

Pathogens, Infection, and Innate Immunity

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Infectious diseases currently cause about one-third of all human deaths in the world, more than all forms of cancer combined. In addition to the continuing heavy burden of ancient diseases such as tuberculosis and malaria, new infectious diseases are continually emerging, including the current pandemic (worldwide epidemic) of *AIDS (acquired immune deficiency syndrome)*, which has already caused more than 25 million deaths worldwide. Moreover, some diseases long thought to result from other causes are now turning out to be associated with infections. Most gastric ulcers, for example, are caused not by stress or spicy food, as was once believed, but by a bacterial infection of the stomach caused by *Helicobacter pylori*.

The burden of infectious diseases is not spread equally across the planet. Poorer countries and communities suffer disproportionately. Frequently, the prevalence of infectious diseases correlates with poor public sanitation and public health systems, which are often further compromised by natural disasters or political upheavals. Some infectious diseases, however, occur primarily or exclusively among industrialized communities: Legionnaire's disease, commonly spread through air-conditioning systems, is a recent example.

Humans have long been both troubled and fascinated with infectious diseases. The earliest written descriptions of how to limit the spread of rabies date back more than 3,000 years. Since the mid-1800s, physicians and scientists have struggled to identify the agents that cause infectious diseases, collectively called **pathogens**. More recently, the advent of microbial genetics and molecular cell biology has greatly enhanced our understanding of the causes and mechanisms of infectious diseases. We now know that pathogens frequently exploit the biological attributes of their host's cells in order to infect them. This understanding can give us new insights into normal cell biology, as well as strategies for treating and preventing infectious diseases.

In a world teeming with hostile, subtle, and rapidly evolving pathogens, how does a fragile and slowly evolving human survive? Like all other multicellular organisms, we have evolved several mechanisms to resist infection by pathogens. First, physical barriers, such as our tough outer layers of skin, and associated chemical defenses, such as acid in the stomach, prevent most microorganisms (microbes) from coming into contact with sterile tissues in our body. Second, individual human cells possess some intrinsic defensive capabilities; for example, cells aggressively degrade double-stranded RNA molecules, which are a hallmark of certain kinds of viral infections. To combat especially powerful pathogens that breach these barricades, vertebrates use two types of immune defense, which are carried out by specialized proteins and cells: **innate immune responses** spring into action immediately after an infection begins and do not depend on the host's prior exposure to the pathogen, while more powerful **adaptive immune responses** operate later in an infection and are highly specific for the pathogen that induced them.

In this chapter, we begin with an overview of the different kinds of organisms that cause disease. We then discuss the cell biology of infection and, finally, consider barriers to infection and innate immunity. Adaptive immunity is the subject of Chapter 25.

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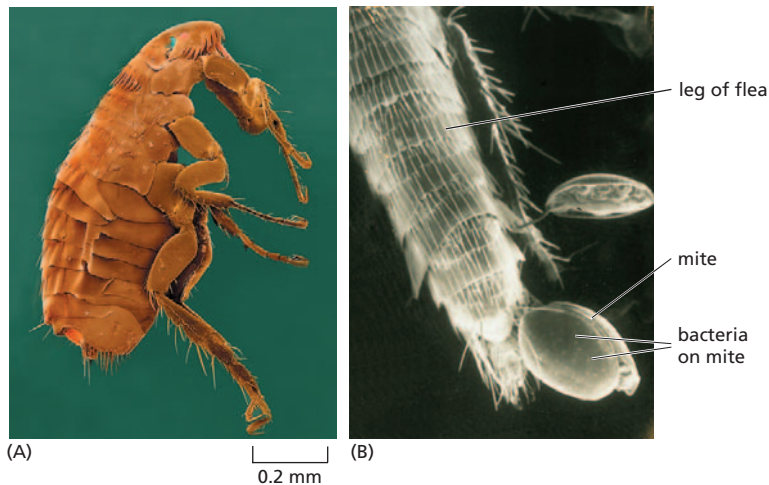


Figure 24–1 Parasitism at many levels. (A) Scanning electron micrograph of a flea. The flea is a common parasite of mammals—including dogs, cats, rats, and humans. It drinks the blood of its host. Flea bites spread bubonic plague by passing the pathogenic bacterium *Yersinia pestis* from the bloodstream of one infected host to that of another. (B) A close-up view of this flea’s leg reveals that it also has a parasite, a type of mite. The mite, in turn, is covered with bacteria. It is likely that *bacteriophages*, which are bacterial viruses, parasitize these bacteria.

Jonathan Swift reported a similar observation in 1733:

So, naturalists observe, a flea
Has smaller fleas that on him prey;
And these have smaller still to bite ‘em;
And so proceed ad infinitum.
(A, courtesy of Tina Carvalho/
MicroAngela; B, courtesy of Stanley
Falkow.)

INTRODUCTION TO PATHOGENS

We normally think of pathogens as hostile invaders that attack our bodies. But a pathogen, like any other organism, is simply fulfilling its biological imperative to live and procreate. Living at the expense of a host organism is a very effective strategy, and it is possible that every living organism on earth is subject to some type of infection (Figure 24–1). A human host is a nutrient-rich, warm, and moist environment, which remains at a uniform temperature and constantly renews itself. It is not surprising that many microorganisms have evolved the ability to survive and reproduce in this desirable niche. In this section, we discuss some of the common features that microorganisms must have in order to be infectious. We then explore the wide variety of organisms that are known to cause disease in humans.

Pathogens Have Evolved Specific Mechanisms for Interacting with Their Hosts

The human body is a complex and thriving ecosystem. It contains about 10^{13} human cells and also about 10^{14} bacterial, fungal, and protozoan cells, which represent thousands of microbial species. These commensal microbes, called the **normal flora**, are usually confined to certain areas of the body, including the skin, mouth, large intestine, and vagina. The normal flora are not just free-loading inhabitants of the ecosystem that is the human body, they can also affect our health. The anaerobic bacteria that inhabit our intestines contribute to the digestion of food, and they are also essential for proper development of the gastrointestinal tract in infants. Normal flora on the skin and elsewhere also help us by competing with disease-causing microorganisms. In addition, humans are always infected with viruses, the vast majority of which rarely become noticeable.

If it is normal for us to live in such close intimacy with a wide variety of microbes, why are some of them capable of causing us illness or death? As we shall see, this question has several answers, and the ability of a particular microorganism to cause obvious damage and disease in a host can depend greatly on external influences. **Primary pathogens**, which can cause overt disease in most healthy people, are usually distinct from the normal flora. They differ from commensal organisms in their abilities to breach barriers and survive in host locations where other microorganisms cannot. Our normal microbial inhabitants only cause trouble if our immune systems are weakened or if they gain access to a normally sterile part of the body, as when a bowel perforation enables gut flora to enter the peritoneal cavity of the abdomen, causing *peritonitis*; occasionally they cause disease if our immune response to them is inappropriately strong. In contrast, primary pathogens do not require an immunocompromised or injured host. Primary pathogens have evolved highly specialized

mechanisms for crossing cellular and biochemical barriers and for eliciting specific responses from the host organism that contribute to the pathogen's survival and multiplication. For some pathogens, these mechanisms are adapted to a unique host species, whereas for others they are sufficiently general that the pathogen can invade, survive, and thrive in a wide variety of hosts.

Some pathogens cause acute epidemic infections and are forced to spread rapidly from one sick or dying host to another; historically important examples include bubonic plague and smallpox. Others cause persistent infections that may last for years in a single individual without necessarily leading to overt disease; examples include Epstein–Barr virus (which can cause the severe flu-like illness mononucleosis in some people), the bacterium *Mycobacterium tuberculosis* (which can cause the life-threatening lung infection tuberculosis), and the intestinal worm *Ascaris*. Although each of these pathogens can make some people critically ill, billions of people who are mostly unaware that they are infected carry each of them in an asymptomatic way. It is hard to draw a line between persistent infection and commensalism. Throughout this chapter, we shall continue to acknowledge the diversity of pathogens and infections while focusing on the cell biological principles that are common to many of them.

In order to survive and multiply in a host, a successful pathogen must be able to: (1) colonize the host; (2) find a nutritionally compatible niche in the host's body; (3) avoid, subvert, or circumvent the host's innate and adaptive immune responses; (4) replicate, using host resources; and (5) exit and spread to a new host. Under severe selective pressure to induce host responses that help to accomplish these tasks, pathogens have evolved mechanisms that maximally exploit the biology of their host organisms. Many of them are therefore skillful and practical cell biologists, metaphorically speaking, and we can learn a great deal of cell biology by observing them.

At the same time, our constant exposure to pathogens has strongly influenced human evolution. The development of the exquisitely precise adaptive immune system in vertebrates, described in Chapter 25, was an important escalation in the arms race that has always existed between pathogens and their hosts. In modern times, humans have upped the ante by deliberately altering our behavior to limit the ability of pathogens to infect us. Improvements in public health measures, including the construction of working sewer systems and clean water supplies, have contributed to the gradual decline in the frequency of total deaths due to infectious disease over the past several centuries. Societies that have dedicated resources to improving childhood nutrition have benefited from generally improved health, including greatly reduced death rates from early childhood infections. Medical interventions such as vaccinations, antimicrobial drugs, and routine testing of blood before using it for transfusion, have also substantially reduced the burden of infectious disease for many humans. As we learn more about the mechanisms by which pathogens cause disease (pathogenesis), our brains will continue to serve as an important extension of our immune systems in fighting infectious diseases.

The Signs and Symptoms of Infection May Be Caused by the Pathogen or by the Host's Responses

Although we can easily understand why infectious microorganisms would evolve to reproduce in a host, it is less clear why they would evolve to cause disease, that is, to damage their hosts. One explanation may be that, in some cases, the pathological responses that microorganisms elicit enhance the efficiency of their spread or propagation and hence clearly have a selective advantage for the pathogen. The virus-containing lesions on the genitalia caused by *herpes simplex* infection, for example, facilitate direct spread of the virus from an infected host to an uninfected partner during sexual contact. Similarly, diarrheal infections are efficiently spread from patient to caretaker. In many cases, however, the induction of disease has no apparent advantage for the pathogen. Some host responses to infection such as lethargy and withdrawal from social interactions would instead seem to inhibit pathogen spread. Infected humans may altruistically try

to avoid infecting their friends and family members, and healthy humans may likewise consciously avoid the sick; similar behavior has also been documented in spiny lobsters. These may be adaptive responses that have been selected in host populations that tend to live in groups.

Many of the symptoms and signs that we associate with infectious disease are actually direct manifestations of the host's immune responses in action. Some hallmark signs at the site of a bacterial infection, including swelling, redness, and the production of pus (mainly dead white blood cells), result from immune system cells attempting to destroy the invading microorganisms. Fever, too, is a defensive response, as the increase in body temperature can inhibit the proliferation of some microorganisms. In extreme cases, the most severe and damaging consequences of an infectious disease are directly caused by an overzealous immune response: the massive tissue destruction seen in cases of leishmaniasis (an infection caused by eucaryotic pathogens that are members of the genus *Leishmania*) is an example. Thus, understanding the biology of an infectious disease requires an appreciation of the contributions of both pathogen and host.

To understand the relative contributions of the infecting microorganism and the host in causing the signs and symptoms of disease, it is useful to consider the cause and extent of damage done to host tissues during an infection. Each interaction between a particular microorganism and a particular host is unique, and the outcome depends on a constantly changing landscape of microbial activity and host immune function. The extent of damage caused to the host depends on the interplay of these factors. In some cases, a particular microorganism may act as a harmless or even beneficial commensal in most people at most times but may cause invasive disease in people with weakened immune systems; this is true for the common skin inhabitant *Staphylococcus epidermidis*, for example (Figure 24–2A). Other microorganisms, such as the virus that causes mumps, will cause severe damage only in the presence of strong immune responses (Figure 24–2B). A very interesting category, nicely illustrating the importance of the interplay between host and microbial factors in causing damage, are the many pathogens that cause severe disease in people with either very weak or very strong immune responses but little if any damage in people with intermediate immune responses (Figure 24–2C). An excellent example is tuberculosis, which currently infects between 1 and 2 billion people on Earth (usually in their lungs), although most are unaware of it because their immune systems have effectively contained the infection. When, however, a person with such a latent *M. tuberculosis* infection becomes immunosuppressed, through drug therapy or infection with the human immunodeficiency virus (HIV), for example, the delicate balance between the bacterium and the immune system is tipped in favor of the bacterium, which now replicates in an uncontrolled manner, leading to serious disease, often with painful cough producing bloody sputum. Conversely, when the immune response to *M. tuberculosis* is overzealous, it can destroy an extensive amount of lung tissue.

Pathogens Are Phylogenetically Diverse

Many types of pathogens cause disease in humans. The most familiar are viruses and bacteria. Viral infections cause diseases ranging from AIDS and smallpox to the common cold. They are essentially fragments of nucleic acid (DNA or RNA) encoding a relatively small number of gene products, wrapped in a protective shell of proteins and (in some cases) membrane (Figure 24–3A). They have no capacity for independent metabolic activity and therefore depend absolutely on metabolic energy supplied by the host. They all use the basic protein synthesis machinery of their host cells for their replication, and many rely on host cell transcription machinery as well.

Of all the bacteria we encounter in our lives, only a small minority are primary pathogens. Much larger and more complex than viruses, bacteria are usually free-living cells, which perform most of their basic metabolic functions themselves, relying on the host primarily for nutrition (Figure 24–3B).

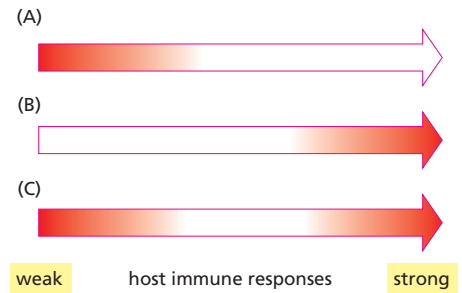


Figure 24–2 Interaction between microbes and immune responses in microbial pathogenesis. The strength of the immune responses increases in the direction of the arrows, while the amount of host damage is indicated by the amount of red shading in the arrows. (A) Normal flora such as the bacterium *Staphylococcus epidermidis* and the fungus *Pneumocystis carinii* only cause disease when the immune system is abnormally weak. (B) For some pathogens, such as the paramyxovirus that causes mumps and the bacterium *Chlamydia trachomatis* that causes a common sexually transmitted disease, it is the immune responses rather than the pathogen that is primarily responsible for the tissue damage. (C) For many pathogens, hosts with normal immune responses may be colonized asymptotically, but hosts with very strong or very weak immune responses may suffer severe damage or even death. Many microorganisms behave in this way, including the bacterium *Mycobacterium tuberculosis*, the fungus *Aspergillus*, and the herpes simplex virus.

Some other infectious agents are eucaryotic organisms. These range from single-celled fungi and protozoa (Figure 24–3C), through large complex metazoa such as parasitic worms. One of the most common infectious diseases on the planet, shared by about a billion people at present, is an infestation in the gut by the nematode worm *Ascaris lumbricoides*. *Ascaris* closely resembles its cousin *Caenorhabditis elegans*, which is widely used as a model organism for genetic and developmental biological research (discussed in Chapter 22). *C. elegans*, however, is only about 1 mm in length, whereas *Ascaris* can reach 30 cm (Figure 24–3D).

Some rare neurodegenerative diseases, including “mad cow” disease, are caused by an unusual type of infectious particle called a *prion*, which is made only of protein. Although the prion contains no genome, it can nevertheless replicate and kill the host.

Even within each class of pathogen, there is striking diversity. Viruses vary tremendously in their size, shape, and content (DNA versus RNA, enveloped or not, and so on), and the same is true for the other pathogens. The ability to cause disease is an evolutionary niche, not a legacy shared only among close relatives.

Each individual pathogen causes disease in a different way, and the same pathogen may cause different diseases in different hosts, making it challenging to understand the basic biology of infection. But, when considering the interactions of infectious agents with their hosts, some common themes of pathogenesis emerge, which are the focus of this chapter.

We now introduce the basic features of each of the major types of pathogens, before we examine the mechanisms that pathogens use to control their hosts and the innate immune responses that hosts use to control pathogens.

Bacterial Pathogens Carry Specialized Virulence Genes

Bacteria are small and appear structurally simple. Most can be classified broadly by their shape as rods, spheres, or spirals (Figure 24–4A) and by their so-called **Gram-staining** properties (Figure 24–4B and C). Their relatively small size and simple range of shapes belies their extraordinary molecular, metabolic, and ecological diversity. At the molecular level, bacteria are far more diverse than eucaryotes, and they can successfully occupy ecological niches in extremes of temperature, salt, and nutrient limitation that would daunt even the most

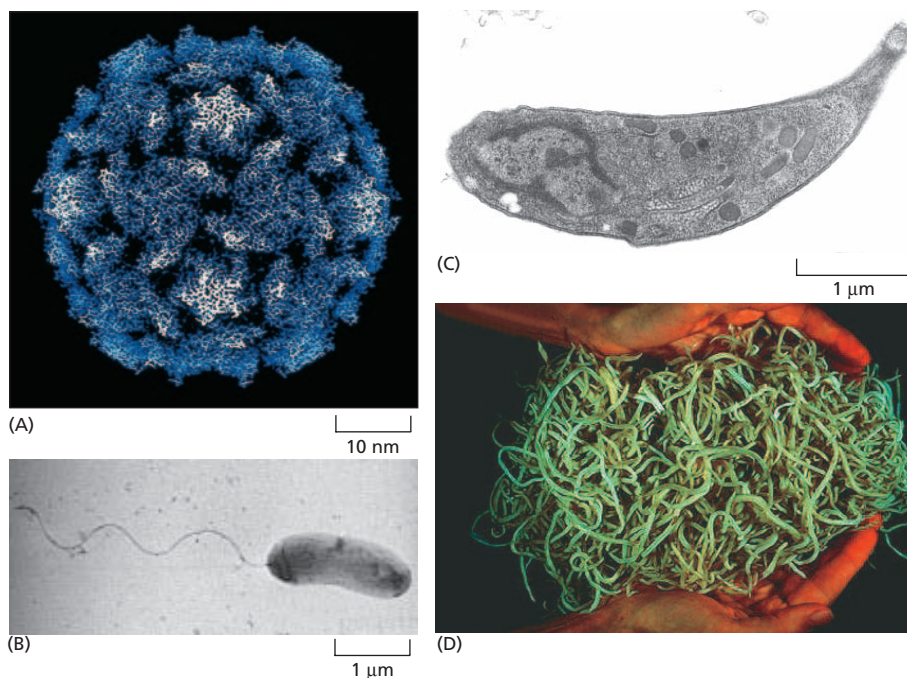


Figure 24–3 Pathogens in many forms.

(A) The structure of the protein coat, or *capsid*, of poliovirus. This virus was once a common cause of paralysis, but the disease (poliomyelitis) has been nearly eradicated by widespread vaccination. (B) The bacterium *Vibrio cholerae*, the causative agent of the epidemic, diarrheal disease cholera. (C) The protozoan parasite *Toxoplasma gondii*. The definitive hosts for this organism are cats, ranging in size from housecats to tigers, but it also can cause serious infections in the muscles and brains of immunocompromised people with AIDS. (D) This clump of *Ascaris* nematodes was removed from the obstructed intestine of a two-year-old boy. (A, courtesy of Robert Grant, Stephan Crainic, and James M. Hogle; B, all attempts have been made to contact the copyright holder and we would be pleased to hear from them; C, courtesy of John Boothroyd and David Ferguson; D, from J.K. Baird et al., *Am. J. Trop. Med. Hyg.* 35:314–318, 1986. Photograph by Daniel H. Connor.)

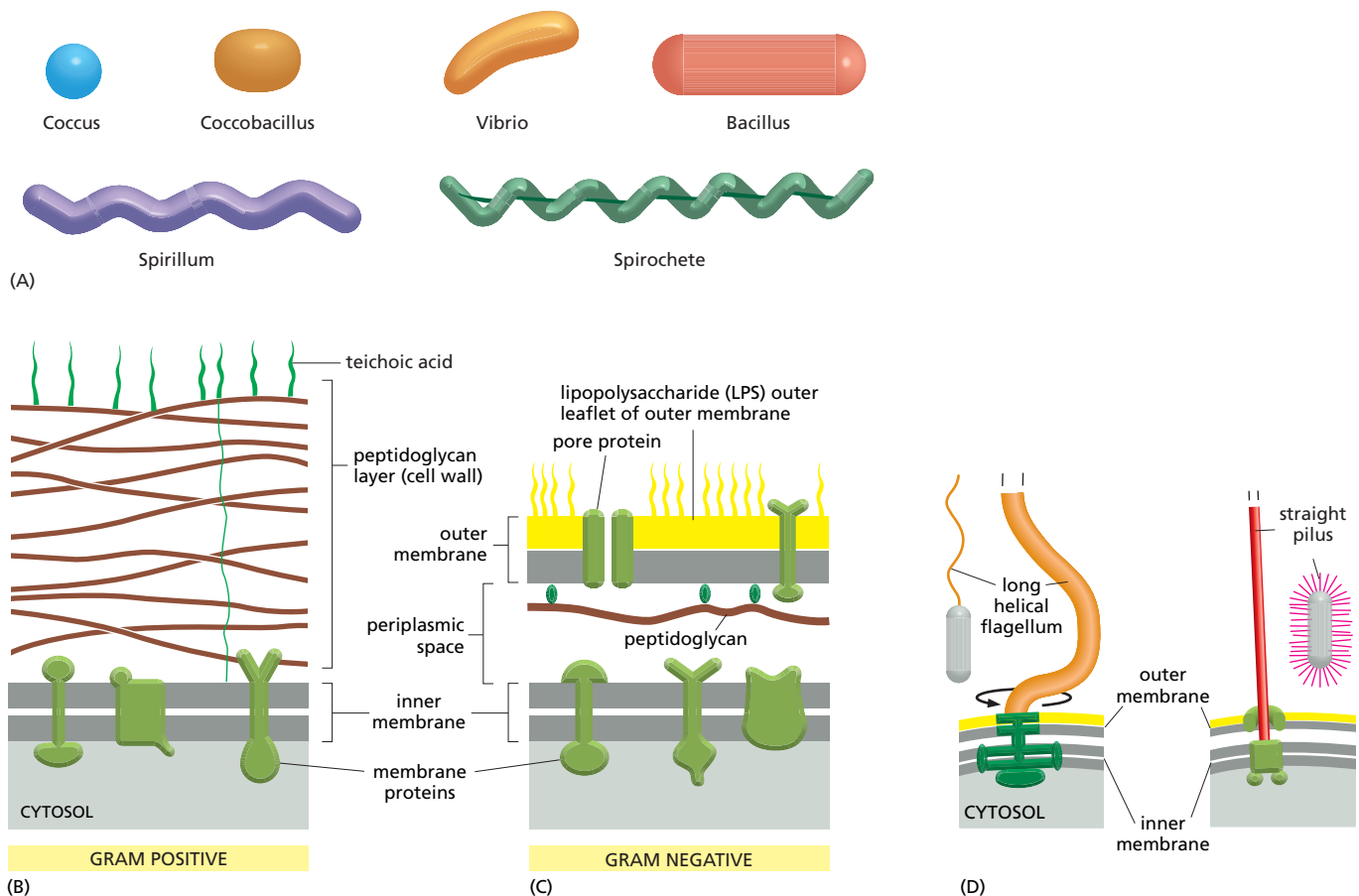


Figure 24-4 Bacterial shapes and cell-surface structures. (A) Bacteria are classified by shape. (B and C) They are also classified as *Gram-positive* or *Gram-negative*. (B) Bacteria such as *Streptococcus* and *Staphylococcus* have a single membrane and a thick cell wall made of cross-linked *peptidoglycan*. They retain the violet dye used in the Gram staining procedure and are thus called Gram-positive. (C) Gram-negative bacteria such as *Escherichia coli* (*E. coli*) and *Salmonella* have two membranes, separated by a *periplasmic space* (see Figure 11-18). The peptidoglycan layer in the cell wall of these organisms is located in the periplasmic space and is thinner than in Gram-positive bacteria; they therefore fail to retain the dye in the Gram staining procedure. The inner membrane of Gram-negative bacteria is a phospholipid bilayer, and the inner leaflet of the outer membrane is also made primarily of phospholipids; the outer leaflet of the outer membrane, however, is composed of a unique glycosylated lipid called *lipopolysaccharide* (*LPS*) (see Figure 24-47). (D) Cell-surface appendages are important for bacterial behavior. Many bacteria swim using the rotation of helical flagella (see Figure 15-71). The bacterium illustrated has only a single flagellum at one pole; others such as *E. coli* have multiple flagella. Straight *pili* (also called *fimbriae*) are used to adhere to various surfaces in the host, as well as to facilitate genetic exchange between bacteria. Some kinds of pili can retract to generate force and thereby help bacteria move across surfaces. Both flagella and pili are anchored to the cell surface by large multiprotein complexes.

intrepid eucaryote. Although they lack the elaborate morphological variety of eucaryotic cells, bacteria display a surprising array of surface appendages, which enable the cells to swim or adhere to desirable surfaces (Figure 24-4D). Their genomes are also small, typically between 1,000,000 and 5,000,000 nucleotide pairs (compared to 12,000,000 for yeast and more than 3,000,000,000 for humans).

As already emphasized, only a minority of bacterial species have the ability to cause disease in humans. Some of those that do cause disease can only replicate inside the body of their host and are called *obligate pathogens*. Others replicate in an environmental reservoir such as water or soil and only cause disease if they happen to encounter a susceptible host; these are called *facultative pathogens*. Many bacteria are normally harmless but have a latent ability to cause disease in an injured or immunocompromised host; these are called *opportunistic pathogens*. As discussed previously, whether or not a particular bacterium causes disease in a particular host depends on a wide variety of factors, including the overall health of the host; many normal flora, for example, can cause severe infections in people with AIDS.

Some bacterial pathogens are fastidious in their choice of host and will infect only a single species or a group of related species, whereas others are generalists. *Shigella flexneri*, for example, which causes epidemic dysentery (bloody diarrhea) in areas of the world lacking a clean water supply, will infect only humans and other primates. By contrast, the closely related bacterium *Salmonella enterica*, which is a common cause of food poisoning in humans, can also infect many other vertebrates, including chickens and turtles. A champion generalist is the opportunistic pathogen *Pseudomonas aeruginosa*, which is capable of causing disease in plants as well as animals.

A relatively small number of genes causes the significant differences between a virulent pathogenic bacterium and its closest nonpathogenic relative. Genes that contribute to the ability of an organism to cause disease are called **virulence genes**, and the proteins they encode are called **virulence factors**. Virulence genes are frequently clustered together, either in groups on the bacterial chromosome called *pathogenicity islands* or on extrachromosomal *virulence plasmids* (Figure 24–5). These genes may also be carried on mobile *bacteriophages* (bacterial viruses). It seems therefore that a new pathogen may arise when groups of virulence genes are transferred together into a previously avirulent bacterium. As more genomes of pathogenic and nonpathogenic bacteria are being completely sequenced, it is becoming clear that the acquisition of large chunks of DNA and other gross chromosomal changes have contributed to bacterial evolution, enabling bacterial species to inhabit new ecological and nutritional niches, as well as to cause disease. Even within a single bacterial species, the amount of chromosomal variation is astonishing; different strains of *E. coli* may differ by as much as 25% in their genomes.

Acquisition of genes and gene clusters can drive the rapid evolution of pathogens and turn nonpathogens into pathogens. Consider, for example, *Vibrio cholerae*—the Gram-negative bacterium that causes the epidemic diarrheal disease cholera. The genes encoding the two subunits of the toxin that cause the diarrhea are carried on a mobile bacteriophage (Figure 24–6A and B). Of the hundreds of strains of *Vibrio cholerae* found in lakes in the wild, the only ones that cause pandemic human disease are those infected with this bacterial virus. As summarized in Figure 24–6C, there have been eight identified pandemics of *V. cholerae* since 1817. The first six were caused by the periodic reemergence of similar strains, called Classical strains. Besides the toxins encoded by the bacteriophage and pathogenicity islands, the Classical strains also shared a similar primary carbohydrate surface antigen, called O1, which is part of the lipopolysaccharide that makes up the outer leaflet of the outer membrane (see Figure 25–4C). In 1961, the seventh pandemic began, caused by a new strain (named “El Tor”), which was markedly different from the Classical strains and appeared to have arisen when an O1-expressing strain in the wild acquired two bacteriophages, as well as at least two new pathogenicity islands not found in Classical strains. El Tor eventually displaced the Classical strains all over the world. In 1991, an eighth pandemic began, this time with the frightening twist that even people who had suffered cholera previously were not immune, as the new strain had a different type of O antigen, rendering the anti-O1 antibodies present in the blood of survivors of previous cholera epidemics ineffective against the new strain. In other respects, the new strain was very similar to El Tor; it had apparently simply acquired a new cassette for synthesis of a different type of O antigen.

What are the genes that can enable a bacterium to cause disease in a healthy host? Many virulence genes encode proteins that interact directly with host cells.

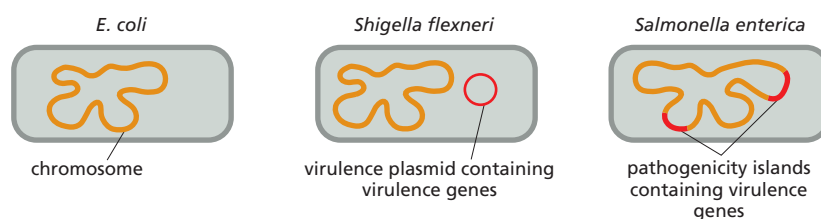


Figure 24–5 Genetic differences between pathogenic and nonpathogenic bacteria. Nonpathogenic *E. coli* has a single circular chromosome. *E. coli* is very closely related to two types of food-borne pathogens—*Shigella flexneri*, which causes dysentery, and *Salmonella enterica*, a common cause of food poisoning. If these three organisms were being named today based on molecular techniques, they would be classified in the same genus, if not in the same species. The chromosome of *S. flexneri* differs from that of *E. coli* at only a few loci; most of the genes required for pathogenesis (virulence genes) are carried on an extrachromosomal virulence plasmid. The chromosome of *S. enterica* carries two large inserts (pathogenicity islands) not found in the *E. coli* chromosome; these inserts each contain many virulence genes.

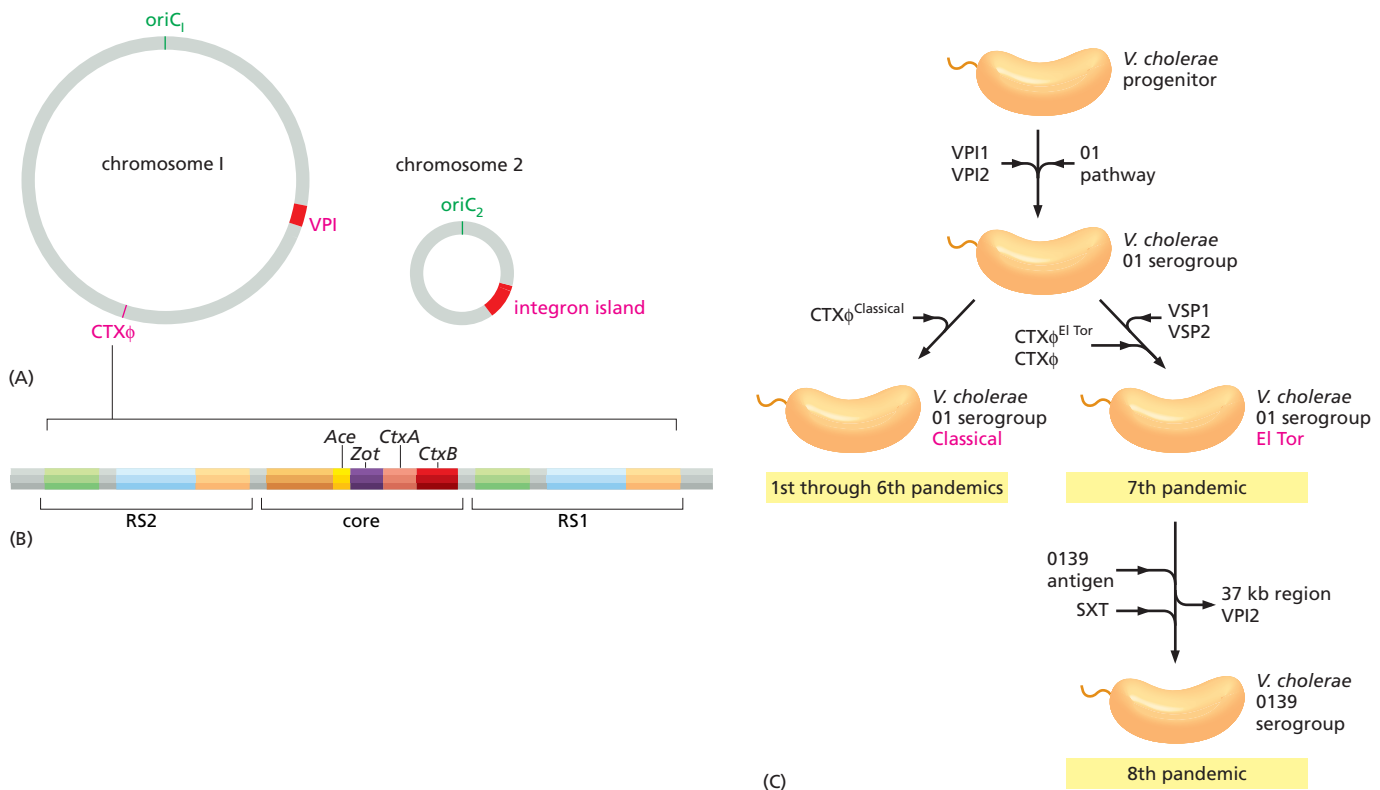


Figure 24–6 Genetic organization of *Vibrio cholerae*. An electron micrograph of *Vibrio cholerae* (*V. cholerae*) is shown in Figure 24–3B. (A) The bacterium is unusual in having two circular chromosomes rather than one. Each chromosome has its own origin of replication (*oriC₁* and *oriC₂*). Three loci in pathogenic strains of *V. cholerae* are absent in nonpathogenic strains and appear to have been acquired relatively recently. *CTX ϕ* on chromosome 1 is an integrated bacteriophage genome that carries the genes encoding cholera toxin. The pathogenicity island *VPI* on chromosome 1 includes genes encoding factors required for intestinal colonization. The *integron island* on chromosome 2 facilitates the insertion of newly acquired DNA fragments downstream of a strong transcriptional promoter and thereby promotes the sequential acquisition of novel genes. Although this integron island has not yet been shown to be required for virulence in *V. cholerae*, similar integron islands in many other pathogens contain virulence genes, as well as genes involved in antibiotic resistance. (B) Map of the *CTX ϕ* locus. The genes encoding the two subunits of cholera toxin are *CtxA* and *CtxB*. Other genes in the core region (*Ace* and *Zot*) are also involved in virulence. The two repeated flanking sequences *RS2* and *RS1* were involved in the insertion of the bacteriophage genome into chromosome 1. (C) Comparative-genomics-based model for the evolution of pathogenic *V. cholerae* strains. Progenitor strains in the wild first acquired the biosynthetic pathway necessary to make the O1 antigen type of carbohydrate chain on the outer-membrane lipopolysaccharide (see Figures 24–4C and 24–47), and perhaps also acquired one or two of the *Vibrio* pathogenicity islands (*VPI1* and *VPI2*). Incorporation of the *CTX ϕ* bacteriophage created the Classical pathogenic strains responsible for the first six worldwide epidemics of cholera between 1817 and 1923. Sometime in the twentieth century, an O1 strain in the environment picked up the *CTX ϕ* bacteriophage again, along with an associated bacteriophage *RS1 ϕ* and two new pathogenicity islands (*VSP1* and *VSP2*), creating the El Tor strain that emerged as the seventh worldwide pandemic in 1961. Before 1991, an El Tor strain picked up a new DNA cassette enabling it to produce the O139 antigen type of carbohydrate chain rather than O1. This altered its interaction with the human immune system, without diminishing its virulence, and triggered the start of the eighth pandemic; this bacterium also picked up a new pathogenicity island (*SXT*) and lost most of the *VPI2* island.

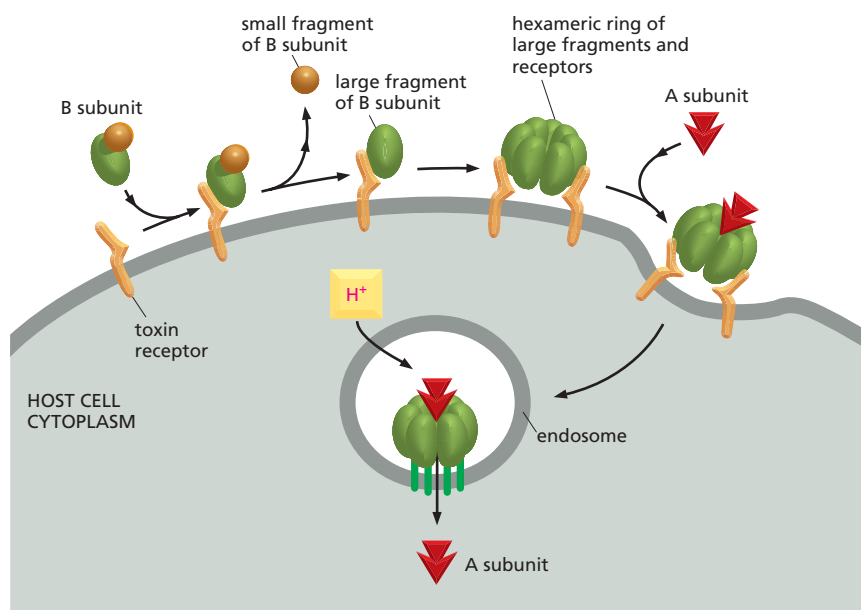
Two carried by the *Vibrio cholerae* phage, for example, encode two subunits of **cholera toxin** (see Figure 24–6B). The B subunit of this secreted, toxic protein binds to a glycolipid component of the plasma membrane of the epithelial cells in the gut of a person who has consumed *Vibrio cholerae* in contaminated water. The B subunit transfers the A subunit through the plasma membrane into the epithelial cell cytoplasm. The A subunit is an enzyme that catalyzes the transfer of an ADP-ribose moiety from NAD^+ to the trimeric G protein G_s , which normally activates adenyl cyclase to make cyclic AMP (discussed in Chapter 15). ADP-ribosylation of the G protein results in an overaccumulation of cyclic AMP and an ion imbalance, leading to the massive watery diarrhea associated with cholera. The infection is then spread to new hosts by the fecal–oral route via contaminated food and water.

Some pathogenic bacteria use several independent mechanisms to cause toxicity to the cells of their host. *Anthrax*, for example, is an acute infectious disease

of sheep, cattle, other herbivores, and occasionally humans. It is usually caused by contact with spores of the Gram-positive bacterium *Bacillus anthracis*. Unlike cholera, anthrax does not spread directly from person to person. Dormant spores can survive in soil for long periods and are highly resistant to adverse environmental conditions, including heat, ultraviolet and ionizing radiation, pressure, and chemical agents. After the spores are inhaled, ingested, or rubbed into breaks in the skin, the spores germinate, and the bacteria begin to replicate. The bacteria secrete two toxins, called **lethal toxin** and **edema toxin**, either of which is sufficient to cause signs of infection. Like cholera toxin, both anthrax toxins are made of two subunits. The B subunit is identical in the two anthrax toxins, and it binds to a host cell-surface receptor protein to transfer the two different A subunits into host cells (Figure 24–7). The A subunit of edema toxin is an adenyl cyclase that directly converts host-cell ATP into cyclic AMP, leading to an ion imbalance that can cause an accumulation of extracellular fluid (*edema*) in the infected skin or lung. The A subunit of lethal toxin is a protease that cleaves several members of the MAP kinase family (see Figure 15–60). Injection of lethal toxin into the bloodstream of an animal causes shock (a fall in blood pressure) and death. The molecular mechanisms leading to death in anthrax remain uncertain.

These examples illustrate a common theme among virulence factors. The factors are frequently either toxic proteins (*toxins*) that interact directly with important host structural or intracellular signaling proteins to elicit a host cell response that is beneficial to pathogen colonization or replication, or they are proteins that are needed to deliver such toxins to the host cell targets. One common and particularly efficient delivery mechanism found in several Gram-negative pathogens, called the **type III secretion system**, acts like a tiny syringe that injects toxic proteins from the cytoplasm of an extracellular bacterium directly into the cytoplasm of an adjacent host cell (Figure 24–8). The effector proteins that these injection devices deliver into the host cell cytoplasm can elicit a variety of host cell responses that enable the bacterium to invade or survive. There is a remarkable degree of structural similarity between the type III syringe and the base of a bacterial flagellum (see Figure 15–71), and many of the proteins in the two structures are homologous. Because flagella are found in a wider range of bacteria than are type III secretion systems and the secretion systems appear to be adaptations specific for pathogenesis, it seems likely that the type III secretion systems evolved from flagella rather than the other way around. Other types of specialized toxin delivery systems found in pathogens appear to have evolved independently. For example, *type IV secretion systems*, used by several pathogens

Figure 24–7 Anthrax toxin entry into host cells. (A) The B subunits of the toxin form a pore to transport the enzymatically active A subunit into the cytoplasm of host cells. The B subunit binds to a receptor protein on the host cell surface and is cleaved autocatalytically, releasing a soluble small fragment and a large fragment that remains associated with the receptor. Seven copies of the large fragment and their associated receptors aggregate on the cell surface to form a heptameric ring. The toxin A subunit then binds to the heptameric ring and is endocytosed along with the ring. As the pH drops in the endosome, the ring undergoes a dramatic conformational change that generates a pore in the endosomal membrane, and the A subunit is delivered into the cytosol of the host cell through the pore. (B) X-ray crystal structure of the B subunit heptameric ring, viewed from the top and from the side. (B, from C. Petosa et al., *Nature* 385:833–838, 1997, with permission from Macmillan Publishers Ltd.)



(A)



(B)

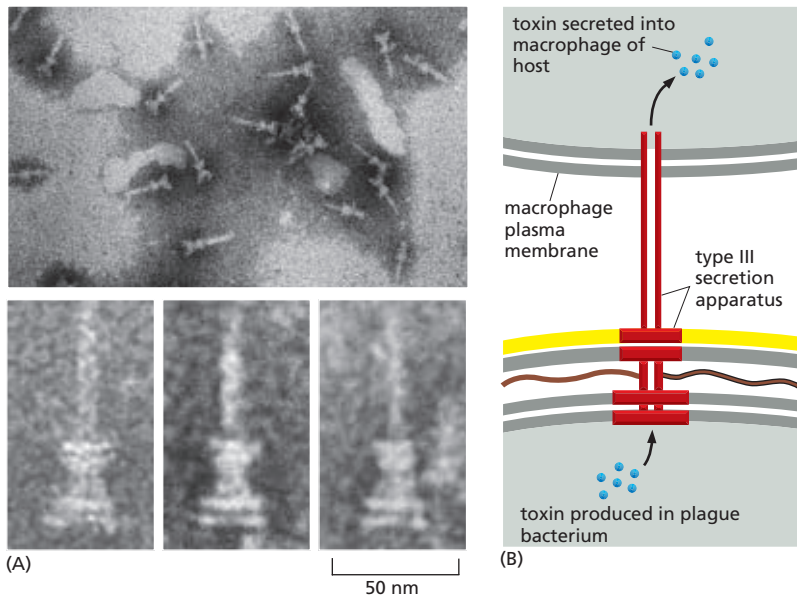


Figure 24-8 Type III secretion systems that can deliver virulence factors into the cytosol of a host cell. (A) Electron micrographs of purified type III secretion systems. About two dozen proteins form the complete structure, which is seen in the three enlarged electron micrographs below. (B) The large lower ring is embedded in the bacterial plasma membrane, and the smaller upper ring is embedded in the bacterial outer membrane. During infection, contact of the hollow tube tip with the plasma membrane of a host cell triggers secretion into the host cell. Here, the plague bacterium, *Yersinia pestis*, delivers toxins to a macrophage. (A, from K. Tamano et al., *EMBO J.* 19:3876–3887, 2000. With permission from Macmillan Publishers Ltd.)

to deliver toxins to the cytoplasm of host cells in a manner analogous to the type III systems, are closely related to the conjugative apparatus that many bacteria use to exchange genetic material.

Fungal and Protozoan Parasites Have Complex Life Cycles with Multiple Forms

Pathogenic fungi and protozoan parasites are eucaryotes. It is therefore more difficult to find drugs that will kill them without killing the host. Consequently, antifungal and antiparasitic drugs are often less effective and more toxic than antibiotics. A second characteristic of fungal and parasitic infections that makes them difficult to treat is the tendency of the infecting organisms to switch among several different forms during their life cycles. A drug that is effective at killing one form is often ineffective at killing another form, which therefore survives the treatment.

The **fungal** branch of the eucaryotic kingdom includes both unicellular yeasts (such as *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*) and filamentous, multicellular molds (like those found on moldy fruit or bread). Most of the important pathogenic fungi exhibit *dimorphism*—the ability to grow in either yeast or mold form. The yeast-to-mold or mold-to-yeast transition is frequently associated with infection. *Histoplasma capsulatum*, for example, grows as a mold at low temperature in the soil, but it switches to a yeast form when inhaled into the lung, where it can cause the disease histoplasmosis (Figure 24-9).

Protozoan parasites are single-celled eucaryotes with more elaborate life cycles than fungi, and they frequently require the services of more than one host. **Malaria** is the most common protozoal disease, infecting 200–300 million people every year and killing 1–3 million of them. It is caused by four species of *Plasmodium*, which are transmitted to humans by the bite of the female of any of 60 species of *Anopheles* mosquito. *Plasmodium falciparum*—the most intensively studied of the malaria-causing parasites—exists in no fewer than eight distinct forms, and it requires both the human and mosquito hosts to complete its sexual cycle (Figure 24-10A). Gametocytes are formed in the bloodstream of infected humans, but they can only differentiate into gametes and fuse to form a zygote in the gut of the mosquito. Three of the *Plasmodium* forms are highly specialized to invade and replicate in specific tissues—the insect gut lining, the human liver, and the human red blood cell. Even within a single host cell type, the *Plasmodium* parasite undergoes a complex sequence of developmental events, reflected in striking morphological changes (Figure

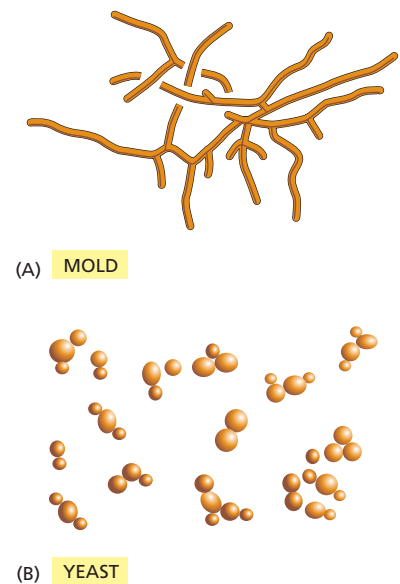


Figure 24-9 Dimorphism in the pathogenic fungus *Histoplasma capsulatum*. (A) At low temperature in the soil, *Histoplasma* grows as a multicellular filamentous mold. (B) After it is inhaled into the lung of a mammal, *Histoplasma* undergoes a morphological switch triggered by the change in temperature. In this unicellular yeast-like form, it closely resembles *Saccharomyces cerevisiae*.

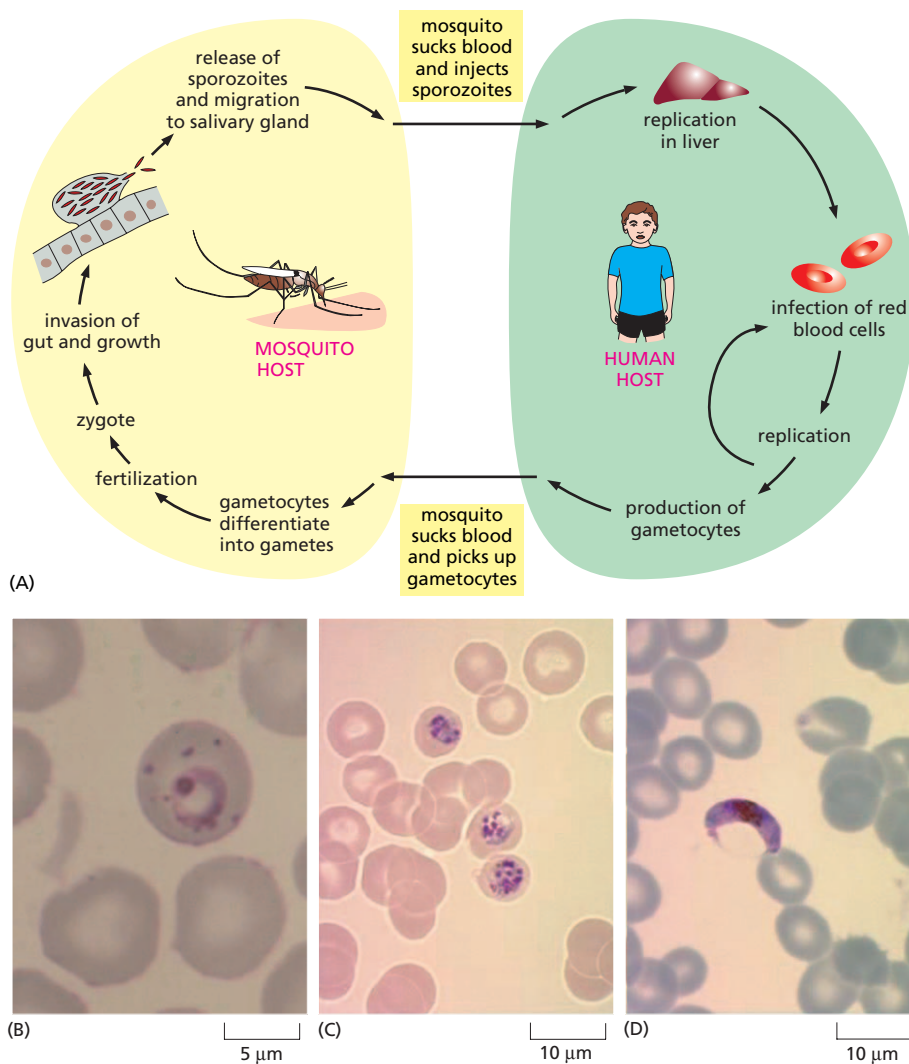
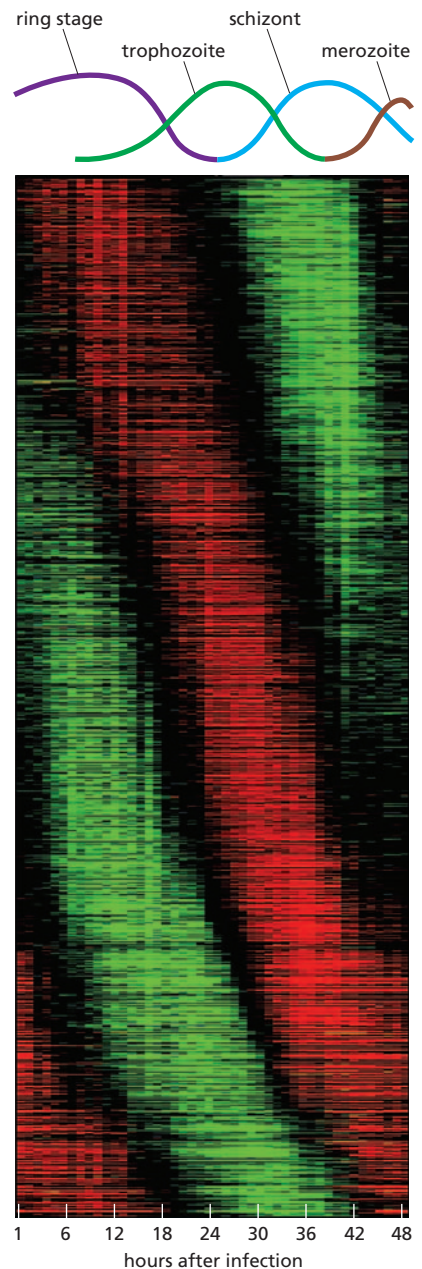


Figure 24–10 The complex life cycle of malaria parasites. (A) The sexual cycle of *Plasmodium falciparum* requires passage between a human host and an insect host. (B)–(D) Blood smears from people with malaria, showing three different forms of the parasite that appear in red blood cells: (B) ring stage; (C) schizont; (D) gametocyte. (Micrographs courtesy of the Centers for Disease Control, Division of Parasitic Diseases, DPDx.)



24–10B, C, D) as well as in stage-specific regulation of a majority of its transcripts (Figure 24–11).

Because malaria is so widespread and devastating, it has acted as a strong selective pressure on human populations in areas of the world that harbor the *Anopheles* mosquito. *Sickle-cell anemia*, for example, is a recessive genetic disorder caused by a point mutation in the gene that encodes the hemoglobin β chain, and it is common in areas of Africa with a high incidence of the most serious form of malaria (caused by *Plasmodium falciparum*). The malarial parasites grow poorly in red blood cells from either homozygous sickle-cell patients or healthy heterozygous carriers, and, as a result, malaria is seldom found among carriers of this mutation. For this reason, malaria has served to maintain the otherwise deleterious sickle-cell mutation at high frequency in these regions of Africa.

Figure 24–11 Time-dependent transcriptional program in malaria parasites developing in red blood cells. RNA was isolated from red blood cells infected with *Plasmodium falciparum* at 1-hour intervals over a total of 48 hours. In this image, each horizontal line represents one of the ~2700 genes in which the transcriptional level changed significantly during the course of infection. Red indicates an increase in mRNA abundance relative to the average, and green indicates a decrease. The genes were arranged in order from top to bottom according to the relative phases of their transcriptional activation. This regular, orderly progression of gene expression parallels the morphological differentiation of the *Plasmodium* parasite through the ring stage, trophozoite stage, schizont stage, and merozoite stage, all observed within red blood cells in infected humans (see Figure 24–10B, C, D). (Adapted from Z. Bozdech et al., *PLoS Biol.* 1: E5, 2003. With permission from Public Library of Science.)

All Aspects of Viral Propagation Depend on Host Cell Machinery

Bacteria, yeast, and protozoan pathogens are cells themselves. Even as intracellular pathogens, they use their own machinery for DNA replication, transcription, and translation, and they provide their own sources of metabolic energy. **Viruses**, by contrast, are the ultimate hitchhikers, carrying little more than information in the form of nucleic acid. The information is largely replicated, packaged, and preserved by the host cells (**Figure 24–12**). Viruses have a small genome, made up of a single nucleic acid type—either DNA or RNA—which, in either case, may be single-stranded or double-stranded. The genome is packaged in a protein coat, which in some viruses is further enclosed by a lipid envelope.

Viruses replicate in various ways. In general, replication involves (1) disassembly of the infectious virus particle, (2) replication of the viral genome, (3) synthesis of the viral proteins by the host cell translation machinery, and (4) reassembly of these components into progeny virus particles. A single virus particle (a *virion*) that infects a single host cell can produce thousands of progeny in the infected cell. Such prodigious multiplication often kills the host cell: the infected cell breaks open (lyses) and thereby allows the progeny virions access to nearby host cells. Many of the clinical manifestations of some kinds of viral infection reflect this *cytolytic effect* of the virus. Both the cold sores formed by *herpes simplex* virus and the lesions caused by the *smallpox* virus, for example, reflect the killing of the epidermal cells in a local area of infected skin. As discussed earlier and again later, some host cell death is also caused by the immune responses to the virus.

Virions come in a wide variety of shapes and sizes, and, unlike cellular life forms, they cannot be systematically classified by their relatedness into a single phylogenetic tree. Because of their tiny sizes, we now have complete genome sequences for nearly all clinically important viruses. The virions of *poxvirus* are among the largest, up to 450 nm long, which is about the size of some small bacteria. Their genome of double-stranded DNA consists of about 270,000 nucleotide pairs. At the other end of the size scale are the virions of parvovirus, which are less than 20 nm in diameter and have a single-stranded DNA genome of under 5000 nucleotides (**Figure 24–13**). The genetic information in a virus can be carried in a variety of unusual nucleic acid forms (**Figure 24–14**).

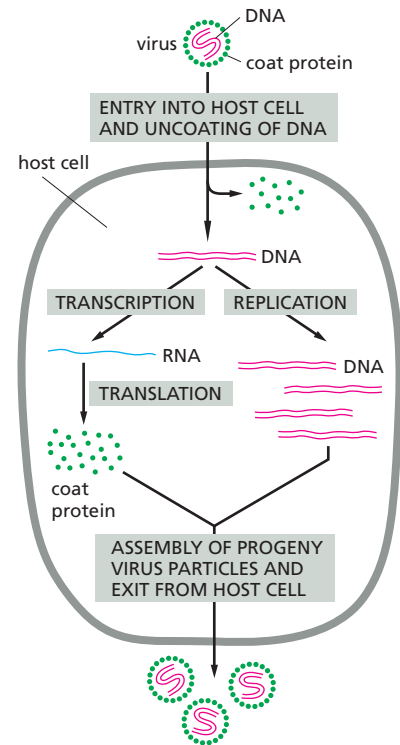


Figure 24–12 A simple viral life cycle. The hypothetical virus shown consists of a small double-stranded DNA molecule that codes for only a single viral capsid protein. No known virus is this simple.

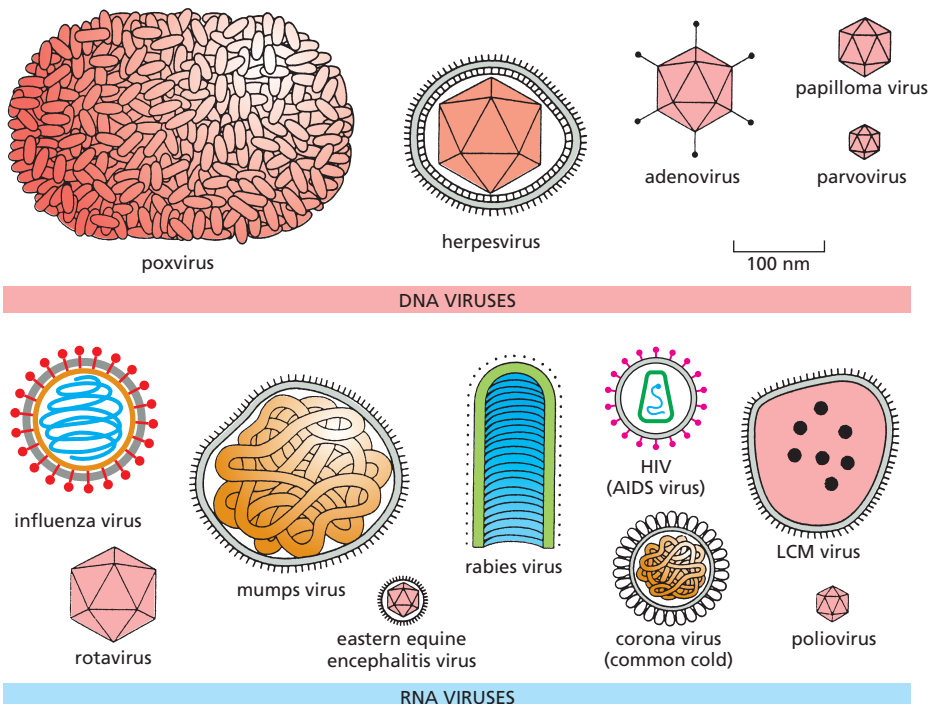


Figure 24–13 Examples of viral morphology. As shown, both DNA and RNA viruses vary greatly in both size and shape.

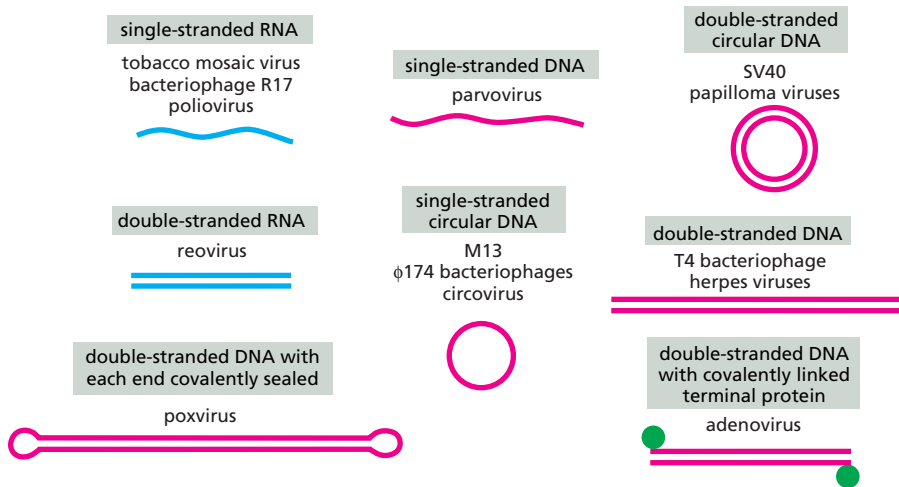


Figure 24-14 Schematic drawings of several types of viral genomes. The smallest viruses contain only a few genes and can have an RNA or a DNA genome. The largest viruses contain hundreds of genes and have a double-stranded DNA genome. The peculiar ends (as well as the circular forms) overcome the difficulty of replicating the last few nucleotides at the end of a DNA strand (discussed in Chapter 5).

The **capsid** that encloses the viral genome is made of one or several proteins, arranged in regularly repeating layers and patterns; the viral genome together with the capsid is called a **nucleocapsid**. In *enveloped viruses*, the nucleocapsid is enclosed by a lipid bilayer membrane that the virus acquires in the process of budding from the host cell plasma membrane (**Figure 24-15**). Whereas *nonenveloped viruses* usually leave an infected cell by lysing it, an enveloped virus can leave the cell by budding, without disrupting the plasma membrane and, therefore, without killing the cell. Enveloped viruses can cause persistent infections that may last for years, often without noticeable deleterious effects on the host.

Despite this variety, all viral genomes encode three types of proteins: proteins for replicating the genome, proteins for packaging the genome and delivering it to more host cells, and proteins that modify the structure or function of the host cell to enhance the replication of the virions (**Figure 24-16**). In the second section of this chapter, we focus primarily on this third class of viral proteins. Many viral genomes also encode a fourth class of proteins, which modulate or subvert the host's normal immune defense mechanisms. Several of these are described in the final section of this chapter.

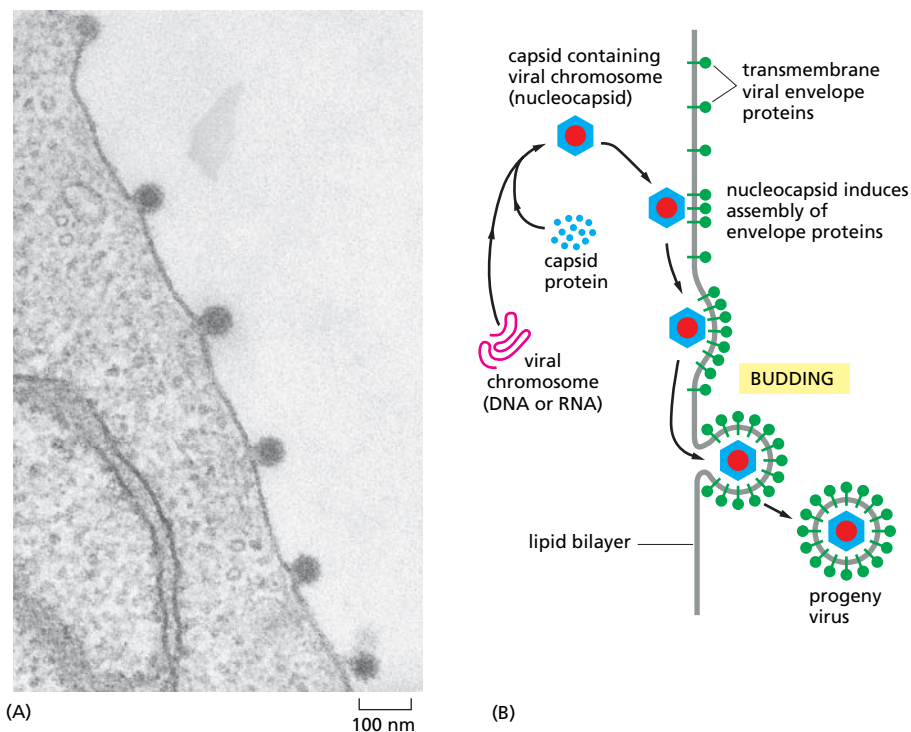
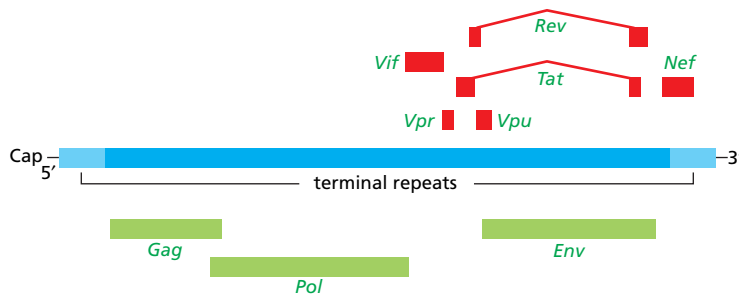


Figure 24-15 Acquisition of a viral envelope. (A) Electron micrograph of an animal cell from which six copies of an enveloped virus (*Semliki forest virus*) are budding. (B) Schematic drawing of the envelope assembly and budding processes. The lipid bilayer that surrounds the viral capsid is derived directly from the plasma membrane of the host cell. In contrast, the proteins in this lipid bilayer (shown in green) are encoded by the viral genome. (A, courtesy of M. Olsen and G. Griffith.)



Since the host cell's machinery performs most of the critical steps in viral replication, the identification of effective antiviral drugs is problematic. Whereas the antibiotic tetracycline specifically poisons bacterial ribosomes, for example, it will not be possible to find a drug that specifically poisons viral ribosomes, as viruses use the host cell's ribosomes to make their proteins. The best strategy for containing viral diseases is to prevent them by vaccinating the potential hosts. Highly successful vaccination programs have effectively eliminated smallpox from the planet, and the eradication of poliomyelitis may be imminent (Figure 24-17).

Prions Are Infectious Proteins

All information in biological systems is encoded by structure. We are used to thinking of biological information in the form of nucleic acid sequences (as in our description of viral genomes), but the sequence itself is a shorthand code for describing nucleic acid structure. The replication and expression of the information encoded in DNA and RNA depend strictly on the structure of these nucleic acids and their interactions with other macromolecules. The propagation of genetic information primarily requires that the information be stored in a structure that can be duplicated from unstructured precursors. Nucleic acid sequences are the simplest and most robust solution that organisms have found to the problem of faithful structural replication.

Nucleic acids are not the only solution, however. **Prions** are infectious agents that replicate in the host by copying an aberrant protein structure. They have been found to occur in organisms ranging from yeasts to sea slugs to humans, and they cause various neurodegenerative diseases in mammals. The most well-known infection caused by prions is *bovine spongiform encephalopathy* (BSE, or mad cow disease), which occasionally spreads to humans who eat infected parts of the cow (Figure 24-18); it can also be transmitted from human to human via blood transfusions. Isolation of the infectious prions that cause the disease *scrapie* in sheep, followed by years of painstaking laboratory characterization of scrapie-infected mice, eventually established that the protein itself is infectious.

Intriguingly, the host makes the infectious prion protein, and the prion's amino acid sequence is identical to that of a normal host protein. Moreover, the prion and normal forms of the protein are indistinguishable in their post-translational modifications. The only difference between them appears to be in their folded three-dimensional structure. The misfolded prion protein tends to aggregate to form regular helical fibers called *amyloid*. The amyloid fibers grow at the

Figure 24-16 A map of the HIV genome. This retroviral RNA genome consists of about 9000 nucleotides and contains nine genes, the locations of which are shown in green and red. Three of the genes (green) are common to all retroviruses: *Gag* encodes capsid proteins, *Env* encodes envelope proteins, and *Pol* encodes both the reverse transcriptase (which copies the RNA into DNA) and the integrase (which inserts the DNA copy into the host cell genome) (discussed in Chapter 5). The HIV genome is unusually complex, because in addition to the three large genes (green) normally required for the retrovirus life cycle, it contains six small genes (red). At least some of these small genes encode proteins that regulate viral gene expression (*Tat* and *Rev*—see Figure 7-103); others encode proteins that modify host cell processes, including protein trafficking (*Vpu* and *Nef*) and progression through the cell cycle (*Vpr*). As indicated by the red lines, RNA splicing (using host cell spliceosomes) is required to produce the *Rev* and *Tat* proteins.

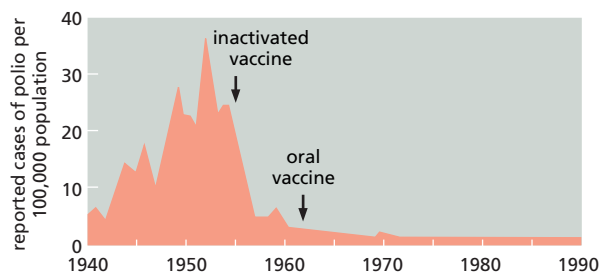


Figure 24-17 Eradication of a viral disease through vaccination. The graph shows the number of cases of poliomyelitis reported per year in the United States. The arrows indicate the timing of the introduction of the Salk vaccine (inactivated virus given by injection) and the Sabin vaccine (live attenuated virus given orally).

ends, much like the cytoskeletal protein filaments discussed in Chapter 16, except that the protein subunits undergo a structural conversion from the normal folded form of the protein to the misfolded form as they become part of the amyloid polymer (see Figure 6–95). In other words, the misfolded prion form has the remarkable capacity to cause the normal protein to adopt its misfolded prion conformation and thereby to become infectious, which is equivalent to the prion's having replicated itself in the host. When one of the amyloid fibrils is broken into smaller pieces, each one can seed the conversion process in a new cell; the prion can therefore propagate as well as replicate. If eaten by another susceptible host, these newly misfolded prions can transmit the infection from organism to organism.

It is not known how most normal proteins are able to find the single, correct, folded conformation, among the billions of other possibilities, without becoming stuck in dead-end intermediates (discussed in Chapters 3 and 6). Prions are a good example of how protein folding can go dangerously wrong. But why are the prion diseases so uncommon? What are the constraints that determine whether a misfolded protein will behave like a prion, or simply get refolded or degraded by the cell that made it? We do not yet have answers to these questions, and the study of prions remains an area of intense research.

Infectious Disease Agents Are Linked To Cancer, Heart Disease, and Other Chronic Illnesses

Thus far, we have considered microorganisms primarily in their roles as causative agents of infectious disease. It is clear, however, that in many cases viral and bacterial infections can contribute to the pathogenesis of important life-threatening illnesses that are not normally classified as infectious diseases. One obvious example is cancer. The oncogene concept, which is that certain altered genes can trigger cell transformation and tumor development, came initially from studies of the *Rous sarcoma virus*, which causes a form of cancer (sarcomas) in chickens. One of the genes encoded by the virus was eventually found to encode an overactive homolog of the host tyrosine kinase Src, which has since been implicated in many kinds of cancer.

Although Rous sarcoma virus does not cause cancer in humans, several human cancers are now known to have a viral origin. *Human papillomavirus*, for example, which causes genital warts, is also responsible for more than 90% of cervical cancers. Worldwide, cervical cancer is the second most common cancer in women and has a mortality rate of ~40%. In wealthy countries, widespread screening using the Pap smear test has reduced the incidence and severity of cervical cancer, but it is still very common in developing countries. The recent development of a vaccine against the most abundant cancer-associated strains of human papillomavirus raises the hope that this form of cancer can be largely prevented worldwide by a simple and cost-effective measure.

The *Epstein–Barr virus (EBV)* provides a more complex example of human cancer linked to a viral infection. Infection by this DNA virus is so common that nearly 90% of adults in the United States over the age of 40 have detectable levels of anti-EBV antibodies in their blood. EBV prefers to invade B cells of the adaptive immune system, especially long-lived memory B cells (discussed in Chapter 25). Most people infected as children have few symptoms and are unaware that they have been infected, but teenagers and young adults infected for the first time often develop *mononucleosis* (also called *glandular fever*), a severe flu-like disease that can lead to high fever, painful swelling of lymph nodes, and fatigue that can persist for several months. After symptoms subside, EBV can remain dormant in the B cells for life, with its genome maintained as an extrachromosomal plasmid in the B cell nucleus. Some of the gene products encoded by the EBV genome inhibit apoptosis and thereby presumably help to prevent the virus from being cleared from the body. Thus, when an infected B cell acquires cancer-promoting mutations, the usual mechanism for eliminating precancerous cells by apoptosis is inhibited, and a form of B cell cancer called Burkitt's lymphoma may develop.

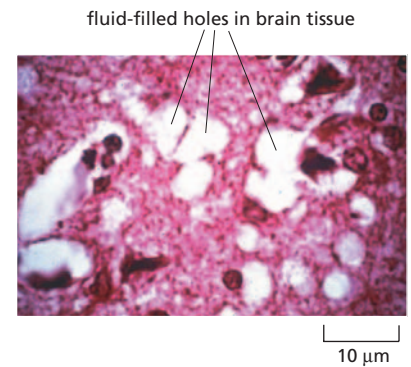


Figure 24–18 Neural degeneration in a prion infection. This micrograph shows a slice from the brain of a person who died of kuru. Kuru is a human prion disease, very similar to BSE, that was spread from one person to another by ritual mortuary practices in New Guinea. The large fluid-filled holes are places where neurons have died. These characteristic holes are why prion-based neurological diseases are called *spongiform encephalopathies*. (Courtesy of Gary Baumbach.)

In some cases, chronic tissue damage caused by infection can increase the likelihood of cancer developing in the infected tissue. The stomach-dwelling bacterium *Helicobacter pylori* has been implicated as a major cause of stomach cancers as well as gastric ulcers, and the *hepatitis viruses* that cause chronic infections in the liver (chronic hepatitis) are associated with more than 60% of liver cancers.

Along with cancer, the other major cause of death in wealthy industrialized nations is cardiovascular disease, frequently brought on by *atherosclerosis*, the accumulation of fatty deposits in blood vessel walls that can block blood flow. The resulting ischemia has dire consequences in the heart and brain. A hallmark of early atherosclerosis is the appearance in blood vessel walls of clumps of strange-looking macrophages, called foam cells because they are loaded with engulfed fatty globules. The foam cells secrete cytokines that recruit other white blood cells into the forming *atherosclerotic plaque*, which also accumulates extracellular matrix. The continued accumulation of cells and matrix can gradually block blood flow, or, alternatively, the plaque can rupture, causing an overlying thrombus to form, which acutely blocks blood flow; moreover, pieces of the thrombus can break off to form emboli that block smaller blood vessels downstream. Interestingly, foam cells in atherosclerotic plaques often contain the bacterial pathogen *Chlamydia pneumoniae*, which commonly causes pneumonia in humans (Figure 24–19). Numerous lines of evidence suggest that *C. pneumoniae* infection is a significant risk factor for atherosclerosis in humans and animal models. DNA from other bacterial species has also been found in atherosclerotic plaques, including DNA from bacteria usually associated with teeth and gums, such as *Porphyromonas gingivalis*. The connection between infectious agents and atherosclerosis is an area of active current research.

In addition to contributing to the life-threatening conditions of cancer and cardiovascular disease, infectious agents are also thought to have a role in many chronic illnesses, although it is often hard to tell whether infection causes these diseases or is a consequence of the diseases. A clear-cut case of an infectious cause for a chronic ailment is *Lyme disease*, a bacterial infection caused by the spirochete *Borrelia burgdorferi*. The infection is acquired by a tick bite and can cause painful chronic arthritis if it is not detected and treated early with antibiotics. Several other bacterial infections, particularly infections by Gram-positive cocci and by small bacteria without a wall, called *Mycoplasma*, can also trigger immune responses leading to arthritis. In some people, *Mycoplasma*, *Chlamydia pneumoniae*, or both, are associated with chronic asthma. As we learn more about the complex interactions between pathogens and the human body, it seems likely that more and more chronic conditions will be found to have a link to an infectious agent. As has been the case with peptic ulcers, curing the infection may cure the disease, or at least alleviate the painful symptoms.

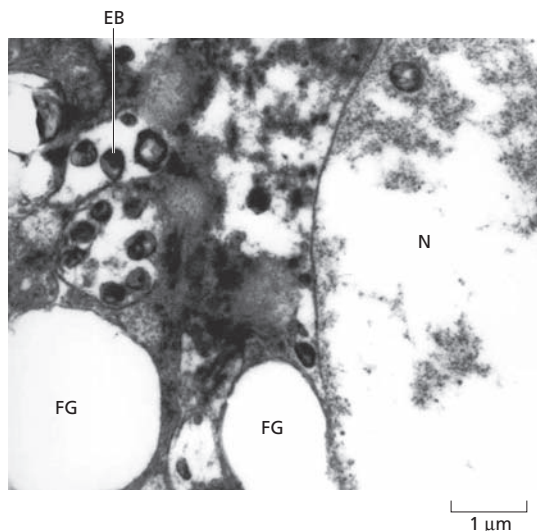


Figure 24–19 *Chlamydia pneumoniae* within a foam cell macrophage in an atherosclerotic plaque. The labels on this electron micrograph indicate: EB, elementary body (bacterium); FG, fat globule; N, macrophage nucleus. (From L.A. Campbell and C.C. Kuo, *Nat. Rev. Microbiol.* 2:23–32, 2004. With permission from Macmillan Publishers Ltd.)

Summary

Infectious diseases are caused by pathogens, which include bacteria, fungi, protozoa, worms, viruses, and even infectious proteins called prions. All pathogens must have mechanisms for entering their host and for evading immediate destruction by the host. Most bacteria are not pathogenic. Those that do contain specific virulence genes that mediate interactions with the host, eliciting responses from the host cells that promote the replication and spread of the pathogen. Pathogenic fungi, protozoa, and other eucaryotic parasites typically pass through several different forms during the course of infection; the ability to switch among these forms is usually required for the parasites to survive in a host and cause disease. In some cases, such as malaria, parasites must pass sequentially through several host species to complete their life cycles. Unlike bacteria and eucaryotic parasites, viruses have no metabolism of their own and no intrinsic ability to produce the proteins encoded by their DNA or RNA genomes; they rely entirely on subverting the machinery of the host cell to produce their proteins and to replicate their genomes. Prions, the smallest and simplest infectious agents, contain no nucleic acid; instead, they are rare, aberrantly folded proteins that replicate by catalyzing the misfolding of normal host proteins with the same amino acid sequence as the prion.

CELL BIOLOGY OF INFECTION

The mechanisms that pathogens use to cause disease are as diverse as the pathogens themselves. Nonetheless, all pathogens must carry out certain common tasks: they must colonize the host, reach an appropriate niche, avoid host defenses, replicate, and exit from the infected host to spread to an uninfected one. In this section, we examine the common strategies that many pathogens use to accomplish these tasks.

Pathogens Cross Protective Barriers to Colonize the Host

The first step in infection is for the pathogen to colonize the host. A thick and fairly tough covering of skin protects most parts of the human body from the environment. The protective boundaries of some other human tissues (eyes, nasal passages, respiratory tract, mouth, digestive tract, urinary tract, and female genital tract) are less robust. In the lungs and small intestine, for example, where oxygen and nutrients, respectively, are absorbed from the environment, the barrier is just a single monolayer of epithelial cells.

Skin and many other epithelial barriers are densely populated by normal flora. Some pathogens also colonize these surfaces and attempt to outcompete the normal flora, but most pathogens avoid such competition by crossing the barriers to gain access to unoccupied niches within the host.

Wounds in barrier epithelia allow pathogens direct access to such niches. This avenue of entry requires little in the way of pathogen specialization, and many members of the normal flora can cause serious illness if they enter through such wounds. Anaerobic bacteria of the genus *Bacteroides*, for example, are carried as harmless flora at very high density in the large intestine, but they can cause life-threatening peritonitis if they enter the peritoneal cavity through a perforation in the intestinal wall caused by trauma, surgery, or infection. *Staphylococcus* from the skin and nose, or *Streptococcus* from the throat and mouth, are also responsible for many serious infections resulting from breaches in epithelial barriers.

Primary pathogens, however, need not wait for a wound to gain access to their host. A particularly efficient way for a pathogen to cross the skin is to catch a ride in the saliva of a biting arthropod. Many insects and ticks nourish themselves by sucking mammalian blood, and a diverse group of bacteria, viruses, and protozoa have developed the ability to survive in arthropods and then use them as *vectors* to spread from one mammalian host to another. As discussed earlier, the *Plasmodium* protozoan that causes malaria develops through several

forms in its life cycle, including some that are specialized for survival in a human and some that are specialized for survival in a mosquito (see Figure 24–10). Viruses that are spread by insect bites include the causative agents for several types of hemorrhagic fever, including yellow fever and Dengue fever, as well as the causative agents for many kinds of viral encephalitis (inflammation of the brain). All these viruses replicate in both insect cells and mammalian cells, as required for their transmission by an insect vector. Bloodborne viruses such as HIV that are not capable of replicating in insect cells are rarely, if ever, spread from insect to human.

The efficient spread of a pathogen via an insect vector requires that individual insects consume blood meals from numerous mammalian hosts. In a few striking cases, the pathogen appears to alter the behavior of the insect so that its transmission is more likely. Like most animals, the tsetse fly (whose bite spreads the protozoan parasite *Trypanosoma brucei*, which causes sleeping sickness in Africa) stops eating when it is satiated. Tsetse flies carrying trypanosomes, however, bite much more frequently and ingest more blood than do uninfected flies. The presence of trypanosomes impairs the function of the insect mechanoreceptors that measure blood flow through the gullet to assess the fullness of the stomach, effectively fooling the tsetse fly into thinking that it is still hungry. The bacterium *Yersinia pestis*, which causes bubonic plague, uses a different mechanism to ensure that a flea carrying it bites repeatedly: it multiplies in the flea's foregut to form aggregated masses that eventually enlarge and physically block the digestive tract. The insect is then unable to feed normally and begins to starve. During repeated attempts to alleviate its hunger by feeding, some of the bacteria in the foregut are flushed into the bite site, thus transmitting plague to a new host (Figure 24–20).

Pathogens That Colonize Epithelia Must Avoid Clearance by the Host

Hitching a ride through the skin on an insect proboscis is just one strategy that pathogens use to pass through host barriers. Whereas many epithelial barriers such as the skin and the lining of the mouth and large intestine are densely populated by normal flora, others, including the lining of the lower lung, the small intestine, and the bladder, are normally kept nearly sterile, despite the presence of a relatively direct route to the outside world. How do these epithelia avoid bacterial colonization? As discussed in Chapter 23, a layer of protective mucus covers the respiratory epithelium, and the coordinated beating of cilia sweeps the mucus and trapped bacteria and debris up and out of the lung. The epithelia lining the bladder and the upper gastrointestinal tract also have a thick layer of mucus, and these organs are periodically flushed by urination and by peristalsis, respectively, which washes away undesirable microbes. The pathogenic bacteria and parasites that infect these epithelial surfaces have specific mechanisms for overcoming these host-cleaning processes. Those that infect the urinary tract, for example, resist the washing action of urine by adhering tightly to the epithelium lining the tract via specific **adhesins**, which are proteins or protein complexes that recognize and bind to host cell-surface molecules. An important group of adhesins in *E. coli* strains that infect the kidney are components of the *P pili* that help the bacteria adhere to the kidney epithelial cells. These surface projections can be several micrometers long and are thus able to span the thickness of the protective mucus layer (see Figure 24–4D). At the tip of each pilus is an adhesin protein that binds tightly to a particular glycolipid disaccharide that is found on the surface of kidney cells. Strains of *E. coli* that infect the bladder rather than the kidney express a second kind of pilus that enables them to adhere to bladder epithelial cells. It is the adhesion specificity of the adhesin proteins on the tips of two types of pili that is responsible for the bacteria's colonizing the different parts of the urinary tract (Figure 24–21). The specificity of the adhesins also restricts the host range for these and many other pathogenic bacteria.

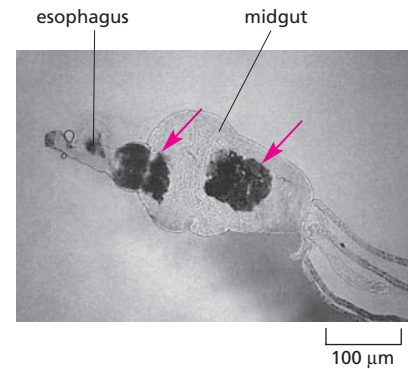


Figure 24–20 Plague bacteria within a flea. This light micrograph shows the digestive tract dissected from a flea that had dined about two weeks previously on the blood of an animal infected with the plague bacterium, *Yersinia pestis*. The bacteria multiplied in the flea gut to produce large cohesive aggregates (red arrows); the bacterial mass on the left is occluding the passage between the esophagus and the midgut. This type of blockage prevents a flea from digesting its blood meals, so that hunger causes it to bite repeatedly, disseminating the infection. (From B.J. Hinnebusch, E.R. Fischer and T.G. Schwann, *J. Infect. Dis.* 178:1406–1415, 1998. With permission from University of Chicago Press.)

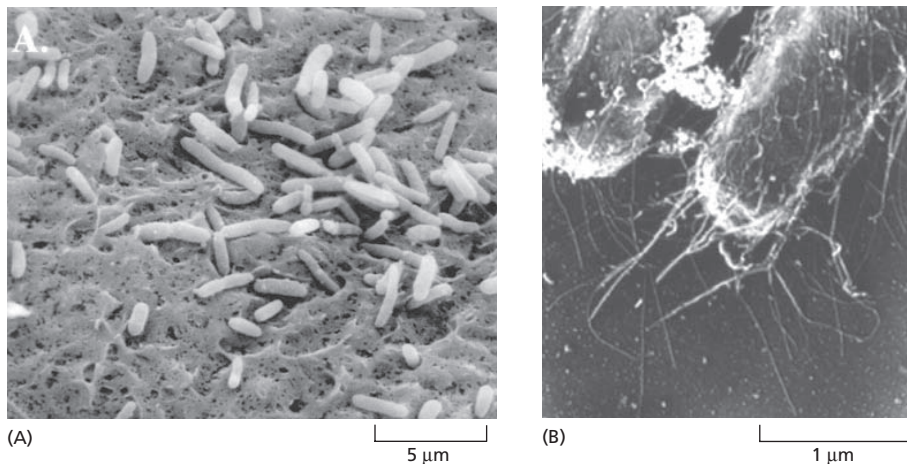


Figure 24-21 Uropathogenic *E. coli* in the infected bladder of a mouse. (A) Scanning electron micrograph of uropathogenic *E. coli*, a common cause of bladder and kidney infections. The bacteria are attached to the surface of epithelial cells lining the infected bladder. (B) A close-up view of one of the bacteria showing the pili on its surface. (A, from G.E. Soto and S.J. Hultgren, *J. Bact.* 181:1059–1071, 1999; B, courtesy of D.G. Thanassi and S.J. Hultgren, *Meth. Comp. Meth. Enzym.* 20:111–126, 2000. With permission from Academic Press.)

One of the hardest organs for a microbe to colonize is the stomach. Besides the thick layer of mucus and peristaltic washing, the stomach is filled with acid (average pH ~2). This extreme environment is lethal to almost all bacteria ingested in food. Nonetheless, the stomach is colonized by the hardy and enterprising bacterium *Helicobacter pylori*, which was recognized only recently as the major causative agent of stomach ulcers and some cases of stomach cancer. Remarkably, it is able to persist for life as a harmless commensal in most of its hosts. Although the older treatments for ulcers (acid-reducing drugs and bland diets) are still used to reduce inflammation, a short and relatively cheap course of antibiotics can now effectively cure a patient of recurrent stomach ulcers. The hypothesis that a persistent bacterial infection of the stomach lining could cause stomach ulcers was initially met with great skepticism. The young Australian doctor who made the initial discovery finally proved the point: he drank a flask of a pure culture of *H. pylori* and developed gastritis. One way in which *H. pylori* survives in the stomach is by producing the enzyme *urease*, which converts urea to ammonia and carbon dioxide; in this way, the bacterium surrounds itself with a layer of ammonia, which neutralizes the acid in its immediate vicinity. The bacteria also express at least five types of adhesins, which enable them to adhere to the stomach epithelium, and they produce several cytotoxins that destroy the stomach epithelial cells, creating painful ulcers. The resulting chronic inflammation promotes cell proliferation and thus predisposes the infected individual to stomach cancer.

Bordetella pertussis, the bacterium that causes whooping cough, provides another remarkable example of active colonization. The first step in a *B. pertussis* infection is colonization of the respiratory epithelium. The bacteria circumvent the normal clearance mechanism that clears the respiratory tract (the *mucoiliary escalator* described in Chapter 23) by binding tightly to the surface of the ciliated cells that line the tract and multiplying on them. *B. pertussis* expresses at least four adhesins that bind tightly to particular glycolipids on the ciliated cells. The adherent bacteria produce various toxins that eventually kill the ciliated cells, compromising the host's ability to clear the infection. The most familiar of these is *pertussis toxin*, which—like cholera toxin—is an ADP-ribosylating enzyme. It ADP-ribosylates the α subunit of the G protein G_i , inhibiting the G protein from suppressing the activity of the host cell's adenylyl cyclase, thereby increasing the production of cyclic AMP (discussed in Chapter 15). This toxin also interferes with the chemotactic pathway that neutrophils use to seek out and destroy invading bacteria (see Figure 16–101). Not content with this, *B. pertussis* also produces an adenylyl cyclase of its own, which is active only when bound to the eucaryotic Ca^{2+} -binding protein calmodulin in the cytoplasm of the host cell. Although both *B. pertussis* and *V. cholerae* drastically increase cAMP levels in the host cells to which they adhere, the symptoms of the diseases differ because the two bacteria colonize different sites in the host: *B. pertussis* colonizes the respiratory tract and causes paroxysmal coughing, whereas *V. cholerae* colonizes the gut and causes watery diarrhea.

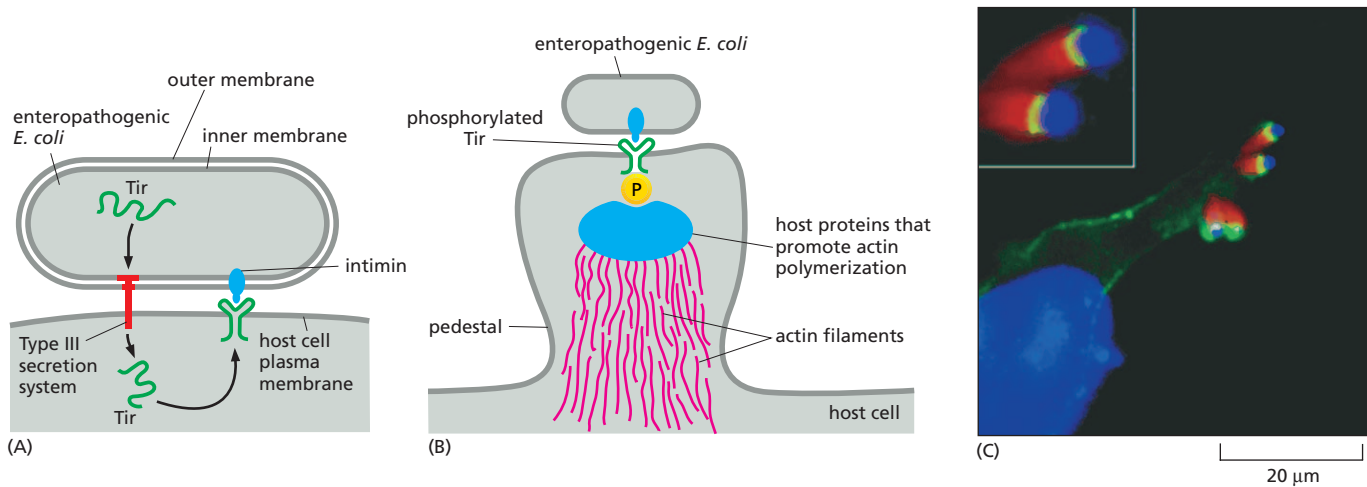


Figure 24–22 Interaction of enteropathogenic *E. coli* (EPEC) with host cells in the gut. (A) When EPEC contacts an epithelial cell in the lining of the human gut, it delivers a bacterial protein called Tir into the host cell through a type III secretion system. Tir then inserts into the plasma membrane of the host cell, where it functions as a receptor for the bacterial adhesin protein intimin. (B) A host cell protein tyrosine kinase phosphorylates the intracellular domain of Tir on tyrosines. Phosphorylated Tir then recruits various host cell proteins in turn, which trigger actin polymerization. Consequently, a dense accumulation of actin filaments assembles underneath the bacterium, forming an actin pedestal. (C) EPEC on a pedestal. In this fluorescence micrograph, the DNA of the EPEC and host cell are labeled in *blue*, Tir protein is labeled in *green*, and host cell actin filaments are labeled in *red*. The inset shows a close up view of the two upper bacteria on pedestals. (C, from D. Goosney et al., *Annu. Rev. Cell Dev. Biol.* 16:173–189, 2000. With permission from Annual Reviews.)

Not all examples of specific colonization require that the bacterium express adhesins that bind to host cell glycolipids or proteins. Enteropathogenic *E. coli*, which causes diarrhea in young children, instead uses a type III secretion system (see Figure 24–8) to deliver its own bacterially produced receptor protein (called Tir) into its host cell (Figure 24–22A). After Tir inserts into the host cell's plasma membrane, a bacterial surface protein binds to the extracellular domain of Tir, triggering a remarkable series of events inside the host cell. First, a host protein tyrosine kinase phosphorylates the Tir receptor protein on tyrosines, which is unusual because bacteria generally do not phosphorylate tyrosines on proteins. The phosphorylated Tir is then thought to recruit a member of the Rho family of small GTPases, which promotes actin polymerization through a series of intermediate steps (discussed in Chapter 16). The polymerized actin then forms a unique cell-surface protrusion, called a *pedestal*, that pushes the tightly adherent bacteria up about 10 µm from the host cell surface (Figure 24–22B, C).

These examples of host colonization illustrate the importance of host–pathogen communication in both the infection process and its evolution. Pathogenic organisms have acquired genes that encode proteins that interact specifically with particular molecules of the host cells. In some cases, such as the *B. pertussis* adenylyl cyclase, an ancestor of the pathogen may have acquired the cyclase gene from its host, whereas in others, such as Tir, random mutation may have produced protein motifs that are recognized by a eucaryotic protein tyrosine kinase.

Intracellular Pathogens Have Mechanisms for Both Entering and Leaving Host Cells

Many pathogens, including *V. cholerae* and *B. pertussis*, infect their host without entering host cells; they are referred to as *extracellular pathogens*. Others, however, including all viruses and many bacteria and protozoa, are **intracellular pathogens**. Their preferred niche for replication and survival is within the cytosol or intracellular compartments of particular host cells. This strategy has several advantages. The pathogens are not accessible to *antibodies* (discussed in

Chapter 25), and they are not easy targets for phagocytic cells (discussed later); furthermore, they are bathed in a rich source of many sugars, amino acids, and other nutrients present in host cell cytoplasm. This lifestyle, however, requires that the pathogen develop mechanisms for entering host cells, for finding a suitable subcellular niche where it can replicate, and for exiting from the infected cell to spread the infection. In the remainder of this section, we consider some of the myriad ways that individual intracellular pathogens exploit and modify host cell biology to satisfy these requirements.

Virus Particles Bind to Molecules Displayed on the Host Cell Surface

The first step for any intracellular pathogen is to bind to the surface of the host target cell. Viruses accomplish this binding through the association of a viral surface protein with a specific receptor on the host cell surface. Of course, no host cell receptor evolved for the sole purpose of allowing a pathogen to bind to it; these receptors all have other functions. The first such “virus receptor” identified was the *E. coli* surface protein that allows the bacteriophage lambda to bind to the bacterium. Its normal function is as a transport protein responsible for the uptake of maltose. Receptors need not be proteins, however; herpes simplex virus, for example, binds to heparan sulfate proteoglycans through specific viral membrane proteins.

Virions that infect animal cells generally use cell-surface receptor molecules that are either very abundant (such as sialic-acid-containing oligosaccharides, which are used by the influenza virus) or uniquely found on those cell types in which the virions can replicate (such as the nerve growth factor receptor, the nicotinic acetylcholine receptor, or the cell–cell adhesion protein N-CAM, all of which are used by rabies virus to infect neurons specifically). Often, many types of virus use a single type of receptor, and some viruses can use several different receptors. Moreover, different viruses that infect the same cell type may each use a different receptor. For example, members of at least six virus families, all of which preferentially replicate in liver cells (hepatocytes), cause hepatitis. Receptors for four of these have been identified, and they all differ. Many virions bind to receptors expressed on cells of the immune system. While seemingly paradoxical, as we might expect that triggering an immune response does not enhance viral survival, invading an immune cell may be a useful way to travel around the body and be taken to lymphoid organs, which are filled with other immune system cells.

Virions often require both a primary receptor and a secondary co-receptor for efficient attachment and entry into host cells. An important example is the AIDS virus *HIV*. Its primary receptor is CD4, a cell-surface protein on helper T cells and macrophages that is involved in immune recognition (discussed in Chapter 25). Viral entry also requires a co-receptor, either CCR5 (a receptor for β -chemokines) or CXCR4 (a receptor for α -chemokines), depending on the particular variant of the virus (Figure 24–23). Macrophages are susceptible only to HIV

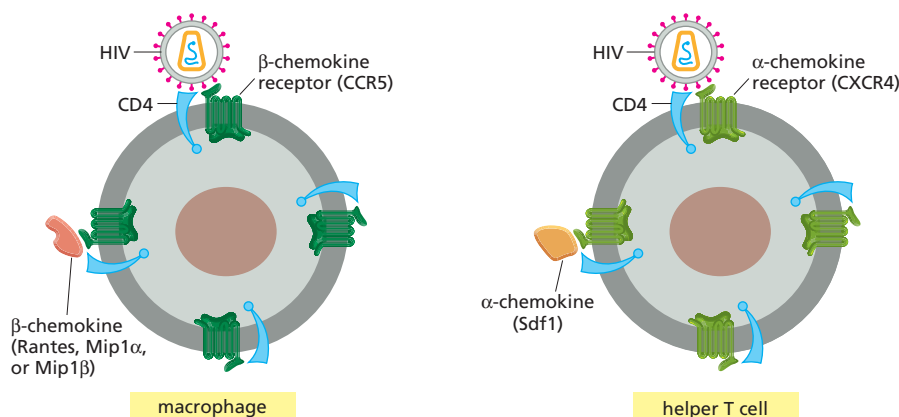
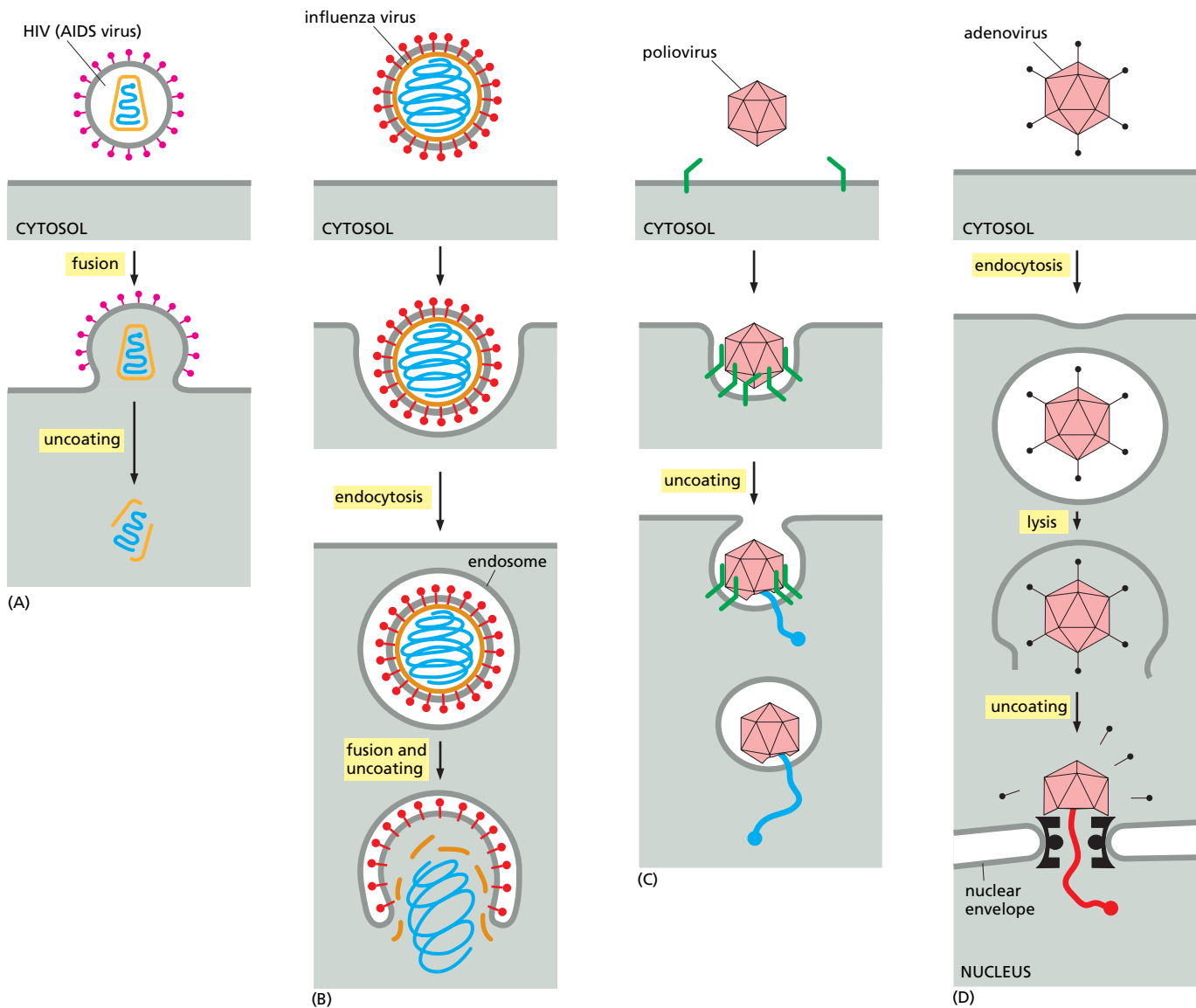


Figure 24–23 Receptor and co-receptors for HIV. All strains of HIV require the CD4 protein as a primary receptor. Early in an infection, most of the viruses use CCR5 as a co-receptor, allowing them to infect macrophages and their precursors, monocytes. As the infection progresses, mutant variants arise that now use CXCR4 as a co-receptor, enabling them to infect helper T cells efficiently. The natural ligand for the chemokine receptors (Sdf1 for CXCR4; Rantes, Mip1 α , or Mip1 β for CCR5) blocks co-receptor function and prevents viral invasion.



variants that use CCR5 for entry, whereas helper T cells are most efficiently infected by variants that use CXCR4. The viruses that are found within the first few months after HIV infection almost invariably require CCR5, which presumably explains why individuals fortunate enough to carry a defective *Ccr5* gene are not susceptible to HIV infection. In the later stages of infection, viruses may either switch to use the CXCR4 co-receptor or adapt to use both co-receptors; in this way, the virus can change the cell types it infects as the disease progresses.

Virions Enter Host Cells by Membrane Fusion, Pore Formation, or Membrane Disruption

After recognition and attachment to the host cell surface, the typical next steps for a virion are to enter the host cell and release its nucleic acid genome from its protective protein coat or lipid envelope. In most cases, the liberated nucleic acid remains complexed with some viral proteins. **Enveloped viruses** enter the host cell by fusing either with the plasma membrane or with the endosomal membrane following endocytosis (Figure 24–24A,B). Fusion is thought to proceed via a mechanism similar to SNARE-mediated fusion of intracellular vesicles during normal vesicular traffic (discussed in Chapter 13). <ATAG>

The virus regulates fusion both to ensure that its particles fuse only with the appropriate host cell membrane and to prevent the particles from fusing with

Figure 24–24 Four virus uncoating strategies. (A) Some enveloped viruses, such as HIV, fuse directly with the host cell plasma membrane to release their genome (blue) and capsid proteins (orange) into the cytosol. (B) Other enveloped viruses, such as influenza virus, first bind to cell-surface receptors, triggering receptor-mediated endocytosis. When the endosome acidifies, the virus envelope fuses with the endosomal membrane, releasing the viral genome (blue) and capsid proteins (orange) into the cytosol. (C) Poliovirus, a nonenveloped virus, binds to a receptor (green) on the host cell surface and then forms a pore (not shown) in the host cell membrane to extrude its RNA genome (blue). (D) Adenovirus, another nonenveloped virus, uses a more complicated strategy. It induces receptor-mediated endocytosis and then disrupts the endosomal membrane, releasing part of the capsid and its DNA genome into the cytosol. The trimmed-down virus eventually docks onto a nuclear pore and releases its DNA (red) directly into the nucleus.

one another. For viruses such as HIV that fuse at neutral pH at the plasma membrane, binding to receptors or co-receptors usually triggers a conformational change in the viral envelope protein to expose a normally buried fusion peptide (see Figure 13–16). Other enveloped viruses, such as influenza, only fuse with a host cell membrane after endocytosis; in this case, it is frequently the acid environment in the early endosome that triggers the conformational change in a viral surface protein that exposes the fusion peptide (see Figure 24–24B). The H⁺ pumped into the early endosome enters the influenza particle through an ion channel and triggers the *uncoating* of the viral RNA, which is directly released into the cytosol as the virus fuses with the endosomal membrane. For some viruses, uncoating occurs after release into the cytosol. In the case of Semliki forest virus, for example, the binding of host ribosomes to the capsid causes the capsid proteins to separate from the viral genome.

It is more difficult to envision how **nonenveloped viruses** enter host cells, as it is not obvious how large assemblies of protein and nucleic acid can cross the plasma or endosomal membrane. Where the entry mechanism is understood, nonenveloped viruses generally either form a pore in the cell membrane to deliver the viral genome into the cytoplasm or they disrupt the endosomal membrane after endocytosis.

Poliovirus uses the first strategy. Binding of poliovirus to its receptor triggers both receptor-mediated endocytosis and a conformational change in the viral particle. The conformational change exposes a hydrophobic projection on one of the capsid proteins, which apparently inserts into the endosomal membrane to form a pore. The viral genome then enters the cytoplasm through the pore, leaving the capsid either in the endosome on the cell surface, or in both places (see Figure 24–24C).

Adenovirus uses the second strategy. It is initially taken up by receptor-mediated endocytosis. As the endosome matures and becomes more acidic, the virus undergoes multiple uncoating steps that remove structural proteins sequentially from the capsid. Some of these steps require the action of a viral protease, which is inactive in the extracellular virus particle (probably because of intrachain disulfide bonds) but is activated in the reducing environment of the endosome. One of the proteins released from the capsid lyses the endosomal membrane, releasing the remainder of the virus into the cytosol. This trimmed-down virus then docks onto the nuclear pore complex, and the viral DNA genome is transported through the pore into the nucleus, where it is transcribed (see Figure 24–24D).

In these various entry strategies, viruses exploit a variety of host cell molecules and processes, which can include cell-surface components, receptor-mediated endocytosis, endosomal maturation steps, and nuclear transport. These strategies illustrate again the sophisticated ways that pathogens have evolved to utilize the basic cell biology of their hosts.

Bacteria Enter Host Cells by Phagocytosis

Bacteria are much larger than viruses, and they are too large to be taken up either through pores or by receptor-mediated endocytosis. Instead, they enter host cells by phagocytosis. Phagocytosis of bacteria is a normal function of macrophages. They patrol the tissues of the body and ingest and destroy unwanted microbes. Some pathogens, however, have acquired the ability to survive and replicate within macrophages after they have been phagocytosed.

Mycobacterium tuberculosis is one such pathogen. As discussed earlier, it causes **tuberculosis**, a serious lung infection that is widespread in some urban populations. It is usually acquired by inhalation of the bacterium into the lungs, where it is phagocytosed by alveolar macrophages. Although the microbe can survive and replicate within macrophages, with the help of the adaptive immune system, the macrophages of most healthy individuals contain the infection within a lesion called a *tubercle*. In most cases, the lesion becomes walled off by a fibrous capsule that eventually undergoes calcification and can then easily be seen on an X-ray of the lungs. Remarkably, *M. tuberculosis* in such

lesions can survive for decades, and, later in life, especially if drugs or disease weaken the immune system, the infection may be reactivated and spread in the lung and even to other organs.

Tuberculosis has infected human populations for thousands of years, but another bacterium that lives within alveolar macrophages was first recognized as a human pathogen only in 1976. *Legionella pneumophila* is normally a parasite of freshwater amoebae, which take it up by phagocytosis. When droplets of water containing *L. pneumophila* or infected amoebae are inhaled into the lung, the bacteria can invade and live inside alveolar macrophages (Figure 24–25), which, to the bacteria, must seem just like large amoebae. This infection leads to the type of pneumonia known as **Legionnaire's disease**. The pathogen is commonly spread by central air-conditioning systems, as the amoebae that are the bacterium's normal host are particularly adept at colonizing air-conditioning cooling towers; moreover, these cooling systems produce microdroplets of water that are easily inhaled. The incidence of Legionnaire's disease has increased dramatically in recent decades, with outbreaks frequently traced to the air-conditioning systems in office buildings, hospitals, and hotels. Other forms of modern aerosolization are sometimes responsible, including decorative fountains and produce sprayers in supermarkets.

Some bacteria invade cells that are normally nonphagocytic. One way in which bacteria can induce such a cell to phagocytose them is by expressing an adhesin that binds with high affinity to a host cell adhesion protein that the host cell normally uses to adhere to another host cell or to the extracellular matrix (discussed in Chapter 19). For example, a bacterium that causes diarrhea, *Yersinia pseudotuberculosis* (a close relative of the plague bacterium *Yersinia pestis*), expresses a protein called *invasin* that binds to $\beta 1$ integrins, and a bacterium that causes a rare but serious form of food poisoning, *Listeria monocytogenes*, expresses a protein that binds to E-cadherin. Binding of the bacterial proteins to these transmembrane host adhesion proteins fools the host cell into attempting to form a cell junction, and it begins moving actin and other cytoskeletal components to the site of bacterial attachment. Since the bacterium is small relative to the host cell, the host cell's attempt to spread over the adhesive surface of the bacterium results in the phagocytic uptake of the bacterium—a process known as the *zipper mechanism* of invasion (Figure 24–26A). The similarity of this form of invasion to the natural process of cell adhesion was revealed when the three-dimensional structure of *invasin* was determined. The bacterial protein has an RGD motif with a structure almost identical to the RGD motif of the integrin-binding site in the extracellular matrix protein laminin (discussed in Chapter 19).

A second pathway by which bacteria can invade nonphagocytic cells is known as the *trigger mechanism* (Figure 24–26B). It is used by various pathogens, including *Salmonella enterica*, which causes food poisoning. When the bacterium injects a set of effector molecules into the host cell cytoplasm through a type III secretion system, it initiates this dramatic form of invasion. Some of these effector molecules activate Rho-family GTPases, which stimulate actin polymerization (discussed in Chapter 16). Others interact with cytoskeletal elements more directly, severing actin filaments and causing the rearrangement of cross-linking proteins. The net effect is to cause dramatic localized ruffling on the surface of the host cell (Figure 24–26C), which throws up large actin-rich protrusions that fold over and trap the bacterium within a large endocytic vesicle called a *macropinosome* (Figure 24–26D). The overall appearance of cells being invaded by the trigger mechanism is similar to the dramatic ruffling induced by some extracellular growth factors, suggesting that similar intracellular signaling pathways are activated in both cases.

Intracellular Eucaryotic Parasites Actively Invade Host Cells

The host cell supplies the energy required for the uptake of viruses by receptor-mediated endocytosis and bacteria by phagocytosis or macropinocytosis. The pathogen is a relatively passive participant, usually providing a trigger to initiate the invasion process. In contrast, intracellular eucaryotic parasites, which are

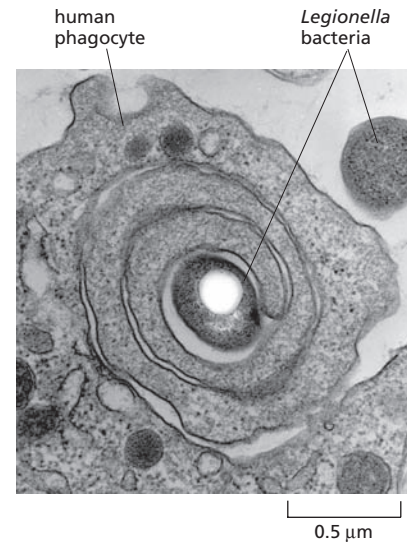


Figure 24–25 Uptake of *Legionella pneumophila* by a human phagocyte. This electron micrograph shows the unusual coil structure induced on the surface of the phagocyte by the bacterium. Some other pathogens, including the bacterium *Borrelia burgdorferi*, which causes Lyme disease, the eucaryotic pathogen *Leishmania*, and the yeast *Candida albicans*, may also sometimes invade cells using this type of coiling phagocytosis. (From M.A. Horwitz, *Cell* 36:27–33, 1984. With permission from Elsevier.)

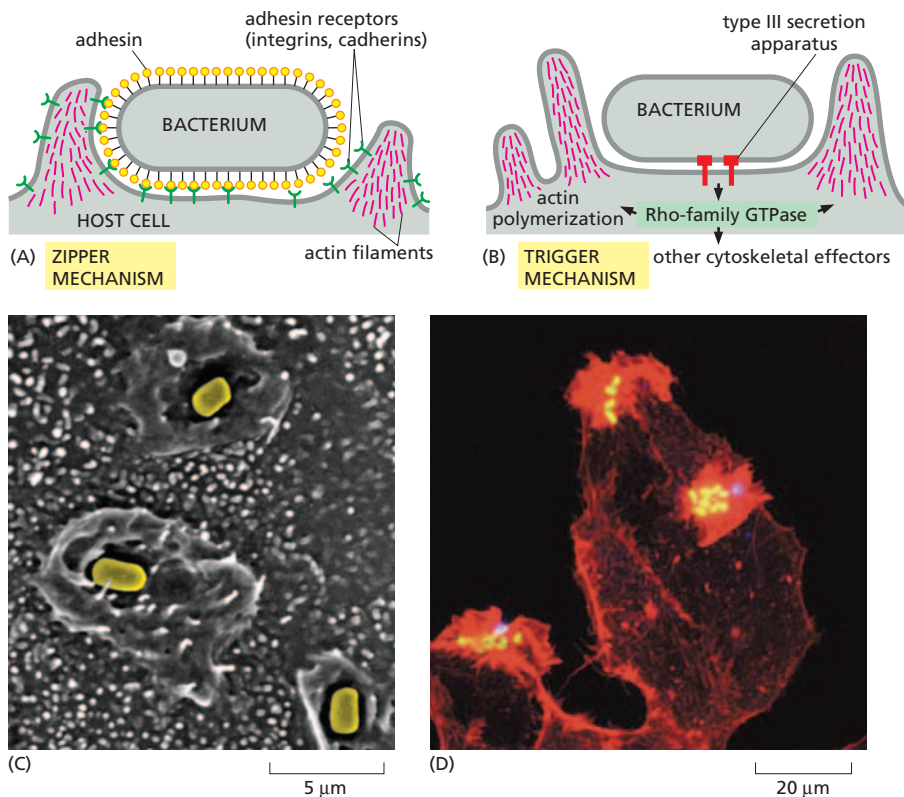


Figure 24-26 Mechanisms used by bacteria to induce phagocytosis by nonphagocytic host cells. (A) The zipper and (B) trigger mechanisms for pathogen-induced phagocytosis both require the polymerization of actin at the site of bacterial entry. (C) A scanning electron micrograph showing a very early stage of *Salmonella enterica* invasion by the trigger mechanism. Each of the three bacteria (pseudocolored yellow) is surrounded by a small membrane ruffle. (D) Fluorescence micrograph showing that the large ruffles that engulf the *Salmonella* bacteria are actin-rich. The bacteria are labeled in green and actin filaments in red; because of the color overlap, the bacteria appear yellow. (From J.E. Galan, *Annu. Rev. Cell Dev. Biol.* 17:53–86, 2001. With permission from Annual Reviews.)

typically much larger than bacteria, invade host cells through a variety of complex pathways that usually require significant energy expenditure on the part of the parasite.

Toxoplasma gondii, the cat parasite that also causes occasional serious human infections, is an example. When this protozoan contacts a host cell, it protrudes an unusual microtubule-based structure called a *conoid*, which it uses to push its way slowly into the host cell. The energy for invasion seems to come entirely from the parasite and requires at least one highly unusual myosin (Class XIV; see Figure 16–57); depolymerizing the actin cytoskeleton in the parasite, but not in the host cell, disrupts this process. As the parasite moves into the host cell, a membrane derived from the invaginated host cell plasma membrane surrounds it. Remarkably, the parasite somehow removes host transmembrane proteins from the surrounding membrane as it forms, so that the parasite is protected in a membrane-enclosed compartment, which does not fuse with lysosomes and does not participate in host cell membrane trafficking processes (Figure 24–27). The specialized membrane allows the parasite to take up metabolic intermediates and nutrients from the host cell's cytosol but excludes larger molecules. Malaria parasites invade red blood cells by using a very similar mechanism.

The protozoan *Trypanosoma cruzi*, which causes multiorgan Chagas disease, mainly in Mexico and Central and South America, uses an entirely different, but no less peculiar, invasion strategy. After attachment to host cell surface receptors, this parasite induces a local elevation of Ca^{2+} in the host cell's cytosol. The Ca^{2+} signal recruits lysosomes to the site of parasite attachment, which fuse with the host cell's plasma membrane during the internalization process, allowing the parasites rapid access to the lysosomal compartment (Figure 24–28). As we discuss below, most intracellular pathogens go to great lengths to avoid exposure to the hostile, proteolytic environment of the lysosome, but *Trypanosoma cruzi* uses the lysosome as its means of entry. In the lysosomal compartment, the parasite secretes an enzyme that removes sialic acid from lysosomal glycoproteins and transfers it to its own surface molecules, thereby coating itself with host cell sugars. Next, the parasite secretes a pore-forming toxin that lyses the lysosome membrane, releasing the parasite into the host cell's cytosol, where it proliferates.

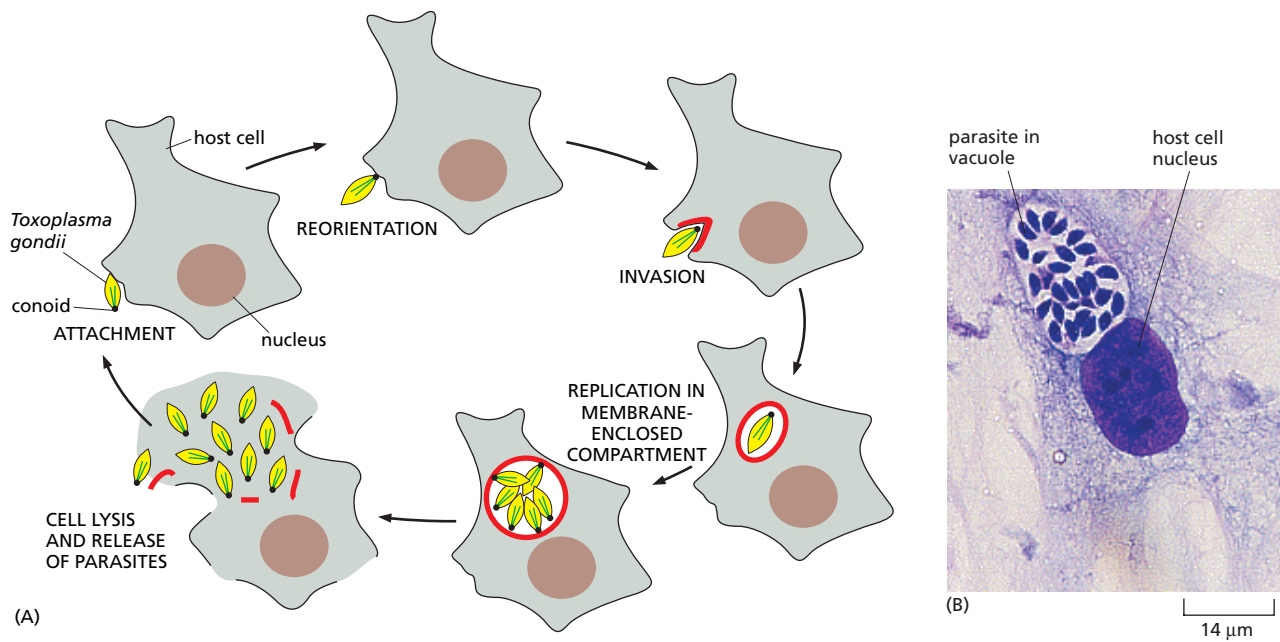
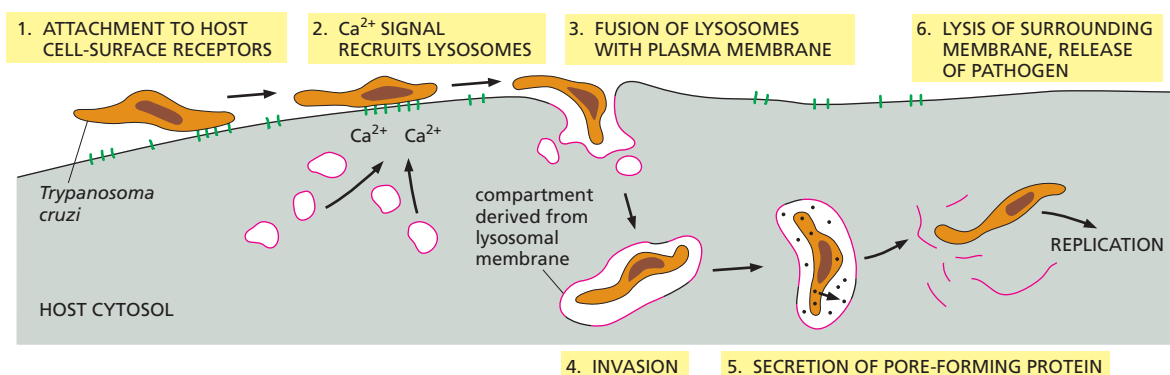


Figure 24–27 The life cycle of the intracellular parasite *Toxoplasma gondii*. (A) After attachment to a host cell, *T. gondii* uses its conoid to push its way into the host cell. As the host cell's plasma membrane invaginates to surround the parasite, the parasite somehow removes the host cell proteins associated with normal endosomes or phagosomes, so that the compartment (shown in red) does not fuse with lysosomes. After several rounds of replication, the parasite causes the compartment to break down and the host cell to lyse, releasing the progeny into the extracellular space from which they can infect other host cells. (B) Light micrograph of *T. gondii* replicating within a membrane-enclosed compartment in a cultured cell. (B, courtesy of Manuel Camps and John Boothroyd.)

The *microsporidia* use perhaps the most bizarre active invasion mechanism. These tiny, obligate, intracellular, eucaryotic parasites are only about 5 μm long and have among the smallest known genomes for a eucaryotic cell, only 2,900,000 nucleotide pairs. Normally, microsporidia cause disease primarily in insects, but they can also cause opportunistic infections in people with AIDS. Having adapted over a long period to a parasitic lifestyle, they depend on their host cells for some metabolic functions and have lost many of the genes and cell structures required for a free-living existence; for example, they no longer have mitochondria or peroxisomes. However, they do have a strange extrusion apparatus, the polar tube, that enables them to invade host cells. In the environmentally resistant spore stage of its life cycle, the polar tube is wound in a coil around the nucleus (Figure 24–29A). On contact with an appropriate host cell, the polar tube discharges explosively, uncoiling in less than 2 seconds to form a mature structure that can be more than ten times the length of the spore. The tip of the discharging polar tube, traveling at a speed of 100 $\mu\text{m}/\text{sec}$, penetrates the host cell and delivers (apparently by osmotic pressure) the internal contents of the spore, including the microsporidian's nucleus, into the cytoplasm of the host cell, where the parasite replicates to form up to a hundred progeny (Figure 24–29B and C). Eventually, the progeny mature into spores, and the host cell

Figure 24–28 Invasion of host cells by *Trypanosoma cruzi*. This parasite recruits host cell lysosomes to its site of attachment. The lysosomes fuse with the invaginating plasma membrane to create an intracellular compartment constructed almost entirely of lysosomal membrane. After a brief stay in the compartment, the parasite secretes a pore-forming protein that disrupts the surrounding membrane, allowing the parasite to escape into the host cell cytosol and proliferate.



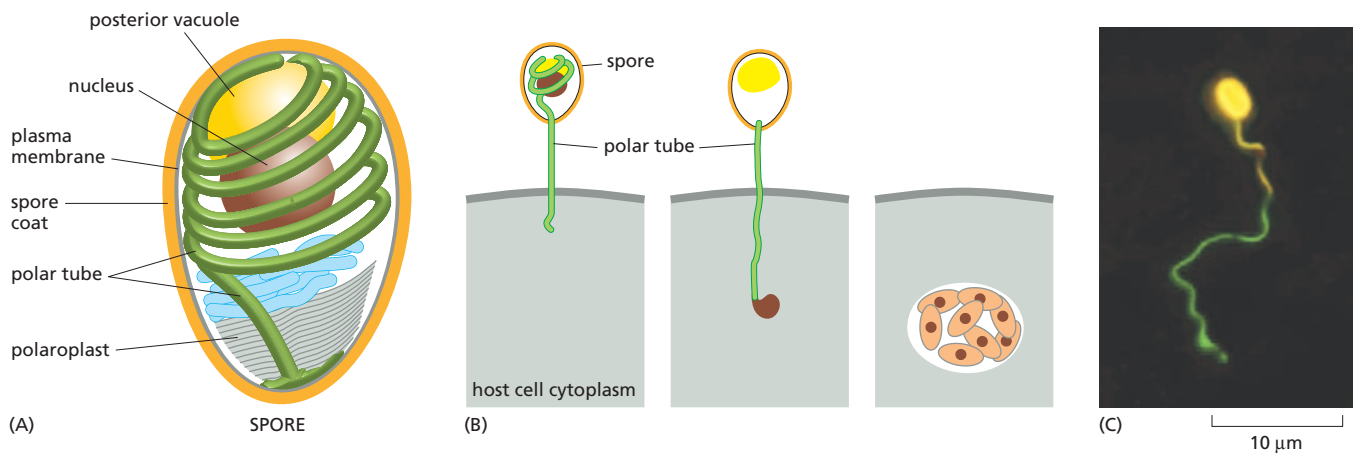


Figure 24-29 Invasion of host cells by microsporidia. (A) The spore form of the parasite is covered with a rigid coat and harbors a coiled polar tube that wraps many times around the nucleus. (B) The polar tube extends explosively when the spore comes into contact with an appropriate host cell, penetrating the host cell and delivering the nucleus and other contents of the spore into the cytoplasm of the host cell. The microsporidian then proliferates in the host cell. (C) Immunofluorescence micrograph of a spore of the microsporidian *Encephalitozoon cuniculi*. The parts of the spore that are outside the host cell are stained yellow, and the parts that are inside the host cell are stained green. The discharged polar tube changes from yellow to green at the point where it has entered the host cell, which is not visible. Scale bar 10 µm. (C, from C. Franzen, *Trends Parasitol.* 20:275–279, 2004. With permission from Elsevier.)

lyses to release them. Microsporidia spores are sufficiently small for macrophages to phagocytose them. When this happens, the spores discharge their polar tube from within the confines of the phagosome, again delivering their contents into the cytosol of the host cell.

Many Pathogens Alter Membrane Traffic in the Host Cell

The three examples of intracellular parasites just discussed raise a general problem that faces all intracellular pathogens, including viruses, bacteria, and eucaryotic parasites. They must deal in some way with membrane traffic in the host cell. After endocytosis by a host cell, they usually find themselves in an endosomal compartment that normally would fuse with lysosomes to form a *phagolysosome*. They therefore must either modify the compartment to prevent its fusion with lysosomes, escape from the compartment before such fusion, escape after fusion but before getting digested, or find ways to survive in the hostile environment of the phagolysosome (**Figure 24-30**).

Most pathogens use the first or second strategy. As we have seen, *Trypanosoma cruzi* uses the escape route, as do essentially all viruses (see Figure 24-24). The bacterium *Listeria monocytogenes* also uses this strategy. It is taken up into cells via the zipper mechanism discussed earlier and secretes a protein called *listeriolysin O* that forms large pores in the phagosomal membrane, releasing the bacteria into the cytosol before they are digested. Once in the

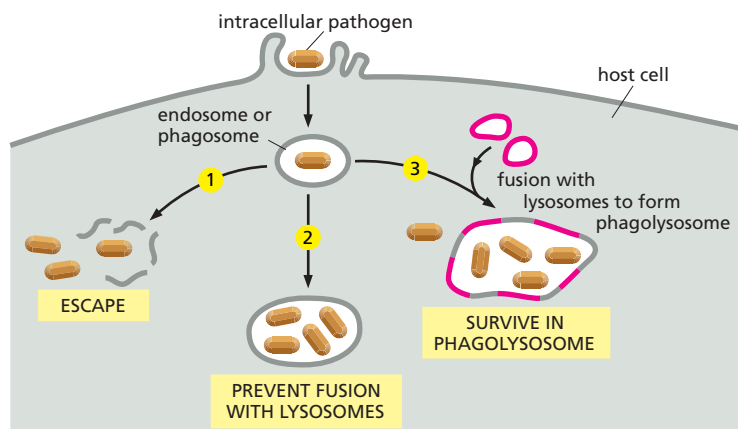
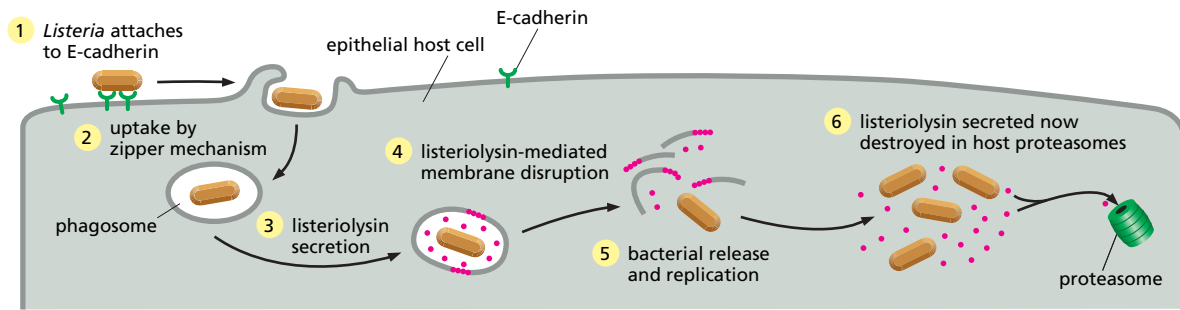


Figure 24-30 Choices that an intracellular pathogen faces. After entry into a host cell, generally through endocytosis or phagocytosis into a membrane-enclosed compartment, intracellular pathogens can use one of three strategies to survive and replicate. Pathogens that follow strategy (1) include all viruses, *Trypanosoma cruzi*, *Listeria monocytogenes*, and *Shigella flexneri*. Those that follow strategy (2) include *Mycobacterium tuberculosis*, *Salmonella enterica*, *Legionella pneumophila*, and *Chlamydia trachomatis*. Those that follow strategy (3) include *Coxiella burnetii* and *Leishmania*.



cytosol, the bacteria continue to secrete listeriolysin O, but it does not destroy other cell plasma membranes for two reasons: first, it is 10 times more active at the acidic pH found in the phagosome than at neutral pH found in the cytosol; second, it is rapidly degraded in the cytosol by host cell proteasomes (see Figure 6–80), which do not have access to the listeriolysin O in the phagosome (Figure 24–31).

If a pathogen is to survive and replicate in a host cell membrane-enclosed compartment, it must modify membrane trafficking in the host cell, and it can use various methods to do this. It must modify the compartment in at least two ways: first, it must prevent lysosomal fusion, and second, it must provide a pathway for importing nutrients from the host cytosol. In addition, many pathogens (particularly viruses) alter membrane trafficking pathways to prevent presentation of their tell-tale foreign antigens on the host cell surface; otherwise, T cells could detect their presence and kill the host cell (discussed in Chapter 25).

Different pathogens have distinct strategies for altering host cell membrane traffic (Figure 24–32). As we have seen, *Toxoplasma gondii* creates a membrane-enclosed compartment that does not participate in normal host cell membrane traffic and yet specifically allows nutrient import. *Mycobacterium tuberculosis* somehow prevents the very early endosome that contains it from maturing, so that the endosome never acidifies or acquires the characteristics of a late endosome or lysosome. Endosomes containing *Salmonella enterica*, in contrast, do acidify and acquire markers of late endosomes, but they arrest their maturation at a stage prior to lysosomal fusion. Other bacteria seem to find shelter in intracellular compartments that are completely distinct from the usual endocytic pathway. *Legionella pneumophila*, for example, replicates in compartments that are enclosed by layers of rough endoplasmic reticulum

Figure 24–31 Selective destruction of the phagosomal membrane by *Listeria monocytogenes*. *L. monocytogenes* attaches to E-cadherin on the surface of epithelial cells and induces its own uptake by the zipper mechanism (see Figure 24–26A). Within the phagosome, the bacterium secretes the hydrophobic protein listeriolysin O, which forms oligomers in the host cell membrane, thereby creating large pores and eventually disrupting the membrane. Once in the host cell cytosol, the bacteria begin to replicate and continue to secrete listeriolysin O. Because the listeriolysin O in the cytosol is rapidly degraded by proteasomes, the host cell's plasma membrane remains intact.

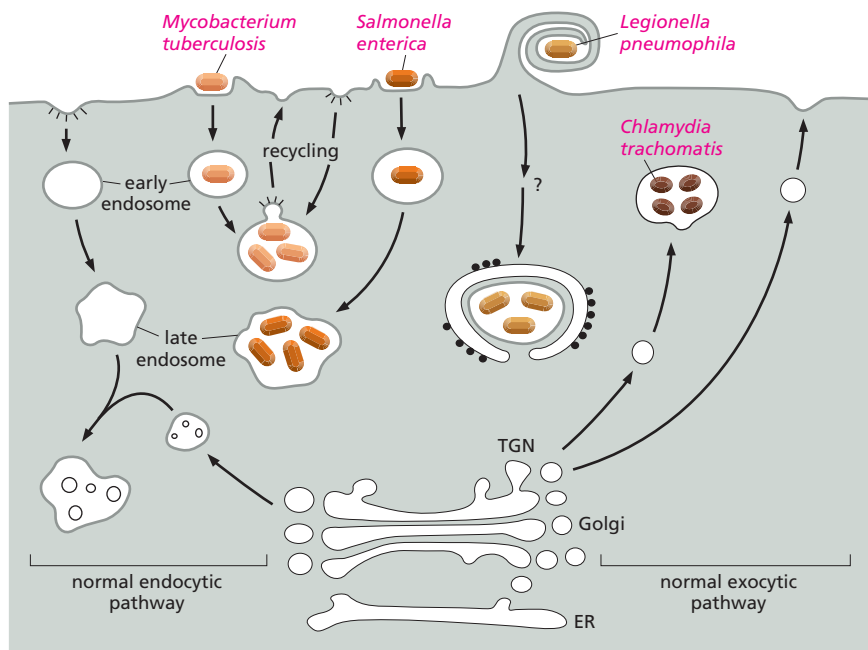
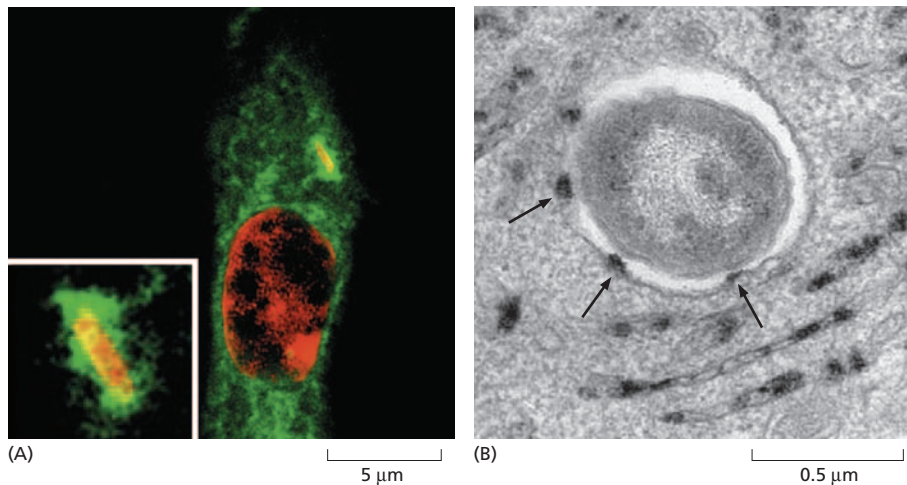


Figure 24–32 Modifications of host cell membrane trafficking by bacterial pathogens. Four intracellular bacterial pathogens, *Mycobacterium tuberculosis*, *Salmonella enterica*, *Legionella pneumophila*, and *Chlamydia trachomatis*, all replicate in membrane-enclosed compartments, but the four compartments differ. *M. tuberculosis* remains in a compartment that has early endosomal markers and continues to communicate with the plasma membrane via transport vesicles. *S. enterica* replicates in a compartment that has late endosomal markers and does not communicate with the plasma membrane. *L. pneumophila* replicates in an unusual compartment that is wrapped in several layers of rough endoplasmic reticulum (ER) membrane; only one layer is shown for simplicity. *C. trachomatis* replicates in an exocytic compartment that fuses with vesicles coming from the trans Golgi network (TGN).



(Figure 24-33). *Chlamydia trachomatis*, a sexually transmitted bacterial pathogen that can cause sterility and blindness, replicates in a compartment that seems similar to part of the exocytic pathway. Some intracellular bacterial pathogens seem to be able to manipulate the location of other membrane-enclosed organelles not in direct physical contact with their own compartment. For example, *Salmonella*-containing late endosomes are normally found in very close apposition to the Golgi apparatus (Figure 24-34). The mechanisms used by these organisms to alter their membrane compartments and other aspects of membrane traffic in the host cell are still poorly understood.

Viruses also often alter membrane traffic in the host cell. Enveloped viruses must acquire their membrane from host cell phospholipids. In the simplest cases, virally encoded proteins are inserted into the ER membrane and follow the usual path through the Golgi apparatus to the plasma membrane, undergoing various post-translational modifications *en route*. The viral capsid and genome then assemble at the plasma membrane and bud off from the cell surface. This is one mechanism used by HIV. Other enveloped viruses interact in complex ways with membrane trafficking pathways in the host cell (Figure 24-35). Even some nonenveloped viruses alter membrane traffic in the host cell to suit their own purposes. For example, a membrane-associated, virus-encoded RNA polymerase carries out poliovirus replication. The replication proceeds more quickly if the surface area of host cell membrane is increased. To accomplish this, the virus induces increased membrane lipid synthesis in the host cell and blocks membrane transport from the ER. ER membrane thereby accumulates, expanding the surface area on which viral RNA replication can occur (Figure 24-36). Many viral pathogens and some bacterial pathogens are frequently found in association with autophagosomes, which form by autophagy (discussed

Figure 24-33 Association of host cell endoplasmic reticulum (ER) membrane with intracellular bacterial pathogens. (A) Shortly after infection with *Legionella pneumophila*, the ER (green) of the host cell is recruited to surround the intracellular bacterium. The DNA of the bacterium and of the host cell are labeled red. The inset shows the close association between the ER and the bacterium. (B) *Brucella abortus*, a Gram-negative bacterium that can cause abortions in cattle, also replicates within an ER-associated compartment. In this electron micrograph, the black smudges within the ER indicate the presence of the ER-specific enzyme glucose-6-phosphatase. The black arrows show concentrations of the enzyme on the membrane enclosing the bacterium, indicating that it has fused directly with the ER. (A, from J.C. Kagan and C.R. Roy, *Nat. Cell Biol.* 4:945–954, 2002. With permission from Macmillan Publishers Ltd; B, from J. Celli and J.P. Gorvel, *Curr. Opin. Microbiol.* 7:93–97, 2004. With permission from Elsevier.)

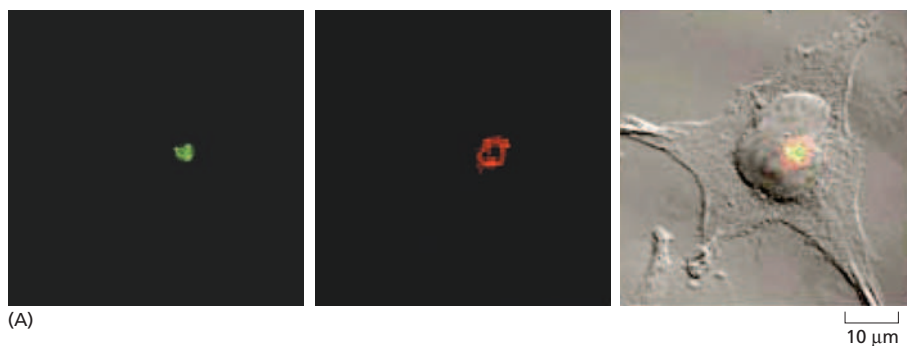
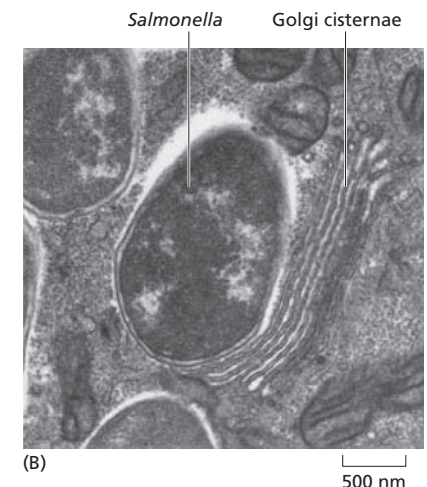


Figure 24-34 Proximity of Golgi stacks to endosomes containing *Salmonella enterica*. (A) In this infected cell, the bacteria are green and the Golgi has been labeled (red) with antibodies against a Golgi-specific structural protein. (B) Electron micrograph showing the very close apposition of a Golgi stack to a late endosome containing the bacterium. (From S.P. Salcedo and D.W. Holden, *EMBO J.* 22:5003–5014, 2003. With permission from Macmillan Publishers Ltd.)



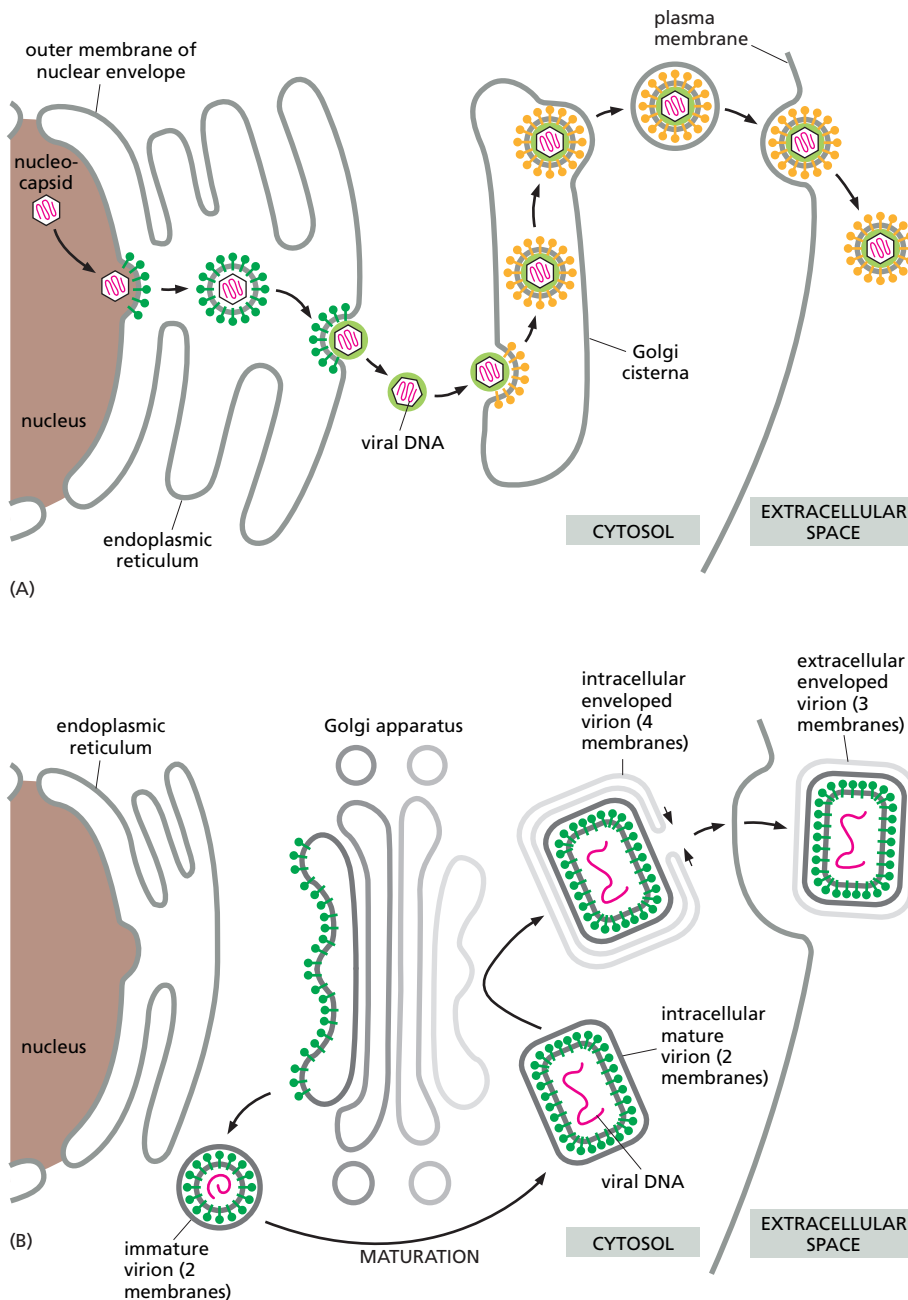


Figure 24–35 Complicated strategies for viral envelope acquisition. (A) Herpes virus nucleocapsids assemble in the nucleus and then bud through the inner nuclear membrane into the space between the inner and outer nuclear membranes, acquiring a membrane coat. The virus particles then apparently lose this coat when they fuse with the outer nuclear membrane to escape into the cytosol. Subsequently, the nucleocapsids bud into the Golgi apparatus and bud out again on the other side, acquiring two new membrane coats. The virus then buds from the cell with a single membrane when its outer membrane fuses with the plasma membrane. (B) Vaccinia virus (which is closely related to the virus that causes smallpox and is used to vaccinate against smallpox) assembles in “replication factories” within the cytosol, far away from the plasma membrane. The first structure that assembles contains two membranes, both acquired from the Golgi apparatus by a poorly defined wrapping mechanism. Some of these viral particles are then engulfed by the membranes of a second intracellular membrane-enclosed compartment; these viral particles have a total of four layers of membrane. After fusion at the plasma membrane, the virus escapes from the cell with three membrane layers.

in Chapter 13). In most cases, it is not clear whether the host cell initiates induction of autophagy as a protective response or the invading pathogen triggers it to assist in the pathogen’s replication.

Viruses and Bacteria Use the Host Cell Cytoskeleton for Intracellular Movement

The cytoplasm of mammalian cells is extremely viscous. It is crowded with organelles and supported by networks of cytoskeletal filaments, all of which inhibit the diffusion of particles the size of a bacterium or a viral capsid. To reach a particular part of the cell to carry out part of its replication cycle, a pathogen must actively move there. As with transport of intracellular organelles, generally, pathogens use the host cell’s cytoskeleton for active movement.

Several bacteria that replicate in the host cell’s cytosol (rather than in membrane-enclosed compartments) have adopted a remarkable mechanism for moving, which depends on actin polymerization. These bacteria, including *Listeria monocytogenes*, *Shigella flexneri*, *Rickettsia rickettsii* (which causes Rocky

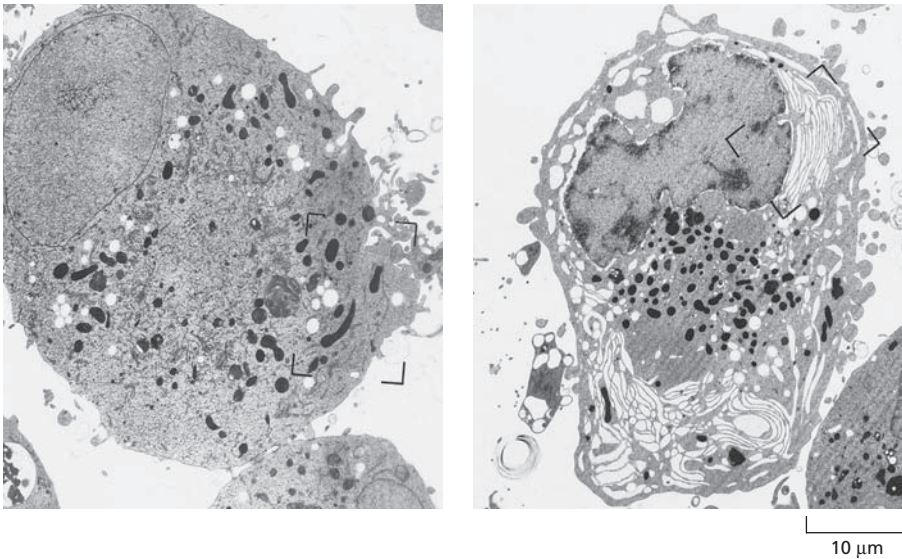
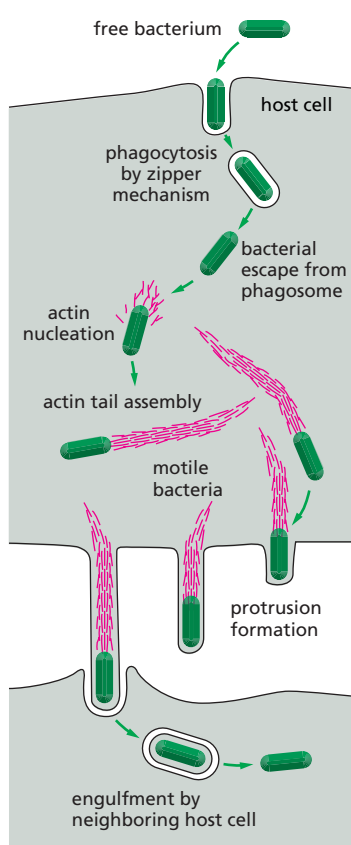
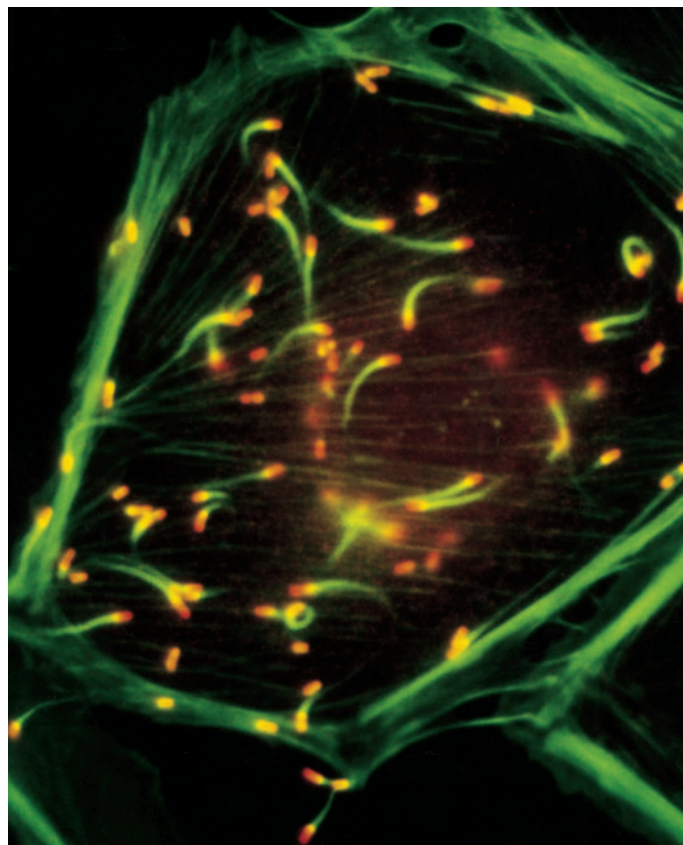


Figure 24-36 Intracellular membrane alterations induced by a poliovirus protein. Poliovirus, like other single-stranded RNA viruses, replicates its RNA genome by using a polymerase that associates with intracellular membranes. Several of the proteins encoded by its genome alter the structure or dynamic behavior of the membrane-enclosed organelles in the host cell. These electron micrographs show an untransfected monkey cell (*left*) and the same type of cell expressing a transgene encoding the 3A protein from poliovirus (*right*). In the transfected cell, the ER is swollen because the transgene-encoded protein inhibits traffic from the ER to the Golgi apparatus. (From J.J.R. Doedens, T.H. Giddings Jr. and K. Kirkegaard, *J. Virol.* 71:9054–9064, 1997.)

Mountain spotted fever), *Burkholderia pseudomallei* (which causes melioidosis), and *Mycobacterium marinum* (a relative of the bacterium that causes tuberculosis), induce the nucleation and assembly of host cell actin filaments at one pole of the bacterium. The growing filaments generate substantial force and push the bacterium through the cytoplasm at rates of up to 1 µm/sec. New filaments form at the rear of each bacterium and are left behind like a rocket trail as the bacterium advances, depolymerizing again within a minute or so as they encounter depolymerizing factors in the cytosol. When a moving bacterium reaches the plasma membrane, it continues to move outward, inducing the formation of a long, thin host cell protrusion with the bacterium at its tip. A neighboring cell often engulfs this projection, allowing the bacterium to enter the neighbor's cytoplasm without exposure to the extracellular environment, thereby avoiding recognition by antibodies produced by the host's adaptive immune system (Figure 24-37).

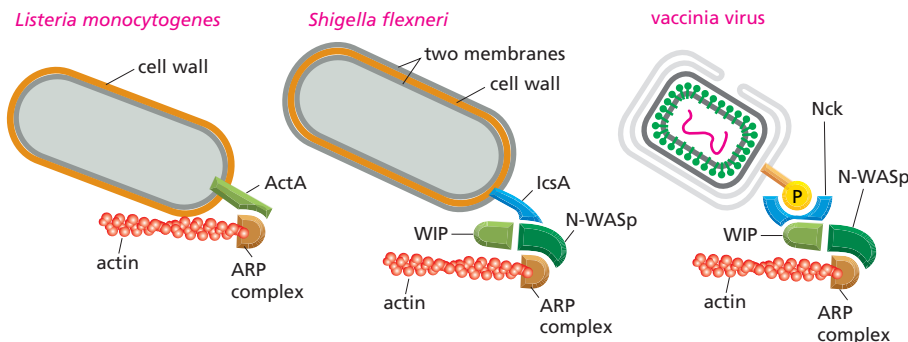


(A)



(B)

Figure 24-37 The actin-based movement of *Listeria monocytogenes* within and between host cells. (A) These bacteria induce the assembly of actin-rich tails in the host cell cytoplasm, enabling the bacteria to move rapidly. Motile bacteria spread from cell to cell by forming membrane-enclosed protrusions that are engulfed by neighboring cells. (B) Fluorescence micrograph of the bacteria moving in a cell that has been stained to reveal bacteria in red and actin filaments in green. Note the comet-like tail of actin filaments behind each moving bacterium. Regions of overlap of red and green fluorescence appear yellow. (B, courtesy of Julie Theriot and Tim Mitchison.)



The molecular mechanism of pathogen-induced actin assembly has been determined for several microorganisms. The mechanisms are different for the different pathogens, suggesting that they evolved independently. Although they all use the same host cell regulatory pathway that normally controls the nucleation of actin filaments, they exploit different points in the pathway. As discussed in Chapter 16, activation of the small GTPase Cdc42 by certain extracellular signals leads to the activation of a protein called N-WASp, which in turn activates an ARP complex, which nucleates the growth of a new actin filament. An *L. monocytogenes* surface protein directly binds to and activates the ARP complex to initiate the formation of an actin tail. *B. pseudomallei* and *R. rickettsii* use a similar strategy, although the sequence of the activating proteins differs in all three cases. In contrast, another unrelated surface protein on *S. flexneri* binds to and activates N-WASp, which then activates the ARP complex. Remarkably, vaccinia virus uses yet another mechanism to move intracellularly by inducing actin polymerization, although it exploits the same regulatory pathway (Figure 24–38).

Other pathogens rely primarily on microtubule-based transport to move within the host cell. Viruses that infect neurons illustrate this movement. The neurotropic alpha herpes viruses, a group that includes the virus that causes chicken pox, provides an important example. The virus enters sensory neurons at the tips of their axons and microtubule-based transport carries the nucleocapsids down the axon to the neuronal nucleus, apparently mediated by attachment of the capsid proteins to the molecular motor protein dynein. After replication and assembly in the nucleus, the enveloped virion is transported along axonal microtubules away from the neuronal cell body, mediated by attachment of a different viral coat component to a kinesin motor protein (Figure 24–39). A

Figure 24–38 Molecular mechanisms for actin nucleation by various pathogens. The bacteria *Listeria monocytogenes* and *Shigella flexneri* and the virus vaccinia all move intracellularly using actin polymerization. To induce actin nucleation, all of these pathogens recruit and activate the ARP complex (see Figure 16–34), although each pathogen uses a different recruitment strategy. *L. monocytogenes* expresses a surface protein, ActA, that directly binds to and activates the ARP complex. *S. flexneri* expresses a surface protein, IcsA (unrelated to ActA), that recruits the host cell signaling protein N-WASp. N-WASp in turn recruits the ARP complex, along with other host proteins, including WIP (WASp-interacting protein). Vaccinia virus expresses an envelope protein that is phosphorylated on tyrosine by a host cell protein tyrosine kinase. The phosphorylated protein then recruits Nck, which binds WIP. WIP binds N-WASp, which recruits and activates the ARP complex. The more complicated mechanism that vaccinia uses is thought to closely resemble how chemotactic factors activate the ARP complex in motile eucaryotic cells. Despite the different molecular strategies used to assemble them, the actin comet tails formed by all three pathogens look very similar, and the pathogens move at similar speeds inside infected cells.

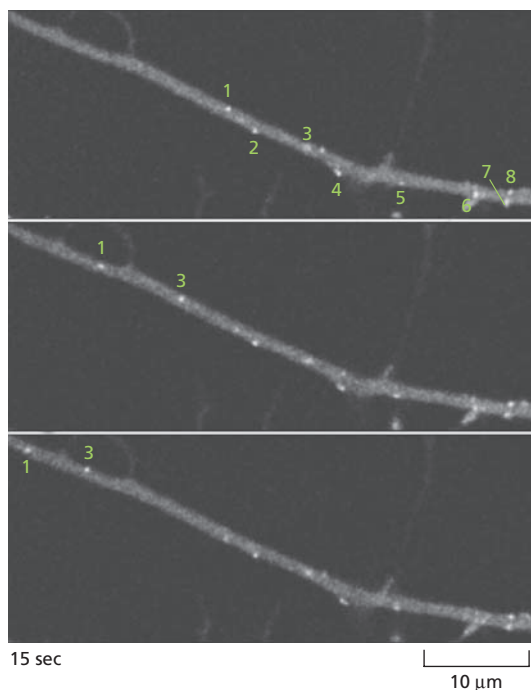
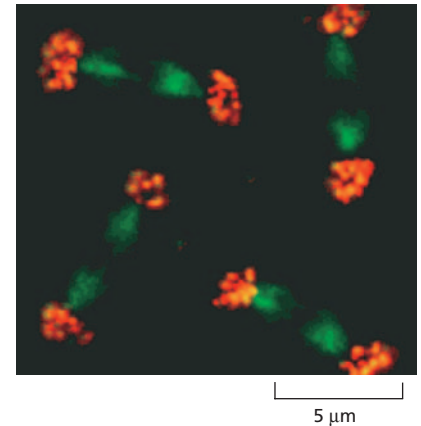


Figure 24–39 Fluorescence micrographs of herpes virus moving in an axon. This nerve cell has been infected with an alpha herpes virus that has been genetically engineered to express green fluorescent protein (GFP) fused to one of its capsid proteins. In this segment of the axon, several viral particles are visible, and two of them (numbered 1 and 3) are moving away from the cell body, which is to the right and out of the picture. (From G.A. Smith, S.P. Gross and L.W. Enquist, *Proc. Natl Acad. Sci. U.S.A.* 98:3466–3470, 2001. With permission National Academy of Sciences.)

Figure 24–40 The association of *Wolbachia* with microtubules. This fluorescence micrograph shows *Wolbachia* (red) associated with the microtubules (green) of four mitotic spindles in a syncytial *Drosophila* embryo. The clumps of bacteria at the spindle poles will segregate along with the chromosomes (not visible at the equator of the spindles) when the chromosomes segregate. (From H. Kose and T.L. Karr, *Mech. Cell Dev.* 51:275–288, 1995. With permission from Elsevier.)



large number of virions, including those of HIV, rabies virus, influenza virus, adenovirus, canine parvovirus, and vaccinia virus (the smallpox relative used for vaccination), have now been shown to associate with dynein or kinesin motor proteins and to undergo directed movement along microtubules at some stage in their replication. A primary function of the microtubule highways laid down in eucaryotic cells is to serve as oriented tracks for membrane traffic; it is not surprising that many viruses have independently evolved the ability to engage these transport systems to enhance their own replication.

One bacterium that is known to associate with microtubules is *Wolbachia*. This fascinating genus includes many species that are parasites or symbionts of insects and other invertebrates, living in the cytosol of each cell in the animal. The infection is spread vertically from mother to offspring, as *Wolbachia* are also present in eggs. The bacteria ensure their transmission into every cell by binding to microtubules, so that the mitotic spindle segregates them simultaneously with chromosome segregation when an infected cell divides (Figure 24–40). As we discuss later, *Wolbachia* infection can significantly alter the reproductive behavior of its insect hosts.

Viral Infections Take Over the Metabolism of the Host Cell

Most intracellular bacteria and parasites carry the basic genetic information required for their own metabolism and replication, and they rely on their host cells only for nutrients. Viruses, in contrast, use the basic host cell machinery for most aspects of their reproduction: they all depend on host cell ribosomes to produce their proteins, and some also use host cell DNA and RNA polymerases for replication and transcription, respectively.

Many viruses encode proteins that modify the host transcription or translation apparatus to favor the synthesis of viral proteins over those of the host cell. As a result, the synthetic capability of the host cell is devoted principally to the production of new virus particles. Poliovirus, for example, encodes a protease that specifically cleaves the TATA-binding factor component of TFIID (see Figure 6–18), effectively shutting off all host cell transcription via RNA polymerase II. Influenza virus produces a protein that blocks both the splicing and the polyadenylation of RNA transcripts, which therefore fail to be exported from the nucleus (see Figure 6–40).

Translation initiation of most host cell mRNAs depends on recognition of their 5′ cap by a group of translation initiation factors (see Figure 6–72). Translation initiation of host mRNAs is often inhibited during viral infection, so that the host cell ribosomes can be used more efficiently for the synthesis of viral proteins. Some viral genomes such as that of influenza virus encode endonucleases that cleave the 5′ cap from host cell mRNAs. Some go even further and then use the liberated 5′ caps as primers to synthesize viral mRNAs, a process called *cap snatching*. Several other viral RNA genomes encode proteases that cleave certain translation initiation factors. These viruses rely on 5′ cap-independent translation of the viral RNA, using internal ribosome entry sites (IRESs) (see Figure 7–108).

A few DNA viruses use host cell DNA polymerase to replicate their genome. Unfortunately for the virions, DNA polymerase is expressed at high levels only during S phase of the cell cycle, and most cells that these viral particles infect spend most of their time in G₁ phase. Adenovirus has evolved a mechanism to drive the host cell to enter S phase, producing large amounts of active DNA polymerase that then replicates the viral genome. The adenovirus genome encodes proteins that inactivate both Rb and p53, two key suppressors of cell-

cycle progression (discussed in Chapter 17). As might be expected for any mechanism that induces unregulated DNA replication, these viruses can promote the development of cancer in some circumstances.

Pathogens Can Alter the Behavior of the Host Organism to Facilitate the Spread of the Pathogen

As we have seen, pathogens often alter the behavior of the host cell in ways that benefit the survival and replication of the pathogen. Similarly, pathogens often alter the behavior of the whole host organism to facilitate pathogen spread, as we saw earlier for *Trypanosoma brucei* and *Yersinia pestis*. In some cases, it is difficult to tell whether a particular host response is more for the benefit of the host or for the pathogen. Pathogens such as *Salmonella enterica* that cause diarrhea, for example, usually produce self-limiting infections because the diarrhea efficiently washes out the pathogen. The bacteria-laden diarrhea, however, can spread the infection to a new host. Similarly, coughing and sneezing help to clear pathogens from the respiratory tract, but they also spread the infection to new individuals. A person with a common cold may produce 20,000 droplets in a single sneeze, all carrying rhinovirus or corona virus.

A frightening example of a pathogen modifying host behavior is seen in rabies, as first described in Egyptian writings over 3000 years ago. Rabies virus replicates in neurons and causes infected people or animals to become “rabid”: they are unusually aggressive and develop a strong desire to bite. The virus is shed in the saliva and transmitted through the bite wound into the bloodstream of the victim, spreading the infection to a new host.

But *Wolbachia* exhibit the most dramatic example of pathogens modifying host behavior. These bacteria manipulate the sexual behavior of their host to maximize their dissemination. As described earlier, *Wolbachia* are passed vertically into offspring through eggs. If they live in a male, however, they hit a dead end, as they are excluded from sperm. In some species of *Drosophila*, *Wolbachia* modify the sperm of their host so that they can fertilize the eggs only of infected females. This modification creates a reproductive advantage for infected females over uninfected females, so that the overall proportion of *Wolbachia* carriers increases. In other host species, a *Wolbachia* infection kills males but spares females, increasing the number of females in the population and thus the number of individuals that can produce eggs to pass on the infection. In a few types of wasp, *Wolbachia* infections enable the females to produce eggs that develop parthenogenetically, without the need for fertilization by sperm; in this species, males have been completely eliminated. For some of its hosts, *Wolbachia* has become an indispensable symbiont, and curing the infection causes death of the host. In one case, humans are making use of this dependence: the filarial nematode that causes African river blindness is difficult to kill with antiparasite medications, but when people with river blindness are treated with antibiotics that cure the nematode’s *Wolbachia* infection, the nematode infection is also arrested.

Pathogens Evolve Rapidly

The complexity and specificity of the molecular interactions between pathogens and their host cells might suggest that virulence would be difficult to acquire by random mutation. Yet, new pathogens are constantly emerging, and old pathogens are constantly changing in ways that make familiar infections difficult to treat. Pathogens have two great advantages that enable them to evolve rapidly. First, they replicate very quickly, providing a great deal of material for the engine of natural selection. Whereas humans and chimpanzees have acquired a 2% difference in genome sequences over about 8 million years of divergent evolution, poliovirus manages a 2% change in its genome in 5 days, about the time it takes the virus to pass from the human mouth to the gut. Second, selective pressures encourage this rapid genetic variation. The host’s adaptive immune system and

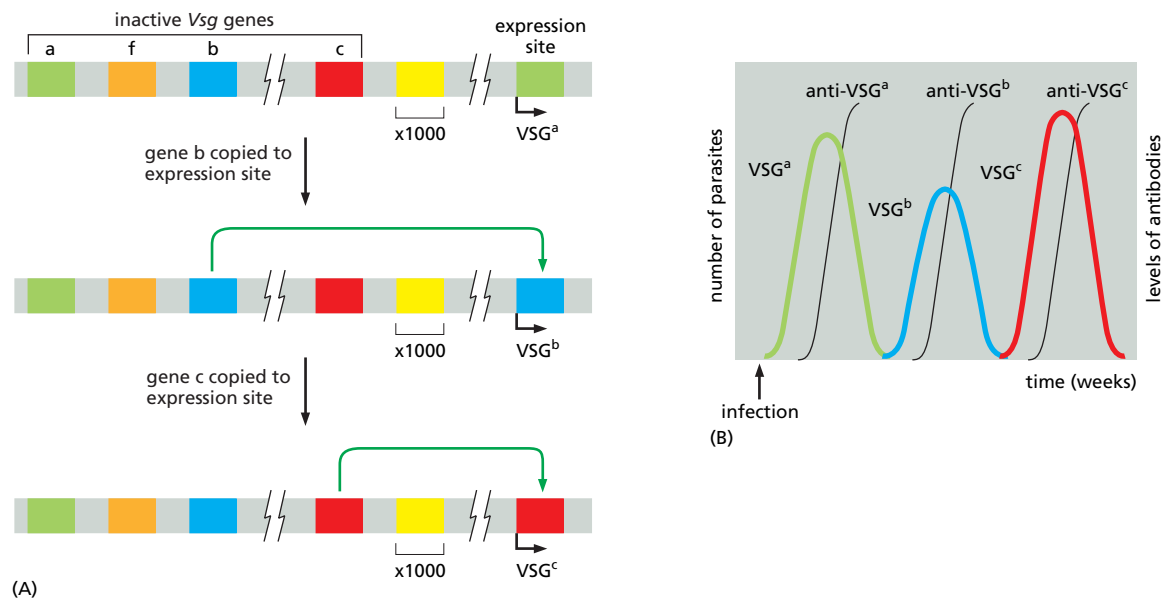
modern antipathogen drugs, both of which destroy pathogens that fail to change, are the source of these selective pressures.

In many cases, changes in human behavior exacerbate the emergence and evolution of new infectious diseases. Crowded and filthy living conditions in medieval cities, for example, contributed to the spread of the bacterium *Yersinia pestis* to humans from its natural rodent host to cause plague. The tendency of modern humans to live at high population densities in large cities has also created the opportunity for infectious organisms to initiate epidemics, such as influenza, tuberculosis, and AIDS, which could not have spread so rapidly or so far among sparser human populations. Air travel can, in principle, allow an asymptomatic, newly infected host to carry an epidemic to any previously unexposed population within a few hours or days.

Antigenic Variation in Pathogens Occurs by Multiple Mechanisms

A small-scale example of the constant battle between infection and immunity is the phenomenon of **antigenic variation**. An important adaptive immune response against many pathogens is the host's production of antibodies that recognize specific molecules (*antigens*) on the pathogen surface (discussed in Chapter 25). Many pathogens change these antigens during the course of an infection, enabling them to evade elimination by antibodies. Some eucaryotic parasites, for example, undergo programmed rearrangements of the genes encoding their surface antigens. The most striking example occurs in African trypanosomes such as *Trypanosoma brucei*, a protozoan parasite that causes sleeping sickness and is spread by an insect vector. (*T. brucei* is a close relative of *T. cruzi*—see Figure 24–28—but it replicates extracellularly rather than inside cells.) *T. brucei* is covered with a single type of glycoprotein, called *variant-specific glycoprotein* (VSG), which elicits a protective antibody response in the host that rapidly clears most of the parasites. The trypanosome genome, however, contains about 1000 *Vsg* genes, each encoding a VSG with distinct antigenic properties. Only one of these genes is expressed at any one time, by being copied into an active expression site in the genome. Gene rearrangements that copy new alleles into the expression site repeatedly change the *Vsg* gene expressed. In this way, a few trypanosomes with an altered VSG escape the antibody-mediated clearance, replicate, and cause the disease to recur, leading to a chronic cyclic infection (Figure 24–41). Many other eucaryotic parasites, including the protozoan *Plasmodium falciparum*, which causes malaria, and the fungus *Pneumocystis carinii*, which causes pneumonia in people with AIDS, use very similar strategies to evade the host adaptive immune responses.

Figure 24–41 Antigenic variation in trypanosomes. (A) There are about 1000 distinct *Vsg* genes in *Trypanosoma brucei*, but there is only one site for *Vsg* gene expression. An inactive gene is copied into the expression site by gene conversion, where it is now expressed. Each *Vsg* gene encodes a different surface protein (antigen). Rare switching events allow the trypanosome to repeatedly change the surface antigen it expresses. (B) A person infected with trypanosomes expressing VSG^a mounts a protective antibody response, which clears most of the parasites expressing this antigen. However, a few of the trypanosomes may have switched to expression of VSG^b, which can now proliferate until anti-VSG^b antibodies clear them. By that time, however, some parasites will have switched to VSG^c, and so the cycle repeats, seemingly indefinitely.



Bacterial pathogens can also rapidly change their surface antigens. Species of the genus *Neisseria* are champions. These Gram-positive cocci can cause meningitis and sexually transmitted diseases. They employ an astonishing variety of mechanisms to promote antigenic variation. First, they undergo genetic recombination very similar to that just described for eucaryotic pathogens, which enables them to vary (over time) the pilin protein they use to make cell-surface pili: by recombination of multiple silent copies of variant *pilin* genes into a single expression locus they can express dozens of slightly different versions of the protein. Second, many cell-surface proteins, as well as many of the biosynthetic enzymes involved in synthesizing cell-surface carbohydrates, have their expression levels continually altered by random slippage and repair of tandem nucleotide repeats in the promoter region or coding sequence of their genes, which modulates transcription or translation. *Neisseria*, for example, have about 10 different genes encoding variants of the Opa family of outer membrane proteins, each of which undergoes random variation of protein expression levels in this way, resulting in a plethora of different surface protein compositions to bewilder the host adaptive immune system. Analysis of the genome sequence for several *Neisseria* species has led to the suggestion that over 100 genes may vary their expression levels using some variation of this mechanism. Third, *Neisseria* are extremely adept at taking up DNA from their environment and incorporating it into their genomes, further contributing to their extraordinary variability. Finally, *Neisseria* lack several of the DNA repair mechanisms present in other bacteria such as *E. coli*, so that the likelihood of their acquiring new mutations through replication error is higher than average. With all these mechanisms working together, it is not surprising that we have not yet developed an effective vaccine against *Neisseria* infections.

Although *Neisseria* is an extreme example, many other bacterial pathogens employ one or more of these techniques to enhance their antigenic variation. Moreover, several studies have shown that pathogenic bacteria isolated from patients with disease symptoms are much more likely to have defects in DNA repair pathways than isolates of the same bacterial species from environmental reservoirs. This intriguing finding suggests that the human immune system may act to accelerate bacterial evolution.

Horizontal gene transfer, rather than point mutations, often causes rapid evolution in bacteria. The acquisition of plasmids and bacteriophages mediates most of this horizontal gene transfer. Bacteria readily pick up pathogenicity islands and virulence plasmids (see Figure 24–5) from other bacteria. Once a bacterium acquires a new set of virulence-related genes, it may quickly establish itself as a new cause of human epidemics. *Yersinia pestis*, for example, is a bacterium endemic to rats and other rodents; it first appeared in human history in 542 A.D., when the city of Constantinople was devastated by plague. Sequence comparisons of *Y. pestis* with those of its close relative *Y. pseudotuberculosis*, which causes a severe diarrheal disease, suggest that *Y. pestis* may have emerged as a distinct strain only a few thousand years ago, not long before its devastating debut as plague.

Error-Prone Replication Dominates Viral Evolution

Error-prone replication mechanisms rather than genomic rearrangements are mainly responsible for antigenic variation in viruses. Retroviral genomes, for example, acquire on average one point mutation every replication cycle, because the viral reverse transcriptase that produces DNA from the viral RNA genome cannot correct nucleotide misincorporation errors. A typical, untreated HIV infection may eventually produce HIV genomes with every possible point mutation. In some ways, the high mutation rate is beneficial for the pathogen. By a microevolutionary process of mutation and selection within each host, most virions change over time from a form that is most efficient at infecting macrophages to one more efficient at infecting T-cells, as described earlier (see Figure 24–23). Similarly, once a patient is treated with an antiviral drug, the viral genome can quickly mutate and be selected for its resistance to the drug. If the

reverse transcriptase error rate were too high, however, deleterious mutations might accumulate too rapidly for the virus to survive. Furthermore, a variant that is successful in one host does not necessarily spread to others, as a mutated virion may not be able to infect a new host. For HIV-1, we can estimate the extent of this constraint by examining the sequence diversity among different infected individuals. Remarkably, only about one-third of the nucleotide positions in the coding sequence of the viral genome are invariant, and nucleotide sequences in some parts of the genome, such as the *env* gene, can differ by as much as 30%. This extraordinary genomic plasticity greatly complicates attempts to develop vaccines against HIV, and it can also lead to rapid drug resistance (discussed below). It has also led to the rapid emergence of new HIV strains. Sequence comparisons between various strains of HIV and the very similar simian immunodeficiency virus (SIV) from a variety of different monkey species suggest that the most virulent type of HIV, HIV-1, may have jumped from chimpanzees to humans as recently as 1930 (Figure 24–42).

Influenza viruses are an important exception to the rule that error-prone replication dominates viral evolution. They are unusual in that their genome consists of several (usually eight) strands of RNA. When two strains of influenza infect the same host, the strands of the two strains can recombine to form a novel type of influenza virus. Prior to 1900, the influenza strain that infected humans caused a very mild disease; a different influenza strain infected fowl such as ducks and chickens, but it only rarely infected humans. In 1918, a particularly virulent variant of avian (bird) influenza crossed the species barrier to infect humans, triggering the catastrophic epidemic of 1918 called the Spanish flu, which killed 20–50 million people worldwide, more than were killed in World War I. Subsequent influenza pandemics have been triggered by recombination, in which a new DNA segment from an avian form of the virus replaced one or more of the viral DNA segments governing human immune response to the virus (Figure 24–43). Such recombination events allow the new virus to replicate rapidly and spread through an immunologically naive human population. Generally, within two or three years, the human population develops immunity to the new recombinant strain of virus, and, as a result, the infection rate drops to a steady-state level. In normal years, influenza is a mild disease in healthy adults but can be life-threatening in the very young and very old. In pandemic years, however, especially in the 1918 pandemic, healthy adults seem unusually susceptible to lethal influenza infection, perhaps because of the tissue damage caused by an overzealous immune response. Because the recombination events are unpredictable, it is not possible to know when the next influenza pandemic will occur or how severe it might be.

Drug-Resistant Pathogens Are a Growing Problem

While human activities such as air travel have promoted the spread of certain infectious diseases, advances in public sanitation and in medicine have prevented or ameliorated the suffering caused by many others. Effective vaccines and worldwide vaccination programs have eliminated smallpox and severely reduced poliomyelitis, and many deadly childhood infections such as mumps and measles are now rarities in wealthy industrialized nations. Nonetheless, there are still many widespread and devastating infectious diseases, such as malaria, for which no effective vaccines are available.

The development of drugs that cure rather than prevent infections has also had a major impact on human health. **Antibiotics**, which kill bacteria, comprise the most successful class. Penicillin was one of the first antibiotics used to treat infections in humans, introduced into clinical use just in time to prevent tens of thousands of deaths from infected battlefield wounds in World War II. Because bacteria form a kingdom distinct from the eucaryotes they infect, much of their basic machinery for DNA replication, transcription, translation, and fundamental metabolism differs from that of their host. These differences enable us to find antibacterial drugs that specifically inhibit these processes in bacteria, without disrupting them in the host. Most of the antibiotics that we use to treat bacterial

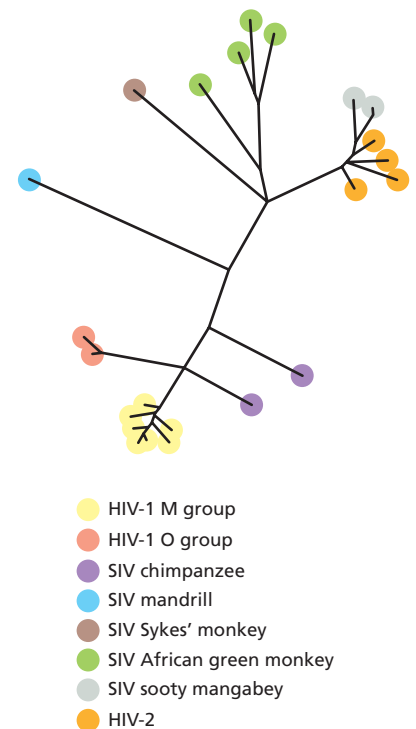


Figure 24–42 Diversification of HIV-1, HIV-2, and related strains of SIV. The genetic distance between any two viral isolates is found by following the shortest path joining them in the tree. HIV-1 is divided into two groups, major (M) and outlier (O). The HIV-1 M group is responsible for the global AIDS epidemic. HIV-1 M is further subdivided into various subtypes (A through G—not named in the figure). Subtype B is dominant in America and Europe; B, C, and E are dominant in Asia; and all subtypes are found in Africa. At least two monkey viruses, chimpanzee and mandrill, are more closely related to HIV-1 than is HIV-2, suggesting that HIV-1 and HIV-2 arose independently of each other. HIV-1 and SIV chimpanzee are estimated to have diverged in about 1930. This tree was constructed from the nucleotide sequences of the *Gag* gene, using a database containing about 16,000 sequences from virus samples isolated around the world.

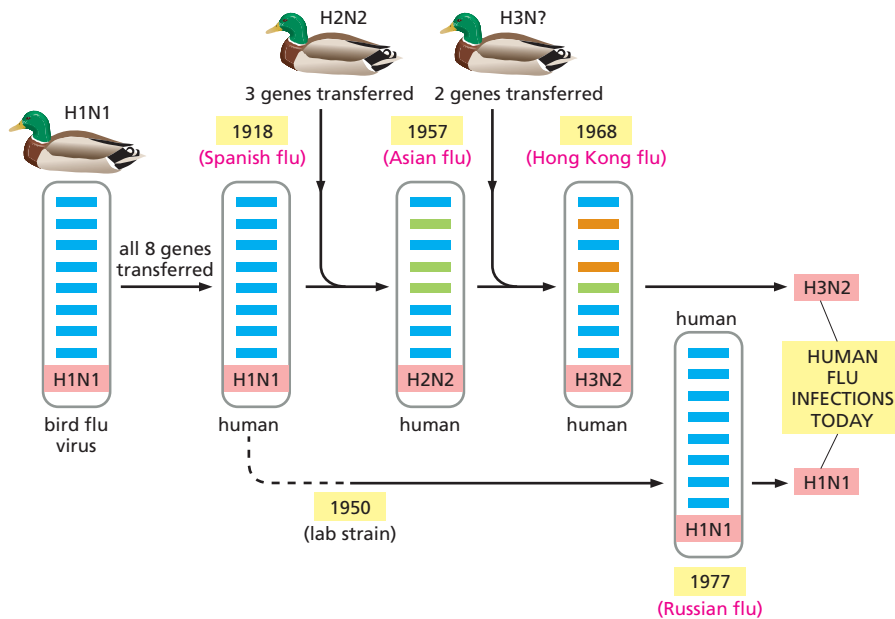


Figure 24-43 Model for the evolution of pandemic strains of influenza virus by recombination. Influenza A virus is a natural pathogen of birds, particularly waterfowl, and is always present in wild bird populations. Before 1900, influenza virus caused sporadic mild disease in humans. In 1918, a particularly virulent form of the virus crossed the species barrier from birds to humans and caused a devastating worldwide epidemic. This strain was designated H1N1, referring to the specific forms of its main antigens, hemagglutinin (HA, or H) and neuraminidase (NA, or N). Changes in the virus, rendering it less virulent, and the rise of adaptive immunity in the human population, prevented the pandemic from continuing in subsequent seasons, although H1N1 influenza strains continued to cause serious disease every year in very young and very old people. In 1957, a new pandemic arose when the genes encoding HA and NA were replaced by equivalent genes from an avian virus (*green bars*); the new strain (designated H2N2) was not effectively cleared by antibodies in people who had previously contracted only H1N1 forms of influenza. In 1968, another pandemic was triggered when the HA gene of H2N2 (but not the NA gene) was replaced from another avian virus; the new virus was designated H3N2. In 1977, there was a resurgence of H1N1 influenza, which had previously been almost completely replaced by the N2 strains. Molecular sequence information suggests that this minor pandemic may have been caused by an accidental release of an influenza strain that had been held in a laboratory since about 1950. As indicated, most human influenza today is caused by H1N1 and H3N2 strains.

infections are small molecules that inhibit macromolecular synthesis in bacteria by targeting bacterial enzymes that either are distinct from their eucaryotic counterparts or are involved in pathways, such as cell wall biosynthesis, that are absent in humans (Figure 24-44 and Table 6-3).

The rapid evolution of pathogens, however, enables bacteria to develop resistance to antibiotics very quickly. The typical lag between the introduction of an antibiotic into clinical use and the appearance of the first resistant strains is only a few years. Similar drug resistance also arises rapidly when treating viral infections with antiviral drugs. The virus population in an HIV-infected person treated with the reverse transcriptase inhibitor AZT, for example, will acquire complete resistance to the drug within a few months. The current protocol for treatment of HIV infections involves the simultaneous use of three drugs, which helps to minimize the acquisition of resistance.

There are three general strategies by which a pathogen can develop drug resistance: (1) it can alter the molecular target of the drug so that it is no longer sensitive to the drug; (2) it can produce an enzyme that destroys the drug; or (3) it can prevent access to the target by, for example, actively pumping the drug out of the pathogen (Figure 24-45).

Once a pathogen has chanced upon an effective drug-resistance strategy, the newly acquired or mutated genes that confer the resistance are frequently spread throughout the pathogen population and may even transfer to pathogens of different species that are treated with the same drug. The highly

Poultry farming brings humans into very close contact with large numbers of birds, which may carry different versions of the influenza virus. These avian viruses occasionally cause fatal disease in humans who have been in direct contact with infected birds (so-called bird flu), but thus far these viruses have not acquired the ability to spread effectively from human to human. Monitoring these cross-species jumps and attempting to predict the origin of the next serious influenza epidemic remain significant public health challenges.

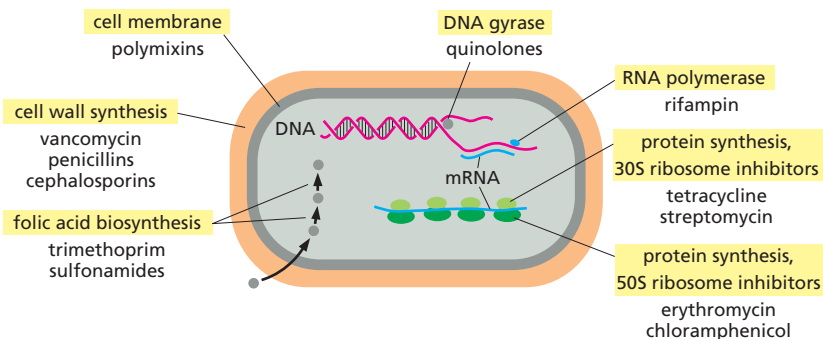


Figure 24-44 Antibiotic targets. Although there are many antibiotics in clinical use, they have a narrow range of targets, which are highlighted in yellow. A few representative antibiotics in each class are listed. Nearly all antibiotics used to treat human infections fall into one of these categories. The vast majority inhibit either bacterial protein synthesis or bacterial cell wall synthesis.

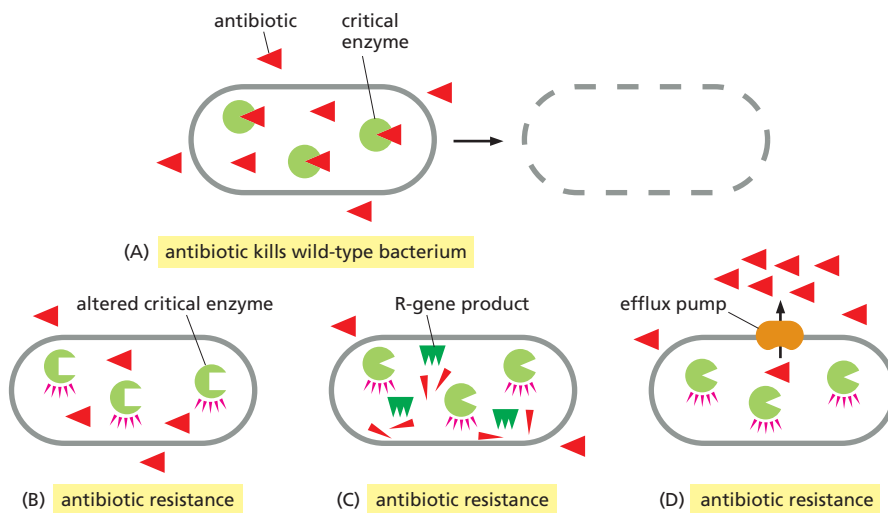


Figure 24-45 Three general mechanisms of antibiotic resistance. (A) Non-resistant bacterial cells bathed in a drug (red triangles) that binds to and inhibits a critical enzyme (light green) will be killed due to enzyme inhibition. (B) Bacteria that have altered the target enzyme so that it no longer binds to the drug will survive and proliferate. In many cases, a single point mutation in the gene encoding the target protein can generate resistance. (C) Bacteria that express a resistance gene (R-factor) that encodes an enzyme (dark green) that either degrades or covalently modifies the drug will survive and proliferate. Some resistant bacteria, for example, make Beta-lactamase enzymes, which cleave penicillin and similar compounds. (D) Yet other resistant bacteria express or up-regulate an efflux pump that ejects the drug from the bacterial cytoplasm using energy derived from either ATP hydrolysis or the electrochemical gradient across the bacterial plasma membrane. Some efflux pumps, such as the TetR efflux pump that confers resistance to tetracycline, are specific for a single drug; others, called multi-drug resistance (MDR) efflux pumps, are capable of exporting a wide variety of structurally dissimilar drugs and so can render a bacterium resistant to a very large number of different antibiotics in a single step.

effective and very expensive antibiotic *vancomycin*, for example, has been used as a treatment of last resort for many severe hospital-acquired bacterial infections that are already resistant to most other known antibiotics. Vancomycin prevents one step in bacterial cell wall biosynthesis, by binding to part of the growing peptidoglycan chain and preventing it from being cross-linked to other chains (see Figure 24-4). Resistance can arise if the bacterium synthesizes a different type of cell wall, using different subunits that do not bind vancomycin. The most effective form of vancomycin resistance depends on a transposon containing seven genes, the products of which work together to sense the presence of vancomycin, shut down the normal pathway for bacterial cell wall synthesis, and generate a different type of cell wall. Although the joining of these genes into a single transposon must have been a difficult evolutionary step (it took 15 years for vancomycin resistance to develop, rather than the typical year or two), the transposon can now be readily transmitted to many other pathogenic bacterial species.

Where do drug-resistance genes come from? Sometimes, when the bacteria are under selective pressure due to drug exposure, resistance genes arise by spontaneous mutation and expand within a population. In many cases, however, they appear in a pathogen's genome as new DNA segments acquired by horizontal transfer, frequently carried on transposons or replicative plasmids. Unlike eucaryotic cells, bacteria commonly exchange genetic material across species boundaries.

Drug-resistance genes acquired by horizontal transfer frequently seem to come from environmental reservoirs, where they play an important part in the competition between microorganisms. Nearly all antibiotics used to treat bacterial infections today are not synthetic creations of chemists; instead, most are natural products produced by fungi or bacteria: penicillin, for example, is made by the mold *Penicillium*, and more than 50% of the antibiotics currently used in the clinic are made by the Gram-positive genus *Streptomyces*. It is believed that these microorganisms produce antimicrobial compounds as weapons in their competition with other microorganisms in the environment. Many of these compounds have probably existed on Earth for at least hundreds of millions of years, which is ample time for other microorganisms, as well as those that produce the antibiotics themselves, to have evolved resistance mechanisms. Unbiased surveys of bacteria taken from soil samples that have never been deliberately exposed to antibiotic drugs reveal that the bacteria are typically already resistant to about seven or eight of the antibiotics widely used in clinical practice. When pathogenic microorganisms are faced with the selective pressure provided by antibiotics treatments, they can apparently draw upon this world-wide and essentially inexhaustible source of genetic material to acquire resistance.

Like most other aspects of infectious disease, human behavior has exacerbated the problem of drug resistance. Many patients choose to take antibiotics

for viral conditions such as influenza, colds, sore throats, and earaches that the drugs do not help. Persistent and chronic misuse of antibiotics in this way can eventually result in antibiotic-resistant normal flora, which can then transfer the resistance to pathogens. Several antibiotic-resistant outbreaks of infectious diarrhea caused by *Shigella flexneri*, for example, originated in this way. The problem is particularly severe in countries where antibiotics are available without a physician's prescription, as in Brazil, where more than 80% of the strains of *S. flexneri* found in infected patients are resistant to four or more antibiotics. Antibiotics are also misused in agriculture, where they are commonly employed as food additives to promote the health of farm animals. An antibiotic closely related to vancomycin was commonly added to cattle feed in Europe; the resulting resistance in the normal flora of these animals is widely believed to be one of the original sources for vancomycin-resistant bacteria that now threaten the lives of hospitalized patients.

Because the acquisition of drug resistance is almost inevitable, it is crucial that we continue to develop innovative novel treatments for infectious diseases. We must also try to do better at delaying the onset of drug resistance.

Summary

All pathogens share the ability to interact with host cells in various ways that promote replication and spread of the pathogen, but these host–pathogen interactions are diverse. Pathogens often colonize the host by adhering to or invading the epithelial surfaces that line the respiratory, gastrointestinal, and urinary tracts, as well as the other surfaces in direct contact with the environment. Intracellular pathogens, including all viruses and many bacteria and protozoa, invade host cells and replicate inside them. They invade by one of several mechanisms. Viruses rely largely on receptor-mediated endocytosis, whereas bacteria exploit cell adhesion and phagocytic pathways; in both cases, the host cell provides the machinery and energy. Protozoa, by contrast, employ unique invasion strategies that usually require significant metabolic expense on the part of the invader. Once inside, intracellular pathogens seek out a cell compartment that is favorable for their replication, frequently altering host membrane traffic and exploiting the host cell cytoskeleton for intracellular movement. Besides altering the behavior of individual host cells, pathogens frequently alter the behavior of the host organism in ways that favor spread to a new host. Pathogens evolve rapidly, so that new infectious diseases frequently emerge, and old pathogens acquire new ways to evade our attempts at treatment, prevention, and eradication.

BARRIERS TO INFECTION AND THE INNATE IMMUNE SYSTEM

Humans are exposed to millions of potential pathogens daily, through contact, ingestion, and inhalation. Our ability to avoid infection depends in large part on our adaptive immune system (discussed in Chapter 25), which remembers previous encounters with specific pathogens and specifically destroys or eliminates them when they attack again. Adaptive immune responses, however, are slow to develop on first exposure to a new pathogen, as specific clones of B and T cells that can respond to it have to become activated and proliferate; it can therefore take a week or so before the responses are effective. By contrast, a single bacterium with a doubling time of 1 hour can produce almost 20 million progeny, a full-blown infection, in a single day. Therefore, during the first critical hours and days of exposure to a new pathogen, we rely on our **innate immune system** to protect us from infection. As we discuss in Chapter 25, we also rely on the innate immune system to help activate adaptive immune responses.

Innate immune responses are not specific to a particular pathogen in the way that the adaptive immune responses are. Generally, there are three lines of innate immune defenses that can prevent an infection or stop it in its tracks before the adaptive immune system needs be called into play. The first of these

are the physical and chemical barriers that prevent easy access of microorganisms to the interior of the human body. These include the thick layer of dead keratinized cells that forms the surface of our skin, the tight junctions between epithelial cells, the acidic pH of the stomach, and components of the mucus layers that inhibit colonization or even kill pathogenic bacteria. The normal flora also have a role in protecting body surfaces against invaders by competing for the same ecological niche and thereby limiting colonization.

The second line of innate defenses comprise cell-intrinsic responses, by which an individual cell recognizes that it has been infected and takes measures to kill or cripple the invader. Most cells that have taken up a bacterium by pathogen-induced phagocytosis (see Figure 24–26), for example, will immediately direct the phagosome to fuse with a lysosome, exposing the invading microorganism to a barrage of digestive enzymes. Another ancient intrinsic defense mechanism is the ability of host cells to degrade double-stranded RNA, which is a common intermediate in viral replication; the infected cells will even degrade any single-stranded RNA that shares sequence identity with the double-stranded trigger. This mechanism not only serves as an effective intrinsic defense against many viral infections, it also enables cell biologists to manipulate gene expression by using the technique of RNA interference (RNAi).

The third line of innate immune defenses depends on a specialized set of proteins and phagocytic cells that recognize conserved features of pathogens and become quickly activated to help destroy invaders. These include professional phagocytic cells such as neutrophils and macrophages, natural killer cells, and the complement system. Whereas the adaptive immune system arose in evolution less than 500 million years ago and is confined to vertebrates, innate immune responses operate in both vertebrates and invertebrates, as well as in plants, and the basic mechanisms that regulate them are similar in these organisms. As discussed in Chapter 25, the innate immune responses in vertebrates are also required to activate adaptive immune responses by producing extracellular signal molecules that help call the adaptive immune system into action.

Epithelial Surfaces and Defensins Help Prevent Infection

In vertebrates, the skin and other epithelial surfaces, including those lining the respiratory, intestinal, and urinary tracts (Figure 24–46), provide a physical barrier between the inside of the body and the outside world. A mucus layer provides additional protection against microbial, mechanical, and chemical insults of the interior epithelial surfaces; many amphibians and fish also have a mucus layer covering their skin. The slimy mucus coating is made primarily of secreted mucin and other glycoproteins, and it physically helps prevent pathogens from adhering to the epithelium. It also facilitates the clearance of pathogens by beating cilia on the epithelial cells (discussed in Chapter 23).

The mucus layer also contains substances that either kill pathogens or inhibit their proliferation. Among the most abundant of these are antimicrobial peptides, called **defensins**, which are found in all animals and plants. They are generally short (12–50 amino acids) and positively charged, and have hydrophobic or amphipathic domains. They constitute a diverse family with a broad spectrum of antimicrobial activities, including the ability to kill or inactivate Gram-negative and Gram-positive bacteria, fungi (including yeasts), parasites (including protozoa and nematodes), and even enveloped viruses such as HIV. Defensins are also the most abundant proteins in neutrophils (see below), which use them to kill phagocytosed pathogens.

It is still uncertain how defensins kill pathogens. One possibility is that they use their hydrophobic or amphipathic domains to insert into the surface membrane of their victims, thereby disrupting the integrity of the membrane. Some of their selectivity for pathogens over host cells may come from their preference for membranes that do not contain cholesterol. After disrupting the membrane of the pathogen, the positively charged peptides may also interact with various negatively charged targets within the microbe, including DNA. Because of the relatively nonspecific nature of the interaction between antimicrobial peptides

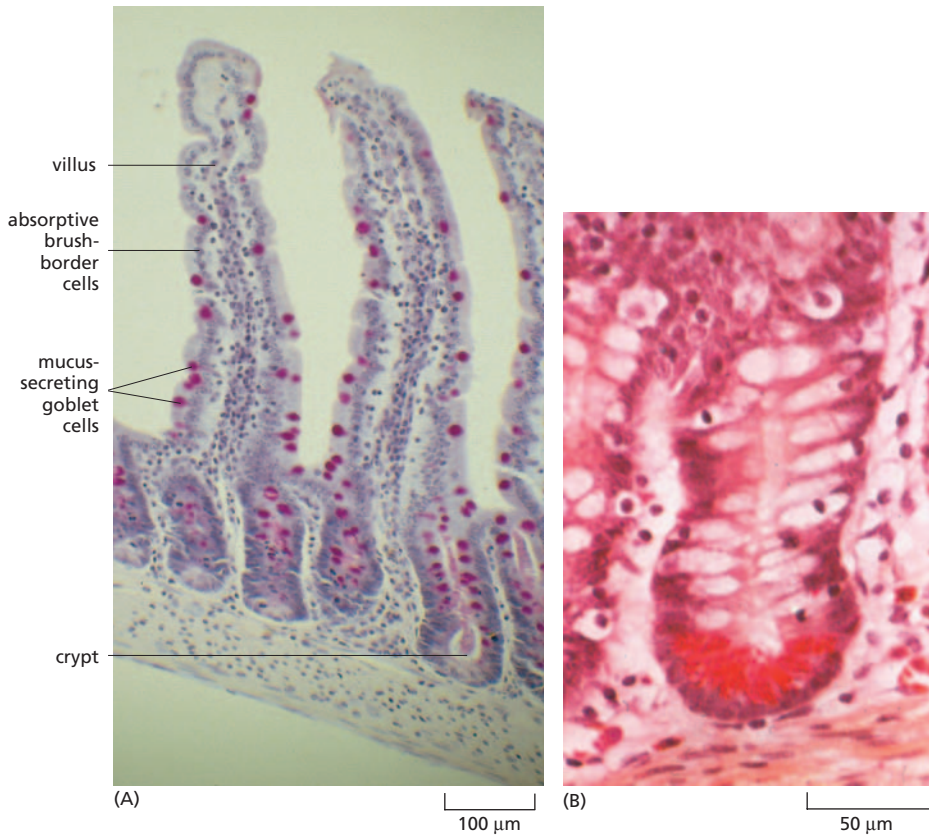


Figure 24-46 Epithelial defenses against microbial invasion. (A) Light micrograph of a cross section through the wall of the human small intestine, showing three villi. Goblet cells secreting mucus are stained *magenta*. The protective mucus layer covers the exposed surfaces of the villi. At the base of the villi lie the *crypts*, where the epithelial cells proliferate. (B) Close-up view of a crypt, stained to show the granules in the Paneth cells (*scarlet*). These cells secrete large quantities of defensins and other antimicrobial peptides into the intestinal lumen. (B, courtesy of H.G. Burkitt, from P.R. Wheater, *Functional Histology*, 2nd ed. London: Churchill-Livingstone, 1987.)

and the microbes they kill, it is difficult for pathogens to acquire resistance to them. Thus, in principle, defensins and other antimicrobial peptides might be useful therapeutic agents to combat infection, either alone or in combination with more traditional drugs.

Human Cells Recognize Conserved Features of Pathogens

Microorganisms do occasionally breach the epithelial barricades. It is then up to the innate and adaptive immune systems to recognize and destroy them, without harming the host. Consequently, the immune systems must be able to distinguish self from nonself. We discuss in Chapter 25 how the adaptive immune system does this. The innate immune system relies on the recognition of particular types of molecules that are common to many pathogens but are absent in the host. These pathogen-associated molecules (called *pathogen-associated* or *microbe-associated immunostimulants*) trigger two types of innate immune responses—*inflammatory responses* (discussed below) and phagocytosis by professional phagocytes (neutrophils and macrophages), and by *dendritic cells*, which activate T cells of the adaptive immune system (discussed in Chapter 25). Both the inflammatory and phagocytic responses can occur quickly, even if the host has never been previously exposed to a particular pathogen.

The **microbe-associated immunostimulants** are of various types. Most are not exclusive to pathogens, but are found in many bacteria, benign as well as harmful. Bacterial translation initiation differs from eucaryotic translation initiation in that *formylated methionine*, rather than regular methionine, is generally used as the first amino acid. Therefore, any peptide containing formylmethionine at the N-terminus must be of bacterial origin. Formylmethionine-containing peptides act as very potent chemoattractants for neutrophils, which migrate quickly to the source of such peptides and engulf the bacteria producing them (see Figure 16-101).

In addition, molecules that do not occur in multicellular hosts compose the outer surface of many microorganisms, and these molecules also act as

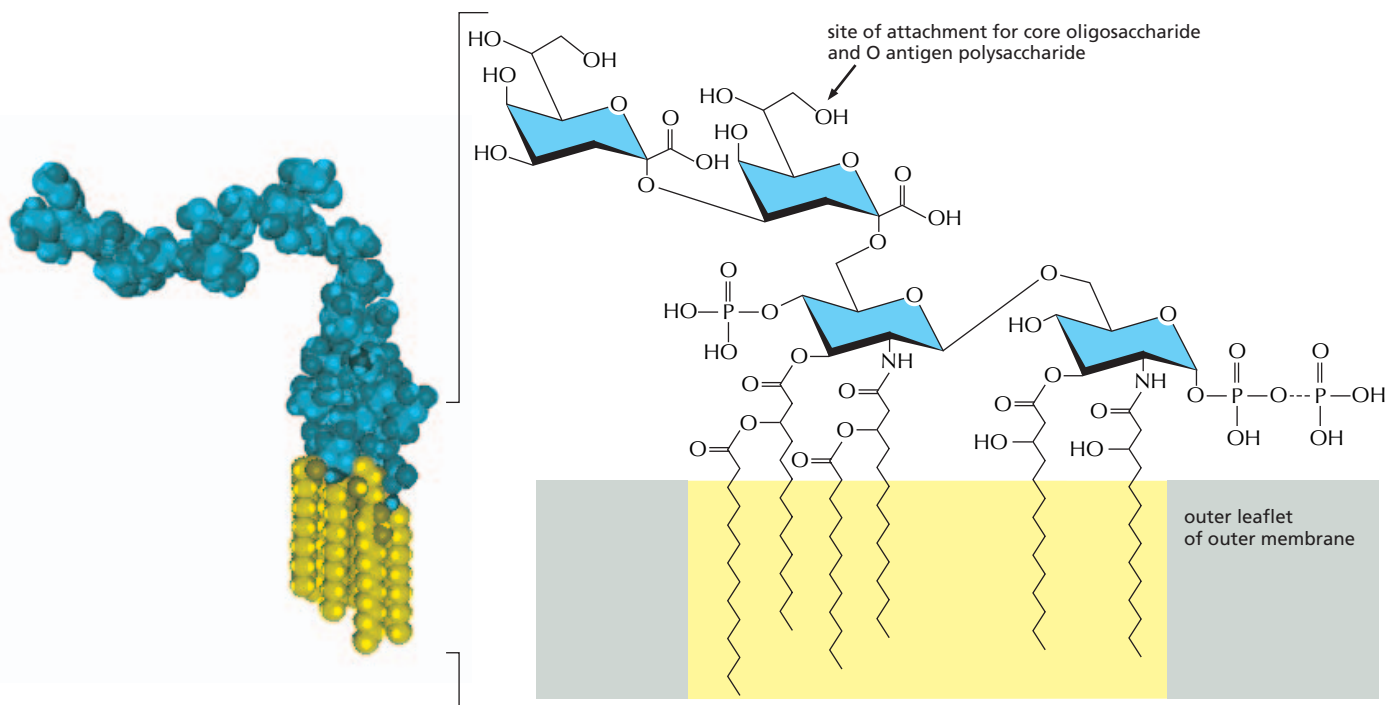


Figure 24-47 Structure of lipopolysaccharide (LPS). On the left is the three-dimensional structure of a molecule of LPS, with the fatty acid tails shown in yellow and the sugars in blue. The molecular structure of the bottom part of LPS is shown on the right. The membrane anchor is made up of two linked glucosamine sugars attached to three phosphates and six fatty acid tails. Attached to this basic structure is a long chain of sugars, which is usually highly branched. This drawing shows the simplest type of LPS that will allow *E. coli* to live; it has just two sugar molecules in the chain, and both are the same (3-deoxy-D-manno-octulosonic acid). At the position marked by the arrow, wild-type Gram-negative bacteria also attach a *core oligosaccharide* made up of 8–12 linked sugars and a long *O antigen polysaccharide* made up of an oligosaccharide unit that is repeated up to 40 times. The sugars making up the core oligosaccharide and O antigen polysaccharide vary from one bacterial species to another, and even among different strains of the same species. All forms of LPS stimulate strong innate immune responses.

immunostimulants. They include the peptidoglycan cell wall and flagella of bacteria, as well as lipopolysaccharide (LPS) on Gram-negative bacteria (Figure 24-47) and teichoic acids on Gram-positive bacteria (see Figure 24-4B). They also include molecules in the cell walls of fungi, including mannan, glucan, and chitin. Many eucaryotic parasites also contain unique membrane components that act as immunostimulants, including glycosylphosphatidylinositol in *Plasmodium*. In order to avoid making unnecessary immune responses, the host must be able to distinguish between microbe-associated immunostimulants produced by pathogens and very similar or identical molecules released from normal flora. In many cases, differences in concentration of the immunostimulant may be sufficient; chronic low concentrations of the molecule may be monitored by the immune system but do not provoke a reaction, while sudden increases in concentration or the appearance of immunostimulants in normally sterile areas of the body will trigger an innate immune response.

Short sequences in bacterial or viral DNA can also act as immunostimulants. The culprit is a “CpG motif,” which consists of the unmethylated dinucleotide CpG flanked by two 5′ purine residues and two 3′ pyrimidines. This short sequence is at least 20 times less common in vertebrate DNA than in bacterial or viral DNA, and it can activate innate immune responses.

The various classes of microbe-associated immunostimulants often occur in repeating patterns and are therefore often called *pathogen-associated molecular patterns (PAMPs)*. Several types of dedicated receptors in the host, collectively called **pattern recognition receptors**, recognize these patterns. These receptors include soluble receptors in the blood (components of the *complement system*, which we discuss below) and membrane-bound receptors on or in host cells (including members of the *Toll-like receptor family*, which we consider

later). The cell-associated receptors have two functions: they initiate the phagocytosis of the pathogen, and they activate a program of gene expression in the host cell responsible for innate immune responses. Some of the complement components also aid in phagocytosis and, in some cases, the direct killing of the pathogen, as we now discuss.

Complement Activation Targets Pathogens for Phagocytosis or Lysis

The **complement system** consists of about 20 interacting soluble proteins that are made mainly by the liver and circulate in the blood and extracellular fluid. Most are inactive until an infection activates them. They were originally identified by their ability to amplify and “complement” the action of antibodies, but some complement components are also pattern recognition receptors that microbe-associated immunostimulants activate directly.

The *early complement components* are activated first. There are three sets of these, belonging to three distinct pathways of complement activation—the *classical pathway*, the *lectin pathway*, and the *alternative pathway*. The early components of all three pathways act locally to activate C3, which is the pivotal component of complement (Figure 24–48). Individuals with a C3 deficiency are subject to repeated bacterial infections. The early components are proenzymes, which are activated sequentially by proteolytic cleavage. The cleavage of each proenzyme in the series activates the next component to generate a serine protease, which cleaves the next proenzyme in the series, and so on. Since each activated enzyme cleaves many molecules of the next proenzyme in the chain, the activation of the early components consists of an amplifying, *proteolytic cascade*.

Many of these cleavages liberate a biologically active small peptide fragment that can attract phagocytic cells such as neutrophils, and a membrane-binding larger fragment. The binding of the large fragment to a cell membrane, usually the surface of a pathogen, helps to carry out the next reaction in the sequence. In this way, complement activation is confined largely to the particular cell surface where it began. The larger fragment of C3, called C3b, binds covalently to the surface of the pathogen, where it recruits fragments of cleaved C2 and C3 to form a proteolytic complex (C4b, C2b, C3b) that catalyzes the subsequent steps in the complement cascade. Specific receptors on phagocytic cells that enhance the ability of these cells to phagocytose the pathogen also recognize C3b. In addition, receptors on B cells recognize C3b, which is the reason that C3b-coated pathogens are especially efficient at stimulating B cells to make

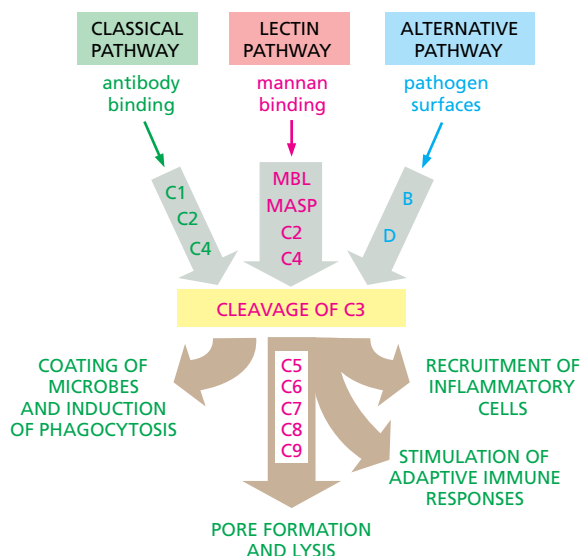


Figure 24–48 The principal stages in complement activation by the classical, lectin, and alternative pathways. In all three pathways, the reactions of complement activation usually take place on the surface of an invading microbe, such as a bacterium, and lead to the cleavage of C3. C1–C9, mannose-binding lectin (MBL), MBL-associated serine protease (MASP), and factors B and D are the central components of the complement system; various other components regulate the system. The early components are shown within gray arrows, while the late components are shown within a brown arrow.

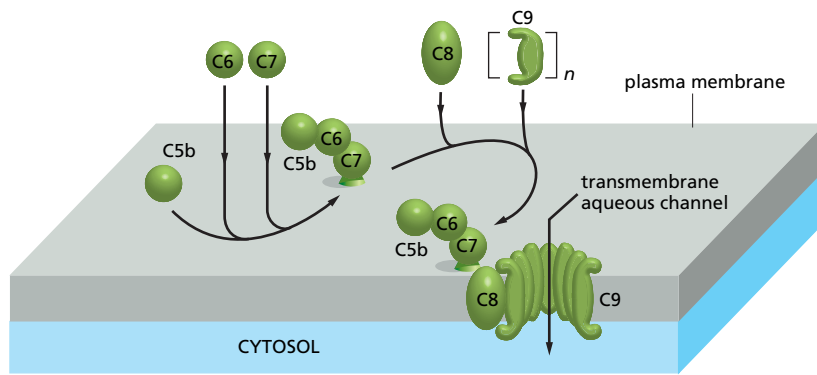


Figure 24–49 Assembly of the late complement components to form a membrane attack complex. When C3b is produced by any of the three complement activation pathways, it is immobilized on a membrane, where it recruits C4b and C2b to form a proteolytic complex. This complex then cleaves the first of the late components, C5, to produce C5a (not shown) and C5b. C5b remains loosely bound to C3b (not shown) and rapidly assembles with C6 and C7 to form C567, which then binds firmly via C7 to the membrane, as illustrated. To this complex is added one molecule of C8 to form C5678. The binding of a molecule of C9 to C5678 induces a conformational change in C9 that exposes a hydrophobic region and causes C9 to insert into the lipid bilayer of the target cell. This starts a chain reaction in which the altered C9 binds a second molecule of C9, which can then bind another molecule of C9, and so on. In this way, a chain of C9 molecules forms a large transmembrane channel in the membrane.

antibodies (discussed in Chapter 25). The smaller fragment of C3 (called C3a), as well as fragments of C4 and C5 (see Figure 24–48), act independently as diffusible signals to promote an inflammatory response by recruiting phagocytes and lymphocytes to the site of infection.

IgG or IgM antibody molecules (discussed in Chapter 25) bound to the surface of a microbe activate the **classical pathway**. **Mannan-binding lectin**, the protein that initiates the second pathway of complement activation, is a serum protein that forms clusters of six carbohydrate-binding heads around a central collagen-like stalk. This assembly binds specifically to mannose and fucose residues in bacterial cell walls that have the correct spacing and orientation to match up perfectly with the six carbohydrate-binding sites, providing a good example of a pattern recognition receptor. These initial binding events in the classical and lectin pathways cause the recruitment and activation of the early complement components. Molecules on the surface of pathogens often activate the **alternative pathway**; activation of the classical or lectin pathways also activates the alternative pathway, forming a positive feedback loop that amplifies the effects of the classical or lectin pathway.

Host cells produce various proteins and surface modifications that prevent the complement reaction from proceeding on their cell surface. The most important of these is the carbohydrate moiety sialic acid, a common constituent of cell surface glycoproteins and glycolipids. Because pathogens generally lack these surface components, they are singled out for destruction, while host cells are spared. At least one pathogen, *Neisseria gonorrhoeae*, the bacterium that causes the sexually transmitted disease gonorrhea, has developed the ability to exploit this feature of host self-protection. Coating itself with a layer of sialic acid, it effectively hides from the complement activation system.

Membrane-immobilized C3b, produced by any of the three pathways, triggers a further cascade of reactions that leads to the assembly of the late complement components to form *membrane attack complexes* (Figure 24–49). These complexes assemble in the pathogen membrane near the site of C3 activation and have a characteristic appearance in negatively stained electron micrographs, where they are seen to form aqueous pores through the membrane (Figure 24–50). For this reason, and because they perturb the structure of the bilayer in their vicinity, they make the membrane leaky and can, in some cases, cause the microbial cell to lyse, much like the defensins mentioned earlier.

The self-amplifying, inflammatory, and destructive properties of the complement cascade make it essential that key activated components be rapidly inactivated after they are generated to ensure that the attack does not spread to nearby host cells. Inactivation is achieved in at least two ways. First, specific inhibitor proteins in the blood or on the surface of host cells terminate the cascade, by either binding or cleaving certain components once they have been activated by proteolytic cleavage. Second, many of the activated components in the cascade are unstable; unless they bind immediately to either another appropriate complement component in the cascade or to a nearby membrane, they rapidly become inactive.

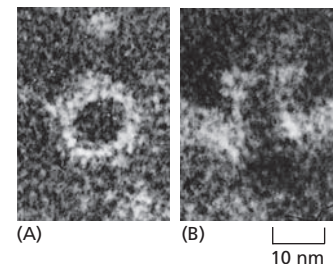


Figure 24–50 Electron micrographs of negatively stained complement lesions in the plasma membrane of a red blood cell. The lesion in (A) is seen *en face*, while that in (B) is seen from the side as an apparent transmembrane channel. The negative stain fills the channels, which therefore look black. This red blood cell has been deliberately sensitized to be susceptible to lysis by complement. (From R. Dourmashkin, *Immunology* 35:205–212, 1978. With permission from Blackwell Publishing.)

Toll-like Proteins and NOD Proteins Are an Ancient Family of Pattern Recognition Receptors

Many of the mammalian pattern recognition receptors responsible for triggering innate immune responses to pathogens are members of the **Toll-like receptor (TLR)** family. *Drosophila* Toll is a transmembrane protein with a large extracellular domain consisting of a series of leucine-rich repeats (see Figure 15–82). It was originally identified as a protein involved in the establishment of dorsoventral polarity in developing fly embryos (discussed in Chapter 22). It is also involved, however, in the adult fly's resistance to fungal infections. The intracellular signal transduction pathway activated downstream of Toll when a fly is exposed to a pathogenic fungus leads to the translocation of the NF κ B protein (discussed in Chapter 15) into the nucleus, where it activates the transcription of various genes, including those encoding antifungal defensins. The leucine-rich repeats found in Toll and TLRs are versatile structural motifs that are useful for binding a wide variety of ligands. Besides their role in pathogen recognition in both animals and plants, proteins with leucine-rich repeats have roles in signal transduction, DNA repair, and cell–cell and cell–matrix adhesion.

Humans have at least 10 TLRs, several of which play important parts in recognizing microbe-associated immunostimulants made by bacteria, viruses, fungi, and parasites. Different ligands activate different TLRs: TLR4, for example, recognizes lipopolysaccharide (LPS) from the outer membrane of Gram-negative bacteria, TLR9 recognizes CpG DNA, and TLR5 recognizes the protein that makes up the bacterial flagellum. Most TLRs are on cell surfaces; they are abundant, for instance, on the surface of macrophages, dendritic cells, and neutrophils, as well as on the surface of epithelial cells lining the respiratory and intestinal tracts. Others, however, are associated with intracellular membranes, where they can detect intracellular pathogens. TLRs act as an alarm system to alert both the innate and adaptive immune systems that an infection is brewing. In mammals, they activate a variety of intracellular signaling pathways, which in turn stimulate the transcription of hundreds of genes, especially those that promote inflammatory responses (discussed later) and help induce adaptive immune responses (Figure 25–51).

A second family of pattern recognition receptors is exclusively intracellular. They are called **NOD proteins** and also have leucine-rich repeat motifs. They are also functionally similar to TLRs but recognize a distinct set of ligands, including

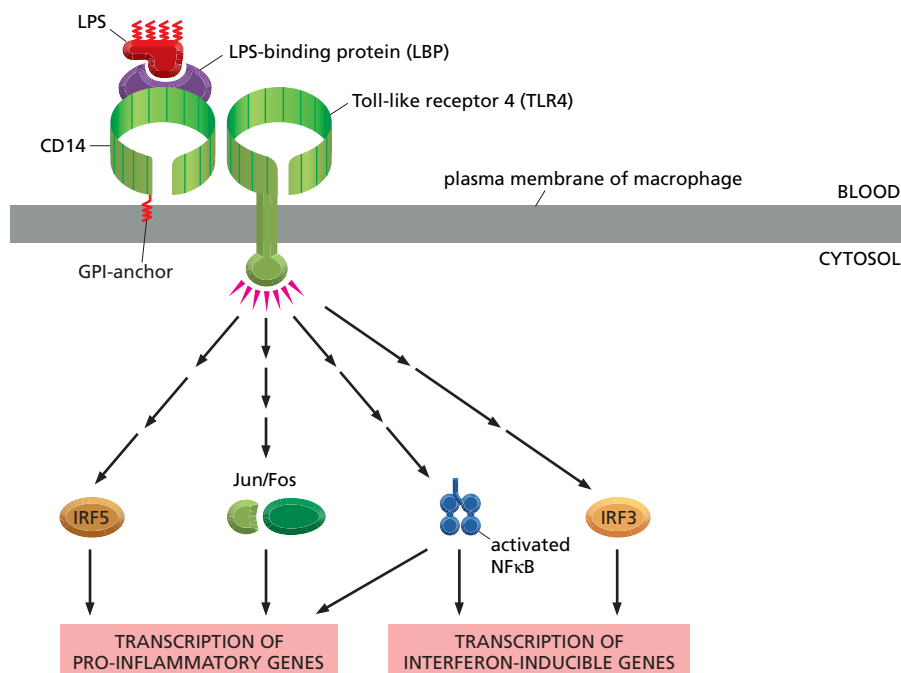


Figure 24–51 The activation of a macrophage by lipopolysaccharide (LPS). LPS binds to LPS-binding protein (LBP) in the blood, and the complex binds to the GPI-anchored protein CD14 on the macrophage surface. The ternary complex then activates Toll-like receptor 4 (TLR4), which activates multiple, downstream, intracellular signaling pathways. As a result, at least four gene regulatory proteins are activated, including NF κ B, an AP1 complex of Jun and Fos, and two interferon regulatory factors, IRF3 and IRF5. This strong, multifaceted transcriptional response results in the production of interferons and pro-inflammatory cytokines, including chemokines that recruit various white blood cells to the site of macrophage activation, reflecting the significant danger that a macrophage perceives when it encounters a high concentration of LPS.



Figure 24-52 Microbial disease in a plant. The tomato leaves shown here are infected with the leaf mold fungus *Cladosporium fulvum*. Resistance to this type of infection depends on recognition of a fungal protein by host cell receptors that are structurally related to TLRs. (Courtesy of Jonathan Jones.)

bacterial cell wall components. The different allelic forms of NODs and TLRs that an individual expresses play an important part in influencing their susceptibility to certain infectious diseases; particular polymorphisms in TLR4 and TLR5, for example, correlate with susceptibility to *Legionella pneumophila*, and members of families that express a particular allele of NOD2 have a greatly increased chance of suffering from Crohn's disease, a chronic inflammatory disease of the small intestine, which is thought to be triggered by bacterial infection.

Proteins related to Toll, TLRs, and NODs are apparently involved in innate immunity in all multicellular organisms. In plants, proteins with leucine-rich repeats and with domains homologous to the cytosolic portion of TLRs are required for resistance to fungal, bacterial, and viral pathogens (Figure 24-52). Thus, at least two families of proteins that function in innate immunity—the defensins and the TLR/NOD families—seem to be evolutionarily very ancient, perhaps predating the split between animals and plants over a billion years ago. Their conservation during evolution underlines the importance of innate immune responses in the defense against microbial pathogens.

Phagocytic Cells Seek, Engulf, and Destroy Pathogens

In all animals, invertebrate as well as vertebrate, the recognition of a microbial invader is usually quickly followed by its engulfment by a phagocytic cell. Plants, however, lack this type of innate immune response. In vertebrates, *macrophages* are professional phagocytes that reside in tissues throughout the body and are especially abundant in areas where infections are likely to arise, including the respiratory and intestinal tracts, for example. They are also present in large numbers in connective tissues, in the liver and spleen. These long-lived cells patrol the tissues and are among the first cells to encounter invading microbes. *Neutrophils* are the second major type of professional phagocytic cells in vertebrates. In contrast to macrophages, they are short-lived cells, abundant in blood but not present in normal, healthy tissues. They are rapidly recruited to sites of infection by activated macrophages, by molecules such as formylmethionine-containing peptides that the microbes themselves release, and by peptide fragments of cleaved complement components. Neutrophils can detect complement-derived chemoattractants at concentrations as low as 10^{-11} M.

Macrophages and neutrophils display a variety of cell-surface receptors that enable them to recognize and engulf pathogens. <TGTA> These include pattern recognition receptors such as TLRs, receptors for antibodies produced by the adaptive immune system, and receptors for the C3b component of complement. Binding to any of these receptors induces actin polymerization at the site of pathogen attachment, causing the phagocyte's plasma membrane to surround the pathogen and engulf it in a large membrane-enclosed phagosome (Figure 24-53). Although some bacteria can actually actively induce a host cell such as an epithelial cell to phagocytose them as a mechanism for invading the cell (see

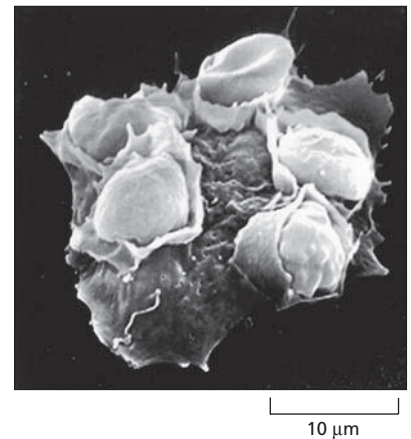


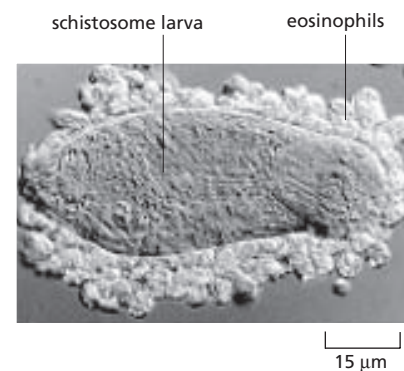
Figure 24-53 Phagocytosis. This scanning electron micrograph shows a macrophage in the midst of ingesting five red blood cells that have been coated with an antibody against a surface glycoprotein. (From E.S. Gold et al., *J. Exp. Med.* 190:1849–1856, 1999. With permission from The Rockefeller University Press.)

Figure 24–25), phagocytosis by a macrophage or neutrophil generally leads to the ingested pathogen's death. Unsurprisingly, some pathogens use specific mechanisms to avoid phagocytosis by macrophages or neutrophils. One strategy is to secrete a thick, slimy layer of polysaccharides, called a *capsule*, which blocks access of complement components to the bacterial surface and also makes it physically difficult for the phagocytic cell to bind to and engulf the bacterium. Another strategy, used by *Yersinia pestis* (the causative bacterium of plague), for example, is to deliver a toxin into the macrophage via a type III secretory system (see Figure 24–8) that disrupts the assembly of the actin cytoskeleton and thereby prevents phagocytosis.

When a macrophage or neutrophil engulfs a pathogen, it unleashes an impressive armory of weapons to kill it. Exposure to both microbe-associated immunostimulants and chemical signals produced by the immune response to the pathogen enhances the phagocytic and killing power of the phagocytes. This exposure is said to “activate” the phagocyte, putting it in a state of high alert, in which not only is it more effective at phagocytosing and killing pathogens, but it also releases cytokines to attract more white blood cells to the site of infection. The location of the phagocyte's weaponry is readily visible in the light or electron microscope as dense membrane-enclosed organelles called *granules*. These specialized lysosomal derivatives fuse with the phagosomes, delivering enzymes such as lysozyme and acid hydrolases that can degrade the pathogen's cell wall and proteins. The granules also contain defensins, the antimicrobial peptides that make up about 15% of the total protein in neutrophils. In addition, the phagocyte assembles *NADPH oxidase complexes* on the phagolysosomal membrane, which catalyze the production of highly toxic oxygen-derived compounds, including superoxide (O_2^-), hypochlorite (HOCl, the active ingredient in bleach), hydrogen peroxide, and hydroxyl radicals. A transient increase in oxygen consumption by the phagocytic cells, called the *respiratory burst*, accompanies the production of these toxic compounds. It is not only these highly reactive oxygen-derived compounds that damage the pathogen trapped within the phagolysosome. The action of NADPH oxidase transports electrons into the phagolysosome and induces a compensatory movement of K^+ along with the electron, which has the net effect of raising the pH. The high pH in the phagolysosome activates a group of potent neutral proteases, which the low pH of the lysosomal granule kept inactive prior to fusion with the phagosome. The neutral proteases quickly destroy the hapless pathogens trapped in the phagolysosome. Whereas macrophages generally survive this killing frenzy and live to kill again, neutrophils usually do not. Dead and dying neutrophils are a major component of the pus that forms in acutely infected wounds. The distinctive greenish tint of pus is due to the abundance in neutrophils of the copper-containing enzyme myeloperoxidase, which is one of the components active in the respiratory burst.

If a pathogen is too large to be successfully phagocytosed (if it is a large parasite such as a nematode, for example), a group of macrophages, neutrophils, or eosinophils (discussed in Chapter 23) will gather around the invader. They secrete their defensins and other bactericidal products contained in their granules by exocytosis, and they also release the toxic products of the respiratory burst (Figure 24–54). This barrage is generally sufficient to destroy the pathogen. In some cases, neutrophils have been observed to eject large parts of their chromatin along with the contents of their granules. The ejected DNA, with its attached histones, form a sticky web that entraps nearby bacteria, preventing their escape (Figure 24–55). Because its sole function is to sacrifice itself to kill invading pathogens, a neutrophil has no hesitation in using every tool available, including its own DNA, to accomplish this task.

Figure 24–54 Eosinophils attacking a schistosome larva. Phagocytes cannot ingest large parasites such as the schistosome larva shown here. When the larva is coated with antibody or complement, however, eosinophils (and other white blood cells) can recognize it and collectively kill it by secreting various toxic molecules. (Courtesy of Anthony Butterworth.)



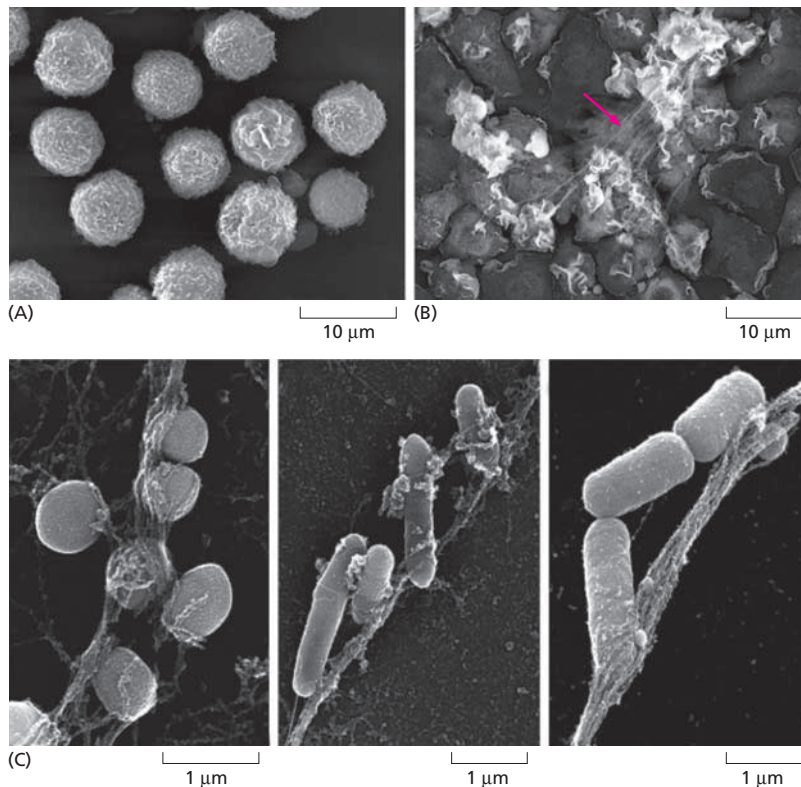


Figure 24-55 Neutrophils eject their chromatin to trap bacteria in a sticky web. (A) Scanning electron micrograph showing resting neutrophils. (B) Activated neutrophils have multiple surface ruffles and are associated with fibrous strands (*arrow*). The strands contain DNA and histones and appear to be chromatin from the neutrophil nucleus that has been ejected during the process of activation. (C) The sticky chromatin can entrap many different kinds of bacteria, including (left to right) *Staphylococcus aureus*, *Salmonella enterica*, and *Shigella flexneri*. (From V. Brinkmann et al., *Science* 303:1532–1535, 2004. With permission from AAAS.)

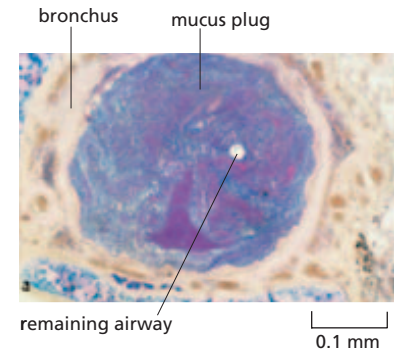
Activated Macrophages Contribute to the Inflammatory Response at Sites of Infection

When a pathogen invades a tissue, it almost always elicits an **inflammatory response**. Changes in local blood vessels cause a response characterized by pain, redness, heat, and swelling at the site of infection (physicians have recognized these four signs of inflammation, in Latin *dolor*, *rubor*, *calor*, and *turgor*, for thousands of years). The blood vessels dilate and become permeable to fluid and proteins, leading to local swelling and an accumulation of blood proteins that aid in defense, including components of the complement cascade. At the same time, the endothelial cells lining the local blood vessels are stimulated to express cell adhesion proteins (discussed in Chapter 19) that facilitate the attachment and extravasation of white blood cells, initially neutrophils, followed later by lymphocytes and monocytes (the blood-borne precursors of macrophages). <CCCC>

Whereas neutrophils usually die at the site of inflammation, macrophages frequently survive their initial encounter with invading pathogens and can migrate to other parts of the body. Pathogens that can survive within the macrophage, such as the bacterium *Salmonella enterica* serovar *Typhi*, for example, can use the macrophages as a transport system to spread a localized infection to distant sites in the body, converting a minor local invasion event in the gut into a severe systemic disease, typhoid fever.

Various signaling molecules mediate the inflammatory response at the site of an infection. Activation of TLRs results in the production of both lipid signaling molecules, such as prostaglandins, and protein (or peptide) signaling molecules, such as cytokines (discussed in Chapter 15), all of which contribute to the inflammatory response, as do the complement fragments released during complement activation. Some of the cytokines produced by activated macrophages are chemoattractants (called *chemokines*). Some of these attract neutrophils, which are the first cells recruited in large numbers to the site of a new infection. Other cytokines trigger *fever*, a rise in body temperature. On balance, fever helps fight

Figure 24–56 Inflammation of the airways in severe asthma. Light micrograph of a section through a bronchus of a patient who died during a prolonged and severe asthmatic attack. There is almost total occlusion of the airway by a mucus plug. The mucus plug is a dense inflammatory infiltrate that includes eosinophils, neutrophils, and lymphocytes. (Courtesy of Thomas Krausz.)



infection, since most bacterial and viral pathogens proliferate better at lower temperatures, whereas adaptive immune responses are more potent at higher temperatures. Still other proinflammatory signaling molecules stimulate endothelial cells to express proteins that trigger blood clotting in local small vessels. By occluding the vessels and cutting off blood flow, this response can help prevent the pathogen from entering the bloodstream and spreading the infection to other parts of the body.

The same inflammatory responses that help control local infections, however, can have disastrous consequences when they occur in response to a disseminated infection in the bloodstream, a condition called *sepsis*. The systemic release of proinflammatory signaling molecules into the blood causes dilation of blood vessels, and loss of plasma volume, which, together, cause a large fall in blood pressure, or *shock*; in addition, there is widespread blood clotting. The end result, known as *septic shock*, is often fatal. Inappropriate or overzealous local inflammatory responses can also contribute to chronic diseases, such as *asthma* (Figure 24–56) and arthritis.

Just as some pathogens have developed mechanisms to avoid the lethal consequences of phagocytosis, so some have acquired mechanisms to either prevent the inflammatory response or, in some cases, take advantage of it to spread the infection. Many viruses, for example, encode potent cytokine antagonists that block aspects of the inflammatory response. Some of these antagonists are simply modified forms of cytokine receptors, encoded by genes that the virus originally acquired from the host genome. They bind the cytokines with high affinity and block their activity. Some bacteria, such as *Salmonella*, induce an inflammatory response in the gut at the initial site of infection, thereby recruiting macrophages and neutrophils that they then invade. In this way, the bacteria hitch a ride to other tissues in the body.

Virus-Infected Cells Take Drastic Measures to Prevent Viral Replication

The microbe-associated immunostimulants on the surface of bacteria and parasites that are so important in eliciting innate immune responses against these pathogens are generally not present on the surface of viruses. Host cell ribosomes construct viral proteins, and host cell lipids form the membranes of enveloped viruses. The only general way that a host cell can recognize the presence of a virus is to detect unusual elements of the viral genome, such as the double-stranded RNA (dsRNA) that is an intermediate in the life cycle of many viruses. DNA virus genomes frequently contain significant amounts of CpG dinucleotide, which can be recognized by the Toll-like receptor TLR9, as discussed earlier.

Mammalian cells are particularly adept at recognizing the presence of dsRNA, and they can mobilize a program of intracellular responses to eliminate it. The program occurs in two steps. First, the cell degrades the dsRNA into small fragments (about 21–25 nucleotide pairs in length), using the enzyme *Dicer*. These double-stranded fragments bind to any single-stranded RNA (ssRNA) in the host cell that has the same sequence as either strand of the dsRNA fragment, leading to the destruction of the ssRNA. This dsRNA-directed ssRNA destruction is the basis of the technique of *RNA interference* (RNAi) that researchers use to destroy specific mRNAs and thereby block specific gene expression (discussed in Chapter 8). Second, the dsRNA induces the host cell to produce and secrete two cytokines—**interferon- α** (IFN α) and **interferon- β** (IFN β), which act in both an

autocrine fashion on the infected cell and a paracrine fashion on uninfected neighbors. The binding of the interferons to their cell-surface receptors stimulates specific gene transcription by the Jak–STAT intracellular signaling pathway (see Figure 15–68), leading to the production of more than 300 gene products, including a large number of cytokines, reflecting the complexity of the cell's acute response to a viral infection.

The interferon response appears to be a general reaction of a mammalian cell to a viral infection, and viral components other than dsRNA can trigger it. In addition to their effects on host cell gene transcription, the interferons activate a latent ribonuclease, which nonspecifically degrades ssRNA. They also indirectly activate a protein kinase that phosphorylates and inactivates the protein synthesis initiation factor eIF-2, thereby shutting down most protein synthesis in the embattled host cell. Apparently, by destroying most of its RNA and transiently halting most of its protein synthesis, the host cell inhibits viral replication without killing itself. If these measures fail, the cell takes the even more extreme step of killing itself by apoptosis to prevent the virus from replicating, often with the help of a killer lymphocyte, as we discuss below and in Chapter 25.

Mammalian cells have a special defense mechanism to help them deal with retroviruses. These viruses activate a family of proteins called APOBEC (named because they are also members of the editing complex that modifies the mRNA for the protein ApoB, which is the major protein component of the low-density lipoprotein, LDL). These enzymes deaminate cytosines in nascent retroviral cDNAs, converting them into uridine and thereby generating large numbers of mutations in the viral genome, leading eventually to the termination of viral replication.

Not surprisingly, many viruses have acquired mechanisms to defeat or avoid these intracellular defense processes. Influenza virus encodes a protein that blocks the recognition of dsRNA by Dicer. HIV encodes a small protein that mediates the ubiquitylation and proteasome-mediated degradation of the APOBEC proteins. Many viruses, including most of those that are able to cause disease in healthy hosts, use various mechanisms to block the activation of the interferon pathway. Some viruses also inhibit host cell apoptosis, which can have the side-effect of promoting the development of cancer; this is one way in which the Epstein–Barr virus occasionally causes Burkitt's lymphoma.

Natural Killer Cells Induce Virus-Infected Cells to Kill Themselves

Interferons have other, less direct ways of blocking viral replication. One of these is to enhance the activity of **natural killer cells (NK cells)**, which are part of the innate immune system. Like cytotoxic T cells of the adaptive immune system (discussed in Chapter 25), NK cells destroy virus-infected cells by inducing the infected cells to kill themselves by undergoing apoptosis. The ways in which cytotoxic T cells and NK cells distinguish virus-infected cells from uninfected cells, however, is different.

Both cytotoxic T cells and NK cells recognize the same special class of cell-surface proteins to detect virus-infected host cells. The proteins are called **class I MHC proteins**, <AAGT> because they are encoded by genes in the major histocompatibility complex; almost all vertebrate cells express these genes, and we discuss them in detail in Chapter 25. Cytotoxic T cells recognize peptide fragments of viral proteins bound to these MHC proteins on the surface of virus-infected cells. By contrast, NK cells monitor the level of class I MHC proteins on the surface of all host cells: high levels inhibit the killing activity of NK cells, so that NK cells selectively kill host cells expressing low levels, which are mainly virus-infected cells and some cancer cells (Figure 24–57).

The reason that class I MHC protein levels are often low on virus-infected cells is that many viruses have developed mechanisms to inhibit the expression of these proteins on the surface of the cells they infect, to avoid detection by cytotoxic T lymphocytes. Adenovirus and HIV, for example, encode proteins that block class I MHC gene transcription. Herpes simplex virus and cytomegalovirus

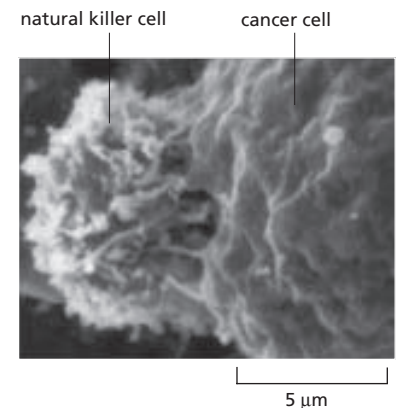


Figure 24–57 A natural killer (NK) cell attacking a cancer cell. The NK cell is the smaller cell on the left. This scanning electron micrograph was taken shortly after the NK cell attached, but before it induced the cancer cell to kill itself. (Courtesy of J.C. Hiserodt, in *Mechanisms of Cytotoxicity by Natural Killer Cells* [R.B. Herberman and D. Callewaert, eds.]. New York: Academic Press, 1995.)

block the peptide translocators in the ER membrane that transport proteasome-derived peptide fragments of viral proteins and host cell proteins from the cytosol into the lumen of the ER; such peptides are required for newly made class I MHC proteins to assemble in the ER membrane and be transported through the Golgi apparatus to the cell surface as peptide–MHC complexes (see Figure 25–59). Cytomegalovirus causes the retrotranslocation of class I MHC proteins from the ER membrane into the cytosol, where they are rapidly degraded in proteasomes. Proteins encoded by still other viruses prevent the delivery of assembled class I MHC proteins from the ER to the Golgi apparatus, or from the Golgi apparatus to the plasma membrane.

By evading recognition by cytotoxic T cells in these ways, however, a virus incurs the wrath of NK cells. The local production of IFN β activates the killing activity of NK cells and also increases the expression of class I MHC proteins in uninfected cells. The cells infected with a virus that blocks class I MHC expression are thereby exposed as being different and become the victims of the activated NK cells. Thus, it is difficult for viruses to hide from both cytotoxic T cells and NK cells simultaneously. Remarkably, however, some large DNA viruses, including cytomegalovirus, encode MHC-like proteins that are expressed on the surface of the host cells they infect. Like *bona fide* class I MHC proteins, these MHC mimics activate inhibitory receptors on NK cells, and block the killing activity of the NK cells.

Both NK cells and cytotoxic T lymphocytes kill infected target cells by inducing them to undergo apoptosis before the virus has had a chance to replicate. It is not surprising, then, that many viruses have evolved mechanisms to inhibit apoptosis, particularly early in infection. As discussed in Chapter 18, apoptosis depends on an intracellular proteolytic cascade, which the cytotoxic cells can trigger, either through the activation of cell-surface death receptors or by injecting a proteolytic enzyme into the target cell (see Figure 24–47). Viral proteins can interfere with nearly every step in these pathways.

Dendritic Cells Provide the Link Between the Innate and Adaptive Immune Systems

Dendritic cells are crucially important cells of the innate immune system that are widely distributed in the tissues and organs of vertebrates. They display a large variety of pattern recognition receptors, including TLRs and NOD proteins, that enable the cells to recognize and phagocytose invading pathogens and to become activated in the process. The dendritic cells cleave the proteins of the pathogens into peptide fragments, which then bind to MHC proteins that carry the fragments to the cell surface. The activated dendritic cells now carry the pathogen-derived peptides, as complexes with MHC proteins, to a nearby lymphoid organ such as a lymph node, where they activate T cells of the adaptive immune system to join in the battle against the specific invader. In addition to the complexes of MHC proteins and microbial peptides displayed on their cell surface, activated dendritic cells also display special, cell-surface co-stimulatory proteins that help activate the T cells. The activated dendritic cells also secrete a variety of cytokines that influence the type of response that the T cells make, ensuring that it is appropriate to fight the particular pathogen. In these ways, dendritic cells serve as crucial links between the innate immune system, which provides a rapid first line of defense against invading pathogens, and the adaptive immune system, which provides slower but more powerful and highly specific ways of attacking an invader.

The battle between pathogens and host defenses is remarkably balanced. At present, humans seem to be gaining a slight advantage, using public sanitation measures, vaccines, and drugs to aid the efforts of our innate and adaptive immune systems. However, infectious diseases are still a leading cause of death worldwide, and new epidemics such as AIDS continue to emerge. The rapid evolution of pathogens and the almost infinite variety of ways that they can invade the human body and elude immune responses will prevent us from ever winning the battle completely.

In the next Chapter, we consider the unique and remarkable strategies that our adaptive immune system has evolved to defend us against such wily opponents. Amazingly, this immune system can mount pathogen-specific responses against pathogens that have never existed before.

Summary

Physical barriers preventing infection, cell-intrinsic responses to infection, and innate immune responses provide early lines of defense against invading pathogens. All multicellular organisms possess these defenses. In vertebrates, innate immune responses can also recruit specific and more powerful adaptive immune responses. Innate immune responses rely on the body's ability to recognize conserved features of microbial molecules that are not made by the host. These microbe-associated immunostimulants include many types of molecules on microbial surfaces, as well as the double-stranded RNA of some viruses. Many of these microbial molecules are recognized by pattern recognition receptors, including the toll-like receptors (TLRs) found in both plants and animals. In vertebrates, microbial surface molecules also activate complement, a group of blood proteins that are activated in sequence to target the microbe for phagocytosis by macrophages and neutrophils, to disrupt the membrane of the microbe, and to produce an inflammatory response. The phagocytes use a combination of degradative enzymes, antimicrobial peptides, and reactive oxygen species to kill the invading microorganism; in addition, they secrete signal molecules that trigger an inflammatory response. Cells infected with viruses produce interferons, which induce a series of cell responses, inhibit viral replication, and activate the killing activities of natural killer cells. Dendritic cells of the innate immune system ingest microbes at sites of infection and carry them and their products to local lymph nodes, where they activate T cells of the adaptive immune system to make specific responses against the microbes.

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