeldin A that trap cytokines within the endoplasmic reticulum) to block cytokine secretion so that these molecules can be immunostained after cellular permeabilization. Cells can then be stained for intracellular cytokines (intracellular cytokine staining; ICS)

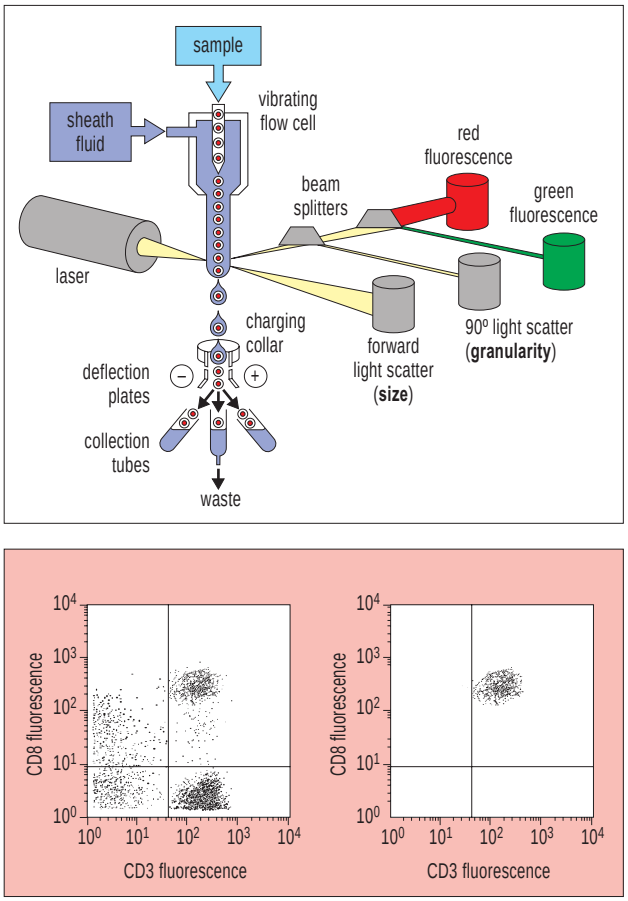


Figure 32.17 Flow cytofluorimetry. Cells in the sample are stained with specific fluorescent reagents to detect surface molecules and then stream one at a time past a laser. Each cell is measured for size (forward light scatter) and granularity (90° light scatter), as well as for red and green fluorescence, to detect two different peripheral blood surface markers – in this instance, CD8 and CD3 respectively (but modern instruments can detect many more different fluorophores). In a cell sorter, the flow chamber vibrates the cell stream, causing it to break into droplets which are then charged according to an arbitrary cut-off 'gate' and can then be steered by deflection plates under computer control to collect different cell populations according to the parameters measured. In the example shown in the left panel, four populations can be seen; after appropriate gating, the CD8 population in the right lower quadrant can be selected; reanalysis gives the plot seen in the lower left panel. (Redrawn from Male D., Brostoff J., Roth D.B., Roitt I. *Immunology*, 7th edition, 2006. Mosby Elsevier, with permission.)

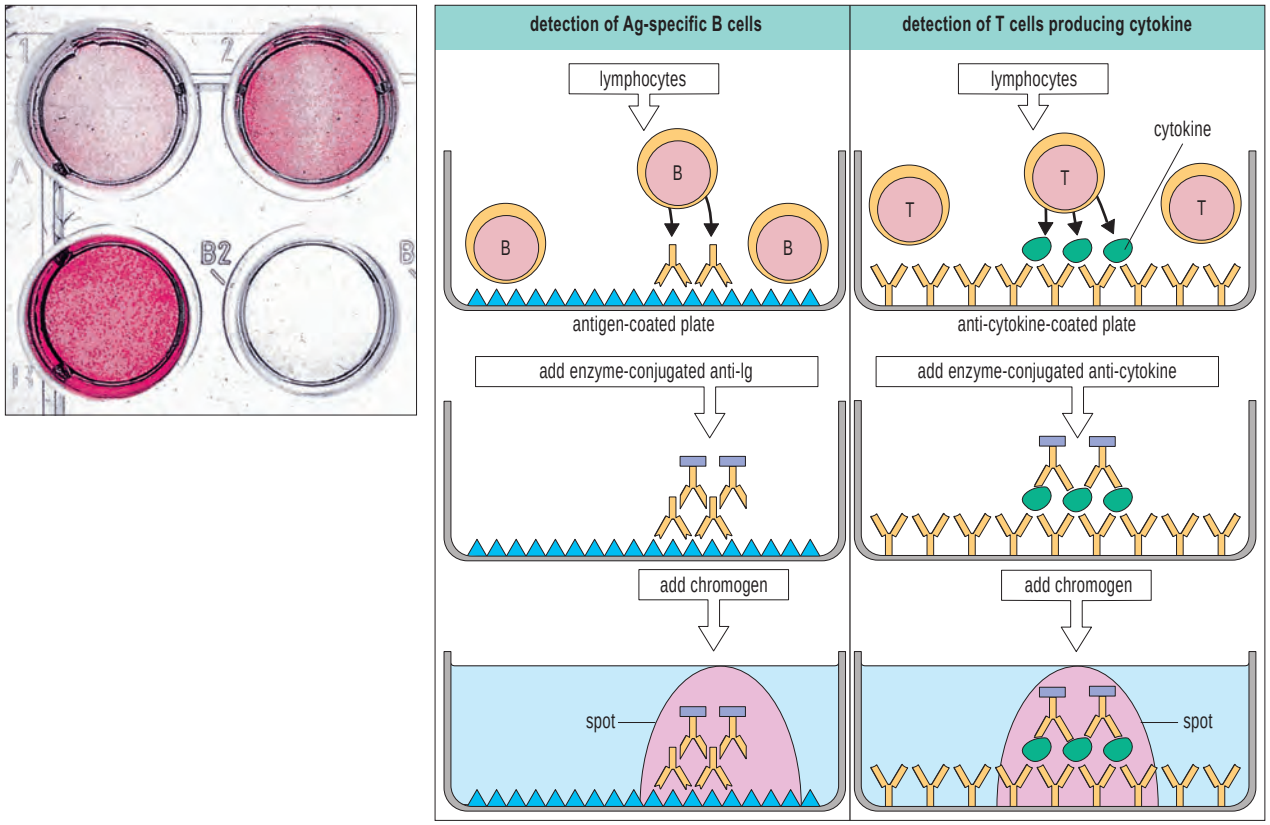


Figure 32.18 The ELISPOT assay for counting lymphocytes secreting antibodies or cytokines. The secreted products (antibodies from B cells and cytokines from T cells) are bound by the solid phase capture molecules immediately beneath the cell and revealed by a colour reagent as a spot corresponding with the secreting cell. Wells with different numbers of ELISPOTs are shown top left. (Redrawn from Male D., Brostoff J., Roth D.B., Roitt I. *Immunology*, 7th edition, 2006. Mosby Elsevier, with permission.)

using specific antibodies, followed by flow cytometric analysis, as described earlier. Another approach makes use of bispecific antibodies that can simultaneously bind to a T-cell surface marker (such as CD4) while the other Fab arm is specific for a cytokine. The cytokines are captured as they are secreted from cells but, due to the bispecific nature of the antibody, the cytokine becomes stably attached to the cell making it and can then be detected with a different cytokine-specific antibody conjugated to a fluorochrome, again using a flow cytometer.

The ability of cytotoxic T cells to attack targets can also be assayed by flow cytometry

The ability of cytotoxic T cells to attack targets such as virally infected cells has been historically accomplished by prelabelling the target with a radioisotope such as ^{51}Cr, and then looking for isotope release into the supernatant from damaged cells. More recently, a variety of more sensitive assays have been developed for use with flow cytometry and immunofluorescent dyes.

PUTTING IT ALL TOGETHER: DETECTION, DIAGNOSIS AND EPIDEMIOLOGY

As seen more fully in Chapter 33, understanding the epidemiology of an infection can help to define the correct strategies for control at the population level. However, this understanding, and decisions about control, both depend heavily upon the ability to recognize outbreaks of disease, to follow their progress and to identify the causative organism concerned. Detection and diagnosis are therefore key activities here, as they are for treatment of infection at the level of the individual.

Descriptive epidemiology involves asking questions about an outbreak of disease that will help to identify the pathogen and the source of infection. It is important to have a *case definition*, which includes the symptoms of the disease as well as details of the individuals involved and the timing of events. Analysis of these data should make it possible to say where and how the outbreak has arisen, who is at risk and what treatment is necessary to control further infection (Box 32.4). Measures used may involve antibiotic treatment of those immediately affected, or vaccination if a large number are at risk (e.g. meningitis outbreaks in university students). For sexually transmitted disease (see above) an important element of detection is to establish contact patterns, or mixing matrices, so that individuals who may acquire an infection can be treated and further transmission prevented.

This approach to outbreaks of known or new disease follows their chance discovery as a result of clinical observations, exemplified by the discovery of AIDS in 1981 through the increased occurrence of *Pneumocystis carinii* (now known as

Box 32.4 Lessons in Microbiology

Importance of DNA sequencing in understanding the Ebola virus outbreak

Nanopore sequencing was used in the 2015–2016 Ebola outbreak in West Africa. By combining data with those of a second group working in Sierra Leone, evidence of frequent transmissions across the border with Guinea was demonstrated. Importantly, data were released regularly throughout the investigation and enabled complicated clinical diagnoses to be discussed with other workers in the study working in different areas of the epidemic. Although the epidemic was officially declared over on 14 January 2016, hours later a new case was confirmed in Sierra Leone. Genomic surveillance is crucial in understanding the sources of new outbreaks, by determining links to previously infected individuals and eliminating any zoonotic connections (also see Ch. 37).

Pneumocystis jirovecii) infection and of Kaposi's sarcoma in MSM (men who have sex with men) males. A more systematic approach to detection relies on a regular notification system – a surveillance system that routinely records episodes of a number of legally notifiable diseases. Such systems operate nationally through government or federal health organizations, as well as internationally through the World Health Organization. Regular monitoring of this kind makes it easier to identify outbreaks, because it provides the baseline against which 'the occurrence of cases in excess of expectancy' (the definition of an epidemic) can be measured.

Once outbreaks of infectious disease have been detected, the pathogen concerned can be identified by conventional diagnostic procedures, to ensure that the appropriate antibiotic or vaccination is given.

KEY FACTS

- Microbiological confirmation of a clinical diagnosis of infection depends upon the collection of high-quality specimens and their rapid dispatch to the laboratory with all the necessary supporting information.
- Laboratory tests detect microorganisms or their products or evidence of a patient's immune response to infection.
- While coming from different perspectives, culture and serological methods are important, cooperative approaches to the identification of clinically important pathogens.
- Newer molecular techniques (e.g. involving PCR and mass spectroscopy) are increasingly used to detect pathogens rapidly; however, antimicrobial susceptibility is most accurately determined and appropriate treatment information provided by isolating organisms in pure culture.
- Growth of bacteria requires at least 18 h (isolation of viruses and of fungi may take much longer); therefore standard culture results cannot be expected in less than 24 h although newer diagnostic tests are more rapid.
- Interpretation of culture results depends upon the source of the specimen. From sites that are normally sterile, any isolated organism is significant. From sites colonized by commensal flora, isolating and identifying the pathogen can be more difficult.
- Good communication between the clinician and the microbiologist is extremely important.

33 Epidemiology and control of infectious diseases

Introduction

Epidemiology is defined as 'The study of the distribution and determinants of health-related states or events in specified populations and the application of this study to the control of health problems' (Porta, M, 2016, *A Dictionary of Epidemiology*).

In epidemiology, we are concerned with populations rather than individuals. What we want to know of a disease in a population is: who, where, when and why. Hepatitis A outbreaks are often associated with institutions, restaurants and specific food. It is therefore important to determine who – which individuals ate potato salad, where – in a nursing home, when – 1 February 2010 – developed hepatitis A, and why – an infectious person who had prepared the food was identified.

The field of epidemiology is divided into observational and interventional epidemiology.

Observational studies are either descriptive, describing the frequency and patterns (who, where, when) of a disease in the population, or analytical (why), investigating associations between risk factors and disease. Disease surveillance describing the number of notifiable disease cases such as measles, meningitis or cholera is an example of observational descriptive epidemiology. Studies showing an association between human papillomavirus infection and cervical cancer are examples of analytical epidemiological studies.

Interventional or experimental epidemiological studies are designed to test a hypothesis by allocating an exposure or intervention to one group of people but not the other and measuring the disease outcome. Examples of intervention studies are randomized controlled trials investigating efficacy of a new vaccine.

Epidemiologists talk about outcomes and exposures. The outcome is usually a disease or event such as death, infection or onset of new symptoms. Sometimes outcomes are laboratory markers, for example C-reactive protein (an acute phase protein) or HIV viral load. These outcomes are sometimes called intermediate outcomes because they may not represent a definite clinically important endpoint. Exposures are either risk factors, for example a specific behaviour or harmful substance, or interventions such as drugs, vaccines or health education.

OUTCOME MEASUREMENTS

It is important to clearly define health-related outcomes. A definition should include the methods used to identify a case, the definition of a case and the unit of analysis.

For example eye disease secondary to *Chlamydia trachomatis* (Ch. 26) is an important public health issue globally. The trachomatous inflammation is graded clinically into whether it involves follicular inflammation of the eyelid, abnormally positioned eyelashes or corneal scarring. When defining a case of trachomatous inflammation, it is important to describe (1) the methods and procedures used to determine a case: clinical examination versus direct immunofluorescence microscopy of conjunctival smear, (2) the definition of a case: e.g. follicular inflammation only compared with including all three clinical grades, and (3) the unit of analysis: in this case one or two eyes.

Disease prevalence and incidence are the two main types of measure of occurrence (disease frequency) used in epidemiology. Prevalence is the number of existing cases in a population at a given point in time. Incidence is the number of new cases occurring in a population during a specified period of time.

Prevalence (P) is influenced by occurrence of new cases (incidence, I) and the duration (D) of each case (i.e. $P=I*D$). Thus prevalence of diseases with short durations such as viral gastroenteritis is mainly influenced by incidence, whereas prevalence of chronic diseases with relatively low mortality is likely to be high even if incidence is low. An example of the interaction between prevalence, incidence and mortality is shown in Box 33.1.

TYPES OF EPIDEMIOLOGICAL STUDIES

Cross-sectional studies

Cross-sectional studies measure the frequency of an outcome and / or exposure(s) in a defined population at a particular point in time (Fig. 33.2A). These studies can be either descriptive, measuring the burden of disease, or analytical, comparing the frequency of disease in people exposed and unexposed to a risk factor.

Box 33.1 Lessons in Microbiology

The interaction between prevalence, incidence, mortality and treatment

When HIV is introduced into an HIV-negative population, HIV prevalence and incidence grow exponentially (Fig. 33.1). As more people become infected, the proportion of individuals not infected decreases. With fewer individuals susceptible to infection the likelihood that an infectious HIV-positive individual will be in contact with an HIV-uninfected individual is reduced. This in turn reduces incidence, but prevalence continues to rise. The median time of survival in the natural course of HIV disease (without antiretroviral treatment) is 6–8 years. Thus, after a time lag, HIV mortality grows, which reduces HIV prevalence. However, if HIV treatment becomes available, survival is prolonged and prevalence grows.

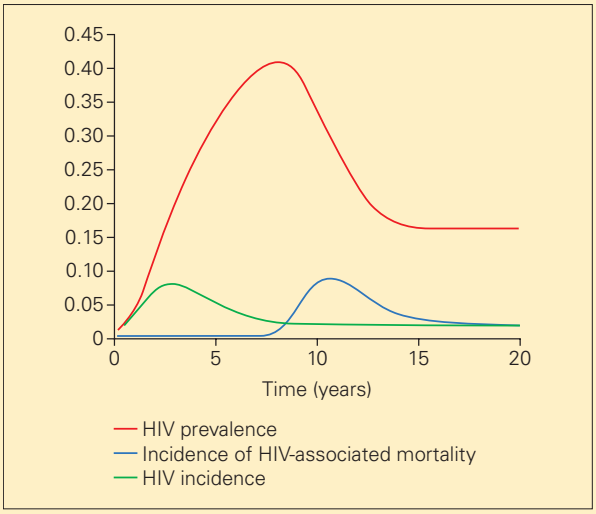


Figure 33.1 HIV prevalence, incidence and mortality in a hypothetical population. (Based on data from: Trends in HIV Incidence and Prevalence: Natural Course of the Epidemic or Results of Behavioural Change? UNAIDS Best Practice Collection in collaboration with Wellcome Trust Centre for the Epidemiology of Infectious Disease, 1999.)

Examples of study questions addressed by cross-sectional studies are:

- What proportion of the population has evidence of a past infection with Lyme disease?
- Is hepatitis B associated with hepatocellular carcinoma?

Cross-sectional studies are relatively cheap and quick to do. They are useful to determine the scale of a problem (prevalence of disease or prevalence of a risk factor in the population), to assess hypotheses for possible causal associations and to evaluate diagnostic tests (Box 33.2). As cross-sectional studies can only measure disease prevalence it is therefore difficult to differentiate between exposures causing the disease or improving the survival. With cross-sectional studies, outcome and exposure are determined at the same time, so there remains uncertainty whether the exposure preceded the outcome, which is a crucial requirement for causality. Sometimes it is difficult to exclude reverse causality (i.e. the outcome caused the 'exposure').

Case–control studies

Case–control studies identify people with the outcome (cases) and a representative group of people in the population from which cases arose but without the outcome (controls). Cases and controls are then compared with regards to differences in their past exposure (Fig. 33.2B). These studies are always analytical studies, as they ask the question, 'Does exposure A cause disease B?'

Examples of study questions addressed by case–control studies are:

- Are women with cervical cancer more likely to be infected with human papillomavirus than women without cervical cancer?
- Is injecting drug use associated with hepatitis C?

Case–control studies are usually less expensive and time consuming than cohort or intervention studies. Rare diseases and diseases with long duration between exposure and outcome are most efficiently investigated using a case–control design, as case–control studies start with diseased and non-diseased individuals. However, when the exposure is rare, case–control studies are impractical. Unbiased ascertainment of exposure is often difficult, especially when it relies on the participant to self-report. Neither disease prevalence nor incidence is measured in a case–control study. Only the increased risk of disease if individuals are exposed compared with if unexposed is measurable. Exposure is determined when the outcome has occurred and thus reverse causality might be the reason for an association between an exposure and disease.

Cohort studies

Cohort studies follow a group of people who do not initially have the outcome of interest and determine whether they develop the disease (descriptive cohort study). Analytic cohort studies classify people at the start of the study as exposed or unexposed to a certain risk factor. Both groups are followed over time and the occurrence of disease is compared between the exposed and unexposed group (Fig. 33.2C).

Examples of study questions addressed by cohort studies are:

- How high is the mortality among patients with methicillin-resistant *Staphylococcus aureus* septicaemia?
- Does infection with human herpes virus 8 cause Kaposi sarcoma in HIV-infected individuals?

Cohort studies measure disease incidence and ascertain risk factors before the outcome occurred. Thus they provide more robust evidence that an association between disease and exposure is likely to be causal. As cohort studies select disease-free exposed and unexposed individuals they are particularly useful to investigate associations between rare exposures and disease, but are inefficient when investigating rare diseases. Minimizing loss to follow-up is sometimes challenging, but important to ensure comparability between exposure groups and validity of the study results. Cohort studies are often expensive in terms of the costs and manpower needed, as well as time consuming unless historical information (e.g. electronic health records of both exposures and subsequent outcomes) is available.

Intervention studies

In an intervention study, disease-free and exposure-free individuals are actively allocated to an exposure (intervention) or no exposure (no intervention) group. The two groups are then followed over a period of time and the frequency of the outcome is compared between the two groups (Fig. 33.2D).

Randomized controlled studies are a subtype of intervention studies and are considered the 'gold standard' type of study because, when rigorously designed and conducted, they provide very strong evidence of causal associations. The intervention is allocated at random, which means that the only reason a participant receives the intervention is by chance

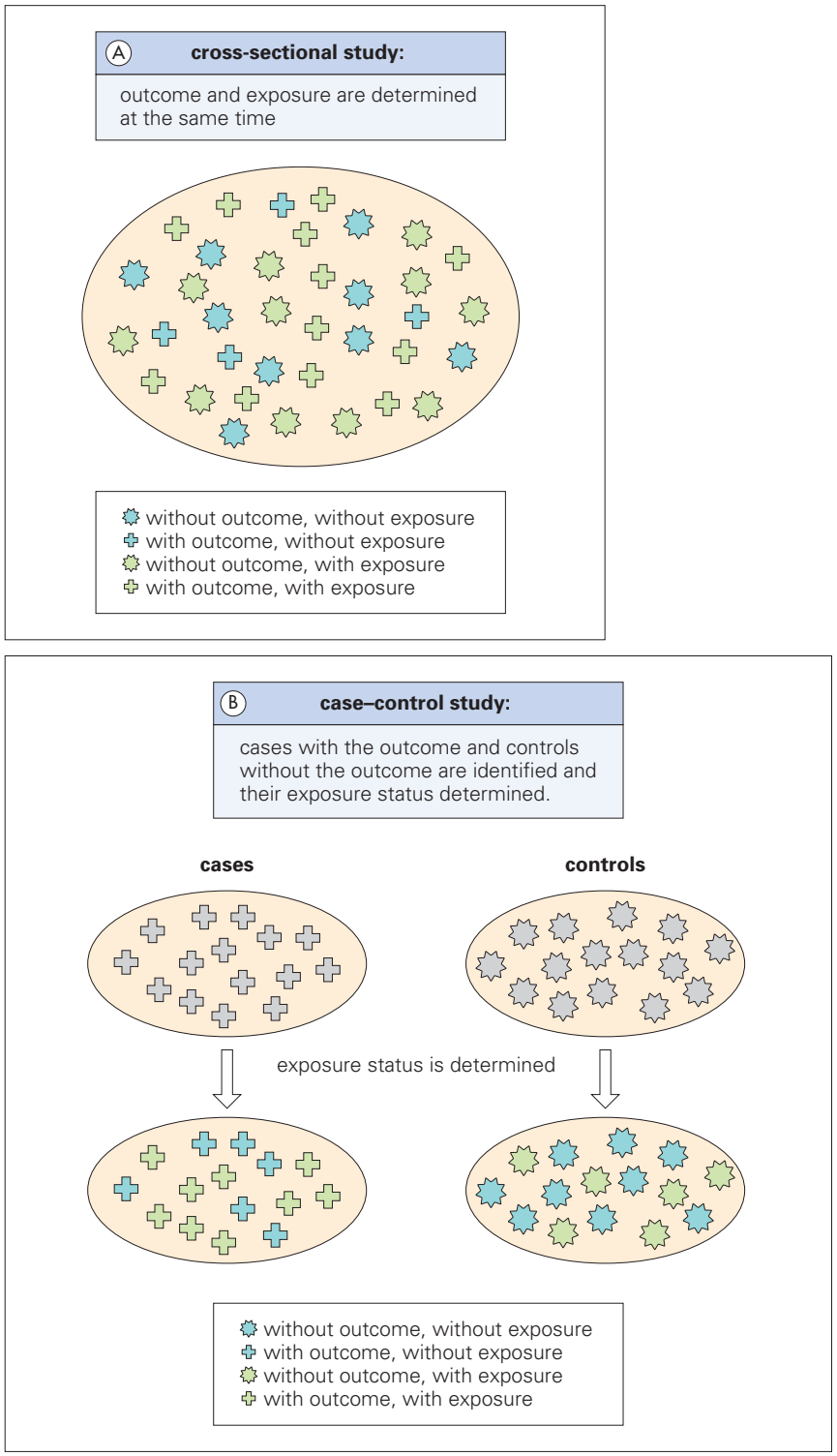


Figure 33.2 (A) Cross-sectional study: outcome and exposure are determined at the same time. (B) Case–control study: cases with the outcome and controls without the outcome are identified and their exposure status determined. *(Continued)*

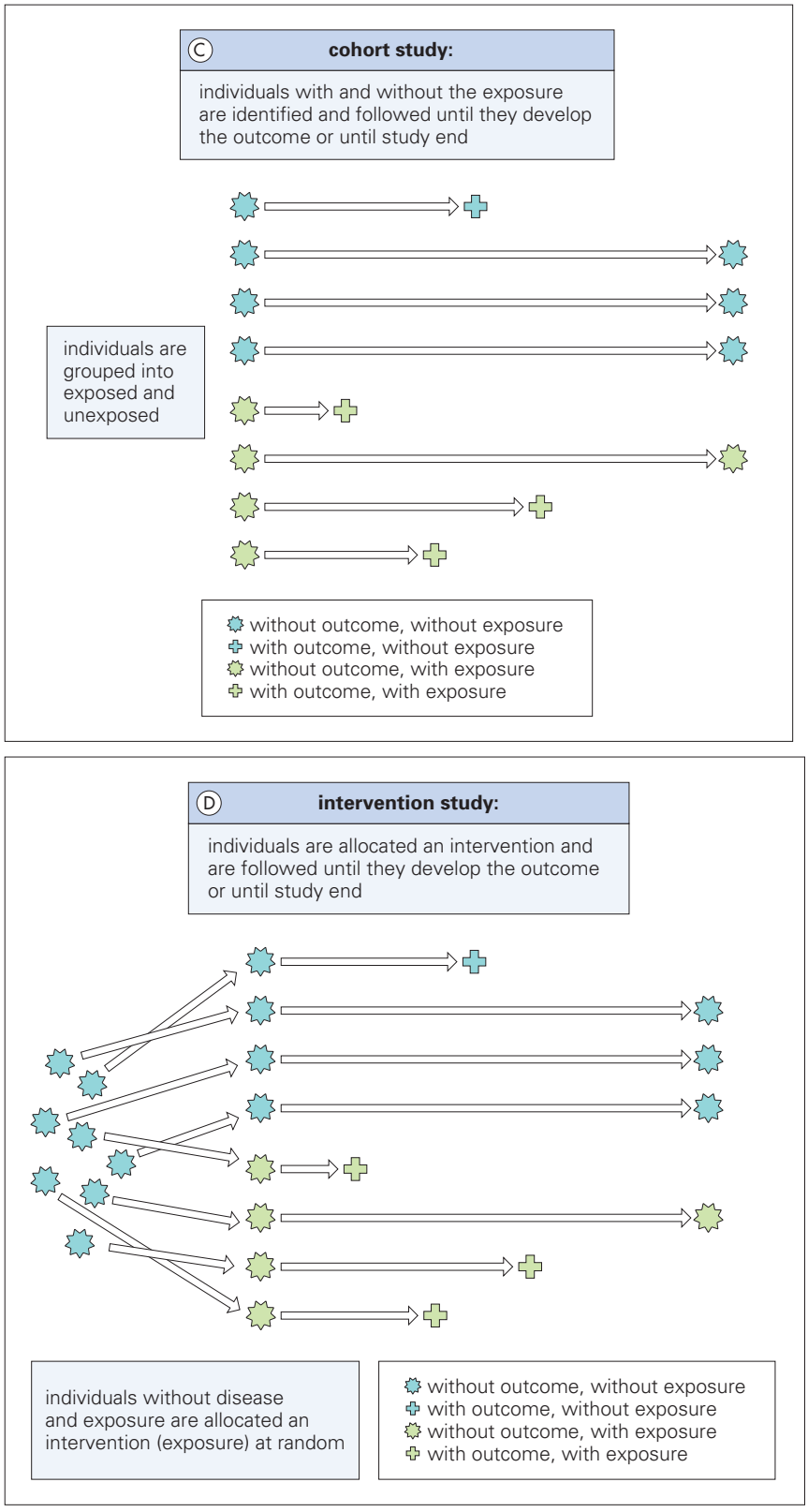


Figure 33.2—Cont'd (C) Cohort study: individuals with and without the exposure are identified and followed until they develop the outcome or until study end. (D) Intervention study: individuals are allocated an intervention (exposure) and are followed until they develop the outcome or until study end.

Box 33.2 Lessons in Microbiology

Sensitivity, specificity, positive and negative predictive value

New diagnostic tests are usually evaluated using a cross-sectional study design. The new test is compared against a gold standard test and sensitivity and specificity are determined.

Sensitivity is the proportion of true positives correctly identified by the new test and specificity is the proportion of true negatives correctly identified by the new test. Both sensitivity and specificity are intrinsic to the test and do not vary according to disease prevalence. However, they can be influenced by operators and environmental conditions.

From the patient's and physician's point of view, the more interesting question is, 'What are the chances for me having the disease if I have a positive test result?' This question is answered by the positive predictive value (PPV), which is the proportion of individuals with a positive test result who actually have the disease. The negative predictive value (NPV) is the proportion of individuals with a negative test result who are free of disease. Both PPV and NPV are related to sensitivity and specificity of a test but also to the prevalence of disease in a population.

The Xpert MTB-RIF is an automated molecular test for diagnosis of *Mycobacterium tuberculosis* (see Ch. 20). Diagnosis of tuberculosis (TB) previously relied on smear microscopy in most resource-limited settings and liquid culture in resource-rich settings. Smear microscopy has a low sensitivity and detects only patients with relatively advanced disease. Liquid culture is the gold standard of TB diagnosis, but takes days to weeks to become positive.

A hypothetical evaluation study in 7000 TB suspects in a high TB prevalence setting revealed a sensitivity of the Xpert MTB-RIF of 92% and a specificity of 98% (Table 33.1A). The prevalence of TB among these 7000 TB suspects was 10%. PPV was 93% and NPV 99%.

The evaluation study was repeated in a population survey with 10 000 participants, among whom TB prevalence was 1%, sensitivity and specificity remained the same, but PPV was 53% and NPV 100% (Table 33.1B).

Table 33.1A Results of the Xpert MTB-RIF evaluation among tuberculosis suspects

		Liquid culture (gold standard)		
		Positive	**Negative**	**Total**
Xpert MTB-RIF	Positive	645	50	695
	Negative	55	6250	6305
	Total	700	6300	7000

Sensitivity = 645 / 700 = 92%
Specificity = 6250 / 6300 = 98%
Positive predictive value = 645 / 695 = 93%
Negative predictive value = 6250 / 6305 = 99%

Table 33.1B Results of the Xpert MTB-RIF evaluation in a population survey

		Liquid culture (gold standard)		
		Positive	**Negative**	**Total**
Xpert MTB-RIF	Positive	92	80	172
	Negative	8	9820	9828
	Total	100	9900	10 000

Sensitivity = 92 / 100 = 92%
Specificity = 9820 / 9900 = 98%
Positive predictive value = 92 / 172 = 53%
Negative predictive value = 9820 / 9828 = 100%

alone. This ensures that the group receiving the intervention and the group not receiving the intervention are equally balanced and comparable. The control group often receives a placebo, such as a tablet or injection containing no active compounds. Some intervention studies are double blinded, which means that neither investigator nor participant knows who receives the active intervention and who receives the placebo.

Examples of study questions addressed by intervention studies are:

- Is a new vaccine effective in preventing pneumococcal disease in children?
- Do steroids improve the outcome in children with meningococcal disease?

Randomized, placebo-controlled, double-blinded studies potentially deal with most problems experienced in observational studies: confounding, recall and observer bias. Confounding occurs when there is unequal distribution of a risk factor between exposed and unexposed individuals and thus the observed association between exposure and disease is due to

this other factor. Recall bias is a systematic error, which occurs when the way a participant answers a question is affected by either the disease status (in case–control or cross-sectional studies) or the exposure status (in cohort studies). Observer bias arises when the accuracy of exposure (in case–control or cross-sectional studies) or outcome (cohort studies) data recorded by the investigator differs systematically between subjects in different outcome or exposure groups. Outcome data are determined prospectively in intervention studies and thus standard case definitions can be applied. Intervention studies may be expensive and time consuming and loss to follow-up can be challenging. Large sample sizes or long follow-up may be needed if disease incidence is low or duration between exposure and disease is long. Allocation of a harmful exposure or withholding of a beneficial intervention is unethical. Intervention studies cannot be conducted under these circumstances.

TRANSMISSION OF INFECTIOUS DISEASE

An infectious disease is transmitted from one person to another either directly or indirectly. Indirect transmission occurs when the infectious agent is transferred from one person to another via an intermediary (e.g. vector or vehicle). The occurrence of a case depends on the occurrence of at least one previous case (source) and each case can itself lead to another case. Disease events in infectious diseases are dependent. We therefore investigate the spread of infectious diseases through a population over time to determine ways to control it (Fig. 33.3).

Infectiousness (Box 33.3)

The infectiousness of a disease in a population depends on several factors:

- the infectious agent: time between infection of a person and becoming infectious
- duration of infectiousness
- the probability of transmission given a contact between an infectious person and a susceptible person
- the environment:
  - the type of contacts between infectious and susceptible individuals
  - the number of contacts
- the characteristics of the individuals in the population:
  - susceptibility of the population (number of susceptible individuals and degree of susceptibility)
  - infectiousness of the infected person.

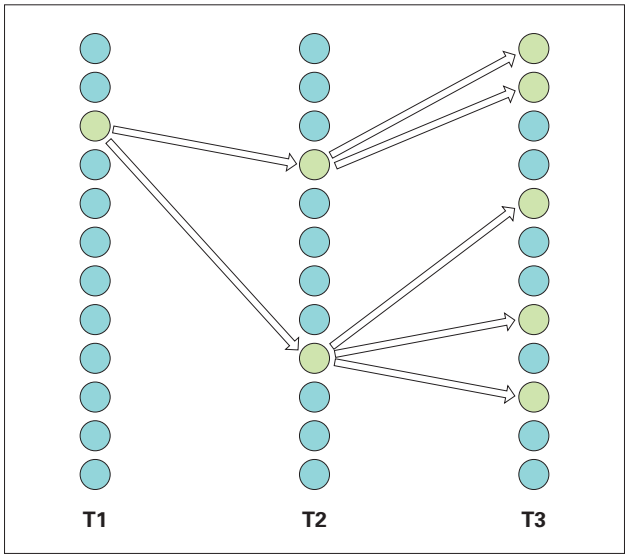


Figure 33.3 Transmission of an infectious disease in a population. One case of disease (source) at T1 transmits the disease to two cases (secondary cases) at T2; those cases transmit the disease to five cases at T3. Note that individuals who had the disease at T1 and T2 do not have the disease at T3 due to immunity.

Time periods of infections

When a susceptible individual becomes infected, he or she enters the latent period (Fig. 3.4, Box 33.4). The latent period is the period between infection and becoming infectious (able to transmit the infection) and hence is often called the pre-infectious period to avoid confusion with the other uses of the term latent (discussed later). This period is followed by the infectious period during which the infected individual

Box 33.3 Lessons in Microbiology

Infectiousness – example syphilis

An individual infected with syphilis develops a painless very infectious sore (chancre) at the site of infection on average 3 weeks following infection. The lesion may persist for 3–6 weeks. The individual cannot transmit the infection before the chancre develops. Thus the duration between infection and becoming infectious is important for transmission. The likelihood of transmitting the infection is increased the more frequently the individual has sexual intercourse and the longer the lesion persists (if the frequency of intercourse remains constant). Therefore the duration of infectiousness and the number of contacts influence transmission. The probability of transmission is reduced if the individual uses condoms.

Box 33.4 Lessons in Microbiology

Terminology: latency

In general, latency is a time delay. The term is frequently used in infectious disease terminology. Strictly speaking, the latent period is the time from infection until the infected individual is able to transmit the disease. However, sometimes the incubation period is called the latent period even though the two periods are differently defined and might differ in duration. A child infected with measles becomes infectious before symptoms occur. Thus the latent period is shorter than the incubation period. In contrast, an individual infected with *P. falciparum* malaria will experience symptoms 7–14 days following infection, but will be infectious only after 24 days. Sometimes, disease stages are called latent, such as latent tuberculosis or syphilis. Latent disease in that context describes periods of inactivity of the disease with regards to signs and symptoms.

is able to transmit the infectious agent. This is followed by the non-infectious period due to death or recovery. If the individual survives, he or she might be immune or remain susceptible to re-infection.

The sum of the average latent and infectious periods is called the average generation time of the infection.

Latent and infectious periods are different for different diseases (Table 33.2). For measles the latent period is 6–9 days followed by an infectious period of 6–7 days. In contrast the latent period of hepatitis B is 13–17 days and the infectious period 19–22 days.

Time periods of infectious disease

Not all infected individuals will develop the disease. Disease and infection differ with regards to symptoms and clinical signs. Infected individuals without symptoms and signs have asymptomatic infections. For some infectious agents such as cytomegalovirus the majority of infections will be asymptomatic.

The incubation period starts with the time of infection and ends when the individual develops symptoms (Fig. 33.4). It is followed by the symptomatic period. The symptomatic period ends with death or recovery.

The incubation period is 8–13 days and 50–110 days for measles and hepatitis B, respectively. Thus an individual infected with measles is infectious before developing symptoms, as persons become infectious after 6–9 days. Therefore importantly, isolation at the time of symptoms will not prevent transmission.

Basic and net reproduction number

The basic reproduction number (R_0) is the average number of infected secondary cases produced by each infectious case in a totally susceptible population.

Disease incidence:

- is static if each case leads to one new case (R_0=1)
- increases if each case leads to more than one infective secondary case (R_0>1)
- decreases if each case leads to less than one infective secondary case (R_0<1), which will result in disease control and eradication.

The basic reproduction number depends on the duration of infectiousness of the case (d), the number of contacts per unit time (c) and the transmission probability (p): $R_0 = c * p * d$. This formula shows that the basic reproduction number is not specific to an infectious agent only, but also to a specific host population at a particular point in time. R_0 for HIV is different for women, men and commercial sex workers. Table 33.3 shows basic reproduction number for different diseases. Measles has a very high basic reproduction number of 15–17, whereas influenza has a basic reproductive rate of 2–3.

A completely susceptible population is unusual. More commonly, a population consists of susceptible and immune individuals. The net reproduction number (R) is the average

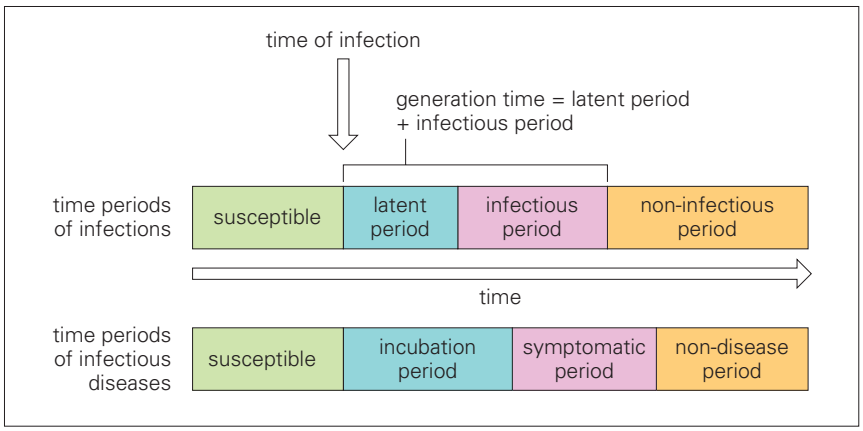


Figure 33.4 Time periods of infections and infectious diseases.

Table 33.2 Latent, infectious and incubation periods for a variety of viral and bacterial infections

Infectious diseases	Latent period (days)	Infectious period (days)	Incubation period (days)
Measles	6–9	6–7	8–13
Mumps	12–18	4–8	12–26
Whooping cough (pertussis)	21–23	7–10	6–10
Rubella	7–14	11–12	14–21
Diphtheria	14–21	2–5	2–5
Varicella	8–12	10–11	13–17
Hepatitis B	13–17	19–22	50–110
Poliomyelitis	1–3	2–3	7–12
Influenza	1–3	2–3	1–3

Table 33.3 Basic reproductive rate for a variety of infectious diseases

Infectious disease	Basic reproductive rate (R_0)
Measles	15–17
Mumps	10–12
Whooping cough (pertussis)	15–17
Rubella	7–8
Diphtheria	5–6
Poliomyelitis	5–6
Influenza	2–3

number of secondary cases in a population where not all individuals are susceptible. The net reproduction number depends on the basic reproduction number (R_0) and the proportion of susceptible individuals (x): $R=R_0*x$. The lower the proportion of susceptible individuals in a population, the lower is the probability that an infectious individual will be in contact with a susceptible individual. Thus, if the proportion of susceptibles (x) is small enough, R will be less than 1 and the disease can be eradicated. The proportion of the population immune to an infection is called herd immunity (HI): HI=1–x. The herd immunity threshold is the proportion of the population that needs to be immune in order for a disease to eventually die out ($R<1$): HIT=R_0-1/R_0. Susceptible individuals become immune once they are vaccinated with a highly effective vaccine. The basic reproduction number allows us to estimate the vaccination coverage which needs to be achieved in order to control an infectious disease. The critical vaccination coverage needs to be very high (92–95%) for measles due to the high reproductive rate (15–17). Rubella has a lower reproductive number (7–8) and thus for disease control vaccination coverage needs to be only 85–87%.

VACCINE EFFICACY

Vaccines protect individuals directly by making them less susceptible (more immune) to the disease. They also protect individuals indirectly (even individuals who did not receive the vaccine) through increased herd immunity.

Vaccine efficacy is the most commonly used measure of effect when evaluating vaccines in randomized controlled trials. Vaccine efficacy is the reduction in the incidence of disease in vaccinated individuals compared with unvaccinated individuals:

Vaccine efficacy=(incidence of disease in unvaccinated individuals−incidence of disease in vaccinated individuals/incidence of disease in unvaccinated individuals). Thus vaccine efficacy measures only the direct effect of the vaccine. Measurement of the indirect effect of vaccines requires more complex study designs.

KEY FACTS

- Epidemiological studies can be observational or interventional.
- Prevalence is the number of existing cases in a population at a given point in time. Incidence is the number of new cases occurring in a population during a specified period of time.
- Infectiousness depends on the infectious agent itself, the environment and the characteristics of the individuals in the population, such as whether they are immune or susceptible.
- Infections can also be characterized by the latent or pre-infectious period, the infectious period, the incubation period and the symptomatic period: individuals can become infectious before they develop symptoms.
- Vaccine efficacy is the reduction in the incidence of disease in vaccinated individuals compared with unvaccinated individuals. When a significant proportion of the community is protected by vaccination, unvaccinated individuals are also less likely to acquire disease; this is called herd immunity.

34 Attacking the enemy: antimicrobial agents and chemotherapy

Introduction

The interactions between host, microbial pathogen and antimicrobial agent can be considered as a triangle, and any alteration in one side will inevitably affect the other two sides (Fig. 34.1). In this chapter, two sides of the triangle will be examined in greater detail:

- the interactions between antimicrobial agents and microorganisms
- the interactions between antimicrobial agents and the human host.

Laboratory aspects of antimicrobial susceptibility tests and assays will also be outlined. The third side of the triangle, the interactions between microorganisms and the human host, has been considered in detail in other chapters. The concluding part of the present chapter will draw together the three sides of the triangle.

SELECTIVE TOXICITY

The term 'selective toxicity' was proposed by the immunochemist Paul Ehrlich (Box 34.1). Selective toxicity is achieved by exploiting differences in the structure and metabolism of microorganisms and host cells; ideally, the antimicrobial agent should act at a target site present in the infecting organism, but absent from host cells. This is more likely to be achievable in microorganisms that are prokaryotes than in those that are eukaryotes, as the former are structurally more distinct from the host cells. (A comparison of the cellular organization of prokaryotic and eukaryotic cells is given in Ch. 1.) At the other end of the spectrum, viruses are difficult to attack because of their obligate intracellular lifestyle. A successful antiviral agent must be able to enter the host cell, but inhibit and damage a virus-specific target. The desirable features of ideal antimicrobial agents are summarized in Box 34.2.

DISCOVERY AND DESIGN OF ANTIMICROBIAL AGENTS

The term 'antibiotic' has traditionally referred to natural metabolic products of fungi, actinomycetes and bacteria that kill or inhibit the growth of microorganisms. Antibiotic production has been historically associated with soil microorganisms and, in the natural environment, is thought to provide a selective advantage for organisms in their competition for space and nutrients. Antibacterial agents derived from natural sources (e.g. penicillins, aminoglycosides) are usually chemically modified (i.e. semi-synthetic) to improve their antibacterial or pharmacological properties. However, some agents are totally synthetic (e.g. sulphonamides, quinolones). Therefore, the term 'antibacterial' or 'antimicrobial' agent is often used in preference to 'antibiotic'. Agents used against fungi, parasites, and viruses can also be included under antimicrobials, but the terms antifungals, antiprotozoans, anthelmintics, and antivirals are more often used.

The discovery of new antimicrobial agents used to be entirely a matter of chance. Pharmaceutical companies undertook massive screening programmes searching for new soil microorganisms that produced antibiotic activity. In the light of our greater understanding of the mechanisms of action of existing antimicrobials, the processes have become rationalized, searching either for new natural products by target-site-directed screening or synthesizing molecules predicted to interact with a microbial target. Genomic approaches to the identification of novel targets have revolutionized this approach. In addition, knowledge of the crystal structure of the key enzymes involved in viral replication such as protease, reverse transcriptase and helicase leads to the design of new drugs. The steps in a rational design programme are summarized in Box 34.3.

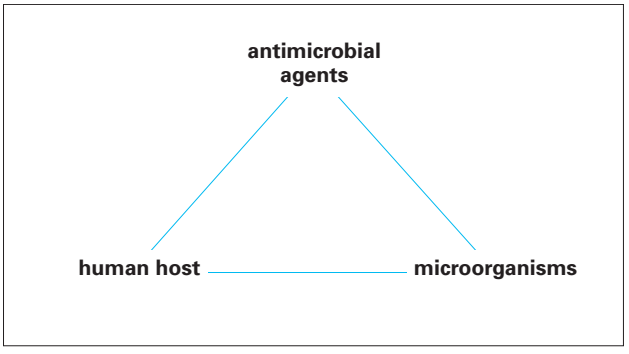


Figure 34.1 The interactions between antimicrobial agents, microorganisms and the human host can be viewed as a triangle. Any effect on one side of the triangle will have effects on the other two sides.

Box 34.1 Lessons in Microbiology

Paul Ehrlich (1854–1915)

Just as Pasteur towers over immunomicrobiology, Ehrlich (Fig. 34.2) is the father figure of immunochemistry. His contributions to the science of medicine at all levels are quite extraordinary. He was the first to propose that foreign antigens were recognized by 'side-chains' on cells (1890), a brilliant insight that took 70 years to confirm. He also discovered the mast cell, invented the acid-fast stain for the tubercle bacillus and devised a method to manufacture and commercialize a strong diphtheria antitoxin. He pioneered the development of antibiotics with his work on '606' (or 'Salvarsan'), a treatment for syphilis, for which he was denounced by the church for interfering with God's punishment for sin.

While working on the treatment of infections caused by trypanosomes he set forth the concept of 'selective toxicity', as illustrated by the following quote: 'But, gentlemen, it should be made clear that in general this task is much more complicated than that using serum therapy. These chemical agents, in contrast to the antibodies, may be harmful to the body. When such an agent is given to a sick organism, a difference must exist between the toxicity of this agent to the parasite and its toxicity to the host. We must always be aware of the fact that these agents are able to act on other parts of the body as well as on the parasites.'

Like Pasteur, he had a grasp of the continuum from the whole body to the cell and the three-dimensional structure of molecules, and throughout his life he stressed the importance of molecular interaction as the basis of all biological function; this is summed up in his famous maxim *corpora non agunt nisi fixata* or 'things do not interact unless they make contact'. A Nobel Prize winner in 1908, his name was systematically eliminated from the records by the Nazi regime on account of his Jewish birth, but he was restored to honour by a reconstruction of his laboratory at the Seventh International Congress of Immunology in Berlin in 1989.

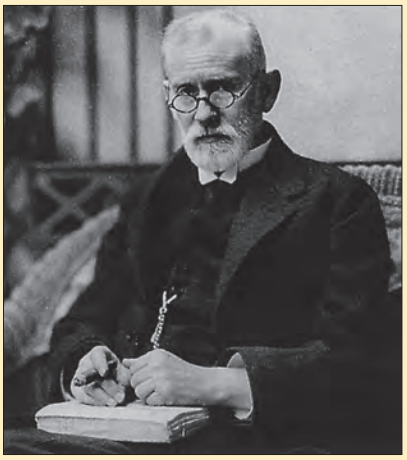


Figure 34.2 Paul Ehrlich (1854–1915).

Box 34.2 Desired Properties of a New Antimicrobial Agent

In the design of new antimicrobial agents, both antimicrobial activity and pharmacological properties of the antibiotic for the host have to be considered.

Antimicrobial properties

- Selectivity for microbial rather than mammalian targets
- Cidal activity (antibacterial and antifungal agents)
- Slow emergence of resistance
- Narrow spectrum of activity[a]

Pharmacological activities

- Non-toxic to the host
- Long plasma half-life (once-a-day dosing)
- Good tissue distribution including CSF
- Low plasma–protein binding
- Oral and parenteral dosing forms
- No interference with other drugs

[a]The desired attribute depends on drug usage. Narrow-spectrum drugs cause less disturbance to the microbiota and may contribute less to emergence of antibiotic resistance, whereas broad-spectrum compounds are more useful for empiric therapy and treatment of polymicrobial infections.
CSF, cerebrospinal fluid.

Box 34.3 Rational Design of an Antimicrobial Agent

The discovery process of new antimicrobial agents has moved away from historical random screening of soil microorganisms towards a rational design programme informed by computer modeling and genomics. From discovery to development and marketing can take over 10 years and cost at least US$1 billion. This list identifies different steps in this programme.

- Select an appropriate target.
- Identify a chemical lead (i.e. a new molecule with inhibitory activity on the target).
- Modify the lead compound to enhance potency.
- Evaluate in vitro activity.
- Evaluate in vivo activity and toxicity.
- Test in clinical trials and develop.

CLASSIFICATION OF ANTIBACTERIAL AGENTS

There are three ways of classifying antibacterial agents:

1. according to whether they are bactericidal or bacteriostatic
2. by target site
3. by chemical structure.

Some antibacterial agents are bactericidal, others are bacteriostatic

Some antibacterial agents kill bacteria (bactericidal), while others only inhibit their growth (bacteriostatic). Thus, the bactericidal process is irreversible, while bacteriostasis is reversible. Nevertheless, bacteriostatic agents are successful in the treatment of some infections because they prevent the bacterial population from increasing and host defence mechanisms can consequently cope with the static population. However, in immunocompromised patients, bacteriostatic drugs may be less efficacious, and certain infections (e.g. endocarditis) require a bactericidal drug even in an immunocompetent patient.

As a means of classification, the distinction between bactericidal and bacteriostatic agents can be somewhat blurred (e.g. some bacteriostatic agents may tend toward bactericidal activity at higher concentrations).

There are five main target sites for antibacterial action

A convenient way of classifying antibacterials is on the basis of their site of action. This classification does not allow an accurate prediction of which antibacterials will be active against which bacterial species, but it does help in the understanding of the molecular basis of antibacterial action, and conversely in the elucidation of many of the synthetic processes in bacterial cells. The five main target sites for antibacterial action are:

- cell wall synthesis
- protein synthesis
- nucleic acid synthesis
- metabolic pathways
- cell membrane function.

These targets differ to a greater or lesser degree from those in the host (human) cells and so allow inhibition of the bacterial cell without concomitant inhibition of the equivalent mammalian cell targets (selective toxicity).

Each target site encompasses a multitude of synthetic reactions (enzymes and substrates), each of which may be specifically inhibited by an antibacterial agent. A range of chemically diverse molecules may inhibit different reactions at the same target site (e.g. protein synthesis inhibitors).

Antibacterial agents have diverse chemical structures

Classification based on chemical structure alone is not of practical use, because there is such diversity. However, a combination of target site and chemical structure provides a useful working classification to organize antibacterial agents into specific families which will be discussed later in this chapter.

RESISTANCE TO ANTIBACTERIAL AGENTS

Resistance to antibacterial agents is a matter of degree. In the medical setting, we define a resistant organism as one that will not be inhibited or killed by an antibacterial agent at concentrations of the drug achievable in the body after normal dosage. 'Some men are born great, some achieve greatness, and some have greatness thrust upon them' (William Shakespeare, *Twelfth Night*). Likewise, some bacteria are born resistant, others have resistance thrust upon them. In other words, some species are innately resistant to some families of antibiotics because they lack a susceptible target, are impermeable to or enzymatically inactivate the antibacterial agent. The Gram-negative rods with their outer membrane layer exterior to the cell wall peptidoglycan are less permeable to large molecules than Gram-positive cells. However, within species that are innately susceptible, there are also strains that develop or acquire resistance.

The genetics of resistance

In parallel with the rapid development of a wide range of antibacterial agents since the 1940s, bacteria have proved extremely adept at developing resistance to each new agent that comes along. This is illustrated for *Staphylococcus aureus* by the timeline shown in Fig. 34.3. The rapidly increasing

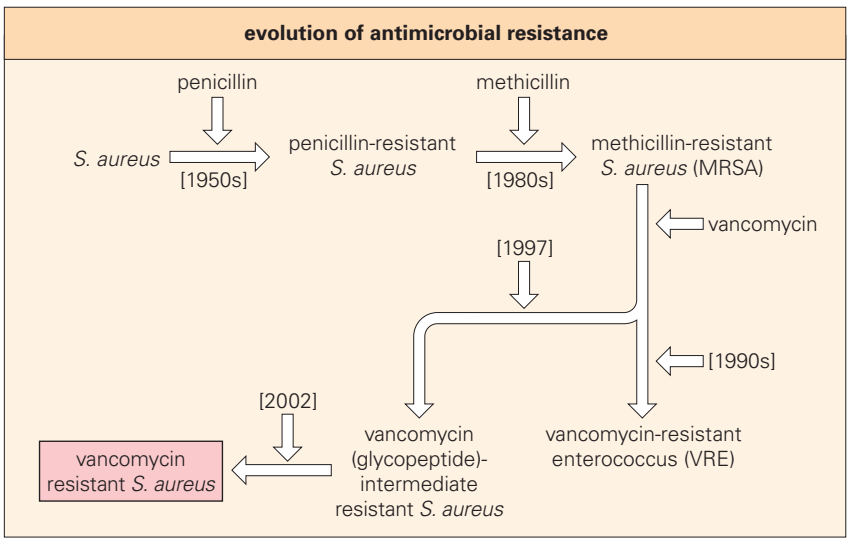


Figure 34.3 'Time line' illustrating the chronological emergence of antibiotic resistance in Gram-positive cocci.

incidence of resistance associated with slowing down in the discovery of novel antibacterial agents to combat resistant strains is now recognized worldwide as a serious threat to the treatment of life-threatening infections.

Chromosomal mutation may result in resistance to a class of antimicrobial agents (cross-resistance)

Resistance may arise from:

- a single chromosomal mutation in one bacterial cell resulting in the synthesis of an altered protein: for example, streptomycin resistance via alteration in a ribosomal protein, or the single amino acid change in the enzyme dihydropteroate synthetase resulting in a lowered affinity for sulphonamides. A mutational event could also alter (i.e. increase or decrease) the production of a protein resulting in increased resistance.
- a series of mutations, for example changes in penicillin-binding proteins (PBPs) in penicillin-resistant pneumococci.

In the presence of antibiotic, these spontaneous mutants have a selective advantage to survive and outgrow the susceptible population (Fig. 34.4A). They can also spread to other sites in the same patient or by cross-infection to other patients and therefore become disseminated. Chromosomal mutations are relatively rare events (i.e. usually found once in a population of 10^6–10^8 organisms) and generally provide resistance to a single class of antimicrobials (i.e. 'cross-resistance' to structurally related compounds).

Genes on transmissible plasmids may result in resistance to different classes of antimicrobial agents (multiple resistance)

Not content with surviving the antibacterial onslaught by relying on random chromosomal mutation, bacteria are also able to acquire resistance genes on transmissible plasmids (Fig. 34.4B; see also Ch. 2). Such plasmids often code for resistance determinants to several unrelated families of antibacterial agents. Therefore a cell may acquire 'multiple' resistance to many different drugs (i.e. in different classes) at once, a process much more efficient than chromosomal mutation. This so-called 'infectious resistance' was first described by Japanese workers studying enteric bacteria, but is now recognized to be widespread throughout the bacterial world. Some plasmids are promiscuous, crossing species barriers, and the same resistance gene is therefore found in widely different species. For example, TEM-1, the most common plasmid-mediated beta-lactamase in Gram-negative bacteria, is widespread in *E. coli* and other enterobacteria and also accounts for penicillin resistance in *Neisseria gonorrhoeae* and ampicillin resistance in *H. influenzae*.

Resistance may be acquired from transposons and other mobile elements

Resistance genes may also occur on transposons; the so-called 'jumping genes', which by a replicative process are capable of generating copies which may integrate into the chromosome or into plasmids (see Ch. 2). The chromosome provides a more stable location for the genes, but they will be disseminated only as rapidly as the bacteria divide. Transposon copies moving from the chromosome to plasmids are disseminated more rapidly. Transposition can also occur between plasmids, for example, from a non-transmissible to a transmissible plasmid, again accelerating dissemination (Fig. 34.4C).

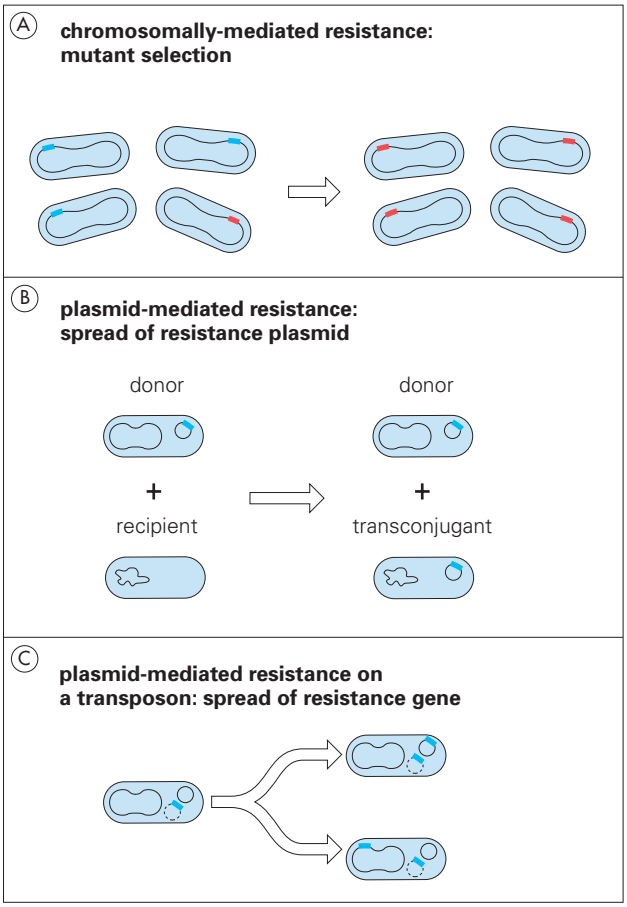


Figure 34.4 A chromosomal mutation (A) can produce a drug-resistant target, which confers resistance on the bacterial cell and allows it to multiply in the presence of antibiotic. Resistance genes carried on plasmids (B) can spread from one cell to another more rapidly than cells themselves divide and spread. Resistance genes on transposable elements (C) move between plasmids and the chromosome and from one plasmid to another, thereby allowing greater stability or greater dissemination of the resistance gene.

'Cassettes' of resistance genes may be organized into genetic elements called *integrons*

As discussed previously, antibiotic-resistance genes may individually reside on plasmids, the chromosome, or on transposons found in both locations. However, in some instances multiple resistance genes may come together in a structure known as an integron. As shown in Fig. 34.5A, the integron encodes a site-specific recombination enzyme (int gene; integrase), which allows insertion (and also excision) of antibiotic-resistance gene 'cassettes' (resistance gene plus additional sequences including an 'attachment' region) into the integron attachment site (att). In classic operon fashion, a strong integron promoter controls transcription of the inserted genes. Based on their integration mechanism (integrase, etc.), integrons have been organized into different classes found in both Gram-negative and Gram-positive organisms. Whether acting as independent mobile genetic elements or inserted

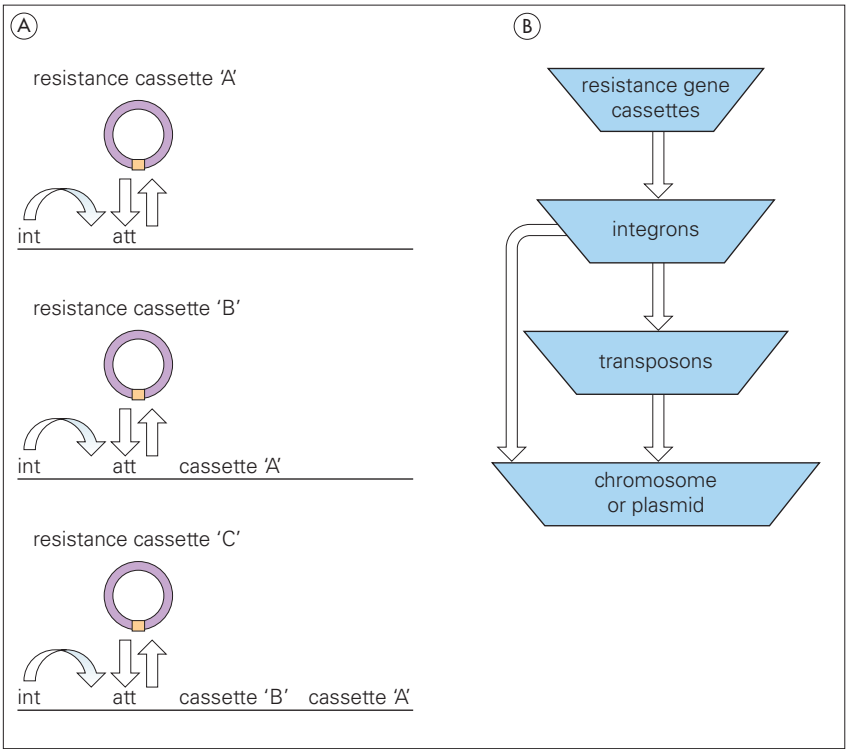


Figure 34.5 (A) Basic integron structure and (B) overall interrelationship between integrons and other DNA elements. *att*, integron attachment site; *int*, integrase.

into transposons, integrons are capable of moving into a variety of DNA molecules, the overall hierarchy of which is depicted in Fig. 34.5B. With their ability to capture, organize and rearrange different antibiotic-resistance genes, integrons represent an important mechanism for the spread of multiple antibiotic resistance in clinically important microorganisms.

Staphylococcal genes for methicillin resistance are organized into a unique cassette structure

Staphylococcal genes responsible for resistance to the antibiotic methicillin (discussed below) are found in a specialized cassette arrangement termed staphylococcal chromosomal cassette *mec* (SCC*mec*). SCC*mec* inserts into a unique target site on the staphylococcal chromosome. The cassette represents a highly recombinogenic region which may not only rearrange internally but also serve as a target for the insertion of other resistance elements (e.g. transposons and plasmids).

Mechanisms of resistance

Resistance mechanisms can be broadly classified into three main types. These are summarized below, in Table 34.1 and described in more detail where relevant for each antibiotic in later parts of this chapter. Where bacterial mechanisms of antimicrobial resistance have been elucidated, they appear to involve the synthesis of new or altered proteins. As mentioned above, the genes encoding these proteins may be found on plasmids or the chromosome.

The target site may be altered

The target may be altered so that it has a lowered affinity for the antibacterial, but still functions adequately for normal metabolism to proceed. Alternatively, an additional (more resistant) target (e.g. enzyme) may be synthesized.

Access to the target site may be altered (altered uptake or increased exit)

This mechanism involves decreasing the amount of drug that reaches the target by either:

- altering entry, for example by decreasing the permeability of the cell wall
- pumping the drug out of the cell (known as an efflux mechanism).

Enzymes that modify or destroy the antibacterial agent may be produced (drug inactivation)

There are many examples of such enzymes especially including:

- beta-lactamases
- aminoglycoside-modifying enzymes
- chloramphenicol acetyl transferases.

These will be described in the relevant parts on these antibiotics.

CLASSES OF ANTIBACTERIAL AGENTS

The following parts of this chapter deal with groups of antibacterial agents based on their target site and chemical structure. In each case, the discussion attempts to summarize the answers to the questions set out in Table 34.2, reviewing the interactions between antibacterial agent and bacteria and between the antibacterial and the host (i.e. two sides of the triangle in Fig. 34.1).

INHIBITORS OF CELL WALL SYNTHESIS

Peptidoglycan, a vital component of the bacterial cell wall (see Ch. 2), is a compound unique to bacteria and therefore provides an optimum target for selective toxicity. Synthesis of peptidoglycan precursors starts in the cytoplasm; wall subunits

Table 34.1 Mechanisms of resistance can be classified into three main types

Antibacterial	Mechanism of resistance		
	Altered target	Altered uptake	Drug inactivation
Beta-lactams	+	+	+
Glycopeptides	+		
Aminoglycosides	+	+	+
Tetracyclines	+	+	
Chloramphenicol		+	+
Macrolides/ketolides	+	+	+
Lincosamides	+		
Streptogramins	+		
Oxazolidinones	+		
Fusidic acid	+		
Sulphonamides/trimethoprim	+	+	
Quinolones	+	+	
Rifampicin	+		
Cyclic lipopeptide	+		

For antibiotics where more than one mode of resistance exists, drugs vary as to which is more frequently encountered.

Table 34.2 In order to understand the nature and optimum use of an antibacterial agent, the questions listed here must be answered

What is it?	Chemical structure: natural or synthetic product
What does it do?	Target site, mechanism of action
Where does it go? (and therefore preferred route of administration)	Absorption, distribution, metabolism and excretion of the drug in the body of the host
When is it used?	Spectrum of activity and important clinical uses
What are the limitations to its use?	Toxicity to the human host; lack of toxicity, i.e. resistance of the bacteria
How much does it cost?	Great variation between agents but cost is a serious limitation on availability of some agents in resource-poor countries

are then transported across the cytoplasmic membrane and finally inserted into the growing peptidoglycan molecule. Several different stages are therefore potential targets for inhibition (Fig. 34.6). The antibacterials that inhibit cell wall synthesis are varied in chemical structure. The most important of these agents are the beta-lactams, the largest group, and the glycopeptides, which are active only against Gram-positive organisms. Bacitracin (primarily used topically) and cycloserine (mainly used as a 'second-line' medication for treatment of tuberculosis, discussed later in this chapter) have many fewer clinical applications.

Beta-lactams

Beta-lactams contain a beta-lactam ring and inhibit cell wall synthesis by binding to penicillin-binding proteins (PBPs)

Beta-lactams comprise a very large family of different groups of bactericidal compounds, all containing the beta-lactam ring. The different groups within the family are distinguished by the structure of the ring attached to the beta-lactam ring – in penicillins this is a five-membered ring, in cephalosporins a six-membered ring – and by the side chains attached to these rings (Fig. 34.7).

PBPs are membrane proteins (e.g. carboxypeptidases, transglycosylases and transpeptidases) capable of binding to penicillin (hence the name PBP) and are responsible for the final stages of cross-linking of the bacterial cell wall structure. Inhibition of one or more of these essential enzymes results in an accumulation of precursor cell wall units, leading to activation of the cell's autolytic system and cell lysis (Fig. 34.8).

Most beta-lactams have to be administered parenterally

Different beta-lactams are administered intramuscularly, intravenously, or orally. Most achieve clinically useful concentrations in the cerebrospinal fluid (CSF) when the meninges are inflamed (as in meningitis) and the blood–brain barrier becomes more permeable. In general, they are not effective against intracellular organisms.

A few of the cephalosporins, notably cefotaxime, are metabolized to compounds with less microbiological activity. Beta-lactams are excreted in the urine, and for some, such

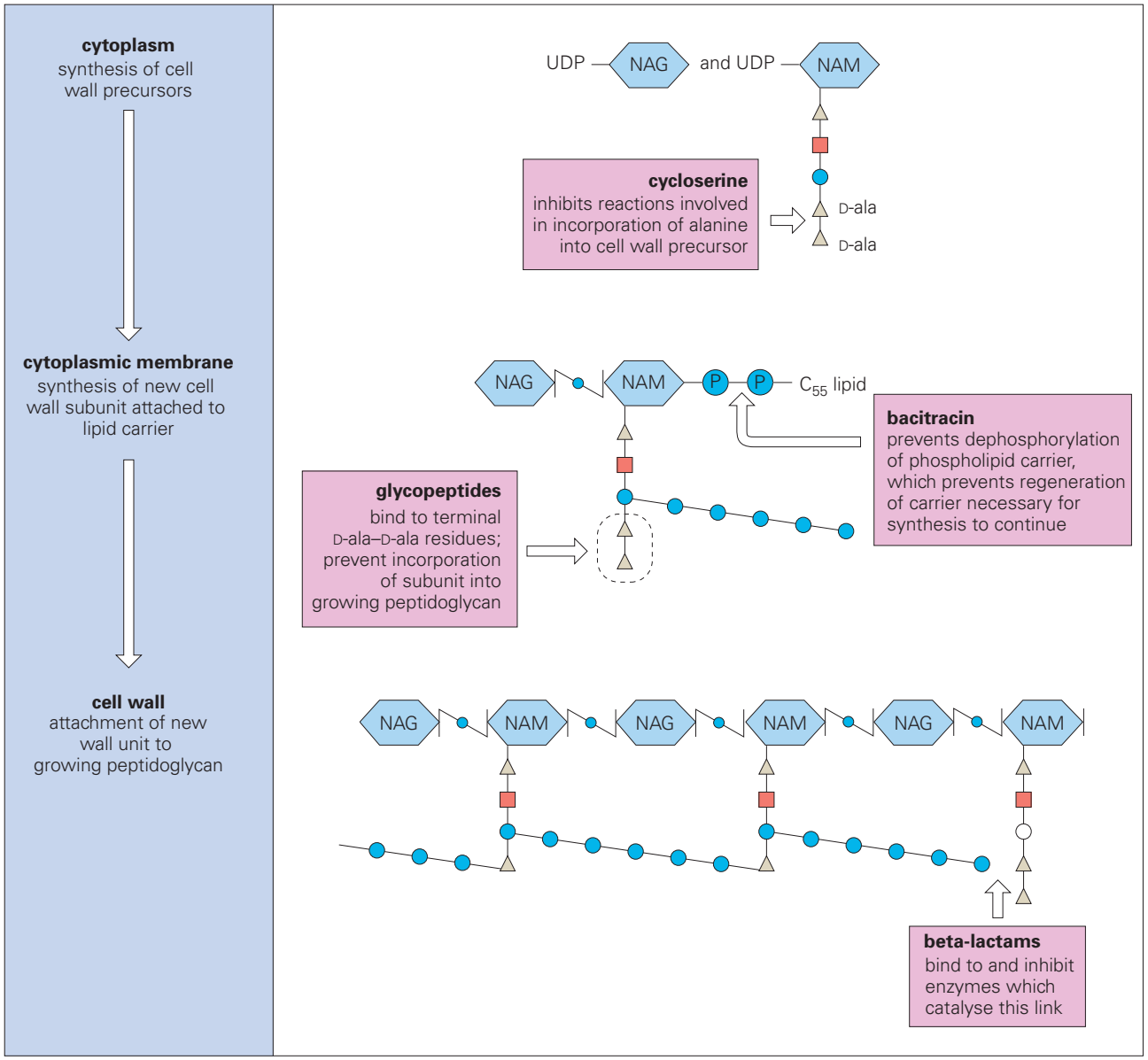


Figure 34.6 The synthesis of peptidoglycan is a complex process that begins in the cytoplasm, proceeds across the cytoplasmic membrane and leads to the attachment of new wall units to the growing peptidoglycan chain. This synthetic pathway can be inhibited at a variety of points by antibacterial agents. NAG, *N*-acetyl glucosamine; NAM, *N*-acetyl muramic acid; UDP, uridine diphosphate.

as benzylpenicillin, this is very rapid; hence the need for frequent doses. Probenecid can be administered concurrently to slow down excretion and maintain higher blood and tissue concentrations for a longer period of time.

Different beta-lactams have different clinical uses, but are not active against species that lack a cell wall

A vast array of beta-lactam antibiotics are currently registered for clinical use. Some, such as penicillin, are active mainly against Gram-positive organisms, whereas others (e.g. semisynthetic penicillins, carboxypenems, monobactams, second-, third-, fourth-, and fifth-generation cephalosporins) have been developed for their activity against Gram-negative rods. Only the more recent beta-lactams are active against innately more resistant organisms such as *Pseudomonas aeruginosa* (Table 34.3).

It is important to remember that beta-lactams are not active against species that lack a cell wall (e.g. *Mycoplasma*) or those with very impenetrable walls such as mycobacteria, or intracellular pathogens such as *Brucella*, *Legionella* and *Chlamydia*.

Resistance to beta-lactams may involve one or more of the three possible mechanisms

Resistance by alteration in target site. Methicillin-resistant staphylococci (e.g. *Staphylococcus aureus, Staph. epidermidis* – MRSA, MRSE, respectively) synthesize an additional PBP (PBP2a), which has a much lower affinity for beta-lactams than the normal PBPs and is therefore able to continue cell wall synthesis when the other PBPs are inhibited. Although the *mecA* gene which codes for PBP2a is present on the chromosome in all cells of a resistant population, in many instances it may only be transcribed in a proportion of the cells, resulting in

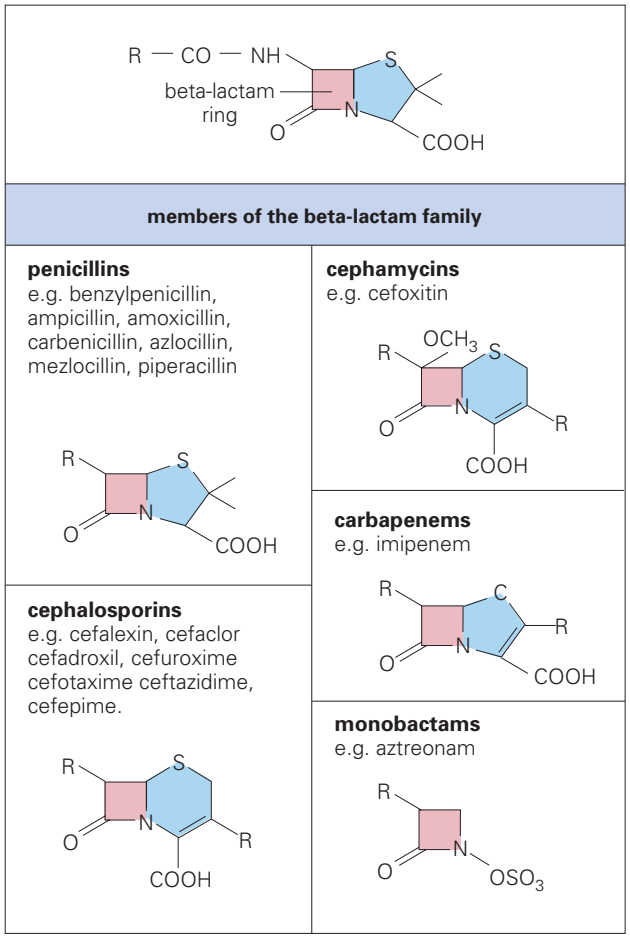


Figure 34.7 The beta-lactam family. The ring structure is common to all beta-lactams and must be intact for antibacterial action. Enzymes (beta-lactamases) that catalyse the hydrolysis of the beta-lactam bond render the agents inactive. The penicillins and cephalosporins are the major classes of beta-lactam antibiotics, but other members of the family and new beta-lactam beta-lactamase inhibitor combinations are the focus of new developments.

a phenomenon known as 'heterogeneous resistance'. In the laboratory, special cultural conditions are used to enhance expression and demonstrate resistance. Methicillin-resistant staphylococci commonly produce beta-lactamase (see below) and are resistant to all other beta-lactams with the exception of ceftaroline, a fifth-generation cephalosporin and the first approved by the US FDA for activity against MRSA. This cephalosporin binds to PBP2a with an affinity 2000-fold better than other beta-lactams, and is thus effective in treating infections caused by MRSA. Another fifth-generation cephalosporin, Ceftabiprole, has a similar spectrum of activity and is available in a number of countries.

Other organisms such as *Streptococcus pneumoniae*, *Neisseria gonorrhoeae* and *Haemophilus influenzae* may also utilize PBP changes to achieve beta-lactam resistance, which may vary depending on the compound employed.

Resistance by alteration in access to the target site. This mechanism is found in Gram-negative cells where beta-lactams gain access to their target PBPs by diffusion through protein channels (porins) in the outer membrane. Mutations in porin genes result in a decrease in permeability of the outer membrane and hence resistance. Strains resistant by this mechanism may exhibit cross-resistance to unrelated antibiotics that use the same porins.

Resistance by production of beta-lactamases. Beta-lactamases are enzymes that catalyse the hydrolysis of the beta-lactam ring to yield microbiologically inactive products. Genes encoding these enzymes are widespread in the bacterial kingdom and are found on the chromosome and on plasmids.

The beta-lactamases of Gram-positive bacteria are released into the extracellular environment (Fig. 34.8A) and resistance will only be manifest when a large population of cells is present. The beta-lactamases of Gram-negative cells, however, remain within the periplasm (Fig. 34.8B).

To date, hundreds of different beta-lactamase enzymes have been described. All have the same function but with differing amino acid sequences that influence their affinity for different beta-lactam substrates. Some enzymes specifically target penicillins or cephalosporins, while others are especially troublesome in broadly attacking most beta-lactam compounds (i.e. extended-spectrum beta-lactamases, ESBLs). Some beta-lactam antibiotics (e.g. carbapenems) are hydrolysed by very few enzymes (beta-lactamase stable), whereas others (e.g. ampicillin) are much more labile. Beta-lactamase inhibitors such as clavulanic acid are molecules that contain a beta-lactam ring and act as 'suicide inhibitors', binding to beta-lactamases and preventing them from destroying beta-lactams. They have little bactericidal activity of their own and are used in combination with beta-lactam antibiotics (Fig. 34.9).

Extended-spectrum beta-lactamases are especially problematic. The extended spectrum of new beta-lactam antibiotics has represented a survival challenge for the affected pathogens, which, unfortunately, they have been fully prepared to meet. Similar to events depicted in Fig. 34.3, new iterations of beta-lactam drugs have been met with mutations in genes encoding beta-lactamases (i.e. Extended-spectrum beta-lactamases; ESBLs) in Gram-negative bacteria. The carriage of such genes on plasmids has allowed their movement both within and between pathogenic species resulting in widespread dissemination of the associated drug resistance. At present, there is a myriad of such enzymes (e.g. TEM, SHV, CTX-M, OXA, beta-lactamases, and IMP, VIM, OXA, KPC, CMY and NDM-1 carbapenemases). An extended spectrum of activity coupled in some instances with resistance to beta-lactam beta-lactamase inhibitor combinations has produced organisms with few if any remaining therapeutic options. For this reason, as noted in the book introduction, carbapenem-resistant ESBL-producing Enterobacteriaceae are now categorized by the World Health Organization as critical and third on the list of 12 pathogens identified as priority for the research and development of new antibiotics.

Side effects

Toxic effects of beta-lactam drugs include mild rashes and immediate hypersensitivity reactions. Statistics regarding allergy to beta-lactam drugs are complicated by the fact that the problem historically involves self-reporting by patients who are often mistaken in their 'diagnosis'. Nevertheless, serious allergy to beta-lactam drugs in the form of an immediate (type 1) hypersensitivity reaction may occur in ca. 0.5–4% of patients, although anaphylaxis occurs much less frequently (c. 0.004 to 0.04% of penicillin treatment courses). Mild

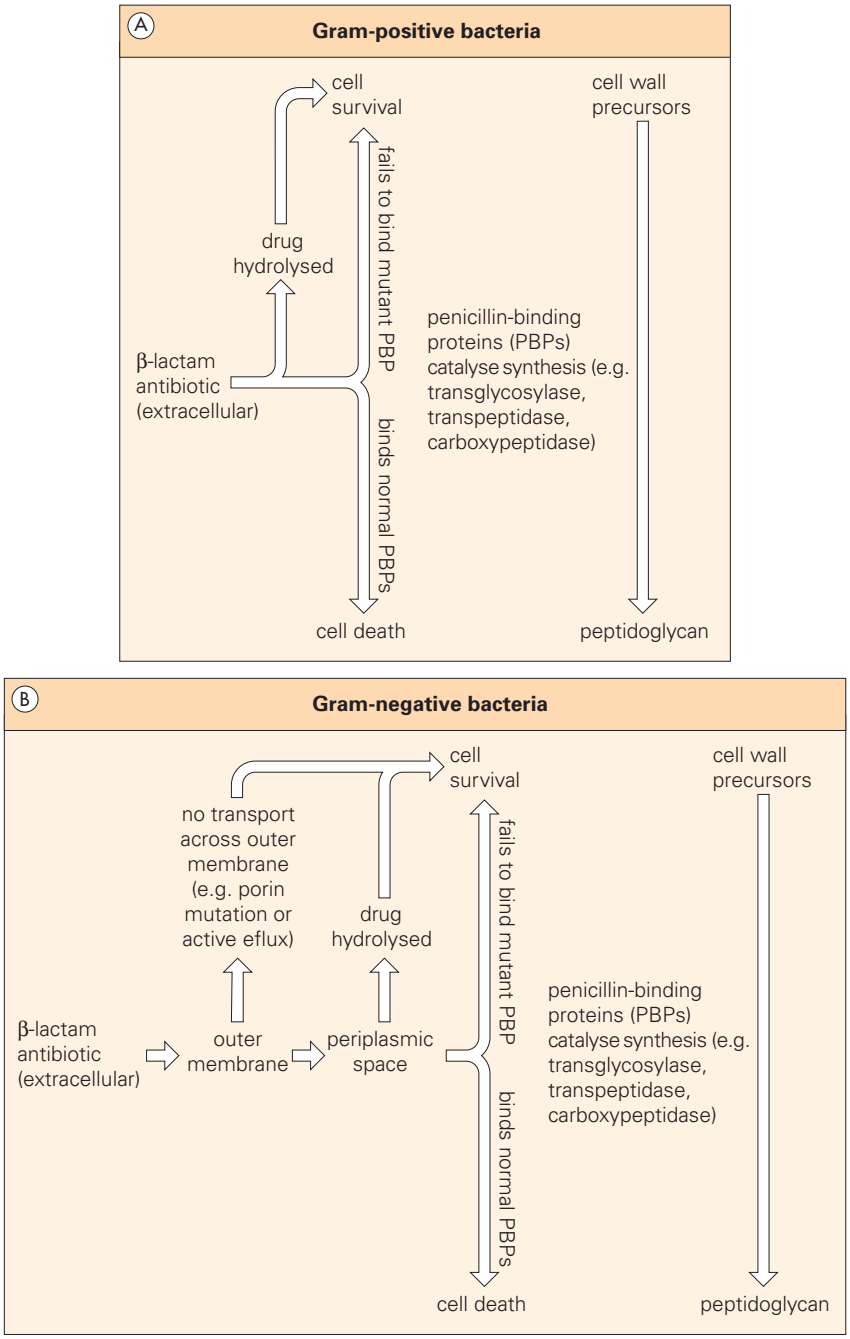


Figure 34.8 Penicillin-binding proteins (PBPs) play a key role in the final stages of peptidoglycan synthesis. They catalyse the cross-linkage of wall subunits, which are then incorporated into the cell wall. Beta-lactams are able to enter the cell (e.g. through pores in the outer membrane of Gram-negatives) and bind to the PBP. This prevents it from catalysing the cross-linkage of subunits, leading to their accumulation in the cell and the release of autolytic enzymes, which causes cell lysis. Within the periplasmic space of Gram-negatives (b1) beta-lactamases can inactivate beta-lactams before they reach their target PBPs, thereby protecting the cell from antibiotic action. Alternatively, mutant PBPs fail to bind with beta-lactams, thus allowing peptidoglycan synthesis to occur. In Gram-positive bacteria (b2) beta-lactams may be extracellularly destroyed by beta-lactamases or rendered ineffective, as in Gram-negatives, by mutant PBPs.

idiopathic reactions, usually in the form of a rash, are more common (>1% of treatment courses), especially with ampicillin. Patients who are allergic to penicillin are often allergic to cephalosporins (less with newer-generation compounds), but aztreonam, a monobactam, shows negligible cross-reactivity.

Neurotoxicity and seizures can occur with all the beta-lactams if improperly dosed for body weight and kidney function, especially in patients with renal impairment. This toxicity is manifest as fits, unconsciousness, myoclonic spasms and hallucinations. Carbenicillin can cause platelet dysfunction and sodium overload (because it is given as a sodium salt), especially in patients with liver failure, renal failure and congestive heart failure.

Glycopeptides

Glycopeptides are large molecules and act at an earlier stage than beta-lactams

Glycopeptides include vancomycin (with structurally related Oritavancin, Telavancin, and Dalbavancin) and teicoplanin. Both vancomycin and teicoplanin are very large molecules and therefore have difficulty penetrating into Gram-negative cells. Teicoplanin is a natural complex of five different but closely related molecules.

Glycopeptides are bactericidal and interfere with cell wall synthesis by binding to terminal D-alanine-D-alanine at the end of pentapeptide chains that are part of the growing bacterial cell wall structure (see Fig. 34.6). This binding inhibits the

Table 34.3 Characteristics of representative beta-lactams

Drug class	Category	General spectrum of activity
Penicillins		
Penicillin G, V[a]	Natural penicillin	Gram-positive bacteria
Nafcillin[a] Oxacillin[a]	Semisynthetic (beta-lactamase resistant) penicillin	Gram-positive bacteria (incl. beta-lactamase producers)
Amoxicillin[a,b] Ampicillin[a,b]	Semisynthetic (amino) penicillin	Gram-positive bacteria Gram-negative bacteria, including spirochetes, *Listeria monocytogenes, Proteus mirabilis* and some *Escherichia coli*
Carbenicillin[a] Mezlocillin Piperacillin[b]	Semisynthetic (carboxy) penicillin Semisynthetic (ureido) penicillin	Gram-positive bacteria Enhanced coverage of Gram-negatives, including *Pseudomonas* and *Klebsiella*
Cephalosporins		
Cefadroxil[a] Cefazolin Cephalexin[a]	First generation	Gram-positive bacteria
Cefaclor[a] Cefprozil[a] Cefuroxime[a]	Second generation	
Cefdinir[a] Cefditoren[a] Cefpodoxime[a] Cefotaxime Ceftazidime Ceftibuten[a] Ceftriaxone	Third generation	
Cefepime	Fourth generation	Improved activity against Gram-negative bacteria
Ceftolozane[b] Ceftabiprole Ceftaroline	Fifth generation	Improved activity against Gram-negative bacteria Improved activity, especially against MRSA
Cephamycin[c]		
Cefotetan Cefoxitin		Gram-positive bacteria Improved activity against *Bacillus fragilis*
Carbapenems		
Ertapenem Imipenem Meropenem Doripenem		Gram-positive and Gram-negative bacteria
Monobactams		
Aztreonam		Gram-negative bacteria including *Haemophilus influenza* and *Pseudomonas aeruginosa*

Although there are many beta-lactam agents available, the most commonly used ones are listed, together with their main indications.
[a]Oral formulation available.
[b]Can be formulated in combination with beta-lactamase inhibitors (see Fig. 34.9).
[c]Often classified with second-generation cephalospoxins.

transglycosylation reaction and prevents incorporation of new subunits into the growing cell wall. As glycopeptides act at an earlier stage than beta-lactams, it is not useful to combine glycopeptides and beta-lactams in the treatment of infections.

Vancomycin and teicoplanin must be given by injection for systemic infections

Vancomycin and teicoplanin are not absorbed from the gastrointestinal tract and do not penetrate the CSF in patients without meningitis. However, bactericidal concentrations are achieved in most patients with meningitis because of the increased permeability of the blood–brain barrier. Excretion is via the kidney.

Both vancomycin and teicoplanin are active only against Gram-positive organisms

Vancomycin and teicoplanin are used mainly for:

- the treatment of infections caused by Gram-positive cocci and Gram-positive rods that are resistant to beta-lactam

drugs, particularly multiresistant *Staphylococcus aureus* and *Staphylococcus epidermidis*
- for patients allergic to beta-lactams
- the treatment of *Clostridium difficile* in antibiotic-associated colitis, although concerns that this may promote emergence of glycopeptide-resistant enterococci in the gut flora have led to the increasing use of alternative compounds.

Resistance

Some organisms are intrinsically resistant to glycopeptides. As mentioned previously, Gram-negative bacteria are 'naturally' resistant to the glycopeptides, since these compounds are too large to efficiently move through the outer membrane to the peptidoglycan. Other organisms have an altered glycopeptide target, such as pentapeptides, terminating in D-alanine-D-lactate (e.g. *Erysiplothrix*, *Leuconostoc*, *Lactobacillus* and *Pediococcus*) or D-alanine-D-serine (e.g. *Enterococcus gallinarum*, *Enterococcus casseliflavus*).

Organisms may acquire resistance to glycopeptides. Historically, the most clinically relevant acquired glycopeptide resistance has been observed in *Enterococcus faecium* and *Enterococcus faecalis* (vancomycin-resistant enterococci; VRE), first reported by investigators in the UK in 1986. Since that time, a variety of resistance phenotypes have been described which can be differentiated by transferability (e.g. plasmid association), inducibility and extent of resistance (Table 34.4). The genes associated with the highest levels of glycopeptide resistance are *vanA*, *vanB*, and *vanD*, which encode ligase-producing pentapeptides terminating in D-alanine-D-lactate.

VanA *is the best understood mechanism of acquired glycopeptide resistance.* *VanA*-type glycopeptide resistance has been the most extensively studied and is characterized by inducible high-level resistance to both vancomycin and teicoplanin. *VanA* is associated with the transposable element *Tn1546* (ca. 11 kb in size), which may be carried either chromosomally or on a plasmid, the latter being transferable in nature.

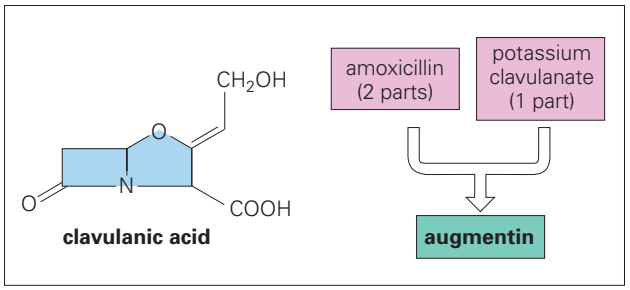


Figure 34.9 Clavulanic acid, a product of *Streptomyces clavuligerus*, inhibits the most common beta-lactamases (e.g. TEM enzymes) and allows amoxicillin to inhibit cells producing these enzymes. Augmentin is the most widely used of these combination drugs. Other combinations include ampicillin and sulbactam, piperacillin and tazobactam, ceftolozane and tazobactam, and ceftazidime and avibactam.

VanB is associated with inducible high-level resistance to vancomycin but not teicoplanin (although teicoplanin resistance can be induced by prior exposure to vancomycin). *VanB* resistance may be chromosomal or plasmid linked and is associated with very large transposable elements such as *Tn1549* (34 kb).

VanD is chromosomal in nature and thus non-transferable, resulting in constitutive resistance to high levels of vancomycin but low levels of teicoplanin.

Glycopeptide resistance in the staphylococci occurs by mutation or by acquisition from the enterococci. Within the coagulase-negative staphylococci (central nervous system [CNS]), *Staphylococcus epidermidis* and *Staph. haemolyticus* are especially prone to development of glycopeptide resistance by mechanisms which remain incompletely understood but likely include cell wall thickening. Resistant clinical and laboratory-generated isolates have been shown to differ from their susceptible counterparts in a variety of ways including changes in glycopeptide-binding capacity, membrane proteins and cell wall synthesis and composition.

Coagulase-positive staphylococci (i.e. *Staph. aureus*) showing decreased susceptibility to glycopeptides (but not fully resistant) were first described by Japanese investigators in 1996. The reduced susceptibility of these vancomycin-intermediate or glycopeptide-intermediate isolates (VISA or GISA, respectively) may be either homogeneously or heterogeneously expressed. In either case, 'resistance' is not associated with *VanA*, *B*, or *D* but, instead, involves other mechanisms affecting cell wall composition (e.g. leading to increased thickness, etc.).

Unfortunately, high-level glycopeptide resistance has also been observed in *Staph. aureus* (vancomycin-resistant *Staph. aureus*; VRSA) due to plasmid-associated movement of the *vanA* gene from VRE. Although highly troubling, this event has fortunately been rare (<20 isolates worldwide).

Side effects

The glycopeptides are potentially ototoxic and nephrotoxic. Vancomycin is usually given by intravenous infusion, administered slowly to avoid 'red-man' syndrome due to histamine release. Particular care must be taken to prevent toxic concentrations accumulating in patients with renal impairment. Oral vancomycin is used for treatment of antibiotic-associated pseudomembranous colitis due to *Clostridium difficile*. Teicoplanin is less toxic than vancomycin and can be given by intravenous bolus and by intramuscular injection.

INHIBITORS OF PROTEIN SYNTHESIS

Although protein synthesis proceeds in an essentially similar manner in prokaryotic and eukaryotic cells, it is possible to

Table 34.4 Characteristics of glycopeptide resistance in enterococci

Type	Resistance	Expression	Transmissible
VanA	Vancomycin Teicoplanin	Inducible	+
VanB	Vancomycin	Inducible	+
VanD	Vancomycin (variable) Teicoplanin (variable)	Constitutive	–

exploit the differences (e.g. 70 S versus 80 S ribosome) to achieve selective toxicity. The process of translation of the messenger RNA (mRNA) chain into its corresponding peptide chain is complex, and a range of antibacterial agents act as inhibitors (Fig. 34.10).

Aminoglycosides

The aminoglycosides are a family of related molecules with bactericidal activity

The aminoglycosides contain either streptidine (streptomycin) or 2-deoxystreptamine (e.g. gentamicin; Table 34.5). The

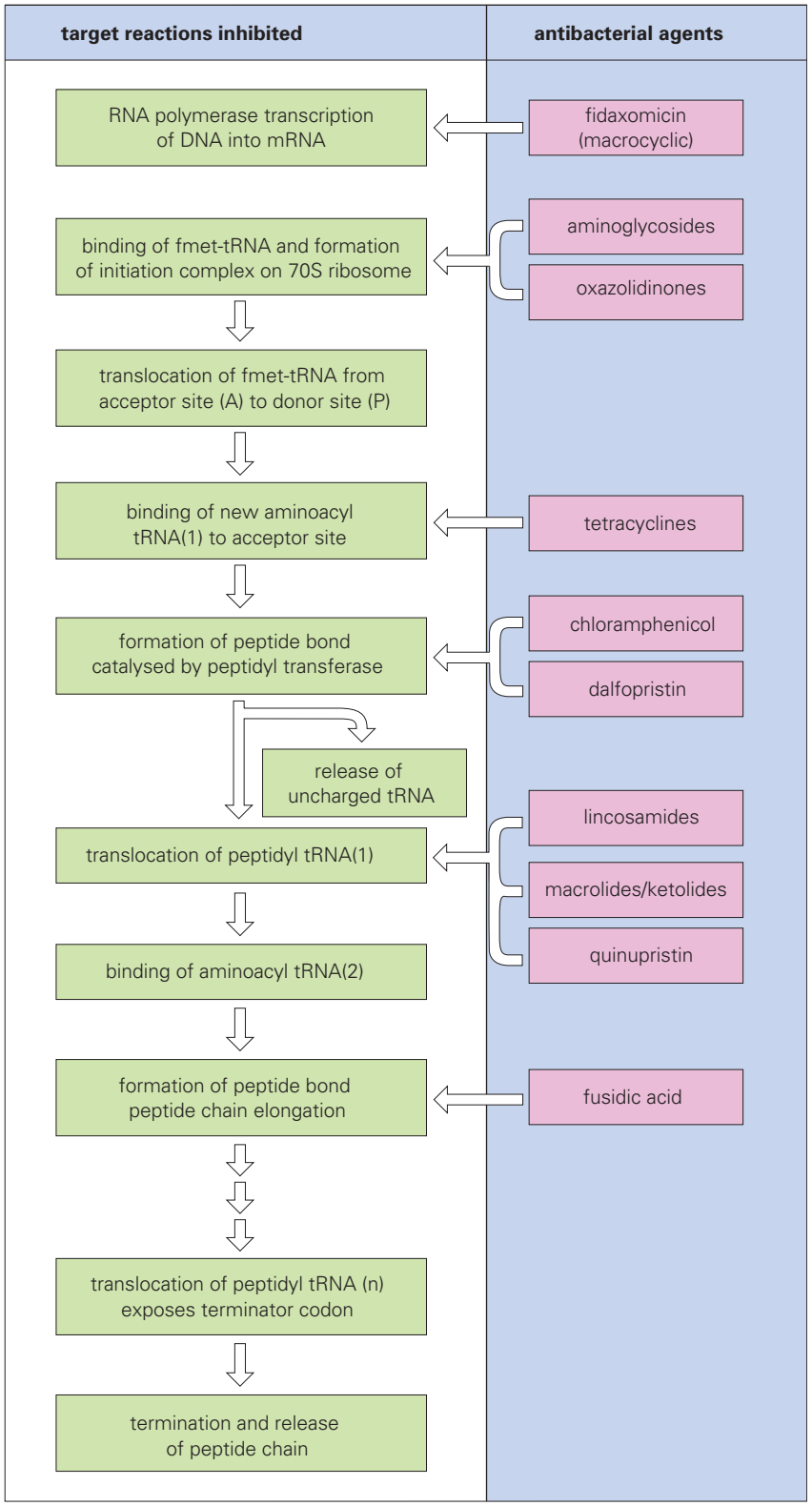


Figure 34.10 The synthetic pathway leading to the production of new protein in bacterial cells is extremely complex. A number of different groups of antibacterial agents act by inhibiting proteins with specific reactions in this synthetic pathway. The macrocyclic drug fidaxomycin inhibits the earliest step (mRNA transcription) while the others can be grouped into those that act on the 30 S subunit of the ribosome (e.g. aminoglycosides and tetracyclines) and those that act on the 50 S subunit (e.g. chloramphenicol, lincosamides, macrolides and fusidic acid). fmet-tRNA, formylmethionyl-transfer RNA.

Table 34.5 Aminoglycoside-aminocyclitol antibiotics classified according to their chemical structure

4,6-distributed 2-deoxystreptamines	
Gentamicin[a]	Complex of three closely related structures; first aminoglycoside with broad spectrum
Tobramycin[b]	Activity very similar to gentamicin but slightly better against *Pseudomonas aeruginosa*
Amikacin	Semisynthetic derivative of kanamycin; active against many gentamicin-resistant Gram-negative rods
4,5-disubstituted 2-deoxystreptamines	
Neomycin[b]	Too toxic for parenteral use but has topical uses in decontaminating mucosal surfaces
Streptidine-containing	
Streptomycin[b]	Oldest aminoglycoside; now use restricted to treatment of tuberculosis

They are also differentiated by the genus of microorganisms that produces them, and this is reflected in the spelling of the names.
[a]Micins from *Micromonospora* species.
[b]Mycins from *Streptomyces* species.

original structures have been modified chemically by changing the side chains to produce molecules such as amikacin and netilmicin that are active against organisms that have developed resistance to earlier aminoglycosides.

Aminoglycosides act by binding to specific proteins in the 30 S ribosomal subunit, where they interfere with the binding of formylmethionyl-transfer RNA (fmet-tRNA) to the ribosome (Fig. 34.10), thereby preventing the formation of initiation complexes from which protein synthesis proceeds. In addition, aminoglycosides cause misreading of mRNA codons and tend to break apart functional polysomes (protein synthesis by multiple ribosomes tandemly attached to a single mRNA molecule) into non-functional monosomes.

Aminoglycosides must be given intravenously or intramuscularly for systemic treatment

Aminoglycosides are not absorbed well from the gut, do not penetrate well into tissues and bone, and do not cross the blood–brain barrier. Thus, they are usually administered as an intravenous infusion. Intrathecal administration of streptomycin is used in the treatment of tuberculous meningitis while gentamicin and amikacin may be administered by this route in the treatment of Gram-negative meningitis in neonates. Aminoglycosides are excreted via the kidney.

Gentamicin and the newer aminoglycosides are used to treat serious Gram-negative infections

Gentamicin, tobramycin, and amikacin are important for the treatment of serious Gram-negative infections, including those caused by *P. aeruginosa* (Box 34.4). They are not active against streptococci or anaerobes, but are active against staphylococci. Against *P. aeruginosa,* amikacin is most active. Amikacin may be active against strains resistant to gentamicin and tobramycin (see below). Streptomycin is now reserved almost entirely for the treatment of mycobacterial infections. Neomycin is not used for systemic treatment, but can be used orally in gut decontamination regimens in neutropenic patients.

Box 34.4 Indications for Aminoglycoside Therapy

Aminoglycosides are valuable additions to the clinician's armamentarium despite their potential toxicity. They are important agents active against Gram-negative facultative bacteria and are often used in combination with beta-lactams to broaden the spectrum to include streptococci and some anaerobes which are not susceptible to aminoglycosides alone. Resistance to aminoglycosides, particularly among enterobacteria and staphylococci, is mediated by the production of aminoglycoside-modifying enzymes, which react with groups on the aminoglycoside molecule to yield an altered aminoglycoside product. This competes with the unmodified aminoglycoside for uptake into the cell and binding to the ribosome.

Basic rule: use only in severe, life-threatening infections

- Gram-negative septicaemia (including *Pseudomonas*) usually in combination with beta-lactam
- Septicaemia of unknown aetiology arising from:[a]
  - healthcare-associated respiratory infections
  - major trauma, major surgery or major burns
  - intravenous catheter
  - complicated urinary catheter-associated infections
- Bacterial endocarditis for synergy with beta-lactam
- *Staphylococcus aureus* septicaemia in combination with beta-lactam
- Pyelonephritis for difficult cases
- Post-surgical abdominal sepsis in combination with anti-anaerobe therapy.

Production of aminoglycoside-modifying enzymes is the principal cause of resistance to aminoglycosides

Although relatively uncommon, resistance to aminoglycoside antibiotics may occur by alteration of the 30 S ribosomal target protein (e.g. a single amino acid change in the P12 protein prevents streptomycin binding). In addition, methylation of 16S ribosomal RNA can prevent aminoglycoside binding to the ribosomal aminoacyl site. Resistance may also arise through alterations in cell wall permeability or in the energy-dependent transport across the cytoplasmic membrane.

Production of aminoglycoside-modifying enzymes is the most important mechanism of acquired resistance (Fig. 34.11). The genes for these enzymes are often plasmid-mediated, located on transposons, and transferable from one bacterial species to another. The enzymes alter the structure of the aminoglycoside molecule, thus inactivating the drug. The type of enzyme determines the spectrum of resistance of the organism containing it.

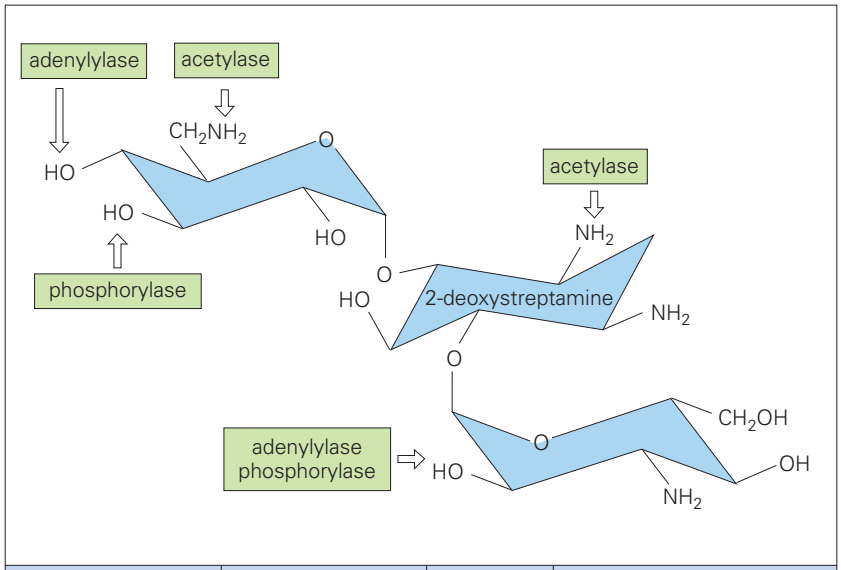


Figure 34.11 Prototype structure of aminoglycoside consisting of aminohexoses linked via glycosidic linkage to a central 2-deoxystreptamine nucleus. Hydroxyl and amino groups are sites at which these compounds can be inactivated by phosphorylation, adenylation or acetylation catalysed by enzymes produced by resistant strains.

aminoglycoside-modifying enzyme	reactive group on aminoglycoside	co-factor	modified aminoglycoside product
acetylase	$-NH_2$	acetyl CoA	–NHAc
adenylylase (nucleotidyl transferase)	–OH	ATP	–O–AMP
phosphorylase	–OH	ATP	$-O-PO_2-OH$

The aminoglycosides are potentially nephrotoxic and ototoxic

The therapeutic 'window' between the serum concentration of aminoglycoside required for successful treatment and that which is toxic is small. Blood concentrations should be monitored regularly, particularly in patients with renal impairment.

Tetracyclines

Tetracyclines are bacteriostatic compounds that differ mainly in their pharmacological properties rather than in their antibacterial spectra

Tetracyclines are a family of large cyclic structures that have several sites for possible chemical substitutions (Fig. 34.12).

Tetracyclines inhibit protein synthesis by binding to the small ribosomal subunit in a manner that prevents aminoacyl transfer RNA from entering the acceptor sites on the ribosome (see Fig. 34.10). While this process may occur with both prokaryotic and eukaryotic ribosomes, the selective action of tetracyclines is due to their much greater uptake by prokaryotic cells.

Tetracyclines are usually administered orally. Doxycycline and minocycline are more completely absorbed than tetracycline and so result in higher serum concentrations and less gastrointestinal upset because there is less inhibition of normal gut flora. Tetracyclines are well distributed and penetrate host cells to inhibit intracellular bacteria. They are excreted primarily in bile and urine.

Tetracyclines are active against a wide variety of bacteria, but their use is restricted due to widespread resistance

Tetracyclines are used in the treatment of infections caused by mycoplasmas, chlamydiae and rickettsiae. Resistance in other genera is common, due partly to the widespread use of these drugs in humans and also to their use as growth promoters in animal feed. The resistance genes are carried on a transposon thus facilitating their spread, and new cytoplasmic membrane proteins are synthesized in the presence of tetracycline. As a result, tetracycline is positively pumped out of resistant cells (efflux mechanism). Although included with the tetracyclines (see Fig. 34.12), tigecycline is a member of a related class of compounds (glycylcyclines), derived from minocycline, with activity against bacteria resistant to tetracyclines.

Tetracyclines should be avoided in pregnancy and in children under 8 years of age

Tetracyclines suppress normal gut flora, resulting in gastrointestinal upset and diarrhoea and encouraging overgrowth by resistant and undesirable bacteria (e.g. *Staph. aureus*) and fungi (e.g. *Candida*).

Interference with bone development and brown staining of teeth occurs in the fetus and in children. Systemic administration may cause liver damage. The potential for photosensitization is another caveat associated with the use of tetracyclines in all patients.

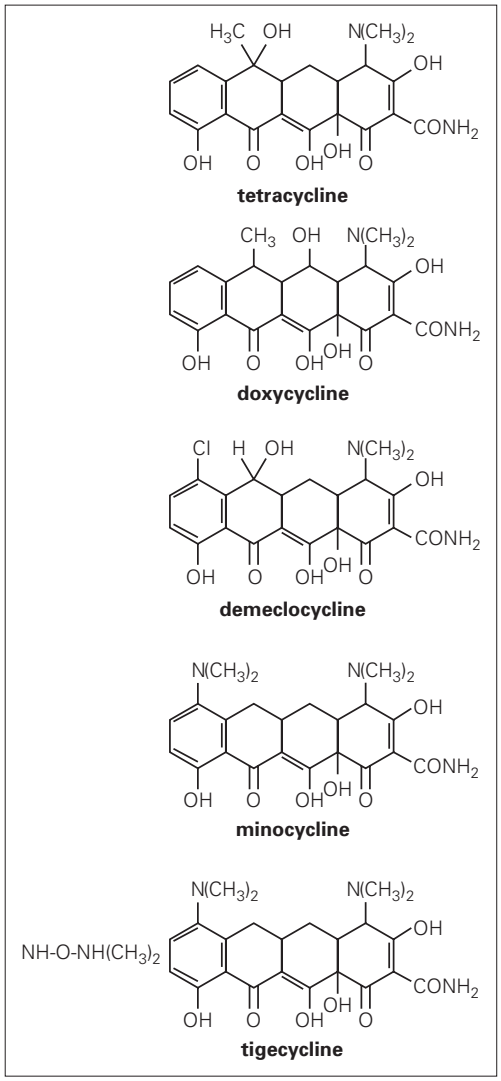


Figure 34.12 Tetracyclines are four-ring molecules with different sites around the rings for substitution, thereby giving rise to a family of molecules with different substituents at different sites. Members of the family differ more in their pharmacological properties than in their spectrum of activity.

Chloramphenicol

Chloramphenicol contains a nitrobenzene nucleus and prevents peptide bond synthesis, with a bacteriostatic result

Chloramphenicol is a relatively simple molecule containing a nitrobenzene nucleus, which is responsible for some of the toxic problems associated with the drug (see below). Other derivatives have been produced, but none is in widespread clinical use.

Chloramphenicol has affinity for the large (50 S) ribosomal subunit where it blocks the action of peptidyl transferase, thereby preventing peptide bond synthesis (see Fig. 34.10). The drug has some inhibitory activity on human mitochondrial ribosomes (which are also 70 S), which may account for some of the dose-dependent toxicity to bone marrow (see below).

Chloramphenicol is well absorbed when given orally, but can be given intravenously if the patient cannot take drugs by mouth. Topical preparations are also available. It is well distributed in the body and penetrates host cells. Chloramphenicol is metabolized in the liver by conjugation with glucuronic acid to yield a microbiologically inactive form that is excreted by the kidneys.

Resistance and toxicity have limited the use of chloramphenicol

Chloramphenicol has been used in the treatment of bacterial meningitis (particularly *H. influenzae*) since the drug achieves satisfactory concentrations in the CSF. Chloramphenicol is active against a wide variety of bacterial species, both Gram-positive and Gram-negative, aerobes and anaerobes, including intracellular organisms. However, its potential serious toxic effects (see below) and issues of resistance have all but eliminated the systemic use of chloramphenicol in countries where alternative agents are readily available.

The most common mechanism of chloramphenicol resistance involves the inactivation of the drug by a plasmid-mediated enzymatic mechanism which is easily transferred within Gram-negative bacterial populations. Chloramphenicol acetyl transferases produced by resistant bacteria are intracellular, but are capable of converting chloramphenicol in the immediate environment of the cell to an inactive form that fails to bind to the ribosomal target.

The most important toxic effects of chloramphenicol are in the bone marrow

Nitrobenzene is a bone marrow suppressant, and the structurally similar chloramphenicol molecule has similar effects. This toxicity takes two forms:

- dose-dependent bone marrow suppression, which occurs if the drug is given for long periods and is reversible when treatment is stopped
- an idiosyncratic reaction causing aplastic anaemia, which is not dose dependent and is irreversible. It can occur after treatment has stopped, but is fortunately rare.

Chloramphenicol is also toxic to neonates, particularly premature babies whose liver enzyme systems are incompletely developed. This can result in 'grey baby syndrome'. Thus, chloramphenicol serum concentrations should be monitored in neonates.

Macrolides, lincosamides and streptogramins

These three groups of antibacterial agents share overlapping binding sites on ribosomes, and resistance to macrolides confers resistance to the other two groups.

Macrolides

Erythromycin is a widely used macrolide preventing the release of transfer RNA after peptide bond formation. The macrolides are a family of large cyclic molecules all containing a macrocyclic lactone ring (Fig. 34.13A) and are bacteriostatic in activity. Erythromycin is the best known but the newer agents, azithromycin and clarithromycin, have fewer side effects and improved activity and pharmacology.

Macrolides bind to the 23 S ribosomal RNA (rRNA) in the 50 S subunit of the ribosome and block the translocation

step in protein synthesis, thereby preventing the release of transfer RNA after peptide bond formation (see Fig. 34.10).

Macrolides are usually administered by the oral route, but can also be given intravenously. They are well distributed in the body and penetrate to reach intracellular organisms. The drugs are concentrated in the liver and excreted in the bile. A small proportion of the dose is recoverable in the urine.

Macrolides are an alternative to penicillin for streptococcal infections, but resistant strains of streptococci are common. Macrolides are active against Gram-positive cocci and an important alternative treatment of infections caused by streptococci in patients allergic to penicillin. They are active against *Legionella pneumophila* and *Campylobacter jejuni*. They are also active against *Mycoplasma* and *Chlamydia* spp. and are therefore important drugs in the treatment of atypical pneumonia and chlamydial infections of the urogenital tract.

Resistance is primarily due to either plasmid-encoded *mef* or *erm* genes, for efflux or alteration in the 23 S rRNA target by methylation of two adenine nucleotides in the RNA, respectively. The methylase enzyme may be either inducible or constitutively expressed. Macrolides are better inducers of resistance than the lincosamides, but strains resistant to macrolides will also be resistant to lincomycin and clindamycin, so-called 'MLS (macrolide-lincosamide-streptogramin) resistance'. Induction also varies between bacterial species, and resistant strains of Gram-positive cocci such as staphylococci and streptococci are common. In contrast to methylation, efflux while active against macrolide and streptogramin B antibiotics does not affect streptogramin A and lincosamide drugs.

Newer macrolide-related (macrocycle) drugs show promise for targeted therapy

The term macrocycle generally refers to compounds with a ring structure containing at least eight atoms. This includes the macrolides but also a newer class of compounds termed macrocyclic antibiotics. Fidaxomicin is a newly approved member of this group. This orally administered bactericidal

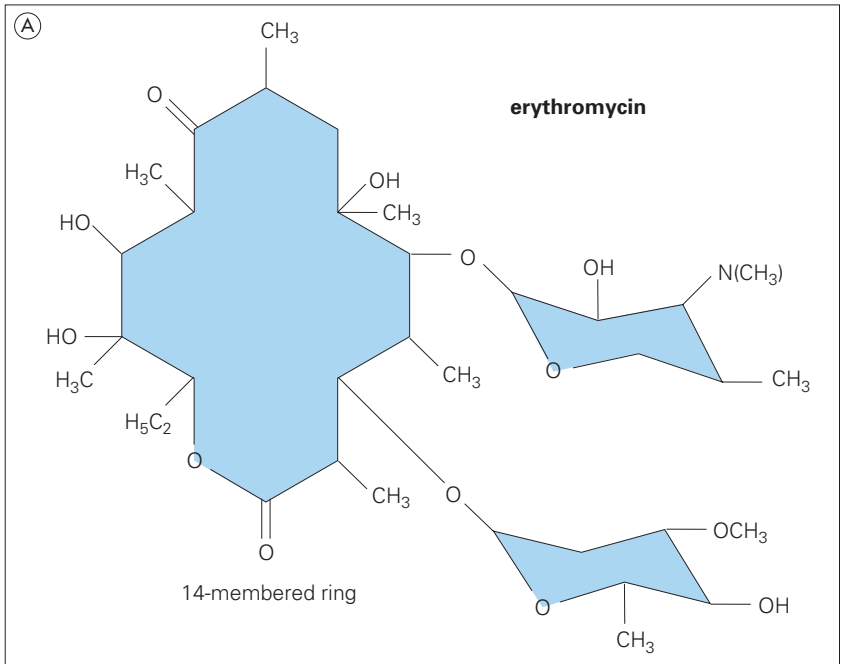
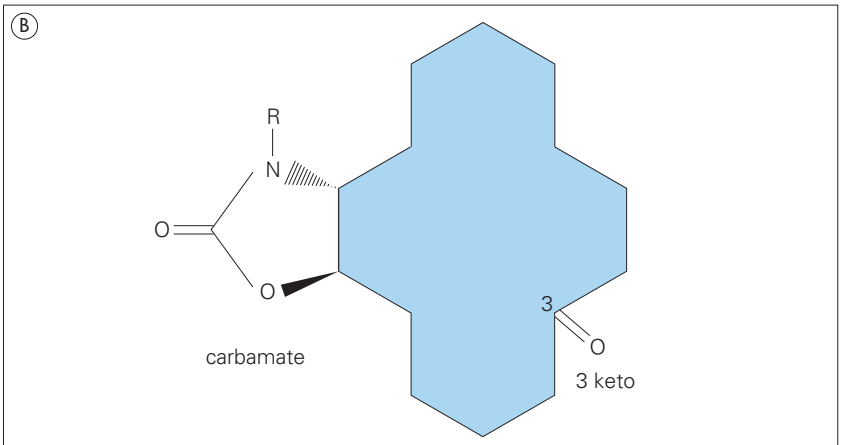


Figure 34.13 (A) The macrolides are antibacterial agents composed of large structures, which may be 14-, 15- or 16-membered rings. Erythromycin is the oldest of these and newer agents with improved activity and fewer side effects are available.

newer macrolides	in vitro activity compared with erthyromycin	human pharmacokinetics
azithromycin (15-membered ring)	improved against Gram-negative bacteria	high tissue concentrations, once-daily administration
clarithromycin (14-membered ring)	improved against Gram-positive bacteria and *Legionella* spp.	improved peak serum concentration compared with erythromycin
new macrocyclic		
fidaxomicin	specifically targets *C. difficile*	minimal systemic adsorption

Figure 34.13—cont'd (B) Major differences in ketolide chemical structure compared with erythromycin (i.e. positions of 3-keto and carbamate on the 'backbone' ring structure).



new ketolide	in vitro activity compared with erthyromycin	human pharmacokinetics
telithromycin (14-membered ring)	improved activity against respiratory pathogens including *Streptococcus pneumoniae*	high and sustained concentrations in bronchopulmonary tissues and fluids with once daily oral dose concerns regarding hepatotoxicity and myasthenia gravis

compound is interesting in its specific targeting of the problem pathogen *Clostridium difficile* (see Ch. 23) without major disturbance of the intestinal microbiota. The drug acts by interfering at the earliest stage of protein synthesis (mRNA transcription) inhibiting bacterial RNA polymerase (Fig. 34.10).

Ketolides are semisynthetic derivatives of erythromycin with improved activity against respiratory pathogens

Modification of the macrolide ring structure (Fig. 34.13B) provides ketolides with increased activity against a variety of Gram-positive (and some Gram-negative) bacteria, especially those associated with respiratory infections. Ketolides are administered orally and act in a manner similar to erythromycin. However, their higher affinity for the 50 S ribosomal subunit allows them to bind to ribosomes, which are resistant to erythromycin. While active against methicillin-susceptible *Staph. aureus* that are either susceptible or inducibly resistant to erythromycin, ketolide activity is poor against erythromycin-resistant MRSA. In addition, telithromycin has had major issues related to hepatotoxicity and exacerbations of myasthenia gravis.

Lincosamides

Clindamycin inhibits peptide bond formation. Clindamycin is a chlorinated more active derivative of the lincosamide lincomycin and represents the most important and most clinically used drug in this class.

Lincosamides bind to the 50 S ribosomal subunit and inhibit protein synthesis in a manner similar to macrolides (see Fig. 34.10), hence the MLS resistance combination noted above. The selectively toxic action results from a failure to bind to the equivalent mammalian ribosomal subunit.

Clindamycin is usually given orally, but can be administered intramuscularly or intravenously. It penetrates well into bone, but not into CSF, even when the meninges are inflamed. Clindamycin is actively transported into polymorphonuclear leukocytes and macrophages. It is metabolized in the liver to several products with variable antibacterial activity, and clindamycin activity persists in faeces for up to 5 days after a dose.

Clindamycin has a spectrum of activity similar to that of erythromycin. Clindamycin is much more active than macrolides against anaerobes, both Gram-positive (e.g. *Clostridium* spp.) and Gram-negative (e.g. *Bacteroides*). However, *C. difficile* is often resistant and may be selected in the gut, causing pseudomembranous colitis (see below). The activity of clindamycin against *Staph. aureus* and its penetration into bone make it an option for the treatment of osteomyelitis. Clindamycin is not active against aerobic Gram-negative bacteria because of poor penetration of the outer membrane.

As clindamycin is a less potent inducer of 23 S rRNA methylase (see MLS resistance above), erythromycin-resistant strains may appear susceptible to clindamycin in vitro. However, resistance will be manifest in vivo.

Pseudomembranous colitis caused by* C. difficile *was first noted following clindamycin treatment. Pseudomembranous colitis caused by *C. difficile* follows treatment with many antibiotics. The pathogenesis of this complication is described in Chapter 23, and it should be treated with drugs such as metronidazole, oral vancomycin, or fidaxomicin.

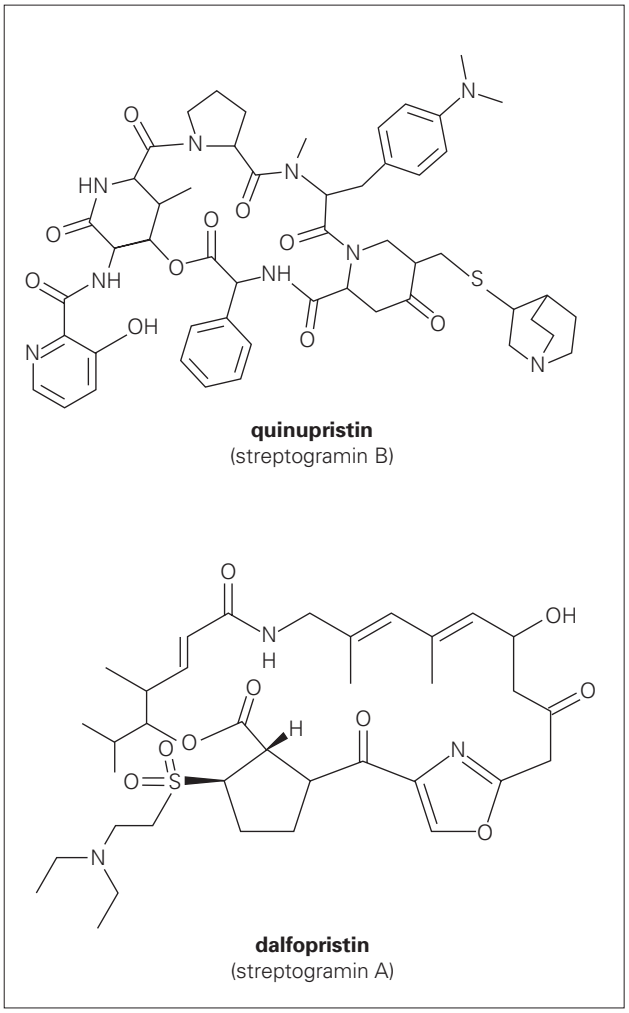


Figure 34.14 Chemical structure of the streptogramins.

Streptogramins

The streptogramin formulation currently available is a mixture of streptogramin B and A compounds – quinupristin and dalfopristin, respectively (Fig. 34.14) – that are bacteriostatic individually but synergistically bactericidal in combination. Both compounds bind to 23 S RNA in the large (50 S) ribosomal subunit (dalfopristin facilitates binding of quinupristin). Dalfopristin inhibits protein synthesis at an earlier stage than quinupristin (see Fig. 34.10), and they together interfere with elongation and extension of peptide chains.

Resistance but may develop by altering the quinupristin binding site (MLS resistance described above), enzymatic inactivation, or efflux.

The quinupristin–dalfopristin combination is active against Gram-positive cocci, including multidrug-resistant isolates. Activity is good against *Enterococcus faecium* but not *E. faecalis* (most probably due to an intrinsic efflux mechanism). However, there has been concern that commercial use of streptogramin compounds (e.g. virginiamycin) to prevent disease and promote growth in poultry could contribute to quinupristin–dalfopristin resistance among Gram-positive pathogens in humans.

Quinupristin–dalfopristin is administered intravenously and primarily metabolized in the liver.

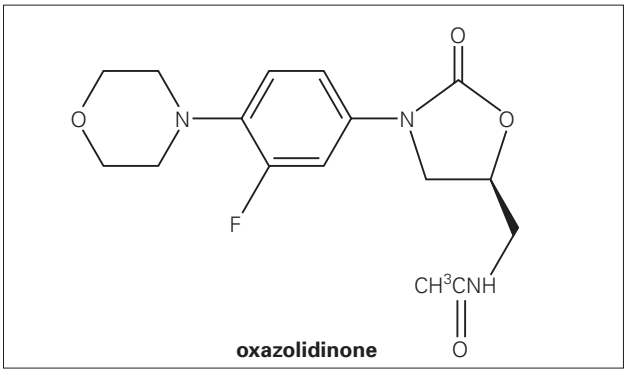


Figure 34.15 Chemical structure of oxazolidinones.

Oxazolidinones

Oxazolidinones are a newer class of synthetic bacteriostatic antimicrobial agents (Fig. 34.15). Linezolid is active against a wide range of Gram-positive bacteria, including multiresistant strains. Linezolid inhibits initiation of protein synthesis (see Fig. 34.10) by targeting 23 S ribosomal RNA in the 50 S subunit in a manner which prevents formation of a functional 70 S complex. Linezolid is administered orally or intravenously and is metabolized in the liver. A newer oxazolidinone tedizolid is administered and acts similarly to linezolid but appears to have lower haematological toxicity. Due to the unique oxazolidinone mechanism of action, emergence of resistance is low.

Fusidic acid

Fusidic acid is a steroid-like compound that inhibits protein synthesis

Fusidic acid is a bacteriostatic agent that inhibits protein synthesis by forming a stable complex with elongation factor EF-G (the bacterial equivalent of the human EF-2), guanosine diphosphate and the ribosome.

Fusidic acid can be administered orally or intravenously. It is well absorbed and penetrates well into tissues and bone, but not into the CSF. Topical preparations are also available, but their use should not be encouraged, because of the rapid emergence of resistance (see below). Fusidic acid is metabolized in the liver and excreted in the bile.

Fusidic acid is a treatment for staphylococcal infections, but should be used with other antistaphylococcal drugs to prevent emergence of resistance

Fusidic acid is active against Gram-positive cocci, and its most important use is in the treatment of staphylococcal infections resistant to beta-lactams or in patients who are allergic to alternative staphylococcal agents. Fusidic acid should be given in combination with another antistaphylococcal agent to prevent the emergence of resistant mutants with altered EF-G, which can emerge rapidly in staphylococcal populations exposed to the drug.

Fusidic acid has few side effects

Occasionally, fusidic acid causes jaundice and gastrointestinal upset.

INHIBITORS OF NUCLEIC ACID SYNTHESIS

Antibacterial agents that act as inhibitors of nucleic acid synthesis do so in one of three main ways, as listed in Box 34.5.

Quinolones

Quinolones are synthetic agents that interfere with replication of the bacterial chromosome

Quinolones represent a large family of bactericidal synthetic agents which, in a manner similar to the cephalosporins, are sometimes discussed in terms of 'generations' based on their spectrum of activity. However, these categories are less clear than for the cephalosporins. Nalidixic acid was the first-generation prototype, but the addition of fluorine at position 6 of the main quinolone ring (i.e. fluoroquinolones – e.g. ciprofloxacin, moxifloxacin) (Fig. 34.16) has improved antibacterial activity, leading to the synthesis of many additional, more commonly used, compounds.

The antibacterial activity of quinolones is due to their ability to inhibit the activity of bacterial DNA gyrase and topoisomerases. During replication of the bacterial chromosome, DNA gyrase produces and removes supercoils in DNA ahead of the replication fork to maintain the proper 'tension' required for efficient DNA duplication. Topoisomerase IV similarly acts to remove supercoils and to separate newly formed DNA 'daughter' strands after replication (Fig. 34.17). These enzymes thus act in concert to insure that the DNA molecule has the proper conformation for efficient replication and packaging within the cell. Quinolones are able to interfere with these essential enzymes in bacteria while not affecting their counterparts in mammalian cells.

Resistance to quinolones is usually chromosomally mediated

Chromosomally mediated resistance is exhibited in two forms:

- mutations, which change the target enzymes in a manner that affects quinolone binding
- changes in cell wall permeability, resulting in decreased uptake, or by efflux. These mechanisms may also lead to cross-resistance to other unrelated agents affected by the same process.

Plasmid-encoded quinolone resistance involves production of a protein (termed qnr) that protects the target DNA from quinolone binding.

Box 34.5 Inhibitors of Nucleic Acid

Inhibition of nucleic acid takes place at different stages in its synthesis and function, and different groups of antimicrobial agents are involved.

Inhibitors of DNA replication

- Quinolones

Inhibitors of RNA polymerase

- Rifampicin

Antimetabolites inhibiting precursor synthesis

- Sulphonamides, trimethoprim

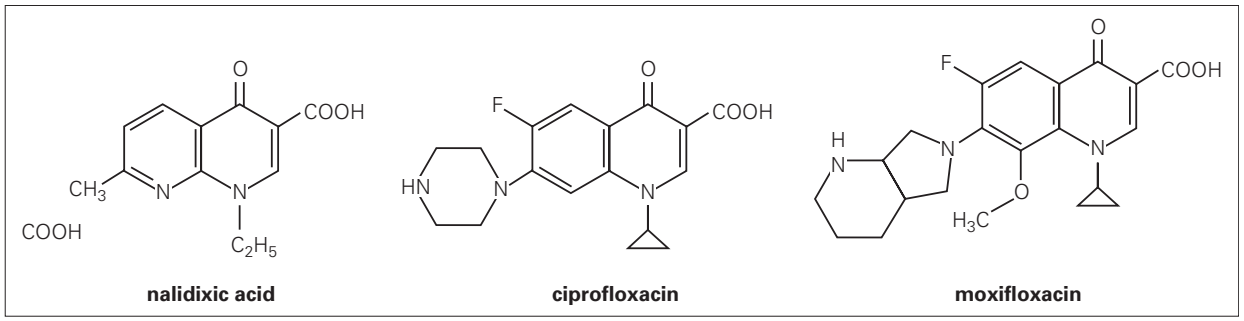


Figure 34.16 The quinolones form a large group of synthetic antibacterial agents.

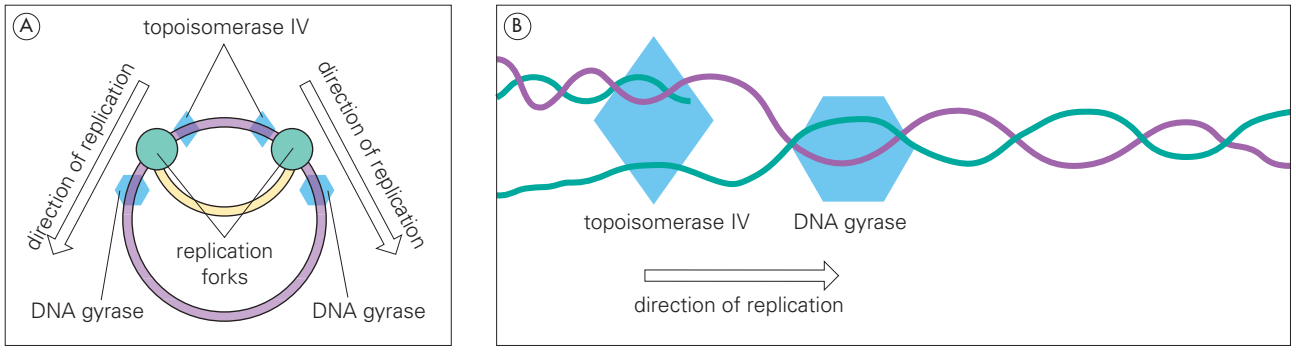


Figure 34.17 (A) An overview and (B) an enlarged view of the role played by bacterial gyrase and topoisomerase enzymes in replication of the bacterial chromosome.

Quinolones are used as alternatives to beta-lactam antibiotics for treating a variety of infections

Quinolones are primarily administered orally since they are readily absorbed from the gastrointestinal tract, achieving significant serum concentrations and good distribution throughout the body compartments.

Ciprofloxacin, gemifloxacin, levofloxacin, moxifloxacin, and ofloxacin are the drugs most commonly used. Excretion is mostly in the urine; however, drugs such as moxifloxacin are excreted to a significant amount in faeces.

The newer quinolones have improved activity against Gram-negative rods, including *P. aeruginosa*. In addition to the treatment of urinary tract infections, the newer quinolones are useful for systemic Gram-negative infections and in the treatment of chlamydial and rickettsial infections. They are also useful in infections caused by other intracellular organisms, such as *L. pneumophila* and *S. typhi*, and in combination with other agents for 'atypical' mycobacteria. They have activity against staphylococci but many strains of methicillin-resistant *Staph. aureus* now exhibit high-level resistance and there is limited use against streptococci and enterococci.

Fluoroquinolones are not recommended for children or pregnant or lactating women because of possible toxic effects on cartilage development

Gastrointestinal disturbances are the most common side effect of quinolones. Neurotoxicity and photosensitivity reactions are less common. All fluoroquinolones have the potential to cause tendon ruptures in active patients who may tend to push their workout regimens. This risk is increased when quinolones and corticosteroids are simultaneously administered.

Rifamycins

Rifampicin is clinically the most important rifamycin and blocks the synthesis of mRNA

Rifampicin is the most important member of the rifamycin family in clinical use. It is a large molecule with a complex structure. Other family members such as rifabutin and rifapentine are also available. All are bactericidal in activity.

Rifampicin binds to DNA-dependent RNA polymerase and blocks the synthesis of mRNA. Selective toxicity is based on the far greater affinity for bacterial polymerases than for the equivalent human enzymes.

Rifampicin is administered orally, is well absorbed and is very well distributed in the body. It crosses the blood–brain barrier and reaches high concentrations in saliva. It also appears to have an affinity for plastics, which can be valuable in the treatment of infections involving prostheses.

Rifampicin is metabolized in the liver and excreted in bile. The compound is red, and the urine, sweat and saliva of treated patients turn orange. This is harmless, although disturbing for the patient, but is good evidence of patient compliance.

The newer rifamycins, rifabutin and rifapentine are excreted more slowly than rifampicin, thereby allowing less frequent administration – a feature particularly attractive in the treatment of tuberculosis.

The primary use for rifampicin is in the treatment of mycobacterial infections, but resistance is a concern

While used primarily against mycobacteria, rifampicin may also be used for the prophylaxis of close contacts of meningococcal and *Haemophilus meningitis*. However, highly resistant meningococcal strains may emerge; thus short courses only (maximum 48 h) should be given.

While staphylococci rapidly develop resistance to rifampicin, the drug can be efficacious if used in combination with another agent, particularly in the treatment of prosthetic valve endocarditis.

Resistance is provided by chromosomal mutations that alter the RNA polymerase target, which then has lowered affinity for rifampicin and escapes inhibition. The prevalence of rifampicin-resistant *M. tuberculosis* is increasing, which is problematic for antituberculosis therapy.

Rashes and jaundice are side effects of rifampicin treatment

Intermittent rifampicin can lead to hypersensitivity reactions.

ANTIMETABOLITES AFFECTING NUCLEIC ACID SYNTHESIS

Several commonly used antimicrobial agents inhibit bacterial metabolic pathways including those which produce precursors for nucleic acid synthesis.

Sulphonamides

Sulphonamides are structural analogues of and act in competition with *para*-aminobenzoic acid

This group of molecules is produced entirely by chemical synthesis (i.e. they are not natural products). In 1935, the parent compound sulphanilamide became the first clinically effective antibacterial agent. The *p*-amino group is essential for activity, but modifications to the sulphonic acid side chain have produced many related agents (Fig. 34.18).

Sulphonamides are bacteriostatic compounds that act in competition with *para*-aminobenzoic acid, PABA, for the active site of dihydropteroate synthetase, an enzyme that catalyses an essential reaction in the synthetic pathway of tetrahydrofolic acid (THFA), which is required for the synthesis of purines and pyrimidines and therefore for nucleic acid synthesis (Fig. 34.19). Selective toxicity depends on the fact that many bacteria synthesize THFA, whereas human cells lack this capacity and depend on an exogenous supply of folic acid. Bacteria that can use preformed folic acid are similarly unaffected by sulphonamides.

Sulphonamides are usually administered orally, often in combination with trimethoprim as co-trimoxazole (see below). Different molecules within the family differ in their solubility and penetrability. Metabolism occurs in the liver, and free and metabolized drug are excreted by the kidneys.

Sulphonamides are useful in the treatment of urinary tract infection, but resistance is widespread

The sulphonamides have a spectrum of activity primarily against Gram-negative organisms (except *Pseudomonas*). They are therefore useful in the treatment of urinary tract infections (see Ch. 21). However, susceptibility cannot be assumed, as resistance is widespread with plasmid-mediated genes coding for an altered dihydropteroate synthetase. This is essentially unchanged in its affinity for PABA, but has a greatly decreased affinity for the sulphonamide. A resistant

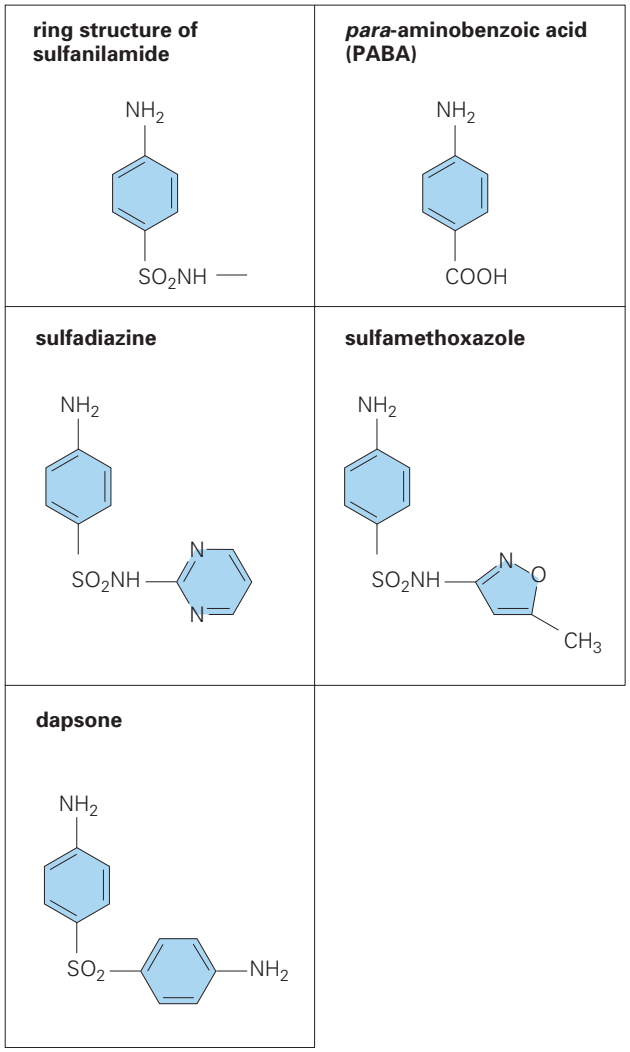


Figure 34.18 The ring structure of the sulphonamides is very similar to the structure of the normal substrate (PABA) of the dihydropteroate synthetase enzyme, which the sulphonamides inhibit. The sulphonamides differ in their pharmacological properties more than in their spectrum of activity. Dapsone is important in the treatment of *Mycobacterium leprae*.

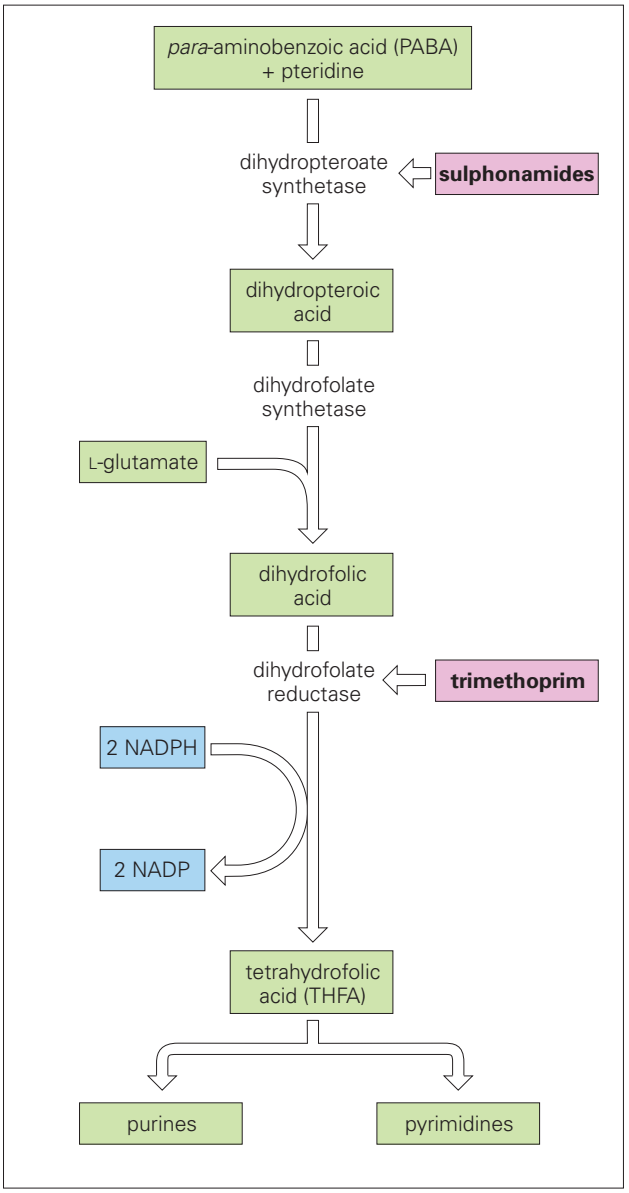


Figure 34.19 Sulphonamides and trimethoprim inhibit in series the steps in the synthesis of tetrahydrofolic acid by interacting with key enzymes in the pathway. NADP, nicotinamide adenine dinucleotide phosphate; NADPH, nicotinamide adenine dinucleotide phosphate reduced form.

cell therefore possesses two distinct enzymes: a sensitive chromosome-encoded enzyme and a resistant plasmid-encoded enzyme.

Rarely, sulphonamides cause Stevens–Johnson syndrome

Sulphonamides are relatively free of toxic side effects, but rashes and bone marrow suppression can occur.

Trimethoprim (and co-trimoxazole)

Trimethoprim is a structural analogue of the aminohydroxypyrimidine moiety of folic acid and prevents the synthesis of THFA

Trimethoprim is one of a group of pyrimidine-like molecules analogous in structure to the aminohydroxypyrimidine moiety of the folic acid molecule (Fig. 34.20). Other agents with a similar structure and mechanism of action include the antimalarial pyrimethamine and the anticancer drug methotrexate.

Trimethoprim, like sulphonamides, prevents THFA synthesis, but at a later stage by inhibiting dihydrofolate reductase (see Fig. 34.19). This enzyme is present in mammalian cells as well as bacterial and protozoan cells, and selective toxicity depends upon the far greater affinity of trimethoprim for the bacterial enzyme.

Trimethoprim is often given in combination with sulphamethoxazole as co-trimoxazole. The advantages of this combination over either drug alone are:

- Mutant bacteria resistant to one agent are less likely to be resistant to the other (i.e. double mutation).

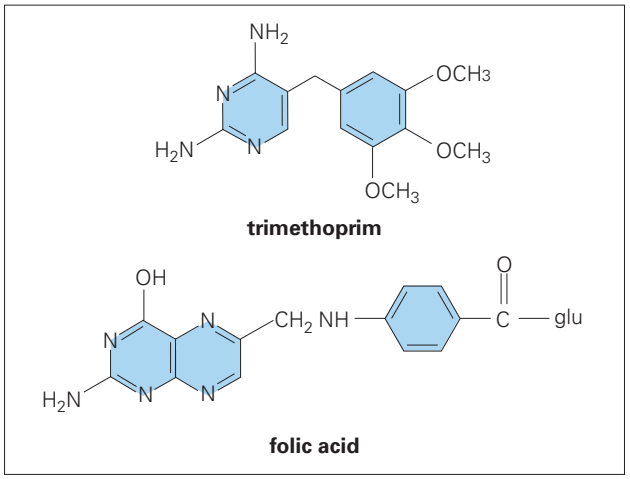


Figure 34.20 Trimethoprim resembles the aminohydroxypyrimidine moiety of folic acid and in this way antagonizes the enzyme dihydrofolate reductase.

- The two agents act synergistically against some bacteria (i.e. the combined action of the two bacteriostatic agents has a bactericidal effect that is greater than the action of either agent alone).

Trimethoprim can be given orally (either alone or as co-trimoxazole) or by intravenous infusion (alone or accompanied by sulphonamide). Trimethoprim is excreted in urine, and in patients with severe renal failure it is excreted more rapidly than sulphonamide so that the synergistic ratio of the combination may be lost.

Trimethoprim is often given with sulphamethoxazole as co-trimoxazole for urinary tract infections

Trimethoprim alone is active against Gram-negative rods with the exception of *Pseudomonas* spp. and its main use is in the treatment (and long-term prophylaxis) of urinary tract infection (see Ch. 21); however, the development of resistance is a concern.

Co-trimoxazole is active against a wide range of urinary tract pathogens and against *S. typhi*. This combination is also valuable for the treatment of pneumonia caused by the fungus *Pneumocystis jirovecii* (formerly *P. carinii*), although pentamidine, another pyrimidine derivative, is probably the preferred alternative. Co-trimoxazole is also useful for the treatment of nocardiosis.

Resistance to trimethoprim is provided by plasmid-encoded dihydrofolate reductases

Plasmid-encoded dihydrofolate reductases with altered affinity for trimethoprim allow the synthesis of THFA to proceed unhindered by the presence of trimethoprim. The 'replacement enzymes' are approximately 20 000-fold less susceptible to trimethoprim while retaining their affinity for the normal substrate. Bacteria that are resistant to sulphonamide and trimethoprim are also resistant to co-trimoxazole.

Trimethoprim and co-trimoxazole

Trimethoprim alone and in combination with sulphamethoxazole can cause neutropenia. Nausea and vomiting may occur.

OTHER AGENTS THAT AFFECT DNA

Nitroimidazoles

While nitroimidazoles are generally known for their antiparasitic activity, metronidazole also exhibits antibacterial properties

After entry into the microbial cell, the molecule is activated by reduction, and the reduced intermediate products are responsible for antimicrobial activity, probably through interaction with, and breakage of, the cell's DNA. The reactive intermediates are short-lived and decompose to non-toxic inactive end-products. Metronidazole is active only against anaerobic organisms because only these can produce the low redox potential necessary to reduce the parent drug.

Metronidazole has also been used as a hypoxic cell sensitizer in radiotherapy.

Metronidazole is usually given orally or rectally. It is well absorbed and well distributed in tissues and CSF. The drug is metabolized and most of the parent compound and metabolites are excreted in the urine.

Metronidazole was originally introduced for the treatment of the flagellate parasite *Trichomonas vaginalis*

Metronidazole is also effective against other protozoan parasites such as *Giardia intestinalis* and *Entamoeba histolytica*. It is an important agent for the treatment of infections caused by anaerobic bacteria.

Metronidazole resistance is of increasing concern in *T. vaginalis*, *G. intestinalis*, and several anaerobic and microaerophilic bacteria, and commonly involves either an alteration in uptake or a decrease in cellular reductase activity, thereby slowing the activation of the intracellular drug. *Helicobacter pylori*, a microaerophilic bacterium causing ulcers and gastritis, has been frequently treated with metronidazole. However, resistance can rapidly develop.

Rarely, metronidazole causes CNS side effects

The most serious side effects of metronidazole involve the CNS and include peripheral neuropathy. However, these are relatively uncommon and usually seen only in patients on large doses or prolonged treatment.

INHIBITORS OF CYTOPLASMIC MEMBRANE FUNCTION

The cytoplasmic membranes that encompass all kinds of living cells perform a variety of vital functions. The structure of these membranes in bacterial cells differs from that in mammalian cells and allows the application of some selectively toxic molecules, but these are few in number compared with those acting at other target sites.

Lipopeptides

Lipopeptides are a newer class of membrane-active antibiotics

Daptomycin is a lipopeptide antibiotic with bactericidal activity against a wide variety of Gram-positive bacteria including vancomycin-resistant *E. faecalis* and *E. faecium* and methicillin-resistant *Staph. aureus* and *Staph. epidermidis*

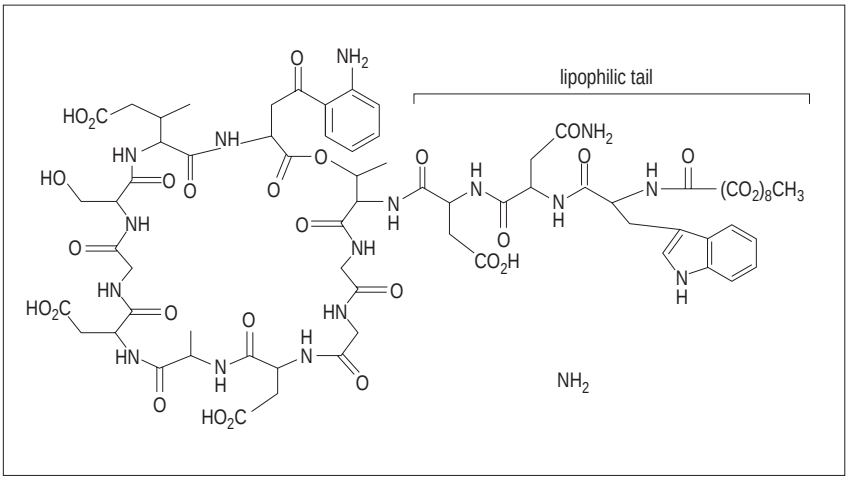


Figure 34.21 Chemical structure of the cyclic lipopeptide, daptomycin, consisting of a 13-member amino acid cyclic lipopeptide with a lipophilic tail, which attacks the bacterial cell membrane, causing depolarization and a potassium iron efflux.

(Fig. 34.21). The drug has been especially useful in treating complicated skin and skin structure infections and bacteremia. The compound acts in a calcium-dependent matter to insert and depolarize the bacterial cytoplasmic membrane leading to a number of consequences including the inability to synthesize ATP and interference with uptake of nutrients. At present, resistance to daptomycin has been relatively rare and seems to occur in a stepwise fashion over time.

Polymyxins

Polymyxins act on the membranes of Gram-negative bacteria

In addition to the polymyxins, the polyene antifungal agents (e.g. amphotericin B, nystatin) also act by inhibiting membrane function (see below). Polymyxins are bactericidal cyclic polypeptides that disrupt the structure of cell membranes.

The free amino groups of polymyxins act as cationic detergents, disrupting the phospholipid structure of the cell membrane. Polymyxin B is the most common member of the family still in clinical use.

In the past, polymyxins have been used systemically, but due to poor distribution in tissues, neurotoxicity and nephrotoxicity, their general use has been superseded by less toxic agents.

There is renewed interest in polymyxins as a last-effort option for treating multiresistant Gram-negative infections

Polymyxins are active against most Gram-negative organisms except *Proteus* spp. They have been especially used topically in ointments. After oral administration, polymyxins are not absorbed from the gut, and polymyxin E (colistin) has been used in some gut decontamination regimens for neutropenic patients, although with caution owing to concerns regarding renal toxicity. Concerns regarding the lack of effective antibiotics for treating multidrug resistant Gram-negative bacteria (especially *Pseudomonas* and *Acinetobacter* spp.) have led to renewed interest in polymixin / colistin combination therapy.

Resistance is due to chromosomally mediated alterations in membrane structure or antibiotic uptake.

URINARY TRACT ANTISEPTICS

Nitrofurantoin and methenamine inhibit urinary pathogens

Nitrofurantoin and methenamine are both synthetic compounds that, when taken orally, are absorbed and excreted in the urine in concentrations high enough to inhibit urinary pathogens. Nitrofurantoin has activity only in acid urine. Methenamine is hydrolysed at acid pH to produce ammonia and formaldehyde; it is the formaldehyde that has the antibacterial activity. Nitrofurantoin is used to treat uncomplicated urinary tract infection, and both agents are used to prevent recurrent urinary tract infections although there are concerns with adverse reactions in the young and elderly. While resistance rarely develops in susceptible bacterial populations, resistance to nitrofurantoin prior to treatment is a concern.

ANTITUBERCULOSIS AGENTS

M. tuberculosis and other mycobacterial infections need prolonged treatment

The treatment of infections caused by *M. tuberculosis* and other mycobacteria presents an enormous challenge to medicine and the pharmaceutical industry because these organisms:

- have a waxy outer layer that makes them naturally very impermeable and difficult to penetrate with antibiotics
- have an intracellular location, often in cells surrounded by a mass of caseous material, that also makes it difficult for antibiotics to get to them
- grow and multiply extremely slowly, and effective inhibition (and therefore cure) takes weeks or months to achieve. Long-term therapy is therefore a challenge for drug delivery, and orally administrable drugs are consequently highly desirable. It also follows that the emergence of resistance among the mycobacteria and toxicity in the patient are more likely than with the 'short sharp shock' treatment more often administered for bacterial infections
- are common and increasing in the wake of the AIDS epidemic in resource-poor countries, where the cost of drug treatment can be prohibitive.

The drugs for first-line therapy of tuberculosis are isoniazid, ethambutol, rifampicin, and pyrazinamide

Treatment regimens vary between countries and the susceptibility of the infecting strain but, where susceptibility is uncertain, an initial course of isoniazid, ethambutol, rifampicin, and pyrazinamide is typically followed for 2 months followed by a two-drug (e.g. isoniazid and rifampicin) continuation phase for an additional 18 weeks. If the strain is susceptible to both isoniazid and rifampicin, ethambutol is then discontinued. The structure and mechanism of action of rifampicin have been described earlier this chapter.

Isoniazid

Isoniazid inhibits mycobacteria and is given with pyridoxine to prevent neurological side effects

Isoniazid is isonicotinic acid hydrazide, a compound that inhibits mycobacteria, but does not affect other species of bacteria or humans to any great extent. Its bactericidal activity results from inhibition of mycolic acid synthesis, which also accounts for its specificity. It is well absorbed after oral administration, and a single daily dose is usually prescribed except in more difficult cases such as meningitis or miliary tuberculosis. The main toxic effects in humans are neurological complications (which can be prevented by the concurrent administration of pyridoxine) and hepatitis.

Ethambutol

Ethambutol inhibits mycobacteria, but can cause optic neuritis

Ethambutol is a synthetic molecule that inhibits, but does not kill, mycobacteria. It acts by inhibiting the polymerization of arabinoglycan, a critical constituent of the mycobacteria cell wall. It is well absorbed after oral administration and well distributed in the body, including the CSF. Resistance appears fairly rapidly if the drug is used alone. Thus, it is combined with other drugs in antituberculosis therapy. An important toxic side effect is optic neuritis, and visual acuity should be monitored during therapy.

Pyrazinamide

Pyrazinamide is a synthetic analogue of nicotinamide which appears to target mycolic acid synthesis. After oral administration, the drug is readily absorbed from the gastrointestinal tract and well distributed in body tissues and fluids. It is primarily metabolized in the liver and excreted by the kidney. As with ethambutol, resistance during monotherapy requires that the drug be used in combination with other first-line agents. The most important toxic side effect of pyrazinamide is hepatotoxicity.

Mycobacterial resistance

Drug resistance and immunocompromised patients complicate tuberculosis therapy

Despite the use of antibiotics in combination, the incidence of resistance among mycobacteria is a persistent and increasing problem. Infections with mycobacteria other than *M. tuberculosis* are on the increase as opportunist infections in people with AIDS, and these organisms tend to be innately more resistant than *M. tuberculosis*.

Treatment of leprosy

The development of resistance during dapsone monotherapy for leprosy has led to its use in combination with rifampicin

Infection caused by *M. leprae* is characterized by persistence of the organism in the tissues for years and necessitates very prolonged treatment to prevent relapse. For many years dapsone, related to the sulphonamides (see Fig. 34.18), has been used. This drug has the advantages that it is given orally and it is cheap and effective. However, monotherapy has resulted in the emergence of resistance, and a combination of dapsone, rifampicin and clofazimine, a phenazine compound (mechanism of action not well understood), is now commonly used as multidrug therapy.

ANTIBACTERIAL AGENTS IN PRACTICE

It is clear from the preceding sections of this chapter that although there are certain 'rules of thumb' about the resistance of bacteria to an antibiotic, it is often impossible to do more than guess in the absence of laboratory tests. Susceptibility tests performed in the laboratory examine the interaction between antibiotics and bacteria in an isolated and rather artificial fashion. At best, the results are a helpful guide to the likely outcome of therapy; at worst, they are misleading. Patient factors such as age, underlying disease, site and type of infection, renal and liver impairment, and drug pharmacodynamics must be taken into account in the antibiotic management of an infection.

Susceptibility tests

Laboratory tests for antibiotic susceptibility fall into two main categories:

- disk diffusion tests
- dilution tests.

Diffusion tests involve seeding the organism on an agar plate and applying filter paper disks containing antibiotics

The isolate to be tested is seeded over the entire surface of an agar plate, and filter paper disks containing the antibiotics are applied. After overnight incubation the plate is observed for zones of inhibition around each antibiotic disk (Fig. 34.22). The amount of antibiotic in the disk is related to, among other things, the achievable serum concentration and therefore differs for different antibiotics. In addition, antibiotics differ in their ability to diffuse in agar, so the size of the inhibition zone (and not simply its presence) is an indicator of susceptibility of the isolate. The zone sizes are compared with those for reference organisms (either tested in parallel or established previously and published in reference tables) and the result recorded as 'S' (susceptible), 'I' (intermediate) or 'R' (resistant). An 'I' result indicates that the isolate is less susceptible than the norm, but may respond to higher doses of antibiotic or in sites where the antibiotic is concentrated (e.g. in urine in the bladder for antibiotics excreted by the kidneys).

A dilution test provides a quantitative estimate of susceptibility to an antibiotic

A more quantitative estimate of the susceptibility of an organism to an antibiotic can be achieved by performing a

MIC (minimum inhibitory concentration) test (i.e. a test to find the lowest concentration that will inhibit visible growth of the bacterial isolate in vitro). Serial dilutions of the test antibiotic are prepared in broth or agar medium and inoculated with a suspension of the test organism. After overnight incubation, the MIC is recorded as the highest dilution in which there is no macroscopic growth (Fig. 34.23). These tests can be performed in a microtitre plate format and form the basis of some automated susceptibility test systems. An alternative approach is the E-test, in which a filter paper strip impregnated with a gradient of antibiotic is laid on an agar plate seeded with the test isolate. The concentration on the strip at which growth is inhibited indicates the MIC.

MIC tests are clearly more time consuming and costly than disk diffusion tests in terms of time and materials and are not routinely performed in the clinical laboratory, but they can yield useful information for the management of difficult infections or for patients who are failing to respond to apparently appropriate therapy.

An advantage of an MIC test is that it can be extended to determine the MBC (minimum bacterial concentration), which is the lowest concentration of an antibiotic required to kill the organism. In order to discover whether the agent has actually killed the bacteria rather than simply inhibited their growth, the test dilutions are subcultured onto a fresh drug-free medium and incubated for a further 18–24 h (Fig. 34.23). The antibacterial agent is considered to be bactericidal if the MBC is equal to or not greater than fourfold higher than the MIC.

Figure 34.22 The antibiotic susceptibility of an organism can be tested by the application of filter paper impregnated with antibiotic onto a lawn of the organisms seeded on an agar plate. After overnight incubation the organism grows and the antibiotics diffuse to produce a zone of inhibition that indicates the degree of susceptibility: disk susceptibility test indicating sulphonamide resistance. SF100 is the sulphonamide disk. (Courtesy of D.K. Banerjee.)

Killing curves provide a dynamic estimate of bacterial susceptibility

One of the disadvantages of MIC and MBC tests is that the result is read at only one point in time. A more dynamic estimate of bacterial susceptibility can be gained by measuring the decrease in viability of the population with time (Fig. 34.24). As with MIC and MBC tests, killing curves are more time consuming and costly than disk diffusion tests and are only typically performed on a research basis. A number of the automated susceptibility test systems use a measure of bacterial viability (e.g. turbidity, electrical impedance) in the presence of an antibacterial as their indicator system. These

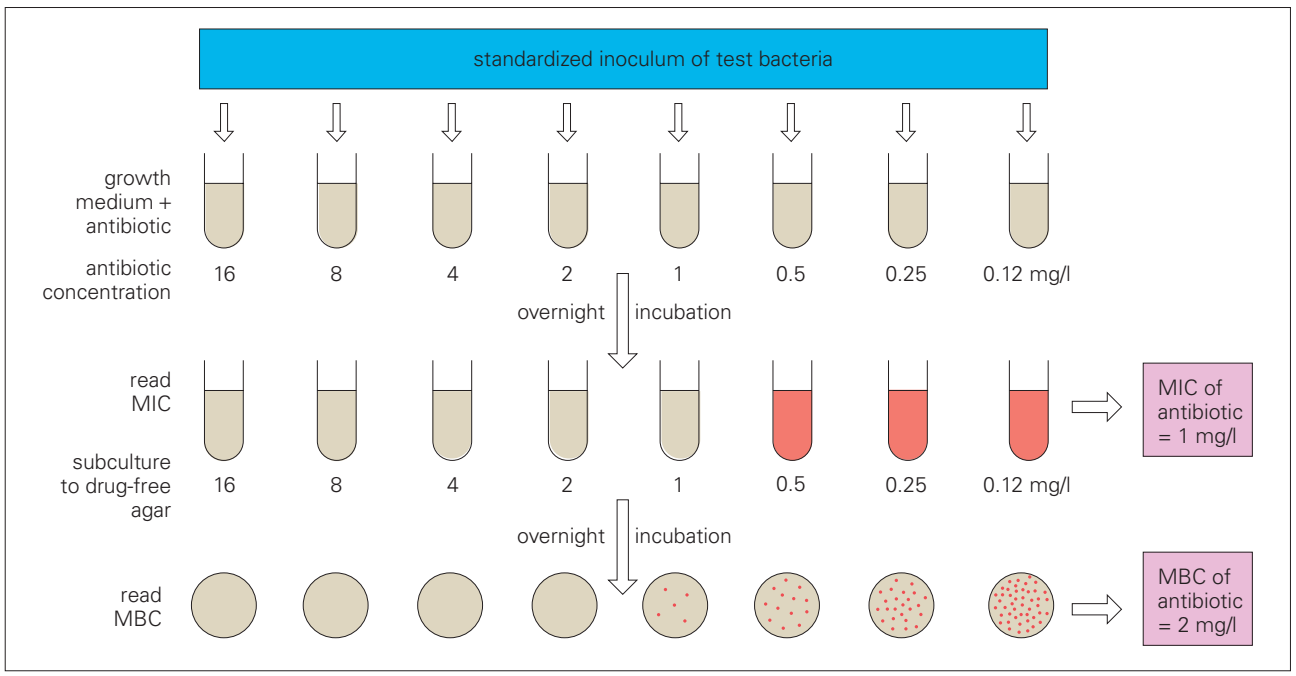


Figure 34.23 More precise measures of the amount of antibiotic required to inhibit and kill a bacterial population can be estimated by establishing the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the antibiotic. Using the standard method as outlined in this illustration, the MIC result is available after 24 h and the MBC result after 48 h. A number of variables such as the inoculum size, the growth medium and the interpretation of the results affect the outcome of MIC tests.

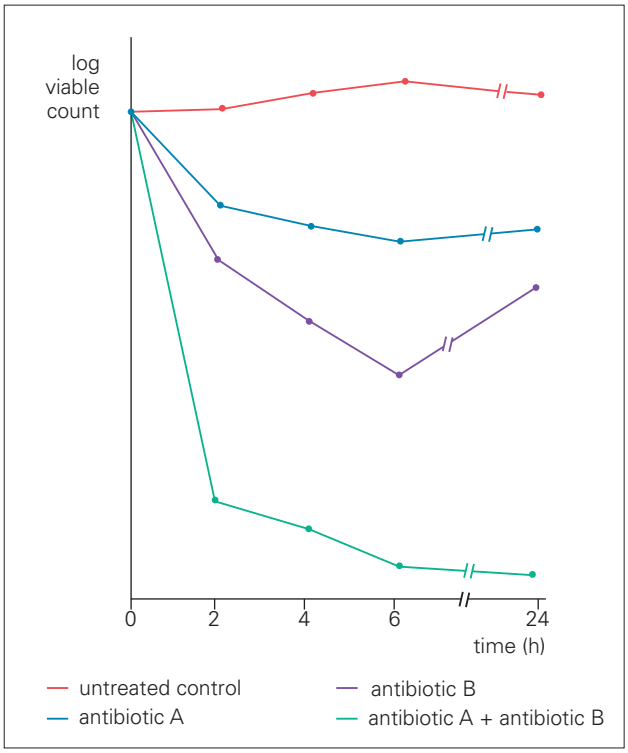


Figure 34.24 A more dynamic picture of the interaction between an antibiotic and a bacterial population can be gained from producing killing curves. In these experiments a culture of 2×10^6 colony forming U/mL was treated with antibiotics A and B alone and in combination. Compared with the untreated control, both A and B inhibit the growth of the bacterial culture, but B is more active than A. However, in combination, the activity of A plus B is synergistic (i.e. it is more active than the sum of the activities of the two antibiotics alone). The combination also prevents the re-growth seen after 6–24 h when the antibiotics are used singly.

machines can produce results more rapidly (e.g. within a few hours) than conventional susceptibility tests. However, fastidious organisms (e.g. *S. pneumoniae*, *N. meningitidis*, etc.) or resistance that is characteristically difficult to detect (e.g. borderline oxacillin MICs in *Staphylococcus aureus*, ESBLs in Gram-negative isolates, etc.) can be problematic.

Combining antibacterial agents can lead to synergism or antagonism

Hospital patients frequently receive more than one antibacterial agent, and these agents may interact with each other (and also with other drugs such as diuretics).

Antibacterial combinations are described as:

- 'synergistic' if their activity is greater than the sum of the individual activities
- 'antagonistic' if the activity of one drug is compromised in the presence of the other.

Both disk diffusion and dilution tests allow the action of combinations of antibiotics to be studied. Although synergy can often be demonstrated in vitro (Fig. 34.25), it is difficult to confirm in vivo. Co-trimoxazole is an example of a combination that is frequently used (see above). Another example is the combination of penicillin (or ceftriaxone) with gentamicin in the treatment of endocarditis caused by a penicillin-susceptible strain, as this combination has been shown to be clearly superior to the effect of the beta-lactam alone (Box 34.6).

Antagonism can be demonstrated between some pairs of antibiotics in vitro but is rarely evident in vivo.

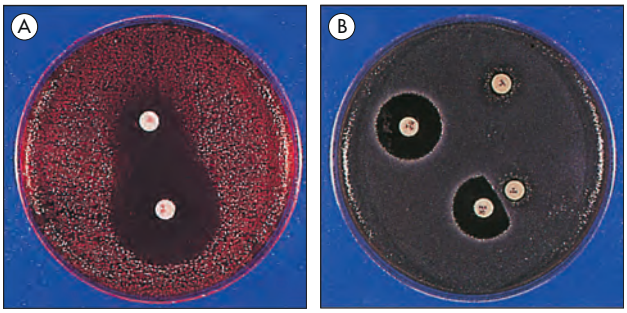


Figure 34.25 (A) Synergy of two antibacterials. Disks containing sulphonamide and trimethoprim have been placed to demonstrate the synergistic activity of these two agents against *E. coli*. Synergy can be recognized by the fact that the zones of inhibition become continuous between the two disks. (B) Antagonism. Nitrofurantoin is capable of antagonizing the activity of nalidixic acid. When the disks are placed far apart, nalidixic acid inhibits the test organism, but when placed close together this inhibition is antagonized by the presence of nitrofurantoin, as demonstrated by the foreshortening of the zone of inhibition.

Box 34.6 Use of Antibiotic Combinations

Reasons for using antibiotic combinations

Ideally, single drugs are used, but antibiotic combinations are justifiable under certain circumstances:

- to obtain a synergistic effect, e.g. co-trimoxazole
- to prevent or delay emergence of persistent organisms, e.g. isoniazid, rifampicin, ethambutol and pyrazinamide for tuberculosis
- to treat polymicrobial infections, e.g. intra-abdominal abscesses where the different microbes have different susceptibilities
- to treat serious infection in the stage before the infectious agent is identified.

ANTIBIOTIC ASSAYS

In the preceding parts of this chapter, the pharmacokinetic properties (i.e. absorption, distribution, excretion) of antibacterial agents have been summarized. Some antibacterials have a narrow 'therapeutic index' (i.e. the concentration required for successful treatment and the concentration toxic to the patient are not very different). The concentrations of such antibiotics should be monitored both to prevent toxicity and to ensure that therapeutic concentrations are achieved. Other less toxic agents should be monitored in some circumstances in some patients (Box 34.7). Serum concentrations are usually measured, but urine, CSF and other body fluids can be assayed if applicable.

Antibiotic assays may be performed by a variety of methods such as high-performance liquid chromatography and direct assays for biological activity (bioassay). However, the most

Box 34.7 Importance of Antibiotic Assays

Assays of antibiotics in clinical practice are particularly important when the antibiotic is potentially toxic, but there are other situations in which assays are important:

- when an antibiotic has a narrow therapeutic index, e.g. aminoglycosides
- when the normal route of excretion of antibiotic is impaired, e.g. in patients with renal failure for agents excreted via the kidney
- when the absorption of the antibiotic is uncertain, e.g. after oral administration
- to ascertain concentrations in sites of infection into which penetration of antibiotics is irregular or unknown, e.g. in cerebrospinal fluid
- in patients receiving prolonged therapy for serious infections, e.g. endocarditis
- in neonates with serious infections
- in patients who fail to respond to apparently appropriate therapy
- to check on patient compliance.

common approach uses immunological methods which can be automated. In this method, the antibiotic in the patient specimen is an 'antigen' that competes with a specific level of labelled 'tracking' antibiotic for binding sites on an 'anti-drug' antibody. Thus, increased antibiotic levels in a patient sample result in decreased binding of tracking antibiotic, etc. Such assays are rapid, require only small volumes of serum, and are highly specific. However, they are obviously only applicable to instances where specific anti-drug antibody is available.

ANTIVIRAL THERAPY

Antiviral drugs do not kill viruses but stop viral replication

The range of targets and number of antiviral agents licensed to treat (unlike James Bond, as they cannot kill) viral infections has been one of the huge successes in clinical virology. Initially, the first antiviral drug, idoxuridine, was approved in 1963 and the armamentarium increased slowly but surely over the next 30 years. However, by 2017 there were nearly 90 antiviral drugs, which included novel combinations and immunomodulators, that were being used to treat human immunodeficiency virus (HIV), hepatitis B and C (HBV, HCV), herpesviruses (including herpes simplex virus [HSV], varicella-zoster virus [VZV] and cytomegalovirus [CMV]), influenza A and B, respiratory syncytial virus (RSV) and human papilloma virus (HPV) (Fig. 34.26). They are all virustatic rather than virucidal, in other words, they do not kill viruses but suppress their replication. Of the increasing array of antiviral agents licensed for treatment, there has been a revolution in treating the viral infections that cause chronic carriage and disease, namely HIV, HBV and HCV infections. Not only have the number and range of drugs increased in a very short time period – around 10 years – but, critically, compliance and ease of taking these treatments has improved, by reducing pill burden by combination therapy and by being able to offer single-tablet oral treatment. Combination antiretroviral therapy (cART) has made HIV infection a chronic, controlled infection, as it has improved survival and reduced hospital admission. It is also being investigated as a way of reducing transmission when given as prophylaxis. As of 2017, there were 14 single-tablet two–four drug combined pills and 25 antiretroviral agents constituting six different classes of drug (summarized in Table 34.6).

However, the most rapid change in the treatment landscape was seen with HCV infection, with new drugs and combinations becoming past history rapidly as others replaced them, leading to sustained viral responses translating into viral clearance after short treatment courses. The problem in developing new antivirals has been mostly due to the difficulty of interfering with viral activity in the cell without adversely affecting the host. This is because viruses are dependent on the host cell's protein synthetic machinery.

Reports have highlighted the importance of making an early diagnosis in short-incubation-period viral infections, such as influenza, in order for antiviral treatment to be successful. Moreover, virus-specific replication steps can be identified (Fig. 34.27), and more of these will doubtless be exploited, such as identifying virus-induced enzymes.

Bearing in mind that antivirals can be used to treat acute and chronic viral infections, and in the latter case may be given for many years or for life, considerations include the length of the treatment course, single versus combination therapy, drug pharmacokinetics and interactions, adverse effects and antiviral resistance. Monitoring viral load as a marker of prognosis and treatment response is important in chronic viral infections such as HIV, HBV and HCV, together with therapeutic drug monitoring and genotypic and phenotypic resistance tests.

Antiviral resistance occurs with varying prevalence in different patient populations; for example, aciclovir-resistant HSV and ganciclovir-resistant CMV are mostly seen in immunocompromised individuals at a low level. Antiretroviral resistance is seen across all the main classes of agents – nucleoside reverse transcriptase inhibitors, non-nucleoside reverse transcriptase inhibitors, and protease inhibitors – with increasing frequency in resource-rich countries. Lamivudine-resistant HBV is well recognized and is usually detected after a couple of years of treatment. Drug resistance involving most of the other agents used to treat HBV carriers also occurs. One issue with antiviral resistance is that the replication fitness of the drug-resistant variants is often less than the wild-type strain. In addition, in the case of a number of viruses, including HBV and HCV, the response varies depending on the viral genotype.

Some viral infections have an immunopathological basis, such as CMV pneumonitis, in which case an antiviral is given in combination with an immunoglobulin preparation. This may be human normal immunoglobulin or virus-specific immunoglobulin (i.e. CMV hyperimmune globulin). Moreover, an immunomodulator may be given in conjunction with an antiviral such as pegylated interferon and ribavirin to treat hepatitis C virus infection.

Palivizumab is an example of a humanized monoclonal antibody produced to prevent infection. It is directed against the RSV fusion protein and has potent neutralizing and fusion

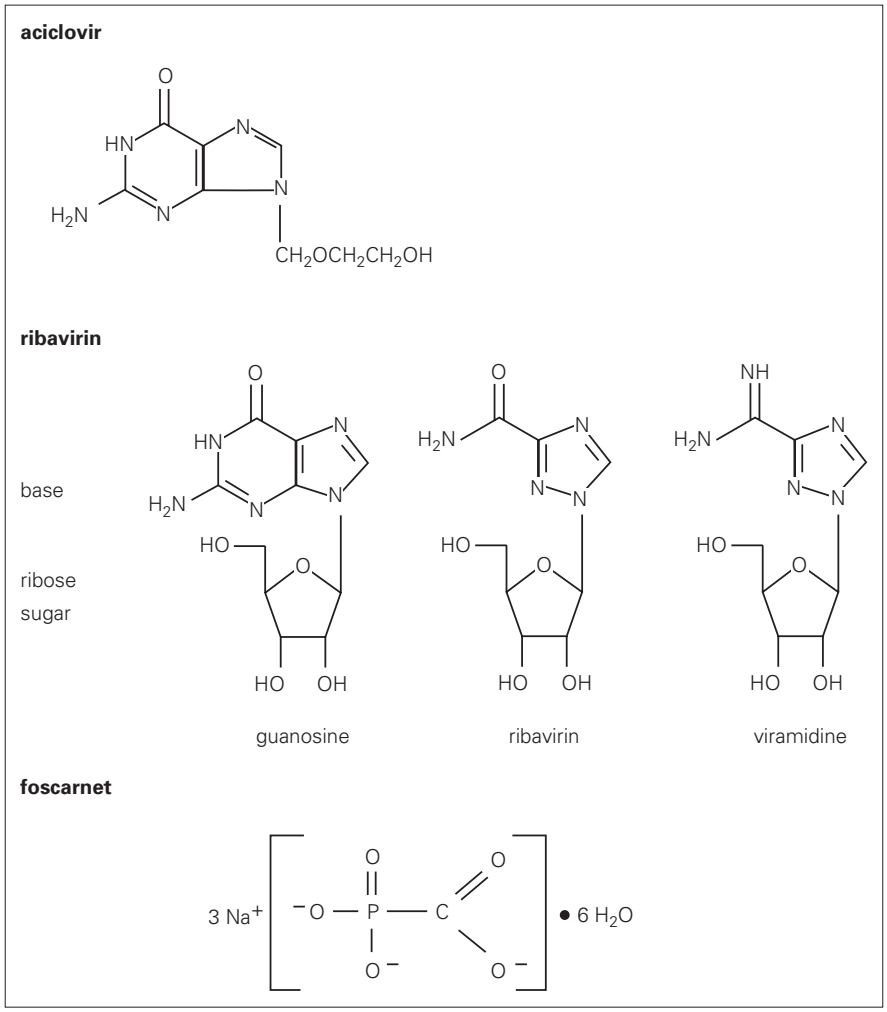


Figure 34.26 Examples of the structures of different antiviral drugs that cause chain termination by being guanosine analogues that can be incorporated into viral DNA and inhibit the viral DNA polymerase (aciclovir), use a similar mechanism but with RNA polymerase (ribavirin, which also has an effect intracellularly to ribavirin monophosphate, which competitively inhibits inosine monophosphate dehydrogenase causing depletion of guanosine triphosphate needed for viral RNA synthesis), or bind at the viral polymerase active site (foscarnet).

Table 34.6 Antiviral drugs

DNA viruses	
CMV	Ganciclovir Valganciclovir Foscarnet Cidofovir
HSV and VZV	Aciclovir Valaciclovir Famciclovir Ganciclovir Foscarnet Cidofovir
HBV	Lamivudine Tenofovir Entecavir Adefovir Emtricitabine Interferon alpha
RNA viruses	
Influenza A and B viruses	Oseltamivir Zanamavir Peramivir

Table 34.6 Antiviral drugs—cont'd

Influenza A viruses	Amantadine Rimantadine
RSV	Ribavirin
HIV	
NRTIs	Abacavir Didanosine (ddI) Emtricitabine Lamivudine (3TC) Stavudine (d4T) Tenofovir Zidovudine (AZT)
Fusion inhibitor	Enfuvirtide (T-20)
CCR5 inhibitor	Maraviroc
NNRTIs	Delavirdine Efavirenz Etravirine Nevirapine Rilpivirine
InSTIs	Dolutegravir Elvitegravir Raltegravir
PIs	Amprenavir Atazanavir Darunavir Fosamprenavir Indinavir Lopinavir + ritonavir (Kaletra) Nelfinavir Ritonavir Saquinavir Tipranavir
HCV	
NS3 PIs	Asunaprevir Boceprivir Paritaprevir Simeprivir Telaprivir
NS5 polymerase inhibitors	Dacaltasvir Elbasvir Ledipasvir Ombitasvir Sofosbuvir Velpatasvir Ribavirin Interferon alpha
Combinations (single tablets)	
HIV	Efavirenz + Emtricitabine + Tenofovir Rilpivirine + Emtricitabine + Tenofovir Elvitegravir + Cobicistat + Emtricitabine + Tenofovir Dolutegravir + Abacavir + Lamivudine Abacavair + Lamivudine Abacavair + Lamivudine + Zidovudine Emtricitabine + Tenofovir Lamivudine + Zidovudine

CMV, cytomegalovirus; HBV, hepatitis B virus; HCV, hepatitis C virus; HIV, human immunodeficiency virus; INSTI, integrase strand transfer inhibitor; NNRTI, non-nucleoside reverse transcriptase inhibitor; NRTI, nucleoside and nucleotide reverse transcriptase inhibitor; NS, non-structural; PI, protease inhibitor; RSV, respiratory syncytial virus; VZV, varicella-zoster virus.

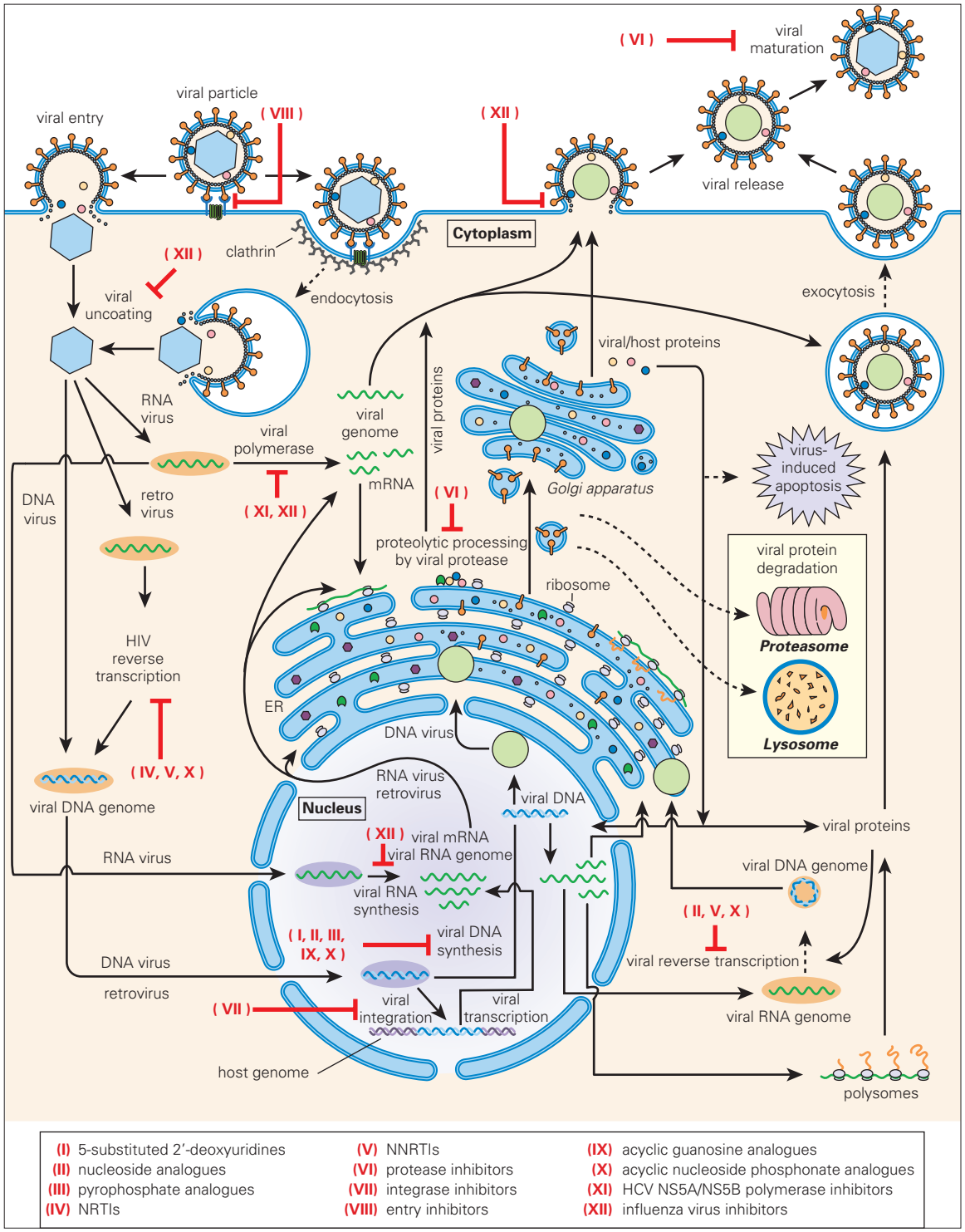


Figure 34.27 Site of action of antiviral agents during the viral life cycle. Twelve groups of drugs are shown at the bottom (red roman numerals). Inhibitory drug action at major stages of the viral life cycle are highlighted (red arrows). Solid black arrows indicate direct biological pathways involving viral replication, and dotted black arrows show biological pathways with intermediate pathways inside host cells. Major viral stages are illustrated including endocytosis, exocytosis, virus entry, reverse transcription, virus integration, viral transcription, viral translation, virus budding/release, virus maturation and other pathways associated with cellular compartments (e.g. Golgi apparatus, mitochondria, endoplasmic reticulum [ER], ribosome, proteasome, polysome and endosome). Replication pathways of DNAviruses (HCMV, HBV, HPV, HSV and VZV), RNA viruses (HCV, RSV and influenza virus), and retroviruses (HIV) diverge after entering host cells. The RNA viruses replicate in the cytoplasm, but DNA viruses and retroviruses replicate in the nucleus. Drug group XIII is not displayed since group acts mainly as immunoregulatory or antimitotic agents not directly targeting viral proteins. Shapes and sizes of proteins and cellular components are not to scale. HCV, hepatitis C virus; NNRTI, non-nucleoside reverse transcriptase inhibitor; NRTI, nucleoside reverse transcriptase inhibitor. (From De Clercq E., Guangdi L. Approved antiviral drugs over the past 50 years. *Clin Microbiol Rev* 2016; 29[3], Fig 4, with permission.)

inhibitory activity. It is used in specific clinical settings to prevent severe lower respiratory tract infections caused by RSV requiring hospitalization in children born at 35 weeks' gestation or less who are less than 6 months old at the onset of the RSV season. In addition, it may be used in children less than 2 years of age with specific respiratory and cardiac conditions such as bronchopulmonary dysplasia.

Finally, in the case of some viral respiratory tract infections, antibiotics are often given to control or act as prophylaxis against a secondary bacterial infection. Influenza infection is an example where staphylococcal and streptococcal pneumonia may occur after the initial virological insult.

It is difficult to group the antiviral drugs in the same way as the antibiotics. One can either look at them as, for example, anti-HIV, anti-HBV and anti-HCV or group them under mechanism of action. The following are classified using the latter heading.

Prodrugs that target the viral DNA polymerase

These include aciclovir, valaciclovir, famciclovir, ganciclovir, valganciclovir and cidofovir.

Aciclovir (acycloguanosine)

Aciclovir inhibits HSV and varicella-zoster virus (VZV) DNA polymerase. Aciclovir is used in the treatment of HSV and VZV infections. A number of other agents include valaciclovir, the L-valyl ester of aciclovir, and famciclovir. Aciclovir is inactive until phosphorylated and is an example of a prodrug. Aciclovir (Fig. 34.28) is phosphorylated by the herpesvirus thymidine kinase and the monophosphate is then converted by cellular kinases to the triphosphate, which inhibits the herpesvirus DNA polymerase. As it is taken up and efficiently phosphorylated by HSV-infected cells, the action on cellular DNA polymerase is minimal and toxic side effects such as neutropenia and thrombocytopenia are rare. The drug is also incorporated into viral DNA, resulting in chain termination. As it is excreted by the kidney, the drug can crystallize in the renal tract in individuals with renal failure, causing acute tubular necrosis. Otherwise, aciclovir has an excellent safety profile.

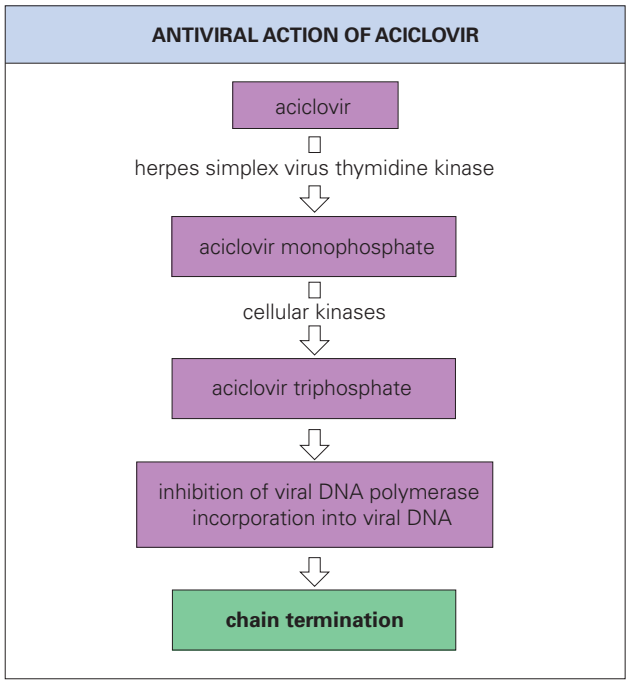


Figure 34.28 The activity of an antiviral agent against different herpes viruses is correlated with the ability of the viruses to induce a thymidine kinase; hence aciclovir is most active against herpes simplex virus and least active against cytomegalovirus.

Systemic aciclovir revolutionized the treatment of HSV encephalitis, and HSV and VZV infections in immunocompromised patients. It is effective in treating primary and recurrent genital herpes. In shingles (herpes zoster), recovery is accelerated and post-zoster pain reduced. As with HSV, the varicella-zoster virus remains latent in ganglia and can reactivate.

As the oral bioavailability is only 15–20%, aciclovir is given intravenously in a number of clinical settings initially. Valaciclovir and famciclovir have improved bioavailability profiles in comparison with aciclovir, resulting in less frequent daily dosages.

Ganciclovir (dihydroxypropoxy-methylguanine, DHPG)

Ganciclovir is structurally similar to aciclovir but has an extra hydroxyl group. The range of activity is broader than that of aciclovir, and the drug is active against CMV infections. CMV does not encode a thymidine kinase, but rather the drug is monophosphorylated by a virus *UL97* gene-specified kinase and then further phosphorylated by cellular kinases. However, selective toxicity is not seen, and it is myelosuppressive, its main adverse effect being bone marrow toxicity. Ganciclovir triphosphate inhibits CMV DNA polymerase. It is given intravenously because of limited oral bioavailability. However, an oral agent, valganciclovir, has improved the outpatient management of individuals with CMV infections, as it has equivalent activity to intravenous ganciclovir.

Ganciclovir is given to treat CMV retinitis, encephalitis and gastrointestinal disease seen in immunocompromised individuals. It is also used as pre-emptive therapy in bone marrow transplant as well as solid organ transplant recipients, who are monitored regularly for the presence of CMV in their blood as this may lead to CMV dissemination.

Valganciclovir

Valganciclovir is the valine ester of ganciclovir, has similar bioavailability but has the advantage of being given orally.

Cidofovir

Cidofovir is another chain terminator that targets the viral DNA polymerase. It is phosphorylated intracellularly to the diphosphate form and is then added to the 3′ end of the viral DNA chain. It is effective against CMV and has been used to treat adenovirus infections. When given topically or intralesionally, it has activity against genital warts and can be used to treat aciclovir-resistant HSV infections. It has to be given intravenously and is nephrotoxic.

Pyrophosphate analogue that blocks the pyrophosphate-binding site on the viral DNA polymerase

Foscarnet

This compound attaches to the pyrophosphate-binding site of the herpesvirus DNA polymerase, preventing nucleotide

binding and therefore inhibiting viral replication. It is used in treating CMV infections and is active against HSV and VZV and can be used to treat aciclovir-resistant HSV infections. It is nephrotoxic and can have compliance issues as it has other adverse effects and is often used as a second-line agent.

Antiretroviral drugs

Antiretroviral drugs are divided into six classes; all are named after their mechanism of action as follows.

Nucleoside and nucleotide reverse transcriptase inhibitors (NRTIs): zidovudine (azidothymidine, AZT), didanosine (ddI), lamivudine (3TC), stavudine (d4T), abacavir, emtricitabine and tenofovir

The aim of antiretroviral therapy is to lower and keep the plasma HIV-1 RNA load below the limit of assay detection and therefore maintain the CD4 count. HIV treatment has become quite complicated owing to the options available. This class of drugs has similar modes of action, can be given singly or combined with each other in some cases, but mostly with other drug classes, such as the non-nucleoside reverse transcriptase inhibitors and protease inhibitors.

Zidovudine (azidothymidine, AZT). Zidovudine is an analogue of the nucleoside thymidine in which the hydroxyl group on the ribose is replaced by an azido group. After conversion to the triphosphate by cellular enzymes (Fig. 34.29) it acts as an inhibitor of, and substrate for, the viral reverse transcriptase. The azido group prevents the formation of phosphodiester linkages. Proviral DNA formation is blocked because AZT triphosphate is incorporated into the DNA, with resulting chain termination.

Zidovudine is given orally. Toxicity is a problem, with bone marrow suppression (macrocytic anaemia, neutropenia, leukopenia) and less commonly nausea, vomiting, headache, myalgia and malaise. This was more often seen in the early days of HIV treatment when the drug was given at a high dose. Other adverse events include lactic acidosis, hyperlipidaemia, lipoatrophy and insulin resistance or diabetes mellitus. Regular blood tests are necessary to detect anaemia and myelosuppression.

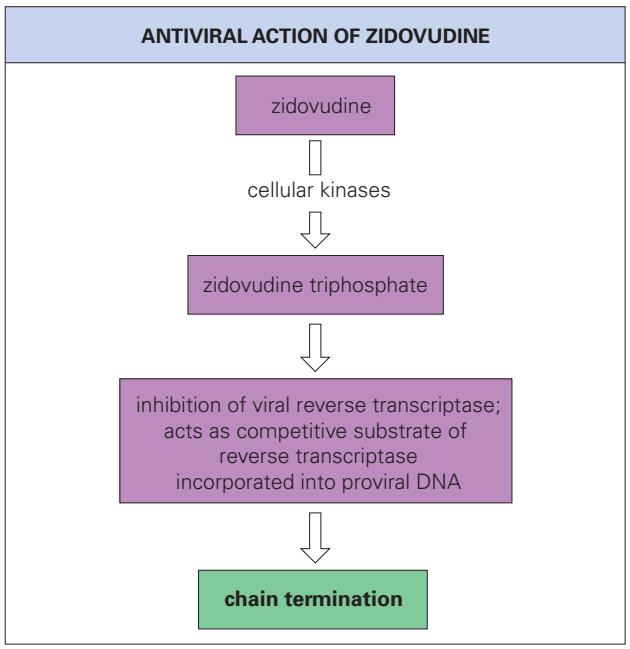


Figure 34.29 HIV reverse transcriptase is 100 times more sensitive than host cell DNA polymerase to zidovudine triphosphate, but toxic effects are not uncommon.

Like zidovudine, the other nucleoside analogues are converted to triphosphates and inhibit the HIV reverse transcriptase. Some of these agents have been combined as fixed-dose treatments such as combivir (AZT and 3TC), truvada (emtricitabine and tenofovir), and trizivir (AZT, 3TC and abacavir).

There are a number of adverse effects shared by this class of drugs but the more specific side effects include pancreatitis (ddI), peripheral neuropathy (d4T, ddI), lipodystrophy (i.e. fatty tissue redistribution from subcutaneous areas such as the face and limbs, to the neck and abdominal viscera [d4T]) and hypersensitivity (abacavir). Mitochondrial toxicity due to inhibition of the mitochondrial DNA polymerase and lactic acidosis is also reported.

Drug resistance can lead to cross-resistance to other nucleoside analogues.

Tenofovir is a nucleotide reverse transcriptase inhibitor and is phosphorylated to the diphosphate form that acts as chain terminator.

The nucleoside and nucleotide RTIs and most of the protease inhibitors can be used to treat HIV-2-infected individuals. The non-nucleoside RTIs cannot be used and the fusion inhibitor, enfuvirtide, has reduced HIV-2 activity.

Non-nucleoside reverse transcriptase inhibitors (NNRTIs)

Nevirapine, efavirenz, delavirdine, etravirine and rilpivirine. These act as non-competitive inhibitors of HIV-1 reverse transcriptase by binding to a hydrophobic pocket proximal to the enzyme catalytic site. They are inactive against HIV-2. The NNRTIs are inducers of cytochrome P450 and it is important to consider potential drug interactions. The most common adverse effect with nevirapine is a skin rash. Efavirenz may cause vivid dreams and sleep disturbance initially and should not be used in the first trimester of pregnancy.

A single mutation in the reverse transcriptase leads to resistance to these drugs, effectively removing this class of drug from the treatment regimen.

Protease inhibitors (PIs)

Nelfinavir, saquinavir, indinavir, ritonavir, lopinavir plus ritonavir (Kaletra), atazanavir, amprenavir, darunavir, fosamprenavir and tipranavir. The protease enzyme acts in the post-translational cleavage of the gag and gag-pol polyproteins into the structural proteins and enzymes critical for viral replication. The result of protease inhibition is the production of immature, defective viral particles. PIs were introduced to HIV treatment combinations in 1996 and had a great effect on the control of HIV infection. Their use led to the term highly active antiretroviral therapy (HAART). They are peptidomimetic inhibitors of the viral protease and prevent the cleavage of the gag and gag-pol polyproteins into functional structural proteins and enzymes. They are very potent drugs which lead to a rapid fall in the plasma HIV RNA load, especially in those individuals with very high HIV loads. Side effects

include gastrointestinal disturbances, lipodystrophy syndrome (body fat redistribution), increased triglycerides, and insulin resistance leading to diabetes.

Drug resistance is well recognized and a number of protease mutations result in cross-resistance. Boosting atazanavir and darunavir with low-dose ritonavir or cobistat, which is only given as a booster, leads to greater virological activity owing to improved pharmacodynamics. However, higher rates of side effects are seen.

Fusion inhibitors

Enfuvirtide, also known as T-20, is a peptide that blocks HIV before it enters the host cell by competitively binding to gp41, the transmembrane glycoprotein, and blocking the post-fusion structure from forming. It therefore should not cross-react with the other classes of antiretroviral drugs. It is given twice daily as a subcutaneous injection and is approved for salvage therapy in those treatment-experienced individuals with resistance mutations to the other drug classes. Adverse events include pain at the injection site and rare hypersensitivity reactions.

Integrase inhibitors (INSTIs)

Dolutegravir, raltegravir, elvitegravir. These are HIV integrase strand transfer inhibitors (INSTI). Integration involves transferring virally encoded DNA into the host chromosome. It is a three-step process including the formation of a preintegration viral DNA complex, 3′ processing and strand transfer. INSTIs inhibit the strand transfer step; it is thought that they interact with divalent cations of the catalytic core of the integrase. INSTIs are also active against HIV strains resistant to other classes of antiretroviral agents. Side effects are mostly gastrointestinal.

Chemokine receptor antagonists

Maraviroc. HIV-1 entry into host cells involves the viral envelope protein binding to the CD4 receptor and subsequently to a chemokine co-receptor. Two co-receptors identified are called CCR5 and CXCR4. Tests that identify the viral phenotype have been used to determine the populations of virus in someone with HIV and these are referred to as R5-tropic, X4-tropic or dual / mixed. Diagnostic laboratories use genotypic tests to predict viral co-receptor tropism, R5 or X4, based on the sequence of the viral envelope on the basis of algorithms.

Maraviroc is a CCR5 chemokine co-receptor antagonist and was approved originally for adults who had been given HAART and had R5 HIV-1 infection.

Treatment combinations

The uses and combinations of these six classes of antiretroviral drugs are too complex to even summarize and treatment guidelines are updated regularly. By 2016, cART was recommended for all individuals with an acute HIV infection as well as all those who were viraemic. The combinations included an NRTI backbone, two NRTIs and an INSTI. Other combinations included NNRTIs or boosted PIs with two NRTIs. The reduction in pill burden by combining drugs together with the increased range of antiretroviral agents, has increased choice and ease of switching regimens to ensure tolerability, compliance, antiretroviral resistance, drug interactions and potential adverse effects such as in pregnancy, chronic viral hepatitis and renal dysfunction. Pre-exposure prophylaxis (PrEP) was being considered as part of HIV prevention, adding to the recommended post-exposure prophylaxis (PEP) in a number of clinical settings.

Inosine monophosphate dehydrogenase inhibitor

Ribavirin

This guanosine analogue is triphosphorylated by cellular enzymes. It has various actions including inhibition of production of guanosine triphosphate pools needed for viral nucleic acid synthesis. Ribavirin can target both RNA and DNA viruses. Once triphosphorylated, it can also interfere with the viral RNA polymerase. It is used clinically as an aerosol for treating severe RSV infection in infants and for arenavirus infections such as Lassa fever (see Ch. 27). Oral ribavirin could be used as postexposure prophylaxis for Lassa fever in the case of high-risk exposure incidents. It is also active against measles virus and hepatitis C and hepatitis E virus infections (see below).

Antivirals targeting influenza viruses

Amantadine, rimantadine, zanamivir, oseltamivir, and peramivir. These drugs have selective activity against influenza viruses and so have been grouped together with this header rather than by their mode of action. Amantadine and rimantadine have activity only against influenza A and are used rarely, if ever. The neuraminidase inhibitors zanamivir, oseltamivir and peramivir have increased the range of activity by inhibiting both influenza A and B viruses.

Amantadine and rimantadine

These are mentioned here only because they are classic drugs that specifically inhibit the replication of influenza A viruses with an interesting mode of action, but have no effect on influenza B and other respiratory viruses. They act by inhibiting the penetration of virus into the cell, or its uncoating. Fusion of the viral envelope with a cell membrane, which normally occurs at a low pH, is prevented. Amantadine acts on the viral matrix protein ion channel, thus stopping hydrogen ion passage, raising the pH in intracellular vacuoles, and therefore blocking infection. The standard dose can cause minor neurological side effects such as insomnia, dizziness and headache, especially in elderly patients, and this has discouraged its widespread use. Amantadine can be given prophylactically during community outbreaks of influenza A. It can also be used for treatment, and if taken within 48 h of symptoms there is a reduction in disease severity. However, rapid emergence of drug-resistant variants can occur, and due to the inactivity against influenza B and CNS side effects, and the development of the neuraminidase inhibitors, this class of drugs is of less importance in the influenza armamentarium.

Neuraminidase inhibitors

Oseltamivir, zanamavir and peramivir. Neuraminidase is one of the two surface glycoproteins studded on the influenza virus surface. It cleaves *N*-acetylneuraminic acid, also known as sialic acid, residues from the host cell, thus releasing the virus and allowing further spread in the respiratory tract.

The neuraminidase inhibitors (NAIs) are *N*-acetylneuraminic acid analogues and act as competitive reversible inhibitors of the neuraminidase enzyme active site. Zanamivir is an inhaled agent and can be given intravenously, oseltamivir is an oral drug and peramivir is an intravenous agent, all of which are cleaved by esterases to the active carboxylate form and act on influenza A and B. The importance of having an increased armamentarium of NAIs was demonstrated during 2007–2008 and 2008–2009 as oseltamivir resistance emerged globally amongst the influenza A H1N1 viruses. In the USA, oseltamivir resistance was seen in around 20% and 90% of influenza A H1N1 viruses tested during both the above seasons, respectively.

These drugs reduce viral shedding, disease severity, duration and symptoms if given early in infection and can be used as prophylaxis. They are effective against the circulating influenza strains including the avian influenza H5N1 virus.

Hepatitis B treatment

The aim of treating individuals with chronic hepatitis B and C virus infections is to reduce the risk of cirrhosis and hepatocellular carcinoma by suppressing HBV DNA and HCV RNA levels, respectively.

Treatment regimens offered to hepatitis B virus carriers include nucleoside and nucleotide reverse transcriptase inhibitors (NRTIs) such as lamivudine, adefovir, entecavir, telbivudine, tenofovir and emtricitabine. After stopping treatment, the antiviral response may be reversed and continuing treatment in the long term may lead to the development of antiviral resistance, although the virus will be less fit than the wild type.

Immunomodulation using pegylated interferon alpha, which has a longer half-life than interferon preparations that do not include polyethylene glycol, enhances the innate immune response by binding to the type 1 interferon receptor. This leads to up-regulation of multiple interferon-stimulated genes limiting viral replication. In hepatitis B (HB)e-antigen-positive and -negative carriers, 48 weeks of pegylated interferon alpha results in HBV DNA loss in 25% and 63% of patients, respectively. However, interferons have a large side effect profile.

Of the nucleoside analogues, lamivudine therapy results in undetectable HBV DNA, improved liver histology and liver enzyme levels in 40–44%, 49–62% and 41–77% of patients, respectively. With entecavir these are 67%, 72% and 68%, respectively. The genetic barrier to resistance is low, as only one mutation is needed to lead to lamivudine resistance, compared with entecavir and tenofovir. The lowest rates of drug resistance are therefore seen with entecavir and tenofovir.

Emtricitabine cannot be used as single-agent therapy owing to high rates of resistance. Telbivudine is effective but has a low genetic barrier to resistance.

Adefovir and tenofovir are acyclic nucleoside phosphonates with tenofovir being more effective than adefovir. They are prodrugs as they need to be phosphorylated to become active and are analogues of adenosine monophosphate. They affect the HBV polymerase by competitively inhibiting deoxyadenosine 5′-triphosphate, resulting in chain termination. The major side effect is nephrotoxicity.

Entecavir is a deoxyguanosine analogue that is one of the more effective drugs. It inhibits the HBV DNA polymerase by preventing the following functions: priming of the HBV DNA polymerase, reverse transcription of the negative strand from the pregenomic messenger RNA and synthesis of positive-strand HBV DNA.

These oral antiviral agents have changed the treatment landscape in chronic hepatitis B carriers, and can be used as combination therapy. The potent effect of the NRTIs has been recorded long term, as seen by a 78% reduction in hepatocellular carcinoma in HBe-antigen-positive carriers in whom the HBV DNA load is higher. However, 6 months after stopping treatment, up to 50% of carriers revert to being HBe antigen positive. Treatment is not recommended for hepatitis B carriers in the following phases of infection – high replication, low inflammation or non-replicative – as both interferon and NRTIs are less likely to result in HBsAg clearance or normalized liver enzymes. There are new therapeutic approaches with direct-acting antivirals targeting other parts of the HBV lifecycle. These include capsid inhibitors, drugs targeting the covalently closed circular DNA (cccDNA), as well as drugs that interfere with the host cell functions that allow viral persistence as assisting the immune response against HBV. This illustrates, once again, the importance of understanding both the pathogen and the host response.

Hepatitis C treatment

The days of pegylated interferon alpha combined with ribavirin as the standard treatment of chronic HCV infection have long gone. However, the aim of antiviral treatment leading to a sustained virological response (SVR) and long-term clinical benefit shown by the serum HCV RNA being below the limit of assay detection is still true – but earlier than 24 weeks after starting treatment, which was also the case previously. 2011 was the year that everything started moving, almost as quickly as Usain Bolt (who needs no introduction), as direct-acting antivirals were shown to improve the SVR rates in even the more difficult to treat HCV infections. The HCV non-structural (NS) protein targets included the NS3 protease inhibitor drugs, starting with telaprevir and boceprivir, which were rapidly replaced by simeprevir, asunaprevir and paritaprevir. The NS5 polymerase inhibitors included daclatasvir, elbasvir, ledipasvir, ombitasvir, sofosbuvir and velpatasvir. By 2015, the antiviral action of sofosbuvir, a nucleotide analogue inhibitor, had been elucidated. Its active form is 2′-F′-2′-C-methyluridine monophosphate. This is incorporated into the growing HCV RNA strand affecting the formation of hydrogen bond networks and stopping the conformational changes in the RNA-dependent HCV polymerase, which disrupts the viral RNA chain. Suddenly, 99% SVRs were being reported across almost all HCV genotypes, the exception being the recalcitrant genotype 3, people previously treated with the older agents as well as those with cirrhosis. If that was not amazing enough, this was achieved with a combination of sofosbuvir and velpatasvir, given orally, once daily for 3 months. As for genotype 3, the SVR was still around 95% in treatment-naive patients and was improved by adding ribavirin.

The development of non-invasive markers of liver fibrosis, serological markers and ultrasound techniques also reduced the need for a liver biopsy as part of the disease staging and management. However, although there is the potential to reduce the hepatologists' workload, making HCV clinics a thing of the past and curing HCV carriers, the DAAs are

expensive, many of those with diagnosed or undiagnosed HCV infection can be difficult to 'access' and there are many with undiagnosed HCV globally.

Finally, HCV is like a puppeteer, pulling the strings of the intracellular environment, using host factors in a positive or negative way, by replicating in an environment of cholesterol and lipid, protected from host ribonucleases, exonucleases and immune responses. In addition, it has been shown that raised 25-hydroxysterol levels are found in HCV infection. This induces a microRNA which affects the cholesterol and lipid environment and reduces HCV replication – a fascinating discovery throwing light on another example of the complex way host and viral factors affect each other.

Interferons – immunomodulatory agents restart

Interferons (see Ch. 10) are natural glycoproteins produced by the innate immune system in response to infections. They have non-virus-specific antiviral and immunomodulatory actions and trigger a cascade of intracellular reactions that activate interferon (IFN)-inducible genes. These genes encode proteins thought to inhibit intracellular virus multiplication by inhibiting translation initiation and assisting RNA degradation. IFNα also binds to immune cells, resulting in class I MHC antigen expression, activation of effector cells and a cytokine cascade. Production of Th1 cells is stimulated, in contrast to Th2 suppressor cells that are reduced. IFNs are generally given as subcutaneous injections and the side effects are significant and include tiredness, headache, myalgia and psychiatric symptoms.

IFNs have been used to treat individuals with chronic HBV and HCV infections and have an effect on HPV infections, given by intralesional injection, but are not used routinely.

When given in the past as monotherapy, success was limited owing to the poor SVR rates for both HBV and HCV infections.

Other targets

Drugs targeting differing parts of the viral life cycle, post-translational processing, virus entry, RNA translation and virus assembly and release as well as host cell-targeting compounds are always being developed. Nucleic-acid-based antiviral agents including antisense oligonucleotides and RNA interference-based agents have been synthesized, as have immunotherapeutic options using antibody-based preparations and therapeutic vaccines.

Clinical management of antiviral therapy

Viral load and antiviral resistance tests as well as therapeutic drug monitoring assist in clinical management

Qualitative and / or quantitative nucleic acid tests are critical in the diagnosis, treatment decision, assessment of response to treatment and prognosis for a number of viral infections. This is true for HIV load testing, together with the CD4 count and percentage. With HCV, it is important to determine the HCV genotype and then monitor the plasma HCV RNA load to look for an SVR. For HBV infection, plasma HBV DNA load and antiviral resistance testing are part of the clinical management strategy. Genotypic analysis is also helpful. Another example is CMV DNA monitoring in post-transplant populations to detect early viraemia in order for pre-emptive treatment to be given.

The main causes of treatment failure in HIV infection are either compliance issues or the development of antiviral resistance.

Combined antiretroviral therapy (cART) has had an enormous impact on HIV disease progression. The development of drug-resistant virus will lead to treatment failure as seen by an increase in HIV load and reduction in CD4 count. Specific mutations can be detected in the drug target sites, that is, reverse transcriptase and protease regions, by nucleic acid sequencing. This is referred to as a genotypic resistance assay. Key mutations known as primary resistance mutations at specific codons have been associated with a reduction in susceptibility to the various clans of antiretroviral drugs. Some mutations are unique to certain drugs, but many confer cross-resistance, resulting in an entire class of drugs (such as the NNRTIs) being removed from the treatment regimen. In addition, viral tropism assays are carried out in diagnostic laboratories to identify co-receptor use, which is critical when deciding on use of chemokine receptor antagonists. HIV-1 entry into lymphocytes and monocytes involves binding of the gp120 envelope glycoprotein to the CD4 receptor, followed by interaction with one of two main co-receptors, CCR5 or CXCR4. This is referred to as viral tropism and whether the virus is X4 or R5 is mainly determined by the amino acid sequence of the V3 region of gp120. Dually tropic strains can use both receptors. In later-stage HIV-1 infection, the CD4 cell count falls and the minority population X4 or R5 / X4 strains rises within the viral quasispecies and can finally emerge as a majority population. HIV-1 tropism can be determined using phenotypic and genotypic methods. Genotypic tropism testing can be carried out in laboratories and predictions of co-receptor use are based on the amino acid sequence of the gp120 V3 loop using interpretative algorithms. Antiretroviral drug regimens are based on the results of antiretroviral resistance sequencing assays as well as viral tropism assays.

As HIV drug resistance can be transmitted, and the prevalence of resistant viruses is increasing in individuals with a new HIV diagnosis, baseline genotypic resistance testing is very important in order to tailor cART appropriately. In addition, this is being used to optimize the treatment regimen during drug failure episodes. Details of the key mutations can be found on specialist HIV websites together with guidelines for managing HIV-infected individuals.

It is important in HIV infection to continue the drugs whilst carrying out resistance tests, as without the 'driver' there is a reversion to the wild-type strain as the minor viral populations that contain the mutations are deselected. Phenotypic analysis may also be helpful.

The effectiveness of cART is dependent on good drug plasma concentrations. Keeping drug concentrations within a therapeutic range is critical and drug interactions and compliance issues may result in high or low drug levels, leading to toxicity or virological failure, respectively. Therapeutic drug monitoring is carried out in specialist laboratories and is helpful in finding and correcting any such problems.

ANTIFUNGAL AGENTS

Compared with antibacterials, the number of suitable antifungal drugs is very limited. Selective toxicity is much

more difficult to achieve in the eukaryotic fungal cells than in the prokaryotic bacteria and, although the available antifungals have greater activity against fungal cells than they do against human cells, the difference is not as marked as it is for most antibacterial agents. Treatment of fungal infections is further hampered by problems of solubility, stability and absorption of the existing drugs, and the search for new agents is a high priority. Drug resistance is also increasing.

Antifungals can be classified on the basis of target site and chemical structure

Like antibacterials, antifungals can be classified on the basis of target site and chemical structure. This immediately reveals a major difference between antibacterial and antifungal agents, with the major antifungals acting on the synthesis or function of the intracellular membranes. The exceptions are flucytosine (5-fluorocytosine) and griseofulvin, which interfere with DNA synthesis, and caspofungin, which inhibits cell wall formation. There are currently no inhibitors of fungal protein synthesis that do not also inhibit the equivalent mammalian pathway.

Azole compounds inhibit cell membrane synthesis

Azole antifungals act by inhibiting lanosterol C14-demethylase, an important enzyme in sterol biosynthesis. Clotrimazole and miconazole are useful as topical preparations. Itraconazole and fluconazole are used in treatment of a variety of serious fungal infections (Table 34.7), and fluconazole is often used in the treatment of *Candida* infections, subject to species identification. Resistance to the azoles is becoming more widespread and threatens to compromise this group of compounds. Newer azole compounds include posaconazole, which is used in aspergillosis unresponsive to amphotericin B, and isavuconazole, which is used in the treatment of invasive mucormycosis.

Echinocandins interfere with cell wall synthesis

The echinocandins caspofungin, micafungin and anidulafungin inhibit the enzyme β-(1,3)-D-glucan synthase, which is required for synthesis of an essential part of the fungal cell wall. This important group of compounds offers new therapeutic options against infections such as invasive *Aspergillus*

Table 34.7 The major therapeutic applications of antifungal drugs

Infection	Antifungal of choice	Route of administration
Superficial mycoses		
Ringworm (dermatophytes)	Topical agents (see text) are used to treat most cases. Systemic therapy is required for scalp ringworm Griseofulvin	Topical Oral
Candidiasis	Clotrimazole Miconazole Nystatin Fluconazole	Topical Topical Topical Oral
Systemic mycoses		
Histoplasmosis	Liposomal amphotericin B then itraconazole	Intravenous Oral
Blastomycosis	Liposomal amphotericin B then fluconazole	Intravenous Oral
Coccidioidomycosis	Fluconazole (Liposomal amphotericin B for severe infection)	Oral Intravenous
Paracoccidioidomycosis	Itraconazole If severe: Liposomal amphotericin B then itraconazole	Oral Intravenous Oral
Aspergillosis	Voriconazole Isavuconazole Liposomal amphotericin B	Oral Oral Intravenous
Candidiasis	Caspofungin Liposomal amphotericin B For ocular or CNS infection or meningitis: Liposomal amphotericin B plus Flucytosine	Intravenous Intravenous Intravenous Oral
Cryptococcosis	Liposomal amphotericin B plus Flucytosine	Intravenous Oral
Mucormycosis	Liposomal amphotericin B	Intravenous
Pneumocystis pneumonia	Trimethoprim-sulfamethoxazole Pentamidine isethionate	Intravenous or oral Intravenous

CNS, central nervous system.

infections, candidaemia and invasive candidiasis and *Pneumocystis*. However, they are not active against *Cryptococcus neoformans*.

Polyenes inhibit cell membrane function

Amphotericin B and nystatin act by binding to sterols in cell membranes, resulting in leakage of cellular contents and cell death. Their preferential binding to ergosterol over cholesterol is the basis for selective toxicity. With a few exceptions, amphotericin remains the drug of choice for the treatment of serious systemic fungal infections despite its serious toxic side effects; lipid formulations have lower toxicity and are increasingly preferred. Nystatin is used only in topical formulations.

Flucytosine and griseofulvin inhibit nucleic acid synthesis

Flucytosine (5-fluorocytosine) is deaminated to 5-fluorouracil, which inhibits DNA synthesis. Selective toxicity is based on the preferential uptake by fungal cells compared with host cells. Flucytosine is active only on yeasts (e.g. *Candida* spp. and *Cryptococcus*). Resistance emerges rapidly to flucytosine given as a single agent, so it should therefore be used in combination with amphotericin B (whereby it is sometimes possible to reduce the dose of amphotericin B and therefore the toxic side effects).

Griseofulvin appears to inhibit nucleic acid synthesis and to have antimitotic activity, possibly by inhibiting microtubule assembly. It may also have effects on cell wall synthesis by inhibiting chitin synthesis. In the host, griseofulvin binds specifically to newly formed keratin and is active in vivo only against dermatophyte fungi (see Chs 4 and 27).

Other topical antifungal agents include Whitfield's ointment, tolnaftate, ciclopirox, haloprogin and naftifine

A variety of agents such as Whitfield's ointment (a mixture of benzoic and salicylic acids), tolnaftate, ciclopirox, haloprogin and naftifine are available as creams for the topical treatment of superficial mycoses. These are usually available over the counter, and there is little to choose between them.

No single antifungal agent is ideal

The main uses and adverse effects of antifungals are summarized in Table 34.7. Although there are several effective preparations available, some conditions such as dermatophyte infection of the nails (onychomycosis) or recurrent vaginal candidiasis may prove intractable to treatment. The number of antifungal agents for systemic fungal infections is limited, and their adverse effects are considerable.

Fungi develop resistance to antifungal agents

Although much less studied than resistance to antimicrobials used against bacteria, there is evidence that many similar mechanisms operate in resistance to antifungals. These include:

- enzyme modification
- target modification
- reduced permeability
- active efflux pumps
- failure to activate antifungal agents.

Resistance involving some or all of these mechanisms has been described in *Aspergillus*, *Candida* and *Cryptococcus*, particularly in the case of the azole compounds.

There is an urgent need for safer more efficacious antifungal agents

Invasive fungal infections are a significant cause of morbidity and mortality in patients undergoing chemotherapy, immune suppression and transplantation. The incidence of these infections is increasing in parallel with the increasing numbers of such patients and their improved survival due to effective antibacterial therapy. New agents to control these infections (e.g. *Aspergillus*) are needed.

ANTIPARASITIC AGENTS

Parasites pose particular problems

Any consideration of antiparasitic agents must take into account the very large number of different parasites capable of infecting humans, the complexities of their life cycles and the differences between them in their metabolic pathways. Thus, drugs acting against protozoa are usually inactive against helminthes, and vice versa. Additionally, protozoa and helminths are eukaryotes and therefore metabolically more similar to humans than are bacteria. Although some antibacterials do have antiprotozoal activity (e.g. metronidazole, tetracycline), in general antibacterials are ineffective against parasites. A major challenge has been to identify targets where there are sufficient differences between host and parasite to facilitate safe drug activity. Some of these targets include:

- unique drug uptake: chloroquine, mefloquine, primaquine in malaria
- differences in folic acid metabolism: pyrimethamine in malaria, sulphonamides in toxoplasmosis, trimethoprim in cyclosporiasis
- polyamine uptake: pentamidine in leishmaniasis
- unique trypanothione-dependent reduction mechanisms: fluoromethylornithine against trypanosomes
- unique neurotransmitters: piperazine, ivermectin, pyrantel against nematodes
- cytoskeletal proteins (tubulin): benzimidazoles against nematodes
- intracellular calcium levels: praziquantel against flukes and tapeworms
- oxidative phosphorylation: niclosamide against tapeworms.

Despite differences between host and parasite in these targets, it remains true that a number of the more effective antiparasite drugs carry the risk of significant toxicity.

The wide array of different antiprotozoal and anthelmintic drugs that have been developed is summarized in Tables 34.8 and 34.9, respectively.

Drug resistance is an increasing problem

As with the antibacterials, drug resistance is a significant problem in the treatment of parasitic infections, particularly with malaria. There are four different indications for antimalarial chemotherapy:

- prophylactic: to prevent infection
- therapeutic: to treat infection (applies to all human malarias)

Table 34.8 Therapeutic applications of the major antiprotozoal drugs

Disease / site	Agent	Route of administration	Comments
Amoebiasis			
Asymptomatic cyst passers	Diloxanide furoate Or paromomycin	Oral Oral	
Invasive (dysentery or liver abscess)	Metronidazole or Tindazole followed by diloxanide furoate or paromomycin	Oral Oral	
Cryptosporidiosis	Nitazoxanide (the agent of choice) Paromomycin (limited activity)	Oral Oral	
Cyclosporiasis	Trimethoprim-sulphamethoxazole	Oral	
Giardiasis	Metronidazole or tinidazole Nitazoxanide Quinacrine (also known as mepacrine)	Oral Oral Oral	
Leishmaniasis			
Cutaneous leishmaniasis	Depending on infecting species, site and number of lesions: Local infiltration with sodium stibogluconate (an antimonial) Intravenous sodium stibogluconate Miltefosine	 Local intralesional injection IV Oral	
Visceral leishmaniasis	Liposomal amphotericin B (agent of choice); or sodium stibogluconate or miltefosine	IV IV Oral	
Malaria			
Blood stages	Chloroquine (for *P. vivax, ovale* or *malariae* **only**) Quinine Mefloquine Atovaquone / proguanil Artemisinin combination therapy (ACT), e.g. artemether / lumefantrine Artesunate Tetracycline	Oral Oral, IV Oral Oral Oral IV Oral	 Used against drug-resistant *P. falciparum* ACTs are the agents of choice for uncomplicated *P. falciparum* malaria. WHO lists five recommended ACTs The agent of choice for severe malaria Used with or after quinine against drug resistant *P. falciparum*
Pre-erythrocytic stages	Primaquine	Oral	Used following chloroquine to kill hypnozoites in the liver and achieve radical cure after chloroquine therapy. Required for *P. vivax* and *P. ovale* **only**. Risk of haemolytic anaemia in G6PD-deficient patients
Toxoplasmosis	Pyrimethamine plus sulfadiazine	Oral	
Microsporidiosis	Albendazole	Oral	Variable, species-dependent response
Trichomoniasis	Metronidazole Tinidazole	Oral Oral	
Trypanosomiasis			
East African	Suramin for haemolymphatic stage Followed by melarsoprol if CNS involved	IV IV	
West African	Pentamidine for haemolymphatic stage Nifurtimox-eflornithine combination if CNS involved	IV Oral nifurtimox IV eflornithine	
American (Chagas disease)	Benznidazole Nifurtimox	Oral Oral	

Several are potentially toxic and must be given under supervision. Some also have antibacterial activity and have been described in detail earlier in the chapter. Drug resistance is a problem, particularly in the treatment of malaria. CNS, central nervous system; G6PD, glucose-6-phosphate dehydrogenase; IV, intravenous.

Table 34.9 Therapeutic applications of the major anthelmintic drugs

Disease	Agent	Comments
Cestodes (tapeworms)		
Adult stage infection	Niclosamide Praziquantel	Avoid praziquantel in intestinal *Taenia solium* infection unless concomitant cerebral cysticercosis has been excluded
Cerebral cysticercosis (larval *T. solium*)	Albendazole plus praziquantel	Under corticosteroid cover
Hydatid disease	Albendazole	Regimen depends on cyst type
Trematodes (flukes)		
Schistosomiasis	Praziquantel	
Intestinal flukes	Praziquantel	
Lung fluke	Praziquantel	
Liver flukes except *Fasciola hepatica*	Praziquantel	
F. hepatica	Triclabendazole	
Nematodes (roundworms)		
Ascariasis and pinworm infection	Mebendazole Albendazole Pyrantel pamoate Piperazine	
Hookworm infection	Mebendazole Albendazole Pyrantel pamoate	
Strongyloidiasis	Ivermectin Albendazole Thiabendazole	Less effective Effective but rarely available. Poorly tolerated due to side-effects
Trichinosis	Albendazole Mebendazole	
Trichuriasis	Albendazole Mebendazole	
Cutaneous larva migrans (infection with animal hookworm)	Ivermectin orally Albendazole orally Thiabendazole paste (rarely available)	
Toxocariasis (visceral larva migrans)	Albendazole	
Lymphatic filariasis	Diethylcarbamazine plus doxycycline	
Onchocerciasis	Doxycycline plus Ivermectin	

All are administered orally except thiabendazole paste for cutaneous larva migrans, which is administered topically. Note that many of these drugs are not safe in pregnancy.

- radical cure: to prevent relapse following the treatment of acute infection (applies to *Plasmodium vivax* and *P. ovale* only)
- killing malarial gametocytes: to prevent transmission.

Plasmodium falciparum malaria resistant to one or more antimalarial agents is now widespread. Chloroquine-resistant falciparum malaria occurs worldwide and *P. vivax* also shows focal resistance to this agent, notably in the Asia-Pacific region. The usual alternative to chloroquine in the tropics was combined sulphadoxine / pyrimethamine but there is now significant resistance to the antifolate compounds. Mefloquine-resistant falciparum malaria is found in parts of SE Asia and parts of South America. Quinine, the original antimalarial, is still used to treat severe malaria if artesunate, the drug of choice, is not available, though quinine requires careful monitoring during treatment to avoid toxicity. Development of antimalarials from natural products has provided new compounds, the most important being derivatives of artemisinin (from the Chinese drug *quinghaosu*, produced from the plant *Artemisia annua*). Intravenous artesunate has supplanted quinine as the agent of choice for the treatment of severe falciparum malaria. Drug combinations are now deployed for the treatment of

falciparum malaria to reduce the chance of developing drug resistance after monotherapy, as happened with chloroquine, and artemisinin combination therapy (ACT) is the first-line treatment of choice. Drug resistance is less of a problem with other protozoa and, although widespread in animal parasitic nematodes, has yet to become a serious issue with human infections.

Protozoa make use of enzyme and target modification to develop resistance (e.g. against antifolates and sulphonamides), but in addition active efflux pumps have been described in resistance of *P. falciparum* to chloroquine and mefloquine. Resistance to benzimidazole anthelmintics involves target modification, arising from mutations in cuticular tubulins.

CONTROL BY CHEMOTHERAPY VERSUS VACCINATION

While vaccination is discussed in detail in Chapter 35, it is important to note here the role both chemotherapy and vaccination play in protecting individuals. An important difference is that chemotherapy is usually given after exposure to infection, whereas vaccination is usually given before exposure. Chemotherapy essentially offers short-term protection, which wanes once the drug is no longer given; vaccination can give long-term protection without repeated treatment. Vaccination is therefore more effective than chemotherapy in protecting populations.

There are, of course, exceptions to these: passive antibody can be used to treat acute infection just as a drug can, while drugs like mefloquine or atovaquone-proguanil combination preparation are used for prophylaxis against malaria, almost as if they were short-term vaccines. However, in most cases there is a clear-cut distinction between the one- or two-shot vaccine, conferring protection for years, and the daily or twice-daily drug dose.

The concept of selectivity, or specificity, is central to both chemotherapy and vaccination

Although they appear so different (Table 34.10), both chemotherapy and vaccination developed together from the intensive study that followed the demonstration in the late 1800s that diseases could be caused by microbes. Louis Pasteur (Box 34.8) showed that killed or weakened microbes (e.g. anthrax, rabies) could be used to induce immunity that was active against that disease, while Ehrlich's work with histological dyes led him to the idea that particular chemicals ('drugs') might bind specifically to particular microbial structures and damage them, thus being active against several diseases. Both therefore established the concept of selectively or specifically targeting infectious organisms within the body as a means of controlling disease.

The specificity of an antimicrobial drug resides in its ability to damage the microbe and not the host

As noted earlier, antimicrobial drugs should ideally bind to a molecule present only in the microbe to ensure specificity for the microbe and not the host. The extent to which this can be achieved varies from microbe to microbe. Bacteria, with their prokaryotic cell structure, are much more remote from humans than fungi, protozoa or worms (which are all eukaryotic). It is not surprising, therefore, that the most effective antibiotics are generally those used against bacteria. As much of the viral life cycle uses host cell components, antiviral chemotherapy has so far been less successful than antibacterial therapy.

Many antimicrobial agents are products of microbes themselves or derivatives of these products. It is presumed that they form part of the self-preservation mechanism by which the microbes prevent overcrowding with their own or other species.

Although it is possible to administer antimicrobial agents in ways that prolong their presence in the body, they are no longer active once concentrations fall below a critical threshold. Continuing antimicrobial activity therefore requires repeated administration as opposed to vaccines, which can provide long-term protection with far less re-administration (see Ch. 35).

CONTROL VERSUS ERADICATION

Control and eradication are different objectives, although eradication is always an ideal endpoint

Many infections can be controlled (at least in some parts of the world) by the use of a combination of strategies, including chemotherapy and vaccination (see Ch. 35; Table 34.10), but are certainly not eradicated, even in those countries where control is most effective. Epidemiological theory (see Ch. 33) predicts that once transmission rates fall below a threshold value the infection should die out, and this may certainly be

Table 34.10 Comparison of chemotherapy and vaccination

	Chemotherapy		Vaccination	
Specificity	Usually high		Very high	
Toxicity	Potentially high		Usually low	
Duration of effect	Usually short		Usually long	
Duration of treatment	May be prolonged		Usually short, but may need boosting	
Effectiveness	Bacteria	High	Viruses	High
	Viruses, Fungi	Moderate	Bacteria, Fungi	Low / moderate
	Parasites	High	Parasites	No vaccine yet marketed for parasitic infections of humans; a malaria vaccine will be the first

Box 34.8 Lessons in Microbiology

Louis Pasteur (1822–1895)

The science of microbiology was established in the nineteenth century by the work of many distinguished scientists. However, one such scientist, Louis Pasteur, may legitimately be regarded as a founding father of this discipline (Fig. 34.30). He, along with Robert Koch, a German doctor (see Ch. 13), was able to show that living organisms or 'microbes' were the cause of disease, and provided a firm scientific basis for their study and control.

Pasteur began work at a time when spontaneous generation was still an accepted explanation for the appearance of microorganisms in decaying material. His elegant experiments showed that sterile organic infusions would not putrefy or ferment if there were no subsequent contact with airborne contaminants, proving that spontaneous generation did not occur, and that all microbes must come from pre-existing microbes. This discovery contributed to many fields of science, both basic and applied. Perhaps most important was the contribution Pasteur made to the work of Lister on antiseptics, which revolutionized approaches to surgery.

Pasteur worked in an amazing variety of microbiological fields, from fermentation in the brewing of beers and production of wines, to identification of silkworm diseases, bringing to each a penetrating scientific insight and making discoveries that brought him national and international renown. His understanding of the roles of organisms in causing diseases, and his acute scientific perception, enabled him to grasp from a series of 'mishaps' with experiments on chicken cholera that attenuated microbes could induce not disease, but immunity from it. His ideas generated powerful opposition, but his belief then was strong enough to encourage him in 1881 to take part in a public trial of his vaccine against anthrax in domestic animals. Later, he used his insight into rabies, caused by organisms he could not see or culture, to develop an attenuated vaccine made from the dried spinal cords of infected rabbits. This was proven effective in humans in 1885, when Pasteur inoculated Joseph Meister, a 9-year-old boy who had been badly bitten by a rabid dog. Meister survived, and Pasteur's views on vaccination became universally accepted.

Pasteur ended his life as a national hero in his native France, and with a worldwide reputation for his work. His name is immortalized not only in the process of sterilization ('pasteurization') that he developed, but also in the Institut Pasteur in Paris, which remains one of the most important international centres of microbiological work.

Figure 34.30 Louis Pasteur (1822–1895).

true at a local level. However, reservoirs of infection persist where treatment is non-existent or ineffective, or infection is re-introduced by the movement of peoples, and new epidemics may therefore develop. To date, only one disease – smallpox – has been taken to the point at which the organism has been eliminated. What are the chances that other infectious diseases will follow smallpox into oblivion? Various factors are important in determining the effectiveness of any eradication programme (Table 34.11).

Realism is required when considering the long-term aims of antimicrobial control strategies

Hopes raised by the early success of antibiotics were soon dashed by the emergence of resistance, and far from the microbial load borne by the human race being diminished in recent years, it has if anything increased. Many infections covered in this book, HIV, Ebola and Zika viruses to name but a few, do not feature in older textbooks of microbiology. Infections previously well controlled by antibiotics have become serious problems in hospitals (MRSA, *C. difficile,* carbapenem-resistant, ESBL-producing Enterobacteriaceae). Approaches to the control of infectious diseases are therefore a matter of identifying priorities such as:

- Which diseases could, with suitable effort, be eradicated?
- Would the cost of eradication be justified?
- Which diseases need urgent measures to stop them getting worse?
- Which diseases are responsible for the most human suffering and economic loss?

Inevitably, some diseases will not feature strongly on any such list, and it must be accepted that they may always be with us.

USE AND MISUSE OF ANTIMICROBIAL AGENTS

Much has been said in this chapter about the interactions between antimicrobial agents and microbes – the mechanisms of selective toxicity and the defences put up by resistant organisms. The distribution, metabolism and excretion of agents by the host have been considered briefly, together with the important toxic side effects of the agents. The choice of antimicrobial for treating specific infections is dealt with in the

Table 34.11 Strategies for control of infectious diseases

General features	Water purification (water-borne diseases) Sewage disposal (enteric infections) Improved nutrition (host defence) Improved housing (less crowding, dirt, etc.)
Food	Cold storage Pasteurization (milk, etc.) Food inspection (meat, etc.) Adequate cooking
Zoonoses and arthropod-transmitted infections	Control of vectors (mosquitoes, ticks, lice, etc.) Control of reservoir animal (rabies, etc.)
Specific disease treatment or prevention	Chemotherapy Vaccines
Miscellaneous measures	Changes in personal habits (reduced promiscuity, use of condoms, improved personal hygiene, etc.) Control of intravenous drug abuse Screening of transfused blood and organs

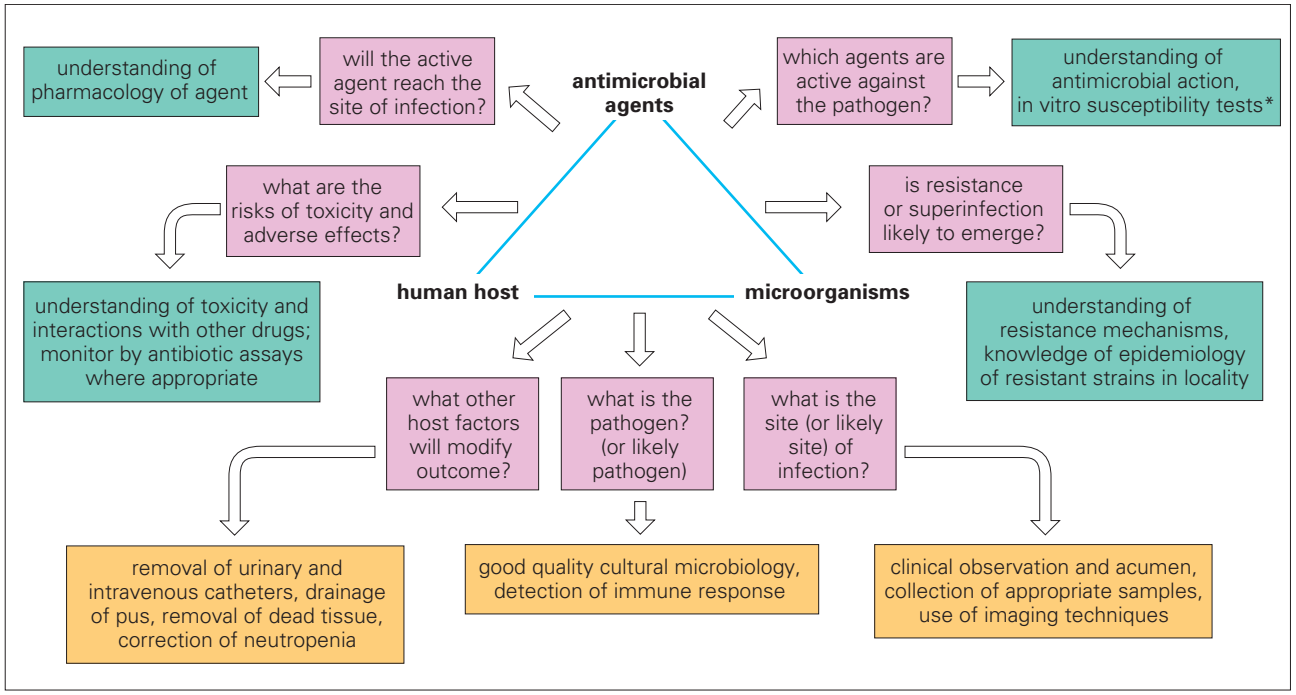


Figure 34.31 The interactions between antimicrobial agents, microorganisms and the human host can be summarized by examining the answers to several questions affecting each side of the triangle of interaction. * Other tests include phenotypic and genotypic antiviral susceptibility tests and viral load tests.

appropriate systems chapter. Dosage regimens have not been included because they vary with the agent, the infection, the age and the underlying condition of the patient, and sometimes from one country to another. Practitioners should consult appropriate local pharmacy guidelines.

Antimicrobial agents should only be used appropriately for prophylaxis or treatment

In conclusion, we should stand back and ask 'Is antimicrobial therapy necessary for this patient, and, if so, which agent is appropriate?' Antimicrobial agents can be used:

- to help prevent infection (prophylaxis)
- to treat infection.

Prophylactic use of antibiotics is appropriate only in a few clearly defined circumstances and is usually of limited duration (e.g. 1–2 days). Specific examples include: (1) patients of normal susceptibility who have been exposed to specific pathogens (e.g. bacterial meningitis or tuberculosis), (2) individuals with increased susceptibility to infection (e.g. neutropenic patients), and (3) perioperative antibiotic 'cover' for patients undergoing surgery.

Antimicrobial use results in the selection of resistant strains

If antibiotic treatment is necessary, several factors must be considered, and these are summarized in Fig. 34.31. It is

important to recognize that during treatment not only the infecting microbe, but also the patient and all his or her normal microbiota are being exposed to the effects of the antimicrobial agent. Use of antimicrobials has been clearly shown to select for resistant strains, both in the individual and in the community, and overuse or inappropriate use only increases this risk. History suggests that microbes will never run out of ways of developing resistance, but we may run out of effective antimicrobials.

KEY FACTS

- Infection is unique among the diseases which afflict humans because it involves two distinct biological systems. Antimicrobial agents are designed to inhibit one system (the microbe) while doing minimal damage to the other (the patient). Antimicrobial agents require selective toxicity.
- Antimicrobial agents are often themselves products of microorganisms (natural products) although most are chemically modified to improve their properties. Other agents are entirely synthetic. Antibacterials are the most numerous; designing antiviral, antifungal and antiparasitic drugs which are selectively toxic provides much greater challenges.
- Antibacterials are classified by their target site and their chemical family; this helps us to understand better their mode of action and the mechanisms of resistance.
- Antibacterials have four possible sites of action in the bacterial cell: cell wall, protein, nucleic acids and cell membrane. Most antibacterials act at the cell wall or inhibit protein or nucleic acid synthesis. At each site there are many different molecular targets (enzymes or substrates) which can be specifically inhibited.
- Development of resistance is the major limiting factor of antibacterials. It arises through random mutation of bacterial chromosomal genes but more importantly through acquisition, from other bacteria, of resistance genes on integrons, transposons and plasmids.
- Mutated or acquired genes confer resistance by altering the target site of the antibacterial, altering the uptake of the drug, or producing drug-destroying enzymes.
- The emergence of AIDS has provided an enormous stimulus to research in antivirals (especially anti-HIV drugs). Selective toxicity is again a major challenge. Drug combinations show promise in the treatment of HIV, but there is no specific therapy for the majority of viral diseases. Effective therapy is available for other viral infections, including hepatitis B and C, influenza A and B, HSV and CMV.
- The number of classes of antifungal molecules is very limited. Toxicity (all), difficulty of formulation (polyenes) and emerging resistance (azoles) make effective treatment of fungal infections a serious challenge.
- Although there are many antiparasitic drugs available, a number show toxicity and others are becoming increasingly ineffective because of the development of resistance. This is particularly so in malaria infections, where parasites show resistance to almost all drugs presently available.
- Bacteria can be tested in the laboratory for their susceptibility to antibacterials. The results of well-controlled tests provide a valuable guide to appropriate treatment. In vitro tests with antifungals are less reliable and are rarely performed with antivirals in the clinical laboratory setting.

35 Protecting the host: vaccination

Introduction

Vaccines are one of the most effective public health tools. This chapter will review how vaccines work, and the vaccines in current use. However, although vaccination is a very cost-effective public health measure that saves an estimated 2–3 million deaths each year, a further 1.5 million people still die each year from a vaccine-preventable disease, as a result of poor vaccine uptake (Fig. 35.1). Many others die from infectious diseases such as HIV for which we have no effective vaccine, so new vaccines are also needed (Table 35.1).

Vaccination exploits the ability of the immune system to develop immunological memory, so that it can rapidly mobilize its forces to fight infection when required. Vaccines can be of different types, including live attenuated organisms, killed organisms, or subunit vaccines. Depending on the vaccine type, more than one dose may be needed to achieve or maintain optimal protection. Adjuvants are often required to increase immunity. The development of new and more effective vaccines is a major area of research, especially with outbreaks of viruses such as Ebola or Zika virus. Successful vaccination also requires an understanding of the epidemiology of disease transmission, to estimate what proportion of the population needs to be vaccinated to produce herd immunity, as discussed in Chapter 33.

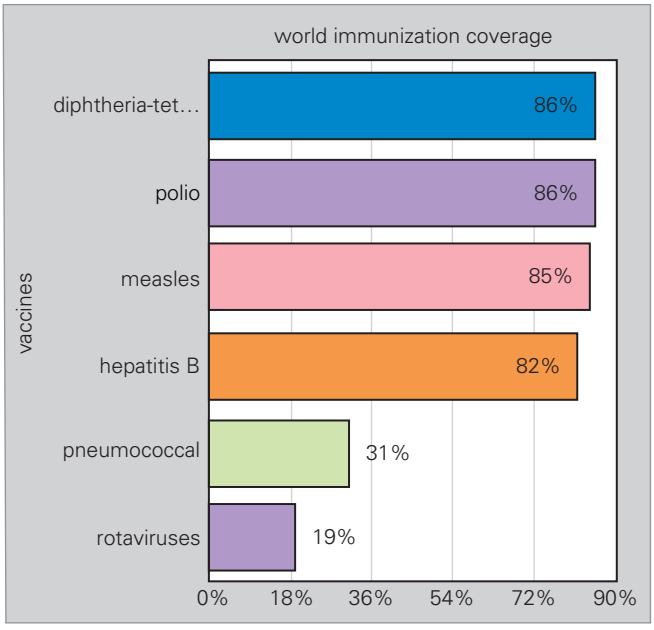


Figure 35.1 Global immunization coverage in 2015. Vaccine coverage is good for some of the older vaccines but many more lives would be saved if available vaccines were more widely used diptheria-tet..., diptheria, pertussis, tetanus. (Source: WHO. http://www.who.int/mediacentre/factsheets/fs378/en/, with permission.)

Table 35.1 Infectious agents that are major killers

Organism	Disease	Estimated annual deaths (millions)
HIV	AIDS	1.1
Mycobacterium tuberculosis	Tuberculosis	1.8
Plasmodium spp.	Malaria	0.4
Total		3.3

We currently lack effective vaccines against these organisms, although bacille Calmette–Guérin (BCG) vaccination can provide protection against disseminated forms of childhood tuberculosis, and pulmonary tuberculosis in some parts of the world. Most of the deaths from HIV are in Africa, and most of the deaths from malaria are in African children.
(*Sources*: Figures for 2015 from WHO.)

VACCINATION – A FOUR HUNDRED YEAR HISTORY

'Never in the history of human progress', wrote the pathologist Geoffrey Edsall, 'has a better and cheaper method of preventing illness been developed than immunization at its best'. The greatest success story in medicine, the elimination of smallpox, began before the existence of microbes or the immune system was even suspected. Due to the pioneering work of Jenner with vaccinia (Box 35.1, Fig. 35.2), all forms of specific, actively induced immunity are now referred to as 'vaccination'.

The principle of vaccination is simple: to prime the adaptive immune system to the antigens of a particular pathogen so that

Box 35.1 ■ Lessons in Microbiology

Edward Jenner (1749–1823)

The English physician Edward Jenner (Fig. 35.2) is regarded as the founder of modern vaccination, but he was by no means the first to try the technique. The ancient practice of 'variolation' dates back to tenth-century China, and arrived in Europe in the early eighteenth century via Turkey. The technique involved the inoculation of children with dried material from healed scabs of mild smallpox cases, and was a striking foretaste of the principles of modern attenuated viral vaccines. This practice was, however, both inconsistent and dangerous, and Jenner's innovation was to show that much safer and more reliable protection could be obtained by deliberate inoculation with cowpox (vaccinia) virus. Milkmaids exposed to cowpox were traditionally known to be resistant to smallpox and so retained their smooth complexions. In 1796, Jenner tested his theory by inoculating 8-year-old James Phipps with liquid from a cowpox pustule on the hand of Sarah Nelmes. Subsequent inoculation of the boy with smallpox produced no disease. Although greeted with skepticism at first, Jenner's ideas soon became accepted, and he went on to inoculate thousands of patients in a shed in the garden of his house at Berkeley, Gloucestershire. He ultimately achieved world fame, though his fellowship of the Royal Society was conferred for a quite different piece of work on the nesting habits of the cuckoo!

Figure 35.2 Edward Jenner (1749–1823).

on first contact with the live organism a rapid and effective secondary immune response will be induced by memory T and B cells. Vaccination therefore depends upon the ability of lymphocytes, both B and T cells, to respond to specific antigens and develop into memory T and B cells, and represents a form of actively enhanced adaptive immunity. The passive administration of preformed elements, such as antibody, is considered in Chapter 36.

AIMS OF VACCINATION

The aims of vaccination can vary from preventing symptoms to eradication of disease

The most ambitious aim of vaccination is eradication of the disease. This has been achieved for smallpox, the eradication of polio is being attempted, and there was a dramatic downward trend in the incidence of many vaccine-preventable diseases from 1950 to 1980 (Fig. 35.3). However, as long as any focus of infection remains in the community, the main effect of vaccination will be protection of the vaccinated individual against infection.

In certain cases, the aim of vaccination may be more limited: namely, to protect the individual against symptoms or pathology. For example, diphtheria and tetanus vaccines induce immunity only against the toxins produced by the bacteria, as it is the effect of these toxins rather than the simple presence of the microbe itself that is harmful.

The importance of herd immunity

Successful vaccination programmes rely not only on the development and use of vaccines themselves, but also on an understanding of the epidemiological aspects of disease transmission. If enough individuals in a population are immunized, this will reduce or stop transmission of the infection. This is called herd immunity. By having your own child immunized, you therefore help protect the whole community – but, conversely, when too many parents decide not to vaccinate their children, because they think the risk of their child getting the disease is low, this may contribute to the disease becoming more common (see Fig. 35.3). It is therefore important to know how many individuals in a population must be immunized to produce herd immunity, and whether immunity should be boosted by revaccination.

VACCINES CAN BE OF DIFFERENT TYPES

Vaccines can be based on whole organisms, either live or inactivated, or components of the infectious agent (Table 35.2). Sometimes two types of vaccine are available for the same disease, and for a good reason.

Live vaccines are designed to induce immunity in a similar way to the actual infection. Most live vaccines use attenuated organisms that were attenuated using culture in eggs, animals or in tissue culture (Fig. 35.4); these attenuated organisms replicate to a limited extent in the vaccinated individual but do not cause disease in healthy people. However, immunosuppression can produce problems with live vaccines. For example, infants with HIV infection given bacille Calmette–Guérin (BCG) vaccination can develop disseminated BCGosis. HIV-infected individuals with severe immunosuppression should not be given live vaccines such as measles or varicella, but they can be given inactivated vaccines.

Inactivated vaccines are safe to use in the immunocompromised, although they may not be as immunogenic, so a good adjuvant may be needed. Inactivation is usually by fixation, for example with formalin. Types of fixatives in use in vaccines are given

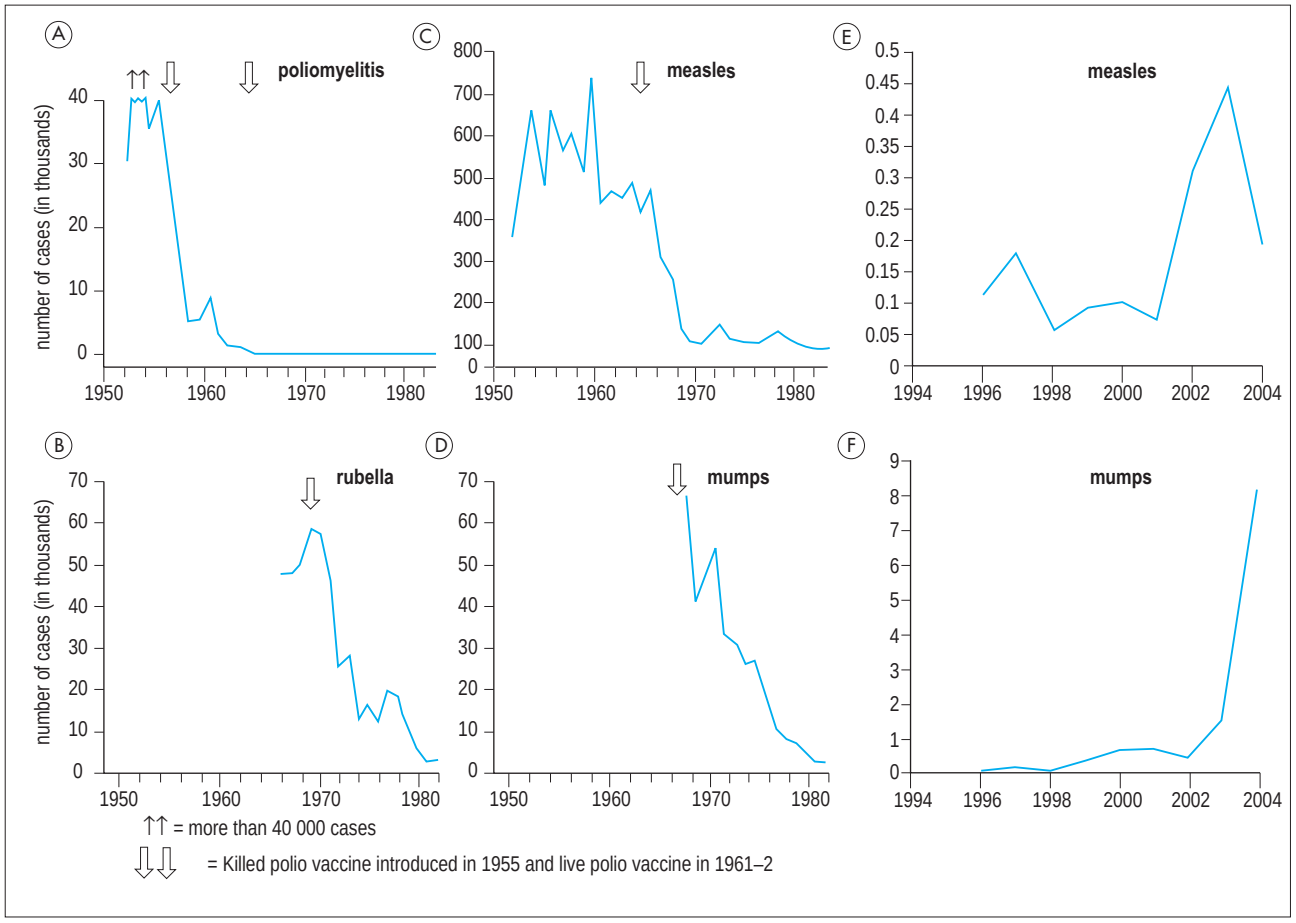


Figure 35.3 The effect of vaccination on the incidence of various viral diseases in the USA and the UK. Most infections (A–D) have shown a dramatic downward trend after the introduction of a vaccine (arrows), but the right-hand panels (E, F) show the resurgence in disease when vaccine uptake is reduced following vaccine 'scares'. (Data from Mims and White and the Health Protection Agency, UK.)

in Table 35.3. Another difference between live and attenuated vaccines is that immunity induced by inactivated vaccines is not affected by circulating antibody. Individual antigens or toxins can also be used as a vaccine, with adjuvant. Purified proteins are used in the acellular pertussis vaccine and recombinant surface antigen protein in the vaccines for hepatitis B. A number of protein antigens can be joined together as a fusion protein, as in some candidate TB vaccines. Polysaccharides form the basis of the pneumococcal vaccine but, as polysaccharide vaccines are not immunogenic in children under 2 years of age, conjugate vaccines that use a polysaccharide linked to a protein have been developed for pneumococcal and meningococcal disease, and for *Haemophilus influenzae* type b (Hib). For some bacteria, it is the toxin that is pathogenic – and this can be inactivated to make a toxoid, as in the tetanus toxoid vaccine. With individual components of an organism, an adjuvant will be needed to boost immune responses. Multiple doses of protein or polysaccharide are usually needed, as these vaccines are less immunogenic than whole organisms.

One or more vaccine antigens can also be delivered by a viral vector, such as modified vaccinia virus Ankara (MVA), which was safely used in humans at the end of the smallpox eradication campaign. Other viral vectors being considered for new vaccines include adenovirus and cytomegalovirus. This type of technology can be used quickly to make new vaccines, and has been exploited to develop vaccines for the Ebola and Zika viruses.

Some vaccines are designed to boost immunity using only selected antigens or by using a different delivery route – called prime boost. For example, some new TB vaccines in development would boost the immunity induced by BCG by giving key antigens delivered by a viral vector (Fig. 35.5), or as a fusion protein with adjuvant.

Recipients of haemopoietic stem cell transplants may need to be revaccinated after the infusion of stem cells, as otherwise antibody titres to vaccine-preventable diseases decline.

Adjuvants

Adjuvants increase the immunity induced by a vaccine in a number of ways. Adjuvants can improve the immune response to the vaccine antigens through inducing activation of Toll-like receptors (TLR) on dendritic cells to improve antigen presentation, or by forming an antigen depot, which allows antigen to persist and to leak out slowly over time. The earliest adjuvants consisted of water-in-oil emulsions; Freund's complete adjuvant, which includes dead mycobacteria in a water-in-oil emulsion, is very effective in animals, although not suitable for use in humans. Other adjuvants increase antigen presentation, or enhance particular types of

Table 35.2 Types of vaccine

Types of vaccine	Examples
Live attenuated	
Viral	Measles, mumps, rubella, varicella, yellow fever, zoster, oral polio, intranasal influenza, rotavirus
Bacterial	BCG, oral typhoid
Inactivated	
Whole virus	Polio, influenza, hepatitis A, rabies, Japanese encephalitis
Whole bacteria	Pertussis, cholera, typhoid
Fractions	
Toxoids	Diphtheria, tetanus
Protein subunits	Hepatitis B, influenza, acellular pertussis, papilloma virus
Polysaccharides	Pneumococcal, meningococcal, *Salmonella typhi* (Vi)
Conjugates	*Haemophilus influenzae* type b (tetanus toxoid, non-toxic diphtheria toxoid or *Neisseria meningitidis* outer membrane protein), pneumococcal (diphtheria toxoid), meningococcal (diphtheria toxoid)

Note that not all types of vaccine are available in all countries. Vaccines are also available for bioterrorism agents such as anthrax and plague, and for vaccinia.

Table 35.3 Fixatives and preservatives used in current vaccines

Fixatives	
Formalin	DTaP / TdaP, Td, HepA, HepB, Hib,* influenza,* Japanese encephalitis, meningococcal,* polio, inactivated typhoid, anthrax
Glutaraldehyde	DtaP, Tdap
Preservatives	
EDTA	Influenza,* rabies,* varicella
Phenol	Hib,* pneumococcal polysaccharide,* inactivated typhoid
2-phenoxyethanol	DtaP, inactivated poliovirus
β-propiolactone	Influenza,* rabies
Sodium deoxycholate	Influenza*
Thiomersal	DT / Td,* influenza,* meningococcal polysaccharide*

*Used in some vaccine formulations and in some multidose vials. TdaP / DtaP, combined tetanus, diphtheria and pertussis; DT / Td, diphtheria and tetanus; HepA, hepatitis A; HepB, hepatitis B; Hib, *Haemophilus influenzae* type b; IPV, inactivated polio vaccine. Thiomersal (Thimerosal) has now been removed from most vaccines because of concerns with having small traces of mercury in the vaccine. Some vaccines can also contain traces of the tissue culture media used to grow the organism or the cell line in which it is grown, for example some influenza vaccines and the yellow fever vaccine have traces of egg proteins.

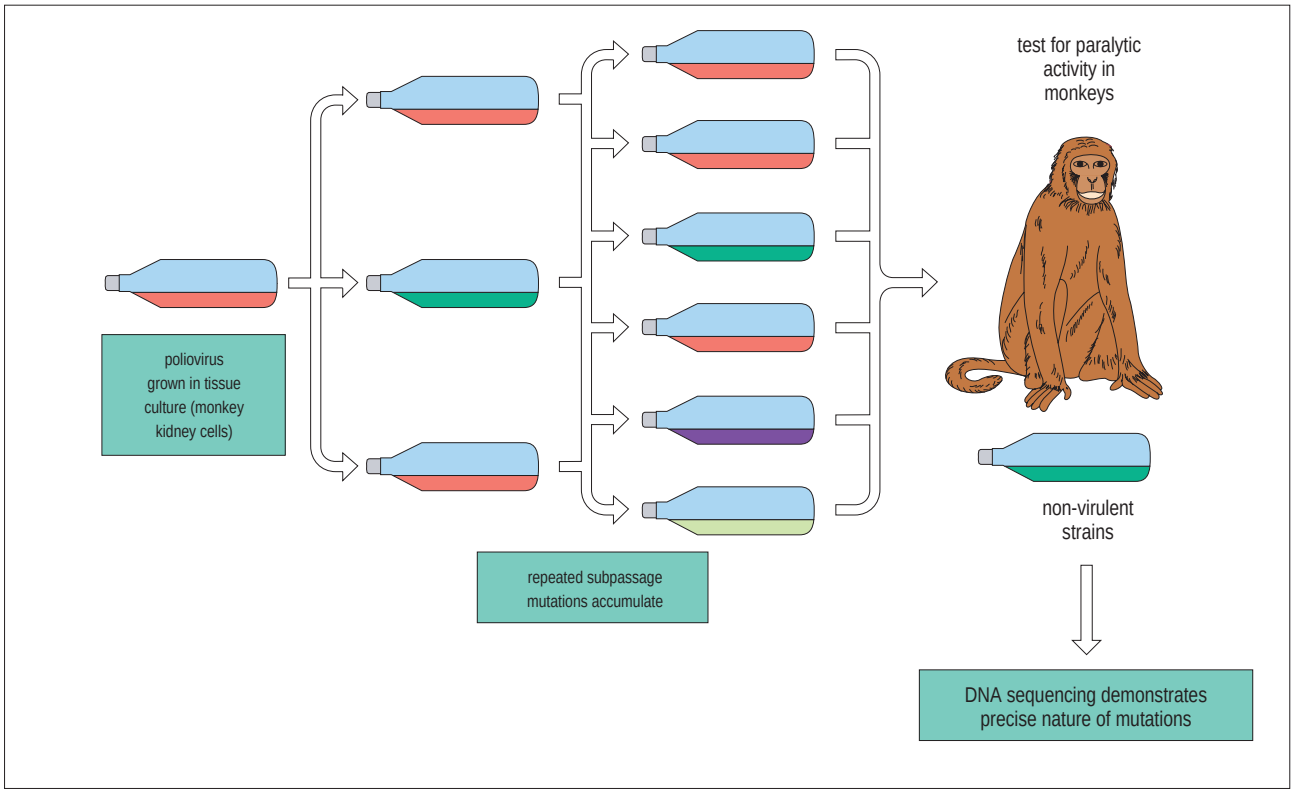


Figure 35.4 Live attenuated vaccines (e.g. polio) were originally produced by allowing viruses to grow in unusual conditions, and selecting the randomly occurring mutants that had lost virulence.

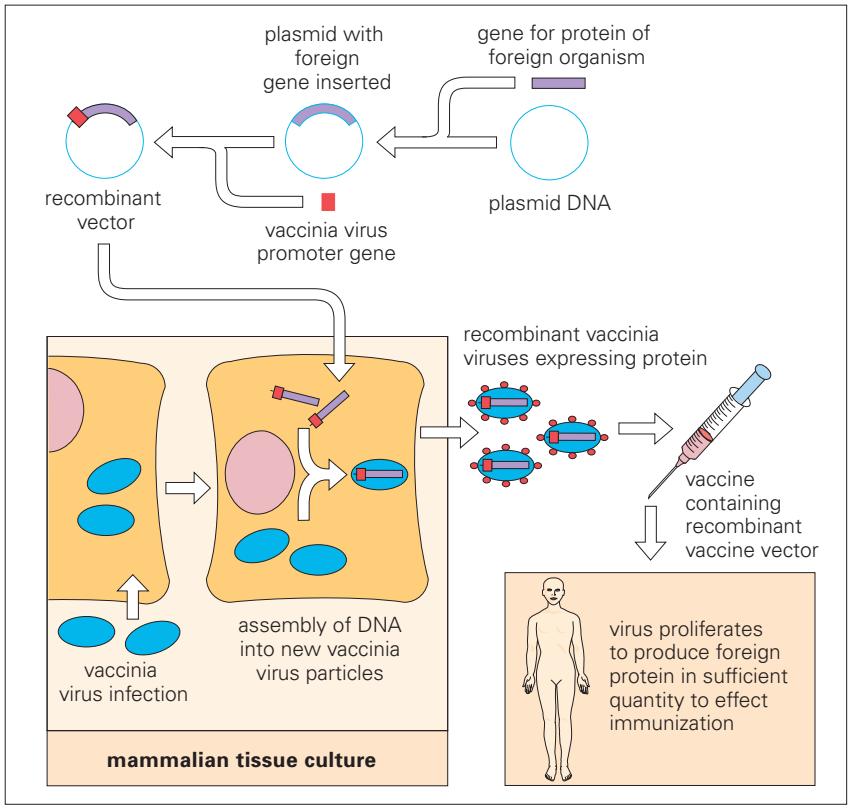


Figure 35.5 It is now possible to insert genes coding for antigens of one or more microorganisms into a large virus such as modified vaccinia virus Ankara (MVA) or adenovirus, so that the virus replicates and antigens are produced in the host. This technology can be exploited to quickly develop new vaccines, for example for Ebola.

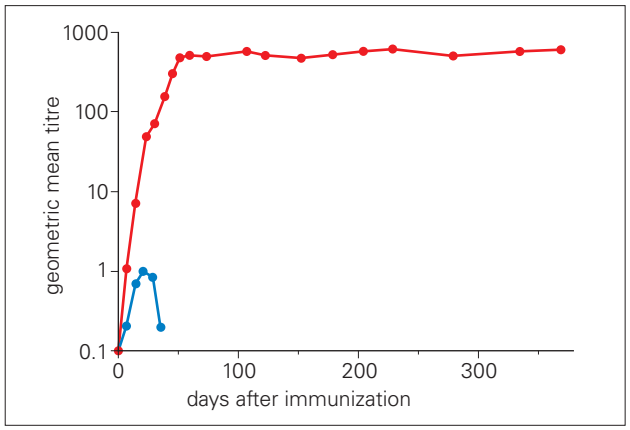


Figure 35.6 Effects of adjuvants on antibody responses of mice to egg albumin. Mice were injected subcutaneously with egg albumin in saline or in Freund's incomplete adjuvant. Antibody titres at intervals over time are shown. The blue symbols represent antigen in saline, and the red symbols antigen in adjuvant. (Redrawn from: Hunter, R. *Vaccine* 2002; 20:S7–S12.)

Box 35.2 Adjuvants in currently used vaccines

Aluminium salts[a]	DTaP, DTaP/IPV/Hib, acellular pertussis, Hib,[b] HepA, HepB, HPV, MenB, PCV-13, Td, Japanese encephalitis
Monophosphoryl lipid A (MPL)	HPV (Cervarix)

[a]Aluminium hydroxide/aluminium hydroxysulphate/aluminium phosphate/aluminium potassium sulphate.
[b]Some formulations.
DTaP, diphtheria tetanus acellular pertussis; HepA/B, hepatitis A/B; Hib, *Haemophilus influenza* type b; HPV, human papilloma virus; IPV, inactivated poliovirus; MenB, meningococcal B vaccine; PCV-13, 13-valent pneumococcal vaccine; Td, tetanus and diphtheria.

immunity, such as antibodies or Th1 immunity. The dramatic effect of adding an adjuvant to a vaccine is shown in Fig. 35.6. Aluminium salts are powerful adjuvants and are still used in many vaccines (Box 35.2); they induce inflammation when cell products from stressed or dying cells (including heat-shock proteins) interact with damage-associated molecular pattern receptors (DAMPS). Experimentally, cytokines such as IL-1, IL-2, IFNγ, IL-12 and IL-18, as well as some chemokines, have been tested as adjuvants. Compounds such as liposomes, lipid-containing vesicles, have also been used, for example 3-*O*-desacyl-4′-monophosphoryl lipid A in the HPV vaccine (Cervarix).

Vaccine safety

As vaccines are given to healthy individuals, it is important that they are safe. In 1926, live *M. tuberculosis* was inadvertently given to healthy children instead of BCG, leading to the Lubeck disaster, and in 1942 US military personnel were vaccinated with yellow fever virus contaminated with hepatitis B virus. Safety testing is now rigorous, requiring extensive quality controls and animal testing, prior to trials or use in humans. Some of the more important issues are summarized in Box 35.3. It is particularly critical that vaccines derived from live

organisms are inactivated to ensure they are safe and that vaccines are preserved appropriately to ensure that vaccine immunogenicity is retained. Examples of fixatives and preservatives used in current vaccines are given in Table 35.3.

Vaccines in current use

Diphtheria, tetanus and pertussis

The diphtheria vaccine consists of the inactivated toxoid. Toxigenic *Corynebacterium diphtheriae* is grown in liquid culture and the filtrate inactivated with formaldehyde to produce the toxoid. This is a highly effective vaccine, giving >90% protection. Three or four doses are required to give good protection, with a booster every 10 years. It is now given in different formulations in combination with other vaccines.

The inactivated tetanospasmin exotoxin from *Clostridium tetani*, inactivated using formaldehyde, is used to vaccinate against tetanus. Tetanus toxoid was first produced in 1924. Again, this is a very effective vaccine, but boosters are required every 10 years. In some developing countries, neonatal tetanus is still a problem; if the mother has been immunized against tetanus this will protect the newborn baby but over 200 000 newborn infants still die each year from neonatal tetanus.

The first vaccine developed against pertussis was a whole cell vaccine, which was available from the mid 1940s and introduced in the UK in 1957 (Fig. 35.7). However, although four doses of vaccine induced 70–90% protection against serious whooping cough, concerns over the safety of the vaccine in the UK and elsewhere in the 1970s led to resurgence of disease and to the development of a safer acellular pertussis vaccine. Current vaccines contain purified filamentous haemagglutinin (FHA) and pertactin as well as pertussis toxin, with some formulations also including fimbriae types 2 and 4, without preservative. However cases of pertussis have been increasing since the switch to the acellular vaccine, often in fully vaccinated children and adolescents, so this is an example where a safer vaccine may not induce as strong immunity.

Global coverage of the combined DTP or diptheria tetanus acellular pertussis (DTaP) vaccines is now good, with an estimated 116 million children receiving three doses of DTP vaccine in 2015, equivalent to 86% coverage. Another formulation for use in adolescents and adults (Tdap) contains tetanus toxoid, with 3–5 pertussis antigens but less diphtheria toxoid than the paediatric DTaP vaccine.

Measles, mumps and rubella vaccines

Live attenuated measles vaccine was introduced in the USA in 1963, using the Edmonston B vaccine, which has since been replaced by the more attenuated Edmonston–Enders strain, grown in chick embryo fibroblast cells. Children should be given two doses of vaccine, as the first dose fails to induce protective antibodies in 5% of those vaccinated. Vaccination is safe and effective, given either on its own or as part of the MMR vaccine with mumps and rubella, or the MMRV vaccine containing measles, mumps, rubella and varicella. However, maternal antibodies inhibit the induction of immunity, so the first dose is generally given at 12–15 months of age, once maternal-derived antibodies have declined, and the second at 4–6 years of age. In lower-income countries where the risk of contracting measles is higher, the vaccine may be given at about 9 months, in an attempt to protect children whose levels of maternal antibodies are declining.

Box 35.3 Problems with vaccine safety

Both living and non-living vaccines require rigorous quality and safety control. Some of the more areas of concern are listed below:

Live attenuated vaccines

- Insufficient attenuation
- Reversion to wild type
- Administration to immunodeficient patients
- Persistent infection
- Contamination by other viruses
- Risk of fetal damage

Non-living vaccines

- Contamination by toxins or chemicals
- Allergic reactions
- Induction of autoimmunity

Genetically engineered vaccines

- Possible inclusion of oncogenes

Weblink: www.who.int/immunization/monitoring_surveillance/data/en/.

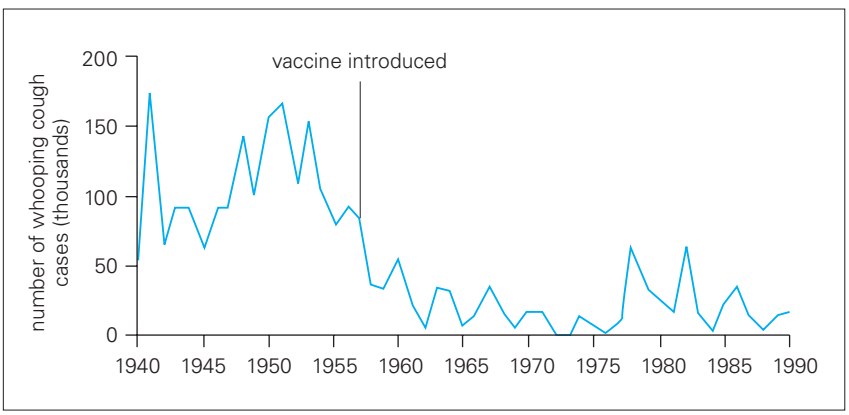


Figure 35.7 The number of cases of whooping cough notified fell steadily after the introduction of mass immunization in the UK in 1958, although epidemics continued to occur at approximately 4-year intervals. Following the scare about the possible adverse effects of pertussis vaccine, the number of cases rose, and there was a large epidemic in the winter of 1978–1979.

Vaccine-induced immunity to measles is long lived and after two doses probably life-long. Between 2000 and 2015, there was an estimated 79% drop in measles deaths worldwide, with an estimated 20 million deaths from measles prevented by vaccination. Nevertheless, WHO has estimated that >134000 people died from measles in 2015, most of whom were children less than 5 years of age. As shown in Fig. 35.3, cases of measles increased in the UK after 2001, following reduced vaccine uptake. This resulted from the suggestion that the MMR (measles, mumps and rubella) vaccine caused autism, as there was an apparent rise in autism in both California and the UK that seemed to coincide with the introduction of the vaccine. However, further studies have failed to show an increased risk of autism after MMR. It is no wonder that parents get worried when bombarded with such scare stories – but they forget that measles infection can kill healthy children. In a measles outbreak in Ireland in 2000, nearly 1500 cases were notified and three children died.

Mumps vaccine

The current mumps vaccine is a live attenuated virus (Jeryl Lynn strain), which was licensed in 1967. Over 97% of those vaccinated make antibodies after a single dose of vaccine, and a study in the UK showed that 88% of those receiving two doses were protected. The importance of receiving two doses of MMR was illustrated by a mumps outbreak in Northern Ireland where 55.4% of the confirmed cases had received one dose of vaccine, compared with 0.9% of those who had received two doses. After two doses, protection should last >25 years and may be life-long. This vaccine is much more effective than an earlier inactivated vaccine – showing how live attenuated viruses induce good immunity.

Rubella vaccine

The current rubella vaccine is a live attenuated virus, strain 27/3, licensed in 1979. The virus was attenuated by 25–30 cell culture passages in human diploid fibroblasts. Over 90% of those vaccinated have at least 15 years of protection from clinical rubella or viraemia. Although rubella itself is a relatively mild infection, it causes real problems if pregnant woman become infected in the first trimester of pregnancy, when congenital rubella syndrome can cause serious damage to the fetus. Thankfully, there has been a dramatic reduction in confirmed cases of congenital rubella syndrome due to vaccination: cases fell by 98% in the Americas between 1998 and 2009.

Polio vaccine

The first polio vaccine was a killed vaccine (inactivated polio vaccine, IPV) developed by Salk; this was first licensed in 1955, and was very effective at reducing the risk of contracting polio. The oral polio vaccine (OPV) developed by Sabin was licensed in the 1960s. Giving the vaccine on sugar lumps or directly into the mouth was much easier than giving it by injection and the live vaccine also gives better intestinal immunity. However, the live polio virus used in OPV vaccine is not genetically stable and can cause vaccine-associated paralytic polio (VAPP) in approximately 1 person per million doses administered (Table 35.4). In addition, it has long been recognized that OPV is transmissible from vaccinees to their close contacts, and it can (on rare occasions) persist in the community as circulating vaccine-derived polio viruses (cVDPV). The global polio eradication initiative that began in 1988 emphasized the use of OPV, but after 2000 most wealthy countries shifted back to IPV, to avoid the risk of VAPP. The eradication initiative has been remarkably successful in reducing the number of polio cases worldwide by >99%, from an estimated 350000 cases in 1988 to 650 wild polio virus cases in 2011 and to 35 in 2016 (Fig. 35.8). As part of the 'endgame' strategy of the eradication programme, countries are switching to IPV to avoid circulation of VDPV, and trivalent OPV has been replaced by bivalent (1–3) OPV. In 2016, only three countries reported cases of polio – Afghanistan, Pakistan and Nigeria.

Table 35.4 Oral and inactivated polio vaccines compared

	Inactivated (IPV)	Attenuated (OPV)
Virus type	Trivalent (types 1–3)	Bivalent types 1 and 3[a] Monovalent type 1 or type 3
Introduced	Salk 1954	Sabin 1957
Route	Injection	Oral
Adjuvant	Alum	None
Advantages	Can be given with other childhood vaccines	Boosts IgA immunity Better immunity in the intestine
Disadvantages	More expensive Requires trained staff to vaccinate	Reversion to virulence[b]

[a]Bivalent OPV used for routine immunization since April 2016.
[b]Although vaccine-associated paralytic polio only occurs in <1/million vaccinated, vaccine-derived polio viruses can circulate within the community.

Pneumococcal vaccines

The challenge in making an effective vaccine against pneumococcal disease is that there are 90 serotypes of *Streptococcus pneumoniae* – but luckily a few serotypes cause most infections. The first vaccine was a pneumococcal polysaccharide vaccine with capsular polysaccharide from 14 serotypes. This was replaced in 1983 with a formulation containing 23 capsular polysaccharides from 23 serotypes, PCV23. However, although this vaccine induced antibodies in >80% of adults, it was not immunogenic in children aged less than 2 years. Two conjugate vaccines are now used: PCV13 in which capsular polysaccharides are conjugated to a non-toxic form of the diphtheria toxin, which is highly immunogenic in infants and young children, and includes the serotypes causing 60% of disease in children under 5 years of age, and the PCV10 vaccine. Animal studies have suggested that attenuated whole bacteria, or specific proteins from *Strep. pneumoniae* (including the detoxified pneumolysin) might also have promise as vaccines. One interesting question is whether the rates of carriage of the different serotypes may be affected by vaccination – in the US, multidrug resistant serotype 35B, a serotype not in the PCV13 vaccines, is becoming more common.

Meningococcal vaccines

As for pneumococcal vaccine, the first vaccine against meningococcal disease caused by *Neisseria meningitides*

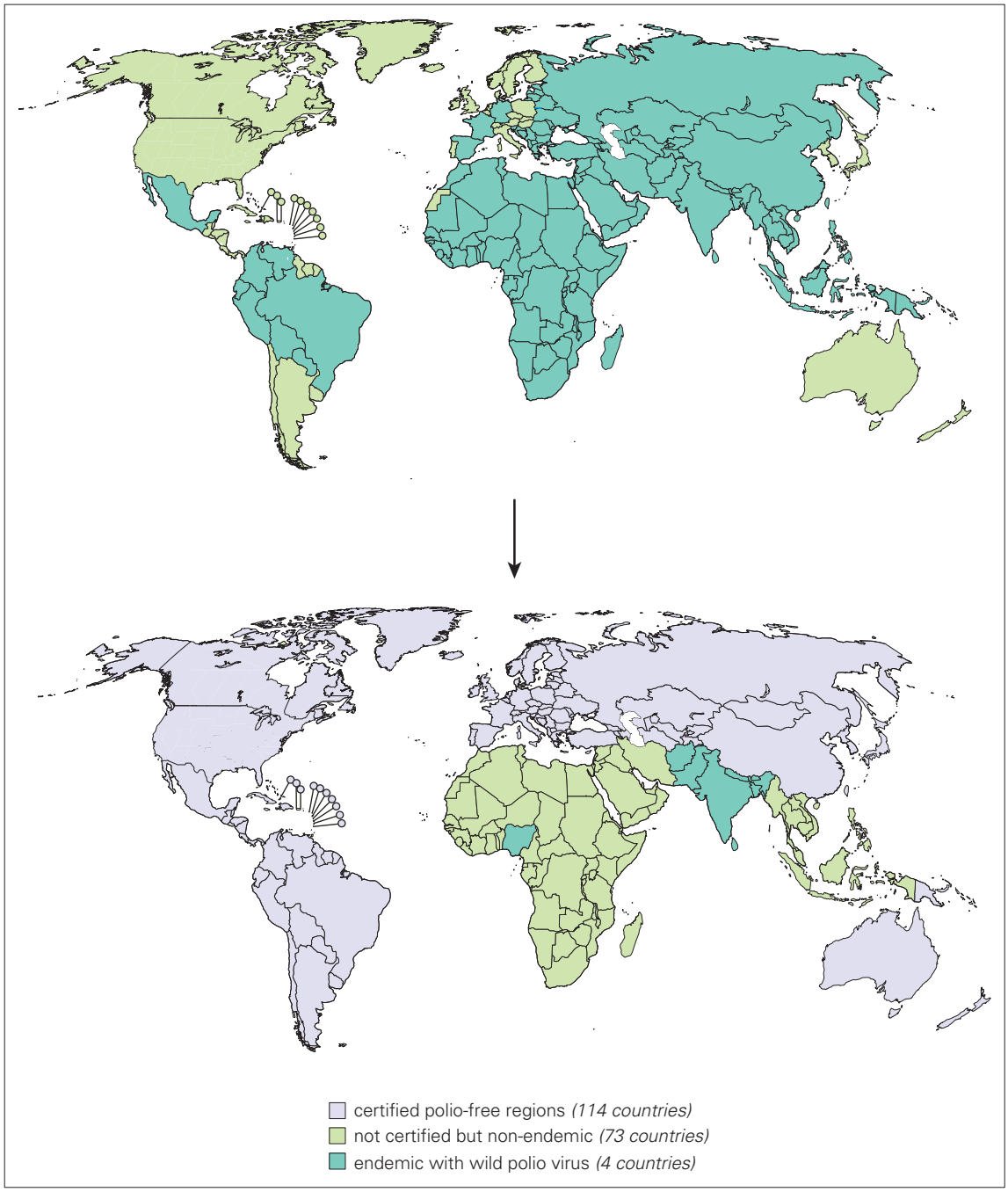


Figure 35.8 Progress towards polio eradication. The progress towards the eradication of polio is illustrated by the increase in certified polio-free countries from 1988 (top map) to 2016 (bottom map). (Redrawn from www.who.int/immunization_monitoring/data/SlidesGlobalImmunization.pdf and data from WHO/Polio database, as at January 2017.)

contained a polysaccharide from serogroup C, but in 1981 this was replaced by a quadrivalent vaccine containing purified capsular polysaccharides for four of the five serotypes, A, C, Y and W-135. Similar to the pneumococcal polysaccharide vaccine, the meningococcal polysaccharide vaccine was not immunogenic in young children, as for other T-independent antigens. A conjugate vaccine containing capsular polysaccharides from the same four serotypes (ACWY) conjugated to diphtheria toxoid is now available, and induces 4X more antibody than the polysaccharide vaccine, with better immunological memory. The B strain is not included in either of these vaccines, as the B-group polysaccharide is poorly immunogenic and may have some cross-reactivity to the human nervous system.

Haemophilus influenzae type b (Hib)

Haemophilus influenzae mainly affects children under 5 years of age. Although there are six capsular serotypes, one, type b composed of a phosphodiester-linked polymer of ribose and ribitol, causes 95% of disease, and so has been the basis of Hib

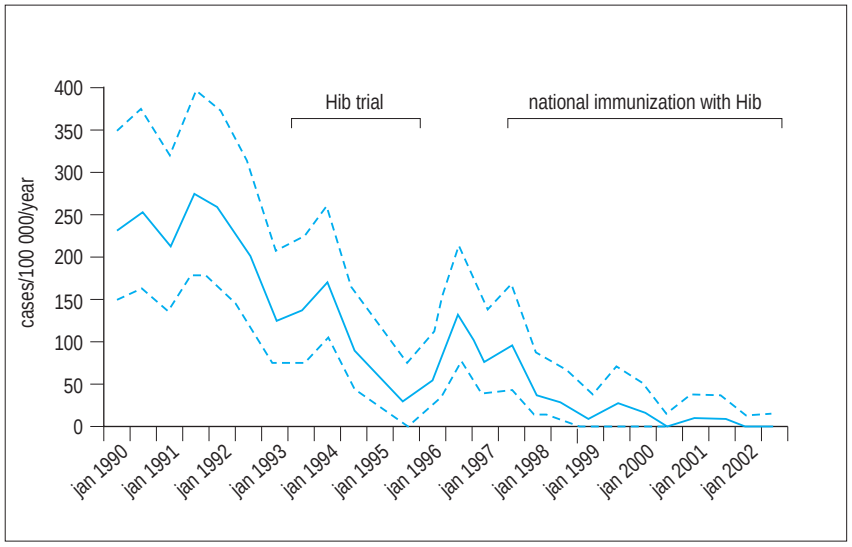


Figure 35.9 Vaccination with a *Haemophilus influenzae* type b (Hib) polysaccharide–tetanus toxoid conjugate vaccine produced a dramatic decrease in the incidence of Hib meningitis in children >1 year old in The Gambia. Dotted lines represent pointwise 90% likelihood-based confidence limits. (Data from: Adegbola R., Secka O., Lahai G. et al. Elimination of *Haemophilus influenzae* type b [Hib] disease from The Gambia after the introduction of routine immunisation with a Hib conjugate vaccine: a prospective study. *Lancet* 2005; 366:144–150.)

vaccines. The introduction of Hib vaccines has dramatically reduced the incidence of Hib bacterial meningitis (Fig. 35.9). The first polysaccharide vaccine introduced in the USA in 1985 was not immunogenic in children under 18 months of age, inducing mostly low-affinity IgM antibodies, similar to other antigens inducing T-cell-independent immune responses. Conjugating the polysaccharide to a T-cell-dependent antigen such as tetanus toxoid, diphtheria toxoid or the meningococcal group B outer membrane protein complex overcame this problem. Even so, three or four doses are needed to induce good immunity, as this is another example of how a subunit vaccine is less immunogenic than a live vaccine.

Influenza

Flu generated a lot of alarm in 2009, when the first flu pandemic since 1968 was caused by a new influenza A (H1N1) virus. The threat from this new virus, and from avian influenza (H5N1), highlighted the limited world capacity to produce new flu vaccines quickly in the quantities needed. Two types of vaccine are currently available: trivalent or quadrivalent inactivated vaccines that can be given to anyone over the age of 6 months by intramuscular or intradermal injection, and a live attenuated influenza vaccine given by intranasal spray to those aged 2–49 years of age who are healthy and not pregnant, that replicates in the mucosa of the nasopharynx.

Flu is a tricky customer, as it changes its haemagglutinin and neuraminidase antigens owing to both point mutations and recombination events, resulting in antigenic drift (Fig. 35.10) and antigenic shift (see Fig.17.10). The recommended compositions of current flu vaccines can be found on the WHO website. The 2016 / 2017 trivalent vaccines for the northern hemisphere contained A/California/7/2009 (H1N1)-like, A/Hong Kong/4801/2014 (H3N2)-like and B/Brisbane/60/2008-like antigens. The influenza A (H1N1) vaccine virus was derived from a 2009 pandemic influenza A (H1N1) virus. Quadrivalent vaccines contain an additional B/Phuket/3073/2013-like virus. Different formulations are recommended for the southern hemisphere, for example in 2017 this used a different influenza A H1N1 virus.

Flu vaccination policy varies in different countries: for example, in the USA, the inactivated vaccine is offered to everyone aged over 6 months, including to pregnant women; the live attenuated virus vaccine is used for those aged 2–49 years. In the UK, vaccination in 2016 / 2017 was restricted to children aged 2–7 years by nasal spray, and as inactivated vaccine to those aged >65 years or in an at-risk group, such as those with asthma and pregnant women. Children aged 6 months to 6 years (in the USA) being vaccinated for the first time are now given two doses of vaccine. A new high-dose trivalent inactivated vaccine is also available for use in those over 65 years of age – but recent evidence suggests it may be just as cost effective to vaccinate more children, increasing herd immunity, as to vaccinate the elderly.

Antibodies provide useful correlates of protection for most of these vaccines

When antibodies provide protection, it is usually possible to determine a quantitative cut-off that is associated with protection. This can be determined by ELISA, by toxin or virus neutralization or in an opsonophagocytosis assay (Table 35.5).

BCG and new vaccines for tuberculosis (TB)

The oldest vaccine still in use is the BCG vaccine, attenuated following extensive culture of *M. bovis* on potato bile medium by Calmette and Guérin. BCG was first used as a vaccine in 1921! Attenuation involved the loss of the RD1 region that encodes the ESAT-6 and CFP-10 antigens used in the currently available commercial diagnostic tests for *M. tuberculosis* infection, the QuantiFERON™ test and the TSPOT-TB ELISPOT™ assay.

BCG is usually given to babies shortly after birth and given to over 100 million children annually. It provides good (and very cost-effective) prevention of the disseminated forms of childhood TB, but variable protection against pulmonary TB in adults. For example, it induced good protection (>80%) in trials in adolescents in the UK, but no protection in South India or Malawi. The reasons for this may include exposure to environmental mycobacteria that can induce a masking or

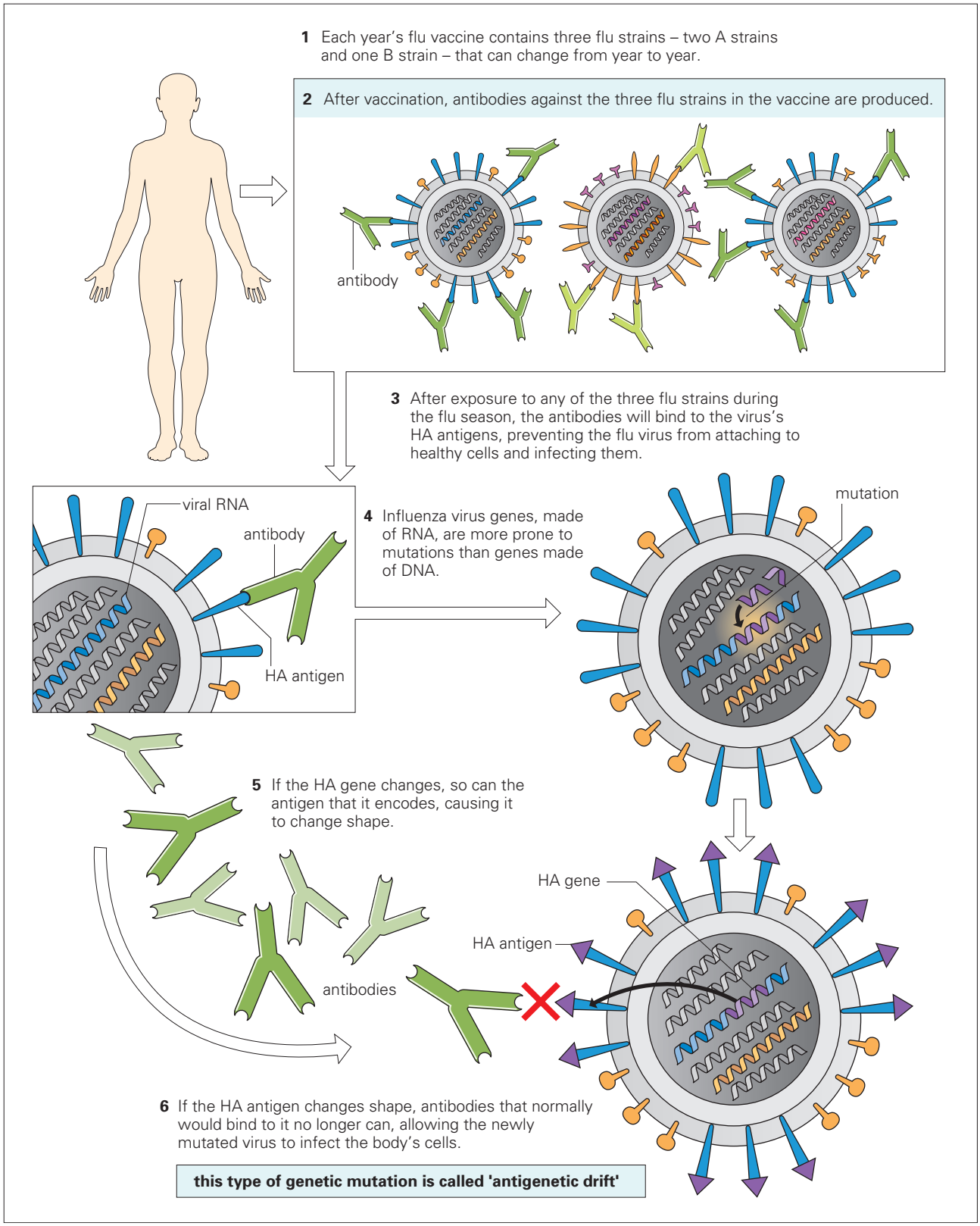


Figure 35.10 Influenza vaccines and antigenic drift. Seasonal influenza vaccines contain three flu strains, two A strains and one B strain. Antibodies to these strains induced by vaccination will protect against infection, but mutations in the influenza genes can cause antigenic drift leading to infection. HA, haemagglutinin. (Modified from National Institute of Allergy and Infectious Diseases. Flu [Influenza]: Antigenic Drift. Bethesda, MD: US Department of Health and Human Services; 2011.)

a blocking effect on the immunity induced by BCG. When BCG is protective, this is associated with induction of a Th1 immune response – although simply measuring IFNγ induced in response to mycobacterial antigens does not provide a correlate of protection. When induced, protective immunity lasts for 10–15 years and in one study lasted for over 50 years. In settings where BCG is protective, it may protect against infection as well as against disease. There is no evidence that revaccination is helpful. In children over 6 years of age, or in those known or likely to have been infected with *M. tuberculosis*, skin testing with *M. tuberculosis* purified protein derivative (Mantoux skin test) should be performed and BCG vaccination given only to those with a negative test result.

Because of the variable protection that BCG vaccination gives against tuberculosis in adults, the search is on for a new TB vaccine. Candidate vaccines in development include genetically modified BCGs, attenuated *M. tuberculosis*, viral vectors expressing key antigens of *M. tuberculosis* and fusion proteins in adjuvant. A modified vaccinia virus Ankara expressing Ag85A, given as a boosting vaccine following BCG (Fig. 35.11), was tested in a phase IIb trial in children and in HIV-infected adults in Africa but was not shown to induce significant protection; new studies are investigating whether giving the vaccine by aerosol might be better. Other promising vaccine candidates include a genetically modified BCG that expresses haemolysin, which might enhance activation of CD8 T cells through escape of antigens into the cytoplasm of the infected macrophage (and that induces improved central memory T-cell responses in mice), and fusion proteins containing various *M. tuberculosis* antigens in adjuvant. Vaccines to be given post-infection to those with latent TB infection, or as therapeutic vaccines for those with drug-resistant TB, are also being developed.

Table 35.5 Serological correlates of protection

Vaccine	Assay	Correlate of protection
Diptheria	Toxin neutralization	0.01–0.1 IU/mL
Hepatitis A	ELISA	10 mIU/mL
Influenza	Haemagglutinin inhibition	1/40 dilution
Pneumococcus	ELISA opsonophagocytosis	0.20–0.35 μg/mL* 1/8 dilution
Polio	Serum neutralization	¼–1/8 dilution
Rubella	Immunoprecipitation	10–15 mIU/mL
Tetanus	Toxin neutralization	0.1 IU/mL

*In children. Some serological tests that provide correlates of protection for vaccines in current use are listed. However sometimes it is secretory antibodies that are more important in protection, and for some vaccines, such as BCG, correlates of protection have not been identified, even though T cells are known to be important. ELISA, enzyme-linked immunosorbent assay. (Data from Plotkin, S.A. *Clinical Infectious Diseases* 2008, 47:401, 2008.)

Vaccines against hepatitis

The first vaccine for hepatitis B virus (HBV) consisted of the surface coat antigen of HBV purified from the plasma of virus carriers. This vaccine was protective, but required very careful purification and inactivation to ensure it was safe, and was expensive to produce. A recombinant hepatitis B surface antigen vaccine was first licensed in the USA in 1986, the first vaccine produced using genetic engineering (Fig. 35.12). Recombinant HBV vaccines produced in yeast have an efficacy of 80–100% against infection or clinical hepatitis, with immunity lasting >20 years after three vaccine doses.

Inactivated whole cell vaccines are available for hepatitis A. The virus is grown in human cells, purified, inactivated with formaldehyde and adsorbed onto alum. Again, these vaccines induce excellent immunity. Combined vaccines for hepatitis A and B are also available but as yet there is no vaccine available for hepatitis C.

Human papillomavirus (HPV)

New HPV vaccines have been introduced in the last decade, due to the association between HPV infection and cervical cancer. The first quadrivalent vaccine (Guardisil™), which induces immunity against four types of HPV, was licensed

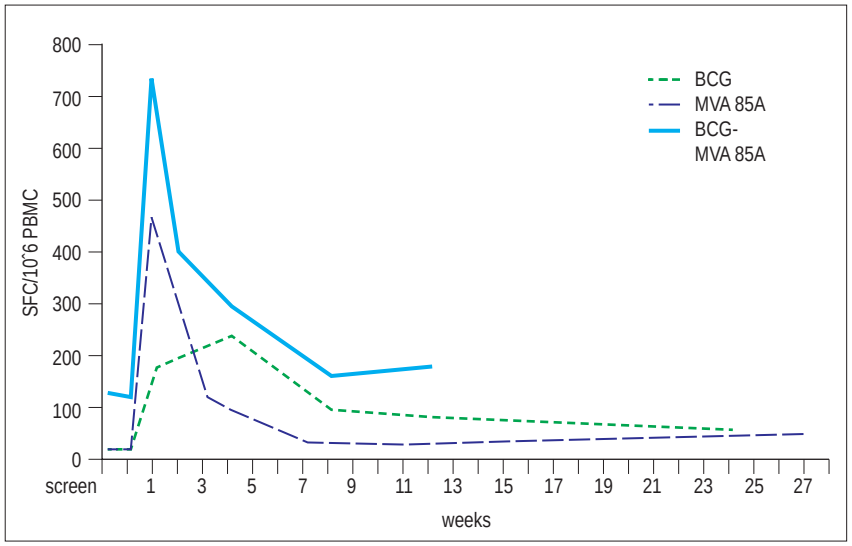


Figure 35.11 The prime boost strategy is being exploited in the design of new vaccines. Modified vaccinia virus Ankara that expresses Antigen 85A (MVA85A) of *M. tuberculosis* was used to boost immunity in people previously vaccinated with bacille Calmette–Guérin (BCG). The prime boost group of vaccinees (BCG-MVA85A) show the greatest numbers of spot-forming cells (SFC) making interferon gamma (IFNγ) in an ELISPOT assay in which peripheral blood mononuclear cells were stimulated with the Antigen 85 protein. (Data from: McShane H., Pathan A.A, Sander C.R. et al. Recombinant modified vaccinia virus Ankara expressing antigen 85A boosts BCG-primed and naturally acquired antimycobacterial immunity in humans. *Nature Medicine* 2004; 10:1240–1244.)

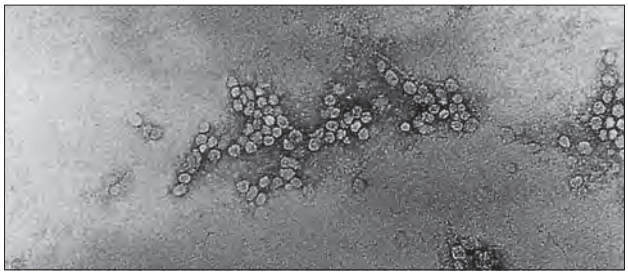


Figure 35.12 Electron micrograph of purified 22 nm hepatitis B surface antigens expressed in yeast cells. (Courtesy of J.R. Pattison.)

in 2006. This contains the L1 capsid protein of HPV from two oncogenic types of virus (HPV16 and HPV18) as well as two non-oncogenic types (HBV6 and HPV11). It is made by recombinant DNA technology, and forms virus-like particles. This vaccine is being given to females aged 11–12 years, before they become sexually active, and can induce antibody responses in >99.5% of vaccinees. The quadrivalent HPV vaccine is also given to males aged 13–21 years of age in the USA, as HPV6 and HPV11 cause ~90% of genital warts. A bivalent HPV vaccine containing L1 from HPV16 and HPV18 was approved for females only, aged between 11 and 12 years in the USA in 2009. This vaccine is cheaper, but most countries have opted for the quadrivalent vaccine, as it also prevents genital warts.

Rotavirus vaccine

Rotavirus causes most serious gastrointestinal disease in infants. Trials of an earlier vaccine were stopped when it caused intussusception, a rare cause of bowel obstruction. Two new live oral vaccines are now in use: the RV5 vaccine (RotaTeq™) contains five reassortant rotaviruses developed from human and bovine parent strains, while the RV1 vaccine (Rotarix™) contains one live attenuated rotavirus strain. The vaccines give 74–87% efficacy against any rotavirus gastroenteritis, and 85–98% protection against severe gastroenteritis. Studies from a number of countries have demonstrated marked reductions in hospitalizations and in GP visits for all-cause acute gastroenteritis in children after the rotavirus vaccine was introduced.

Typhoid

Two vaccines are available for typhoid. The live oral vaccine contains a live attenuated mutant strain of *Salmonella typhi*, Ty21a in coated capsules, and the Vi capsular polysaccharide vaccine is injected intramuscularly. These show a difference in immunogenicity – the oral vaccine needs 3–4 doses compared with a single injection of the polysaccharide. However, the oral vaccine may be inducing immunity in the right place.

Varicella

A live attenuated viral vaccine is available against varicella, or chickenpox. The virus isolated from the vesicular fluid of a child with varicella was attenuated by culturing in three different types of cell lines; the vaccine can be given to children aged >12 months. Older individuals are susceptible to developing shingles or post-herpetic neuralgia, and a new vaccine containing a much higher dose of live attenuated varicella-zoster virus (19400 plaque-forming units [PFUs] compared with 1350 PFU in the infant vaccine) is now available for those aged >50 years. This is not fully effective but does reduce the risk of shingles by ~50%.

Vaccines that are required for entry into particular countries, or for particular regions

The yellow fever vaccine is required for entry into certain countries. A vaccination certificate may be required for all those entering a particular country, or for individuals coming from a country where yellow fever is endemic. Luckily this is a very immunogenic vaccine and a vaccination certificate is now valid for the life of the person vaccinated. Vaccination against meningitis ACWY is compulsory for pilgrims visiting Mecca in Saudi Arabia for the Umrah and Haj pilgrimages, as there was a *N. meningitis* W-135 outbreak in pilgrims in 2000.

Travellers spending longer periods in areas of rural Asia, where Japanese encephalitis (JE, a mosquito-transmitted flavivirus) is common, can be vaccinated with an inactivated JE virus vaccine. A live attenuated (recombinant) tetravalent dengue vaccine is now licensed in some countries, having shown 79% protection against severe dengue in two phase 3 trials; this contains yellow fever viruses expressing surface membrane and pre-envelope proteins for the four dengue serotypes, with further vaccine candidates in development. However, it is important that the vaccine does not predispose the vaccinees to developing the severe forms of dengue haemorrhagic fever that can occur when someone is re-infected with dengue (see Ch. 18). Finally, two inactivated viral vaccines for tick-borne encephalitis have been developed and are available in some countries.

Vaccines for subgroups at high risk

Rabies vaccination is available for those exposed to rabies, or whose work or travel puts them at increased risk. Two types of vaccine are available: inactivated virus from cell cultures (from human diploid or chick embryo cells). The cell-culture-derived vaccines are considered safer than earlier brain tissue-based vaccines.

A vaccine has been produced for those working with *Bacillus anthracis*, such as laboratory or animal workers, or some military personnel. To ensure protection, five doses of vaccine are given and a yearly booster is necessary.

Complexity of vaccine schedules

An increasing number of vaccines are being given to infants – at a time when their immune system is not fully mature. However, studies have shown that pre-term babies can still be vaccinated safely at the right chronological age for vaccination. Table 35.6 gives an overview of vaccines being given to infants, children and adolescents in the UK and the USA. The current recommended vaccine schedules for the rest of the world can be found on the WHO website (www.who.int).

It is important to ensure that all these vaccines do not interfere with each other, and thus reduce vaccine-induced immunity, so testing for non-interference is required before a new vaccine is introduced.

There may be other factors that affect how well a vaccine works in the real world. Some studies have reported sex differences in vaccine-induced immunity, or seasonal effects, and some vaccines do not induce equivalent immunity in all

Table 35.6 Examples of vaccination schedules in the UK and the USA

Vaccine	UK	USA
Diphtheria, tetanus, acellular pertussis	2, 3, 4 months, 3 years 4 months	2, 4, 6, 15–18 months, 4–6 years
Inactivated polio vaccine	2, 3.4 months, 3 years 4 months	2, 4, 6–18 months, 4–6 years
Haemophilus influenzae type b	2, 3, 4 months, 1 year	2, 4, 6, 12–15 months
Pneumococcal conjugate vaccine	2, 4 months, 1 year	2, 4, 6, 12–15 months
Meningitis B	2, 4 months, 1 year	10 years
Meningitis C	1 year	11–12 years, 16 years
Meningitis AWCY	14 years	2 or 9 months; men CY from 6 weeks[a]
Measles, mumps and rubella	1 year, 3 years 4 months	12–15 months, 4–6 years
Hepatitis A	Not used	12–13 months, 18–19 months
Hepatitis B	Not used	0, 1–3 months, 6–18 months later
Human papillomavirus[a]	12–13 years ×2	11–12 years×3[b]
Varicella	Not used	12–15 months, 4–6 years
Rotavirus	2, 3 months	2, 4, (6) months[c]
Influenza	From 6 months (seasonal vaccine)	From 6 months (seasonal vaccine)

Note that the schedules and vaccines given may differ. These indicative schedules are based on recommendations in January 2017; up-to-date schedules can be found at: http://www.nhs.uk/conditions/vaccinations/pages/vaccination-schedule-age-checklist.aspx for the UK, www.cdc.gov/vaccines/schedules/index.html for the US and www.who.int/immunization/policy/immunization_tables/en/ for all other countries. Routine BCG vaccination is given shortly after birth in most countries outside Europe and the USA.

[a]For human papillomavirus vaccine, the vaccine is routinely given to girls, but can be given to boys to prevent genital warts.

[b]Three doses given at 0, 1–2 months and 6 months.

[c]Different formulations of rotavirus vaccine require two or three doses.

settings. Vaccination is a very powerful public health tool, but not all infants and children will get their vaccines at the right ages or in the recommended order. Vaccines for developing countries therefore need to be tested in the populations most at risk, where other factors and infections such as malaria or intestinal helminths may modulate vaccine-induced immunity.

Changes in demography means new vaccine strategies are needed

In many countries, the proportion of older individuals is increasing. With age, immunity can be lost, and in particular, T-cell immunity is weakened. Hospitalizations for infections such as pneumonia and influenza in older people place a burden on health systems. One strategy is to vaccinate older individuals against flu and pneumococcal disease – and in the USA those >65 years of age are also recommended to have varicella-zoster vaccination. However, due to the reduced efficiency of the immune system in old age, new vaccine strategies may be needed. If the elderly are vaccinated with the live attenuated varicella-zoster vaccine or the inactivated flu vaccine, they are given 14 times the colony-forming unit (CFU) of the varicella-zoster virus, or four times the dose of the haemagglutinin antigen used in the flu vaccine for children, in an attempt to improve immunogenicity.

New vaccines in development

Improved coverage with available vaccines is reducing child deaths (Fig. 35.13), but if effective vaccines were developed against HIV / AIDS, malaria and tuberculosis then many more lives could be saved. The development of new vaccines against tuberculosis was covered above – but what about HIV and malaria?

HIV vaccines

HIV has proved to be a real challenge in terms of vaccine development. Since 1987, over 30 vaccines have been tested in phase I or phase II trials but, despite all this effort, no effective vaccine is yet available. The first trials used recombinant gp120 with adjuvant, hoping to induce neutralizing antibodies but failed to protect; recombinant adenovirus was then used to deliver the *gag*, *pol* and *nef* genes, to induce CD8 T-cell responses, but this vaccine slightly increased the rate of infection. Pre-existing or even vaccine-induced immunity against the viral vector used may be a problem, although so far a combination of priming with DNA and boosting with an adenovirus has not improved protection. The RV144 trial in Thailand using a priming canary pox vaccine encoding the HIV *gag*, *pol* and *env* genes and a boosting vaccine with recombinant gp120 showed modest protection of 31.2%, associated with the presence of non-neutralizing antibodies. Part of the problem is that the gp120 molecule mutates and circulating HIV viruses are highly variable; the killed virus is not sufficiently immunogenic to use as a vaccine, and the route of infection, mostly through the genital tract, means localized mucosal immunity is needed. This illustrates that, despite huge advances in molecular biology and immunology, it can be difficult to design a protective vaccine. Some new strategies being investigated include novel antigen design strategies to induce non-neutralizing antibodies to the V2 loop of Env, broadly neutralizing antibodies to Env, the use

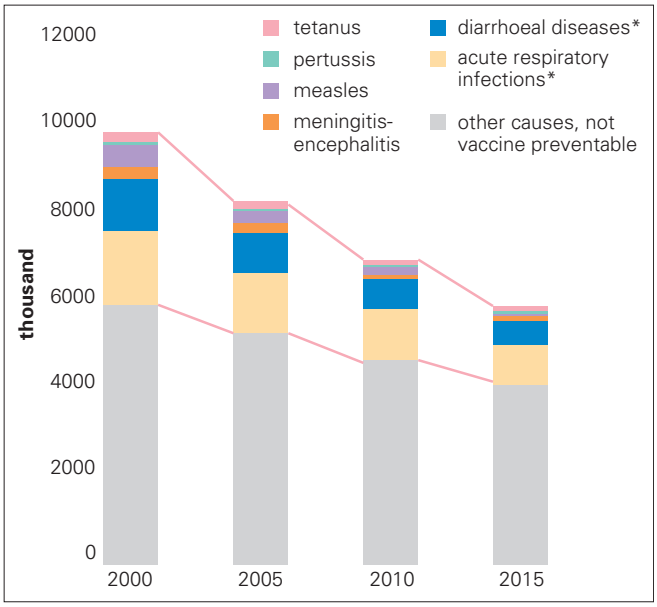


Figure 35.13 Vaccination has reduced deaths in children. The effect of vaccination on deaths in children under 5 years of age, from 2000 to 2015 is shown. However, not all diarrhoeal diseases or acute respiratory infections can be prevented by vaccination. (Source: WHO www.who.int/immunization/global_vaccine_action_plan/SAGE_GVAP_Assessment_Report_2016_EN.pdf?ua=1.)

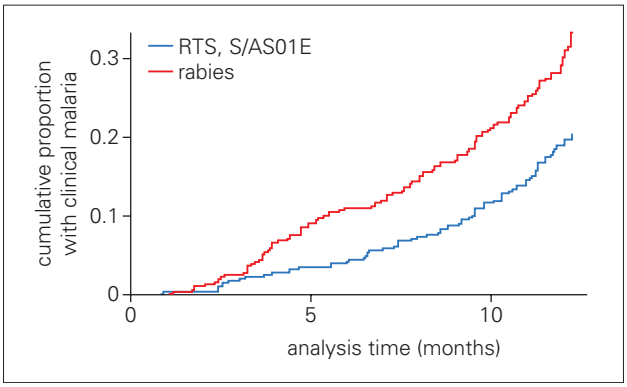


Figure 35.14 A new candidate vaccine for malaria that uses parts of the circumsporozoite protein fused to the hepatitis B surface antigen reduces the prevalence of malaria infection in young African children. Children given three doses of the RTS,S vaccine had a longer delay before they developed clinical malaria infection compared with controls given rabies vaccine. If a fourth boosting vaccination was given 18 months after the primary vaccine schedule, efficacy in children aged 5–17 months was 36.3%. (Reprinted with permission from Elsevier. Efficacy and safety of RTS,S/AS01 malaria vaccine with or without a booster dose in infants and children in Africa: final results of a phase 3, individually randomised, controlled trial. *The Lancet* 2015; 386 [9988]: 31–45.)

of new vectors such as rhesus cytomegalovirus (CMV) to induce T-cell memory in the mucosa or using mosaic antigens to generate antibodies to a broad range of HIV epitopes from viruses worldwide.

Malaria

Malaria has been another challenging disease against which to develop an effective vaccine. The RTS,S vaccine, based on the circumsporozoite protein and designed to combat the invasive sporozoite stage either in the skin or as it invades the liver, showed promise in early trials (Fig. 35.14) and has reduced the numbers of cases of clinical malaria by 36.3% in a phase IIII trial in Africa, when a fourth boosting vaccination was given 18 months after the initial 3 doses. WHO has now recommended large-scale pilot implementation of the vaccine in African children aged 5–9 months. Irradiated sporozoites can also give good protection, but this would not be an easy vaccine to produce in bulk. Other approaches include delivering key pre-erythrocytic or blood-stage antigens by viral vectors, as virus-like particles, or trapped within liposomes. For long-term efficacy, a vaccine may need to induce immunity against malaria antigens that are not normally immunogenic and that may be under less immune pressure to evolve. Finally, a vaccine against the sexual forms of malaria that are infectious to mosquitos could help reduce transmission and two candidates are in early trials.

Vaccines for neglected tropical diseases are also needed

Some infections such as leishmaniasis, leprosy and helminth infections are described as neglected tropical diseases – neglected while most emphasis is put on HIV, malaria and TB. Schistosomiasis, oncocerciasis, hookworm, leishmaniasis and trachoma are all examples of neglected tropical diseases where there is currently no vaccine available, although a glutathione-S-transferase antigen in alum vaccine for *Schistosoma haematobium* is in phase II / II trials and vaccines for *S.mansonii*, oncocerciasis and hookworm are in early clinical trials. Leprosy also has no vaccine, but luckily the BCG vaccine has been shown to provide partial immunity to leprosy – hopefully any new TB vaccine will do even better.

How quickly can a new vaccine be produced?

The time to make and introduce a new vaccine is critical, as delays mean lives lost. The recent outbreaks of Ebola in West Africa illustrated how vaccine development can be accelerated. Luckily several potential vaccine candidates were available, existing virus vectors could be used, and regulatory processes were accelerated. Within 3 years of the start of the West African Ebola outbreak, results from a phase III trial of a recombinant vesicular stomatitis virus expressing an Ebola glycoprotein, rVSV-ZEBOV, showed the vaccine to be 100% protective. A ring vaccination design was used in which all those exposed to a confirmed Ebola case were vaccinated immediately or 28 days later. Another four vaccines underwent accelerated development and were assessed for immunogenicity.

New delivery systems and technologies for future vaccines

Adenoviruses are being tested as vaccine vectors, as they induce good CD8 T-cell responses, but too many individuals already have antibodies to some adenovirus strains, which may reduce the efficacy of the vaccine. For example, although only 20% of individuals in the Netherlands have antibodies to type 5 adenovirus, this rises to 80% in sub-Saharan Africa, so some new vaccine trials are using the Ad35 strain instead,

as seroreactivity to Ad35 is lower in Africa. Delivery by adenovirus is being used to design some of the new candidate vaccines for TB.

Genetic engineering can be used to make more effective vaccines. Viral recombinant vaccines are being developed as new RSV vaccines – using parainfluenza virus expressing key RSV proteins. Codon optimization can be used, for example, for poliovirus, where reversion to virulence can be reduced by altering the codon usage. Virus-like particles can be made that express key viral proteins, yet are replication deficient. The latest papillomavirus vaccines are virus-like particles made from recombinant HPV coat proteins. This approach is also being used for blue-tongue virus vaccine for sheep, and for malaria. Genetically modified or transgenic plants can be used to produce immunogens, including glycosylated proteins, and even full virus-like particles.

DNA vaccines were thought to hold great promise – but so far have not been licensed for use. They are good at priming the immune system and can induce good memory responses and Th1 responses in immunologically naive recipients.

New routes of vaccination

The oral polio vaccine was not the first vaccine to be given orally – the BCG vaccine was originally given by mouth. Dissolvable tablets or wafers may be used under the tongue in future. Some new work is even investigating expressing vaccine antigens in edible fruit or vegetables, such as tomatoes or lettuce!

If protection is needed in the mucosal-associated lymphoid tissues, then to prime cells in this region is very sensible, and nasal sprays can be used, as in one seasonal flu vaccine formulation. Another approach is to use skin patches – these deliver the vaccine antigens through the transcutaneous route. Nozzle jet or powder injectors are also being investigated as a means of delivery. Vaccines of the future may use nanoparticles, or be injected using dissolving microneedles, designed to deliver the antigens to cutaneous antigen-presenting cells, and said to be relatively painless. This is clearly an area where molecular science and technological developments can make a real impact.

KEY FACTS

- Vaccination aims to prime the adaptive immune system to the antigens of a particular pathogen so that a first infection induces a secondary immune response.
- Vaccines can use live attenuated organisms, killed whole organisms, subcellular fractions or antigens produced artificially by gene cloning or chemical synthesis.
- In general, live vaccines are more effective than other types, but carry the risk of reverting to virulence or inducing disease in immunocompromised patients.
- The details of vaccine formulation, route, dose and risks have to be considered for each disease individually.
- Overall, vaccination is a very effective public health tool, but many challenges remain, including the effective implementation of existing vaccines worldwide and the design of new vaccines against those infections for which they are not yet available.

36 Active, passive and adoptive immunotherapy

Introduction

Immunotherapy involves any manipulation of the host immune response that results in attenuating or preventing an infection. Vaccines, as described previously, are either inactivated / killed or live attenuated, with the aim of producing long-term protection against that pathogen by inducing an antigen-specific immunological memory which is stimulated when the host meets the antigen again. Other forms of defence are needed if the host is immunocompromised, as live vaccines could be life threatening because, although the pathogen is attenuated, the host response is blunted. In addition, due to the immunosuppression, the host may not respond. Moreover, if a person is already infected and antimicrobial agents may be unavailable or are ineffective, other forms of immunotherapy are needed.

The role of immunotherapy is to activate immune effector genes but without enhancing any deleterious effects they could have, as there are myriad events set in motion when activating innate and adaptive immunity.

Immunotherapy strategies are divided into four approaches

1. Active immunotherapy – systemic and non-specific activation of immune responses.
2. Active and specific immunotherapy – T- or B-cell activation of specific antigen recognition pathways.
3. Adoptive immunotherapy – cells with antigen-specific or non-specific effector responses are expanded in vitro and given to the host.
4. Passive immunotherapy – preformed specific or non-specific antibodies are given to the host.

Active and specific immunotherapy are concepts discussed in previous chapters and involve, for example, interferons, DNA vaccines expressing specific genes, the products of which may clear the infection, and cytokines that enhance T-cell expansion and activate antigen-presenting cells that may control the infection. The focus of the next section will be on adoptive and passive immunotherapy.

ADOPTIVE IMMUNOTHERAPY

T cells recognize and kill target cells and so adoptive T-cell therapy has been investigated as a way of targeting cells with latent and integrated viral infections. These include herpesvirus and retroviral infections, as well as hepatitis B virus (HBV) infections.

In allogeneic bone marrow transplantation programmes, recipients are at high risk of herpesvirus reactivation, including cytomegalovirus (CMV) and Epstein–Barr virus (EBV) in particular. Some of the others, including herpes simplex virus (HSV) and varicella-zoster virus (VZV), can be suppressed by aciclovir, an antiviral that is relatively free of side effects. However, those used to suppress CMV have numerous adverse effects, so other ways of managing CMV reactivations have been investigated. An antiviral drug that is effective in reducing EBV replication has yet to be licensed; only the anti-CD 20 monoclonal antibody, rituximab, is really of use, but depletes the B-cell population. As a result, producing donor-derived CMV or EBV or adenovirus-specific T cells given as a donor lymphocyte infusion, either prophylactically or as treatment, has been reported. If these are not available, donors that have the most common human leukocyte antigen (HLA) alleles can be used as a source. One concern is that graft-versus-host disease (GVHD) can occur with these infusions.

Chimeric-antigen receptor (CAR) modified T cells are being used in haemato-oncology, but were originally investigated as a way of treating individuals with human immunodeficiency virus (HIV) infection.

The HIV envelope CD4 receptor protein was the CAR component and the idea was that these modified T cells would attack the HIV-infected T cells. It was shown that the CAR T cells found their way to reservoirs of infection in the body, including mucosa, and persisted for years on follow-up.

In 2009, the scientific world was surprised by a report of an HIV-positive person with acute myeloid leukaemia being apparently cured of HIV infection having had an allogeneic bone marrow transplant. A donor had been selected who was homozygous for the CCR5 Δ32 mutation, which confers genetic resistance to HIV infection.

There could be some intriguing treatment plans involving CAR T cells and combined antiretroviral therapy (cART). With

respect to intriguing combinations, there is the some-may-think humorous and hypothetical combination of EAGA, the UK Expert Advisory Group on AIDS, and BHIVA, the British HIV Association, which could have been named EAGA BHIVA.

In addition, gene-editing strategies could be used to affect the *CCR5* gene in T cells, which could then be infused into HIV-positive individuals, adding to their T-cell portfolio and reducing *CCR5* expression.

PASSIVE IMMUNOTHERAPY

Certain diseases are treated by a passive transfer of immunity, which can be life saving

Before the introduction of antibiotics, acute infectious diseases were often treated by the injection of preformed antibody on the principle that the patient was already ill and it was too late for 'active' vaccination. Indeed, the demonstration that immunity to tetanus and diphtheria could be transferred to mice with serum from vaccinated rabbits was a key experiment in the discovery of antibody in the 1890s. Subsequently, the production of antiserum for the passive treatment of diphtheria, tetanus and pneumococcal pneumonia, and against the toxic effects of streptococci and staphylococci, became an important industry, and generations of horses that had retired from active duty were kept on as the source of 'immune serum'. The introduction of antitetanus serum in the early months of the First World War reduced the incidence of tetanus dramatically, by up to 30-fold (Fig. 36.1).

The advent of penicillin and other antibiotics changed the picture considerably, and passive immunotherapy is now used for only a select group of diseases (Table 36.1). The serum may be specific or non-specific and of human or animal origin. Convalescent human serum from influenza A and Ebola virus disease survivors has been used to treat those with severe infections, especially if antiviral drugs have been ineffective or unavailable.

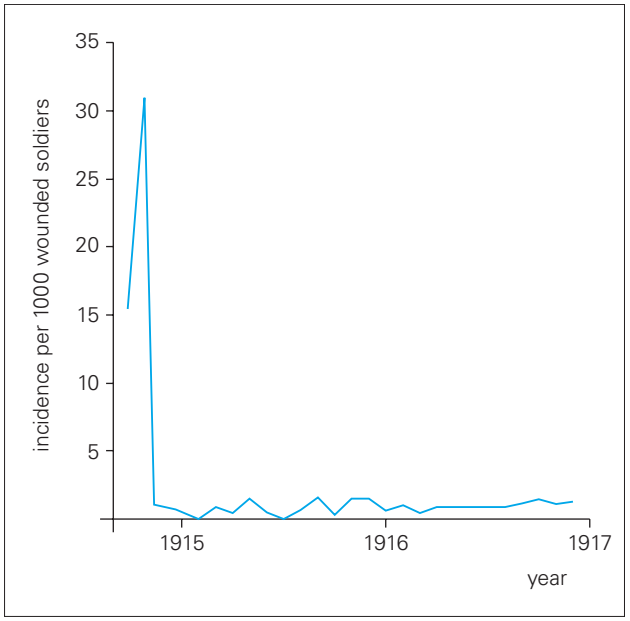


Figure 36.1 Passive immunization significantly reduced the incidence of tetanus in the early months of the First World War. The figure shows the incidence of tetanus per 1000 wounded soldiers in British hospitals during 1914–1916. There was a dramatic fall after the introduction of anti-tetanus serum in October 1914.

The use of antiserum raised in animals can cause serum sickness

The use of antiserum raised in horses or rabbits has largely been abandoned because of the complications resulting from the immune response to the antibody, which is of course a foreign protein. These include progressively more rapid elimination and therefore reduced clinical effectiveness as well as serum sickness due to immune complex deposition in, for example, the kidney and skin (see Ch. 18) and even anaphylaxis. These complications can be avoided by using human serum collected during convalescence or following vaccination – to prevent infection after exposure, such as in rabies, hepatitis B and chickenpox virus infections.

Antibody in pooled serum can provide protection against infection

With common infections, it can be assumed that most immunocompetent people have antibody to the pathogen in their serum. The clearest proof of this is that patients with hypogammaglobulinaemia can be kept free of recurrent infection by regular injections of immunoglobulin G (IgG) from pooled normal serum, and that immunodeficient children can be protected against measles in the same way (Box 36.1). Immunoglobulin is prepared from batches of plasma from 1000–6000 healthy donors after screening for a number of infections, including hepatitis B and C, HIV and treponemal infection. Other infections may be included in the screening tests depending on the prevalence in that country. Intravenous or intramuscular injections may be used.

Table 36.1 Specific passive immunotherapy with antibody

Infection	Source of antibody	Indication
Diphtheria	Human, horse	Prophylaxis, treatment
Tetanus	Human, horse	
Varicella-zoster virus	Human Varicella Zoster Immunoglobulin (VZIG)	Prophylaxis in those susceptible and at high risk (includes bone marrow and solid organ transplant recipients, pregnancy)
Gas gangrene Botulism Snake bite Scorpion bite	Horse	Post-exposure
Rabies virus	Human Rabies Immunoglobulin (RIG)	Post-exposure plus vaccine
Hepatitis B virus	Human Hepatitis B immunoglobulin (HBIG)	Post-exposure and vaccine may be given
Measles	Pooled human immunoglobulin	Post-exposure

Box 36.1 Indications for Normal Immunoglobulin Therapy

Sufficient antibody to protect immunocompromised patients against common infections can be obtained from pooled human normal plasma.

- X-linked agammaglobulinaemia / hypogammaglobulinaemia
- Common variable deficiency
- Wiskott–Aldrich syndrome
- Ataxia telangiectasia
- IgG subclass deficiency with impaired antibody response
- Chronic lymphocytic leukaemia
- Post-bone marrow transplantation for CMV pneumonitis in conjunction with an antiviral agent

The immunity conferred by mothers on their newborn infants by placental transfer of IgG and subsequently by colostral IgA (though the latter is not absorbed, but remains in the intestine) is further evidence for the protective effect of relatively small amounts of antibody.

An effective therapy is provided by one or more monoclonal antibodies specific for a known target antigen

The first monoclonal antibody (MAb) was licensed in 1986, having been generated in mice in 1975 using a hybridoma method (see Ch. 12). MAbs are monovalent antibodies produced by one lymphocyte clone and bind to one epitope. Hybridomas are made by immunizing mice, for example, against a specific epitope on an antigen and then harvesting the B cells from the spleen. These B cells are fused with an immortal cell line creating the hybridoma, which is cultured and the B-cell clones secrete individual antibodies (MAbs) into the medium. A serious complication is that they are highly immunogenic in humans and give rise to human anti-mouse antibodies (HAMA), which accelerate clearance of the MAb from the blood and possibly cause hypersensitivity reactions; they also prevent the mouse antibody from reaching its target, which, in some cases, block its binding to antigen. Different expression systems were therefore developed.

Engineering antibodies

Monoclonal antibodies can be generated by phage display techniques

An important strategy based on bacteriophage expression and *selection* then achieved a prominent position. In essence, mRNA, preferably from primed human B cells, is converted to cDNA and the antibody genes or fragments are expanded by the polymerase chain reaction (PCR). Single constructs are then made in which the light and heavy chain genes are allowed to combine randomly as *Fab* or single-chain *Fv* (scFv) fragments in tandem with the bacteriophage coat protein gene. This *combinatorial library* encodes a huge repertoire of antibody fragments expressed as fusion proteins with a filamentous coat protein on the bacteriophage surface. The extremely high number of phages produced by *Escherichia coli* infection can now be panned on solid phase antigen to select those bearing the highest-affinity antibodies attached to their surface (Fig. 36.2). Because the genes which encode these highest-affinity antibodies are already present within the selected phage, they can readily be cloned and the antibody fragment expressed in bulk.

It should be recognized that this selection procedure has an enormous advantage over techniques which employ *screening*, because the number of phages which can be examined is several logs higher. Although a 'test-tube' operation, this approach to the generation of specific antibodies does resemble the affinity maturation of the immune response in vivo in the sense that antigen is the determining factor in selecting out the highest-affinity responders. In order to increase the affinities of antibodies produced by these techniques, antigen can be used to select higher-affinity mutants produced by random mutagenesis or even more effectively by site-directed replacements at mutational hot spots, again mimicking the natural immune response, which involves random mutation and antigen selection.

Single-domain variable region fragments have several advantages

Phage libraries have been created which express just single heavy or light chain variable region domains (V_H or V_L dAbs). When selected from large naive human phage libraries and fine-tuned by random mutation and further selection, dAbs of surprisingly high affinity, sometimes in the low nanomolar range, can be obtained, clearly without the need for prior immunization. Camelids are immunologically curious in that one-half of their antibodies are conventionally composed of heavy and light chains but the other half are just heavy chains, albeit with unusual complementarity-determining regions (CDRs) which can subserve high-affinity interactions with antigen. Thus a parallel technology was developed in which high-affinity V_{HH} (variable domains from heavy chain antibodies) were selected from immunized llamas.

Both human and llama V_H dAbs have several advantages. They are easy to engineer in bulk cheaply, they can readily be custom tailored by molecular biological manipulations, and they are small and robust in their ability to withstand variations in temperature and acidity, making them relatively insensitive to environmental conditions and the need for refrigeration, and permitting their use for oral therapy and for repeated affinity chromatographic purification of antigens. Another advantage is their low immunogenicity.

Antibody fragments lacking the Fc structures required for secondary activity obviously will not provide protection where complement fixation, phagocytic uptake or extracellular killing is required to eliminate a pathogen. Where they are effective is in blocking cognate enzyme–substrate, hormone or toxin–receptor and microbial addressin–epithelial cell receptor interactions. The latter situation is particularly relevant to mucosal infections, where specific adherence to a cognate epithelial receptor is an essential initial step in the infectious process. Initial studies demonstrated the efficacy of dAbs in preventing experimental rotavirus infection and vaginal candidiasis (Fig. 36.3).

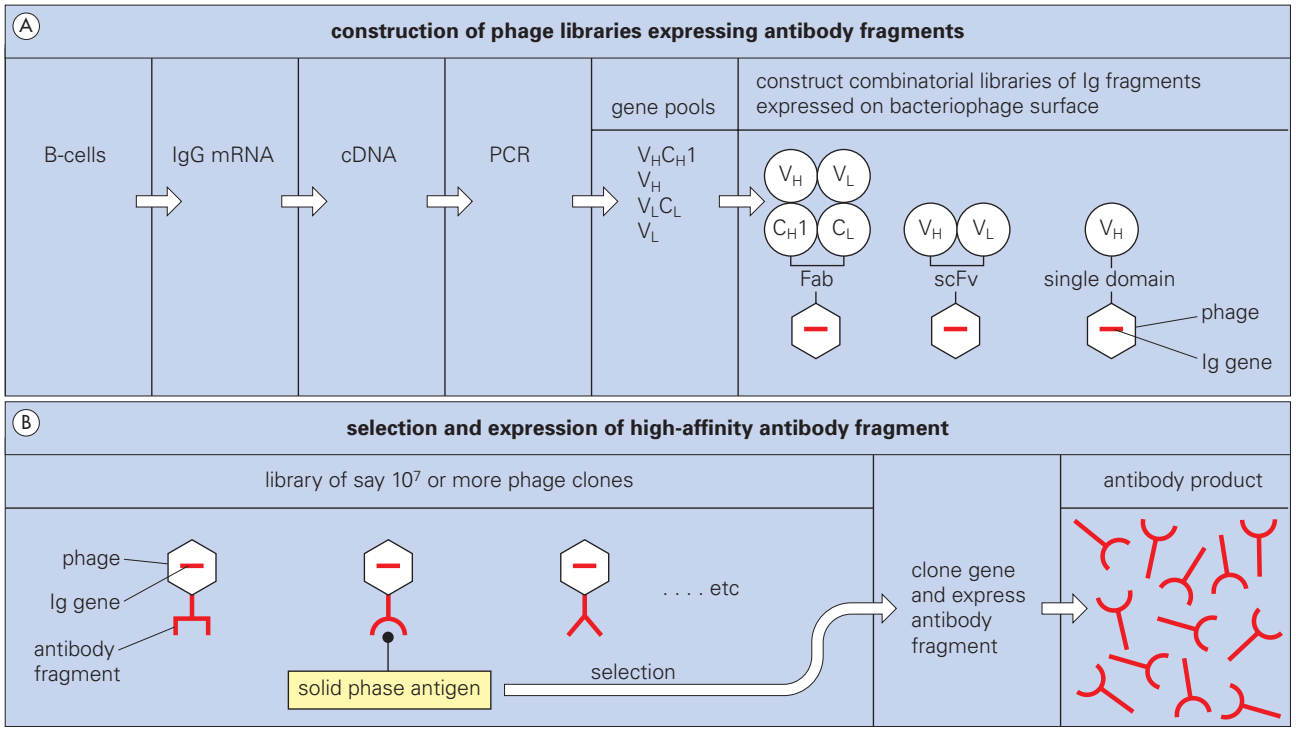


Figure 36.2 Pools of genes encoding Ig domains derived from IgG mRNA are randomly combined and expressed as either *Fab* or single-chain *Fv* (scFv) fragments on the surface of the bacteriophage. Libraries expressing single domains of the heavy chain variable region (V_H) (human or llama, usually) can also be constructed. Phage clones containing genes encoding high-affinity antibody fragments can be selected from these extremely large libraries using solid phage antigen. The appropriate Ig genes can then be cloned and expressed in suitable vectors to produce abundant antibody fragments.

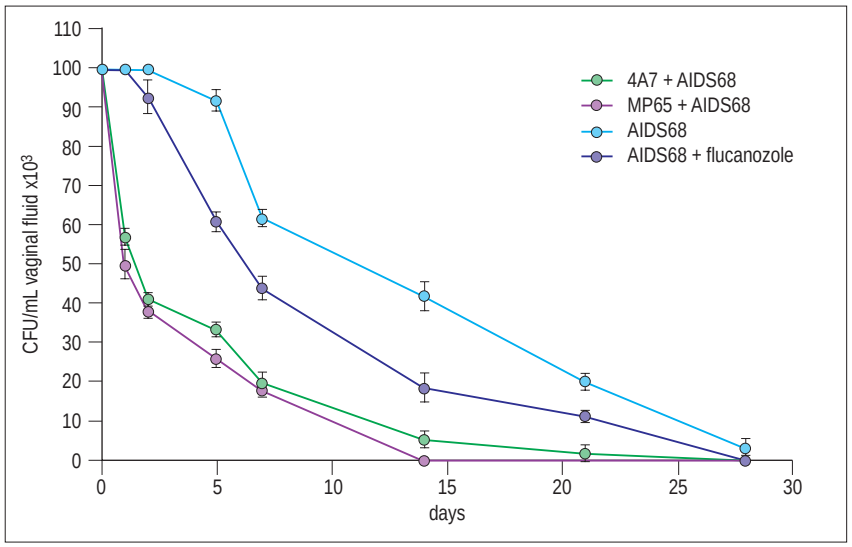


Figure 36.3 Protective activity of an anti-Sap2 (4A7) and an anti-MP65 human variable region single-domain antibody (dAb) against rat vaginal infection by a *C. albicans* fluconazole-resistant strain (AIDS68). Five rats per group were used. Each animal was administered intravaginally 20 μg of each dAb 30 min before intravaginal challenge with 10^7 fungal cells. Fluconazole was used as a single intravaginal dose of 50 μg, 30 minutes before challenge. Irrelevant dAbs were not protective. Efficacy in protection against infection paralleled the ability of the dAb to inhibit the adherence of *Candida* to cultures of epithelial cells. *CFU*, colony-forming unit. (Courtesy of F. de Bernadis and A. Cassone et al.)

Monoclonal antibodies were then made more efficacious and are increasingly used in the clinical setting

Improvements were made by focusing on areas that included pharmacokinetics, immunogenicity, antigen-binding affinity and the effector functions. By pegylating MAbs, using polyethylene glycol, the plasma half-life increased. MAbs can be made more immunogenic by humanizing them, reducing the chance of developing HAMA, for example. There are also ways to improve effector function and antigen-binding affinity.

Many human MAbs have been evaluated for clinical use against a number of infections in vitro and in animal models. Those licensed include palivizumab, for preventing respiratory syncytial virus (RSV) infections in high-risk infants and raxibacumab, for prophylaxis and treatment of anthrax. By November 2016, there were at least 38 MAbs in active

clinical development for infections that included *Clostridium botulinum* and *C. difficile*, Ebola virus disease, hepatitis B and C viruses, Hendra virus, herpes simplex virus (HSV), human immunodeficiency virus (HIV), influenza viruses, rabies virus, RSV, *Staphylococcus aureus* and *Staph. epidermidis*.

Not only can the genes for a monoclonal antibody be engineered for expression in bulk in the milk of lactating animals, but also plants can be exploited for this purpose, even producing secretory IgA. So-called 'plantibodies' have been expressed in bananas, potatoes and tobacco plants. Just imagine a high-tech farmer with one field growing anti-tetanus toxoid, and another anti-meningococcal polysaccharide. Although seemingly in science fiction territory, ZMapp was used to treat individuals with Ebola virus infections in 2014. It was an experimental drug made by inserting the genes coding for three MAbs, part of the Ebola virus surface glycoprotein, into viral vectors that then infected tobacco plants and the MAbs were then extracted and purified from the plants.

NON-SPECIFIC CELLULAR IMMUNOSTIMULATION

Cytokines and other molecular mediators stimulate the immune system

The demonstration by William Coley almost one century ago that crude extracts of bacteria could induce remission and sometimes cure cancers indicated the extent to which the immune system can be non-specifically 'overstimulated', with potentially beneficial results. Many of the compounds used in this way were of microbial origin, but the induction of cytokines and other molecular mediators was probably the basis of action of the older crude materials (Box 36.2).

Most of the applications of this type of immunostimulation have been in oncology, but some infectious diseases respond to treatment with cytokines. Foremost among these are the interferons (IFNs), notably IFNα, which is effective in a number of virus infections, though less than might have been predicted from the importance of its normal role in inhibiting viral replication. IFNγ has been found to benefit many cases of chronic granulomatous disease (CGD). However, the unpleasant side effects of high-dose therapy with interleukins or IFNs restrict their use and include:

- fever
- malaise leading to fatigue
- muscle pain
- toxicity to the kidney, liver, bone marrow and heart.

There is an interesting 'grey area' where immunostimulation and nutrition overlap

A variety of plant products such as saponins, ginseng and Chinese herbal remedies appear to improve resistance to infection and in some cases also act as adjuvants when combined with vaccines, but the complexity and variability of the extracts makes the active components difficult to track down.

CORRECTION OF HOST IMMUNODEFICIENCY

Antibody defects are the easiest to treat

This subject is discussed in more detail in Chapter 31, and will be only briefly summarized here:

- Antibody defects are the easiest to treat, since immunoglobulin can be given as an intravenous infusion and has a reasonably long half-life (about 3 weeks for IgG).
- Treatment of T-cell defects is more difficult, though thymus or bone marrow grafting has been tried in certain cases with some success.
- Phagocytic defects are the most difficult to correct, and in practice antibiotics remain the mainstay of therapy, though the future may lie in gene replacement.

Gene defects have recently been identified in certain serious immunodeficiency diseases including hyper-IgM syndrome, CGD and Bruton's agammaglobulinaemia.

PROBIOTICS

Probiotics are dietary supplements containing potentially beneficial bacteria or yeast, of which lactic acid bacteria are the most common microorganisms used. Therefore, they are live microbial compounds that may have a beneficial effect on the host. They may be given with prebiotics, which are non-digestible fibres that stimulate bacterial growth, a combination known as synbiotics. The gut flora can be thrown out of balance by a wide range of circumstances including the use of antibiotics or other drugs, excess alcohol, stress, disease, exposure to toxic substances or even the use of antibacterial soap. In cases like these, the 'friendly' bacteria, which work well with our bodies, may decrease in number, so allowing harmful competitors to thrive to the detriment of our health. Probiotic bacterial cultures are intended to assist the body's naturally occurring flora within the digestive tract to re-establish themselves. They are sometimes recommended after a course of antibiotics or as part of the treatment for candidiasis. Many probiotics are present in natural sources such as yoghurt, commonly used bacteria being *Lactobacillus acidophilus* and *Bifidobacterium bifidum*.

A range of potentially beneficial medicinal uses for probiotics have been explored and these include managing lactose intolerance, preventing colon cancer, lowering cholesterol, improving immune function and preventing infections and reducing inflammation. It is also possible to

Box 36.2 Non-Specific Immunostimulators

A variety of foreign and endogenous materials have been used in an attempt to raise the general level of immunological competence.

Microbial

- Coley's toxin (filtered cultures of *Streptococci* and *Serratia marcescens* used against tumours)
- BCG (bacillus Calmette–Guérin)
- Streptococcal-derived OK432 (possible immunomodulator in cancer immunotherapy)

Endogenous

- Thymus factors and hormones
- Cytokines, such as interferons used to treat chronic viral hepatitis B and C infections

increase and maintain a healthy gut flora by increasing the amounts of prebiotics in the diet, such as inulin, raw oats and unrefined wheat; a combination of the two should prove to be synergistic since prebiotics are effective only in the large intestine, whereas probiotics exert their influence in the small bowel. A systematic review and meta-analysis of randomized control trials in 2016 reported that, of 20 trials with nearly 1500 participants, the results suggested that probiotics / synbiotics given to adults undergoing elective abdominal surgery reduced the risk of surgical site infections compared with placebo or standard of care. It was also reported that there were benefits in other bacterial infections, as well as in HIV-positive individuals and in preventing *Candida* colonization in preterm neonates.

KEY FACTS

- Transfusion of pooled IgG is the most widely practised type of passive immunotherapy and is used to treat most forms of antibody deficiency.
- Specific antibodies can be used for certain defined conditions. Such antibodies can be produced as mouse or human monoclonals.
- T cells recognize and kill target cells and so adoptive T-cell therapy has been investigated as a way of targeting cells with latent and integrated viral infections.
- Antibodies can be engineered for expression in bulk in conventional vectors in vitro, or in vivo in the milk of lactating animals or in plants.
- Fab, single-chain Fv (scFv) or heavy chain variable region domain fragments can be selected by antigen from expression libraries of bacteriophages bearing the antibody fragments as a surface protein.
- These fragments are effective in blocking cognate interactions such as microbial adherence to mucosal epithelial cells as a precursor to invasion.
- Non-specific stimulation of T-cell-mediated immunity involves cytokines and IFN for viral infections.

37 Infection control

Introduction

Infections associated with healthcare settings are an increasingly complex issue

Amassing sick people together under one roof has many advantages, but some disadvantages, notably the easier transmission of infection from one person to another. In the past, the major environment for this interaction has been the hospital, which led to the term 'nosocomial infection' (i.e. any infection acquired while in hospital). Increasing numbers of individuals in skilled nursing and homecare settings have prompted the more recent use of the term 'healthcare-associated infections' (HAI). Nevertheless, hospitals remain the major environment associated with HAI. Hospital infection is generally defined as any infection acquired while in hospital (e.g. occurring 48 h or more after admission and up to 48 h after discharge). Most of these infections become obvious while the patient is in hospital, but some (e.g. postoperative wound infections) may not be recognized until after the patient has been discharged. Earlier discharges, encouraged to reduce costs, contribute to these unrecognized infections, although a shorter preoperative stay reduces the chance of acquiring hospital pathogens (see below).

Healthcare-associated infection may be acquired from:

- an exogenous source (e.g. from another patient – cross-infection – or from the environment)
- an endogenous source (i.e. another site within the patient – self- or auto-infection).

An infection that is incubating in a patient when he or she is admitted into hospital is not a hospital infection. However, community-acquired infections brought into hospital by the patient may subsequently become hospital infections for other patients and hospital staff.

Many healthcare-associated infections are preventable

In 1850, Semmelweiss demonstrated that many hospital infections are preventable when he made the unpopular suggestion that puerperal fever (an infection in women who have just given birth, see Ch. 24) was carried on the hands of physicians who came directly from attending an autopsy to the delivery ward, without washing. The death rate was reduced by introducing the simple measure of hand washing before and after any clinical examination. Recent studies demonstrate that healthcare-associated infections are significantly less frequent in resource-rich countries compared with those with more limited resources (Fig. 37.1). However, regardless of geographic location, a significant number of these infections can be prevented (e.g. 20–30% in the United States) with success related to the type of infection and available intervention methods. Current US estimates place HAI costs associated with hospital infection at approximately 2 million infections leading to nearly 100 000 deaths at a cost of US$20 billion annually.

COMMON HOSPITAL INFECTIONS

Hospital infections are frequently associated with indwelling devices

The infections most commonly acquired in hospitals are:

- surgical wound infection
- respiratory tract infection (pneumonia)
- gastrointestinal infection (e.g. *Clostridium difficile*; Ch. 23)
- urinary tract infection (UTI)
- bacteraemia.

The relative frequencies of these infections are illustrated in Fig. 37.2. A significant number of these infections are associated with medical devices (e.g. catheters and ventilators). Infections may arise from a variety of sources. For example, bacteraemia may be:

- primary – due to the direct introduction of organisms into the blood from, for example, contaminated intravenous fluids or via an indwelling device
- secondary to a focus of infection already present in the body (e.g. UTI).

Some infections (e.g. gastroenteritis and hepatitis) may contribute to outbreaks in the hospital setting.

IMPORTANT CAUSES OF HOSPITAL INFECTION

Staphylococci and *Escherichia coli* have traditionally been the most important Gram-positive and Gram-negative causes of infection, respectively, however the list is expanding

Almost any microbe can cause a hospital infection, though protozoal infections are rare. The pattern of hospital infection has changed over the years, reflecting advances in medicine and the development of antimicrobial agents. In the

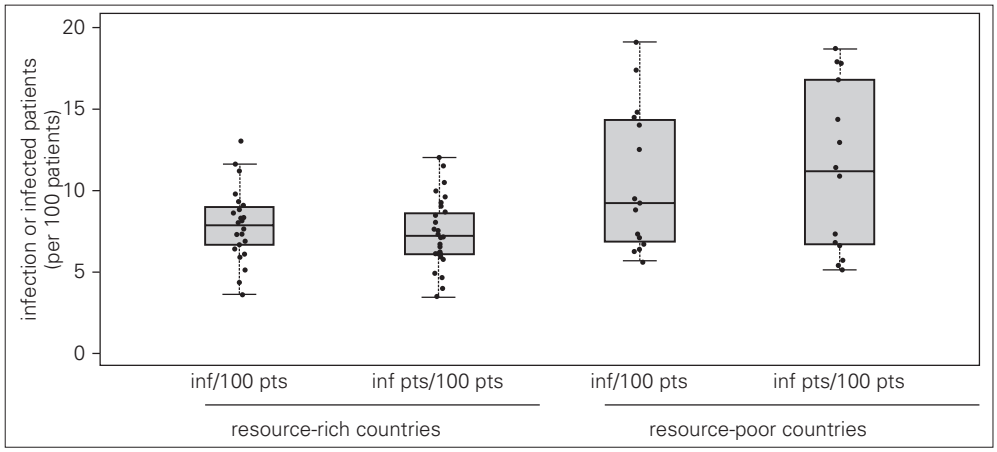


Figure 37.1 The prevalence of healthcare-associated infections in resource-rich versus resource poor countries, 1995–2010. (Taken from *Report on the Burden of Endemic Health Care-Associated Infection Worldwide*, WHO, 2011. http://apps.who.int/iris/bitstream/10665/80135/1/9789241501507_eng.pdf.)

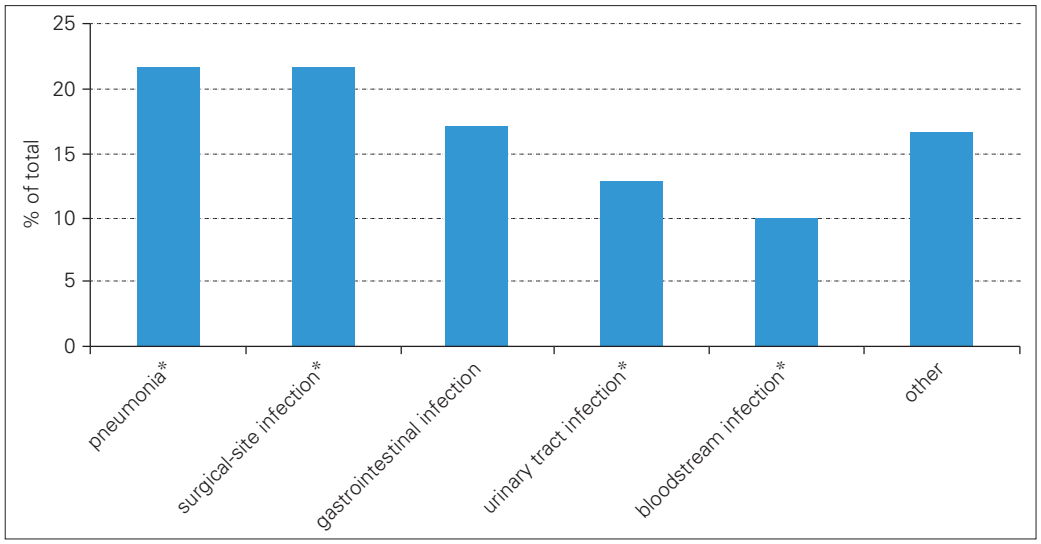


Figure 37.2 The relative frequencies of different kinds of hospital infection vary in different patient groups, but are most frequently associated with medical devices (e.g. catheters, ventilators; denoted with an asterisk). (Data taken from www.cdc.gov/hai/surveillance/index.htm.)

pre-antibiotic era, the majority of infections were caused by Gram-positive organisms, particularly *Streptococcus pyogenes* and *Staphylococcus aureus*. With the advent of antibiotics active against staphylococci, Gram-negative organisms such as *Escherichia coli* and *Pseudomonas aeruginosa* emerged as important pathogens. More recently, the development of more potent and broad-spectrum antimicrobials and the increase in invasive medical techniques has been accompanied by an increase in the incidence of:

- antibiotic-resistant Gram-positive organisms such as coagulase-negative staphylococci, enterococci (especially those resistant to vancomycin; VRE), methicillin-resistant *Staph. aureus* (MRSA) and *C. difficile*.
- multidrug-resistant Gram-negative organisms, especially including carbapenem-resistant Enterobacteriaceae (e.g. *Klebsiella* spp. and *E. coli*) (see Ch. 34), which in some instances are resistant to the vast majority (if not all) available antibiotics
- *Candida*.

Many of these organisms are considered as 'opportunists' – microbes not usually causing disease in healthy people with intact defence mechanisms, but able to cause infection in compromised patients or when introduced during the course of invasive procedures. While organisms such as *Staph. aureus* are a major contributor to healthcare (and hospital) infection, predominant pathogens can vary depending on the specific type of infection. (Box 37.1).

Some infections historically associated with hospitals are now increasingly seen outside of the healthcare setting

Recent reports in numerous countries have documented the emergence of virulent MRSA strains causing infection

Box 37.1 Order of Pathogen Importance

The general rank order of pathogen importance is listed for the different infection categories. Although a few species are the most important in all kinds of hospital infection, predominant pathogens vary in different infections. *Staphylococcus aureus* is very important in surgical wound infections and bacteraemia, but much less important in urinary tract infections. The importance of Gram-negative rods has increased since the advent of broad-spectrum antibiotics because these organisms often carry multiple and broad-spectrum antibiotic resistance.

Urinary tract infections

- *E. coli*
- *Klebsiella pneumoniae*
- *Staphylococcus saprophyticus*
- *Enterococcus* spp.
- Other (e.g. *P. aeruginosa*, *Proteus mirabilis*, *Staph. aureus*, *Candida* spp.)

Surgical wound infections

- Staphylococci (*Staph. aureus* and coagulase negative)
- Enterococci
- *E. coli*, *P. aeruginosa* (other Gram-negatives to a lesser extent)

Pneumonia

- *Staph. aureus*
- *P. aeruginosa* (other Gram-negatives to a lesser extent)

Bloodstream infections

- *Staphylococci* (*Staph. aureus* and coagulase-negative)
- Enterococci
- *Candida*
- *K. pneumoniae* (other Gram-negatives to a lesser extent)

Gastrointestinal infections

- *C. difficile*

Box 37.2 Criteria for Distinguishing Community-Associated MRSA (CA-MRSA) From Healthcare (Including Hospital)-Associated MRSA (HA-MRSA)

Individuals with MRSA infections that meet all of the following criteria probably have CA-MRSA infections:

- Diagnosis of MRSA was made in the outpatient setting or by a culture positive for MRSA within 48 h after admission to the hospital
- No medical history of MRSA infection or colonization
- No medical history in the past year of:
  - Hospitalization
  - Admission to a nursing home, skilled nursing facility, or hospice
  - Dialysis
  - Surgery
- No permanent indwelling catheters or medical devices that pass through the skin

in individuals outside of the healthcare system. These community-associated MRSA (CA-MRSA) can be transported into the healthcare environment, thus blurring the distinction between community-associated and healthcare-associated infection. This has prompted guidelines for differentiating the increasing number of CA-MRSA infections from those associated with healthcare, summarized in Box 37.2.

Viral infections probably account for more hospital infections than previously realized

These affect both patients and healthcare workers and include:

- viruses acquired by the respiratory route, especially influenza, as well as respiratory syncytial virus (RSV), parainfluenza.
- viruses acquired by contact with vesicular lesions such as varicella-zoster virus (VZV) and herpes simplex virus (HSV)
- viruses acquired by contact with contaminated fomites such as noroviruses and rotavirus
- viruses acquired by contact with blood-contaminated fomites, needlestick injury or splash on mucous membranes, such as hepatitis B virus (HBV), hepatitis C virus (HCV), human immunodeficiency virus (HIV) and human T-cell lymphotropic virus (HTLV). These may also be acquired in countries where blood and blood products are not screened, or in the rare instance where the blood donor was in the early incubation (window) period of infection, thereby escaping detection by the screening assay.

The risk of hospital infection is a sum of the transmissibility of the virus and the susceptibility of the patient group. Some viruses, such as varicella-zoster, are of low risk in general, but very important in paediatric units and particularly in immunocompromised children.

SOURCES AND ROUTES OF SPREAD OF HOSPITAL INFECTION

Sources of hospital infection are people and contaminated objects

As stated above, the source of infection may be:

- *human* from other patients or hospital staff, and occasionally visitors
- *environmental* from contaminated objects ('fomites'), food, water or air.

The source may become contaminated from an environmental reservoir of organisms – for example, contaminated antiseptic solution distributed for use into sterile containers (Fig. 37.3). Eradication of the source will also require eradication of the reservoir.

Human sources may be:

- people who are themselves infected
- people who are incubating an infection
- healthy carriers.

The time period for which a human source is infectious varies with the disease. For example, some infections can be spread during their incubation period, others in the early stages of clinical disease, whereas others are characterized by a prolonged carrier state even after clinical cure (e.g. typhoid fever) (Fig. 37.4). Carriers of virulent strains of, for example, *Staph. aureus* or *Strep. pyogenes* may act as sources of hospital infection, although they themselves do not develop clinical disease. The carrier state may persist for a long time and go unnoticed unless there is an outbreak or, depending on the significance of the organism, a single case of infection that is traced to the carrier (e.g. a healthcare worker with chronic hepatitis B).

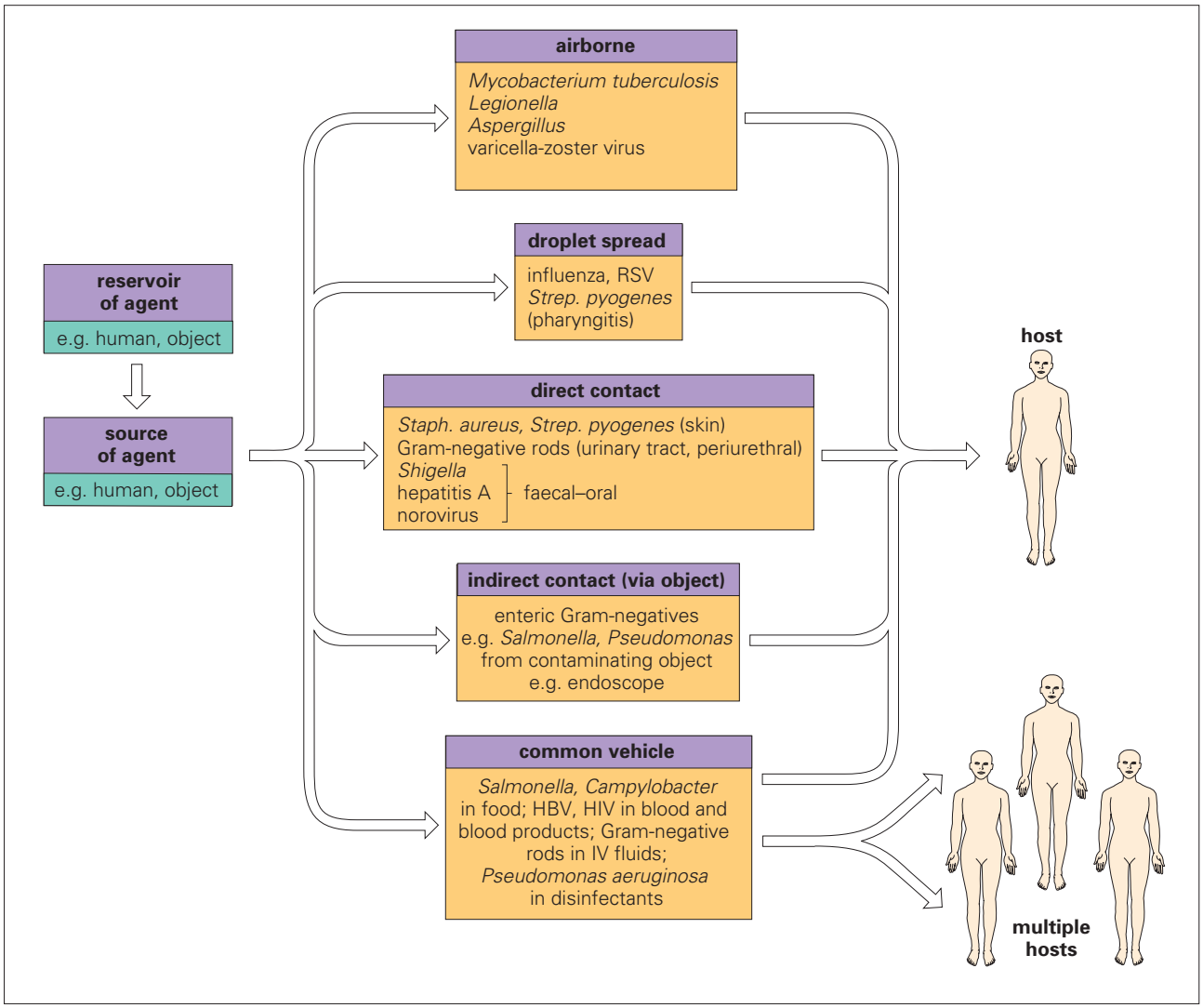


Figure 37.3 Hospital infections are spread by the same routes as infections spread in the community. The reservoir and the source of infection may be human or inanimate and may be one and the same (e.g. a nurse with an infected skin lesion). If the reservoir and source are distinct (e.g. contaminated distilled water supply used to prepare a variety of pharmaceuticals), both must be eliminated if the spread of infection is to be halted, otherwise the reservoir may continue to contaminate new sources. HBV, hepatitis B virus; HIV, human immunodeficiency virus; IV, intravenous; RSV, respiratory syncytial virus.

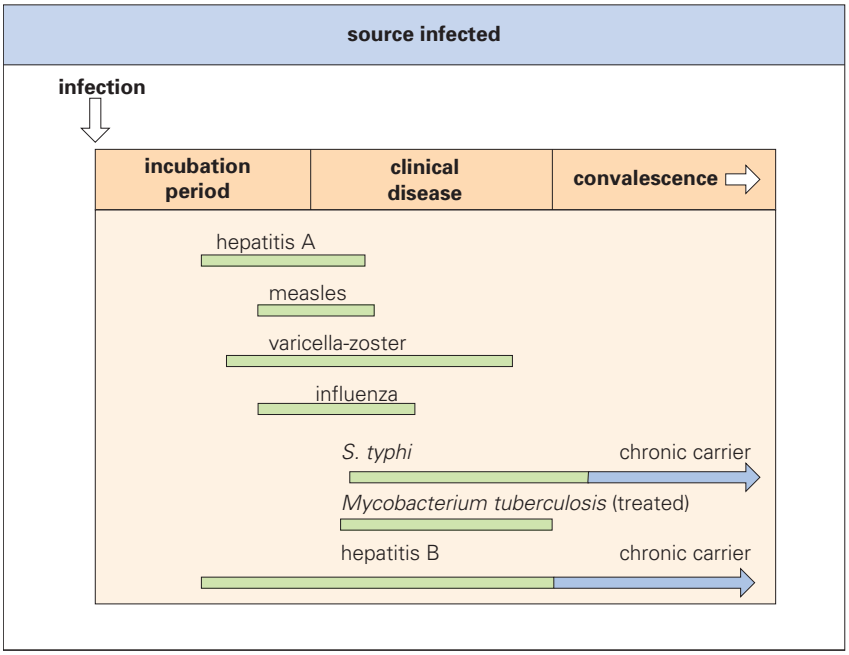


Figure 37.4 Pathogens differ in the time periods for which they can be disseminated from an infected person. For some, it is during the incubation period when infected people may not realize they are ill and infectious. Some people continue to carry organisms such as *Salmonella typhi* and hepatitis B virus long after they have recovered from the clinical disease. Opportunist pathogens are often members of the normal microbiota and may therefore be carried for long periods without the host experiencing any adverse effects.

Hospital infections are spread in the air and by contact and common vehicle

The important routes of spread of infection in hospitals are those common to all infections: airborne, contact and common vehicle. Examples of organisms spread by these routes in hospitals are illustrated in Fig. 37.3. Although theoretically possible, vector-borne spread is very unusual in the healthcare setting, as is sexually transmitted infection. It is important to remember that the same organism may be spread by more than one route. For example, *Strep. pyogenes* can be spread from patient to patient by the airborne route in droplets or dust, but is also transmitted by contact with infected lesions, for example on a nurse's hand. In addition, a patient or healthcare worker with shingles can transmit VZV to a susceptible person having direct contact with rash blisters.

HOST FACTORS AND HOSPITAL INFECTION

Underlying disease, certain treatments and invasive procedures reduce host defences

Host factors play a fundamental role in the infection equation, particularly in hospitals because of the high proportion of hospital patients with compromised natural defences against infection. The spread of an infectious agent to a new host can result in a spectrum of responses: from colonization, through subclinical infection, to clinically apparent disease, which may be fatal. The degree of host response differs in different people depending upon their degree of compromise. The very young are particularly susceptible because of the immaturity of their immune system. Likewise, the elderly suffer a greater risk of infection because of predisposing underlying disease, impaired blood supply and immobility, which contribute to stasis and therefore to infection in, for example, the lungs. In all age groups, underlying disease and the treatment of that disease (e.g. cytotoxic drugs, steroids) may predispose to infection while invasive procedures allow organisms easier access to previously protected tissues. The important host factors to be considered in hospital infection are summarized in Table 37.1. Infections in the compromised host are discussed in more detail in Chapter 31.

A variety of factors predispose to wound infection

Wound infection or wound sepsis is characterized by the presence of inflammation, pus and discharge in addition to the isolation of organisms such as *Staph. aureus*. Extensive studies of postoperative wound infection have identified a number of predisposing factors:

- Prolonged preoperative stay increases the opportunity for the patient to become colonized with antibiotic-resistant hospital pathogens.
- The nature and length of the operation also have an effect (Table 37.2; see also Ch. 27).
- Wet or open wounds are more liable to secondary infection.

From these studies, it has been possible to identify the patients and operations with greatest risk and apply preventive measures such as prophylactic antibiotic regimens and ultra-clean air in orthopaedic operating theatres (see below).

CONSEQUENCES OF HOSPITAL INFECTION

Hospital infections affect both the patient and the community

Hospital infection may result in:

- serious illness or death
- prolonged hospital stay, which costs money and results in a loss of earnings and hardship for the patient and his or her family
- a need for additional antimicrobial therapy, which is costly, exposes the patient to additional risks of toxicity, and increases selective pressure for resistance to emerge among hospital pathogens
- the infected patient becoming a source from which others may become infected, in the hospital and in the community.

Table 37.1 Factors which predispose patients to hospital infection

Age	Patients at extremes of age are particularly susceptible
Specific immunity	Patient may lack protective antibodies to, e.g. measles, chickenpox, whooping cough
Underlying disease	Other (non-infectious) diseases tend to lead to enhanced susceptibility to infection, e.g. hepatic disease, diabetes, cancer, skin disorders, renal failure, neutropenia (either as a result of disease or of treatment)
Other infections	HIV and other immunosuppressing virus infections; patients with influenza prone to secondary bacterial pneumonia; herpes virus lesions may become secondarily infected with staphylococci
Specific medicaments	Cytotoxic drugs (including post-transplant immunosuppression) and steroids both lower host defences; antibiotics disturb normal flora and predispose to invasion by resistant hospital pathogens
Trauma Accidental Intentional	 Burns, stab or gunshot wounds, road traffic accidents Surgery, intravenous and urinary catheters, peritoneal dialysis } Disturb natural/host defence mechanisms

Hospital patients are not all at equal risk of infection. Some factors that predispose to infection can be influenced by e.g. treating underlying disease, improving specific immunity and avoiding inappropriate use of antibiotics. Other factors such as age are unalterable.

Table 37.2 Risk factors for postoperative infections

Length of preoperative stay	Longer stay – more likely to become colonized with virulent and antibiotic-resistant hospital bacteria and fungi
Presence of intercurrent infection	Operating on an already infected site more likely to cause disseminated infection
Length of operation	Longer – greater risk of tissues becoming seeded with organisms from air, staff, other sites in patient
Nature of operation	Any operation which results in faecal soiling of tissues has higher risk of infection (e.g. postoperative gangrene), 'adventurous' surgery tends to carry greater risks
Presence of foreign bodies	For example, shunts, prostheses, impair host defences
State of tissues	Poor blood supply encourages growth of anaerobes; inadequate drainage or presence of necrotic tissue predisposes to infection

The risks of infection after surgery have been studied in considerable detail, and surgeons are consequently much more aware of the problems. However, 'high-tech' surgery is often long and difficult, increasing the potential for postoperative infection.

PREVENTION OF HOSPITAL INFECTION

There are three main strategies for preventing hospital infection

For the reasons outlined above, the prevention of hospital infection deserves a very high priority, and the three main strategies are:

- excluding sources of infection from the hospital environment
- interrupting the transmission of infection from source to susceptible host (breaking the chain of infection)
- enhancing the host's ability to resist infection.

Exclusion of sources of infection

Exclusion of inanimate sources of infection is achievable, but it can be difficult to avoid contamination by humans

Exclusion of inanimate sources of infection is both desirable and, to a large extent, achievable. Examples include the provision of sterile instruments and dressings, sterile medicaments and intravenous fluids, clean linen and uncontaminated food, and the use of blood and blood products screened for infectious agents. However, many of the sources of infection are human or are objects that become contaminated by humans, in which case exclusion is more difficult. Hospitals must attempt to prevent patient contact with staff who are carriers of pathogens. The problem is the identification of staff who are carriers of pathogens and their relocation to less hazardous positions.

Staff must undergo health screening before employment and should have regular health checks. For example, in the UK all new healthcare workers (HCWs) are offered testing for HIV and hepatitis C. Hepatitis B immunization is offered and HCWs must know whether they responded post-immunization and are therefore protected. Any HCW found to be a hepatitis B carrier would have the hepatitis B e markers tested in addition to an HBV DNA load test. In the UK, an HCW has to have an HBV load of 1000 genome equivalents / mL or less before carrying out exposure-prone procedures (EPPs). It is critical that those carrying out EPPs who either do not know their post-immunization status or have not responded to the hepatitis B vaccine are checked to ensure that they either do not have a current HBV infection or have a protective level of hepatitis B surface antibody. This is because HBV could be transmitted to the patients if the HCW carrying out EPPs is a hepatitis B carrier and also because the unprotected HCW is at risk of infection from a hepatitis B carrier patient. There are also guidelines for HCWs with current HIV and HCV infections.

Hospitals have blood-borne virus exposure policies for the management of healthcare workers and others who may have been exposed to viruses, including HBV, HCV and HIV, having sustained a needlestick injury or mucous membrane splash from a potentially infected source. Prophylaxis includes active and / or passive immunization against hepatitis B and a 4-week course of antiretroviral prophylaxis for HIV exposure (see Ch. 22). The risk of transmission is highest for HBV transmission at around 30–33% in unimmunized recipients, around 0.3% for HIV transmission and for HCV is thought to be between 1% and 3%, but may be higher. However, as reporting of exposure incidents and follow-up of the recipient improves, so too does our understanding of the outcomes of the incident itself. In the UK, it was reported in 2014 that those HCWs who were found to have developed an HCV infection after an exposure incident recovered either spontaneously or after receiving ribavirin and pegylated interferon treatment.

In general, staff should be encouraged to report any incidence of infection (e.g. an infected cut or a bout of diarrhoea). Appropriate immunizations should be offered and in some instances made mandatory. While work restrictions for personnel with infectious diseases are important, healthy carriers of, for example, virulent staphylococci are difficult to identify unless bacteriological screening is undertaken, which is not feasible on a routine basis. In addition, staff members are sources of opportunist organisms such as coagulase-negative staphylococci or enterobacteria, which are part of their normal microbiota and cannot be excluded.

Breaking the chain of infection

There are two elements to be considered in breaking the chain of infection: the structural and the human. The structure of the hospital and its equipment can play a role in preventing airborne spread of infection and in facilitating aseptic practices by the staff, but this is of no avail if staff members do not use the facilities correctly and do not themselves act positively to prevent the spread of infection.

Control of airborne transmission of infection

Ventilation systems and air flow can play an important role in the dissemination of organisms by the airborne route. Wards comprising separate rooms have been shown to afford some protection against airborne spread, and rooms with controlled ventilation are even better. However, neither prevents the carriage of organisms into the room on staff members and their clothing, and some studies suggest that this is a more important route of infection than airborne spread. However, *Legionella* infection is acquired by the airborne route, and air-conditioning systems throughout the hospital should be maintained so as to prevent the multiplication of these organisms (see Ch. 20). *Aspergillus* infection in hospitals has been attributed to dissemination of the spores in hospital air, especially when building work is ongoing in the locality.

Ventilation systems in operating theatres must be properly installed and maintained to prevent the ingress of contaminated air and to minimize air currents carrying organisms from the HCWs in the operating room to the operation site. 'Ultra-clean' air is air passed through high-efficiency filters to remove bacteria and other particles and has been shown to contribute positively to a reduction in the number of postoperative wound infections developing after long orthopaedic operations.

Airborne transmission of infection can be reduced significantly by isolating patients. Patient isolation may be carried out:

- to protect a particularly susceptible patient from exposure to pathogens (i.e. protective isolation)
- to prevent the spread of pathogens from an infected patient to others on the ward (i.e. source isolation).

Isolation also helps to prevent the transmission of infection by other routes by limiting access to the patient and reminding staff of the importance of contact in the spread of infection.

Protective isolation can be provided by a single room on a ward or by enclosing the patient in a plastic isolator. With appropriate positive-pressure ventilation, air should flow from the 'clean' patient area out of the room or isolator. Staff entering the room or in contact with the patient should wear sterile gowns, gloves and masks to prevent organisms they are carrying or have picked up from other patients from coming in contact with the patient.

While source isolation historically involved patient accommodation in an isolation unit in a separate building (e.g. the tuberculosis sanatoria), hospital isolation is typically arranged in a separate ward or in side rooms off the main ward. To prevent airborne transmission of organisms from the patient's room to the ward, air should flow from the ward to the isolation room. In practice, it is difficult to maintain the correct air flows without sophisticated designs, including double doors and air locks.

Facilitation of aseptic behaviour

A general state of cleanliness throughout the hospital is essential, and the design of hospital facilities affects the ease with which the environment can be kept clean and the staff can practise good techniques.

Bacteriologically effective hand washing is one of the most important ways of controlling hospital infection. The hands of staff convey organisms to patients from septic lesions and healthy carrier sites of other patients, from equipment contaminated by these sources and from carrier sites of the staff themselves (Table 37.3 and Fig. 37.5).

Staff should therefore wash their hands:

- before any procedure for which gloves or forceps are necessary
- after contact with an infected patient or one who is colonized with multiply resistant bacteria
- after touching infective material.

Although soap and water are adequate in many circumstances, emphasis is shifting to the use of fast-drying alcohol-based gels and solutions, which are easier to use and appear to have a greater antibacterial effect. A mandate from the US Centers for Disease Control, for example, has put this approach into practice in US hospitals. Drying hands after any washing procedure is important. A more prolonged and thorough hand decontamination is required before commencing surgery.

The design of taps, soap dispensers and other washing facilities, including bedpan washers, has reached a high degree of sophistication. However, human behaviour can be influenced by architectural design to only a limited degree, and there is often a disappointingly low compliance with the

Table 37.3 Contact spread of opportunist pathogens

Patient	Nursing activity	Number of *Klebsiellae* recovered per hand[a]
A	Physiotherapy	10–100
	Taking blood pressure and pulse	100–1000
	Washing patient	10–100
	Taking oral temperature	100–1000
B	Taking radial pulse	100–1000
	Touching shoulder	1000
	Touching groin	100–1000
C	Touching hand	10–100
D	Extubation	100–1000
	Touching tracheostomy	1000

Nursing procedures involving skin contact resulting in contamination of staff hands. These data are derived from experiments performed during an outbreak of *Klebsiella* infection among urology patients.
[a]Control hand washings taken prior to procedure yielded no *Klebsiellae*.
(Data from Casewell M, Phillips I. Hands as route of transmission for Klebsiella species. *British Medical Journal*. 1977;2[6098]:1315–1317.)

Figure 37.5 Gram-negative rods are not usually part of the resident skin flora except in moist environments, but are readily carried on hands and can be transferred from a source to a susceptible patient. This picture shows an impression of a hand that was inoculated with approximately 1000 *Klebsiella* aerogenes.

simple technique of hand washing. Therefore, training and regular reinforcement in appropriate behaviour are essential.

Enhancing the host's ability to resist infection

Host resistance can be enhanced by boosting immunity and reducing risk factors

Although attempts can and should be made to control and prevent hospital infection by removing sources of infection and preventing transmission from sources to susceptible hosts, neither of these strategies is fail-safe. In addition, they do not protect the host from endogenous infection. A way of tipping the balance in favour of the host is to enhance his or her ability to resist infection, both by boosting specific immunity and by reducing personal risk factors. The following aspects should be considered:

- boosting specific immunity by active or passive immunization
- the appropriate use of prophylactic antibiotics
- care of invasive devices that breach the natural defences (e.g. urinary catheters, intravenous lines)
- attention to the risks predisposing to postoperative infection.

Boosting specific immunity

Passive immunization provides short-term protection. Boosting specific immunity by immunization has been discussed in Chapters 35 and 36. The problem for the immunocompromised patient is that they may not be able to mount an antibody response.

Appropriate use of prophylactic antibiotics

There are well-documented uses for prophylaxis, but antibiotics tend to be misused. This is discussed in Chapter 34. There are several well-documented uses for prophylactic antibiotics in 'dirty' surgery and when the consequences of infection would be disastrous (e.g. in cardiac, neuro- and transplant surgery). However, there is a tendency to misuse antibiotics:

- first, by using them too often or for too long, thereby increasing the selection pressure for the emergence of resistant organisms
- second, by choosing inappropriate agents.

Treatment (as opposed to prophylaxis) of patients and staff who are carriers of pathogens such as *Staph. aureus* or *Strep. pyogenes* has been used successfully to prevent endogenous infection and to control outbreaks of infection with these organisms. Topical preparations of antibiotics such as pseudomonic acid (mupirocin), a fermentation product of *Pseudomonas fluorescens*, have been shown to be efficacious. However, resistance (both low and high level) to the drug has occurred.

Gut decontamination regimens and selective bowel contamination aim to reduce the reservoir of potential pathogens in the gut. Gut decontamination regimens to reduce the aerobic Gram-negative flora of neutropenic patients has been practised for some time. With some patients (e.g. liver transplant) in intensive care units (ICU), selective bowel decontamination (SBD) has been employed. The aim is to reduce the reservoir of potential pathogens in the gut by oral administration (or via a nasogastric tube) of a high concentration of a mixture of antibiotics. At the present time, there is still controversy about the efficacy and safety of SBD.

Care of invasive devices

Care of invasive devices is essential to reduce the risk of endogenous infection. It is essential to take care of intravascular devices so as to reduce the risk of endogenous infection from skin organisms, and of catheters so as to reduce the risk that the periurethral flora will cause endogenous infection of the

bladder in catheterized patients. Guidelines for the care of urinary catheters are discussed in Chapter 21.

The majority of hospital-associated bacteraemias and candidaemias are infusion related. These infusion-related bacteraemias and candidaemias derive mainly from vascular catheters. Most bacteraemias associated with invasive devices are caused by the patient's own skin flora, although this may be a more resistant flora acquired during the patient's stay in hospital replacing susceptible resident bacteria. Coagulase-negative staphylococci are the most common aetiological agents, but enterococci, *Candida*, and various Gram-negative rods are also implicated. These infections are largely preventable if appropriate steps are taken.

Reducing the risks of postoperative infection

Prevention of postoperative infection involves minimizing the risks. Reducing the risks of postoperative infection involves an understanding of the risks and the ways in which they can be circumvented. For example:

- The preoperative length of stay in hospital should be kept to a minimum.
- Intercurrent infections should be treated appropriately before surgery whenever possible (e.g. treatment of UTI before resection of the prostate).
- Operations should be kept to the minimum duration consistent with good operating technique.
- Adequate debridement of dead and necrotic tissue is essential, together with adequate drainage and maintenance or re-establishment of a good blood supply to provide the body's natural defences with optimum working conditions.
- Prevention of pressure sores and stasis by good nursing techniques and active physiotherapy minimizes the risks of developing respiratory tract infection or UTI.

INVESTIGATING HEALTHCARE-ASSOCIATED INFECTION

Many of the epidemiological principles outlined in Chapter 33 apply to the investigation of healthcare-associated infection. Outbreaks within hospitals are epidemics – they are detected because the incidence of an infection is seen to be above normal levels for that institution. Investigation therefore must determine the extent of the problem, identify the source of infection and the way in which it is spread, identify those at risk, and propose effective methods for control. As with infectious diseases in general, the application of statistical techniques (e.g. calculation of risk ratios) and mathematical modelling has helped to provide an analytical and predictive framework for such infections, but day-to-day investigations still require the application of proven microbiological approaches.

Hospital infections, like community infections, can involve all the major groups of pathogens, from viruses to arthropods. However, a particular problem with hospital infections, compared with those commonly occurring in the community, is the transmission of antibiotic-resistant bacteria, the emergence of which, and their spread, is favoured by the hospital environment. The recent surge in community-associated MRSA infections is an unfortunate exception to this trend. Epidemiological investigations of infections place great importance on molecular (typing) methods to identify and characterize the causative organism. Such molecular epidemiology can make a very important contribution to tracking and controlling infection.

In many hospitals, the responsibility for investigating hospital infection falls on the infection control committee, which includes an infection control officer (who may be a physician or microbiologist) and at least one nurse. The roles of the infection control committee include:

- the surveillance of hospital infection
- the establishment and monitoring of policies and procedures designed to prevent infection (e.g. catheter care policy, antibiotic policy, disinfectant policy, blood-borne virus exposure incidents, including needlesticks and blood splashes)
- the investigation of outbreaks – tracking the source and routes of transmission.

Surveillance

Surveillance allows early recognition of any change in the number or type of hospital infections

National and international surveys continue to highlight the prevalence and importance of hospital infection. By maintaining local surveillance, the infection control team can establish the normal trends in their hospital and proactively recognize any change in the number or type of infections. Sources of surveillance data are as follows:

- *Microbiology laboratory reports.* These can be used for general surveillance, for example, monitoring haemodialysis patients regularly for hepatitis B surface antigen and HCV antibody, as outbreaks of HBV and HCV infection have been reported in renal units around the world, or monitoring for 'sentinel' or 'alert' organisms such as *Staph. aureus,* VRE, and Enterobacteriaceae producing expanded-spectrum beta-lactamases (ESBLs) (Ch. 34).
- *Ward rounds.* New cases of infection can be identified by direct inspection, and previously identified cases of infection can be followed up. Surveys can also be carried out on the wards (e.g. of wound infections after different practices or procedures).
- *Other sources*. These include autopsy reports, staff health records and surveys of patients after discharge from hospital.

Investigation of outbreaks

When an outbreak (or epidemic) occurs or when routine surveillance highlights an increase in the incidence of infection, the infection control team should initiate an investigation. There is no universally applicable routine for finding the cause of an outbreak, but in principle each investigation has an epidemiological element and a microbiological element.

There must be a description of an outbreak in epidemiological terms

This involves obtaining information about a number of relevant factors:

- How many people are infected?
- When were they admitted?
- When did they develop their infection?

- Are they all on the same ward?
- Are they all treated by the same medical or surgical team?
- Have they all been exposed to the same treatments?

The causative organism needs to be isolated and/or detected in all patients in the outbreak

It is the role of the microbiology laboratory to attempt to isolate the causative organism and to show that it occurs in all patients in the outbreak (i.e. they are all infected with organisms that are indistinguishable – see below). The identity of the infecting organism can provide clues as to the possible source:

- Respiratory and intestinal viruses implicate the source of infection as a patient or attending medical staff.
- Hepatitis indicates spread via contaminated blood products or hypodermics.
- An outbreak of wound infection with *Staph. aureus* is likely to be associated with contact spread from staff in theatre or on the ward.
- An outbreak of *Salmonella gastroenteritis* is more likely to originate in the kitchen.
- Infections with *Legionella* or *Pseudomonas* are likely to reflect environmental (especially water) contamination.

In addition, the location of the outbreak, whether in a general ward, a surgical ward, a paediatric unit or intensive care unit (once described as the epicentre of hospital infections) may also provide valuable clues.

Stages in tracking infection

Once the problem has been identified clinically, appropriate specimens should be collected from the patients and, if the indicators are that medical staff are involved, from hospital personnel (see Ch. 32). Likely sources of environmental contamination (surfaces, materials, equipment, water) should also be sampled. This is an important step, as data (using a non-infectious DNA marker as an experimental infectious organism) have shown that after release there is a rapid spread from hands of medical staff to almost all available surfaces (computers, charts, telephones, control knobs, door handles, heater controls, patient monitors). Once samples have been collected, the microbiology laboratory then has the task of identifying and typing the organisms concerned.

While the investigation is proceeding, steps should be taken to contain the outbreak and prevent spread to other patients. Infected patients must be isolated and treated appropriately. Staff who show a similar infection, or who are subsequently found to be carriers, must be suspended from duty until they have been treated. At the end of the investigation, the relevant procedures must be reviewed to try and prevent the reoccurrence of a similar outbreak.

Epidemiological typing techniques

Bacteria are the commonest causes of nosocomial infections and of the greatest concern because of the prevalence of antibiotic resistance. For example, in 2011 there were over 700 000 nosocomial infections in US acute care hospitals resulting in approximately 75 000 deaths. Of the pathogens involved most were bacteria (see Box 37.1). Tracking infection is therefore disproportionately concerned with bacterial pathogens, although molecular techniques are also applied to monitoring viral infections.

A variety of phenotypic and genotypic characters are used to 'fingerprint' bacteria for epidemiological purposes

In epidemiological studies of the spread of hospital infections, as in the investigation of outbreaks in the community, it is necessary to identify isolates of the infectious organisms to determine whether or not they are distinct (one commonly does not say that two organisms are the same, only that they are indistinguishable). In the case of bacteria, if the species is a regular member of the normal human flora or is found frequently in the environment, it is necessary to distinguish the 'outbreak' strain from other strains of the same species that are not involved in the outbreak, but that may also be isolated during the course of the investigation. Essentially, typing is used to look for evidence of a clonal spread of a particular pathogen.

To be valuable in this context, good typing techniques must:

- be discriminatory (i.e. able to show differences between strains of the same species)
- be reproducible (i.e. the same strain gives the same result when tested on different occasions and in different places)
- have a high degree of typability (i.e. capable of assigning a type to all strains).

Antibiotic susceptibility patterns

Antibiotic susceptibility testing is readily performed in the diagnostic laboratory (see Chs. 32, 34) and is useful as a preliminary clue as to whether two isolates are indistinguishable. However, discrimination is poor: many susceptibility patterns are common, and quite different strains may have the same pattern. Conversely, during an outbreak, strains may gain or lose plasmids carrying antibiotic resistance markers. More specialized typing techniques are commonly performed in reference laboratories. This has the advantage that quality assurance can be optimized, but also means that there is an inevitable delay in reporting the results and therefore in learning whether an outbreak of hospital infection is caused by a single strain.

Specialized typing techniques

Serotyping distinguishes between strains, using specific antisera

This classic technique distinguishes between strains by a difference in their antigenic structure, which is recognized by reaction with specific antisera. The 'O' somatic antigens and 'H' flagellar antigens are therefore used to divide salmonellae into types (sometimes referred to as species; see Ch. 23). *Strep. pneumoniae*, *Neisseria meningitidis* and *Klebsiella aerogenes* can be typed on the basis of their capsular (K) antigens, and *Strep. pyogenes* on the basis of its M- and T-cell wall proteins. However, serotyping requires the production and maintenance of appropriate banks of reagents (e.g. antisera), which is both time consuming and costly. Therefore this approach, when employed, is usually restricted to reference laboratories.

Bacteriophage (phage) typing has been used to type *Staph. aureus*, *Staph. epidermidis* and *Salmonella typhi*

This technique compares the pattern of lysis obtained when isolates (grown as lawns on agar plates) are exposed to a standard series of phage suspensions. In the past, this method has been important for typing *Staph. aureus*, *Staph. epidermidis*

and *Salmonella typhi*, but has also been applied to other species such as *P. aeruginosa*. However, as with serotyping, phage typing requires a reference laboratory for the production, maintenance and testing of the standard phage suspensions and has thus fallen out of favour.

Molecular typing

Molecular typing techniques involve characterizing an organism's DNA

The above methods have been of great use in the epidemiological analysis of nosocomial pathogens, but are all variations on the phenotypic characterization of isolates. As the chromosome represents the most fundamental 'molecule of identity' in the cell, genotypic approaches are used for characterization, often referred to as 'molecular epidemiology'.

Plasmid profiles are an example of 'first-generation' molecular epidemiology

Agarose-gel electrophoresis of lysed cell suspensions allows a comparison of plasmid carriage in different isolates. However, the method is useful only for those species that carry a variety of plasmids and it suffers from the drawback that what is actually being characterized is the plasmid and not the organism containing it. Different Gram-negative rods may acquire the same plasmids by conjugation between different species. However, this method has also been used to map the spread of antibiotic-resistant plasmids among hospital pathogens.

Restriction enzymes and probes represent 'second-generation' molecular epidemiology

Restriction enzyme digestion of total cellular DNA from isolates results in a pattern of different-sized fragments, which can be separated and compared by agarose gel electrophoresis–restriction enzyme analysis (REA). All bacterial cells possess chromosomal DNA and can theoretically be analysed by this process. However, the DNA sequences recognized by most restriction enzymes, such as *Eco*RI, *Hin*dIII, etc., are present in hundreds of copies throughout a typical bacterial chromosome. Thus, the challenge is to accurately compare electrophoretic patterns comprising hundreds of restriction fragments which often co-migrate in clusters of similar size, and may include resident plasmid DNA.

The principle of complementary DNA sequences hybridizing with each other (e.g. Southern hybridization; named after its inventor, Ed Southern) has led to applications where specific-DNA appropriately labelled 'probes', complementary to 'target' sequences found at various chromosomal locations, are hybridized against isolate REA patterns. Northern blotting is similar in principle but characterizes RNA sequences. Antibiotic resistance genes, and a variety of repeated sequences (e.g. transposons), have been especially useful targets in this context. The result is a pattern of hybridization with different restriction fragments, commonly termed restriction fragment length polymorphism (RFLP) analysis, corresponding to the chromosomal location of the probed sequences, which provides an indication of chromosomal relatedness between different isolates (Fig. 37.6A). For example, copies of the genes for ribosomal RNA (5 S, 16 S and 23 S rRNA) are found at different locations on the chromosome of many medically important bacteria. These highly conserved sequences (i.e. very similar sequences in different species) allow RFLP analysis using a common probe (i.e. ribotyping). However, discrimination between strains of the same species may be less because of the conserved nature of the target sequences. The greatest success with RFLP analysis has primarily involved probes for insertion sequences that provide sufficient coverage (i.e. in number and diversity of chromosomal location) to reflect epidemiologically relevant interrelationships. The use of IS6110 probes in the RFLP analysis of *Mycobacterium tuberculosis* isolates is an example of a successful use of this approach. While superior to REA alone, RFLP analysis remains only moderately discriminatory for epidemiological analysis.

PFGE and PCR are 'third-generation' approaches to molecular epidemiology

Instead of using frequently 'cutting' restriction enzymes, chromosomal DNA may be digested using enzymes with rare recognition sites in bacterial chromosomes (e.g. *Not*I, *Sfi*I, *Spe*I and *Xba*I in most Gram-negatives; *Asc*I, *Rsr*II, *Sgr*AI and *Sma*I in most Gram-positives). The extremely large DNA fragments produced are too large to be separated by conventional agarose gel electrophoresis but may be resolved by electrophoretic current 'pulsed' in different directions for different lengths of time-pulsed field gel electrophoresis (PFGE). PFGE has proved to be a powerful epidemiological tool. The macro restriction patterns produced by PFGE provide a sense of 'global' chromosomal monitoring – genetic events that affect distances between rare restriction site sequences can be inferred from changes in restriction fragment size (Fig. 37.6B). To date, the major disadvantage to PFGE analysis has been the extra time and effort involved in producing unbroken chromosomal molecules necessary for reproducible macro restriction fragment patterns. For many years the overall success with which PFGE analysis has been employed made it the method of choice – the 'gold standard' – for the epidemiological analysis of most pathogens of clinical concern.

Economy, speed and the relatively low level of technical expertise required by the polymerase chain reaction (PCR) (Ch. 32) have led to a wealth of amplification-based applications for epidemiological analysis. One of the earliest and most common PCR-based approaches has been randomly amplified polymorphic DNA (RAPD), also called arbitrarily primed PCR (AP-PCR). The method is based on the use of relaxing conditions affecting the stringency (i.e. specificity) with which PCR primers bind to DNA templates. PCR primers are allowed to bind randomly to chromosomal sequences of varying homology, resulting in products which can be comparatively analysed by agarose gel electrophoresis. A group of clinical isolates representing inter-patient transfer of a single strain would thus be expected to exhibit the same degree of 'randomness', resulting in identical PCR products (Fig. 37.6C). However, numerous studies have shown that this method is especially prone to artefactual and inter- and intra-laboratory variation. Nevertheless, the overall simplicity and utility of PCR has driven commercialization of this approach, although remaining issues of sensitivity and specificity have hampered its widespread use.

'Fourth-generation' molecular epidemiology is based on DNA sequence analysis

Since the chromosome is the most fundamental molecule of identity in the cell, a comparison of actual chromosomal

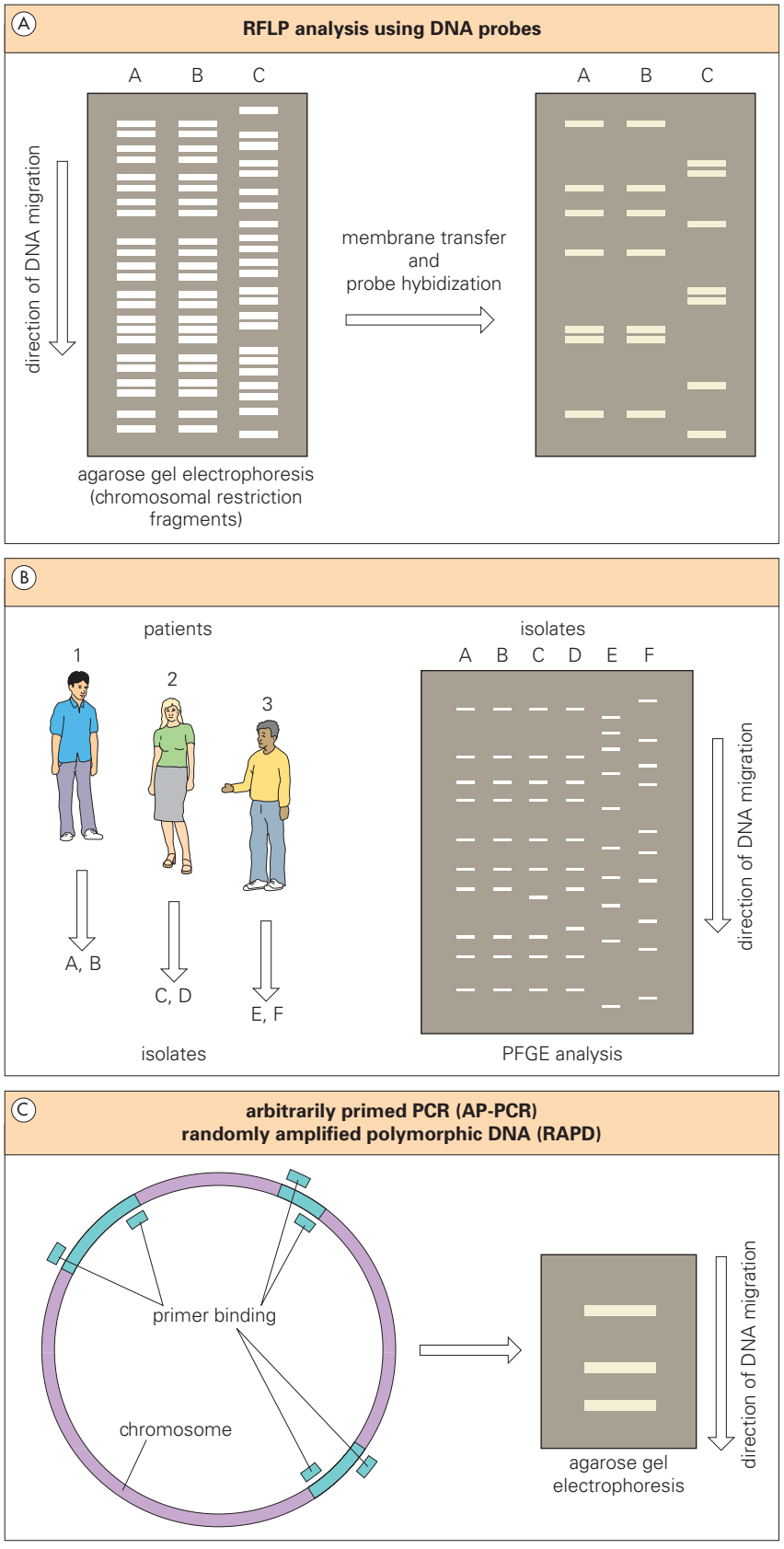


Figure 37.6 (A) Restriction fragment length polymorphism (RFLP) analysis using DNA probes. An illustration of three nosocomial isolates (A and B epidemiologically related; C unrelated) analysed by restriction enzyme analysis and subsequently by a specific DNA probe. (B) Pulsed field gel electrophoresis (PFGE) analysis of two bacterial isolates from each of three patients. Isolates in the first two patients are highly related (although slightly different in patient 2). Isolates from patient 3 are epidemiologically unrelated. (C) In the RAPD / AP-PCR approach to epidemiological analysis, PCR products result from the random binding of PCR primers to chromosomal sequences, and the pattern is expected to be similar in epidemiologically related isolates. AP-PCR, arbitrarily primed polymerase chain reaction; RAPD, randomly amplified polymorphic DNA.

sequences is the most fundamental means of assessing potential interrelationships in nosocomial isolates. Thus, one could consider sequenced-based analysis fourth-generation molecular epidemiology. While recent years have seen a variety of sequence-based approaches to assessing microbial relatedness, technology now exists to generate and compare the entire chromosomal sequence of isolates via bench-top instrumentation (whole genome sequencing [WGS]). In the most common approach a library of extracted genomic DNA is sequenced resulting in multiple copies of short regions (reads) several hundred base pairs in length. Computer algorithms then either *de novo* assemble the overlapping sequence reads with the goal of reproducing the original sequence or align the sequence reads to a related chromosomal template (reference mapping) (see Fig. 2.22). In either case, bacterial genomes can be divided into core and accessory regions. The core genome represents conserved genes, which are found in all members of a bacterial species while the presence or absence of other (accessory) genomic regions is variable. Taken together, all the core and variable sequences found in members of a bacterial species are termed the pan genome. Bioinformatic analysis of chromosomal sequences then allows a genomic comparison of isolate relatedness either on the basis of single-nucleotide base differences (single-nucleotide polymorphisms; SNPs) or gene-by-gene differences in the core genome (core-genome multi-locus sequence typing; cgMLST).

Molecular techniques for epidemiological fingerprinting have many advantages

Although molecular techniques may require expertise and equipment, they have several advantages. They can be extremely precise, can be performed rapidly, in some instances do not involve handling infectious organisms and provide the potentially most fundamental (i.e. chromosomal) assessment of isolate relatedness.

Investigation of viral infections

Nosocomial viral infections usually occur via the airborne route, contaminated fomites or blood-to-blood contact as outlined previously with, for example, RSV, noroviruses or hepatitis B, respectively. These are investigated mostly by detecting virus in samples from symptomatic patients and then, depending on the clinical setting, may involve collecting samples from asymptomatic patients to include in a cohort for broader analysis. In general, only identification of the microbe as a virus is required in outbreaks of viral gastroenteritis, as the management is the same for all the viral causes of gastroenteritis. However, in this setting it is important from an epidemiological perspective to identify the cause of the outbreak. Surveillance is critical to monitor any changes in the virus as these alterations to parts of its genome may result in the virus evading detection as the primers used in the diagnostic test may no longer match the complementary sequence of the template. In addition, for those viruses for which we have a vaccine, it is important to know which strains are circulating currently so as to ensure a good antigenic match with the vaccine strains.

In an outbreak of respiratory infection, identification and typing of the virus are important not only for epidemiological purposes but also for issues of treatment and prophylaxis.

Molecular detection and typing methodologies such as sequencing may be required, usually for epidemiological purposes rather than direct management of patients. However, in a setting such as postoperative acute hepatitis B infection, an investigation will be carried to determine possible routes of transmission. This may include investigating blood products, healthcare workers who were involved in exposure-prone procedures, other patients on the operating list, sexual contacts, and other risk activities involving potentially blood-contaminated needles. Once the potential sources have been identified, serological tests may be carried out to seek evidence of current, recent or past hepatitis B infection. Genome detection methods can play an important role in screening blood samples from the individual with acute hepatitis B, as well as the potential source or sources, to help confirm the transmission event or events.

Corrective / preventive measures

Once tracking is complete, corrective and preventive measures can be introduced

Typing of the aetiological agent responsible for the outbreak and knowledge of its characteristics and mode of transmission allow preventive measures to be taken. What these include depends to a great extent on the pathogen involved, but all must aim to improve basic hygiene, from more effective hand washing and improved general cleaning to more effectively regulated sterilization of equipment. Hygiene is a crucial factor as agents of nosocomial infection can be spread between patients by hospital staff. With some organisms that are widely distributed in the environment (e.g. *P. aeruginosa*) or occur in water supplies (e.g. *Legionella*), corrective measures may involve radical improvements to facilities.

As noted earlier, awareness of the risks of being exposed to blood-borne virus infections in a hospital setting is important to prevent blood-borne virus exposure incidents. Important protective measures include immunization of HCWs, wearing appropriate personal protective equipment (PPE) for procedures that could result in a break in the skin or exposure of mucous membranes, and appropriate post-exposure steps in the case of an incident.

Nosocomial transmission of SARS (see Ch. 20) has shown how easily airborne infection can be transmitted in a hospital setting. The use of PPE that included an N95 respirator, eye protection, mask, gloves and gown was mandatory to reduce the chance of transmission. Disposable second layers of clothing were also used, for example outer gloves, a gown and hand and foot covering.

STERILIZATION AND DISINFECTION

It is clear that the prevention of hospital infection depends in part upon the availability of clean, and where necessary sterile, equipment, instruments and dressings, isolation facilities and the safe disposal of infected material. Sterilization and disinfection are often talked about by microbiologists in relation to the production of sterile culture media and other laboratory activities, but it must be stressed that the concept of sterility is central to almost all areas of medical practice. An understanding of the rationale of sterilization and disinfection will aid intelligent use of the range of sterile equipment (from

needles to prostheses) and techniques (from surgery to hand washing) employed in medical practice.

Definitions

Sterilization is the process of killing or removing all viable organisms

An item that is sterile is free from all viable organisms – in this sense, viable means capable of reproducing. Sterilization is achieved by physical or chemical means, either by the removal of organisms from an object or by killing the organisms in situ, sometimes leaving toxic breakdown products (pyrogens) in the object.

Disinfection is a process of removing or killing most, but not all, viable organisms

Disinfection employs either:

- a chemical 'disinfectant', which kills pathogens but may not kill viruses or spores, or
- a physical process such as boiling water or low-pressure steam, which reduces the bioburden (i.e. the load of viable organisms).

Antiseptics are used to reduce the number of viable organisms on the skin

Antiseptics are a particular group of disinfectants. Some act differentially, destroying the transient flora but leaving untouched the normal skin flora deep in the skin pores and hair follicles. It is impossible to sterilize the skin, but thorough washing with antiseptic soaps can reduce the numbers of organisms on the surface considerably and therefore reduce contact spread of infection (see above). However, the resident bacteria in the hair follicles and ducts of sweat glands can recolonize the skin surface within hours.

Pasteurization can be used to eliminate pathogens in heat-sensitive products

Pasteurization reduces the total numbers of viable microbes in bulk fluids such as milk and fruit juices without destroying flavour and palatability. It does not affect spores, but is effective against intracellular organisms such as *Brucella* and mycobacteria and many viruses.

Since the beginning of recorded history, various other techniques have been used to prevent the multiplication of microorganisms, such as drying and salting of food.

Deciding whether sterilization or disinfection should be used

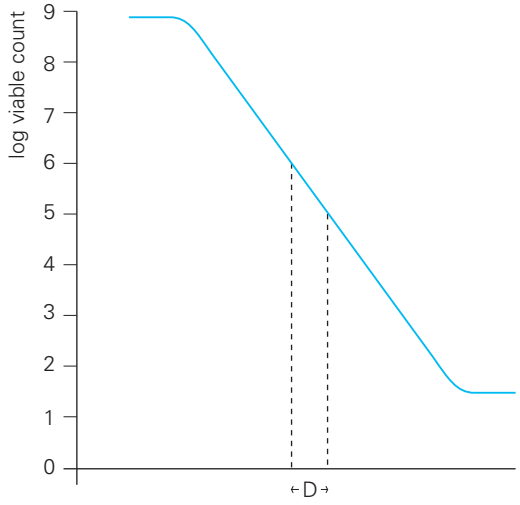
Sterilization and disinfection processes are costly, and so it is important to choose the appropriate method and the one that causes the least damage to the material involved. A variety of considerations influence the choice of method. The detailed mechanisms of the death process of microorganisms may vary with the sterilizing technique used, but the net effect is similar in that essential cell constituents (nucleic acids or proteins) are inactivated.

It is easier to sterilize a clean object than a physically dirty one

This is because organic matter protects microbes and hinders penetration of heat or chemicals and may inactivate certain chemicals. In other words, a low bioburden is a prerequisite for cost-effective sterilization.

The rate of killing of microorganisms depends upon the concentration of the killing agent and time of exposure

The number of organisms surviving sterilization can be expressed by the equation: $N \propto 1 / CT$, where N is the number of survivors, C is the concentration of agent and T is time of exposure to the agent. If a population of microbes is exposed to a sterilizing technique, and the number of survivors, expressed as a logarithm, is plotted against time, the slope of the graph defines the death rate (Fig. 37.7). These lines may be sigmoid or have shoulders, indicating that individual cells respond slightly differently, some being killed more easily than others. In the case of bacteria, the physiological state of the organisms influences the shape of the killing curve: young, replicating cells are usually more vulnerable than stationary or decline-phase organisms or those that are sporing. Graphs like



D = decimal reduction time (i.e. time required to reduce the population by 90% at a specified temperature)

Bacillus cereus	D_{121} = 2.4 min
Bacillus stearothermophilus	D_{122} = 3.4 min
Clostridium botulinum	D_{104} = 5.5 min
Clostridium perfringens	D_{104} = 2.3 min

Figure 37.7 Theoretically, there is a straight-line relationship between the log viable count of a bacterial population and time when the population is exposed to a lethal temperature. In practice, these lines are usually sigmoid. The *D* value is the time required to reduce the population by 90% at a specified temperature. *Bacillus stearothermophilus* spores are used as biological indicators of effective heat sterilization by including filter paper strips carrying a standard number of spores into the autoclave cycle. The strips are then incubated to attempt to recover viable organisms. The usual autoclave cycle of 121°C for 15 min is adequate to kill *B. stearothermophilus* with a margin of safety.

those shown in Fig. 37.7 can be used to predict the conditions necessary to achieve sterility. However, these experimental data are usually based on pure cultures in the laboratory (bacterial spores are often used as model systems), whereas in real life the bioburden is mixed. Therefore, predictions from such data may be inappropriate for mixed populations.

Techniques for sterilization

Sterilization may be achieved by:

- heat
- irradiation (gamma or ultraviolet)
- filtration
- chemicals in liquid or gaseous phase.

Other techniques of doubtful efficiency include freezing and thawing, lysis, desiccation, ultrasonication and the use of electrical discharges, but these are not applied in hospital practice.

Ultraviolet irradiation is inefficient as a sterilant, and its important uses in the hospital setting are in inhibiting growth of bacteria in water, in complex apparatus such as auto-analysers, and in safety hoods in microbiology laboratories. The potential for damage to the cornea and skin precludes the wider use of ultraviolet irradiation. It should be remembered that the agents of Creutzfeldt–Jakob disease (CJD), bovine spongiform encephalopathy (BSE) and scrapie are highly resistant and are not completely inactivated by formalin, ultraviolet irradiation, ionizing radiation or regular autoclaving. Sterilization can be achieved by boiling in 1 N NaOH for 10 min at atmospheric pressure followed by autoclaving at a higher than normal temperature for a longer period than usual (134°C for 18 min), but obviously this technique cannot be applied to living tissues or materials that are damaged at high temperatures.

Heat

Heat, as a way of transferring energy, is the preferred choice for sterilization on the grounds of ease of use, controllability, cost and efficiency.

Dry heat sterilizes by oxidation of the cell components. Incineration and the use of the laboratory Bunsen burner are examples of sterilization by dry heat. Glassware can be sterilized in a hot air oven at 160–180°C for 1 h.

The most effective agent for sterilization is saturated steam (moist heat) under pressure. This can be achieved using an autoclave. Steam under pressure aids penetration of heat into the material to be sterilized (such as dressings), and there is a direct relationship between temperature and steam pressure. Steam under pressure has a temperature in excess of 100°C, which results in increased killing of microbes.

Sterilizing efficiency is improved by evacuating all of the air from the autoclave chamber. The subsequently introduced high-pressure steam rapidly penetrates to all parts of the chamber and its load, and results in predictable rises in temperature in the centre of articles to be sterilized. The length of an autoclave cycle is determined by the holding time plus a margin of safety, and is derived from the thermal death curves for heat-resistant pathogens such as Clostridia. Therefore, the usual cycle of 121°C for 15 min is sufficient to kill the spores of *Clostridium botulinum* with an adequate margin of safety. However, the spores of some bacterial species, especially soil organisms, are able to withstand this temperature. The safety margin is reduced in the presence of large numbers of organisms because there is a greater probability of more heat-resistant individuals existing in a large population – hence the importance of cleaning instruments, whenever possible, before sterilization.

Moist heat in an autoclave is used to sterilize surgical instruments and dressings and heat-resistant pharmaceuticals. A method for the sterilization of heat-sensitive instruments such as endoscopes uses a solution of 0.55% ortho-phthalaldehyde.

Many of these processes are carried out in a pressure vessel usually available in the hospital central sterile supply department.

Immersion in boiling water for a few minutes can be used as a rapid emergency measure to disinfect instruments. Immersion in boiling water for a few minutes will kill vegetative bacteria and many, but not all, spores.

Pasteurization uses heat at 62.8–65.6°C for 30 min. This technique was devised by Pasteur to prevent the spoilage of wine by heating it to 50–60°C. It is now used for fluids such as milk to reduce the number of bacteria. This helps to eliminate pathogens present in small numbers and to improve the shelf-life of milk. The fluid is held at a temperature of 62.8–65.6°C for 30 min, or may be 'flash' pasteurized at 71.7°C for 15 s. After either process, the fluid should be kept at a temperature below 10°C to minimize subsequent bacterial growth.

Irradiation

Gamma irradiation energy is used to sterilize large batches of small-volume items. The use of gamma irradiation energy for sterilization is an industrial process that works well with products such as needles, syringes, intravenous lines, catheters and gloves, and even to prevent food spoilage. Although the capital cost of the equipment is high, the process is continuous and 100% efficient. Articles are sterilized while sealed in their original packaging, without any heat gain. The process must be conducted in a suitably constructed building, usually at a location distinct from the hospital and usually outside the hospital administration. However, irradiation can cause materials to deteriorate and is thus not suitable for resterilization of equipment. The killing mechanism involves the production of free radicals, which break the bonds in DNA. Irradiation kills spores, but at a higher dose than vegetative cells because of the relative lack of water in spores.

Sterilization using ultraviolet irradiation is discussed above.

Filtration

Filters are used to produce particle- and pyrogen-free fluid. Solutions that are heat sterilized will contain pyrogens. These heat-stable breakdown products of microbes are capable of inducing fever and are therefore undesirable in products such as intravenous fluids. Filtration or separation of the product from the contamination has a long history in the clarification of water and wine. Modern filters composed of compounds such as nitrocellulose or mixed cellulose ester work by electrostatic attraction and physical pore size to retain organisms or other particles. The resulting fluid should be particle free. Filtration is used in some parts of the world to purify drinking water.

Filtration techniques are also used to recover very small numbers of organisms from very large volumes of fluid (e.g.

Legionella from cooling tower water) and can be used as a method for quantifying bacteria in fluids.

Chemical agents

The gases ethylene oxide and formaldehyde kill by damaging proteins and nucleic acids. Ethylene oxide and formaldehyde are examples of alkylating gases:

- Ethylene oxide has been widely used to sterilize single-use medical requisites such as heart valves. However, it is toxic and potentially explosive.
- Formaldehyde is not explosive, but has an extremely unpleasant odour and is an irritant to mucous membranes. It has been used as a disinfectant to decontaminate rooms (such as isolation rooms) and in the laboratory to disinfect exhaust-protective cabinets. A high relative humidity is essential for effective killing.

The liquid glutaraldehyde is used to disinfect heat-sensitive articles. Glutaraldehyde is less toxic than formaldehyde and can be stabilized in solution to remain active for up to several weeks at in-use concentration. It is used for the disinfection of, but does not sterilize, heat-sensitive articles such as endoscopes and for inanimate surfaces.

Many different antimicrobial chemicals are available, but few are sterilant. Some, like the derivatives of pine and turpentine, have been known since ancient times, and chloride of lime and coal tar fluids were in use before the germ theory of disease was established. Most fall into the category of disinfectant or antiseptic, but a few are capable of rendering articles sterile. Factors that affect their efficacy include:

- physical environment (e.g. porous or cracked surfaces)
- presence of moisture
- temperature and pH
- concentration of the agent
- hardness of water
- the bioburden on the object to be disinfected
- the nature and state of the microbes in the bioburden
- the ability of the microbes to inactivate the chemical agent.

It is obvious that the above factors are difficult to control in every circumstance. The main groups of chemical agents are shown in Table 37.4. They act by causing chemical damage to proteins, nucleic acids or cell membrane lipids. The activity of a given disinfectant may result from more than one pathway of damage.

Table 37.4 Examples of disinfectants for use in hospitals

Group	Examples	Advantages and disadvantages
Phenolics	Clear-soluble phenolic compounds, white fluids	General-purpose disinfectants used less frequently than in the past; not readily inactivated by organic matter; active against wide range of organisms including *Mycobacterium*; not sporicidal
	Chloroxylenols	Inactivated by hard water and organic matter; *Pseudomonas* grows readily in chloroxylenol solutions; limited activity against other Gram-negatives
Halogens	Hypochlorites (chloramine)	Cheap, effective, act by release of free chlorine; active against viruses and therefore recommended for disinfection of equipment soiled with blood (because of hepatitis and HIV risk); inactivated by organic material, corrode metals
	Iodine and iodophors	Useful skin disinfectants; sporicidal
Quaternary ammonium compounds	Benzalkonium chloride, didecyl dimethyl ammonium bromide	Have detergent properties; low concentrations are bacteriostatic, high concentrations are bacteriocidal
Diguanides	Chlorhexidine	Useful disinfectant for skin and mucous membranes, inactivated by many materials and too expensive for environmental use, alcoholic solutions are less easily contaminated, combinations of chlorhexidine and detergent highly effective for disinfection of hands
Alcohols	Ethyl alcohol, isopropyl alcohol	Good choice for skin disinfection and for clean surfaces, sometimes used in combination with iodine or chlorhexidine (see above); water must be present for bacterial killing (i.e. 70% ethanol best); isopropyl preferred for skin and articles in contact with patient
Aldehydes	Formaldehyde/formalin	Too irritant for use as general disinfectant
	Glutaraldehyde	Kills vegetative organisms, including mycobacteria, slowly but effectively; more active, less toxic than formaldehyde; sporicidal (within 6 h when fresh); slightly irritant; used in alkaline solution which is stable for 1–2 weeks; expensive, limited use, e.g. disinfection of endoscopes
Chlorinated bisphenols	Triclosan	A polychloro phenoxy phenol used in bacteriostatic concentrations in personal products

Note that no one group of disinfectant has all the properties desirable for use both on skin and on inanimate surfaces.

Controlling sterilization and disinfection

In general, it is preferable to control the process rather than the product

This means that it is better to run checks on the technique while it is in operation rather than attempting to recognize process failure by isolating microorganisms from the product. Trying to discover whether one or a few viable organisms remain is analogous to trying to find a needle in a haystack. It is known that damaged bacteria can recover, given time and special nutrient recovery media, but it may not be feasible to hold back a batch of product for such tests. In addition, how many samples of the product should be tested? If too few are examined, the likelihood of missing a failed sample is high; if too many are examined, too much of the batch is used up in quality control to be economically sensible.

The usual process controls are either physical or chemical checks on the technique – for example, tests showing that the autoclave reached the desired temperature for the desired time. They do not show that there are no viable organisms remaining after the process, but this is assumed if the process satisfies the controls. However, the stringency of the controls can be altered intentionally or accidentally to give either an undersensitive or an oversensitive test.

Disinfectants can be monitored by microbiological 'in-use' tests

These tests involve challenging the solution with a bacterial suspension and withdrawing samples, which are then treated to prevent carryover of the disinfectant and cultured. However, these tests are rarely performed in the hospital setting, where the use of disinfectants is guided largely by the manufacturer's recommendations.

KEY FACTS

- Realization that infection can be associated with a variety of institutional settings has resulted in a preference for the term 'healthcare-associated infection' rather than 'hospital-acquired infection'.
- Nosocomial infection refers to infection acquired in hospital.
- Hospital infections often have serious consequences for the individual, for the hospital community and for the community at large. They may be caused by almost any organism, but a few species cause the vast majority of infections.
- The hospital environment favours the survival of resistant strains and therefore infections are often caused by organisms with limited antibiotic susceptibility.
- MRSA, traditionally viewed as a problem in hospital infection, is increasingly seen in community-acquired infections in the absence of healthcare contact.
- Most common hospital infections are UTIs, respiratory tract infections, surgical wound infections and bacteraemia (septicaemia).
- The most important bacterial causes are Gram-positive cocci (*staphylococci* and *streptococci*) and Gram-negative rods (e.g. *E. coli*, *Pseudomonas*). Multiple antibiotic-resistant organisms are common. *Candida* is the significant fungal cause, and viruses probably cause more hospital infections than has been previously recognized.
- Infecting organisms originate from the patient's own flora (endogenous infection) or from other human or inanimate sources (exogenous or cross-infection). Airborne spread and contact spread are the most important routes of transmission.
- Host factors are of critical importance in determining susceptibility to infection.
- Surveillance should be an ongoing activity to facilitate early recognition of outbreaks of infection. Investigation of outbreaks involves both epidemiological and microbiological expertise. Molecular techniques to 'fingerprint' the causative organism are becoming increasingly sophisticated.
- Prevention of hospital infections by excluding sources, interrupting transmission and enhancing the patient's resistance is fundamental to improving patient care and reducing costs.
- Sterilization and disinfection are key processes in the control and prevention of hospital infections as well as being central to many areas of medical practice.

Bibliography – list of useful websites

USEFUL WEBSITES

ACGM Compendium of guidance
www.hse.gov.uk/biosafety/gmo/acgm/acgmcomp/
AJIC – *American Journal of Infection Control*
www.ajicjournal.org
AMEDEO The Medical Literature Guide
www.amedeo.com
(free medical literature guide)
American Society for Microbiology
www.asm.org
Association for Professionals in Infection Control and Epidemiology
https://apic.org/Resources/Overview
Bioterrorism Emergency Preparedness and Response
https://emergency.cdc.gov/bioterrorism/index.asp
BMJ publishing group reviews of clinical evidence for medical practice
www.clinicalevidence.org
British Society for Antimicrobial Chemotherapy
www.bsac.org.uk
(contains latest advice on susceptibility testing)
Centers for Disease Control and Prevention (CDC):
www.cdc.gov
CDC – Emerging Infectious Diseases journal homepage
www.cdc.gov/ncidod/eid
CDC – vaccines
www.cdc.gov/vaccines/ index.html
Epidemiology and Prevention of Vaccine-preventable Diseases (The Pink Book): www.cdc.gov/vaccines/pubs/pinkbook/index.html
Directory of medical and veterinary ectoparasites and endoparasites
www.southampton.ac.uk/~ceb/
Doctor's Guide Personal Edition
www.docguide.com
Electronic guidelines on effective healthcare
www.eguidelines.co.uk
Federation of European Societies for Chemotherapy and for Infections
www.fesci.net
Fitfortravel – travel health information
www.fitfortravel.scot.nhs.uk
Food Standards Agency
www.foodstandards.gov.uk
Free Medical Journals
www.freemedicaljournals.com
Global Alliance to Eliminate Lymphatic Filariasis
www.filariasis.org
Health Protection Agency England, Wales and Northern Ireland
www.gov.uk/government/organisations/public-health-england
Hospital Infection Society
www.his.org.uk
Human Microbiome Project
hmpdacc.org
Infection Prevention Society – UK
www.ips.uk.net/
Infectious Diseases Society of America (IDSA)
www.idsociety.org/
International travel and health
www.who.int/ith/en/
Intestinal worms
www.who.int/intestinal_worms/more/en/
Isabel Differential Diagnosis tool
www.isabelhealthcare.com
ISRCTN registry
www.isrctn.com
(Metaregister of controlled trials)
Johns Hopkins Division of Infectious Diseases – Hopkins ABX Guide
www.hopkins-abxguide.org
(guide to pathogenic bacteria, antibiotics and diagnosis)
Leprosy elimination info
www.who.int/lep/en/
Morbidity and Mortality Weekly Report (MMWR)
www.cdc.gov/mmwr/
National Center for Emerging and Zoonotic Infectious Diseases (NCEZID)
www.cdc.gov/ncidod/dvbid/index.html
National Institute for Health and Care Excellence (NICE) summary of guidance issued to the NHS in England and Wales
www.nice.org.uk
National Travel Health Network and Centre, UK, for online information on vaccines required for travellers
nathnac.net
Roll Back Malaria
www.rollbackmalaria.org
Royal College of Pathologists website for information on courses, links to other learned societies and discussion sites
www.rcpath.org
The Sanford Guide – antimicrobial therapy & HIV / AIDS therapy
www.sanfordguide.com

Society for Healthcare Epidemiology of America (SHEA)
www.shea-online.org
Stop TB Partnership website
www.stoptb.org
TDR homepage: the UNICEF–UNDP–World Bank–WHO Special Programme for Research and Training in Tropical Diseases
www.who.int/tdr/en/
UK National Health Service – vaccine schedules in United Kingdom
www.nhs.uk/Conditions/vaccinations/pages/vaccination-schedule-age-checklist.aspx?tabname=NHS%20vaccination%20schedule
UK NEQAS Microbiology home page
www.ukneqasmicro.org.uk
UK Public Health England:
Immunization against infectious disease (The Green Book)
www.gov.uk/government/collections/immunisation-against-infectious-disease-the-green-book
Malaria reference laboratory (Malaria RL)
www.gov.uk/government/collections/malaria-reference-laboratory-mrl
Update on current literature and meetings reports, can be focused to infectious diseases
www.medscape.com
US Clinical Virology News
www.clinical-virology.org
US vaccine schedules
www.cdc.gov/vaccines/schedules/
Wellcome Trust Sanger Institute
www.sanger.ac.uk
World Health Organization (WHO/OMS):
www.who.int/en/
WHO emergencies preparedness, response
www.who.int/csr/en/
WHO expanded programme of immunization schedules
www.who.int/immunization/policy/immunization_tables/en/
WHO general information on immunization, vaccines and biologicals
www.who.int/immunization/en/
WHO international travel and health
www.who.int/ith/en/
WHO tuberculosis – prevention and control
www.who.int/gtb/
WHO Weekly Epidemiological Record (WER)
www.who.int/wer/

Pathogen parade

The reader should refer to the index to find the relevant chapters for all pathogens.

Viruses

Adenoviruses

Characteristics	***Virus family*** **Adenoviridae**	***Type*** **dsDNA**	***Envelope*** **no**	***Shape*** **icosahedral**	***Size (nm)*** **70–90**	***Nucleocapsid*** **icosahedral**
	At least 51 types of adenovirus share a common group-specific antigen. Rod-like structures called fibres are topped by knobs projecting from the vertices of particles and these attach virus to different receptors on the cell surface.					
Replication	After attachment, endocytosis and uncoating, viral DNA is transcribed within the nucleus by cellular DNA-dependent RNA polymerase. RNA transcripts corresponding to several genes (less than the whole genome) undergo cleavage and splicing to form monocistronic mRNA. Early mRNA codes for enzymes needed for replication; late mRNA (after viral DNA synthesis) for structural proteins. Particles are assembled in the nucleus and released from the damaged cell.					
Diseases	Cause pharyngoconjunctival fever; epidemics of acute respiratory disease, including pneumonia; intestinal illness including gastroenteritis, mesenteric adenitis, intussusceptions; keratoconjunctivitis; hepatitis and disseminated disease in immunosuppressed individuals; haemorrhagic cystitis; central nervous system (CNS) disease.					
Transmission	Via respiratory droplets, faeces, and sometimes from eye to eye via contaminated hands, towels or eye drops.					
Pathogenesis	Adenoviruses infect epithelium of respiratory tract and eyes, and probably intestine. Spread to involve lymphoid tissues and can persist for long periods in tonsils and adenoids of children. Viral protein interferes with immune defences by blocking action of interferon and Tc cells.					
Laboratory identification	DNA detection in a range of samples in different clinical settings by polymerase chain reaction (PCR). Virus isolation in cell culture, antigen detection by immunofluorescence and electron microscopy and antibody detection in serum by complement fixation tests are becoming classical tests used in fewer laboratories worldwide.					
Treatment and prevention	Ribavirin and cidofovir have been used to treat severe adenovirus infections, especially in the immunocompromised. Live oral vaccine (types 4 and 7 in enteric-coated capsules) has been used in military recruits to prevent outbreaks of respiratory infection and is used in the USA for this group.					

Arenaviruses

Characteristics	***Virus family*** **Arenaviridae** ***Genus*** **Arenavirus**	***Type*** **ssRNA –ve sense**	***Envelope*** **yes**	***Shape*** **spherical**	***Size (nm)*** **50–300**	***Nucleocapsid*** **helical**
	Species include Old World arenaviruses: Lassa virus, lymphocytic choriomeningitis (LCM) virus, and New World arenaviruses: Junin virus, Machupo virus, Sabia virus, Tacaribe virus. Host ribosomes visible as granules inside the envelope (Latin: *arena*, sand).					
Replication	Virions containing two circular RNA segments, one negative and one ambisense. Viral RNA-dependent RNA polymerase produces positive-strand RNA that is translated to form a nucleoprotein and two glycoproteins. Maturation is by budding with no cytopathic effect on the cell.					

Continued

Arenaviruses—cont'd

Characteristics						
	Virus family **Arenaviridae** ***Genus*** **Arenavirus**	***Type*** **ssRNA –ve sense**	***Envelope*** **yes**	***Shape*** **spherical**	***Size (nm)*** **50–300**	***Nucleocapsid*** **helical**
Diseases	Febrile illness, sometimes complicated by aseptic meningitis (LCM), or by severe haemorrhagic disease (Lassa fever, Argentinian and Bolivian haemorrhagic fevers).					
Transmission	Cause inapparent persistent infections in the natural rodent host and can result in zoonotic spread to humans via contact with rodent excreta. LCM virus occurs worldwide, comes from mice and hamsters; Lassa fever virus in West Africa from the bush rat *Mastomys natalensis*; Junin and Machupo viruses from bush mice (*Calomys* spp.) in South America causing Argentinian and Bolivian haemorrhagic fevers.					
Pathogenesis	Natural rodent host is infected in utero or neonatally and non-cytopathic virus remains in all tissues throughout life. In human host, virus spreads systemically causing meningitis or haemorrhagic disease by local or general replication plus immunopathology.					
Laboratory identification	Specialist reference laboratory tests: detect specific antibody, viral RNA detection by PCR and virus isolation.					
Treatment and prevention	Ribavirin can be used as treatment and prophylaxis for Lassa fever. Vaccines for routine use are not available.					

Bunyaviruses

Characteristics						
	Virus family **Bunyaviridae**	***Type*** **ssRNA –ve sense**	***Envelope*** **yes**	***Shape*** **spherical**	***Size (nm)*** **80–120**	***Nucleocapsid*** **helical**
	Genera include *Hantavirus*, *Nairovirus*, *Orthobunyavirus* and *Phlebovirus*. Contains more than 100 different viruses. Viral genome has three unique negative or ambisense ssRNA called L (large), M (medium) and S (small). Bunyamwera is a locality in Africa where the prototype Bunyamwera virus was isolated. Included in the *Orthobunyavirus* genus is La Crosse virus (Californian encephalitis virus). Other human infections include *Hantavirus* (SE Asia, USA): Hantaan virus, Seoul virus, Puumala virus and Sin Nombre virus; *Phlebovirus*: Rift Valley fever (RVF) virus (Africa), Toscana virus (sandfly fever Naples virus); and *Nairovirus*: Crimean–Congo haemorrhagic fever virus, Dugbe virus.					
Replication	After attachment to cell receptors and endocytosis, nucleocapsid enters cytoplasm by fusion with endosomal membranes; the three segments of RNA are transcribed into mRNA and translated. Virus RNA is then transcribed and replicated. Glycoproteins are synthesized and glycosylated in endoplasmic reticulum, entering the Golgi complex, where budding takes place, with release by exocytosis or lysis of the cell.					
Diseases	Cause febrile illnesses, generally mild. RVF can cause haemorrhagic phenomena, sometimes with a lethal outcome; La Crosse virus can cause encephalitis and hantaviruses can cause renal and/or a severe pulmonary syndrome.					
Transmission	With the exception of the hantaviruses, which are acquired from urine of infected rodents, bunyaviruses are transmitted by mosquitoes, ticks or sandflies, and there is a bird or mammal reservoir.					
Pathogenesis	After a viraemia, virus disseminates to the CNS, liver and kidneys and multiplies in vascular endothelium (hantaviruses).					
Laboratory identification	Specialist reference laboratory tests: antibody detection, viral RNA detection and virus isolation.					
Treatment and prevention	Ribavirin may be effective in hantavirus haemorrhagic fever with renal syndrome if given early. Prevention is by avoiding contact with arthropod vector (RVF virus, La Crosse virus) or with infected rodents (hantaviruses). Vaccines have been developed for RVF.					

Noroviruses

Characteristics	***Virus family*** **Caliciviridae**	***Type*** **ssRNA +ve sense**	***Envelope*** **–**	***Shape*** **spherical**	***Size (nm)*** **27–40**	***Nucleocapsid*** **icosahedral**
	Norovirus is the genus name for the group of viruses previously called 'Norwalk-like viruses' (NLV) or small round structured viruses (SRSV).					
Replication	RNA-dependent RNA polymerase. Subgenomic mRNAs are transcribed from the full-length negative strand, and the products undergo post-translational modifications.					
Diseases	Acute gastroenteritis, sporadic and outbreaks especially in hospitals, care homes and cruise ships.					
Transmission	Faecal–oral route. Incubation period is up to 48 h.					
Pathogenesis	Replication in small intestine mucosal epithelium likely leading to flattened short villi. Mucosal cell damage, atrophic villi, loss of digestive enzymes; reduced absorption leads to diarrhoea.					
Laboratory identification	Faecal material: viral RNA detection; electron microscopy; antigen detection by ELISA available but a less sensitive method of detection.					
Treatment and prevention	Supportive. Good hygiene and sanitary disposal of waste.					

Coronaviruses

Characteristics	***Virus family*** **Coronaviridae**	***Type*** **ssRNA +ve sense**	***Envelope*** **yes**	***Shape*** **spherical**	***Size (nm)*** **120–160**	***Nucleocapsid*** **helical**
	Virions have a characteristic fringe of surface projections (Latin: *corona*, crown). Coronaviruses infect mammals and birds.					
Replication	Virions attach to cell surface receptors by their spikes; viral envelope fuses with host plasma membrane. Viral RNA-dependent RNA polymerase uses genomic positive strand to produce negative-strand RNA, which acts as template for new positive strands. Nucleocapsids bud into endoplasmic reticulum, from which they are released by exocytosis.					
Diseases	Common-cold-type illness in humans caused by the human coronavirus OC43, 229E, NL63 and HKU1. Gastrointestinal infections. Severe acute respiratory syndrome (SARS) and Middle East respiratory syndrome (MERS)-associated coronavirus infection are severe lower respiratory tract infections. SARS originally in bats spread to palm civets, ferret badgers and raccoon dogs in China, which were eaten/handled in the wet markets and transmitted to humans. MERS has a camel reservoir.					
Transmission	Respiratory droplets.					
Pathogenesis	Replication in cells lining upper respiratory tract. Optimum growth temperature 33–35°C.					
Laboratory identification	Specialist reference laboratory tests: viral RNA detection by PCR, antibody tests, electron microscopic examination of various sample types, viral isolation.					
Treatment and prevention	No antivirals or vaccines available.					

Hepatitis D (Delta) Virus (HDV)

Characteristics	*Family* Deltavirus	*Type* partially ssRNA	*Envelope* + (HBsAg)	*Shape* spherical	*Size (nm)* 35–37	*Nucleocapsid* heterogeneous satellite
	Classified as a subviral satellite as it requires hepatitis B to provide the envelope proteins to assemble infectious virus. About 20 million people infected worldwide.					
Replication	RNA-directed RNA synthesis. Two forms of the delta antigen, small and large, required for genome replication and assembly of ribonucleoprotein particles into new virions.					
Diseases	Acute and chronic hepatitis as a co-infection or superinfection with hepatitis B virus (HBV). Most HDV co-infections are cleared. Superinfections can result in chronic infection, cirrhosis, and liver failure.					
Transmission	Spread via blood. Incubation period is 3–7 weeks.					
Pathogenesis	Virus spreads via blood to liver and replicates in hepatocytes.					
Laboratory identification	HDV-specific IgM and IgG, detect delta antigen or viral RNA.					
Treatment and prevention	Mostly indirect by treating hepatitis B. Prevented by hepatitis B immunization.					

Filoviruses

Characteristics	*Virus family* Filoviridae	*Type* ssRNA –ve sense	*Envelope* yes	*Shape* tubular but may be branched, circular or filamentous	*Size (nm)* 80–14 000	*Nucleocapsid* helical
	Genera include *Ebolavirus* and *Marburgvirus*, Ebola and Marburg viruses.					
Replication	Enters cell cytoplasm after attaching to cell surface receptor and is transcribed, resulting in subgenomic mRNAs and full-length positive-strand copies made with virions released by budding.					
Diseases	Viral haemorrhagic fever, conjunctivitis, neurological symptoms.					
Transmission	Person to person. Probable fruit bat reservoir. Incubation period 2–21 days.					
Pathogenesis	Virus spreads to multiple sites of the body. Generalized haemorrhage in most organs.					
Laboratory identification	Specialist reference laboratory tests: antibody and antigen detection, viral RNA detection, virus isolation, electron microscopy.					
Treatment and prevention	Supportive, especially rehydration and electrolyte replacement. Isolation of patients. Range of experimental treatments includes antiviral drugs and monoclonal antibodies but no definitive trial and evidence base for whether they are effective as of 2017. Vaccine trials have been reported and other vaccine candidates in preparation.					

Flaviviruses

Characteristics	***Virus family*** **Flaviviridae**	***Type*** **ssRNA +ve sense**	***Envelope*** **yes**	***Shape*** **spherical**	***Size (nm)*** **40–60**	***Nucleocapsid*** **icosahedral**
	The *Flavivirus, Pestivirus, Hepacivirus* and *Pegivirus* genera have been placed in the Flaviviridae family. Of these, it is only the members of the *Flavivirus* genus that are transmitted by arthropod vectors (arthropod-borne viruses also called arboviruses). These include dengue, Japanese encephalitis, yellow fever, tick-borne encephalitis, West Nile and Zika viruses. Hepatitis C virus has been placed in the *Hepacivirus* genus. Pestiviruses cause veterinary diseases around the world and pegiviruses are persistent virus infections detected in humans and pathogenicity is yet to be established.					
Replication	The positive-strand RNA is translated into structural and non-structural proteins, the latter including the RNA-dependent RNA polymerase, which replicates the viral genome by directing the formation of a negative-strand template and thus giving rise to positive-strand progeny. Full-length RNA is formed, with rapid cleavage of the resulting polyprotein. After assembly, the virus exits from the cell, budding from the endoplasmic reticulum.					
Diseases	The arboviruses cause febrile illnesses, which may be severe when there is involvement of liver (yellow fever) or CNS (West Nile virus, Japanese and tick-borne encephalitides, Zika virus), or when there are immunopathological complications (dengue haemorrhagic fever). Hepatitis C virus (HCV) causes acute and chronic hepatitis (the former being mostly asymptomatic), cirrhosis and hepatocellular carcinoma.					
Transmission	Infected tick or mosquito bites for the arboviruses. Routes of transmission for hepatitis C include contaminated blood products, blood-contaminated needles or equipment (i.e. injecting drug use, tattoos, body piercing). Vertical and sexual transmission infrequent.					
Pathogenesis	Initial infection via skin (arboviruses) causes no detectable local lesion. Virus spreads to local lymph nodes and blood, liver (yellow fever), or CNS (mononuclear cells often infected in dengue). Immunopathology important in dengue haemorrhagic fever. Ingested virus infects gut epithelium of mosquitoes and ticks and spreads to and multiplies in salivary glands. HCV-associated liver disease is probably due to direct cytopathic infection of hepatocytes in addition to the host immune/inflammatory responses. Maternal infection with Zika virus can result in fetal microcephaly. In addition, Zika virus infections in general can cause other neurological problems including Guillain–Barré syndrome.					
Laboratory identification	Hepatitis C antibody and HCV RNA detection. HCV RNA load and genotype for monitoring disease progression and treatment response. Serological methods, viral RNA detection by PCR and, to a lesser extent, virus isolation are used to diagnose the arbovirus infections.					
Treatment and prevention	There is no antiviral therapy for arbovirus infections. A live attenuated virus vaccine prevents yellow fever, tick-borne and Japanese encephalitis viruses. Vector control measures limit virus transmission. HCV treatment options have progressed in a rapid period of time and involve a host of directly acting antivirals that act by inhibiting viral non-structural proteins/protease and can eradicate the infection.					

Hepatitis B Virus (HBV)

Characteristics	***Virus family*** **Hepadnavirida**	***Type*** **partially dsDNA**	***Envelope*** **yes**	***Shape*** **spherical**	***Size (nm)*** **42**	***Nucleocapsid*** **icosahedral (circular)**
	Infectious Dane particles consist of an envelope, the hepatitis B surface antigen (HBsAg), surrounding the core or nucleocapsid. There are a number of genotypes, A–G. The virus is unusual as it is the only human virus, other than the retroviruses, that encodes a reverse transcriptase.					
Replication	After attachment to hepatocytes, particles are endocytosed and uncoated. In the nucleus, viral DNA polymerase converts viral DNA into a complete circular dsDNA. Negative-strand DNA is transcribed by cellular RNA polymerase to form a single positive RNA strand, which moves to the cytoplasm, is translated into protein, and is then encapsulated into cores together with viral DNA polymerase. The positive RNA strand is then used to synthesize negative-strand DNA (reverse transcriptase activity). A small fragment from the 5′ end of the positive RNA strand primes synthesis of all but about one-third of the positive DNA strand. Hence, complete particles contain dsDNA with an ssDNA region. Release from cell is by budding. Viral DNA integrates into host DNA.					

Continued

Hepatitis B Virus (HBV)—cont'd

Characteristics	*Virus family* Hepadnavirida	*Type* partially dsDNA	*Envelope* yes	*Shape* spherical	*Size (nm)* 42	*Nucleocapsid* icosahedral (circular)
Diseases	Acute and chronic hepatitis including fulminant liver failure, cirrhosis and hepatocellular carcinoma. Persistent infection, known as the carrier state, is common after infection in infancy or early childhood and non-icteric infection in adults. There are 250 million carriers worldwide.					
Transmission	Spread via HBV-infected blood (e.g. by contaminated needles and blood products, by sexual intercourse and from mother to baby). Median incubation period 10–12 weeks, range 6 weeks to 6 months.					
Pathogenesis	Virus spreads via blood to liver and replicates in hepatocytes. Immunopathological disease is due to cytotoxic T cells lysing HBsAg-bearing hepatocytes. Immune complex formation can cause rash and arthritis. Virus integrates in hepatocyte genome and can therefore reactivate if the individual is immunocompromised.					
Laboratory identification	Neutralizable HBsAg in blood indicates either acute or persistent infection. HBc IgM is detected in acute and recent infection but may also be seen in individuals with high viral replication. Presence of HBe antigen means blood is highly infectious. Detection of HBc antibody and HBs antibody in the absence of HBsAg indicates previous infection and immunity. Serum HBV DNA quantification assists monitoring of the carrier state and response to antiviral treatment. The virus cannot be grown in cell culture.					
Treatment and prevention	Since 2000, six drugs have been approved for treating chronic HBV infection including interferon, an immunomodulator, and the nucleoside/nucleotide inhibitors, namely lamivudine, telbivudine, adefovir, entecavir and tenofovir. Although initial treatment involves monotherapy using pegylated interferon, entecavir or tenofovir, interferon is used less often. The genetically engineered HBsAg-based vaccine is highly effective. Post-exposure prophylaxis also involves using hepatitis B immunoglobulin.					

Hepatitis E Virus (HEV)

Characteristics	*Virus family* Hepeviridae	*Type* ssRNA +ve sense	*Envelope* –	*Shape* icosahedral	*Size (nm)* 27–34	*Nucleocapsid* icosahedral
	Hepevirus genus, found worldwide, about 20 million HEV infections annually. Four hepatitis E virus genotypes: 1 and 2 in humans, 3 and 4 in various animals including pigs.					
Replication	RNA-dependent RNA polymerase. Two subgenomic mRNAs are transcribed from the full-length negative strand and the products undergo post-translational modifications.					
Diseases	Sporadic acute and sometimes fulminant hepatitis, especially in pregnancy. Epidemic enterically transmitted hepatitis. Self-limiting but can cause chronic infection in immunocompromised hosts. A zoonotic infection that in 2017 was being increasingly identified in individuals with jaundice.					
Transmission	Spread via fecal–oral route as well as by blood transfusion. Mean incubation period is 6 weeks; range is 2–10 weeks.					
Pathogenesis	Virus spreads to liver and replicates in hepatocytes.					
Laboratory identification	HEV-specific IgM and IgG and HEV RNA.					
Treatment and prevention	Supportive, but ribavirin or interferon may be used to treat immunocompromised patients. Recombinant subunit HEV vaccine registered in China.					

Herpesviruses

Characteristics	*Virus family*	*Type*	*Envelope*	*Shape*	*Size (nm)*	*Nucleocapsid*
	Herpesviridae	**dsDNA**	**+**	**icosahedral**	**180–200**	**icosahedral**

Includes herpes simplex (HSV), varicella-zoster (VSV), cytomegalovirus (CMV), Epstein–Barr virus (EBV), human herpesvirus (HHV)-6, -7 and -8.

Replication

Virus attaches to specific receptor on cell and enters by fusion of envelope with plasma membrane. Nucleocapsid moves to nucleus; viral DNA is uncoated at nuclear pores and then transcribed by cellular RNA polymerase so that the five sets of viral genes are sequentially activated. 'Immediate-early' gene products stimulate synthesis of second wave of 'early' gene products that are involved in genome replication and include the DNA polymerases. After DNA replication, the remaining 'late' gene products are expressed and are involved in assembly. In the nucleus, viral DNA is inserted in capsids, and resulting nucleocapsids attach to sites on inner nuclear membrane where envelope proteins are present and budding takes place between inner and outer nuclear membranes. Enveloped virus particles are transported through the cytoplasm and released by reverse phagocytosis. Replication cycle about 36 h. Generally persist for long periods in body, often in latent form (in neurones, monocytes, T cells, B cells), and can reactivate.

Diseases	Type	Clinical features include
	HHV-1 (HSV-1) HHV-2 (HSV-2)	Gingivostomatitis, cold sores, genital herpes, meningitis, encephalitis
	HHV-3 (VZV)	Varicella (chickenpox) that may include pneumonitis and encephalitis zoster (shingles)
	HHV-4 (EBV)	Mononucleosis (glandular fever), hepatitis, encephalitis, Burkitt's lymphoma, post-transplant lymphoproliferative disease, nasopharyngeal carcinoma
	HHV-5 (CMV)	Mononucleosis, hepatitis, pneumonitis, congenital CMV
	HHV-6	Exanthem subitum, mild febrile illness, encephalitis
	HHV-7	Exanthem subitum, mild febrile illness, encephalitis
	HHV-8	Kaposi's sarcoma, primary effusion lymphoma and Castleman's disease

Transmission

HSV: saliva, vesicle fluid, sexual contact, birth canal in neonate. VZV: respiratory droplets, vesicle fluid. EBV: saliva, organ transplants. CMV: saliva, urine, semen, cervical secretions, milk; also via organ transplants and across placenta. HHV-6, -7: saliva. HHV-8: saliva, semen.

Pathogenesis

HSV: vesicular lesions – oral, skin, genitals; axonal travel to latency sites in sensory ganglia; reactivation (cold sores). VZV: respiratory infection, systemic spread to skin, axonal travel to latency sites in sensory ganglia, reactivation (zoster). EBV: pharyngeal infection, systemic spread, latency in B cells, epithelium; subclinical reactivation. CMV: pharyngeal infection, systemic spread, latency in mononuclear cells; reactivation. HHV-6, -7: present in T cells. HHV-8: infects endothelial cells in Kaposi's sarcoma.

Laboratory identification

DNA detection by PCR methods using a range of samples, i.e. whole blood in EDTA, swabs, vesicle and other fluids including cerebrospinal fluid (CSF). Mostly vesicle fluid / swabs in HSV and VZV infections. Detecting intranuclear inclusions in tissue biopsies, multinucleated cells. For EBV and CMV infection: lymphocytosis and / or atypical lymphocytes in blood films; serology: EBV VCA IgM (heterophile antibody [Monospot] testing is less sensitive and specific especially if under 16 years old) and CMV IgM.
HHV-6, -7, -8 mostly DNA detection by PCR methods.

Treatment and prevention

Aciclovir, valaciclovir, famciclovir (HSV, VZV); ganciclovir, valganciclovir, foscarnet (CMV but also second line for HSV and VZV), foscarnet (HHV-6 infection in immunocompromised and / or encephalitis). Varicella-zoster immune globulin (VZIG) is used to prevent or attenuate chickenpox when susceptible immunocompromised individuals are exposed to infection. Live attenuated varicella vaccine licensed for prevention of chickenpox and shingles, the latter is a higher dose vaccine for specific age groups.

Orthomyxoviruses

Characteristics	*Virus family*	*Type*	*Envelope*	*Shape*	*Size (nm)*	*Nucleocapsid*
	Orthomyxoviridae	**ssRNA, linear, –ve sense, eight segments**	**+**	**pleomorphic**	**80–120**	**helical**

Influenza viruses. Envelope glycoproteins: haemagglutinin (H) attaches virus to sialic-acid-containing receptor on cell and, after exposure to endosomal acid, acts as fusion protein; neuraminidase (N) cleaves sialic acid from glycoproteins and is involved in release of virus from cell surface.
Influenza A: widespread in birds, horses, pigs, humans. Genetic reassortment between animal and human strains produce subtypes with novel combinations of H and N genes referred to as antigenic shift; new strains can cause pandemics. Antigenic drift also occurs and is a gradual alteration by developing point mutations in H to generate new strains. Influenza B: occurs only in humans; undergoes antigenic drift and can cause epidemics. Influenza C: of doubtful pathogenicity in humans.

Replication
Virus binds to cell via its H, enters after envelope fuses with lysosome wall. After uncoating, viral polymerase transcribes genome into eight mRNAs, which are translated in the cytoplasm. Progeny RNA synthesized in nucleus and nucleocapsids assembled in cytoplasm. Viral matrix protein joins nucleocapsid to viral envelope components in the cell wall and maturation takes place by budding.

Diseases
Fever, myalgia, malaise, nasal discharge, sore throat, cough, pneumonia.
Notable influenza A viruses include:
- that caused the pandemic in 1918 and the pandemic in 2009
- that caused the pandemic in 1957
- that caused the pandemic in 1968
- that was first reported in Hong Kong in 1997 and isolated cases are still reported in South-East Asia
- outbreak in poultry farms and in 89 people in the Netherlands in 2003
- infections in humans in China in 1988 and detected for the first time in the UK in 2001/2002
- mostly seen in birds in China with a few children infected in Hong Kong in 2009
- and H7N3 outbreaks in poultry farms
- infections in poultry and an increase in human infections in China since 2016.

Transmission
Incubation period 2–3 days. Via respiratory droplets.

Pathogenesis
Mainly infection of respiratory tract but can cause neurological and gastrointestinal symptoms. Cytokines contribute to symptoms and secondary bacterial infection well recognized.

Laboratory identification
Detection of viral RNA by PCR is the most sensitive diagnostic test. Some laboratories around the world may use viral antigen detection by immunofluorescence or virus isolation in cell culture. Serology: acute and convalescent sera can be tested by complement fixation test, but this is not widely used as it has been replaced owing to the need to make a rapid diagnosis.

Treatment and prevention
Neuraminidase inhibitors such as zanamivir and oseltamivir are effective against both influenza A and B viruses. Amantadine was used for treatment and prophylaxis for influenza A infection only. A killed vaccine has been complemented by a nasal spray. Live attenuated vaccine for children containing current circulating A and B strains prevents disease in susceptible at-risk individuals. Also, trivalent and quadrivalent vaccines.

Papovaviruses

Characteristics	*Virus family*	*Type*	*Envelope*	*Shape*	*Size (nm)*	*Nucleocapsid*
	Papovaviridae	**dsDNA**	**–**	**icosahedral**	**45–55**	**icosahedral (circular)**

Include papillomaviruses, John Cunningham (JC) and BK viruses.

Replication
Virus attaches to cellular receptors and enter epithelial cells by endocytosis; viral mRNA is transcribed in the nucleus by a cellular transcriptase; early gene products initiate viral DNA replication, transcription, transformation; late genes, L1 and L2 products are structural proteins. Of the early genes, E6 and E7 are viral oncogenes and their products modify the cell cycle by inactivating two tumour-suppressing proteins, p53 and pRb (retinoblastoma protein). The Papovaviridae include papillomaviruses, polyomaviruses and simian vacuolating viruses (e.g. SV40), with at least 150 human papillomaviruses and two polyomaviruses (BK and JC viruses). These viruses persist in latent form and can reactivate causing complications in immunocompromised patients.

Papovaviruses—cont'd

Characteristics	*Virus family* Papovaviridae	*Type* dsDNA	*Envelope* –	*Shape* icosahedral	*Size (nm)* 45–55	*Nucleocapsid* icosahedral (circular)
Diseases	Papillomaviruses cause warts on skin and genital regions. There are low-risk (types 6 and 11) and high-risk (types 16 and 18) types. The latter cause cervical cancer and carcinoma of penis, vulva and rectum. Human papillomavirus (HPV)-6 and -11 can cause laryngeal papillomatosis in children (infected via birth canal). Polyomaviruses on primary infection cause mild upper respiratory illness. In immunocompromised patients, JC virus causes progressive multifocal leukoencephalopathy (PML) and BK virus is excreted in the urine and can cause nephropathy, in particular haematuria.					
Transmission	Papillomaviruses: from skin to skin by direct or indirect contact, and between mucosae by sexual intercourse. Polyomaviruses: from the upper respiratory tract by droplets and perhaps by contact with other infected body fluids.					
Pathogenesis	Papillomavirus infection of epithelial cells and local multiplication results in a wart after an incubation period of up to 1–2 months. The wart regresses over the course of many months; there is no spread to deeper tissues, but viral DNA remains in basal epithelial cells. Viral genome integration into the host cell DNA can lead to the E6 and E7 genes being expressed, and cellular proliferation is promoted together with the risk of malignant change. Polyomaviruses spread from the upper respiratory tract and localize in tubular epithelium in the kidney (excretion in urine) or in oligodendrocytes to cause PML.					
Laboratory identification	HPV DNA detection and typing is usually carried out using molecular methods such as PCR in specialist laboratories. Serology tests are of little diagnostic help. Vacuolated or inclusion-bearing cells (koilocytosis) detected by histo/cytopathology. Papanicolaou staining; virus particles visible (urine or tissues) by electron microscopy. Virus culture is either difficult (polyomaviruses) or impossible (papillomaviruses).					
Treatment and prevention	Skin warts can be destroyed by freezing (liquid nitrogen) and areas of cervical dysplasia (genital warts) by laser treatment. Slower methods include using podophyllin or salicylic acid. Cidofovir has been used to treat immunosuppressed individuals, as has intralesional interferon. Preventive measures include the HPV vaccines, of which there were three types by 2017, all containing HPV-16 and -18 with one adding types 6 and 11 (quadrivalent) and another adding another five types to those four (nine valent), socks/shoes to cover plantar warts and condoms for genital warts.					

Paramyxoviruses

Characteristics	*Virus family* Paramyxoviridae	*Type* ssRNA, non-segmented –ve sense	*Envelope* +	*Shape* pleomorphic	*Size (nm)* 150–250	*Nucleocapsid* helical
	Includes measles virus, mumps virus, respiratory syncytial virus (RSV), human metapneumovirus, parainfluenza viruses, Nipah virus and Hendra virus. Envelope glycoproteins are H (haemagglutinin), N (neuraminidase), F (fusion), and G. H and N are combined (HN) in paramyxoviruses (mumps, parainfluenza 1–4), morbillivirus (measles) has H, and pneumovirus (RSV) has G (no H or N).					
Replication	Virus particle binds via its attachment protein (HN, H or G) to cell surface, penetrates and is uncoated. Viral polymerase transcribes genome into mRNAs, which are translated into viral proteins. Nucleocapsid is assembled and matrix protein joins it to the envelope proteins forming on the plasma membrane of the infected cell. Release then occurs by budding.					
Diseases	Measles: fever, conjunctivitis, nasal discharge, rash (very rarely encephalitis, subacute sclerosing panencephalitis); incubation period 10–14 days. Mumps: parotitis, aseptic meningitis (rarely orchitis, encephalitis); incubation period 12–25 days. Parainfluenza viruses: common cold; bronchiolitis, pneumonia; incubation period 3–6 days. RSV: common cold (adults), bronchiolitis, pneumonia (infants); incubation period 2–8 days. Metapneumovirus: mild upper respiratory tract disease, severe bronchiolitis and pneumonia in children. Nipah virus: encephalitis or pneumonia. Hendra virus: pneumonia and encephalitis.					
Transmission	Respiratory droplets. Contact with body fluids of infected animals, including urine and faeces, for Nipah and Hendra viruses.					

Continued

Paramyxoviruses—cont'd

Characteristics	*Virus family* Paramyxoviridae	*Type* ssRNA, non-segmented –ve sense	*Envelope* +	*Shape* pleomorphic	*Size (nm)* 150–250	*Nucleocapsid* helical
Pathogenesis	Initial infection via respiratory tract. RSV and parainfluenza virus infections: local replication and disease. Measles and mumps: no lesions at site of initial infection, spread to local lymph nodes, blood and invasion of skin and mucosa (measles) or salivary glands, CNS (mumps). Zoonotic infection: contact with infected animals' body fluids (Hendra, Nipah).					
Laboratory identification	Viral RNA detection by PCR in combined nose and throat swabs for respiratory virus infection, mouth swabs/oral fluid for measles and mumps and CSF, and respiratory samples for Nipah and Hendra virus infections. Virus isolation in cell culture and demonstration of viral antigen by immunofluorescence can be carried out. Serology: measles and mumps IgM and IgG, and also for Hendra and Nipah virus infections.					
Treatment and prevention	Aerosolized ribavirin for infants and highly immunocompromised individuals with severe RSV infections (oral and intravenous ribavirin are also available). No antiviral drugs had been effective in treating symptomatic highly immunocompromised individuals with parainfluenza infection by 2017. Measles and mumps prevented by live attenuated virus vaccines. No vaccines in routine use for RSV and parainfluenza viruses in 2017 although clinical trials had been carried out on RSV vaccines.					

Parvovirus B19 Human Bocavirus

Characteristics	*Virus family* Parvoviridae	*Type* ssDNA	*Envelope* –	*Shape* icosahedral	*Size (nm)* 18–26	*Nucleocapsid* icosahedral
	Includes human parvovirus B19 (single serotype), human bocavirus and the adeno-associated viruses (AAV). Human bocavirus (HBoV), discovered in 2005, is a member of the Parvoviridae subfamily. It is closely related to parvovirus B19. The name bocavirus is derived from *bo* (bovine) and *ca* (canine) virus, as there are other bocaviruses found in cattle and dogs. AAV are defective, requiring concurrent infection of the cell with 'helper' adenovirus or herpesvirus; positive DNA strands and negative DNA strands are carried in separate particles. B19 is autonomous but requires mitotically active cells.					
Replication	Occurs in the nucleus. Viral DNA replication takes place only when cell DNA replication is occurring (i.e. during the S phase of the cell cycle). Cellular transcriptase forms a cDNA strand to give dsDNA, and transcripts produce mRNAs.					
Diseases	B19 parvovirus causes a mild disease, erythema infectiosum, in children, with a 'slapped cheek' rash. Aplastic crisis may occur in those with sickle cell anaemia. Arthropathy is seen in adults. Intrauterine infection may result in fetal death with hydrops fetalis. Bocavirus: thought to cause upper and lower respiratory tract in infants and young children. The adeno-associated viruses are not known to cause disease.					
Transmission	Via respiratory droplets. Bocavirus: likely to be via respiratory droplets. Seasonal – mostly autumn, winter and spring.					
Pathogenesis	Parvovirus spreads from respiratory tract and can infect haemopoietic cells in bone marrow. Bocavirus: not known.					
Laboratory identification	Detection of parvovirus-specific IgM and IgG, B19 DNA.					
Treatment and prevention	There is no specific antiviral treatment and no vaccine, although the parvovirus B19 host cell receptor is known. Supportive measures include fetal exchange transfusion in hydrops fetalis and using human intravenous immunoglobulin, which contains B19 IgG, to damp down viral replication in infected immunosuppressed patients with recurrent episodes of anaemia. No antiviral agents or vaccine for bocavirus infections.					

Picornaviruses

Characteristics	*Virus family* Picornaviridae	*Type* ssRNA +ve sense	*Envelope* –	*Shape* spherical	*Size (nm)* 22–30	*Nucleocapsid* icosahedral
	Includes enteroviruses, coxsackieviruses, echoviruses, rhinoviruses, parechoviruses, polioviruses and hepatitis A virus. Hepatitis A is the only member of the *Hepatovirus* genus of this family and is distinct from the other picornaviruses. Human parechovirus is a member of the *Parechovirus* genus and is also distinct from the other picornaviruses. Enteroviruses are members of the picornavirus family. The enterovirus genus comprises 12 species that themselves are made up of serotypes that include coxsackieviruses, echoviruses, enteroviruses, rhinoviruses and polioviruses.					
Replication	Virus binds to cell via receptor molecule intercellular adhesion molecule-1 (ICAM-1), resulting in endocytosis and uncoating. The positive-sense viral ssRNA acts as mRNA, which is translated into a single polyprotein, cleaved by virus-coded protease into separate proteins. These include the RNA polymerase that makes negative-strand cRNA, which in turn acts as template for positive strands of viral RNA. RNA and capsid proteins assemble in the cytoplasm to form nucleocapsids, which are released on death of the cell. General features: four viral capsid proteins (VP1–VP4). There is no envelope.					
Diseases	Rhinoviruses: more than 100 serotypes; common cold symptoms include fever, rhinitis, sore throat, coughing and sneezing. Enteroviruses: respiratory and neurological illness with enterovirus D68 and enterovirus 71, respectively. Enterovirus 71 also associated with hand, foot and mouth disease. Neurological illness includes aseptic meningitis and polio-like syndrome. Conjunctivitis with enterovirus 70. Poliovirus types 1–3: aseptic meningitis, paralytic poliomyelitis. Echoviruses (enterocytopathic human orphan viruses): 32 types; aseptic meningitis/meningoencephalitis/encephalitis, rashes. Coxsackieviruses A and B: 29 types – herpangina, hand, foot and mouth disease (coxsackie A) myopericarditis and aseptic meningitis (coxsackie B). Parechovirus: respiratory illness. Hepatitis A virus: acute hepatitis.					
Transmission	Respiratory droplet spread for rhinoviruses and certain group A coxsackieviruses. Polioviruses, other enteroviruses and hepatitis A spread by the faecal–oral route.					
Pathogenesis	Rhinoviruses (acid-labile, optimal growth 33°C) replicate in upper respiratory tract. Enteroviruses (resist pH 3.0–9.0) replicate in pharynx and gastrointestinal tract, often with spread to lymph nodes, blood and then to CNS (e.g. polio, echoviruses), heart and muscle (coxsackie B), or liver (hepatitis A).					
Laboratory identification	Hepatitis A IgM and IgG detection. The rest of the picornavirus diagnoses involve viral RNA detection by PCR involving various samples, particularly faeces, CSF and combined nose and throat swabs. Virus isolation in cell culture from a range of samples in reference laboratories, generally. Serology is of limited use because of multiple serotypes, mostly IgM tests which lack sensitivity.					
Treatment and prevention	No specific treatment. Poliomyelitis prevented by vaccination with live attenuated (Sabin) or killed (Salk) vaccine. Hepatitis A prevented by inactivated virus vaccine has replaced the use of human normal immunoglobulin that was used but, in many countries where the hepatitis A prevalence had declined, had to be supplemented with hepatitis A IgG to make it effective. No vaccines for other picornaviruses.					

Poxviruses

Characteristics	***Virus family*** **Poxviridae**	***Type*** **dsDNA**	***Envelope*** **+**	***Shape*** **brick or ovoid**	***Size (nm)*** **250×300**	***Nucleocapsid*** **complex structure**
	Includes the genera *Molluscipoxvirus, Orthopoxvirus* and *Parapoxvirus* that infect humans, as well as animals in the latter two. The largest viruses; dermatotrophic, causing 'pocks' on the skin.					
Replication	Attach to the cell surface by electrostatic interaction with glycosaminoglycans followed by fusion of viral and cellular membranes and entry of the viral core into the cytoplasm (unlike other DNA viruses). Viral DNA-dependent RNA polymerase is used to synthesize mRNA. Transcripts are translated directly into proteins, some of which undergo post-translational cleavage to give functional molecules. After assembly, infectious virions are released as the cell disintegrates, some of them acquiring an envelope in the Golgi complex.					
Diseases	Molluscum contagiosum causes a mild infection with nodular skin lesions. Cowpox or milkers' nodules virus lesions on cow udders can cause vesicular lesions on the skin of milkers. Orf virus is responsible for contagious pustular dermatitis in sheep and those in contact with infected animals may develop vesicular skin lesions. After exposure to monkeys infected with monkeypox virus (monkeys are a favourite food in some parts of the Ivory Coast), humans develop a smallpox-like disease, which is distinguishable from smallpox by laboratory tests. Smallpox was eradicated in late 1979.					
Transmission	By direct contact with virus from skin lesions. Molluscum contagiosum is transmitted between humans, but monkeypox, cowpox, orf and milkers' nodules viruses are zoonoses, transmissible from the animal host to humans.					
Pathogenesis	Infection generally initiated in skin with local replication to form virus-rich vesicles; limited spread to local lymph nodes. Smallpox infection was via the respiratory tract and spread via blood to cause severe disease with disseminated skin and mucosal lesions.					
Laboratory identification	Viral DNA detection by PCR. Characteristic poxvirus particles are seen on electron microscopic examination of scrapings or biopsies of skin lesions. Cell culture methods for virus isolation and antibody tests are not available routinely. However, due to potential bioterrorist threats, smallpox detection by PCR can be carried out in specialist laboratories.					
Treatment and prevention	The lesions will gradually disappear and antivirals are not available. Cryotherapy with liquid nitrogen can be used to treat molluscum contagiosum. Orf lesions can develop secondary bacterial infections which need antibiotic treatment. In the past, methisazone was used to treat the serious side effects very occasionally caused by vaccination against smallpox with live vaccinia virus. Vaccination was the principal method used to eradicate smallpox. Vaccinia was the first virus to be used as an expression vector for live recombinant vaccines.					

Reoviruses

Characteristics	***Virus family*** **Reoviridae**	***Type*** **dsRNA, 9–12 segments depending on the genus 10 (reo) or 11 (rota) segments**	***Envelope*** **–**	***Shape*** **icosahedral**	***Size (nm)*** **60–80**	***Nucleocapsid*** **icosahedral (double layered)**
	Wide host range. Human infections include rotaviruses and Colorado tick fever virus.					
Replication	Virion resists acid pH, drying and detergents. Host protease in intestinal phagolysosome cleaves outer capsid protein to produce a fully infectious particle, which binds to cell membrane via receptor that may be histo-blood group antigens. Core enters cytoplasm; viral RNA-dependent RNA polymerase (one molecule for each genome segment) synthesizes 10–11 mRNAs (not polyadenylated), which direct synthesis of proteins, one of which is an RNA polymerase. The latter produces negative-strand viral RNA; positive strands are formed, and the assembled virus is released by cell lysis.					
Diseases	Mild or subclinical reovirus infections. The word reovirus derives from respiratory enteric orphan virus (orphan because initially not associated with any disease). Rotavirus infections in humans (types A–D): diarrhoeal illness, especially in infancy and childhood, and sometimes respiratory symptoms. Colorado tick fever virus endemic in the Rocky Mountains (orbivirus group): acute febrile illness, leukopenia, gastrointestinal and neurological symptoms, rash and, rarely, severe haemorrhagic disease.					

Reoviruses—cont'd

Characteristics	***Virus family*** **Reoviridae**	***Type*** **dsRNA, 9–12 segments depending on the genus 10 (reo) or 11 (rota) segments**	***Envelope*** **–**	***Shape*** **icosahedral**	***Size (nm)*** **60–80**	***Nucleocapsid*** **icosahedral (double layered)**
Transmission	Orthoreoviruses: faecal–oral and possibly respiratory spread. Rotaviruses: faecal–oral spread (virus survives drying and stomach acid). Colorado tick fever virus: by bite of an infected tick, mostly rodent reservoir.					
Pathogenesis	Orthoreoviruses: entry via respiratory or gastrointestinal tract (M cells) and spread to local lymphoid tissue. Rotaviruses: infection of enterocytes with no spread to deeper tissues; causes gastrointestinal illness with shortening and flattening of villi and interference with transport mechanisms. Colorado tick fever: virus enters skin via tick bite, spreads to local lymph nodes and blood, infects erythrocytes, and causes febrile illness.					
Laboratory identification	Viral RNA detection by PCR. Virus particles in faeces by electron microscopy. Viral antigen detection by ELISA or particle agglutination is less sensitive. Detection of viral antigens on erythrocytes by immunofluorescence or specific IgM (Colorado tick fever). Virus isolation not generally used.					
Treatment and prevention	No antiviral agents routinely available. Rotavirus diarrhoea is treated supportively by replacing water and electrolytes. Cross infection prevented by improved hygiene. Live attenuated rotavirus vaccines introduced by 2016 as routine immunization programmes in 90 countries. Anti-tick measures protect against Colorado tick fever.					

Retroviruses

Characteristics	***Virus family*** **Retroviridae**	***Type*** **dsRNA, ssRNA diploid +ve sense**	***Envelope*** **+**	***Shape*** **truncated cone shape**	***Size (nm)*** **80–100**	***Nucleocapsid*** **icosahedral**
	Includes human immunodeficiency virus (HIV)-1 and -2 (lentiviruses); human T-cell lymphotropic virus (HTLV)-1 and -2 (oncoviruses); human foamy virus (spumavirus), which causes foamy change in cells, but little else known; endogenous retroviruses, which exist as sequences in human genome.					
Replication	HIV binds to CD4 cell surface receptor and chemokine co-receptors, enters and is uncoated. Virion RNA-dependent DNA polymerase (reverse transcriptase) transcribes viral genome into dsDNA (provirus), which is then integrated into host cell DNA by viral integrase. Transcription is by host RNA polymerase; genomic and viral mRNA are formed, and translated into structural and regulatory proteins. Viral genes *gag, pol, env* code for structural proteins, and other gene products have regulatory functions. Nucleocapsids assemble in cytoplasm and are released by budding.					
Diseases	HIV: mild early illness with mononucleosis; sometimes aseptic meningitis; progressing to AIDS, with multiple opportunistic infections including Kaposi's sarcoma (HHV-8), primary cerebral lymphoma (EBV), *Pneumocystis jiroveci* pneumonia (PCP), oral candidiasis. HTLV-1: tropical spastic paraparesis, adult T-cell leukaemia (ATLL). HTLV-2: neurological disease including myelopathy.					
Transmission	HIV: sexual intercourse, blood-borne, breastfeeding, mother-to-baby transplacental transfer. HIV-1 worldwide; HIV-2 mainly in West Africa. HTLV-1 and -2: via breastfeeding, blood-borne and sexual intercourse. HTLV-2 has spread among injecting drug users. HTLV-1 occurs in certain islands in Caribbean and Japan, and in parts of South America and Africa. HTLV-2: in West Africa and South America.					
Pathogenesis	HIV: initial entry via mucosal route with infection of CD4-positive cells (helper T cells, dendritic cells, monocytes, macrophages). Spread through body including CNS, placenta. Action on immune cells results in severe immunosuppression leading to opportunist infections and reactivations (viral, bacterial, protozoal). Also Kaposi's sarcoma associated with presence of HHV-8. HTLV-1: pathogenesis of CNS disease not clear. Leukaemia (mean 30 years after infection) results from multistage process initiated by tat gene product in infected T cells stimulating transcription of host genes that control cell division.					

Continued

Retroviruses—cont'd

Characteristics	*Virus family* Retroviridae	*Type* dsRNA, ssRNA diploid +ve sense	*Envelope* +	*Shape* truncated cone shape	*Size (nm)* 80–100	*Nucleocapsid* icosahedral
Laboratory identification	HIV: combination assays detect antibodies and p24 antigen with type differentiation by immunoblot. HIV-1 RNA and HIV-2 RNA load assays to monitor disease progression and treatment responses. HIV-1 proviral DNA qualitative detection. Genotypic antiretroviral resistance tests, tropism assays and integrase resistance assays too. HTLV-1 and -2: antibody tests with type differentiation by immunoblot.					
Treatment and prevention	HIV: various classes of antiretroviral drugs, referred to as combined antiretroviral therapy (cART), inhibit virus replication and arrest disease progress without eliminating virus from body (viral DNA transcripts remain in infected cells). The main classes of antiretroviral drugs are nucleoside, nucleotide, and non-nucleoside reverse transcriptase inhibitors (e.g. zidovudine, tenofovir and nevirapine respectively), protease inhibitors (e.g. darunavir), fusion inhibitors (e.g. enfuvirtide) and integrase inhibitors (e.g. raltegravir). Various combinations of classes and within classes can be used and combining three drugs in one pill has revolutionized treatment. Treatment of opportunist infections. Various vaccines have and are undergoing clinical trials. Prevention by increasing HIV screening, avoiding blood-borne virus (e.g. needle exchange programmes, treatment of blood and blood products) and practising safe sex (e.g. education, condoms). HTLV-1 and -2: antiretroviral drug regimens have been investigated and bone marrow transplantation has been used in ATLL patients.					

Rhabdovirus

Characteristics	*Virus family* Rhabdoviridae *Genus* Lyssavirus	*Type* ssRNA –ve sense	*Envelope* +	*Shape* bullet	*Size (nm)* 180×75	*Nucleocapsid* helical
	Rabies virus.					
Replication	Viral G protein attaches to nicotinic acetylcholine receptor, neural cell adhesion molecule (CD56) and low-affinity nerve growth factor receptor (NTR75) on cells; virus is endocytosed and uncoated. Virion RNA polymerase synthesizes five mRNAs, and virus-coded RNA polymerase replicates viral RNA. After assembly of nucleocapsid, the envelope is acquired by budding from the plasma membrane without detectable cell damage. Rabies virus can infect all mammals. Present in wild animals in all continents except Australia and Antarctica. Only one serotype.					
Diseases	Incubation period 2–10 weeks but may be longer. CNS symptoms and signs include excitement, confusion, lethargy, hydrophobia, progression to seizures, paralysis, coma and death.					
Transmission	Via bite of infected dog, cat, skunk, raccoon, bat in low- or high-risk countries. Human-to-human transmission is rare and has been reported in the organ transplantation setting.					
Pathogenesis	Virus replicates at site of bite, ascends axons to CNS where it spreads, and then descends down peripheral nerves to skin, salivary glands.					
Laboratory identification	Viral RNA detection by PCR in corneal scrapings, biopsy of hair-bearing skin, cerebrospinal fluid, brain tissue (at autopsy). Also examined for presence of inclusions (Negri bodies) and for rabies antigen by immunofluorescence.					
Treatment and prevention	No specific treatment although there is one famous report using the Milwaukee protocol – a chemically induced coma to reduce cerebral activity together with ribavirin and amantadine antiviral drugs given to a 15-year-old girl with rabies who survived. Post-exposure prophylaxis by washing wound, giving human rabies-specific immunoglobulin and a course of inactivated vaccine produced in human diploid cells.					

Togaviruses

Characteristics	***Virus family*** **Togaviridae**	***Type*** **ssRNA +ve sense**	***Envelope*** **+**	***Shape*** **spherical**	***Size (nm)*** **60–70**	***Nucleocapsid*** **icosahedral**

Includes rubella, chikungunya, o'nyong-nyong, Ross River, Eastern and Western encephalitis viruses.

Human togaviruses include the non-arthropod-borne rubella virus (genus *Rubivirus*), and the arboviruses of the *Alphavirus* genus. The latter occur in all parts of the world, often have exotic names (Kyasanur Forest disease virus, India; Omsk haemorrhagic fever virus, Russia), and replicate in the arthropod vector as well as in the vertebrate host. Most have animal reservoirs. The alphaviruses include chikungunya virus, o'nyong-nyong virus, Western equine encephalomyelitis (WEE), Eastern equine encephalomyelitis (EEE), Venezuelan encephalitis virus, Ross River virus, Semliki Forest virus.

Replication

The positive-strand RNA is translated into structural and non-structural proteins, the latter including the RNA-dependent RNA polymerase, which replicates the viral genome by directing the formation of a negative-strand template and thus giving rise to positive-strand progeny. Full-length and subgenomic-length RNA is formed. After assembly, the virus exits from the cell, budding from the plasma membrane.

Diseases

Rubella causes a mild rash. In adults, it may be complicated by arthralgia. A primary infection in pregnancy can result in fetal infection and congenital malformations known as congenital rubella syndrome. The latter includes heart, brain, eye and hearing defects. The remaining togaviruses cause febrile illnesses which may be severe when there is involvement of the CNS (equine encephalitides).

Transmission

Rubella is transmitted between humans by respiratory droplets; the rest are transmitted by the bite of infected arthropods.

Pathogenesis

Initial infection via respiratory tract (rubella) or skin (arthropod-borne viruses) causes no detectable local lesion. Virus spreads to local lymph nodes and blood, multiplying in respiratory tract, placenta and fetus (rubella) or CNS (equine encephalitides). Mononuclear cells often infected (rubella). Arboviruses – in mosquitoes, ingested virus infects gut epithelium, spreads to salivary glands, and multiplies there.

Laboratory identification

Serology includes rubella-specific IgG and IgM as well as IgG avidity and rubella virus RNA detection. Arthropod-borne togavirus infection diagnosis: serological methods, viral RNA detection by PCR. Virus isolation may be carried out in some reference laboratories too.

Treatment and prevention

There is no antiviral therapy. A live attenuated rubella virus vaccine is incorporated with the mumps and measles virus vaccines formulated as MMR in many parts of the world. Vaccines are not available for the other arthropod-transmitted togaviruses, but horses can be protected from WEE and EEE with veterinary vaccines.

Prion Diseases

Characteristics

Not viruses. Prions are transmissible proteins that cause spongiform encephalopathies causing progressive neurodegenerative disorders. These affect humans and animals. Aetiological agents of the human prion diseases share the same general features and are sporadic, inherited or acquired. The diseases are Creutzfeldt–Jakob disease (CJD), Gerstmann–Straússler–Scheinker syndrome (GSS), fatal familial insomnia (FFI), variant CJD and kuru. The animal diseases are bovine spongiform encephalopathy (BSE), chronic wasting disease, scrapie, transmissible mink encephalopathy, feline spongiform encephalopathy and ungulate spongiform encephalopathy, The prototype agent, scrapie, causes CNS disease in sheep. Host-coded prion protein (PrP) in slightly altered form, PrP^{Sc} is transmissible and causes abnormal folding of cellular proteins leading to spongiform change and neuronal death. Highly resistant to heat (special autoclaving procedures required for destruction), chemical agents and irradiation. Very slow replication, very long incubation period (up to 20 years in humans). Infect a variety of mammals and can be transmitted to cows, mink, cats and mice, for example, when food contains infected material.

Diseases

'Spongiform encephalopathies', 'prion diseases'. Kuru: fatal neurological diseases in Papua New Guinea still occur but are very rare. CJD: rare chronic encephalopathy, occurs worldwide; 10% cases familial with mutated prion protein gene. GSS and FFI.

Continued

Prion Diseases—cont'd

Transmission	Kuru: from eating infected human brain during ritualistic feasts (may be consumption or due to transmission via lesions in skin). CJD: in most cases unknown; occasionally transmitted from infected human brain but also by blood and by medical and surgical procedures (iatrogenic CJD known as iCJD); familial cases genetically transmitted. Variant CJD (vCJD) from consumption of BSE (bovine spongiform encephalopathy)-infected food.
Pathogenesis	Infectious agent replicates inexorably in lymphoid tissues and then in brain cells, where it produces intracellular vacuoles and deposition of altered host prion protein. Uniformly fatal if host lives long enough.
Investigations and laboratory identification	Brain MRI shows hyperintense regions in the cerebral cortex, basal ganglia and thalamus. EEG shows generalized slowing and polyspike-wave complexes and sharp waves. Lumbar puncture – cerebrospinal fluid positive for 14-3-3 protein and raised total tau protein and neurone-specific enolase. However, it is not definitive as they may be as a result of neuronal death/injury in general. Positive prion protein gene genetic test – mutations at codon 129 of the prion protein gene. Intracellular vacuoles (spongiform change) visible histologically in brain biopsy. Also see neuronal loss and astrogliosis. PrP^{Sc} detected by immunohistochemistry is a definitive diagnostic test. Isolation of agent requires experimental animals and is lengthy, difficult and not routinely undertaken. No specific immune responses.
Treatment and prevention	No treatment or vaccine. Kuru died out when cannibalism ceased. Iatrogenic transfer of CJD preventable (e.g. when genetically engineered growth hormone became available).

Virus Families

Virus	Family
Adenoviruses	Adenoviridae
Lassa, Junin, Machupo, Sabia, Tacaribe, lymphocytic choriomeningitis viruses	Arenaviridae
Hantaan, Seoul, Puumala, Sin Nombre, Rift Valley, Toscana, Crimean–Congo haemorrhagic fever, Dugbe, Bunyamwera, La Crosse viruses	Bunyaviridae
Noroviruses	Caliciviridae
Coronaviruses OC43, 229E, NL63, HKU1, SARS, MERS	Coronoviridae
Hepatitis D virus	Deltavirus
Ebola, Marburg viruses	Filoviridae
Dengue, Japanese encephalitis, yellow fever, tick-borne encephalitis, West Nile, Zika, hepatitis C viruses	Flaviviridae
Hepatitis B virus	Hepadnaviridae
Hepatitis E virus	Hepeviridae
Herpes simplex types 1 and 2, cytomegalovirus, varicella-zoster, Epstein–Barr, human herpesvirus (HHV)-6, -7, -8 viruses	Herpesviridae
Influenza A, B and C viruses	Orthomyxoviridae
Papillomaviruses, JC and BK viruses	Papovaviridae
Measles, mumps, respiratory syncytial virus, human metapneumovirus, parainfluenza types 1–4, Nipah and Hendra viruses	Paramyxoviridae
Parvovirus B19, human bocavirus	Parvoviridae
Enteroviruses, coxsackieviruses, echoviruses, rhinoviruses, parechoviruses, polioviruses, hepatitis A virus	Picornaviridae
Molluscum contagiosum, orf, cowpox, monkeypox, smallpox	Poxviridae
Rotaviruses, Colorado tick fever virus	Reoviridae
Human immunodeficiency virus types 1 and 2, human T-cell lymphotropic virus types 1 and 2, human foamy virus	Retroviridae
Rabies virus	Rhabdoviridae
Rubella, chikungunya, o'nyong-nyong, Ross River, Eastern and Western encephalitis viruses	Togaviruses

Bacteria

Gram-positive Cocci

Genus *Staphylococcus*

Genus contains over 30 species, of which three are of greatest medical importance: *Staph. aureus, Staph. epidermidis, Staph. saprophyticus.*

Major distinguishing features of medically important staphylococci

Test	*Staph. aureus*	*Staph. epidermidis*	*Staph. saprophyticus*
Coagulase production[a]	+	–	–
Protein A on cell surface	+	–	–
Production of recognized exotoxins	+	–	–
Haemolysin production	+[b]	–[b]	+
Resistance to novobiocin (5 μg)[c]	–	–	+

[a]Note that the coagulase-negative species *Staph. epidermidis* and *Staph. saprophyticus* and other less commonly isolated coagulase-negative species (e.g. *Staph. capitis* and *Staph. haemolyticus*) are often referred to simply as 'coagulase-negative staphylococci' without further identification.
[b]Usual result, but not for all strains.
[c]Useful for distinguishing between *Staph. epidermidis* and *Staph. saprophyticus.*

Staphylococcus aureus

Characteristics
Gram-positive coccus; cells in clusters (reflecting ability to divide in more than one plane); individual cells approximately 1 mm in diameter. Some strains produce capsules. Non-fastidious; capable of aerobic and anaerobic respiration.

Laboratory identification
White or golden colonies on blood agar. Catalase positive, coagulase positive; most strains ferment mannitol anaerobically. Kits available for biochemical characterization.

Diseases
Boils; skin sepsis; postoperative wound infection; scalded skin syndrome; catheter-associated infection; food-borne infection; septicaemia, endocarditis; toxic shock syndrome; osteomyelitis; pneumonia.

Transmission
Normal habitat: humans (and animals associated with them); skin, especially nose and perineum (carriage rates higher in hospital patients and staff). Spread is by contact and airborne routes. Organism survives drying; tolerant of salt and nitrites.

Epidemiological analysis
Whole genome sequencing is replacing pulsed-field gel electrophoresis and other molecular techniques.

Pathogenesis
Virulence multifactorial, and most factors shown below are present in some strains.
Present in all strains:
- mucopeptide
- coagulase.

Present in some strains:
- cell-associated: capsule, protein A, fibronectin-binding protein, collagen-binding proteins
- extracellular products: enterotoxins, epidermolytic toxin, toxic shock syndrome toxin, membrane-damaging toxins (haemolysins), leukocidin, staphylokinase
- many strains have protein A bound to the mucopeptide of the cell wall. This protein interacts non-specifically with host IgG antibodies reducing opsonization and causing local activation of complement.

Treatment and prevention
In susceptible isolates, antibiotics of choice are beta-lactamase-stable penicillins; however, the vast majority of hospital isolates are beta-lactamase producers. Multiple drug resistance (including methicillin and tolerance or resistance to vancomycin) is a worldwide problem. Mupirocin can be used for topical treatment of carriage. Prevention of spread by isolation and/or treatment of carriers in high-risk areas in hospital. No vaccine available.

Staphylococcus epidermidis

Characteristics	As for *Staph. aureus*.
Laboratory identification	White colonies on blood agar; catalase positive, coagulase negative, mannitol not fermented anaerobically. Kits available for biochemical characterization.
Diseases	Opportunist pathogen associated with device-related sepsis (e.g. catheter-related sepsis; prosthetic valve endocarditis; infection of artificial joints; shunt infections); urinary tract infections (UTIs); sternal wound osteomyelitis.
Transmission	Normal habitat: skin (carriage rate approximately 100%). Spread by contact with self, other patients or hospital personnel. Almost all infections acquired in hospital, but may be endogenous. Survives drying; salt tolerant.
Epidemiological analysis	Whole genome sequencing is replacing pulsed-field gel electrophoresis and other molecular techniques.
Pathogenesis	Extracellular slime production may be a marker of virulence and aid in the colonization of plastic implants (e.g. intravenous catheters and prostheses).
Treatment and prevention	Antibiotic resistance: often multiresistant (including penicillin and methicillin). Prevention of infection: catheter care; no vaccine available.

Staphylococcus saprophyticus

Characteristics	As for *Staph. aureus*.
Laboratory identification	White colonies on blood agar; catalase positive, coagulase negative, mannitol not fermented anaerobically. Kits available for biochemical characterization.
Diseases	Urinary tract infection in previously healthy women (associated with intercourse).
Transmission	Normal habitat: skin and genitourinary mucosa. Endogenous spread to urinary tract in colonized women.
Epidemiological analysis	Whole genome sequencing, pulsed-field gel electrophoresis and other molecular techniques.
Pathogenesis	Virulence factors unknown, but organism has the ability to colonize periurethral skin and mucosa.
Treatment and prevention	Urination after intercourse helps to wash organisms out of the bladder and prevent infection.

Genus *Streptococcus*

A large group of Gram-positive cocci distributed widely in humans and animals, mostly forming part of the normal flora, but some species responsible for some major infections. Individual cells 0.5–1 μm diameter and, because they divide in one plane only, occur in pairs and chains. The medically significant streptococci may be divided on the basis of either haemolysis on blood agar (complete haemolysis, beta; partial haemolysis, alpha; no haemolysis, gamma) or by the presence or absence of a group-specific carbohydrate antigen (i.e. the Lancefield group labelled alphabetically A to V).

Beta-haemolytic Streptococci

Streptococcus pyogenes (Group A Streptococci)

Characteristics	Gram-positive cocci in chains, cells less than 1 μm diameter, non-motile, non-spore-forming.
Laboratory identification	Grown on blood agar. Pronounced haemolytic activity (enhanced anaerobically). Catalase negative. Bacitracin (0.04 units); all strains are susceptible; detection of group-specific carbohydrate (A antigen); detection of L-pyrrolidonyl arylamidase (PYR).
Diseases	Infections of upper respiratory tract and of skin and soft tissue (e.g. pharyngitis, cellulitis, erysipelas, lymphadenitis). Toxic manifestations include scarlet fever. Non-suppurative sequelae (acute glomerulonephritis and rheumatic fever) are important complications of both skin and throat infections.
Transmission	Normal habitat is the human upper respiratory tract and skin. Spread by airborne droplets and by contact. Survival in dust may be important. Epidemiological typing of strains (see below) useful in outbreaks.

Streptococcus pyogenes (Group A Streptococci)—cont'd

Epidemiological analysis	Antigen from cell wall reacting with specific antisera (rabbit), either in a grouping precipitin or latex agglutination reaction. In addition to this group-specific polysaccharide, type-specific M and T antigens can be detected for epidemiological purposes. Pulsed-field gel electrophoresis and other molecular techniques are commonly used for epidemiological analysis.
Pathogenesis	*Strep. pyogenes* elaborates many enzymes and exotoxins, which may play a role in infection: erythrogenic toxin (lysogenic phage mediated); streptolysins; streptokinase A and B (therapeutic applications); deoxyribonuclease; hyaluronidase ('spreading factor').
Treatment and prevention	Penicillin is the drug of choice. Vaccines are not available. Oral cephalosporin or vancomycin is an alternative for penicillin-allergic patients.

Streptococcus agalactiae (Group B Streptococci)

Characteristics	Gram-positive cocci in chains.
Laboratory identification	Beta-haemolytic on blood agar; colonies larger than *Strep. pyogenes* frequently pigmented after anaerobic incubation on Columbia agar (Islam's medium). Grow in the presence of bile on MacConkey agar. Biochemical tests include hippurate hydrolysis (positive), aesculin hydrolysis (negative). Possess group B Lancefield capsular antigen. Group-specific carbohydrate and commercially available molecular tests for definitive identification. Positive CAMP (Christie, Atkins, Munch–Peterson) test.
Diseases	Neonatal meningitis and septicaemia. Mastitis in bovines.
Transmission	Normal habitat; gut and vagina. Babies acquire organism from colonized mother at birth or by contact spread between babies in nursery after birth.
Pathogenesis	Virulence factors not clearly identified.
Treatment and prevention	Susceptible to penicillin, but less so than *Strep. pyogenes*; combination of penicillin and gentamicin for serious infections. Screening pregnant women recommended; prophylactic antibiotics may be given to babies (especially premature) of carriers.

Other Beta-Haemolytic Streptococci of Medical Importance

Streptococci of Lancefield groups C and G may sometimes cause pharyngitis. Group D streptococci include the *Streptococcus bovis* group and organisms now classified in the genus *Enterococcus* (see below).

Streptococcus milleri Group

Microaerophilic streptococci that often form small colonies (formerly termed *Strep. milleri*) and carry Lancefield group A, C, F or G antigens. Have a propensity for abscess formation (especially in liver and brain).

Alpha-haemolytic Streptococci

Streptococcus pneumoniae

Characteristics	Gram-positive coccus characteristically appearing in pairs (diplococci) in Gram films. Cells approximately 1 μm diameter, often capsulate. Requires blood or serum for growth. Capable of aerobic and anaerobic respiration; growth may be enhanced in CO_2.
Laboratory identification	On blood agar alpha-haemolytic 'draughtsman' colonies that may autolyse within 48 h at 35°C. Catalase negative. Susceptible to bile (bile solubility test) and Optochin (ethyl hydrocupreine hydrochloride; available in paper disks). Polysaccharide capsules can be demonstrated by appropriate staining techniques. They are antigenic and in the presence of specific antiserum appear to swell (quellung reaction).

Continued

Streptococcus pneumoniae—cont'd

Diseases	Pneumonia, septicaemia and meningitis. Otitis and related infections in children. Capsular type III frequently associated with pneumonia.
Transmission	Normal habitat is the human respiratory tract; c. 5% of population may carry in small numbers. Transmission via droplet spread.
Pathogenesis	Capsule protects the organism from phagocytosis. Pneumolysin may have a role as a virulence factor, but to date no known exotoxins. Splenectomy may predispose to infection. Viral infection may be a precursor to pneumonia.
Treatment and prevention	Penicillin remains the antibiotic of choice, but resistance is increasing rapidly, and susceptibility test results should be used to guide therapy. Vaccine available.

Oral Streptococci

There are several other species of alpha-haemolytic streptococci that in the past have been lumped together under the colloquial heading 'viridans streptococci'. These and some of the non-haemolytic streptococci have now been reclassified. Most species are commensal in the mouth. *Strep. mutans* is strongly associated with dental caries. Several species are capable of causing bacterial endocarditis. Most strains are susceptible to penicillin; however, moderate to high resistance has also been observed. Moderately resistant isolates may be treated with penicillin plus an aminoglycoside while highly resistant strains require a broad-spectrum cephalosporin or vancomycin. It is important to distinguish these streptococci from *Strep. pneumoniae* in cultures from the respiratory tract.

Genus *Enterococcus* (Faecal Streptococci)

	Formerly classified in the genus *Streptococcus*, with which they share many characteristics; there are more than 30 species of enterococci. *E. faecalis* and *E. faecium* are the most important clinically and are considered together.
Characteristics	Gram-positive cocci, cells often in pairs and chains; more ovate appearance than streptococci. Non-fastidious; capable of aerobic and anaerobic respiration.
Laboratory identification	On blood agar may produce alpha, beta or no haemolysis. Resistant to 40% bile salts and Optochin; relatively heat tolerant (grow at 45°C), and salt tolerant (grow in 6.5% NaCl); hydrolyze esculin. Kits available for biochemical identification. Carry Lancefield's group D antigen, but the antigen is teichoic acid rather than polysaccharide.
Diseases	Urinary tract infection; endocarditis; infrequent, but severe septicaemia after surgery and in the immunocompromised.
Transmission	Normal habitat is the gut of humans and animals. Most infections thought to be endogenously acquired, but cross-infection may occur in hospitalized patients.
Pathogenesis	No toxins or other virulence factors convincingly demonstrated. Plasmid-mediated haemolysin may play a role.
Treatment and prevention	Penicillins used in combination with aminoglycosides. Resistant to cephalosporins and incidence of resistance to vancomycin (VRE) is a problem. Linezolid, daptomycin, and quinupristin/dalfopristin (only *E. faecium*) may be used in treatment. Patients with known heart defects should be given prophylactic antibiotics to prevent endocarditis before dentistry or surgery on gut or urinary tract.

Gram-positive Rods

Genus *Corynebacterium*

This genus contains many species, is widely distributed in nature. Although cell wall structure has similarities to *Mycobacterium* and *Nocardia*, the short-chain mycolic acids present do not confer acid-fast staining. The species of major importance is *C. diphtheriae*. This and other pathogens within the genus need to be distinguished from commensal corynebacteria.

Corynebacterium diphtheriae

Characteristics	Gram-positive, non-capsulate, non-spore-forming, non-motile rods, 2–6 μm in length. In Gram-stained films, cells arranged as 'Chinese letters' or palisades and showing irregular staining or granule formation are characteristic. Non-fastidious, but growth enhanced by inspissated serum (Loeffler medium). Capable of aerobic and anaerobic respiration.
Laboratory identification	Grows on blood agar, but identification aided by a selective medium (e.g. blood tellurite) on which characteristic black colonies form within 48 h at 35°C (but many other organisms may produce black colonies). Clinically, most important biotypes of *C. diphtheriae* are mitis and gravis, and they have characteristic colony morphology. *C. diphtheriae* is catalase positive and reduces nitrate. Species identification is established on the basis of biochemical tests or species-specific sequencing. Toxin production has been traditionally demonstrated by the Elek test. A polymerase chain reaction (PCR) assay for the toxin gene is available. It is important to demonstrate toxigenicity to confirm diphtheria diagnosis, but non-toxigenic strains may also be associated with disease (e.g. septicaemia, endocarditis).
Diseases	Diphtheria caused by toxigenic strains of *C. diphtheriae*. Focus of infection may be the throat or the skin.
Transmission	Normal habitat: usually nasopharynx, occasionally skin of humans. Infection is usually spread by aerosol. Patients may carry toxigenic organisms for up to 2–3 months after infection.
Pathogenesis	Disease is due to production of diphtheria toxin controlled by the *tox* gene, which is integrated into the bacterial chromosome on a lysogenic (β) phage. When concentration of exogenous inorganic iron (Fe^{3+}) is very low, exotoxin production is maximal; the selective advantage to the organism is unknown. The mode of action of the toxin is to block protein synthesis of the host cells by inactivating an elongation factor.
Treatment and prevention	Urgent supportive therapy to maintain airway essential in throat diphtheria. Antitoxin neutralizes toxin, penicillin kills organisms; antibiotics have little effect since diffusion of toxin is not influenced by inhibition of organisms at local site. In outbreak, carriers treated with erythromycin or penicillin. Immunization effective in prevention of diphtheria; in areas where immunization rates reach 85%, herd immunity sufficient to protect whole population. Circulating antibody after immunization neutralizes test dose of standardized toxin (Schick test). Positive result (i.e. skin reaction) equates with insufficient antibody. Babies acquire immunity from immune mothers for a few months.

Other Corynebacteria

C. ulcerans has been found in diphtheria-like disease. It produces two toxins, one of which is neutralized by diphtheria antitoxin, the other is similar to that produced by *C. pseudotuberculosis*. *C. jeikeium* is isolated from blood cultures and wounds in immunosuppressed patients. It is usually detected by its relative resistance to antibiotics other than glycopeptides such as vancomycin. *C. pseudotuberculosis* is a significant pathogen of horses and sheep. *C. xerosis* and *C. pseudodiphtheriticum* are skin inhabitants, and many other coryneforms may also be found on skin. These, and other related genera such as *Brevibacterium* and *Rhodococcus*, are lipophilic and require lipids for optimal growth.

Genus *Bacillus*

This genus contains more than 70 species, most of which are soil organisms. There are two species of major medical importance: *B. anthracis* and *B. cereus*.

Bacillus anthracis

Characteristics	Large (4–10 μm) Gram-positive spore-forming encapsulated rods. Spores are formed only after the organism is shed from the body. Respires aerobically.
Laboratory identification	In smears of body fluids, the capsule can be stained with polychrome methylene blue McFadyen reaction or direct fluorescent antibody is diagnostic of *B. anthracis*. Amplification-based molecular tests (e.g. PCR) are also available. The species is non-fastidious; grows well on simple media. Characteristic colonies (Medusa head) are probably related to chaining of the long rods. Non-haemolytic on horse blood agar (many of the other species are haemolytic). Growth in CO_2 encourages the formation of the capsule and smooth colonies. Biochemical reactions are unhelpful except in expert hands.

Continued

Bacillus anthracis—cont'd

Diseases	Anthrax is a significant disease in both domesticated and wild animals. It is a zoonosis and humans are usually infected by contact with infected hides or bones. Intestinal anthrax is rare in humans. Woolsorter's disease (i.e. respiratory or inhalation anthrax) is also rare. However, the potentially lethal effect of anthrax infections has especially attracted interest as an aspect of biological warfare.
Transmission	Soil organisms: *B. anthracis* can survive in competition with other organisms for many years depending on the temperature and humidity. The carcasses of animals dying with anthrax are buried 6 feet deep to prevent organisms being carried to the surface. Humans are accidental hosts, and infection is usually acquired when spores enter abrasions on the skin or are inhaled.
Pathogenesis	The polyglutamic acid capsule is antiphagocytic. In addition, an exotoxin encoded on a temperature-sensitive plasmid is produced. Toxin has three components: oedema factor, lethal factor and protective antigen. Individually, the components have no biological effect, but toxicity is produced by either of the first two factors together with the antigen. The toxin acts locally in the skin and lung. Pasteur used heat attenuation to produce a virulent strain that could be used as an attenuated vaccine.
Treatment and prevention	Ciprofloxacin is the drug of choice but (depending on susceptibility and especially in the case of inhalation anthrax) may be combined with other antibiotics (e.g., penicillins, doxycycline). Prevention includes control measures such as formalin disinfection of hides, strict control of infected domestic animals, and the immunization of veterinarians and laboratory workers at risk.

Bacillus cereus

Characteristics	Large Gram-positive spore-forming rod. This and many other *Bacillus* species are similar to *B. anthracis* in many respects except most are motile and non-capsulate. Respires aerobically.
Laboratory identification	Non-fastidious. Produces haemolysis on horse and sheep blood agar. Lecithinase production and inability to utilize mannitol are used as distinguishing features on a specially designed selective medium.
Diseases	*B. cereus* causes food poisoning, the commonest association being with reheated cooked rice and pulses. Two different syndromes are recognized, due to different toxins (see below). The organism is also a rare cause of bacteraemia, especially in immunocompromised hosts.
Transmission	*B. cereus* spores are found on many foods, especially rice, pulses and vegetables. Infection / symptoms occur following ingestion of organisms or toxin.
Pathogenesis	Some strains produce heat-stable toxin in food associated with spore germination; this gives rise to a syndrome of vomiting within 1–5 h of ingestion. Others produce a heat-labile enterotoxin after ingestion, which causes diarrhoea within 10–15 h.
Treatment and prevention	The majority of illness is short-lived and self-limiting, and antibiotic treatment is not indicated. Bacteraemia in immunocompromised patients and other *B. cereus* infections should be treated promptly with gentamicin, vancomycin, ciprofloxacin, or clindamycin. As with other food-borne infections, hygienic preparation of food is paramount. Cooked food should be stored in a refrigerator and reheated thoroughly before serving.

Genus *Listeria*

	These organisms were included with the genus *Corynebacterium* in older classifications. They also share antigenic relationships with enterococci and lactobacilli. *L. monocytogenes* is the species of major medical importance.

Listeria monocytogenes

Characteristics	Short Gram-positive rods, often coccobacillary in clinical material (must avoid confusion with streptococci in chains); frequently Gram variable. Motile at 25°C with a characteristic 'tumbling' movement; non-motile at 37°C.
Laboratory identification	Haemolytic on sheep or horse blood agar. Selective medium aids recovery of these organisms, especially from food samples (fish, chicken and cheeses). Cold enrichment at +4°C for several weeks is also an effective selective technique. On translucent, non-blood-containing agar, colonies appear green-blue in oblique light. Catalase positive, nitrate reduction negative; coupled with motility at room temperature these results are useful identifying features. Biochemical and serological tests provide definitive identification.
Diseases	Meningitis and sepsis in neonates. Infections in the immunocompromised (particularly meningitis) and in pregnant women.
Transmission	Widely distributed in nature, survives well in cold. Reaches food chain via silage as well as more directly via for example vegetables. Excreted in large numbers in cows' milk. Humans may carry *Listeria* in gut as normal flora. Infection may be acquired by ingestion or transplacentally to the baby in utero. While 13 different serotypes exist, pulsed-field gel electrophoresis and other molecular techniques are routinely used to investigate outbreaks.
Pathogenesis	Virulent strains produce internalins (cell attachment factors), haemolysins, and a motility protein; organism can survive in phagocytes.
Treatment and prevention	Treatment with penicillin or ampicillin, often in combination with gentamicin. Widespread distribution of organism in nature makes prevention of acquisition difficult. Pregnant women have been advised against eating uncooked food thought to be of particular risk (e.g. coleslaw, pâté, soft cheese, unpasteurized milk).

Genus *Clostridium*

This genus contains many species of Gram-positive anaerobic spore-forming rods; a few are aerotolerant. Widely distributed in soil and in the gut of humans and animals. The spores are resistant to environmental conditions. The major diseases associated with species of the genus are gangrene, tetanus, botulism, food poisoning and pseudomembranous colitis. In each of these, the production of potent protein exotoxins is an important cause of pathology, and in several species the genes encoding toxins are carried by plasmids or bacteriophages.

Clostridium perfringens

Characteristics	Anaerobic Gram-positive rods; spore forming, but spores rarely seen in infected material. More tolerant of oxygen than other clostridia.
Laboratory identification	Haemolytic colonies on blood agar incubated anaerobically. Identification confirmed by demonstration of alpha-toxin (lecithinase) production in the Nagler's test. Germination of heat-resistant spores (with subsequent toxin production) may be responsible for food poisoning. Five types of *C. perfringens* (A–E) identified on the basis of toxins produced; type A strains can be further divided into several serotypes.
Diseases	Gas gangrene resulting from infection of dirty ischaemic wounds. Food poisoning following ingestion of food contaminated with enterotoxin-producing strains.
Transmission	Spores and vegetative organisms widespread in soil and normal flora of humans and animals. Infection acquired by contact; may be endogenous (e.g. wound contaminated from patient's own faecal flora) or exogenous (e.g. contamination of a wound with soil, ingestion of contaminated food).
Pathogenesis	In ischaemic wounds, production of numerous toxins and tissue-destroying enzymes allows organism to establish itself and multiply in wound. Local action of toxins produces necrosis thereby further impairing blood supply and keeping conditions anaerobic, and aiding spread of organism into adjacent tissues. Food poisoning results from the ingestion of large numbers of vegetative cells, which sporulate in the gut and release enterotoxin.
Treatment and prevention	Gangrene requires rapid intervention with extensive debridement of the wound. Penicillin plus clindamhycin or tetracycline are examples of appropriate therapies. Hyperbaric oxygen may also be helpful. Food poisoning does not usually require specific treatment.

Clostridium tetani

Characteristics	Gram-positive spore-forming rod with terminal round spore (drumstick). Strict anaerobe.
Laboratory identification	Grows on blood agar in anaerobic conditions as a fine spreading colony; 'ground glass' appearance (hand lens inspection of cultures important). Has very little biochemical activity useful for identification purposes. Demonstration of toxin in a specimen is possible in a two-mouse model in which one animal is protected with antitoxin, the other unprotected (performed in Public Health reference laboratories).
Diseases	Tetanus (lockjaw). Severe disease characterized by tonic muscle spasms and hyperflexia, trismus, opisthotonos and convulsions.
Transmission	Organism widespread in soil. Acquired by humans by implantation of contaminated soil into wound. Wound may be major (e.g. in war, in road traffic accident) or minor (e.g. a rose-thorn puncture while gardening). No person-to-person spread.
Pathogenesis	Tetanus results from neurotoxin (tetanospasmin) produced by organisms in wound. Toxin genes are plasmid-encoded. The organism is non-invasive, but the toxin spreads from site of infection via bloodstream and acts by binding to ganglioside receptors and blocking release of inhibitory neurotransmitters. Causes convulsive contractions of voluntary muscles.
Treatment and prevention	Antitoxin is available (hyperimmune human gamma globulin; tetanus immune globulin). Metronidazole and spasmolytic drugs indicated. Prevention readily available and effective in form of immunization with toxoid. Usually given in childhood, but if immunization status of injured patient is unknown, toxoid is given in addition to antitoxin.

Clostridium botulinum

Characteristics	Anaerobic Gram-positive rods. Not easily cultivated in competition with other organisms. Produces most potent toxins known to man. Seven immunologically distinct toxins (A to G) produced by different strains of *C. botulinum*. Types A, B, E and F are most commonly associated with human disease: serotypes A and B linked to a variety of foods (e.g. meat), serotype E especially associated with fish.
Laboratory identification	Requires strictly anaerobic conditions for isolation. Grows on blood agar, but very rarely isolated from human cases of disease. Detection of the toxin or organisms in the food or detection of the toxin or organisms in the serum or faeces of the patient, respectively, is the way of confirming the diagnosis.
Diseases	Major pathogen of birds and mammals, rare in humans. Botulism acquired by ingesting preformed toxin. Disease entirely due to effects of toxin. Infant botulism results from ingestion of organisms and production of toxin in infant's gut. Associated with feeding honey contaminated with spores of *C. botulinum*. Wound botulism: toxin produced by organisms infecting a wound. Extremely rare.
Transmission	Soil is the normal habitat. Intoxication most often by ingestion of toxin in foods that have not been adequately sterilized (e.g. home-preserved foods) and improperly processed cans of food. Toxin is associated with germination of spores. There is no person-to-person spread.
Pathogenesis	Toxin released from organism as inactive protein and cleaved by proteases to uncover active site. It is acid stable and survives passage through stomach. Taken up through stomach and intestinal mucosa into bloodstream. Acts at neuromuscular junctions inhibiting acetylcholine release. Results in muscle paralysis and death from respiratory failure.
Treatment and prevention	Supportive therapy is paramount. Trivalent antitoxin is available. In the rare cases of infant and wound botulism (i.e. when the organism is growing in vivo), penicillin and metronidazole are effective. Prevention relates to good manufacturing practice. The toxin is not heat stable, therefore adequate cooking of food before consumption will destroy it.

Clostridium difficile

Characteristics	Slender Gram-positive anaerobic rod; spore former; motile.
Laboratory identification	Difficult to isolate in ordinary culture because of overgrowth by other organisms; selective medium CCFA (cycloserine–cefoxitin–fructose agar) may be helpful; however, mere presence of the organism is not indicative of infection. Diagnosis by detection of toxin in faeces (e.g., immunoassay) or molecular detection of toxin genes.
Diseases	Pseudomembranous colitis (antibiotic-associated diarrhoea). Can be rapidly fatal especially in the compromised host.
Transmission	Component of normal gut flora; flourishes under selective pressure of antibiotics. May also be spread from person to person by the faecal–oral route.
Pathogenesis	Toxin-mediated damage to gut wall. Produces both an enterotoxin (toxin A) and cytotoxin (toxin B).
Treatment and prevention	Oral vancomycin or metronidazole. Other antibiotics should be withheld if possible. Prevention of cross-infection in hospitals depends upon scrupulous attention to hygiene.

Genus *Mycobacterium*

	Mycobacteria are widespread both in the environment and in animals. The major human pathogens are *M. tuberculosis* and *M. leprae*, but awareness of the importance of other species (e.g. *M. avium* complex) is increasing with their recognition as pathogens in AIDS and other immunocompromised patients.
Characteristics	Aerobic rods with a Gram-positive cell wall structure, but stain with difficulty because of the long-chain fatty acids (mycolic acids) in the cell wall. Acid fastness can be demonstrated by resistance to decolorization by mineral acid and alcohol (Ziehl–Neelsen stain). Mycobacteria grow more slowly than many other bacteria of medical importance, but the genus can be divided into: rapid growers (form visible colonies within c. 3–7 days); slow growers (form visible colonies only after c. 2 weeks to 2 months' incubation).
Laboratory identification	Staining and microscopic examination of specimens for acid-fast rods are important because of the time required for culture results. All species except *M. leprae* can be grown in artificial culture, but they require complex media. Identification is based on rate of growth (rapid or slow), optimum temperature of growth and pigment production. Scotochromogenic species produce pigment in the absence of light whereas photochromogenic species require exposure to light before pigment becomes apparent. Further biochemical tests are required for full specification. Polymerase chain reaction methods, DNA probes and sequence-based approaches are available for identification purposes.
Diseases	*M. tuberculosis* causes tuberculosis in humans and animals. *M. leprae* is restricted to humans and causes leprosy. Mycobacteria other than tuberculosis (MOTT) are associated with a range of conditions, usually in immunocompromised hosts. *M. avium–intracellulare* (*M. avium* complex) has important associations with AIDS patients in the USA; in Africa *M. tuberculosis* is more common.
Transmission	Droplet spread aided by ability of organisms to survive in the environment (*M. tuberculosis, M. leprae*). Unpasteurized milk from cattle infected with *M. bovis* has been responsible for human infections in the past. Social and environmental factors and genetic predisposition all have a role. Leprosy requires close and prolonged contact for spread.
Pathogenesis	Both *M. tuberculosis* and *M. leprae* are intracellular parasites surviving within macrophages. They give rise to slowly developing, chronic conditions in which much of the pathology is attributable to host immune responsiveness rather than to direct bacterial toxicity.
Treatment and prevention	Prolonged treatment with combinations of antimycobacterial drugs is required. Bacille Calmette-Guérin (BCG) vaccination is valuable for prevention in endemic areas. Isoniazid prophylaxis used for contacts of cases of tuberculosis. Pasteurization of milk and improvement of living conditions have played a major role in prevention.

Genus Actinomyces

The actinomycetes are true bacteria, although they have in the past been considered to resemble fungi because they form branching filaments. They are related to the corynebacteria and mycobacteria in the chemical structure of their cell walls. It is important to differentiate them from fungi because infections with actinomycetes should respond to antibacterial agents whereas similar clinical presentations caused by fungi are resistant to antibacterials (and extremely refractory to treatment by antifungal agents). This genus contains many species, some of which are important to humans as producers of antimicrobial agents. A few are pathogenic to humans and animals; *A. israelii* is a key cause of actinomycosis.

Actinomyces israelii

Characteristics	Gram-positive anaerobic filamentous branching rods. Non-spore-forming, non-acid fast.
Laboratory identification	Forms 'sulphur granules' composed of a mass of bacterial filaments in pus. These can be identified by washing pus, squashing granules and observing in stained microscopic preparations. Gram-positive branching rods also visible in stained pus. Forms characteristic breadcrumb or 'molar tooth' colonies on blood agar after 3–7 days anaerobic incubation at 35°C.
Diseases	Actinomycosis follows local trauma and invasion from normal flora. Hard non-tender swellings develop which drain pus through sinus tracts. Cervicofacial lesions are most common, but abdominal lesions after surgery and infection related to intrauterine contraceptive devices also occur.
Transmission	*A. israelii* is part of normal flora in mouth, gut and vagina. Infection is endogenous. There is no person-to-person spread.
Pathogenesis	Virulence factors not described.
Treatment and prevention	Penicillin is the drug of choice. Prolonged treatment is required, accompanied by surgical drainage.

Genus *Nocardia*

Characteristics	Aerobic Gram-positive rods that form thin branching filaments. Widespread in the environment. *N. asteroides* complex represents the important human pathogens.
Laboratory identification	Gram stains of pus may reveal Gram-positive filaments or rods. Sulphur granules not seen. Grow as 'breadcrumb' colonies on blood agar within 2–10 days' incubation. Often weakly acid fast. Catalase positive.
Diseases	*N. asteroides* complex are opportunistic pathogens especially infecting immunocompromised patients; primarily a pulmonary infection, but secondary spread to form abscesses in brain or kidney is common. *N. brasiliensis* is the cause of actinomycetoma in Central and South America.
Transmission	Infection is acquired from the soil by the airborne route. Outbreaks of infection in renal transplant units have been associated with local building work. Actinomycetoma is acquired by implantation of organisms into wounds and progressive destruction of skin, fascia, bone and muscle.
Pathogenesis	Appears to be related to organism's ability to survive the host's inflammatory responses. Infection is controlled by cell-mediated immunity, but this may be defective in immunocompromised patients.
Treatment and prevention	Nocardiosis is often difficult to treat, but most regimens include sulphonamides as the drug of choice.

Gram-negative Rods

Enterobacteriaceae

Most numerous facultative anaerobes in the human gut, comprising approximately 10^9/g of faeces. Outnumbered only by Gram-negative anaerobes (e.g. *Bacteroides*), which are present in numbers approximately 10 times those of the enterobacteria. Genera of the family Enterobacteriaceae share features that distinguish them from other families; can be distinguished from each other by biochemical tests.

Genus *Escherichia*

Genus contains only one species of medical importance: *E. coli*.

Escherichia coli

Characteristics	Gram-negative rod; motile; with or without capsule; non-fastidious, facultative anaerobe; bile tolerant; capable of growth at 44°C.
Laboratory identification	Grows readily on routine laboratory media and on bile-containing selective media. Lactose fermenter. Kits available for full identification.
Diseases	UTIs; diarrhoeal diseases; neonatal meningitis; septicaemia.
Transmission	Normal habitat is gut of humans and animals; may colonize lower end of urethra and vagina. Spread is by contact and ingestion (faecal–oral route); may be food-associated; may be endogenous. Possesses O (somatic), H (flagellar), K (capsular) and F (fimbrial) antigens, which can be used to characterize strains by serotyping (e.g. O157:H7 EHEC strains, see below). Pulsed-field gel electrophoresis and whole genome sequencing used for epidemiological analysis.
Pathogenesis	A variety of virulence factors have been identified, particularly in strains associated with diarrhoeal disease: • endotoxin: present in all strains • adhesins: P fimbriae (pili) associated with UTIs; colonization factors (e.g. CFA I, II and III, K88, K99) associated with gastrointestinal tract infection in humans and animals • capsule present in some strains; may be associated with adhesion; K1 capsular type associated with neonatal meningitis • enterotoxins associated with diarrhoeal disease: ETEC (enterotoxigenic *E. coli*) produce heat-stable (ST) and cholera-like heat-labile (LT) toxins; EIEC (enteroinvasive *E. coli*) produce shiga-like cytotoxin; EHEC (enterohaemorrhagic *E. coli*) produce verotoxin-associated with haemolytic uraemic syndrome.
Treatment and prevention	Wide range of antibacterial agents potentially available, but incidence of resistance variable and often plasmid-mediated; must be determined by susceptibility testing. Specific treatment of diarrhoeal disease usually not required. No currently available vaccine.

Genus *Proteus*

	This genus contains several species, of which two are of medical importance: *P. mirabilis* and *P. vulgaris*.
Characteristics	Gram-negative rod; non-fastidious; facultative anaerobe; bile tolerant; likes alkaline pH; characteristic unpleasant odour; highly motile and swarms on some media.
Laboratory identification	Lactose non-fermenter; produces urease; kits available for full identification. Species can be distinguished by indole test: *P. mirabilis*, indole negative; *P. vulgaris*, indole positive. O (somatic) and H (flagellar) antigens characterize. Proteus strains OX-19, OX-2 and OX-K share antigens with Rickettsiae in the typhus and spotted fever groups and are agglutinated by antibodies produced by patients with these rickettsial infections (Weil–Felix test). Serological response to *Proteus* infection not useful diagnostically.
Diseases	Urinary tract infection; hospital-acquired wound infection, septicaemia, pneumonia in the compromised host.
Transmission	Normal habitat is human gut, soil and water. Contact spread; infection often endogenous.
Pathogenesis	Characterized virulence factors include endotoxin and urease; possible role for bacteriocins.
Treatment and prevention	Range of agents available. Prevention is by good aseptic technique in hospitals. No vaccine available.

Genus *Klebsiella* and Related Enterobacteria *Serratia* and *Enterobacter*

	Unlike *E. coli*, species of the genera *Klebsiella, Serratia* and *Enterobacter* are rarely associated with infection except as opportunists in compromised patients.
Characteristics	Gram-negative rods, sometimes capsulate (usual for *Klebsiella*), non-fastidious growth requirements. Capable of aerobic and anaerobic respiration.
Laboratory identification	Lactose-fermenting, bile-tolerant organisms. Grow readily on routine laboratory media. Oxidase negative. Full identification based on biochemical reactions (commercial kits available).
Diseases	Opportunist infections in the compromised (usually hospitalized) host. Urinary and respiratory tracts are most common sites of infection. Distinction between colonization and infection can sometimes be difficult.
Transmission	Normal habitat is gut of humans and animals and moist inanimate environments, especially soil and water. Infection may be endogenous or acquired by contact spread. *Klebsiella* have remarkable capacity for survival on hands. Pulsed-field gel electrophoresis most commonly used for epidemiological investigation of healthcare-associated infection.
Pathogenesis	All possess endotoxin and fimbriae or other adhesins. Capsules, where present, are important in inhibiting phagocytosis.
Treatment and prevention	Multiple antibiotic resistance, usually plasmid-mediated, is common, and susceptibility must be determined by laboratory tests if treatment is indicated. Prevention depends upon scrupulous attention to aseptic techniques and to hand washing in hospitals.

Genus *Salmonella*

Unlike other members of the Enterobacteriaceae, *Salmonella* and *Shigella* are not normal inhabitants of the human gut (except in post-infection carriers). Both genera are responsible for diarrhoeal disease, which may be severe; *Salmonella* may also cause bacteraemia (most commonly associated with *S. typhi, S. paratyphi* and *S. choleraesuis*).

Regarding taxonomy, the Kauffmann–White classification recognizes each serologically distinct *Salmonella* (of which there are over 2000) as a species (a convention also retained here). These designations are arranged in groups based on the serological identification of O (somatic) and H (flagellar) antigens. DNA hybridization studies now indicate only two *Salmonella* species. Within *S. enterica* (the most important for human infection) six subgroups (A, B, C1, C2, D and E) can be distinguished. Distinction on the basis of infection is between *S. typhi, S. paratyphi, S. schottmuelleri* (formerly *S. paratyphi B*) and *S. hirschfeldii* (formerly *S. paratyphi C*), which cause enteric fevers, and other serotypes (e.g. *S. enteritidis*), which cause diarrhoeal disease.

Kauffmann–White classification

Group	Name*	Somatic (O) antigen	Flagella (H) antigen phase I	Flagella (H) antigen phase II
A	*S. paratyphi A*	1, 2, 12	a	
B	*S. schottmuelleri* *S. typhimurium*	1, 4, 5, 12 1, 4, 5, 12	B i	1, 2 1, 2
C1	*S. choleraesuis*	6, 7	c	1, 5
D	*S. typhi* *S. enteritidis*	9, 12, Vi 1, 9, 12	d g, m	

*Examples of a few important species only.

Characteristics

Gram-negative, motile non-spore-forming rods. All except *S. typhi* are non-capsulate. Capable of aerobic and anaerobic respiration.

Laboratory identification

Bile tolerant. Non-fastidious. Oxidase negative. Lactose non-fermenters. Produce acid and gas from glucose (except *S. typhi*, which is anaerogenic). Combination of biochemistry (commercial kits available) and serotyping required for full identification; important to distinguish enteric fever salmonellae from others. Detection of circulating antibody (Widal test) may aid diagnosis of enteric fevers. While serotyping (and phage typing of most important serotypes) is useful for investigation of outbreaks, molecular approaches such as pulsed-field gel electrophoresis are more definitive.

Diseases

Vast majority cause diarrhoeal disease; very occasionally invasive (particularly *S. choleraesuis*). Sickle cell disease predisposes to osteomyelitis. *S. typhi*, *S. paratyphi*, *S. schottmuelleri* (formerly *S. paratyphi B*) and *S. hirschfeldii* (formerly *S. paratyphi C*) cause systemic disease, typhoid and paratyphoid (enteric fevers).

Transmission

Widespread in animals; encountered in food chain (especially in poultry, eggs, meat, milk, and cream). Acquired by ingestion of contaminated food or person to person via faecal–oral route. *S. typhi* and *S. paratyphi* are human pathogens only. Spread via faecal–oral route, usually via contaminated water or food. Carriers are important source of organisms.

Treatment and prevention

S. typhi and *S. paratyphi* infections should be treated with systemic antibiotics based on susceptibility tests. Antibiotic resistance is an increasing problem in many countries (important implications for travellers). Salmonella diarrhoea should not be treated with antibiotics unless there is evidence of invasive disease. Prevention depends upon interrupting faecal–oral transmission and on eliminating opportunities for transmission via the food chain. Vaccines are available to protect against *S. typhi* and *S. paratyphi*.

Genus *Shigella*

	Contains four species of importance to humans as causes of bacillary dysentery: *S. dysenteriae, S. boydii, S. flexneri* and *S. sonnei* (in descending order of severity of symptoms).
Characteristics	Gram-negative rods. Non-motile (in contrast to salmonellae). Non-capsulate. Capable of aerobic and anaerobic respiration.
Laboratory identification	Non-fastidious, bile-tolerant. Lactose non-fermenters. Full identification requires use of biochemistry (commercial kits available) and serological tests for O antigens. Serodiagnosis of disease not applicable.
Diseases	Bacillary dysentery.
Transmission	Human pathogens spread by faecal–oral route, especially in crowded conditions. Small infective dose.
Pathogenesis	Invasion of ileum and colon causes damage, which results in diarrhoea. Intense inflammatory response involving neutrophils and macrophages characteristic. *S. dysenteriae* produces an exotoxin (Shiga toxin) causing damage to intestinal epithelial cells. In fewer instances, the toxin results in damage to glomerular endothelial cells, leading to haemolytic uremic syndrome (HUS).
Treatment and prevention	Antibiotic therapy (e.g. fluoroquinolones, trimethoprim-sulphamethoxazole) should be given only for severe diarrhoea; usually not required. Many strains carry multiple antibiotic resistances, usually on plasmids; thus susceptibility testing is important. Prevention depends upon interrupting faecal–oral spread; hand hygiene important. No vaccine available.

Genus *Pseudomonas* and Related Organisms *Burkholderia*, *Stenotrophomonas* and *Acinetobacter*

This group contains a large number of species, a few of which are human pathogens, some are animal pathogens and others are important pathogens of plants. Species also widely distributed and may contaminate the hospital environment and cause opportunist infections. Most important in humans are:

- *P. aeruginosa*, an important opportunist in a variety of compromised patients
- *B. pseudomallei*, cause of melioidosis, a disease of restricted geographical distribution
- *B. cepacia*, commonly associated with nosocomial infection and respiratory tract infections in cystic fibrosis patients
- *S. maltophilia*, an opportunistic pathogen also commonly associated with nosocomial infection
- *A. baumannii* (and other species), opportunistic pathogens causing a variety of infections (e.g. wound, respiratory tract, urinary tract); frequently antibiotic resistant.

Pseudomonas aeruginosa

Characteristics	Aerobic Gram-negative rod, motile by means of polar flagella. Able to utilize a very wide range of carbon and energy sources and to grow over a wide temperature range. Does not ferment carbohydrates. Does not grow anaerobically (except when nitrate is provided as a terminal electron acceptor).
Laboratory identification	Grows readily on routine media including bile-containing selective media. Produces irregular iridescent colonies and a characteristic smell. Most strains produce a blue-green pigment (pyocyanin; unique to *P. aeruginosa*) and a yellow-green pigment (pyoverdin). Pigment production is enhanced on special media (King's A and B); oxidase positive.
Diseases	*P. aeruginosa* is an opportunist pathogen that can infect almost any body site given the right predisposing conditions. It causes infections of skin and burns, it is a major lung pathogen in cystic fibrosis, and can cause pneumonia in intubated patients. It can also cause UTIs, septicaemia, osteomyelitis and endocarditis.
Transmission	Carriage as part of the normal gut flora occurs in a small percentage of normal healthy people and in a higher proportion of hospital inpatients. Thus, endogenous infection may occur in compromised patients. *P. aeruginosa* is widespread in moist areas in the environment; patients usually become infected by contact spread, directly or indirectly, from these environmental sites.
Pathogenesis	A number of virulence factors have been identified, including endotoxin and exotoxin A, which acts as an inhibitor of elongation factor in eukaryotic protein synthesis. Extracellular polysaccharide capsule helps to prevent phagocytosis (e.g. massive amounts of alginate produced by strains specifically in cystic fibrosis patients). Pigments may have a role in pathogenicity, and pyoverdin acts as a siderophore.

Pseudomonas aeruginosa—cont'd

Treatment and prevention	Resistant to many antibacterial agents; propensity to develop resistance during therapy. Combination antimicrobial chemotherapy based on susceptibility testing is required (e.g. aminoglycoside and beta-lactam antibiotic). Prevention depends upon good aseptic practice in hospitals, avoidance of unnecessary or prolonged broad-spectrum antibiotic treatment and prophylaxis.

Curved Gram-Negative Rods

There are several genera of curved Gram-negative rods containing species that occur in humans as pathogens. Three of the most important are *Vibrio*, *Campylobacter* and *Helicobacter*.

Genus *Vibrio*

	Most important species is *V. cholerae*.
Characteristics	Curved Gram-negative rods, highly motile by means of single polar flagellum. Capable of aerobic and anaerobic respiration (facultatively anaerobic). Many species are salt (NaCl) tolerant; some salt requiring.
Laboratory identification	Grow in alkaline conditions (can be selected from other gut flora in alkaline peptone water). Oxidase positive. Grow on thiosulphate citrate bile salts sucrose (TCBS) medium to form yellow colonies (*V. cholerae*) or green colonies (other species). Biochemical tests and use of specific antisera required for complete identification.
Diseases	Cholera caused by *V. cholerae*. *V. parahaemolyticus* causes diarrhoeal disease. *V. vulnificus* causes wound infections and bacteraemia.
Transmission	*V. cholerae* is a human pathogen; no animal reservoir, but El Tor biotype survives better in the inanimate environment than classic *V. cholerae*. Infection is acquired from contaminated water (usually) or food (sometimes). *V. parahaemolyticus* and *V. vulnificus* acquired from consumption of contaminated fish and seafood.
Pathogenesis	*V. cholerae* possesses several virulence factors (e.g. mucinase, adhesins and, most importantly, enterotoxin). Chromosomally encoded subunit toxin produced after cells bind to intestinal epithelium enters cells and binds to ganglioside receptors activating adenyl cyclase and causing fluid loss, resulting in massive watery diarrhoea. *V. parahaemolyticus* produces a cytotoxin (which also haemolyses human red blood cells – the Kanagawa test). *V. vulnificus* produces cytolytic compounds and antiphagocytic polysaccharides.
Treatment and prevention	For cholera, fluid replacement (oral rehydration therapy: ORT) is of prime importance. Tetracycline shortens symptoms and duration of carriage. Some vaccine protection. Prevention of cholera depends upon provision of a clean (chlorinated) water supply and adequate sewage disposal. Specific treatment not indicated for *V. parahaemolyticus* diarrhoea. Combination treatment (e.g. a tetracycline plus fluoroquinolone) used in treatment of *V. vulnificus*, *V. parahaemolyticus* and *V. vulnificus* infections. Can be prevented by adequate cooking of seafood.

Genus *Campylobacter*

Curved Gram-negative rods once classified as vibrios, campylobacters are primarily pathogens of animals, but several species also cause infections in humans. *C. jejuni* is a major cause of bacterial gastroenteritis in resource-rich countries. At a much lower frequency, *C. coli* also causes gastroenteritis. The infections caused by these organisms have an essentially identical clinical presentation, and laboratories generally do not distinguish between them.

Campylobacter jejuni

Characteristics	Slender, curved (seagull-shaped) Gram-negative rods. Motile by means of a polar flagellum at one or both ends. Microaerophiles. Do not utilize carbohydrate.
Laboratory identification	Require enriched media and moist microaerophilic environment (10% O_2) for growth. Incubation at 42°C for 24–72 h. Colonies resemble water drops. Full identification by biochemical tests and characteristic antibiotic susceptibility pattern.

Continued

Campylobacter jejuni—cont'd

Diseases	Diarrhoea. Can invade to give septicaemia. Guillain–Barré syndrome infrequently associated with *Campylobacter* disease.
Transmission	Animal reservoir. Organisms acquired from contaminated food and milk (but do not multiply in these vehicles). Person-to-person spread is rare.
Pathogenesis	Little known, but cytotoxin implicated. Also invasion and local destruction of gut mucosa.
Treatment and prevention	No specific treatment necessary for diarrhoea. First-line agents for treatment for invasive disease include fluoroquinolones or azithromycin. Prevention depends upon good food hygiene. No vaccine.

Helicobacter pylori

Characteristics	Associated with gastritis and duodenal ulcers; originally named *C. pylori* but now moved into the genus *Helicobacter*. Overall cellular morphology similar to *Campylobacter*.
Laboratory identification	Require enriched media and moist microaerophilic environment (10% O_2) for growth. Incubation at 37°C for 24–72 h produces translucent colonies. Differentiated from *Campylobacter* by tests such as nitrate reduction (*C. jejuni*, positive; *H. pylori*, negative) and urease (*C. jejuni*, negative; *H. pylori*, positive). Full identification by biochemical tests and characteristic antibiotic susceptibility pattern. Organism in endoscopic biopsy specimens; positive urease test from endoscopic biopsy specimens or labelled urea breath-test also very useful.
Diseases	Gastritis and duodenal ulcers, associated with gastric carcinoma.
Transmission	Person-to-person transmission (faecal–oral) likely. Infections observed in multiple family members.
Pathogenesis	Both bacterial and host factors involved. Protease affects gastric mucosa; urease produces ammonia and buffers stomach acid. Some invasion of intestinal epithelium.
Treatment and prevention	Proton pump inhibitor plus antibiotics (e.g. clarithromycin, metronidazole, tetracycline).

Gram-Negative Non-Spore-Forming Anaerobes

Historically, all short Gram-negative anaerobic rods or coccobacilli have been classified in the genus *Bacteroides* and longer rods with tapering ends in the genus *Fusobacterium*. Recent applications of new techniques to the *Bacteroides* have resulted in the definition of two additional genera: *Porphyromonas* and *Prevotella*. The genus *Bacteroides* is now restricted to species found among the normal gut flora. *Prevotella* contains saccharolytic oral and genitourinary species, including *P. melaninogenica* (formerly *B. melaninogenicus*), which produces a characteristic black-brown pigment. The genus *Porphyromonas* contains asaccharolytic pigmented species, which form part of the normal mouth flora (*P. gingivalis*) and may be involved in endogenous infection within the oral cavity. The most important non-spore-forming anaerobe causing infection is *B. fragilis* although others are much more common (e.g. in gingivitis and other endogenous oral infections).

Bacteroides fragilis

Characteristics	Small pleomorphic Gram-negative rods or coccobacilli. Capable only of anaerobic respiration. Non-spore-forming, non-motile.
Laboratory identification	Grows on blood agar incubated anaerobically and in other media designed for isolation of anaerobes. Plates may require up to 48 h incubation at 35°C for colonies to become visible. Cultures have a foul odour due to the fatty acid end-products of metabolism. These can be used as identifying characteristics by analysis of culture supernates by gas–liquid chromatography (GLC). The major products of *Bacteroides* are acetate and succinate. Full identification in the diagnostic laboratory is based on biochemical tests and antibiogram. Commercial kits are available.
Diseases	Intra-abdominal sepsis; liver abscesses; aspiration pneumonia; brain abscesses; wound infections. Infections often mixed with aerobic and microaerophilic bacteria.
Transmission	Endogenous infection arising from contamination by gut contents or faeces is most common route of acquisition.

Bacteroides fragilis—cont'd

Pathogenesis	Little is known about the virulence factors of *B. fragilis*. A polysaccharide capsule and production of extracellular enzymes (e.g. enterotoxin) are important features. An anaerobic environment is essential and in mixed infections growth of aerobic organisms probably helps the growth of *Bacteroides* by using up available oxygen.
Treatment and prevention	Metronidazole, imipenem, or beta-lactam–beta-lactamase inhibitor combinations used in therapy. Many strains produce beta-lactamases and thus susceptibility to penicillin and ampicillin is unreliable. Prevention of endogenous infection is difficult; good surgical technique and appropriate use of prophylactic antibiotics are important in abdominal surgery.

Gram-negative Cocci

Genus *Neisseria*

	This genus contains several more or less fastidious species of which two, *N. gonorrhoeae* and *N. meningitidis*, are important human pathogens.
Characteristics	Non-motile Gram-negative diplococci with fastidious growth requirements: capnophilic; *N. meningitidis* is capsulate, *N. gonorrhoeae* is not.
Laboratory identification	Gram stains of pus or cerebrospinal fluid may reveal Gram-negative kidney-shaped diplococci, often intracellular (in polymorphs). Require supplemented media for growth (chocolate agar). *N. gonorrhoeae* easier to isolate on enriched media containing antibiotics to inhibit other organisms of normal flora from sample sites. The two species are differentiated by sugar utilization pattern. Kits available to detect *N. gonorrhoeae* nucleic acid in specimens. Latex agglutination test available for *N. meningitidis*.
Diseases	*N. gonorrhoeae*: gonorrhoea, and pelvic inflammatory disease and salpingitis in females; ophthalmia neonatorum in infants born to infected mothers. *N. meningitidis*: meningitis; occasionally septicaemia in absence of meningitis.
Transmission	Human pathogens; no animal reservoir. *N. gonorrhoeae* may be carried in genital tract, nasopharynx and anus. Spread by sexual or intimate contact. *N. meningitidis* carried in pharynx. Carriage rate in population increases during epidemics. Droplet spread. *N. meningitidis* has several immunologically distinct capsular types (e.g. A, B, C, Y, W135).
Pathogenesis	Several virulence factors have been identified. *N. gonorrhoeae*: pili or fimbriae act as adhesins; endotoxin; outer membrane proteins; protease production; resistance to lytic activity of serum; IgA proteases. *N. meningitidis*: the polysaccharide capsule is antiphagocytic; endotoxin and IgA protease also implicated.
Treatment and prevention	*N. gonorrhoeae*: resistance to first-line drugs now widespread; usual choice is beta-lactamase-stable cephalosporin (e.g. ceftriaxone). *N. meningitidis*: penicillin, ceftriaxone (or equivalent cephalosporin), or chloramphenicol. Prevention of gonorrhoea requires education, contact tracing. No vaccine available. Rifampicin is used for prophylaxis of close contacts of *N. meningitidis* meningitis. Bivalent, trivalent, and tetravalent (types A, C, Y, W135) vaccines available.

Genus *Moraxella*

M. catarrhalis, previously classified as *Branhamella catarrhalis*, is a Gram-negative coccus morphologically similar to *Neisseria*, but with less fastidious growth requirements. Formerly regarded as a commensal in the respiratory tract, it has been associated with a variety of infections, including bronchitis, bronchopneumonia, sinusitis and otitis media. The majority of strains produce beta-lactamase and may be involved in the 'protection' of more obvious pathogens, especially in the respiratory tract, by destroying penicillin or ampicillin administered as treatment.

Genus *Haemophilus*

The genus contains many species; *H. influenzae* and *H. ducreyi* are of medical importance.

Haemophilus influenzae

Characteristics	Small Gram-negative rods, frequently coccobacillary. Non-motile. Fastidious, capnophilic, facultative anaerobe. May be capsulate when isolated from site of infection.
Laboratory identification	Requires both haematin (X factor) and nicotinamide adenine dinucleotide (NAD, V factor) for growth (other species require one factor only). Grows on blood-containing enriched media. Larger colonies around colonies of other organisms that secrete V factor (e.g. *Staph. aureus*) (satellitism). Dependence on X and V used as indicator of identity. *H. influenzae* can also be distinguished from other species by its inability to produce porphyrin. Six antigenically distinct capsular types recognized (a–f). Although type b has been most frequently found in disease, this has changed with the introduction of vaccines against the type b strains. Capsulate organisms can be agglutinated by specific antisera and detected directly (e.g. by latex agglutination) in specimens.
Diseases	Capsular type b *H. influenzae* causes meningitis, osteomyelitis, epiglottitis and otitis. All are more common in children than in older age groups. Non-capsulate strains associated with acute exacerbations of chronic bronchitis. Invasive disease due to types c and f isolates has increased.
Transmission	Normal habitat is upper respiratory tract in humans and associated animals. Transmitted from person to person by airborne route. Osteomyelitis probably follows septicaemia from respiratory focus.
Pathogenesis	Polysaccharide capsule is important virulence factor. Outer membrane proteins and endotoxin may play a part, but no known exotoxin.
Treatment and prevention	Frequent beta-lactamase-producing strains. Ampicillin (or amoxicillin) may be used if isolates are susceptible. Third-generation cephalosporins (e.g. cefotaxime or ceftriaxone) are the usual alternatives. All children should be immunized with Hib vaccine. Rifampicin prophylaxis recommended for close contacts of *Haemophilus* meningitis.

Haemophilus ducreyi

Cause of the genital tract infection 'soft chancre'. Slender Gram-negative rods appearing in pairs or chains. Direct microscopic examination of smear from chancre can be diagnostic. Organism very susceptible to dehydration; inoculate plates in clinic. Requires enriched medium (as for *H. influenzae*, but with addition of antibiotics to inhibit growth of other genital tract organisms).

Genus *Bordetella*

	There are three species, of which one, *B. pertussis*, is of greatest medical importance.
Characteristics	Small Gram-negative coccobacilli. Slow growing and fastidious in its growth requirements.
Laboratory identification	Requires enriched medium (e.g. Bordet–Gengou or blood charcoal agar). Intolerant of fatty acids in medium. Fails to grow on routine blood agar (i.e. 5–7% blood). Requires 3–7 days' incubation in moist atmosphere. Iridescent bisected pearl colony type characteristic on Bordet–Gengou. Further identification by reaction with specific antisera. Nucleic acid amplification tests in development.
Diseases	Whooping cough (pertussis).
Transmission	Human pathogen spread by airborne route from cases of disease (healthy carriage not documented).
Pathogenesis	Several virulence factors, including tracheal cytotoxin, fimbrial antigen and endotoxin. Stimulates a lymphocytic response.
Treatment and prevention	Macrolides erythromycin or clarithromycin for cases and close contacts of whooping cough. Antibacterial therapy has little effect on clinical course, but may reduce infectivity and incidence of superinfection. Vaccine administered to young children in five doses together with diphtheria and tetanus toxoids.

Genus *Brucella*

	There are several species of the genus *Brucella*, each characteristically associated with an animal species. Four species: *B. abortus* from cattle, *B. suis* from pigs, *B. canis* from dogs and *B. melitensis* from goats, are most often found causing human zoonotic infections.
Characteristics	Small Gram-negative coccobacilli. Intracellular pathogens. Growth enhanced by erythritol in placenta of animals (not in humans).
Laboratory identification	Some strains slow-growing and fastidious, requiring complex growth media. Isolation from blood cultures improved by use of biphasic systems (e.g. Castaneda bottles containing both broth and agar). Usually require 3–5 days' incubation in CO_2-enriched environment, but some strains of *B. abortus* may take up to 4 weeks – important in investigation of fever of unknown origin (FUO). Identification is by biochemical reactions, patterns of resistance to certain dyes, and serological tests. The disease may be diagnosed by examination of patient's serum for antibodies.
Diseases	Undulant fever (brucellosis). Patients frequently present with FUO. Infection may become chronic if not adequately treated.
Transmission	Zoonotic infections transmitted to humans through consumption of contaminated milk or other unpasteurized dairy products (increasingly seen in individuals who prefer untreated products) and by direct contact (occupational hazard for veterinarians, abattoir workers and farmers).
Pathogenesis	Virulence associated with ability to survive intracellularly, especially in bone marrow, liver and spleen, and thus 'hide' from host defences. Erythritol is a growth stimulant for the organism in animals and accounts for the tropism of the organisms to the placenta and fetus. This is not true in humans.
Treatment and prevention	Doxycycline alone or in combination (e.g. with rifampin). Tetracyclines may not be tolerated during long treatment courses required; trimethoprim-sulphamethoxazole also effective. Recrudescence of infection is common. Prevention depends upon eliminating the disease from domestic animals by vaccination and pasteurization of milk.

Francisella tularensis

Characteristics	Small Gram-negative coccobacilli. Strict aerobe. Intracellular pathogen. The organism is found worldwide and occurs in a variety of wild and domestic animals.
Laboratory identification	Requires specialized medium (e.g. chocolate agar plates supplemented with cysteine) and lengthy incubation. Identification is by reaction with specific (i.e. anti-*Francisella*) antiserum. The diagnosis of disease may be aided by examination of patient's serum for antibodies. However, the long-term persistence of antibody may cloud discrimination of current from past disease. Antibody against *Brucella* may cross-react with *Francisella*.
Diseases	*F. tularensis* causes tularaemia (also known as glandular fever, deerfly fever or tick fever). Human disease is most commonly acquired from bite of an infected tick or contact with an infected animal (e.g. infected squirrels and rabbits). Tularaemia quickly develops after a short period of incubation (e.g. 3–4 days), potentially leading to high fever, chills, myalgia and malaise depending on the specific form of the disease (i.e. ulceroglandular, glandular, oculoglandular, oropharyngeal, pneumonic, gastrointestinal, typhoidal).
Transmission	Zoonotic infections transmitted to humans through contact with infected animals, the bite of infected fleas or ticks, or ingestion of contaminated meat.
Pathogenesis	Virulence associated with an antiphagocytic capsule and the ability to survive intracellularly in macrophages. *F. tularensis* is highly infectious with as few as 10 organisms causing disease. For this reason, the public health agencies, such as the World Health Organization and the US Centers for Disease Control, are concerned about its potential use as an agent of bioterrorism.
Treatment and prevention	Aminoglycosides are generally recommended. Prevention depends upon avoiding the vectors and reservoirs of infection and use of protective clothing and gloves. In the USA, a live attenuated vaccine is available for at-risk individuals (e.g. laboratory workers, hunters, trappers, etc.).

Pasteurella multocida

Characteristics	Facultatively anaerobic, small Gram-negative coccobacilli. Occurs as a commensal in the upper respiratory tract of many animals including livestock, poultry and domestic pets.
Laboratory identification	Gram stain of pus or other fluid specimen. Organisms grow well on ordinary bacteriological media at 37°C. Oxidase positive and catalase positive. Bipolar staining enhanced by Wright, Giemsa or Wayson stain.
Diseases	Infected animal (e.g. cat or dog) bite. Acute onset of redness, pain and swelling.
Transmission	Zoonotic (animal bite) infection.
Pathogenesis	Capsule.
Treatment and prevention	Treat animal bite as polymicrobial infection (e.g. a beta-lactam antibiotic such as amoxicillin combined with a beta-lactam inhibitor).

Genus *Yersinia*

A member of the family Enterobacteriaceae. This genus contains a variety of species, only a few of which are considered important human pathogens.

Yersinia pestis

Characteristics	Gram-negative rods, facultatively anaerobic, zoonotic.
Laboratory identification	Exhibits bipolar staining with special (e.g. Wright–Giemsa, Wayson) stains. Grows best on media containing blood or tissue fluids. Tentative identification by biochemical reactions. Definitive identification by immunofluorescence.
Diseases	Bubonic plague results from multiplication within monocytes with production of antiphagocytic proteins. On their reaching the lymph nodes an intense haemorrhagic inflammation develops. Dissemination via the bloodstream leads to haemorrhagic and necrotic lesions in multiple organs. Pneumonic plague results from inhalation leading to haemorrhagic consolidation and sepsis.
Transmission	Zoonotic infection transmitted to humans through the bite of fleas carried by rodents.
Pathogenesis	Multiple virulence factors including lipopolysaccharides with endotoxic activity, antiphagocytic envelope protein, and plasmid-encoded virulence factors. Concern has been expressed regarding the possible use of this organism as an agent of bioterrorism.
Treatment and prevention	Broad-spectrum cephalosporins, doxycycline, trimethoprim-sulphamethoxazole. Control and eradication of infected animals is important.

Yersinia enterocolitica

Characteristics	Gram-negative rods, zoonotic. A multitude of serotypes exist; however, depending on geographical origin, most causing human disease are serotype 03, 08, or 09.
Laboratory identification	Non-lactose-fermenting Gram-negative rods; urease positive and oxidase negative. Bipolar staining. Facultative anaerobe that grows best and is motile at 25°C but non-motile at 37°C. Diagnosis involves isolation of the organism from the patient's faeces or other body fluid (blood, vomit, etc.). Confirmation by biochemical and serological tests.
Diseases	*Y. enterocolitica* most commonly causes enterocolitis although extraintestinal infections may also (rarely) occur.
Transmission	Infection results from ingestion of contaminated food and drink (e.g. unpasteurized milk, raw pork, etc.). The organism adheres to and penetrates the terminal ileum, leading to non-specific ileocolitis with potential lymph node infection and bacteraemia. Symptoms include fever, abdominal pain and diarrhoea, which may be watery or bloody. *Y. enterocolitica* can grow at refrigeration temperatures and transmission by blood transfusion has been observed.
Pathogenesis	Multiple virulence factors including plasmid-encoded proteins related to adherence and invasion.
Treatment and prevention	Most enteric infections are self-limited. When necessary, treatment is usually with doxycycline, aminoglycosides, trimethoprim-sulphamethoxazole, or third-generation cephalosporins. Prevention includes avoiding contaminated food and drink.

Genus *Legionella*

	In the overall history of microbiology, this is one of the more recent discoveries, originally demonstrated by techniques used for virus isolation (e.g. growth in embryonated hens' eggs). In free-living state, can grow in water, but is difficult to cultivate on routine laboratory media. *L. pneumophila* is the pathogen of greatest medical importance.

Legionella pneumophila

Characteristics	In tissue appear as Gram-negative coccobacilli; pleomorphic on laboratory media; stain poorly with Gram's stain (and therefore easily missed). Fastidious growth requirements in laboratory.
Laboratory identification	Direct fluorescent antibody tests performed on sputum samples have the advantage of specificity, distinguishing *L. pneumophila* from environmental contaminants. However, relatively few organisms may be present in expectorated sputum. Silver-staining techniques are better than standard Gram-staining method. Require enriched media containing iron and cysteine and absorbents to remove fatty acids. Most require incubation for 3–5 days for growth. Produces small tenacious colonies. Further identification based on requirement for cysteine and serological characteristics. Diagnosis is often based on antibody detection rather than culture.
Diseases	Legionnaires' disease; one of the causes of atypical pneumonia. Pontiac fever, which may be caused by other species, is a less severe flu-like illness.
Transmission	Environmental saprophyte acquired by inhalation of contaminated water from showers, air-conditioning systems, cooling towers.
Pathogenesis	Virulence factors unclear, but intracellular survival in alveolar macrophages important. Host predisposition (e.g. immunocompromised, chronic lung disease) important.
Treatment and prevention	Fluoroquinolone or newer macrolide (azithromycin). No vaccine available; prevention depends upon maintenance of hot-water and air-conditioning systems, particularly in large buildings such as offices, hospitals and hotels.

Gardnerella vaginalis

Characteristics	Formerly known as *Haemophilus vaginalis* and *Corynebacterium vaginalis*. Gram-variable facultatively anaerobic rods.
Laboratory identification	Special culture requirements (e.g. increased levels of carbon dioxide). Vaginal epithelial cells covered with 'clue cells' (Gram-variable coccobacilli) and the amine or 'whiff' test (i.e. presence of fishy odour after addition of potassium hydroxide to a sample of vaginal discharge) helpful in diagnosis.
Diseases	Cause a variety of genitourinary infections but one of a number of organisms commonly associated with bacterial vaginosis. To a lesser extent may also be associated with genitourinary infections (i.e. lower urinary tract) in men.
Transmission	Transmitted by sexual contact.
Pathogenesis	Poorly understood.
Treatment and prevention	Metronidazole, clindamycin. Condom use may aid in prevention.

Spiral Bacteria

There are three genera of medical importance: *Treponema*, *Leptospira* and *Borrelia*.

Genus *Treponema*

	Regularly coiled spirochetes with a longer wavelength than *Leptospira*. Several species and subspecies are important human pathogens; others are members of the normal flora, especially in the mouth. *T. pallidum*, its subspecies *pertenue* and *T. carateum* are the most important species.
Characteristics	Individual cells too small to visualize by direct light microscopy; can be seen with dark-ground (dark-field) illumination or after silver impregnation or immunofluorescent staining. Cells are actively motile by means of flagella contained within the periplasmic sheath.
Laboratory identification	*T. pallidum* and closely related species cannot be grown in artificial media; diagnosis of infection depends upon microscopic examination of fluid from primary lesions and on serology.
Diseases	*T. pallidum*: syphilis. *T. pallidum-pertenue* and *T. carateum*: the non-sexually transmitted treponematoses, yaws and pinta.
Transmission	Very susceptible to heat and drying, so successful transmission depends upon very close contact. *T. pallidum* is spread by close sexual contact and may also be vertically transmitted in utero. Yaws and pinta spread by direct contact from infected skin lesions. No animal reservoir.
Pathogenesis	Study of virulence factors hampered by the inability to grow *T. pallidum* in artificial culture media. Disease presents characteristically in three phases: after local primary infection, organisms widely disseminate in the body and may become quiescent for months or years. Immunopathology plays a major role in causing damage to the host, particularly in the tertiary stage of disease.
Treatment and prevention	Penicillin is the treatment of choice for syphilis. Doxycycline or tetracycline may be given to penicillin-allergic patients. Prevention depends upon detection and treatment of cases, contact tracing and serological testing of pregnant women. Possible cross-reactions between *T. pallidum* and the species causing yaws and pinta must be noted.

Genus *Leptospira*

	Two species: *L. interrogans* and *L. biflexa*; the former is parasitic, the latter contains free-living species. Within the species *interrogans* there are several different serogroups and serovars responsible for disease in humans and animals.

Leptospira interrogans

Characteristics	Finely coiled spirochetes with hooked ends. Cells 0.1–0.2 μm in diameter, up to 20 μm in length. Not visible by direct light microscopy unless stained by silver impregnation or immunofluorescent methods. Dark-ground (dark-field) microscopy reveals rotational and directional motility by means of periplasmic flagella.
Laboratory identification	Direct microscopy of blood and urine possible, but difficult to interpret. *Leptospira* can be grown, with difficulty, in special serum-containing media. Serological diagnosis is usual. Commercial kits available.
Diseases	Leptospirosis or Weil's disease in humans and animals.
Transmission	Leptospirosis in humans is a zoonosis, usual hosts being rodents, bats, cattle, sheep, goats and other domestic animals. Leptospires excreted in urine contaminate food and water. Infection occurs by contact either through occupation (e.g. sewer workers, farmers, abattoir workers) or recreation (e.g. canoeing, windsurfing on inland waters). Organisms may penetrate unabraded skin and conjunctiva.
Pathogenesis	After initial invasion, there is haematogenous spread before the organisms localize in various organs including the liver and kidney. Subclinical infection is common in endemic areas.
Treatment and prevention	Doxycycline or azithromycin are normally used. Disease may be prevented after exposure by doxycycline.

Genus *Borrelia*

	Two species of *Borrelia* are of importance in humans: *B. burgdorferi* causes Lyme disease; *B. recurrentis* causes relapsing fever.
Characteristics	Less finely coiled than the leptospires. Cells 0.2–0.5 μm in diameter; stain readily, so are visible by light microscopy.
Laboratory identification	Microaerophilic, complex nutritional requirements, long growth time (weeks) thus culture is not routinely used for identification. *B. recurrentis* demonstrated in blood smears by staining with Giemsa or acridine orange. *B. burgdorferi* much more difficult to visualize. Culture from biopsy material possible, but difficult; diagnosis usually by serology.
Diseases	In relapsing fever, the relapsing element may be due to antigen switching. Lyme disease slowly progressive rather than relapsing. Characteristic 'bull's eye' skin lesion (erythema chronicum migrans) commonly occurs. Joint pains and fatigue common and later, in untreated cases, neurological and cardiac manifestations.
Transmission	*B. recurrentis* spread from person to person by lice. Lyme disease is a zoonosis transmitted to humans by hard ticks (*Ixodes* spp.) associated with deer. Ticks are found on bracken and undergrowth and attach to exposed skin. Tick bite is often unnoticed, but less than a minute is required for the organisms to enter the host.
Pathogenesis	Little is known about the pathogenesis of either disease. Antigen switching in *B. recurrentis* presumably allows evasion of host's antibody response.
Treatment and prevention	Penicillins and tetrayclines used successfully. Prevention depends upon avoiding contact with vectors (e.g. protective clothing for walkers and forestry workers).

Other Bacteria

Mycoplasmas

Characteristics	Distinguished from other prokaryotes and placed in the class Mollicutes because they lack a true cell wall and consequent rigidity. This is a stable characteristic exhibited by genera such as *Mycoplasma* and *Ureaplasma* and is distinct from cell-wall-deficient and L-forms of other species. The outer membrane, the outermost layer, functions as the major antigenic interface. It is a flexible triple-layered structure of proteins and lipids. Many species also contain cholesterol in the membrane, which is absent from other bacterial cells. The important species is *M. pneumoniae*, but *M. hominis* and *U. urealyticum* may cause genital tract infections.
Laboratory identification	Many species are fastidious, and complex media and soft agar may be required for satisfactory culture. Cultures incubated for at least 7 days, although some species (e.g. *M. hominis*) grow readily on moist blood agar plates within 48 h. Cells variable in size (up to 100 μm but many smaller than 0.5 μm) and morphology; cannot be stained by Gram stain (no cell wall), but impressions of colonies can be stained with Dienes or Romanowsky stains. Diagnosis of infection is based on serology because of difficulties of culture.
Diseases	*M. pneumoniae* is an important cause of 'atypical pneumonia'. Mycoplasmas are also associated with genital infections (e.g. non-gonococcal urethritis) and with joint and other inflammatory infections. Other mycoplasmas are important pathogens of animals and birds.
Transmission	Transmission of *M. pneumoniae* is from person to person by airborne route. Other mycoplasmas and ureaplasmas can be transmitted by sexual contact.
Pathogenesis	Surface protein adhesin binds *M. pneumoniae* to sialoglycolipids on respiratory epithelium of host. Other virulence factors are not yet clearly understood.
Treatment and prevention	Doxycycline or erythromycin (note that the lack of cell wall target means lack of susceptibility to beta-lactams). No vaccine currently available. Prevention by interruption of spread is difficult.

Rickettsiae

Characteristics	These organisms have requirement for coenzyme A, NAD and adenosine triphosphate (ATP), which they cannot supply themselves, and are therefore obligate intracellular parasites; with rare exceptions they need to be grown in cell cultures or experimental animals.
Laboratory identification	Small (0.7–2 μm diameter), Gram-negative bacteria. Isolation in laboratory is difficult for the reasons outlined above (and may carry a high risk of laboratory-acquired infection); therefore rarely attempted outside specialized facilities. Diagnosis of infection based on serology.
Diseases	Typhus; Rocky Mountain, Mediterranean and other spotted fevers; Q fever.
Transmission	Maintained in animal reservoirs and transmitted by bites of ticks, fleas, mites and lice. In contrast, *Coxiella burnetii* (a related organism now moved to a separate genus) survives drying and is transmitted in aerosols from animals or materials contaminated by infected animals and inhaled.
Pathogenesis	Mechanisms unclear, but organisms have a predilection for endothelial cells, giving rise to characteristic primary skin lesion (in spotted fevers) and vasculitis. The intracellular habitat is important to the organism's survival in the face of host defences.
Treatment and prevention	Tetracyclines generally used. Beta-lactams ineffective. Infection prevented by avoiding contact with vectors. Vaccines available for at-risk groups (e.g. veterinarians, farm workers).

Chlamydiae

Characteristics	Obligate intracellular parasites (unable to synthesize ATP) with distinct life cycle involving elementary bodies and reticulate bodies. Small cells with genome approximately 25% of that of *E. coli*. Important species are *Chlamydia trachomatis*, *Chlamydophila psittaci* and *Chlamydophila pneumoniae*.
Laboratory identification	Must be grown in cell culture, so cultural techniques are limited to specialized laboratories. In cell cultures, *C. trachomatis* forms characteristic, glycogen-containing inclusion bodies, which can be stained with iodine. Both *C. psittaci* and *C. trachomatis* contain specific surface antigens that allow detection by immunofluorescent antibody techniques. Nucleic-acid-based tests for *C. trachomatis* are also available. *C. pneumoniae* is currently detectable only by serology.
Diseases	*C. trachomatis* causes trachoma (eye infection), urethritis and other infections of the genital tract, and pneumonitis in newborns, acquired during birth from infected mothers. *C. pneumoniae*, described more recently, is now recognized as an important cause of atypical pneumonia. *C. psittaci* causes the atypical pneumonia, psittacosis.
Transmission	*C. pneumoniae* and *C. psittaci* are acquired by inhalation, the latter from infected birds or contaminated bird litter. *C. trachomatis* is spread by direct contact and is sexually transmitted.
Pathogenesis	Virulence factors remain unclear, but the intracellular habitat and different life cycle forms help organisms to evade host defences. Uptake into cells may be by parasite-encoded mechanisms.
Treatment and prevention	Tetracyclines, erythromycin (tetracycline should not be used in children). Vaccines not available and may not be useful because of the immunopathological element of the infections.

Fungi

Superficial Mycoses

Dermatophytes

	General term for species invading superficial layers of skin. Of the many species involved, those belonging to *Epidermophyton*, *Microsporum* and *Trichophyton* are of greatest importance.
Characteristics	Filamentous fungi invading surface keratinized structures: skin, hair, nails. Hyphae penetrate between cells.
Laboratory identification	Examination of KOH-treated skin scrapings for hyphae; fluorescence under Wood's lamp. Culture on media useful in identifying species. Both Sabouraud dextrose agar (SDA) and dermatophyte test medium (DTM) can be used.
Diseases	Tinea, ringworm, athlete's foot.
Transmission	By fungal material on skin scales.
Pathogenesis	Skin inflammation, pruritus – sometimes localized hypersensitivity reactions.
Treatment and prevention	Topical (imidazoles) and oral antifungal agents (griseofulvin, itraconazole, terbinafine). Improved skin care and hygiene.

Sporothrix schenckii

Characteristics	Dimorphic fungus (capable of growing as both single-celled yeast and multicelled hyphae). Occurs in external environment. Invades subcutaneous tissues.
Laboratory identification	Budding cells in inflammatory exudate from lesions. Culture on SDA.
Diseases	Sporotrichosis.
Transmission	Direct fungal contamination of wounds in skin (e.g. those made by thorns).
Pathogenesis	Ulceration or abscess formation in draining lymphatics.
Treatment and prevention	Itraconazole.

Deep Mycoses

Aspergillus

	A. fumigatus is the most important of three common species, the others being *A. flavus* and *A. niger*.
Characteristics	Filamentous fungi causing opportunistic infections in immunocompromised patients. Occur widely in external environment. Invade lungs and blood vessels.
Laboratory identification	Presence of hyphae in tissues. Culture on SDA. PCR. Serology.
Diseases	Aspergillosis.
Transmission	Inhalation of airborne stages (conidia).
Pathogenesis	Causes thrombosis and infarction when blood vessels invaded. Partial blockage of airways from fungal mass. Allergic bronchopulmonary reactions.
Treatment and prevention	Amphotericin B or its lipid complexes. High doses needed for neutropenic patients. Voriconazole is an alternative.

Blastomyces dermatitidis

Characteristics	Dimorphic fungus. Invades through lungs, can become widely disseminated in body.
Laboratory identification	Yeast cells in sputum or skin lesions. Culture on SDA.
Diseases	Blastomycosis.
Transmission	Inhalation of airborne spores.
Pathogenesis	Fungal infection in lungs. Presentation may be confused with tuberculosis. Skin involved in 40–80% of cases. Can produce abscesses.
Treatment and prevention	If needed (e.g. progressive disease) amphotericin B, itraconazole.

Candida albicans

Characteristics	Dimorphic fungus, occurring as yeast on mucosal surfaces as component of normal flora, but forms hyphae when invasive. Produces opportunistic infections in stressed, suppressed and antibiotic-treated individuals. *Paracoccidioides brasiliensis* in central and South America has many similarities.
Laboratory identification	Fungal stages in tissues. Culture on SDA. Isolates may be typed by molecular techniques. Serological methods can be used for disseminated disease, but less helpful in neutropenic patients.
Diseases	Candidiasis, thrush.
Transmission	Part of normal flora of skin, mouth and intestine.
Pathogenesis	Localized mucocutaneous lesions; invasion of all major organs in the disseminated condition.
Treatment and prevention	Topical and oral antifungals (e.g. nystatin, miconazole). Fluconazole, itraconazole, amphotericin B for disseminated disease.

Coccidioides immitis

Characteristics	Dimorphic fungus, growing as hyphae in soils, but as yeast-like endospores within capsules (spherules) in tissues. Invasion through lungs; can become widely disseminated in body.
Laboratory identification	In sputum or tissues. Culture on SDA. PCR. Serology.
Diseases	Coccidioidomycosis. Indigenous to the Americas.
Transmission	Inhalation of airborne stages (arthroconidia).
Pathogenesis	Lung infections give mild, influenza-like condition, but serious illness may follow dissemination.
Treatment and prevention	Amphotericin B, itraconazole, fluconazole for at-risk patients.

Cryptococcus neoformans

Characteristics	Encapsulated yeast-like fungus common in soils where there are bird droppings. Invades through lungs; can spread to CNS.
Laboratory identification	Encapsulated yeast cells in sputum or cerebrospinal fluid. Culture on SDA. Molecular methods. Serology.
Diseases	Cryptococcosis.
Transmission	Inhalation of airborne cells.
Pathogenesis	Lung infection may result in influenza-like condition or pneumonia. In immunocompromised patients, CNS involvement leads to meningitis.
Treatment and prevention	Amphotericin B plus flucytosine, followed by azole therapy.

Histoplasma capsulatum

Characteristics	Dimorphic fungus, growing as hyphae in soil where there are bird droppings. Invades through lungs and grows as yeast cells, which can survive intracellularly after phagocytosis. Can become widely disseminated in body.
Laboratory identification	Yeast cells in sputum or tissues. Culture on SDA. Molecular typing of isolates. Serology.
Diseases	Histoplasmosis.
Transmission	Inhalation of airborne spores.
Pathogenesis	Many infections are asymptomatic. Can produce acute and chronic pulmonary disease. Serious illness results from dissemination into other organs.
Treatment and prevention	Amphotericin B, itraconazole.

Pneumocystis jirovecii (carinii)

Characteristics	Respiratory organism previously classed as a sporozoan protozoan, now classified as a fungus. Lives extracellularly within alveoli.
Laboratory identification	Histological identification of organisms in sputum, bronchial lavage or tissues. PCR.
Diseases	Pneumonia-like condition, severe in immunocompromised patients. Worldwide distribution.
Transmission	Assumed to be by droplets.
Pathogenesis	Inflammation in lung.
Treatment and prevention	Trimethoprim-sulphamethoxazole or pentamidine.

Protozoa

Cryptosporidium hominis and *C. parvum*

Characteristics	Intestinal coccidian, invades and reproduces in epithelial cells of small intestine. Forms oocysts, which are passed in faeces.
Laboratory identification	Small (5 μm) oocysts in faeces, detected by acid-fast staining and/or immunofluorescent staining. PCR.
Diseases	Cryptosporidiosis. Worldwide distribution.
Transmission	Faecal–oral. Swallowing infective oocysts, usually in contaminated water. Animal reservoirs of infection for *C. parvum*.
Pathogenesis	Invasion of epithelial cells causes diarrhoea; can be profuse in immunocompromised patients.
Treatment and prevention	Nitazoxanide. Paromomycin is of limited value. Self-limiting in those with normal immunity. Treatment often required in immunocompromised patients, but antiparasitic drugs less effective in that group. Improved sanitation.

Cyclospora cayetanensis

Characteristics	Intestinal coccidian. Forms oocysts, which are passed in faeces.
Laboratory identification	8–10 μm oocysts with two sporocysts found in faeces, detected by microscopy of faecal concentrate and/or acid-fast staining. PCR in some centres.
Diseases	Cyclosporiasis.
Transmission	Faecal–oral. Swallowing infective oocysts in contaminated food. It is unclear whether or not animals act as reservoir hosts.
Pathogenesis	Diarrhoea. Infection can be serious in immunocompromised patients.
Treatment and prevention	Infections may be self-limiting. Trimethoprim-sulphamethoxazole if treatment required. Washing of fruit and vegetables.

Entamoeba histolytica/dispar

Characteristics	Intestinal amoeba, lives in intestine as trophozoite; produces resistant cysts, which are passed in faeces. *E. histolytica* and *E. dispar* have morphologically identical faecal cyst stages, but only *E. histolytica* is pathogenic.
Laboratory identification	Microscopic identification of cysts in faeces. ELISA or PCR to distinguish *E. histolytica* from *E. dispar*. Only *E. histolytica* produces haematophagous trophozoites in the faeces. Serology.
Diseases	Amoebic dysentery, liver abscess. Worldwide distribution, commonest in tropical and subtropical countries.
Transmission	Faecal–oral. Swallowing cysts in contaminated water or food.
Pathogenesis	Invasion of large bowel mucosa causes ulceration and diarrhoea, often bloody. Spread to liver causes formation of bacteriologically sterile abscess.
Treatment and prevention	Metronidazole or tinidazole to kill invasive amoebae, followed by diloxanide furoate to kill amoebae in gut lumen. Hygiene and sanitation.

Giardia intestinalis (Formerly *G. lamblia*)

Characteristics	Intestinal flagellate; lives on mucosa of small bowel. Produces cysts, which are passed in faeces.
Laboratory identification	Trophozoites in faeces, detected in fixed stained smears. Cysts in faeces seen in faecal concentrates. Direct recovery of trophozoites from duodenal aspirate. PCR.
Diseases	Giardiasis. Worldwide distribution.
Transmission	Faecal–oral. Swallowing cysts, usually in contaminated water. Animal reservoirs of infection.
Pathogenesis	Large numbers of trophozoites can cause severe diarrhoea and impaired absorption. Most severe in immunocompromised patients.
Treatment and prevention	Metronidazole, tinidazole. Improved sanitation, water treatment.

Genus *Leishmania*

	Genus contains several species, of which *L. (Viannia) braziliensis* and *L. donovani* complex cause serious disease.
Characteristics	Tissue flagellates living intracellularly in macrophages as amastigote stage. Transmitted by phlebotomine sandflies.
Laboratory identification	Presence of amastigotes in stained biopsy material, in-vitro culture of tissue specimens to obtain promastigotes. PCR of tissue specimens.
Diseases	Visceral (donovani complex), cutaneous (tropica, major) and mucosal (*Viannia*) leishmaniasis. Disease also known by many local names (e.g. kala-azar, Oriental sore, espundia). Commonest in tropical and subtropical countries.
Transmission	By bite of infected sandfly.
Pathogenesis	Visceral: hepatosplenomegaly from invasion of macrophages in liver and spleen; sometimes dermal nodules after treatment. Cutaneous: localized ulcers, which resolve. Mucosal: progressive invasion of mucosal tissues in nose and mouth.
Treatment and prevention	Liposomal amphotericin for visceral disease, Oral miltefosine in some cases. Local or systemic antimonials for cutaneous form. Avoidance of vectors.

Genus *Plasmodium*

	Genus contains five species causing disease: *P. falciparum*, *P. malariae*, *P. ovale*, *P. vivax* and *P. knowlesi*. *P. falciparum* and *P. vivax* are commonest. *P. falciparum* is by far the most dangerous, but *P. knowlesi* can cause illness of equal severity.
Characteristics	Sporozoa living intracellularly in liver and primarily in red blood cells.
Laboratory identification	Parasites in red blood cells in stained blood smear. Rapid antigen detection tests. PCR.
Diseases	Malaria. Commonest in tropical and subtropical countries.
Transmission	By bite of infected anopheline mosquito.
Pathogenesis	Bursting of infected red cells causes periodic fevers. In falciparum malaria, sequestration of infected cells in brain capillaries can cause fatal cerebral malaria; this infection is sometimes associated with intravascular haemolysis. Infection with *P. malariae* can lead to nephritis due to immune complex deposition.
Treatment and prevention	Many antimalarial drugs, but *P. falciparum* parasites show considerable drug resistance. Avoidance of vectors. Mosquito control. Field trials of vaccines underway.

Toxoplasma gondii

Characteristics	Coccidian living intracellularly, forming large tissue cysts. Natural host is cat, where parasite has enteric cycle, producing oocysts in faeces. In humans, organisms can invade many tissues.
Laboratory identification	Serology; need repeated tests to establish current infection or estimate when infection may have taken place. PCR in special cases.
Diseases	Toxoplasmosis. Worldwide distribution.
Transmission	Swallowing oocysts passed by cats; ingestion of tissue cysts in raw or undercooked meat; transplacental.
Pathogenesis	In adults, many cases are asymptomatic. Also causes mild influenza-like illness; lymph nodes may be enlarged. Symptoms more severe in immunocompromised patients. Congenital infections can damage eye or brain and prove fatal.
Treatment and prevention	In adults with normal immunity, specific treatment is not usually required. Pyrimethamine plus sulfadiazine plus folinic acid when indicated. Hygiene, cooking of meat.

Trichomonas vaginalis

Characteristics	Flagellate living in genitourinary system of females and males. Trophozoite form only, no cyst.
Laboratory identification	Identification of trophozoites in stained material from vaginal smears. Culture. PCR.
Diseases	Trichomoniasis. Worldwide distribution.
Transmission	Venereal.
Pathogenesis	Mild in males; causes vaginitis with discharge in females.
Treatment and prevention	Metronidazole or tinidazole. Use of condoms.

Genus *Trypanosoma*

	Genus contains three species that cause disease: *T. brucei gambiense*, *T. brucei rhodesiense* (African trypanosomiasis) and *T. cruzi* (American trypanosomiasis).
Characteristics	Flagellates living in blood and tissues. *T. cruzi* has intracellular stages.
Laboratory identification	Organisms in blood or cerebrospinal fluid (African) or blood or tissue biopsy (American). Serology (especially for *T. cruzi*). PCR.
Diseases	African trypanosomiasis (sleeping sickness): sub-Saharan Africa. American trypanosomiasis (chagas disease): South America.
Transmission	By introduction of trypanosomes in the bite of an infected tsetse fly (African). American transmitted by trypanosomes in faeces of infected reduviid bug entering the site of its bite.
Pathogenesis	African: infection of CNS causing meningoencephalitis. American: destruction of infected cells, especially neurones, megacolon, megaoesophagus, sudden death from arrhythmia or ruptured cardiac aneurysm, cardiac failure.
Treatment and prevention	Various antitrypanosomal drugs, all toxic (e.g. arsenicals). Avoidance of vectors. Vector control.

Microsporidia (Contains a Number of Species)

Characteristics	Intracellular pathogens, in intestine and other organs, characteristic spores.
Laboratory identification	Detection of organisms in faeces, urine, biopsies. Gram, modified trichrome and other stains can be used. PCR.
Diseases	Microsporidiosis.
Pathogenesis	Diarrhoea, other symptoms dependent on organ infected. Infection can be serious in immunocompromised patients.
Treatment and prevention	Albendazole (response is species variable and may be poor). Hygiene and sanitation.

Cystoisospora belli

Characteristics	A coccidian parasite infecting epithelial cells of the small intestine.
Laboratory identification	Diagnosis of infection based on observation of oocysts in faeces. Internal morphology of the oocyst helpful in identification.
Diseases	Chronic intestinal disease especially in immunocompromised patients. Symptoms of infection include diarrhoea, malaise and abdominal pain.
Transmission	Infection occurs by ingestion of sporulated oocysts from the faeces of another host.
Pathogenesis	Invasion of epithelial cells in the small intestine. Cycles of reproduction (asexual to sexual reproduction) with additional epithelial cell invasion and further production of oocysts, which are excreted in the stool.
Treatment and prevention	Trimethoprim-sulphamethoxazole. Hygiene and improved sanitation.

Helminths

Tapeworms

Diphyllobothrium latum

Characteristics	Large adult tapeworm in intestine. Scolex with sucking grooves not suckers. Eggs released and passed in faeces.
Laboratory identification	Faecal concentrates. Eggs in faeces have characteristic operculum (lid).
Diseases	Diphyllobothriasis (fish tapeworm). Worldwide distribution. Commonest where freshwater fish eaten raw.
Transmission	Larval stages in fish. Adult worm acquired when infected fish eaten raw or undercooked.
Pathogenesis	Usually harmless; may be associated with vitamin B_{12} deficiency.
Treatment and prevention	Niclosamide, praziquantel. Cooking of fish. Sanitation.

Echinococcus granulosus

Characteristics	Large fluid-filled (hydatid) cysts, in liver (approximately 66%), lungs (approximately 25%). Can occur in any organ or system, e.g. bone, CNS.
Laboratory identification	Scans, serology.
Diseases	Hydatidosis, hydatid disease. Worldwide distribution, commonest in sheep-rearing countries.
Transmission	Swallowing eggs released from adult tapeworms in dogs. Natural cycle is adult (dog), larval cysts (sheep).
Pathogenesis	Cysts exert pressure on internal organs. Release of cyst fluid can cause anaphylaxis.
Treatment and prevention	Albendazole. Surgical removal or therapeutic injection of cysts. Prevention of dogs eating infected viscera from sheep. Hygiene after handling dogs.

Hymenolepis nana and *Hymenolepis diminuta*

Characteristics	Small (2–4 cm) adult tapeworms in intestine. Scolex with suckers and hooks. Eggs passed in faeces. Life cycle is direct for *H. nana* and via rat flea intermediate host for *H. diminuta*.
Laboratory identification	Microscopy of faecal concentrates. Thin-shelled eggs in faeces.
Diseases	Hymenolepiasis (dwarf tapeworm). Worldwide distribution.
Transmission	Swallowing eggs (*H. nana*). Accidental ingestion of larvae in rat fleas (*H. diminuta*).
Pathogenesis	Usually harmless. Numbers of *H. nana* can build up by autoinfection in children and enteritis may result.
Treatment and prevention	Niclosamide, praziquantel. Hygiene and sanitation.

Genus *Taenia*

	Two species of this genus infect humans: *T. saginata* and *T. solium*.
Characteristics	Large (metres) adult tapeworms in intestine. Scolices with suckers (saginata) or suckers and hooks (solium). Proglottids (segments) passed in faeces. Small cysts (larval stages of solium) in muscles, CNS, and eyes.
Laboratory identification	Proglottids in faeces. Species identifiable on basis of number of branches to uterus (*T. saginata* 15–30; *T. solium* 7–12). Eggs in faeces (morphologically identical for both species). Scans and serology for cysticercosis.
Diseases	Taeniasis (beef and pork tapeworms). Cysticercosis (larval stages in tissue) occurs with *T. solium* only. Worldwide distribution.
Transmission	Adult worms acquired by eating raw or undercooked meat (beef, *T. saginata*; pork, *T. solium*) from animals infected with larval stages. Cysticercosis via ingestion of *T. solium* eggs in food contaminated with faecal matter from a person infected with adult *T. solium*.
Pathogenesis	Adult worms essentially asymptomatic. In cysticercosis, cysts in brain can result in convulsions or other neurological symptoms.
Treatment and prevention	Niclosamide, praziquantel for intestinal worms. Albendazole plus praziquantel under steroid cover for cerebral cysticercosis. Adequate cooking of meat. Prevention of human faeces contaminating grazing and feeding areas of cattle and pigs. Hygiene.

Flukes

Clonorchis sinensis

Characteristics	Liver fluke. Narrow elongated worms in bile ducts.
Laboratory identification	Eggs in faecal concentrates.
Diseases	Clonorchiasis (Asia).
Transmission	Larval stages in fish; adult flukes acquired when infected fish eaten raw or undercooked.
Pathogenesis	Inflammation and distortion of bile ducts. Long-standing heavy infections can result in cholangiocarcinoma.
Treatment and prevention	Praziquantel. Cooking of fish. Sanitation.

Paragonimus westermani

Characteristics	Lung fluke. Thick fleshy worms living as pairs in cysts.
Laboratory identification	Eggs in sputum or faeces.
Diseases	Paragonimiasis (Asia).
Transmission	Larval stages in crabs; adult flukes acquired when infected crabmeat eaten raw or undercooked.
Pathogenesis	Inflammation of lungs, secondary bacterial infections. Cavities may be confused with tuberculosis.
Treatment and prevention	Praziquantel. Cooking of crabmeat. Sanitation.

Genus *Schistosoma*

	Genus contains several species able to infect humans. Three are of major importance: *S. haematobium, S. japonicum* and *S. mansoni*.
Characteristics	Blood flukes; adult worms in blood vessels around intestine (*S. japonicum, S. mansoni*) or bladder (*S. haematobium*). Eggs in tissues.
Laboratory identification	Microscopy of faecal concentrates and urine for eggs. Spined eggs in faeces (*S. japonicum*, small lateral spine; *S. mansoni*, large lateral spine). Eggs in urine (*S. haematobium*, terminal spine). Serology.
Diseases	Schistosomiasis. Widely distributed in tropical / subtropical countries (*S. mansoni*, Africa, S. America; *S. haematobium*, Africa, Middle East; *S. japonicum*, Asia).
Transmission	Larvae released from eggs infect aquatic snails. These release infective cercariae, which actively penetrate human skin.
Pathogenesis	Hypersensitivity responses to eggs cause inflammation, granuloma formation, fibrosis and obstructive disease in intestine, bladder and liver.
Treatment and prevention	Praziquantel. Avoidance of infected waters. Snail control. Sanitation.

Nematodes

Ascaris lumbricoides

Characteristics	Large (up to 30 cm) intestinal roundworm; migratory stages pass through the lungs.
Laboratory identification	Microscopy of faecal concentrates. Thick-shelled eggs in faeces; worms also passed occasionally.
Diseases	Ascariasis. Worldwide distribution. Commonest in tropical and subtropical countries.
Transmission	Swallowing infective eggs in contaminated soil, food or water.
Pathogenesis	Migrating larvae cause pneumonitis. Adults can obstruct intestine, interfere with digestion and absorption of food, and migrate into bile or pancreatic ducts. Allergic symptoms may occur in the acute phase.
Treatment and prevention	Mebendazole, piperazine. Hygiene and sanitation.

Enterobius vermicularis

Characteristics	Small (1 cm) roundworm in large bowel. Worms emerge from anus at night to lay eggs.
Laboratory identification	Eggs recovered from perianal skin by saline swab or adhesive slide test; adult worms in faeces.
Diseases	Enterobiasis, threadworm, pinworm. Worldwide distribution. Commonest in children.
Transmission	Swallowing eggs, which can be carried on fingers and in dust. Eggs infective when laid, so direct re-infection is common.
Pathogenesis	Perianal pruritus.
Treatment and prevention	Mebendazole, pyrantel, piperazine. Hygiene.

Filarial Nematodes

	Large group. Most important species living in lymphatic tissues (*Wuchereria bancrofti*, *Brugia malayi*) or in skin (*Onchocerca volvulus*).
Characteristics	Adults very long, thin worms, living in lymphatics with microfilariae (larvae) in blood (*Wuchereria*, *Brugia*) or in subcutaneous nodules with microfilariae in skin (*Onchocerca*).
Laboratory identification	Detection of microfilariae in stained blood smear, fresh filtered blood or fresh skin snip. Serology less helpful. Antigen detection for *Wuchereria*.
Diseases	Lymphatic filariasis (*Wuchereria*, *Brugia*). Onchocerciasis or river blindness (*Onchocerca*).
Transmission	Microfilariae taken up by blood-feeding insects (mosquitoes, *Wuchereria*, *Brugia*; *Simulium* blackflies, *Onchocerca*) develop to infective stage and re-introduced into humans at the next blood meal. Widely distributed in tropical and subtropical countries.
Pathogenesis	In lymphatic filariasis, adult worms cause inflammation of lymph nodes and blockage of lymphatics, sometimes causing elephantiasis (big leg). In onchocerciasis, hypersensitivity to microfilariae leads to skin and eye lesions.
Treatment and prevention	Diethyl carbamazine (lymphatic) and ivermectin (onchocerciasis). Avoidance of vectors. Vector control.

Hookworms

	General term for intestinal bloodsucking worms. Two major species: *Ancylostoma duodenale* and *Necator americanus*.
Characteristics	Small (1 cm) intestinal roundworms; migratory stages pass through skin and lungs. Adult worms have expanded mouths for attachment to intestinal mucosa.
Laboratory identification	Microscopy of faecal concentrates. Thin-shelled eggs in faeces.
Diseases	Hookworm disease. Widespread in tropical and subtropical countries.
Transmission	Infective larvae penetrate skin (both species) or mucous membranes after ingestion (*Ancylostoma*).
Pathogenesis	Bloodsucking of worms can lead to anaemia and protein loss. Larval penetration associated with local dermatitis ('ground itch').
Treatment and prevention	Mebendazole, pyrantel. Hygiene and sanitation.

Strongyloides stercoralis

Characteristics	Minute (2 mm) intestinal roundworm, living in humans only as larvae and parthenogenetic females. Migratory stages pass through skin and lungs, often seen in lungs in hyperinfestation. Eggs hatch in intestine; rhabditiform larvae in faeces may become infective filariform larvae directly or initiate a free-living generation in soil, from which infective filariform larvae develop.
Laboratory identification	Larvae in fresh faecal specimens. Charcoal culture. Serology.
Diseases	Strongyloidiasis. Widespread in tropical and subtropical countries.
Transmission	Infective larvae penetrate skin.
Pathogenesis	In immunocompromised patients, repeated autoinfection (development of larvae released from females in the intestine) can lead to hyperinfection (disseminated strongyloidiasis), with larvae invading all body tissues. Hyperinfection can be fatal. Diarrhoea and malabsorption accompany heavy intestinal infections.
Treatment and prevention	Ivermectin. Hygiene and sanitation.

Toxocara canis

Characteristics	Invasion of larvae of roundworm *T. canis* normally maturing in intestine of dogs.
Laboratory identification	Serology.
Diseases	Toxocariasis, visceral larva migrans, ocular larva migrans. Worldwide distribution.
Transmission	Swallowing infective eggs passed by dogs in contaminated soil, food or water.
Pathogenesis	Invasion of body tissues causing granulomatous inflammatory responses. Larvae in liver may lead to hepatomegaly, in lungs to pneumonitis, and in CNS to convulsions. Ocular granulomas may cause unilateral visual loss.
Treatment and prevention	Albendazole. Hygiene. Routine deworming of puppies and pregnant bitches.

Trichinella spiralis

Characteristics	Minute (2–3 mm) roundworms, living as adults in the intestine. Coiled larvae in muscles. Low host specificity; infects and matures in wide variety of mammals.
Laboratory identification	Clinical signs, serology, muscle biopsy.
Diseases	Trichinellosis (trichinosis). Worldwide distribution.
Transmission	Acquired by eating raw or undercooked meat (usually pork) containing infective larvae.
Pathogenesis	Diarrhoea during intestinal phase. Allergic symptoms, skeletal muscle pain, myocarditis; can be fatal.
Treatment and prevention	Early treatment with albendazole or mebendazole. Adjunctive corticosteroids if symptoms severe. Cooking of meat.

Trichuris trichiura

Characteristics	Medium size (3–5 cm) roundworms in large bowel. Body of characteristic 'whipworm' form, with long thin anterior and short, thicker posterior.
Laboratory identification	Microscopy of faecal concentrate. Eggs in faeces have characteristic shape, oval with plug at each pole.
Diseases	Trichuriasis. Worldwide distribution. Commonest in tropical and subtropical countries.
Transmission	Swallowing infective eggs in contaminated soil, food or water.
Pathogenesis	Light infections asymptomatic. Heavy infections may produce trichuris dysentery syndrome with rectal prolapse, rectal bleeding, anaemia, growth stunting and growth retardation in children.
Treatment and prevention	Mebendazole. Hygiene and sanitation.

Vaccine parade

Vaccine	Abbreviation	Disease	Vaccine type	Delivery	Composition	EPI	UK	US	Notes
Bacillus Calmette–Guérin	BCG	Tuberculosis	Live attenuated *Mycobacterium bovis* BCG bacteria	ID (needle) PC (multipuncture device)	Live bacteria	Yes	HRG	No	
Diphtheria	D	Diphtheria	Toxoid	IM	Formaldehyde inactivated toxoid, alum	Yes	Yes	Yes	Can be formulated with other vaccines*
Influenza virus	IIV (inactivated influenza virus); LAIV (live attenuated influenza vaccine)	Influenza A and B virus infections	Inactivated virus or live attenuated virus	IM or ID (IIV); IN (LAIV)	Trivalent or quadrivalent formulations with haemagglutinin and neuraminidase antigens	No	Yes	Yes	Seasonal composition**
Haemophilus influenzae	Hib	Meningitis, pneumonia, epiglottitis	Polysaccharide b	IM	Polysaccharide conjugated to tetanus toxoid / diphtheria toxoid / meningococcal b outer membrane protein	Yes	Yes	Yes	Can combine as Hib-MenCY or with HepB as Hib-HepB
Hepatitis A virus	HepA	Hepatitis A virus infection	Inactivated virus	IM	Formaldehyde-inactivated, alum	No	No	Yes	Combination hepatitis A and B vaccine available; also travellers to high-risk regions
Hepatitis B virus	HepB	Hepatitis B virus infection	Inactivated recombinant hepatitis B surface antigen	IM	HBsAg made in yeast; aluminium hydroxide adjuvant	Yes	Yes	Yes	Different immunization schedules for specific circumstances; combination hepatitis A and B vaccine available
Herpes zoster virus	HZV	Shingles (herpes zoster)	Live attenuated	IM or SC	Live attenuated Oka / Merck strain of varicella-zoster virus	No	Yes	Yes	Older adults only
Human Papilloma virus	HPV	Cervical cancer (genital warts)	Subunit with major capsid protein L1	IM	Quadrivalent (HPV types 6, 11, 16, 18) or bivalent (HPV types 16, 18); alum adjuvant	Yes	Yes	Yes	Sometimes to girls only
Measles virus	MCV	Measles	Live attenuated: measles virus (Edmonston–Enders)	IM	Live attenuated virus	Yes	Yes	Yes	Separate measles virus vaccine given through EPI
Measles, mumps and rubella viruses	MMR	Measles, mumps and rubella virus infections	Live attenuated: measles virus (Edmonston–Enders); mumps virus (Jeryl Lynn); rubella virus (Wistar RA)	IM or SC	Live attenuated viruses	Yes	Yes	Yes	
Meningococcus B	MenB	Meningitis	Recombinant Factor H binding protein strain B	IM	Recombinant factor H binding protein	No	Yes	HRG	
Meningococcus C	MenC	Meningitis	Men C polysaccharides	IM	Polysaccharides conjugated to diphtheria toxoid	No	Yes	Yes	
Meningococcus ACWY	Men ACWY (MCV4) or MPSV4	Meningitis	Men ACWY polysaccharides	IM (MenACW) or SC (MPSV4)	Polysaccharides conjugated to diphtheria toxoid (MenACWY) or free polysaccharides (MPSV4)	HRG	Yes	Yes	

Continued

Vaccine	Abbreviation	Disease	Vaccine type	Delivery	Composition	EPI	UK	US	Notes
Mumps virus		Mumps	Live attenuated virus (Jeryl Lynn)	IM	Live virus; can be given as MMR	No	Yes	Yes	
Pertussis		Whooping cough	Whole inactivated bacteria or acellular protein (filamentous haemagglutinin, pertactin, pertussis toxin, can have fimbriae types 2 and 4)	IM	Inactivated (formalin); subunit with aluminium salts as adjuvant	Yes	Yes	Yes	
Pneumococcal vaccines	PCV7, PCV10, PCV13, PPSV23	Pneumococcal pneumonia	Subunit (capsular polysaccharide)	IM	Polysaccharides conjugated to diphtheria toxoid; phenol as preservative (children); or polysaccharide for adults (PPSV23)	Yes	Yes	Yes	Different vaccines given at different ages
Polio virus	IPV or OPV	Poliomyelitis	Inactivated poliovirus (IPV) or live oral poliovirus (OPV)	IM (SC) or oral (live vaccine)	Formalin inactivated; can have 2-phenoxyformalin as preservative; or live virus	Yes	Yes	Yes	Live oral poliovirus vaccine being withdrawn
Rotavirus	RV1 (monovalent) RV5 (pentavalent)	Rotavirus gastroenteritis	Live attenuated virus	Oral	Live virus	Yes	Yes	No	Not given before 6 weeks of age and not after 6–8 months
Rubella virus		Rubella	Live attenuated virus	IM	Live virus; can be given as MMR	Yes	Yes	Yes	
Tetanus	TT	Tetanus	*Clostridium tetani* inactivated exotoxin	IM	Formaldehyde inactivated; can have aluminium salts	Yes	Yes	Yes	Boosters required
Typhoid		Typhoid fever	Live mutant bacteria (Ty21a) or capsular polysaccharide (Vi)	Oral (Ty21a) or IM (Vi)		No	No	No	Travellers to high-risk regions
Varicella virus	VAR	Chickenpox	Live attenuated virus (Oka strain)	SC	No preservative	No	HRG	Yes	May be given with MMR as MMRV (i.e. in USA)
Yellow fever virus		Yellow fever virus infection	Live virus (17D strain of yellow fever virus)	SC or IM		HRG	HRG	HRG	Travellers to/ those living in high-risk regions

ID, intradermal; IM, intramuscular; IN, intranasal; HRG, high-risk groups; MMR, measles, mumps, rubella; SC, subcutaneous.
*Combined diphtheria pertussis and tetanus vaccine formulations can vary. Formulations of adsorbed diphtheria and tetanus toxoids, (DT, Td), with acellular pertussis vaccine (DTaP, Tdap), with inactivated poliovirus vaccine (DTaP-IPV), with hepatitis B vaccine and inactivated poliovirus vaccine (DTaP-HepB-IPV), with inactivated poliovirus vaccine (DTaP-IPV) or with inactivated poliovirus vaccine and *Haemophilus influenzae* type b conjugate vaccine (DTaP-IPV/Hib are available.
**2017–18 composition for northern hemisphere 2017–18 season H1N1, H3N2 and influenza B (two influenza B viruses in the quadrivalent vaccine).
Vaccines for dengue, Japanese encephalitis and tick-borne encephalitis are available in some countries.
Current vaccine schedules are available at www.who.int/immunization/policy/immunization_tables/en/, www.who.int/immunization/policy/immunization_tables, www.nhs.uk/vaccinations and www.cdc.gov/vaccines/schedules/.