chapter 2:

PATHOGENS AND VIRULENCE

OBJECTIVES

After studying this chapter you should be able to:

- define the terms pathogen, parasite and infection;
- list the types of parasites and pathogens;
- outline the types and effects of microbial virulence factors.

2.1 INTRODUCTION

The body is exposed to many pathogenic microorganisms and multicellular parasites. Most microorganisms found associated with the body are harmless and live as commensals or symbionts but others cause disease and are known as **pathogens**. **Infection**, from the Latin 'inficere' (to put in), is the successful persistence and/or multiplication of the pathogen on or within the host. In this respect, **pathogenesis** may be defined as the molecular and biochemical mechanisms that allow pathogens to cause diseases. Mummies preserved in ancient Egypt and elsewhere display evidence that infectious diseases have always been a threat. The emergence of new pathogens and the development of resistance to current treatments for existing pathogens means that infectious diseases will probably always be with us.

Many antibiotic drugs (*Chapter 3*) are available to treat infectious diseases and, in many cases, will effect a cure. It is, however, possible for them to exacerbate the problem since the drug can remove commensals allowing antibiotic resistant pathogens to flourish. Also, an adequate immune response is often necessary, since drugs alone may fail to eliminate the infection. Thus all pathogens must overcome the defense systems present in their hosts (*Chapter 4*).

Some pathogens regularly cause diseases while others do not. For example, *Pseudomonas aeruginosa* (*Figure 2.1*) can cause overwhelming disease in patients whose defense systems are compromized but not in those with intact defenses. It is likely that any microorganism with the ability to live in or on humans will sometimes become an **opportunistic pathogen** especially if the balance between the usual microorganisms present and the immune

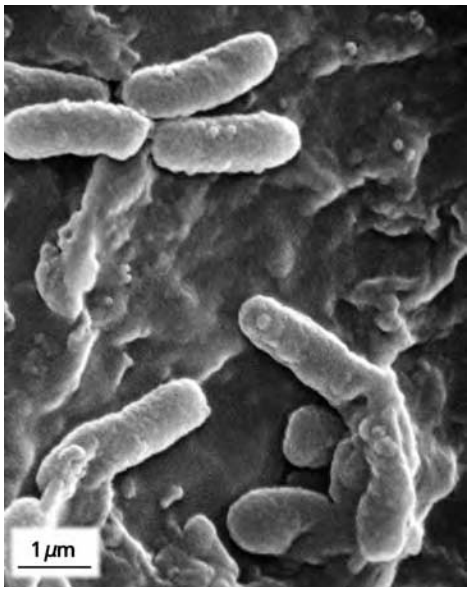

Figure 2.1 Electron micrograph of ***Pseudomonas aeruginosa***. Courtesy of Dr J. Carr, Public Health Image Library, Centers for Disease Control and Prevention, USA.

Figure 2.2 Electron micrograph of dividing *Escherichia coli* cells. Courtesy of Dr A. Curry, Manchester Royal Infirmary, UK.

Margin Note 2.1

Communicable pathogens are transmitted from host to host and, in general, cannot live for extended periods outside a host. **Noncommunicable pathogens** normally live in the abiotic environment but may infect a host if they are transmitted to him or her. **Contagious pathogens** are those that are easily transmitted. The sources of pathogens can be abiotic: soil, water, or animals or other humans.

system is disturbed. Thus bacteria which are normally harmless, but which are opportunistic pathogens, can cause infections under certain conditions. For example, wounds can become badly infected with bacteria that normally exist on the skin, and bacteria that normally live in the gut can cause serious infections if peritonitis (*Chapter 11*) allows the gut contents to enter the peritoneum. In general, these infections are not transferable to other healthy humans.

The success of pathogenic microorganisms depends on their ability to colonize host tissues and to counter the host's defense mechanisms. Virulence is measured by the **infective dose** and the severity of the disease caused. For example, as few as 10 to 100 *Shigella dysenteriae* cells can cause shigellosis but more than 10 000 cells are needed of the less virulent salmonella or cholera bacteria. True pathogens are equipped with a range of **virulence factors**. The strains of some bacterial species, such as pathogenic forms of *Escherichia coli* (*Figure 2.2*) can produce different virulence factors that cause, for example, diarrhea, urinary tract infections or sepsis. Other strains, however, do not produce virulence factors or do so to a lesser extent and are therefore not pathogenic, except when they infect an immunocompromized host.

A pathogen must be transmitted from a source to the patient. Direct contact between hosts is the most obvious form of transmission but coughs and sneezes (aerosols), food, water and arthropod vectors are all used by various pathogens. The long-term survival of pathogenic microorganisms also depends on maintaining their infectivity during transmission from host to host. Diseases that are transmitted from animals to humans are called **zoonoses**, while humans who harbor a pathogen but are asymptomatic are called **carriers.**

2.2 TYPES OF PATHOGENS

Infectious diseases are caused by pathogens that have the ability to infect humans. They may be subcellular, such as prions and viruses, single-celled prokaryotic bacteria, single-celled eukaryotic protozoa and yeasts, or multicellular organisms such as, fungi, certain worms, such as nematodes and flukes (generally referred to as **helminths**) and arthropods, such as mites. The term **parasite**, an organism that lives at the expense of another, is often applied to viruses, protozoa and helminths, although the terms pathogen and parasite are virtually interchangeable.

PRIONS

Spongiform encephalopathies or prion diseases (*Chapter 15*) are all fatal diseases for which there is no cure. They include Creutzfeldt-Jacob disease (CJD), Gerstmann-Sträussler-Scheinker syndrome (GSS) and fatal familial insomnia (FFI). These diseases generally develop slowly over 10 to 20 years in older individuals. Prion diseases occur sporadically, or they can be familial, that is genetic, or they can be acquired, that is, infectious.

Prions are degenerate host proteins. The normal form of the protein adopts a largely α helical conformation that is harmless, but can refold to a β sheet-rich form that is a pathological conformation. Such misfolded proteins aggregate to form deposits in the brain leading to a lethal spongiform condition where holes develop in the brain. A misfolded prion protein, in some poorly understood way, induces a conformational change in a native α prion protein to produce a β type conformation. This new misfolded protein, in turn, can catalyze conformational changes in other native proteins, eventually forming a chain reaction and produces deposits of prions in the brain. The sporadic form of the disease occurs in individuals with mutations in the prion gene that predisposes them to produce the misfolded form of the protein. Since

the diseases usually only occur after reproductive life is over, they can run in families giving the familial form. If misfolded prions enter the body in the diet, they resist digestion. They may also enter by iatrogenic means, through surgery or blood transfusions for instance, and can initiate the infectious form of the disease.

VIRUSES

Viruses are obligate intracellular parasites. They are complexes of proteins, which form a **capsid,** and nucleic acid (RNA or DNA), comprising their genome, that together form a viral particle or virion (*Figure 2.3*). Some viral particles also have a lipid membrane or **envelope** acquired when the viral particle leaves its host cell (*Figures 1.4* and *2.7*). Viruses must enter a target or host cell to replicate. They bind to the target cell by attaching to specific proteins or carbohydrates on the cell's surface (*Figure 2.4*). For example, the human immunodeficiency virus (HIV) attaches to a protein called CD4 found on the surfaces of certain T lymphocytes and macrophages of the immune system (*Chapter 4*). Other examples of viruses and the cellular receptors to which they bind are shown in *Table 2.1*.

Once infected, the host cell then manufactures new viral particles. In some cases, replication may include an inactive latent state. For example, the virus *Varicella zoster* (*Figure 2.5*), which causes chicken pox, enters nerve cells and, after the initial infection, remains dormant. If, however, the host immune system becomes weakened, *Varicella* can reactivate and cause painful attacks of shingles in the area served by that nerve.

In some cases the viral nucleic acid can be integrated into that of the host and eventually lead to cell transformation and the formation of cancers (*Chapter 17*). Thus, for example, hepatitis B virus can contribute to primary hepatocellular carcinoma, while certain strains of the human papillomavirus that cause genital warts may contribute to the development of cervical carcinoma.

Virus	Cell membrane protein used as virus receptor
HIV	CD4 (*Chapter 4*)
Human rhinovirus 91	ICAM (Intracellular adhesion molecule) I
Sindbis virus	High density laminin receptor
Coxsackie A	ICAM I
Human coronavirus 229E	aminopeptidase N
Hepatitis B virus	HBV Binding factor (a metalloprotease)

Table 2.1 Some cell membrane proteins used for viral attachment

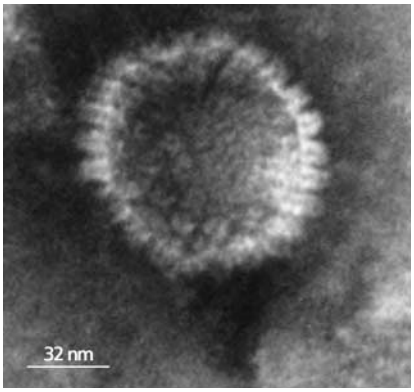

Figure 2.5 Electron micrograph of *Varicella zoster*. Courtesy of H. Cotterill, Manchester Royal Infirmary, UK.

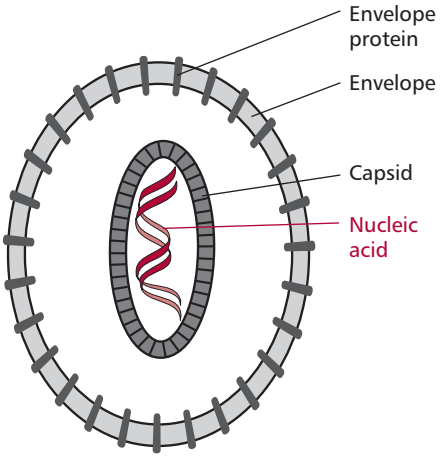

Figure 2.3 Schematic showing the structure of a typical viral particle.

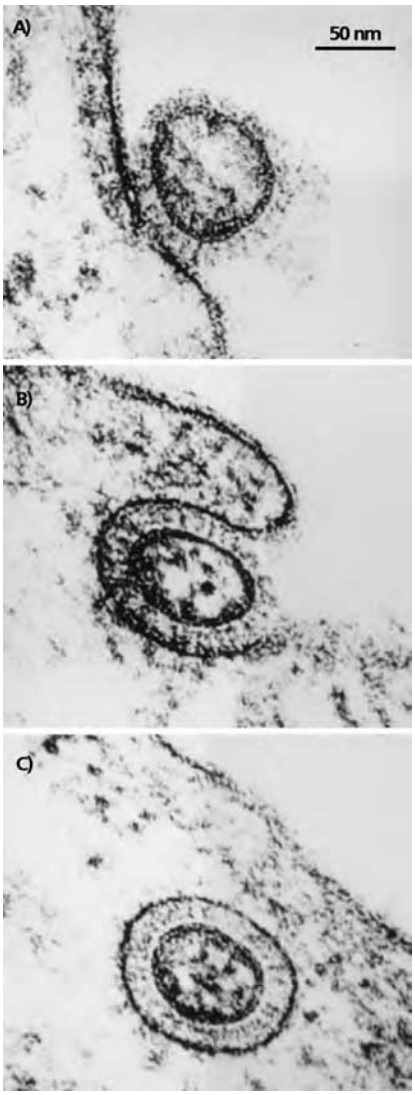

Figure 2.4 Electron micrographs showing (A) the binding, (B) enveloping and (C) internalization of an influenza virus. Courtesy of Dr J.J. Shekel, National Institute for Medical Research, London.

BOX 2.1 Infectious diseases in history

The earliest human skeletons show evidence of a variety of infectious diseases. Evidence from mummies preserved in ancient Egypt 4000 years ago shows that the population suffered from diseases such as tuberculosis, trachoma and dental caries. The skeletons of monks buried in medieval Northern monasteries show the characteristic signs of syphilis!

Perhaps the best known example of an infectious disease is **plague,** a term originally applied to any widespread disease causing great mortality. The name is now confined to bubonic plague, an infectious disease of animals and humans caused by the bacterium *Yersinia pestis* (*Figure 2.6*). This bacterium mainly affects rodents but their fleas can transmit the bubonic form of the disease to humans when they bite to feed on blood. Transmission occurs readily in crowded urban areas with poor hygiene. Infection usually results from a bite from a rodent flea carrying the plague bacterium or by handling an infected animal. Once humans are infected, they infect others very rapidly. Plague causes fever and painful swellings of the lymph glands called buboes, which is how the disease derives its name. It also causes spots on the skin that are initially red but then turn black. The coughing and sneezing of infected individuals spreads pneumonic plague; a much more lethal form of the disease. Bubonic plague is fatal in about 30% of cases but is readily treatable with antibiotics. In contrast, pneumonic plague is often fatal even with antibiotic therapy.

Bubonic plague has had a profound impact on humans throughout recorded history. In AD 541, the first great plague **pandemic**, that is a disease affecting patients over a wide geographical area, spread throughout the world from its origins in Egypt. It is thought to have killed between 50% and 60% of the population over four years, being spread by the flea-infested rats that inhabited human homes and workplaces and by human sufferers of the disease. In the early 1330s, a second pandemic originated in China. At the time China was one of the world's busiest trading nations and the disease rapidly spread to western Asia, the Middle East and Europe. It entered Europe in October 1347 when several Italian merchant ships, returning from a trip to the Black Sea (a key link in trade with China) arrived in Sicily with many people on board already dying of plague. Within days the disease spread to the city and the surrounding countryside. In August the following year, the plague had spread as far north as England, where people called it the **Black Death** because of the black spots on the skins of patients. Medieval medicine was ineffective in treating the disease. However, in winter the disease declined simply because the fleas that carried the bacteria were dormant. Each spring, the plague returned. After five years more than 13 million people in China and 20–30 million in Europe, one third of the European population, were dead.

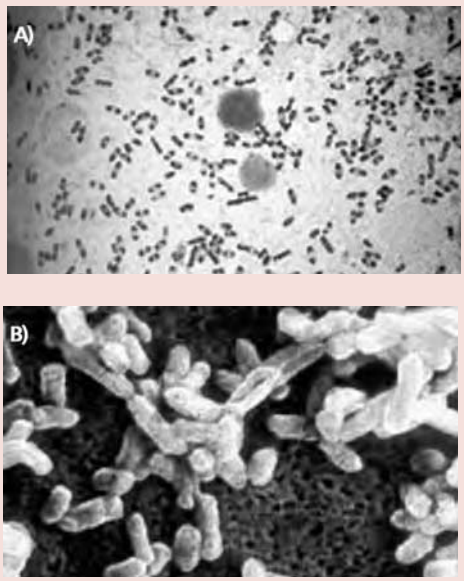

Figure 2.6 (A) Light and (B) electron micrographs of *Yersinia pestis*. The cells are 1 to 3 μm in length. Courtesy of Dr M.K. Khalid, Editor, *Middle East Journal of Emergency Medicine* and Dr M. Schneider, *Kryptozoologie* respectively.

In addition to the terrible fatalities, the Black Death produced enormous social changes from which medieval society never recovered. The resulting serious labor shortages all over Europe led to a demand for higher wages by workers and peasant revolts broke out in Belgium, England, France and Italy. The end of the 1300s saw the eventual collapse of the prevailing social system of tied serfs.

Even when the Black Death pandemic ended, smaller outbreaks of bubonic plague continued for centuries. The Great Plague of London in 1664 to 1666 is estimated to have killed 70 000 people out of a total population of 460 000. In 1894, outbreaks occurred in Canton and Hong Kong in which 80 000 to 100 000 died. Within 20 years the plague had spread and killed 10 million people worldwide.

Advances in living conditions, public health and antibiotic therapy make future pandemics of *Yersinia pestis* unlikely. However, if an infected person is not treated promptly, the disease is likely to cause severe illness or death. Outbreaks of plague still occur in some rural communities or in some cities that are still associated with infected rats and their fleas. The World Health Organization reports 1000 to 3000 cases of plague annually. It is unlikely that the disease will ever be completely eradicated because wild animals hold a huge reservoir of the bacterium.

In more recent years, pandemics of a number of other diseases have occurred. The most recent is associated with the human immunodeficiency virus (HIV) virus (*Figures 1.4* and *2.7* and *Chapter 4*), although the most numerous are associated with influenza (*Figure 2.8*). The influenza pandemic of 1918–1919, named Spanish Flu though the name has little relevance, killed up to 40 million people, many more than the nine million fatalities of World War 1 (WW1). It was one of the most devastating epidemics in history and is comparable with the Black Death in sheer numbers of people killed. In two years, a fifth of the world's population was infected. The virus had a mortality rate of 2.5% compared with the previous influenza epidemics of less than 0.1%. Further, most deaths were of people aged 20 to 40 years, which is unusual given that influenza normally kills the elderly and the very young. Indeed, the death rate for 15- to 34-year-olds from the influenza and associated pneumonia were 20 times higher in 1918 than in previous years.

The origin of this influenza variant is uncertain but is thought to have originated in China where mutations led to an influenza virus with novel surface proteins making it relatively unrecognizable to immune defense. The influenza pandemic swiftly followed trade routes and shipping lines. The mass movement of people as a result of WW1 also enabled the virus to spread rapidly. Outbreaks swept through North America, Europe, Asia, Africa, Brazil and the South Pacific. The shortage of medical facilities created by WW1 accentuated problems. As with the Black Death, social problems occurred with shortages of coffins, morticians and gravediggers so that bodies had to be stored in piles until they could be buried.

The genes of all influenza viruses are maintained in wild aquatic birds. Periodically these viruses are transmitted to other species. Thus the potential for outbreaks of influenza is still present. Influenza viruses mutate constantly but usually in such a way that one year's vaccine offers some protection against the next year's strain. However, every 10 to 20 years, a major mutation produces a particularly new virulent strain against which current vaccines offer little protection. Such viruses are associated with epidemics and pandemics like that of 1918 to 1919. Indeed, in 1997 epidemiologists and public health officials recognized a new variety of influenza virus, known as subtype H5N1 from its surface proteins. Given its lethal effects on poultry it was called Chicken Ebola, but is now more commonly called 'bird flu'. When it infected the human population of Hong Kong it killed six of the first 18 confirmed cases. Fortunately the H5N1 subtype cannot be transmitted through the air from one human host to another. In Hong Kong, bird-to-human contact is relatively easy given the often close proximity of the two and is believed to have been the route of transmission and Hong Kong officials ordered the slaughter of Hong Kong's entire poultry population in 1997. However, by 2005/6, cases of bird flu were being reported in diverse parts of the world.

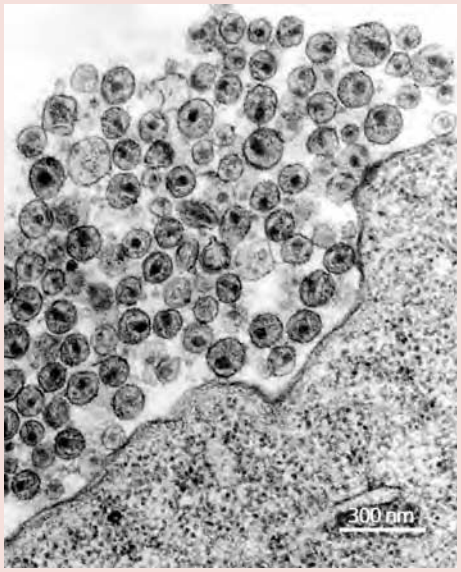

Figure 2.7 HIV viral particles being released from the surface of a human cell. Courtesy of H. Cotterill, Manchester Royal Infirmary, UK.

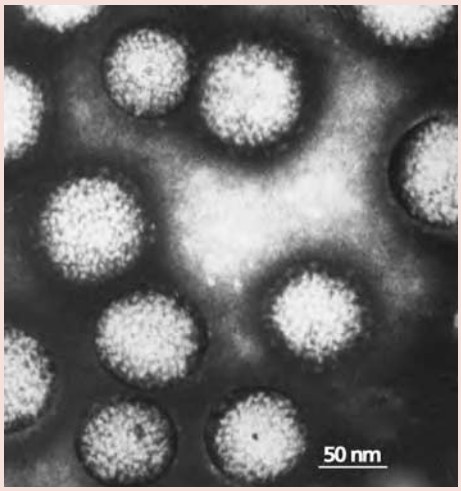

Figure 2.8 Electron micrograph of influenza viral particles. Courtesy of H. Cotterill, Manchester Royal Infirmary, UK.

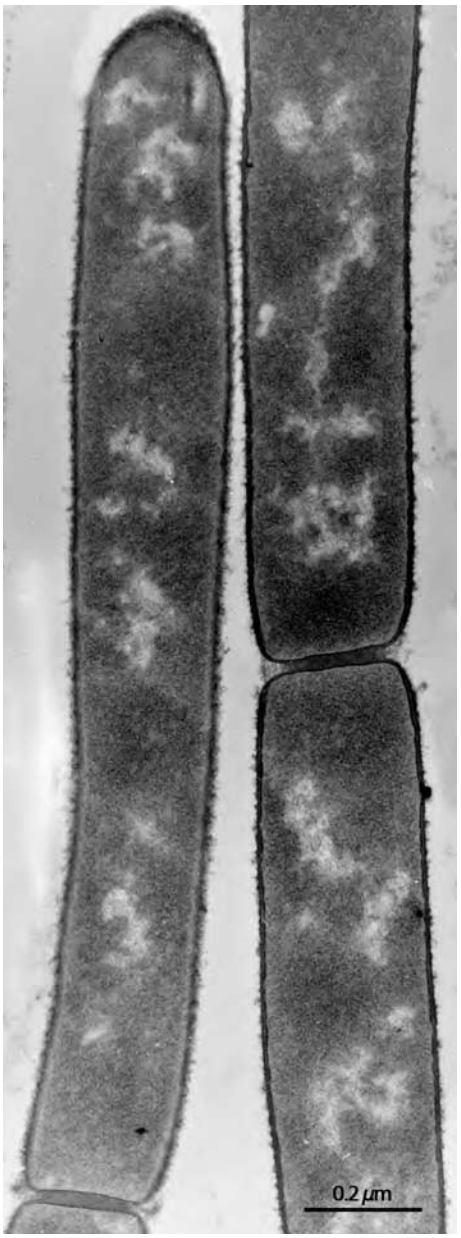

Figure 2.9 Electron micrograph of bacterial cells. Note the lighter staining nuclear material free in the cytoplasm and not contained in a nucleus. Courtesy of Dr I.D.J. Burdett, National Institute for Medical Research, London.

BACTERIA

Bacteria are cellular microorganisms that do not have a discrete nucleus and are described as prokaryotic (*Figure 2.9*). They are responsible for a large proportion of all infectious diseases and although prokaryotes share considerable biochemistry with eukaryotes, their metabolism differs in numerous ways, making them susceptible to chemical agents that do not significantly damage human cells. This is the basis of antibiotic therapy (*Chapter 3*). While most species of pathogenic bacteria do not enter host cells and are described as extracellular pathogens, some significant groups of bacteria are intracellular parasites. Examples of the latter include Mycobacteria and Listeria.

PROTOZOA, FUNGI AND HELMINTHS

Protozoa, fungi and helminths are eukaryotic organisms. They therefore share many biochemical features with humans. Also, they have many seemingly sophisticated ways of countering the host immune system. This often makes them difficult to eradicate when they cause disease. Fungi, protozoa and helminth parasites are responsible for a large proportion of infective diseases, particularly in the developing world.

Protozoa consist of single eukaryotic cells although in some species these may group together as loose aggregates. Malaria, caused by species of *Plasmodium*, is easily the most clinically important protozoal infection worldwide and is responsible for many millions of deaths annually (*Box 2.2*). Protozoa are, however, also the causative agents for a number of other infections including amebiasis, giardiasis, leishmaniasis, toxoplasmosis, trichomoniasis and trypanosomiasis.

Fungi are a heterogeneous group of organisms, ranging from unicellular yeasts to elongated chains of cells, known as hyphae (*Figure 2.10*). Fungal infections, or mycoses, are relatively common and may be superficial or systemic. Fungi cause disease in humans by invading tissues, by being toxic or by initiating an allergic response. Clinically important fungal infections include those of *Epidermophyton*, *Microsporum* and *Trichophyton* species that cause ringworm, athlete's foot and nail infections (*Figure 2.11*). Infections with yeasts and other fungi can result in, for example, candidiasis,

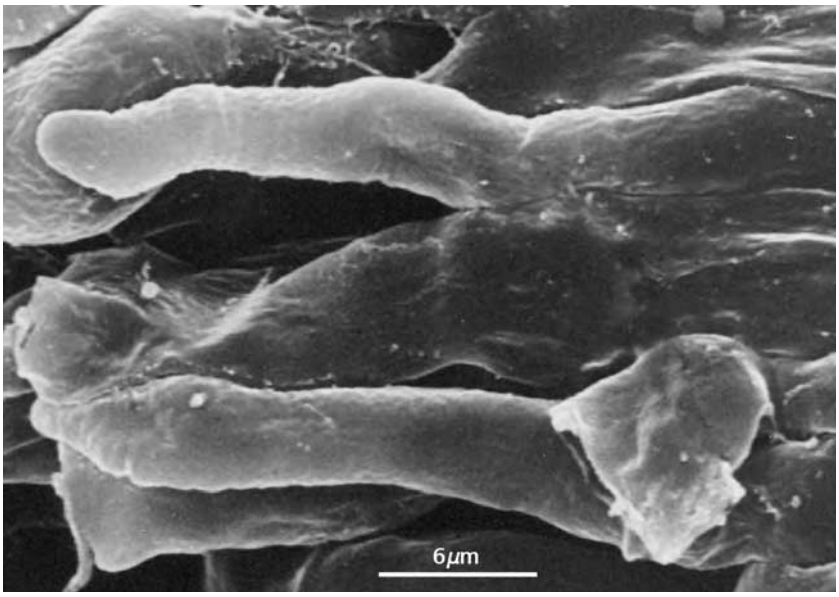

Figure 2.10 Electron micrograph of fungal hyphae.

aspergillosis and cryptococcosis. Systemic fungal infections are, in general, much more common in immunocompromized individuals, for example those with AIDS (*Chapter 3*), those undergoing cancer chemotherapy (*Chapter 17*) or those being treated with immunosuppressive drugs to prevent rejection of a transplant (*Chapter 6*).

The clinically important helminths (worms) can be divided into three main groups: nematodes (roundworms), cestodes (flatworms) and trematodes (flukes). Helminths often have complex life cycles that may involve other hosts in addition to humans. In humans, they may infect the alimentary canal, blood vessels, lymphatics or other tissues such as skeletal muscle. Helminths are significant parasites in tropical climates. Examples of disease-causing helminths are the pork tapeworm *Taenia solium* (*Figure 2.12*) that can live in the gut and *Schistosoma* the cause of bilharzia.

ARTHROPODS AND VERTEBRATES

Arthropods may be directly parasitic, but many are also relevant to infectious diseases as vectors of pathogens. The mite *Sarcoptes scabiei* lives in the outer layers of the skin and can cause scabies while fleas (*Pulex*) and head and pubic lice, *Phthirus capitans* and *pubis* respectively, are blood sucking parasites (*Figure 2.13*). Houseflies and cockroaches are noted carriers of food poisoning organisms. More specific vectors include ticks that transmit *Borrelia burgdorferi*, the cause of Lyme disease. *Yersinia pestis*, the bubonic plague organism is spread by the fleas on black rats (*Box 2.1*). Malarial parasites are spread by female *Anopheles* mosquitos (*Box 2.2*) and tsetse flies are vectors for *Trypanosoma brucei* which causes sleeping sickness. Several disease-causing organisms use mammals as vectors with perhaps the best known being the rabies virus.

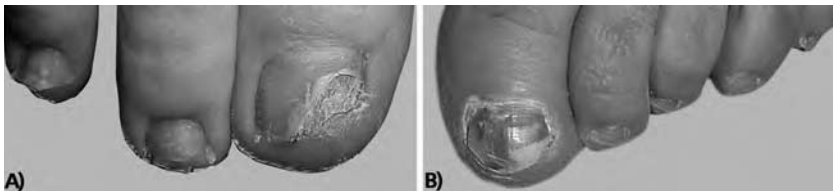

Figure 2.11 Infections of the toenails (onychomycosis) with (A) *Trichophyton tonsurans* and (B) *Trichophyton rubrum* with secondary infections with the fungus, *Scopulariopsis brevicaulis* and the yeast, *Candida guilliermondii*. Courtesy of Dr Pavel Dubin, Israeli Board Certified Dermatologist.

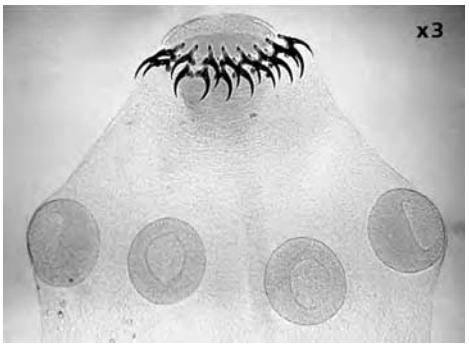

Figure 2.12 The 'head' of the pork tapeworm, *Taenia solium*. Note the hooks and suckers that allow it to remain attached to the intestine wall. Courtesy of Public Health Image Library, Centers for Disease Control and Prevention, USA.

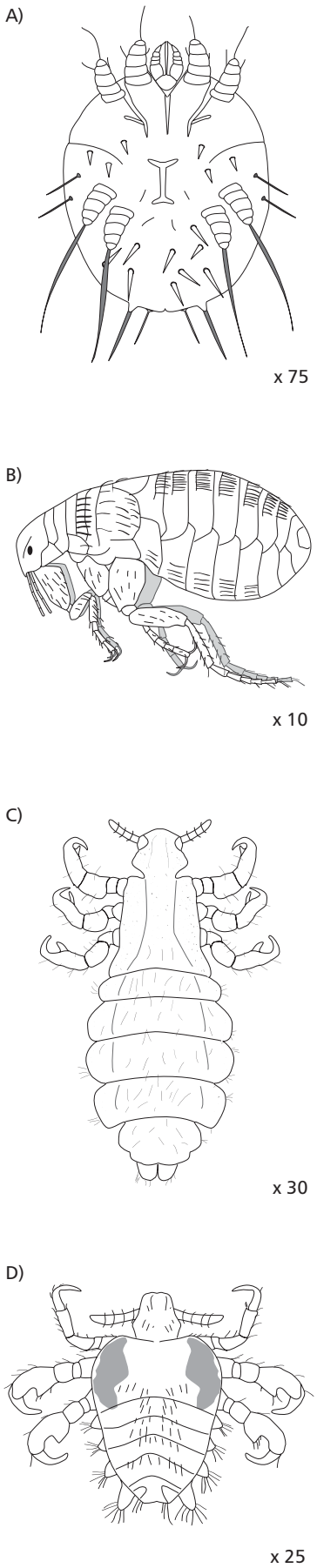

Figure 2.13 Drawings of (A) the scabies mite, (B) flea, (C) head and (D) pubic lice.

BOX 2.2 Malaria – the bad air disease

Malaria infects hepatocytes and blood. It is named malaria from the eighteenth-century Italian 'mala aria', meaning bad air, from the belief the disease was caused by the unwholesome air of swampy districts. Malaria is caused by four main *Plasmodium* species, *falciparum, malariae, ovale* and *vivax*, and is responsible for a significant proportion of mortality and morbidity worldwide, particularly in tropical climates.

The life cycle of *Plasmodium* (*Figure 2.14*) is complicated by any standards! A feeding female *Anopheles* mosquito infects a human with a haploid stage of the parasite called a sporozoite. These infect liver cells, replicate and develop, and when released as merozoites can infect other liver cells or erythrocytes. Here the parasite, usually called a trophozoite, also grows and divides leading to the eventual lysis of the blood cell. The released trophozoites can invade other erythrocytes in 48 h cycles of invasions and lyses that cause the characteristic fevers and chills associated with malaria. The symptoms of the disease are usually most severe during the lysis of erythrocytes. However, eventually some trophozoites develop into male and female gametocytes that can be ingested by a feeding mosquito. Within the insect gut, the gametocytes fuse forming a diploid zygote. The zygote enters the gut wall forming a cyst. Within the cyst, the zygote develops and forms sporozoites that migrate to the salivary gland. Thus the cycle of events can be repeated when the mosquito next feeds on a human.

The symptoms of malaria may present after a variable incubation period and include headaches, general malaise, sweating, muscular pains and rigors and anorexia. The clinical course and symptoms of different forms of malaria are variable although infections are characterized by the recurring attacks of chills and fevers described above.

A variety of antimalarial drugs is available (*Chapter 3*). Some prevent the hepatic forms of *Plasmodium* from invading erythrocytes; others destroy the erythrocytic or gametocyte forms of the parasite in the patient's blood preventing transmission of the parasite by the mosquito. Chloroquine is the usual choice for treating malaria because it is cheap, safe and normally effective. However, chloroquine-resistant strains of *Plasmodium falciparum* are now endemic in sub-Saharan Africa and elsewhere. Drugs available to treat these resistant parasites include halofantrine, mefloquine, quinine and quinidine and others. However, in many cases the molecular mechanisms of action of antimalarial drugs are not well understood.

The remarkably complicated life cycle of the malarial parasites with their prehepatic, hepatic, pre-erythrocyte and erythrocyte stages, means there is a large choice of antigenic targets to use for vaccine development (*Chapter 3*). Despite a number of efforts and trials to develop an effective malarial vaccine, to date none has been successful, although the complete sequencing of its genome in 2005 should assist in this.

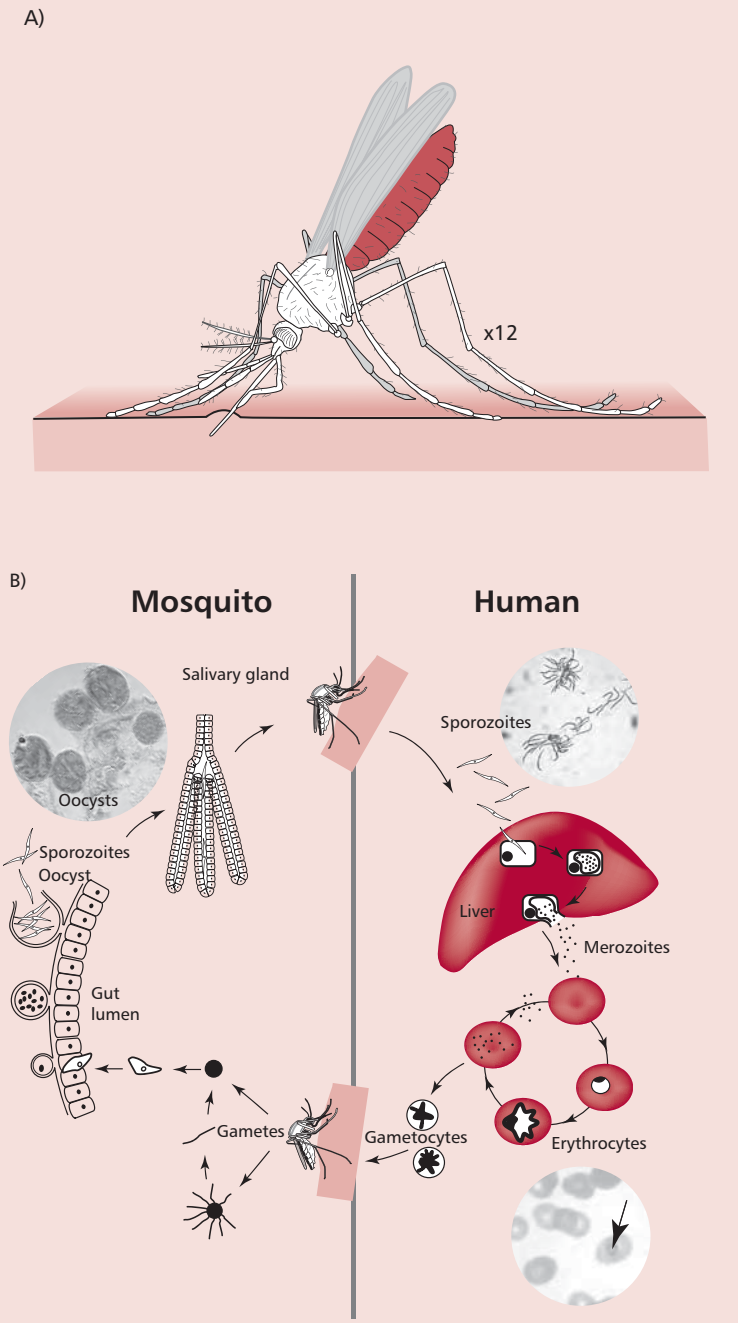

Figure 2.14 (A) Drawing of an *Anopheles* mosquito. Courtesy of Public Health Image Library, Centers for Disease Control and Prevention, USA. (B) Diagram showing the life cycle of *Plasmodium*. See text for details. **The three inserts are light micrographs showing from top left in a clockwise direction: cysts in the *Anopheles* gut, free trophozoites in the blood of a human patient and a young *P. falciparum* trophozoite in an erythrocyte. Note its characteristic signet ring shape.** Courtesy of J.R. O'Kecha, Homerton University Hospital, London.

2.3 VIRULENCE FACTORS

Evolution has provided pathogens and parasites with a wide range of factors that allow them to invade and colonize their host while at the same time avoiding and/or neutralizing host defense mechanisms. Many virulence factors of pathogens have been identified; some are relatively nonspecific in action: some microorganisms, for example, possess specialized iron uptake systems. Microorganisms require iron for oxygen transport, mitochondrial energy metabolism, electron transport, the synthesis of nucleic acids and gene expression. However, although an essential element, iron is often only available in limited quantities and microorganisms that possess a variety of iron uptake systems are able to grow in regions of the host that would otherwise be expected to be sterile since little iron is available. Other virulence factors have rather more defined defensive or offensive actions.

DEFENSIVE VIRULENCE FACTORS

Numerous pathogens evade the host's defenses by producing slime layers or possessing polysaccharide capsules (*Figure 2.15*). Slime layers consist of exopolysaccharides (EPSs) that bind large quantities of water. Slime production is particularly important in bacteria that form biofilms since it forms a protective coat around the bacterial population. For example, the biofilm formed by the opportunistic *Pseudomonas aeruginosa* in the respiratory tract of cystic fibrosis patients protects it from the immune system and antibiotics.

Capsules generally consist of a single polysaccharide structure that also binds considerable quantities of water and forms a protective layer around the bacterial cell. The polysaccharide is often negatively charged which renders it resistant to uptake by phagocytic cells. Capsules can also protect the bacterium from attack by the immune system (*Chapter 4*). Some polysaccharide capsules are molecular mimics of host cell surface structures. The capsule of *Escherichia coli* K1 and the type B capsule of *Neisseria meningitidis* consists of α-2, 8-*N*-acetylneuraminic acid residues. This is identical to neuraminic acid residues on the neuronal adhesion molecule N-CAM and other sialylated molecules of the nervous system. Consequently host immune systems do not recognize the bacteria as foreign and both pathogens can invade the CNS causing meningitis.

Other examples of molecular mimicry are the many proteins of pathogenic bacteria that are homologous to specific regions of host proteins. *Yersinia* induces the production of antibodies that cross-react with part of a particular variant of a host protein called HLA-B27 (*Chapters 4 and 5*). Cross-reactivity between other bacterial species and HLA-B27 is thought to be involved in the development of types of arthritis known as Reiter's syndrome and ankylosing spondylitis.

Some bacteria directly or indirectly activate or suppress actions of the immune system by producing pathogenicity factors called modulins or microkines. The P fimbriae of the uropathogenic *Escherichia coli*, for example, induces an increase in the release of interleukin 4 (IL-4) by uroepithelial cells. Modulation of cytokine production may lead to increased pathogenicity (*Chapter 4*).

A number of microorganisms prevent their hosts mounting an effective immune response by changing their surface antigens. This can occur in a number of ways. For example, several viruses, including influenza and HIV, have genes coding for surface proteins that mutate at relatively fast rates. This is referred to as hypermutability. Thus, the antigenic structure of these surface proteins is prone to change at intervals, leaving a population that is no longer immune to that virus. Other microorganisms that undergo antigenic variation include the trypanosome that causes sleeping sickness. These regularly change the structure of their surface glycoproteins during the course of an

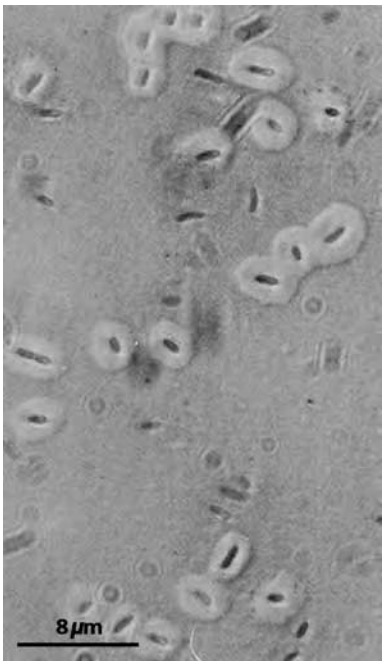

Figure 2.15 Light micrographs of ***Bacteroides fragilis*** **an obligately anaerobic bacterium that is normally found in the gastrointestinal tract. It is most frequently isolated from clinical infections such as peritonitis (*Chapter 11*). Note the prominent capsules surrounding each cell.** Courtesy of Dr S. Patrick, Queen's University Belfast, UK.

infection. The malarial parasites *Plasmodium* also express different surface antigens during the infection process. Some parasitic worms, for example, schistosomes, become coated with patient antigens, such as MHC molecules and common blood group antigens, and so avoid recognition by the host.

OFFENSIVE VIRULENCE FACTORS

Bacterial offensive virulence factors include adhesins, invasins and toxins. Adhesins are proteins found on the surfaces of microbial cells that bind to specific sites on the cells of the host. The best studied are those in the pili of, for example, certain strains of *Escherichia coli* and *Vibrio cholerae*. Pili are fibers about 2 μm long and 2 to 8 nm in diameter that extend from some bacterial cells (*Figure 2.16*). They consist of about 1000 protein molecules and include a type of adhesin that belongs to a group of biomolecules called lectins. These are glycoproteins that bind specifically to certain sugars or the glycosidic bonds found in some carbohydrates. In the case of *Escherichia coli* and *Vibrio cholerae*, these are mannose and fucose sugar residues respectively which may be found on the surfaces of host cells.

Invasins are also proteins. They allow pathogens that have bound to the host to be internalized, that is, enter the host cell preventing it from being removed by ciliary action or washing and ensuring that the pathogen is protected from direct immune attack. Once internalized, the microorganisms may remain in membrane-bound vesicles. Others escape into the cytosol and so avoid the killing mechanisms associated with phagocytosis. Some microorganisms are so adapted to intracellular life that they are unable to reproduce outside the host cell. These include species of *Chlamydia*, *Rickettsia* and some mycobacterial pathogens. These organisms are therefore obligate intracellular parasites and may cause infectious diseases, for example, *Chlamydia pneumoniae*, *Rickettsia typhi* and *Mycobacterium leprae*. However, others, such as strains of Listeriae, Salmonellae, Shigellae and Yersiniae, are facultative and can live outside their host cells.

Pathogenic microorganisms produce different types of toxin. They can be classified into two types: cell-associated toxins, for example endotoxins, and those secreted by the bacterium called exotoxins.

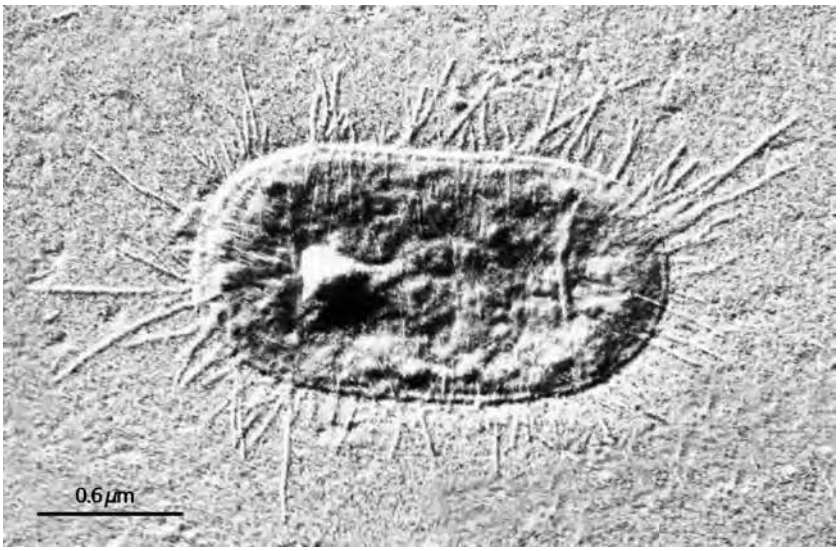

Figure 2.16 Electron micrograph of an *Escherichia coli* cell with numerous pili.

Endotoxins are produced by bacteria with Gram-negative cell walls and are lipopolysaccharides embedded in the outer membrane of the cell walls of Gram-negative bacteria. The toxic portion is called lipid A and the damage it causes varies with the susceptibility of the host. Fever is common because endotoxins stimulate host cells to release cytokines that affect the thermoregulatory center of the hypothalamus. In serious cases, endotoxic shock can result.

Exotoxins are proteins secreted by both Gram-positive and negative bacteria. They may be subdivided into three groups: those that damage membranes, those with specific host targets and superantigens.

Exotoxins that damage membranes cause the cell to lose water and ions, disrupting ion gradients across the membrane. In high doses cell lysis occurs, hence they are sometimes called hemolysins or cytolysins. A clinically significant feature of such toxins in an infection is their antiphagocytic activity. Some of the hemolysins and cytolysins of bacteria, such as *Staphylococcus aureus*, *Streptococcus pyogenes* and *Bordetella pertussis* are polypeptides that aggregate in the membranes of host cells forming pores. Thiol-activated lysins are predominantly produced by Gram-positive bacteria. These toxins are proteins that contain a large number of cysteine residues. They bind to cholesterol molecules in the membranes of target cells in oligomers of 25 to 100 toxin molecules. These form large toxin-lined aqueous pores in the membrane that constitute the lesions of membrane damage.

Phospholipases catalyze the hydrolysis of phospholipids in the membranes of host cells. For example, the α toxin of *Clostridium perfringens* (*Figure 2.17*) is a phospholipase C which catalyzes the following reaction:

$$\text{Phosphotidylcholine} + H_2O \xrightarrow{\text{Phospholipase C}} \text{phosphocholine} + \text{diacylglycerol}$$

Similarly, the β hemolysin of *Staphylococcus aureus* is a sphingomyelinase C that catalyzes the following reaction:

$$\text{Sphingomyelin} + H_2O \xrightarrow{\text{Sphingomyelinase C}} \text{phosphocholine} + \text{ceramide}$$

The degradation of membrane lipids, naturally, results in a loss of membrane integrity and function. Exotoxins that target specific sites in the host do so in a wide variety of ways, for example, they may act on cells to deregulate or kill them or they may have an extracellular target. Gram-negative bacteria, such as Enterobacteriaceae (*Escherichia coli*, *Citrobacter freundii*, *Yersinia enterocolitica*), secrete heat-stable enterotoxins as small as M_r 2000 (*Figure 2.18*). These toxins bind to specific receptors that are part of a cyclic GMP-dependent signal transduction system of enterocytes in the upper intestinal epithelium. This system regulates the concentration of intracellular cyclic GMP that, in turn, is involved in the activation of intracellular enzymes, for example, protein kinase G (*Chapter 7*). The binding of the toxins interrupts the secretion of Na^+ and Cl^- and this results in a watery diarrhea.

A number of exotoxins consist of two dissimilar polypeptides usually referred to as the A and B subunits. The B subunit recognizes and binds to specific target cells and facilitates entry of the A subunit which has an intracellular toxicity. The tetanus and botulinum toxins are Zn-dependent proteases (*Figure 2.19*) that act as neurotoxins. They catalyze the hydrolysis of synaptobrevin 2, a protein involved in docking and fusion of vesicles containing neurotransmitters. Thus their actions inhibit the release of neurotransmitters. Following its internalization into neurons of the CNS, the tetanus A subunit, tetanospasmin, migrates to peripheral nerve endings by

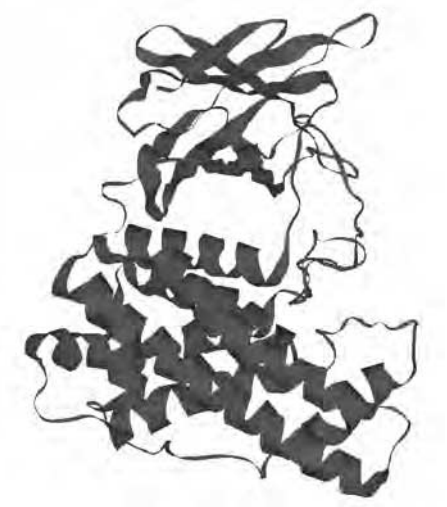

Figure 2.17 Molecular model of the α toxin of *Clostridium perfringens*. PDB file 1CA1.

Figure 2.18 Molecular model of the heat stable enterotoxin of *Escherichia coli*. The bars represent disulfide bonds. PDB file 1EHS.

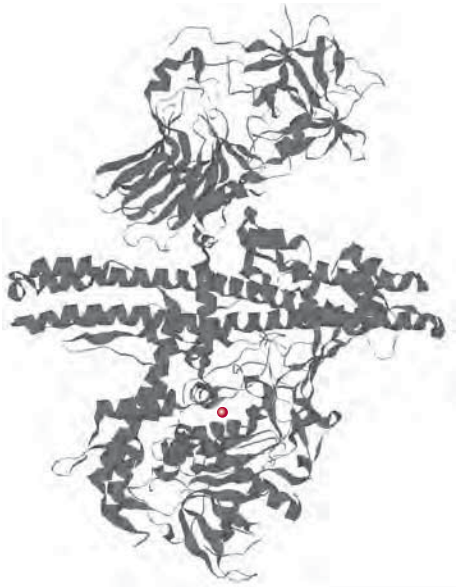

Figure 2.19 Molecular model of the botulinum toxin. The red sphere represents a bound Zn. PDB file 1S0F.

retrograde axonal transport. Here it is released by postsynaptic dendrites and diffuses to the presynaptic neurons where its action prevents the release of the inhibitory neurotransmitters, γ-aminobutyric acid and glycine. This leads to unchecked excitatory impulses with a continuous stimulation of muscles and spastic paralysis. In contrast, *botulinum toxin* is absorbed from the gastrointestinal tract and is transported to susceptible neuromuscular and peripheral autonomic synapses where it inhibits the release of acetylcholine, causing flaccid paralysis.

Some exotoxins are ADP ribosyl transferases. The cholera, pertussis and diphtheria toxins (*Figure 2.20*) use NAD^+ as a donor substrate so that the ADP ribosyl portion of NAD^+ is transferred to the target protein releasing nicotinamide. Cholera and pertussis toxins attack G proteins and interfere with signal transduction so that receptor-mediated signal transduction pathways are activated or inhibited (*Chapter 7*).

ADP ribosylation by cholera toxin fixes the Gα protein in its active form. This leads, in turn, to a long-lasting activation of adenylate cyclase and synthesis of cyclic AMP and activation of protein kinase A. The net result is a long-lived opening of the chloride channel of the cystic fibrosis transmembrane conductance regulator, (CFTR, *Chapter 16*), that increases secretion of hydrogen carbonate (HCO_3^-) and Cl^- into the intestinal lumen but which inhibits the absorption of Na^+ and Cl^-. The resulting osmotic effect causes a massive leakage of intracellular water into the intestinal lumen and subsequent diarrhea. This is called **fulminant cholera**.

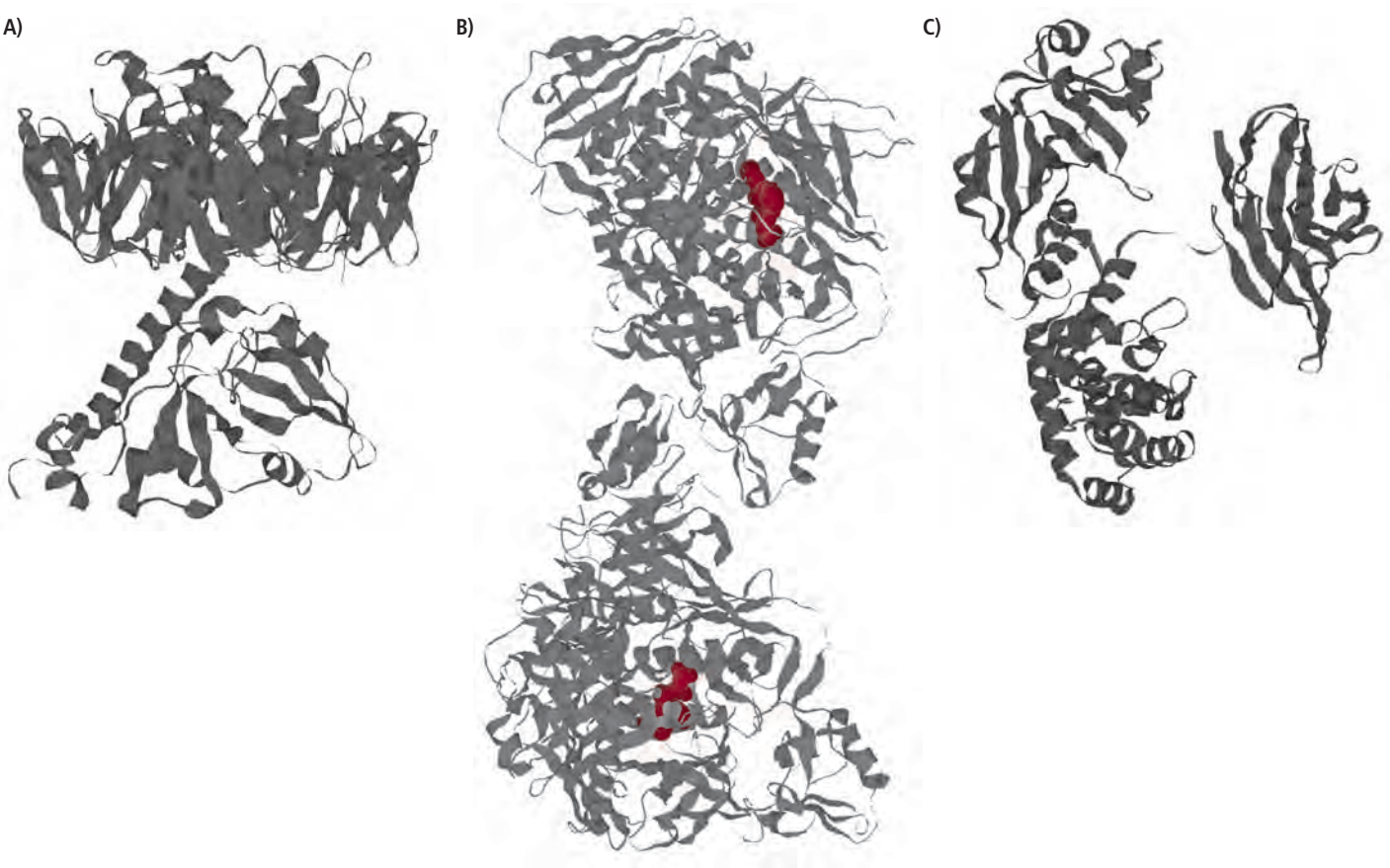

Figure 2.20 Molecular models of (A) cholera, (B) pertussis, with the bound ATP shown in red, and (C) diphtheria toxins. The structure of many bacterial toxins are known, which helps in understanding how they function and in the design of antidotes. PDB files 1XTC, 1BCP and 1DDT respectively.

Diphtheria toxin kills cells by blocking protein synthesis. The target of the toxin is a single protein, elongation factor EF2, involved in protein synthesis. Its activities are inhibited by ADP ribosylation. The Shiga family of toxins produced by *Shigella dysenteriae* and *Escherichia coli* (*Figure 2.21*) also inhibit protein synthesis but by a different mechanism. They are *N* glycosidases that hydrolyze an *N*-glycosidic bond between specific adenine and ribose residues of the 28S ribosomal RNA of the 60S ribosomal subunit.

Microorganisms secrete a number of toxins that are enzymes which degrade components of the connective tissues. Staphylococci and Streptococci secrete enzymes that degrade the extracellular matrix allowing them to invade and colonize tissues. *Clostridium perfringens* secretes a variety of carbohydrases and proteases that degrade connective tissues, allowing it to colonize and spread through subcutaneous tissues. If the bacteria invade the deeper muscles tissue they may cause necrosis and gas production from anaerobic fermentations (gas gangrene). This type of infection is extremely painful and can spread rapidly. Much of the direct damage is caused by the α toxin described above. Other pathogens secrete proteases that hydrolyze specific components of the immune defense, such as IgA or enzymes that detoxify catalases and superoxidase dismutases (SODs) that are used by some immune cells to kill microbial pathogens (*Chapter 4*).

Superantigens are polypeptides synthesized by Gram-positive pathogens, such as some strains of *Staphylococcus aureus* and *Streptococcus pyogenes*. They are extremely effective and potent stimulators of the immune system because of their unique ability to stimulate large numbers of its cells simultaneously. This leads to a massive release of molecules called cytokines that activate numerous physiological systems, such as the temperature regulatory system. The superantigen of *Streptococcus pyogenes* is responsible for streptococcal toxic shock syndrome (STSS). The fever, shock and tissue damage associated with STSS is thought to be the result of an overproduction of the cytokines Tumor Necrosis Factor α, Interleukin 1β and Interleukin 6. The superantigen of *Staphylococcus aureus*, toxic shock syndrome toxin 1 (*Figure 2.22*) causes symptoms that can lead to a rapid failure of many body organs.

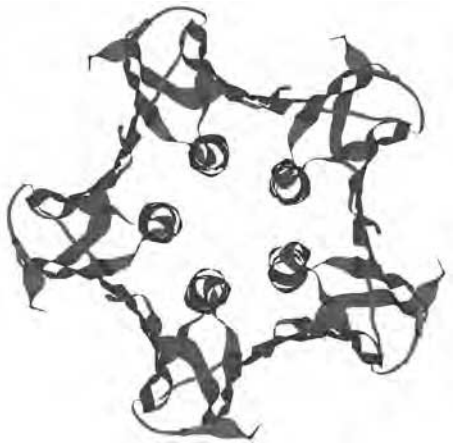

Figure 2.21 Molecular model of a Shiga-like toxin subunit. PDB file 1CZG.

Figure 2.22 Molecular model of TSST-1 from *Staphylococcus aureus*. PDB file 2QIL.

2.4 COURSE OF INFECTION

The course of an infection can be considered to follow up to four major stages namely: adhesion, entry, localized infection and generalized infection.

A virulent pathogen is one that is well adapted to establish an infection. Most pathogens are adapted to adhere to cells, usually epithelial, that line the site of entry. Adherence is the first stage of infection, given that it prevents the pathogen being swept away and eliminated from the body.

Pathogens enter the body through one of a number of so-called **portals of entry**. These include the skin and conjunctiva, respiratory tract, gastrointestinal tract (GIT), urogenital system and, in the case of fetuses, the placenta. Once entry has been gained, conditions for growth, such as temperature, nutrients, must be favorable, but the pathogen must also be able to overcome the local defenses. Pathogens are often adapted to enter their hosts through a single portal of entry and do not cause infectious diseases if they enter through a different portal.

A localized infection acts as a focus of infection and, indeed, many remain local in nature or are prevented from spreading by host defenses. If the pathogenic organisms penetrate tissues and reach the blood or lymphatic systems or enter cells such as phagocytes, they can be distributed throughout the body and infect other tissues and organs causing a generalized infection. Again,

certain pathogens are particularly well adapted to infect specific target tissues and organs. During infections, the pathogen multiplies and can be shed from the host into the environment through **portals of exit**. In localized infections these are the same as that of entry; in a generalized infection, other portals are used. These may include loss from the skin in pus, scales and blood, from the respiratory tract as droplets and aerosols of mucus and saliva, from the GIT in feces and bile, from the urogenital system in urine, mucus and genital secretions and, in pregnancy, from the placenta with direct transfer to the fetus.

A pathogen may be eliminated at any stage of the infective process. In some cases, the growth may be held in check but not eliminated. Such latent infections can be activated later giving recurring infections. In some cases, individuals can recover from a disease but the pathogen may remain in the body for considerable periods. Such people are symptomless carriers and are reservoirs of the disease.

Some viruses which exhibit latency, with or without sporadic reactivation are shown in *Table 2.2*.

Microorganism	Disease
Herpes simplex virus types 1 and 2 (HSV-1 and HSV-2)	Oral and genital herpes
Epstein-Barr virus	Glandular fever
Hepatitis B virus	Hepatitis Hepatocellular carcinoma
Varicella zoster	Chicken pox, shingles
Measles virus	Measles, subacute sclerosing panencephalitis (SSPE)

Table 2.2 Some viruses that exhibit latency

CASE STUDY 2.1

A 38-year-old male, Brian was admitted into hospital two weeks after spending a year working in several African countries. Seven days before admission Brian had developed coughing, muscular pains and recurrent chills and fevers approximately three times daily. He was also slightly jaundiced.

Questions

(a) What is the most likely disease affecting Brian?

(b) Which organism(s) causes this disease? How could this be confirmed?

(c) How could this disease have been prevented?

2.5 SUMMARY

Organisms that cause diseases in humans are found in all microbial groups, including viruses, bacteria as well as fungi and helminths. In addition, prion proteins can cause infectious disease since these aberrant proteins can be passed on, for example, in food. A number of microorganisms are not normally pathogenic, but can become so when a new 'niche' becomes available to them, for example a burn wound, or when the host is immunocompromized in some way. Pathogenic microorganisms display a variety of virulence factors that aid entry into the host and which help them to overcome host defenses. These virulence factors can have a generalized

action or be more specifically defensive or offensive in nature. An example of the former is the possession of hypermutable genome that leads to changes in the nature of the surface antigens of the microorganism and enables them to escape the host's immune system. Examples of offensive virulence factors include the production of exotoxins.

QUESTIONS

1. To which class of pathogen or parasite do the following belong:

   Trypanosoma brucei, *HIV*, *Microsporum* species *Treponema pallidum*, *Candida albicans*?

2. Arrange the two following lists into their most appropriate pairings.

Clostridium perfringens	shigellosis
Epstein-Barr virus	sleeping sickness
HIV	gas gangrene
Human rhinovirus 91	chicken pox
Microsporum spp, *Trichophyton* species	bubonic plague
Plasmodium	CD4
Sarcoptes scabiei	malaria
Shigellae dysenteriae	glandular fever
Trypanosoma brucei	scabies
Varicella zoster	intracellular adhesion molecule
Yersinia pestis	athlete's foot

3. Compare and contrast the actions of endo- and exotoxins.

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Useful web site:

www.ph.ucla.edu/epi/snow.html