

Molecular Biology of **THE CELL**

Fifth Edition



ALBERTS

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Bruce Alberts

Alexander Johnson

Julian Lewis

Martin Raff

Keith Roberts

Peter Walter

With problems by

John Wilson

Tim Hunt

Garland Science

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Bruce Alberts received his Ph.D. from Harvard University and is Professor of Biochemistry and Biophysics at the University of California, San Francisco. For 12 years, he served as President of the U.S. National Academy of Sciences (1993–2005). **Alexander Johnson** received his Ph.D. from Harvard University and is Professor of Microbiology and Immunology and Director of the Biochemistry, Cell Biology, Genetics, and Developmental Biology Graduate Program at the University of California, San Francisco. **Julian Lewis** received his D.Phil. from the University of Oxford and is a Principal Scientist at the London Research Institute of Cancer Research UK. **Martin Raff** received his M.D. from McGill University and is at the Medical Research Council Laboratory for Molecular Cell Biology and the Biology Department at University College London. **Keith Roberts** received his Ph.D. from the University of Cambridge and is Emeritus Fellow at the John Innes Centre, Norwich. **Peter Walter** received his Ph.D. from The Rockefeller University in New York and is Professor and Chairman of the Department of Biochemistry and Biophysics at the University of California, San Francisco, and an Investigator of the Howard Hughes Medical Institute.

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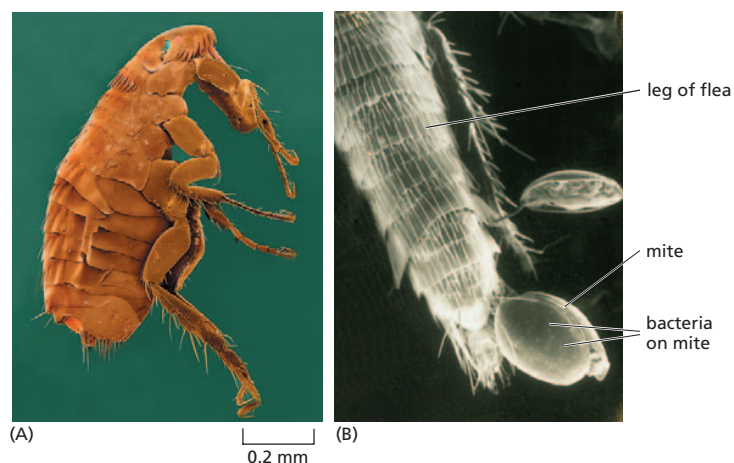


Figure 24-1 Parasitism at many levels.

(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.

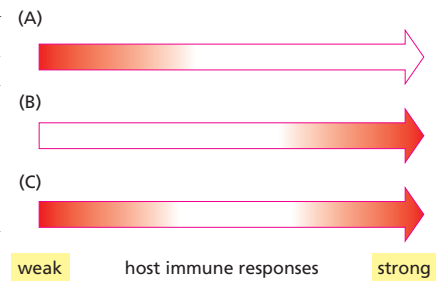


Figure 24–2 Interaction between microbes and immune responses in microbial pathogenesis. The strength of the immune responses increases in the direction of the arrows, while the amount of host damage is indicated by the amount of red shading in the arrows. (A) Normal flora such as the bacterium *Staphylococcus 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.

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).

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

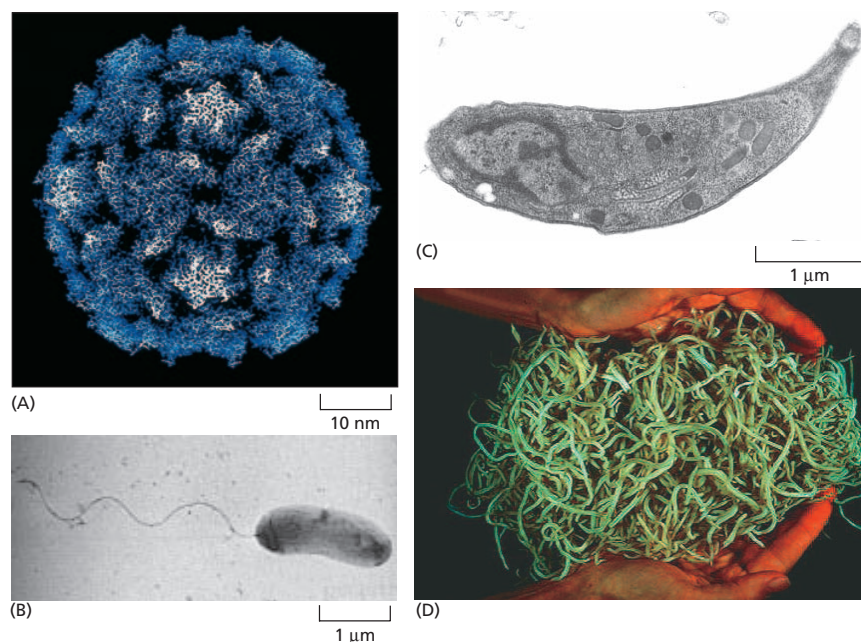


Figure 24–3 Pathogens in many forms.

(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.)

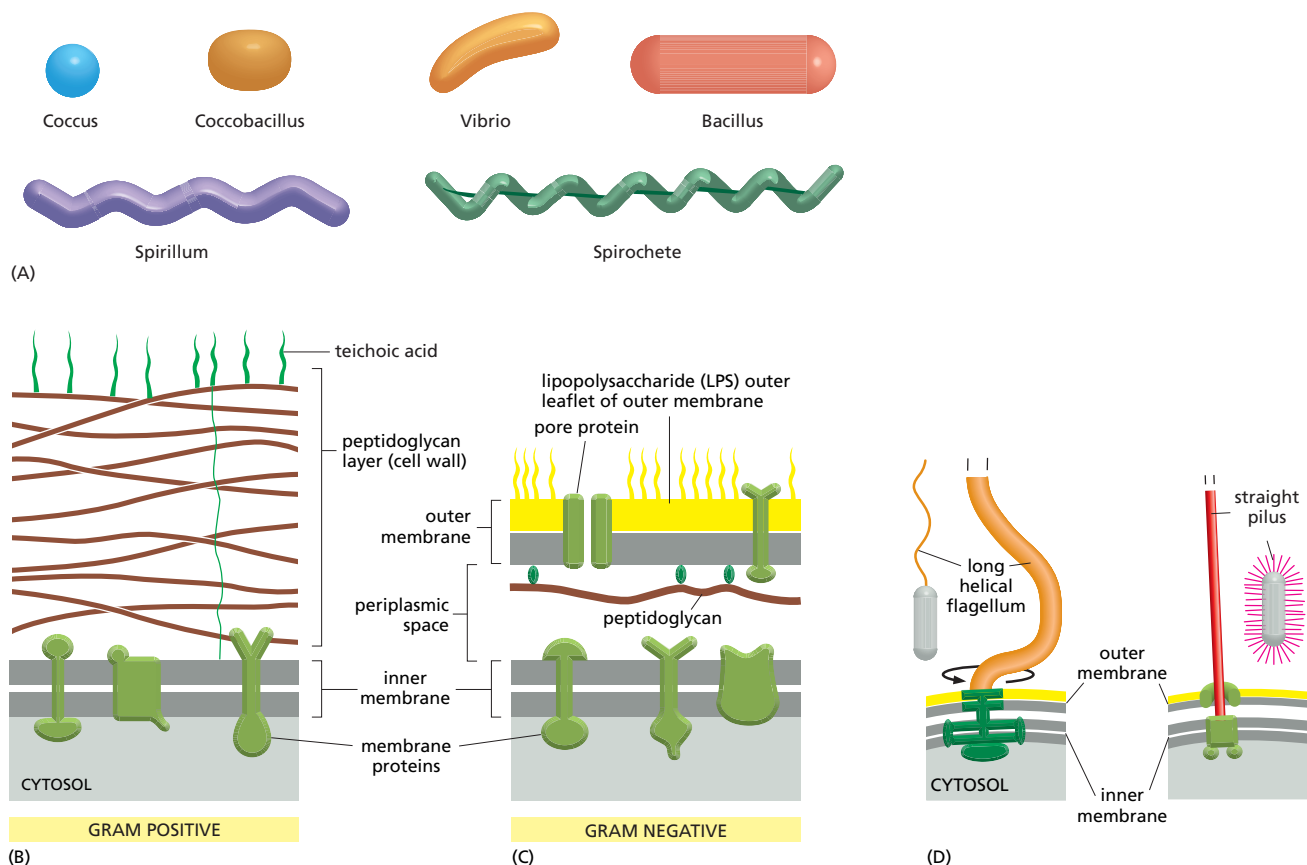


Figure 24-4 Bacterial shapes and cell-surface structures. (A) Bacteria are classified by shape. (B and C) They are also classified as *Gram-positive* or *Gram-negative*. (B) Bacteria such as *Streptococcus* and *Staphylococcus* have a single membrane and a thick cell wall made of cross-linked *peptidoglycan*. 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.

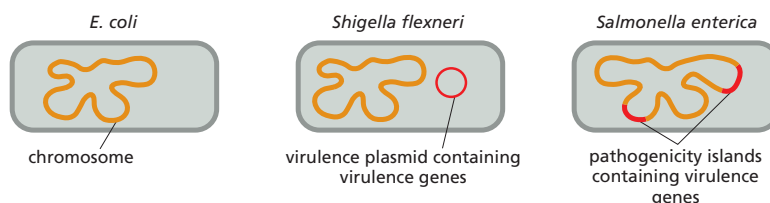


Figure 24–5 Genetic differences between pathogenic and nonpathogenic bacteria.

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.

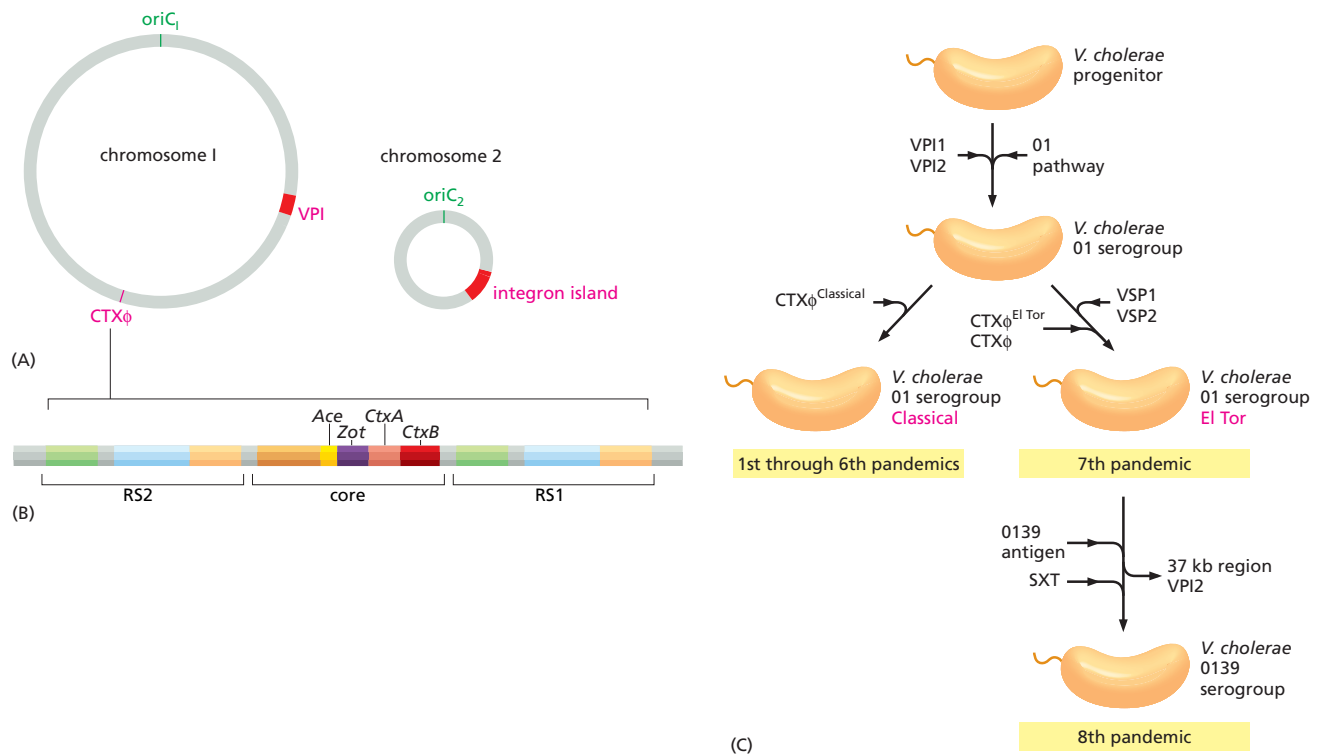


Figure 24–6 Genetic organization of *Vibrio cholerae*. An electron micrograph of *Vibrio cholerae* (*V. cholerae*) is shown in Figure 24–3B. (A) The bacterium is unusual in having two circular chromosomes rather than one. Each chromosome has its own origin of replication (*oriC₁* and *oriC₂*). Three 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.

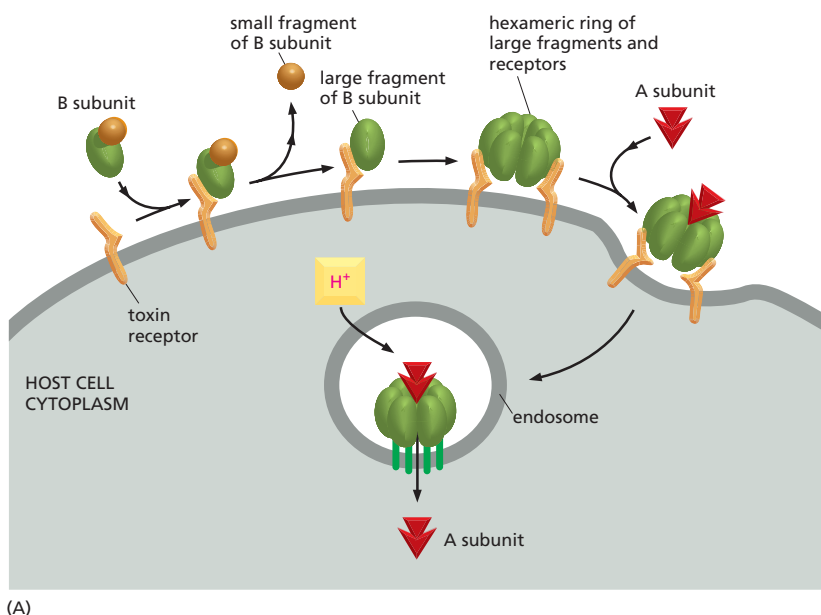
Two carried by the *Vibrio cholerae* phage, for example, encode two subunits of **cholera toxin** (see Figure 24–6B). The B subunit of this secreted, toxic protein binds to a glycolipid component of the plasma membrane of the epithelial cells in the gut of a person who has consumed *Vibrio cholerae* 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 adenylyl 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 adenylyl 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 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.)



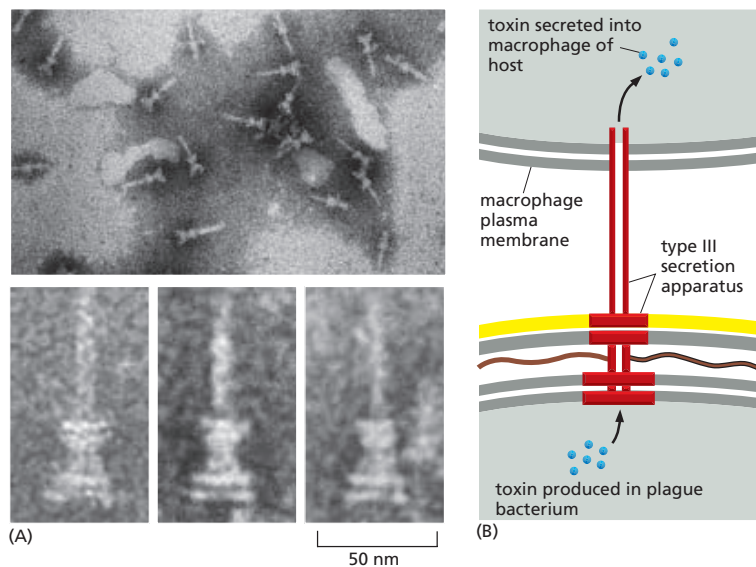


Figure 24-8 Type III secretion systems that can deliver virulence factors into the cytosol of a host cell. (A) Electron micrographs of purified type III secretion systems. About two dozen proteins form the complete structure, which is seen in the three enlarged electron micrographs below. (B) The large 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

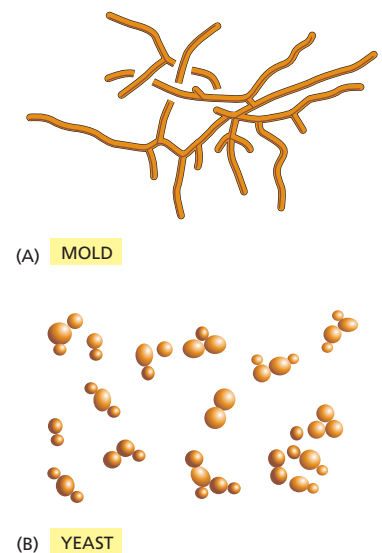


Figure 24-9 Dimorphism in the pathogenic fungus *Histoplasma capsulatum*. (A) At low temperature in the soil, *Histoplasma* grows as a multicellular filamentous mold. (B) After it is inhaled into the lung of a mammal, *Histoplasma* undergoes a morphological switch triggered by the change in temperature.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

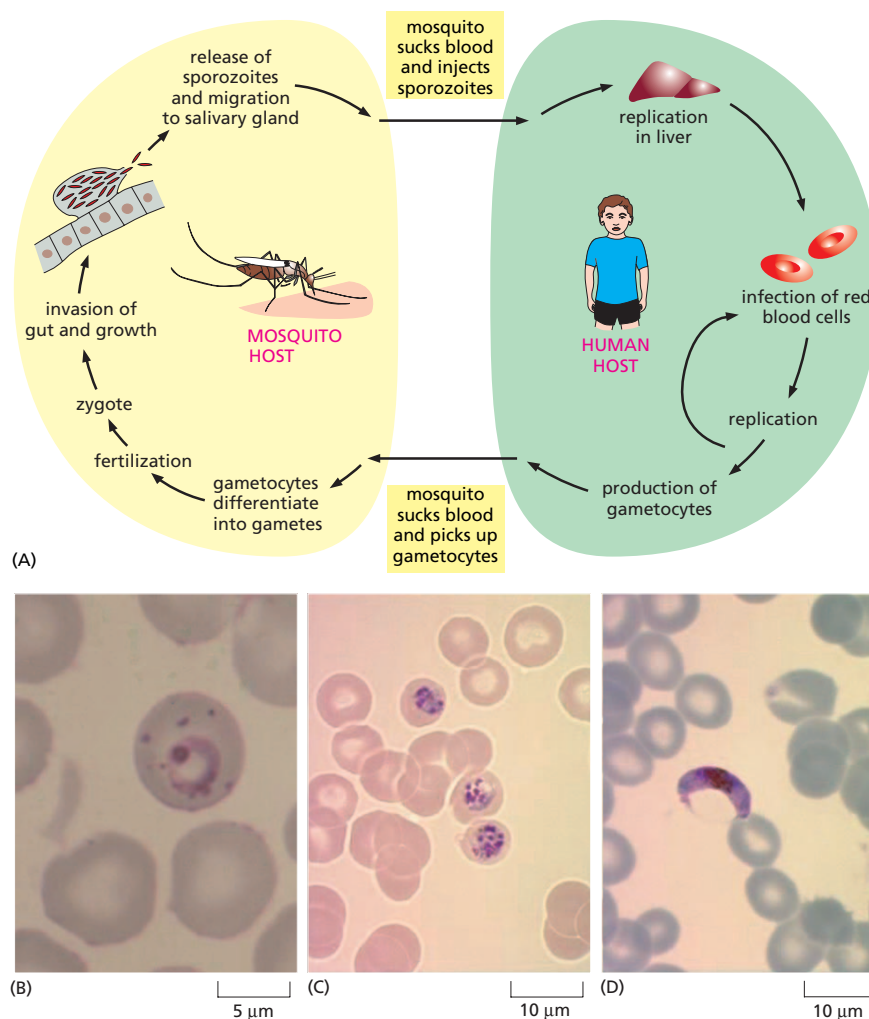
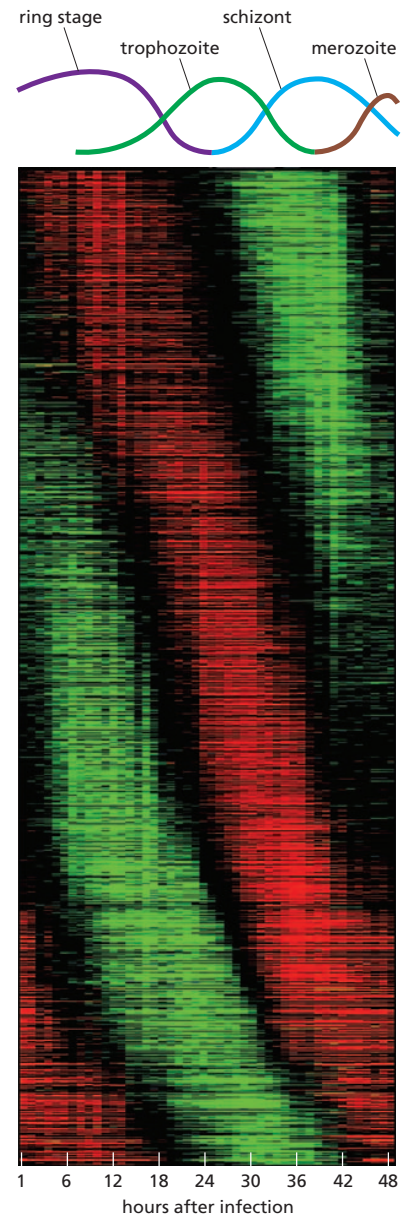


Figure 24-10 The complex life cycle of malaria parasites. (A) The sexual cycle of *Plasmodium falciparum* requires passage between a human host and an insect host. (B)–(D) Blood smears from people with malaria, showing three different forms of the parasite that appear in red blood cells: (B) ring stage; (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).

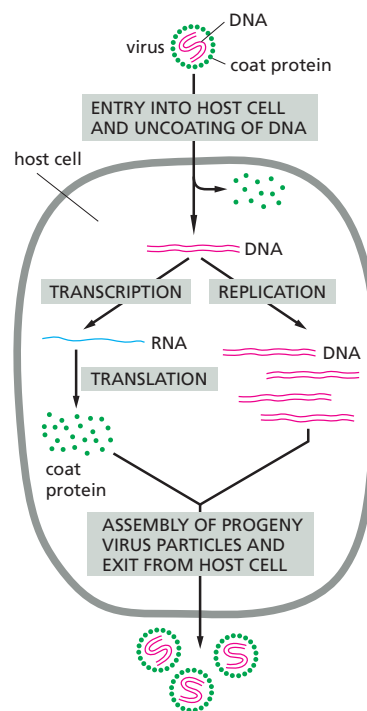


Figure 24–12 A simple viral life cycle. The hypothetical virus shown consists of a small double-stranded DNA molecule that codes for only a single viral capsid protein. No known virus is this simple.

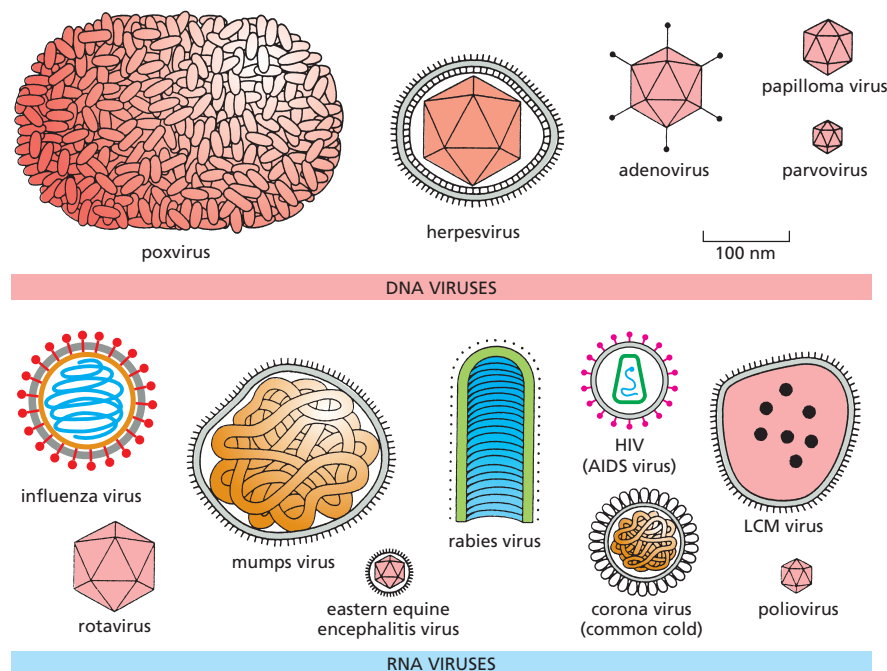


Figure 24–13 Examples of viral morphology. As shown, both DNA and RNA viruses vary greatly in both size and shape.

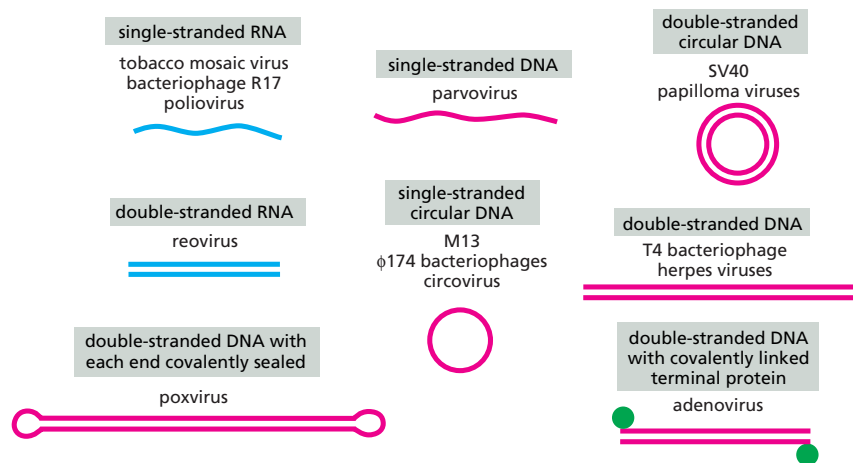


Figure 24–14 Schematic drawings of several types of viral genomes. The smallest viruses contain only a few genes and can have an RNA or a DNA genome. The largest viruses contain hundreds of genes and have a double-stranded DNA genome. The peculiar ends (as well as the circular forms) overcome the difficulty 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.

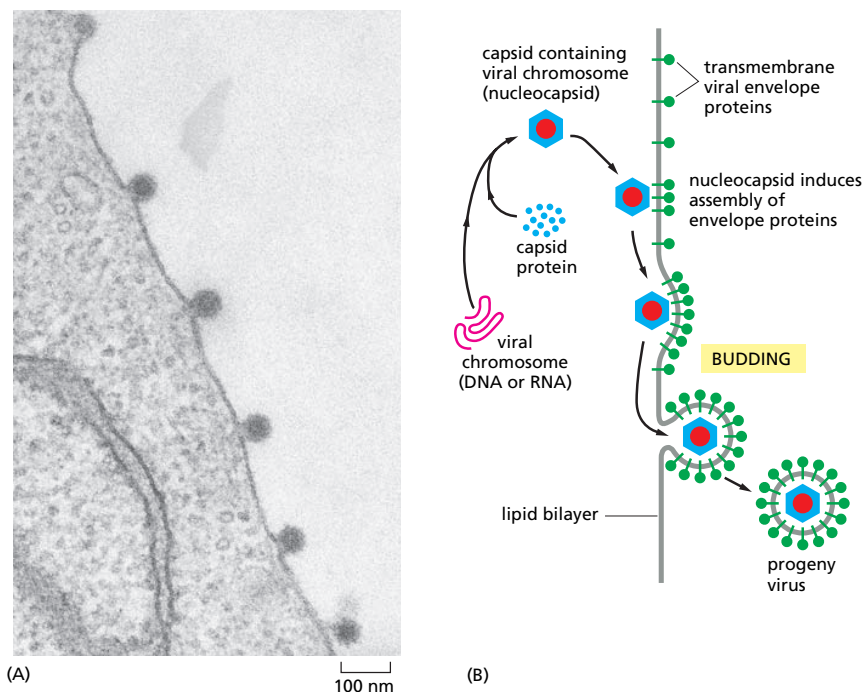


Figure 24–15 Acquisition of a viral envelope. (A) Electron micrograph of an animal cell from which six copies of an enveloped virus (*Semliki forest virus*) are budding. (B) Schematic drawing of the envelope assembly and budding processes. The lipid bilayer that surrounds the viral capsid is derived 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.)

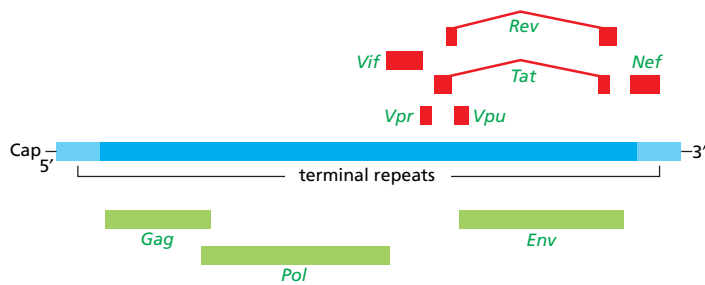


Figure 24–16 A map of the HIV genome.

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.

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

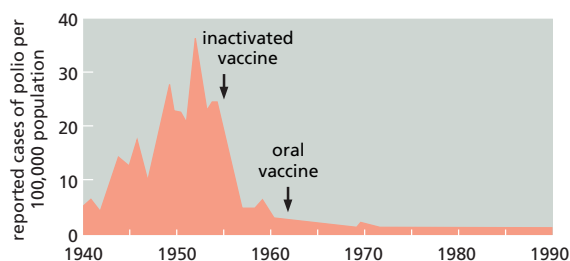


Figure 24–17 Eradication of a viral disease through vaccination. The graph shows the number of cases of poliomyelitis reported per year in the United States. The arrows indicate the timing of the introduction of the Salk vaccine (inactivated virus given by injection) and the Sabin vaccine (live attenuated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

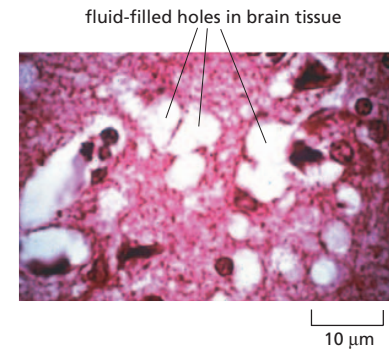


Figure 24–18 Neural degeneration in a prion infection. This micrograph shows a slice from the brain of a person who died of kuru. Kuru is a human prion disease, very similar to BSE, that was spread from one person to another by ritual mortuary practices in New Guinea. The large fluid-filled holes are 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.

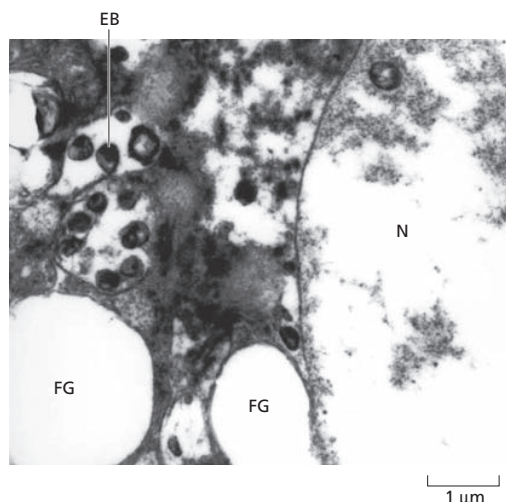


Figure 24–19 *Chlamydia pneumoniae* within a foam cell macrophage in an atherosclerotic plaque. The labels on this electron micrograph indicate: EB, elementary body (bacterium); FG, fat globule; N, macrophage nucleus. (From L.A. Campbell and C.C. Kuo, *Nat. Rev. Microbiol.* 2:23–32, 2004. With permission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

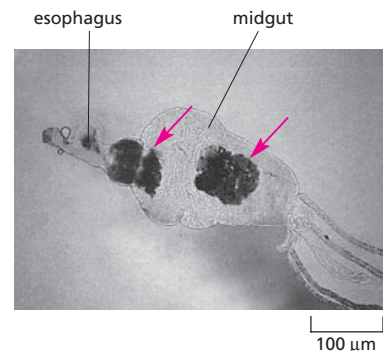


Figure 24–20 Plague bacteria within a flea. This light micrograph shows the digestive tract dissected from a flea that had dined about two weeks previously on the blood of an animal infected with the plague bacterium, *Yersinia pestis*. The bacteria multiplied in the flea gut to produce large cohesive 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.)

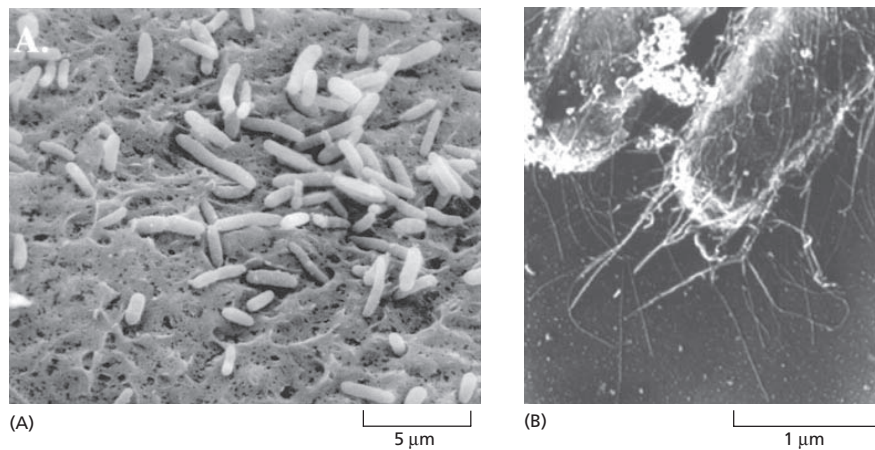


Figure 24-21 Uropathogenic *E. coli* in the infected bladder of a mouse.

(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 *mucociliary 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.

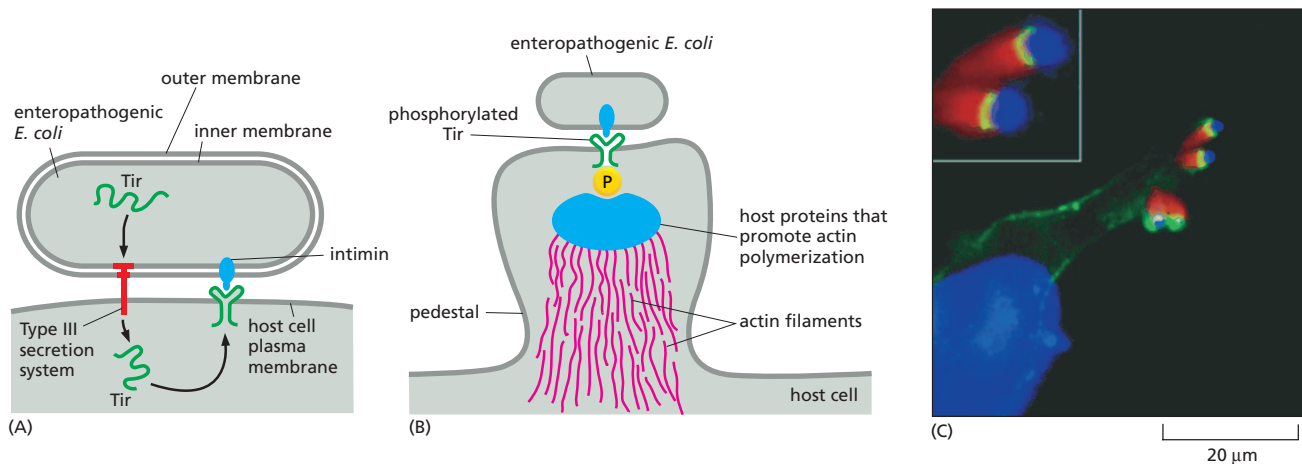


Figure 24-22 Interaction of enteropathogenic *E. coli* (EPEC) with host cells in the gut. (A) When EPEC contacts an epithelial cell in the lining of the human gut, it delivers a bacterial protein called Tir into the host cell through a type III secretion system. Tir then inserts into the plasma membrane 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* adenyl 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

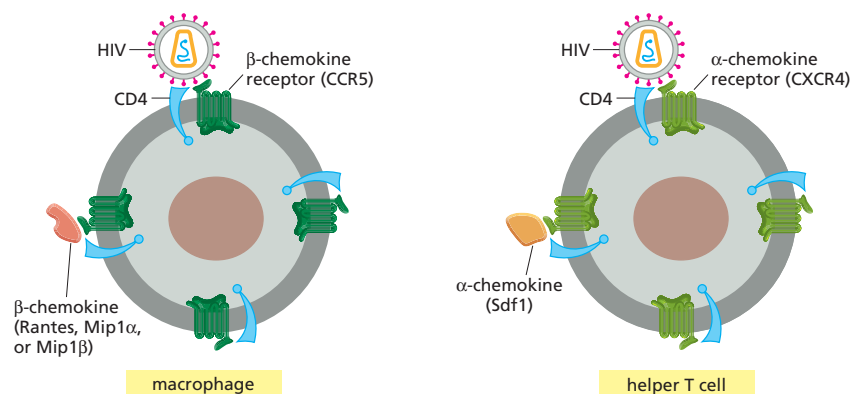
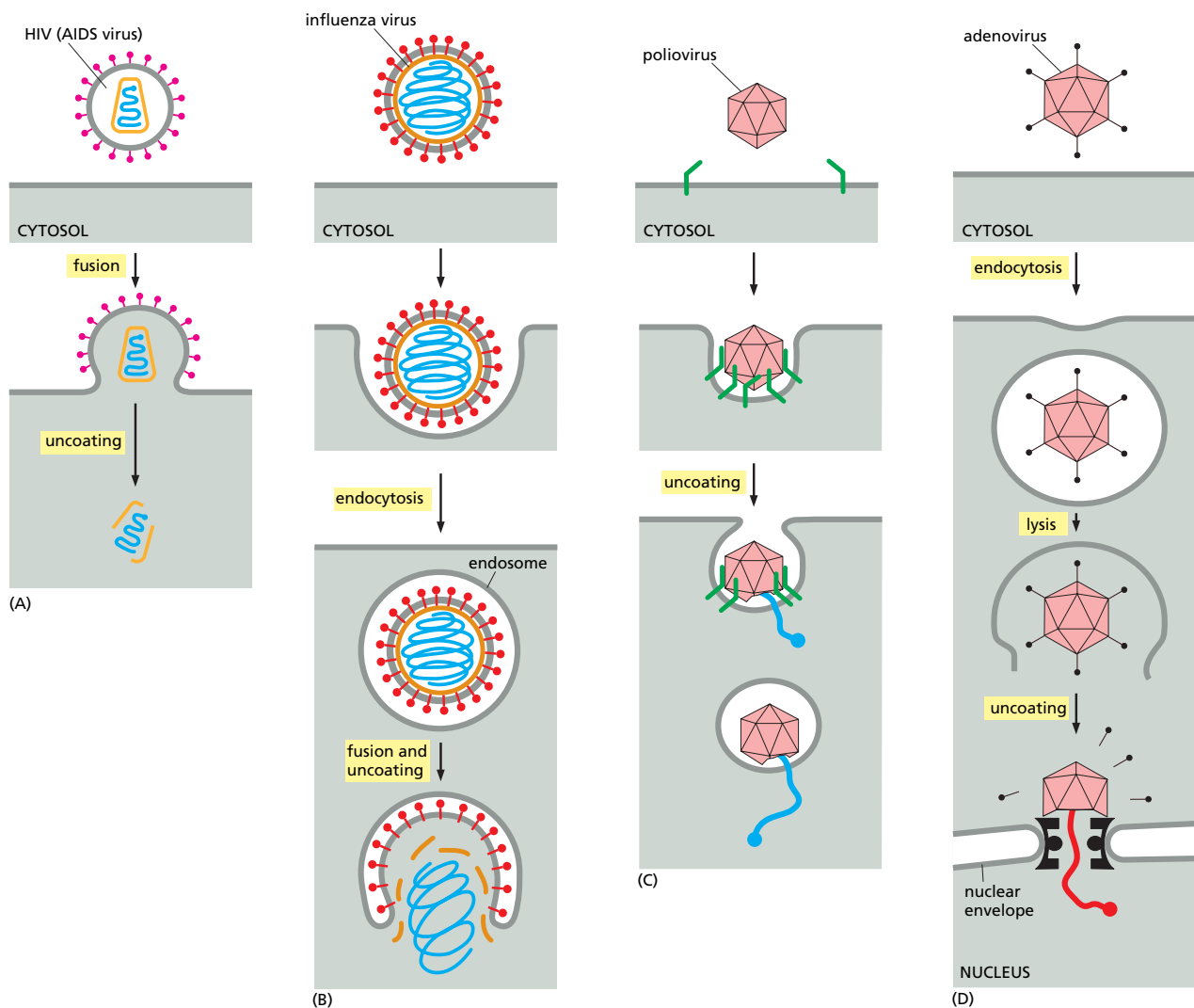


Figure 24–23 Receptor and co-receptors for HIV. All strains of HIV require the CD4 protein as a primary receptor. Early in an infection, most of the viruses use CCR5 as a co-receptor, allowing them to infect macrophages and their precursors, monocytes. As the infection progresses, mutant variants arise 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 intra-chain 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

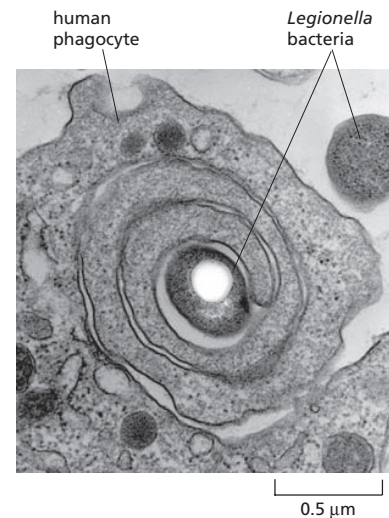


Figure 24–25 Uptake of *Legionella pneumophila* by a human phagocyte. This electron micrograph shows the unusual coil structure induced on the surface of the phagocyte by the bacterium. Some other pathogens, including the bacterium *Borrelia burgdorferi*, which causes Lyme disease, the eucaryotic pathogen *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.)

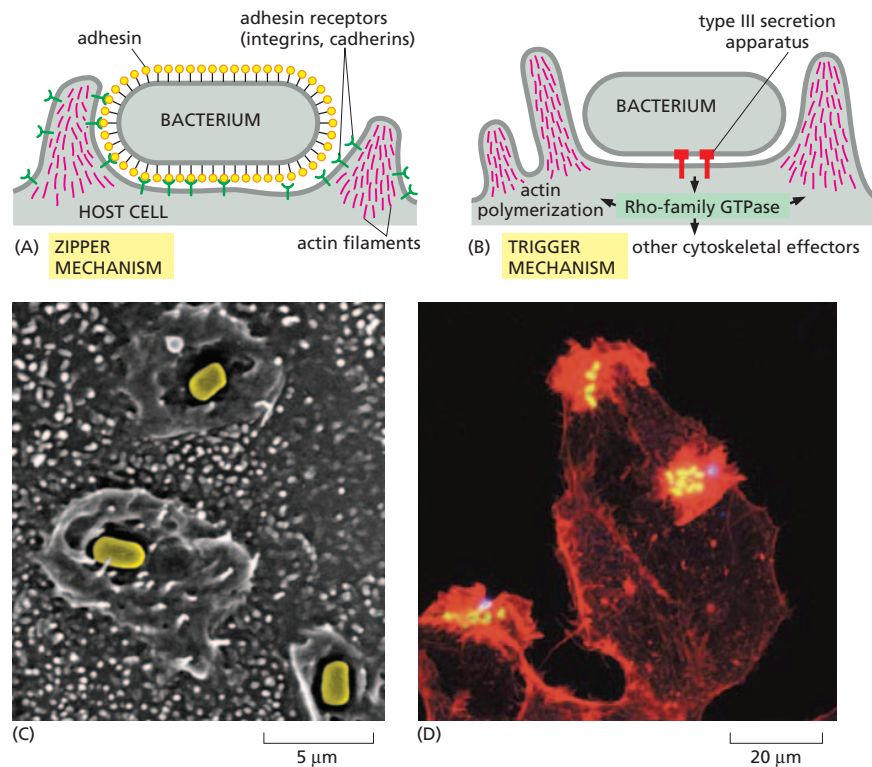


Figure 24–26 Mechanisms used by bacteria to induce phagocytosis by nonphagocytic host cells. (A) The zipper and (B) trigger mechanisms for pathogen-induced phagocytosis both require the polymerization of actin at the site of bacterial entry. (C) A scanning electron micrograph showing a very early stage of *Salmonella enterica* invasion by the zipper 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.

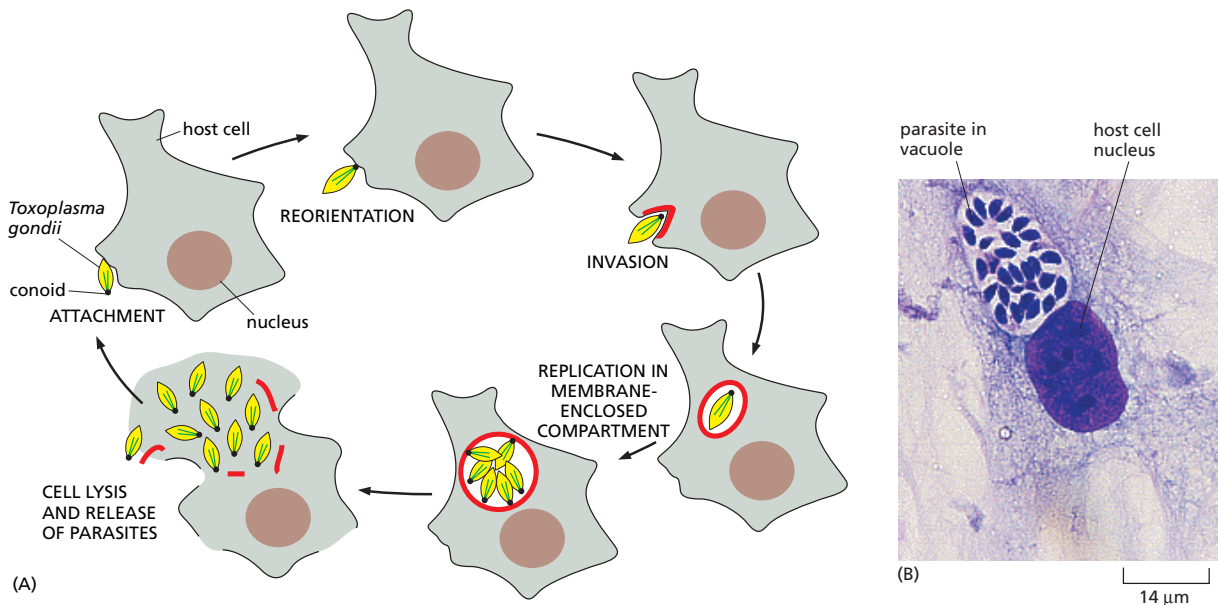
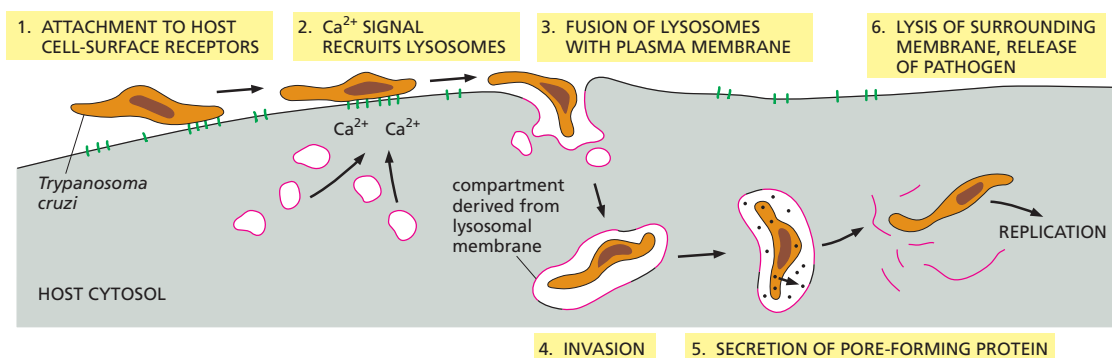


Figure 24-27 The life cycle of the intracellular parasite *Toxoplasma gondii*. (A) After attachment to a host cell, *T. gondii* uses its conoid to push its way into the host cell. As the host cell's plasma membrane invaginates to surround the parasite, the parasite somehow removes the host cell proteins 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.



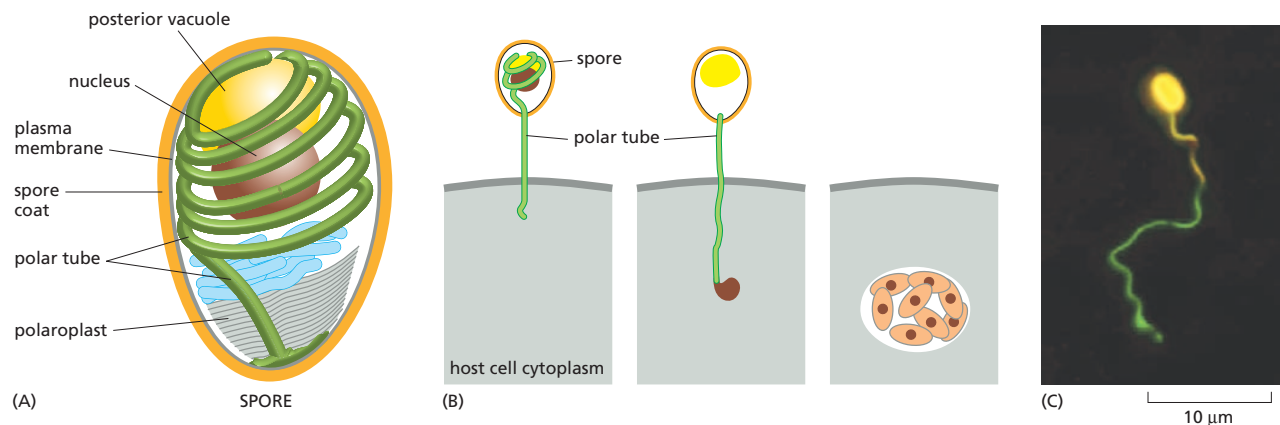


Figure 24-29 Invasion of host cells by microsporidia. (A) The spore form of the parasite is covered with a rigid coat and harbors a coiled polar tube that wraps many times around the nucleus. (B) The polar tube extends explosively when the spore comes into contact with an appropriate host cell, penetrating 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

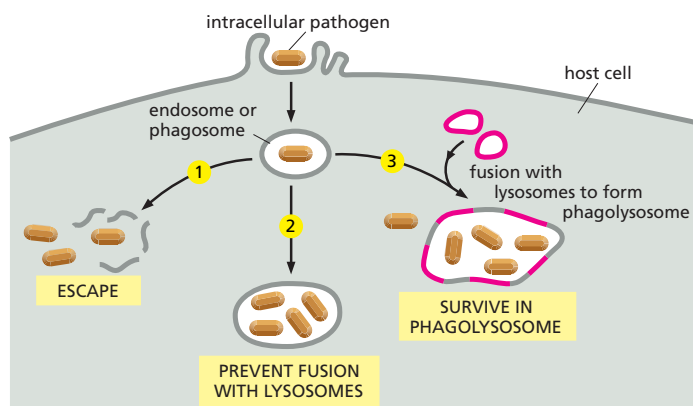
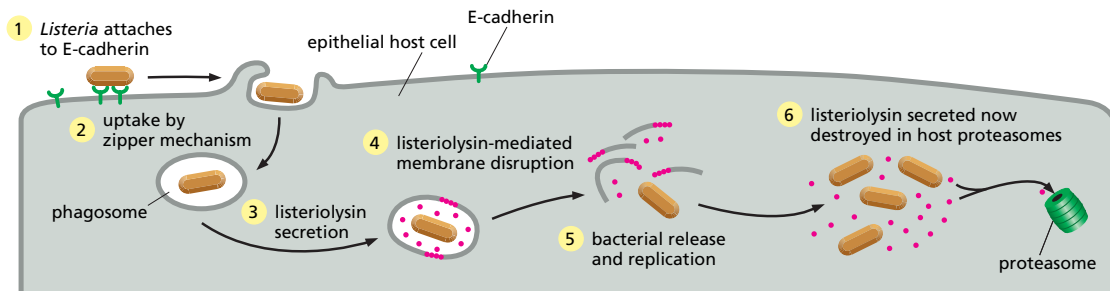


Figure 24-30 Choices that an intracellular pathogen faces. After entry into a host cell, generally through endocytosis or phagocytosis into a membrane-enclosed compartment, intracellular pathogens can use one of three strategies to survive and replicate. Pathogens that follow strategy (1) include all 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.

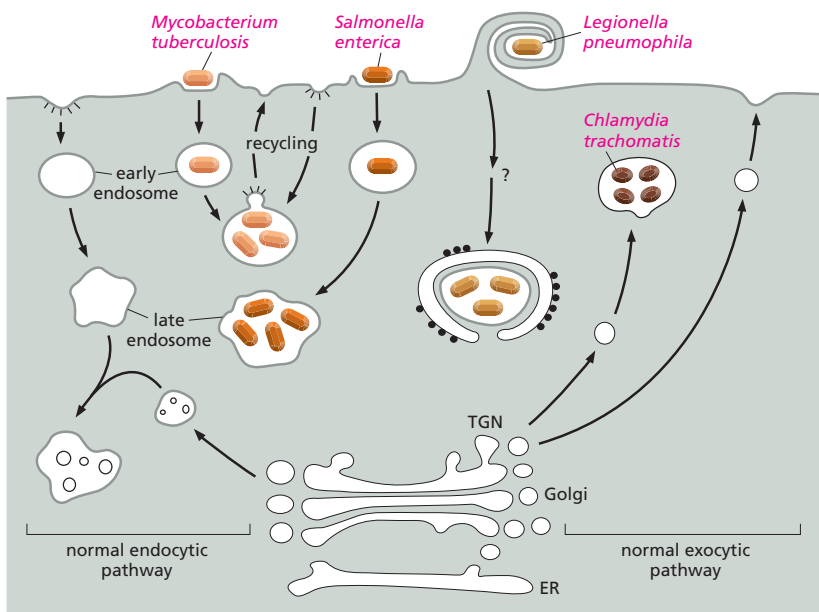
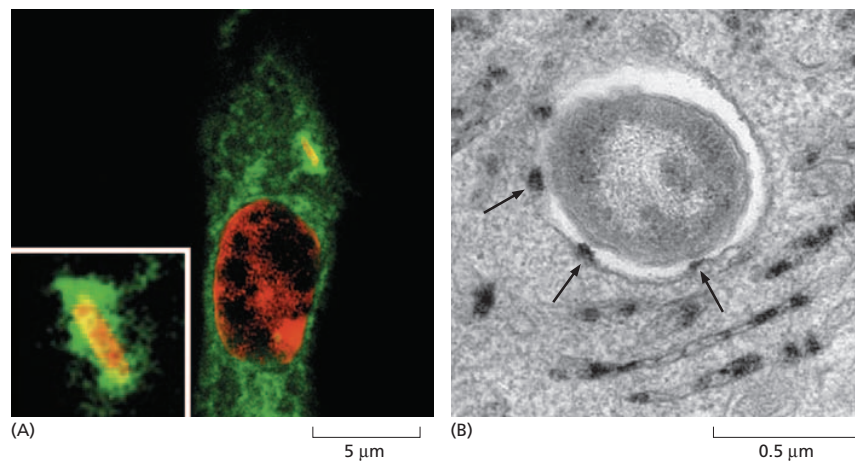


Figure 24–32 Modifications of host cell membrane trafficking by bacterial pathogens. Four intracellular bacterial pathogens, *Mycobacterium tuberculosis*, *Salmonella enterica*, *Legionella pneumophila*, and *Chlamydia trachomatis*, all replicate in membrane-enclosed compartments, but the four compartments 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.)

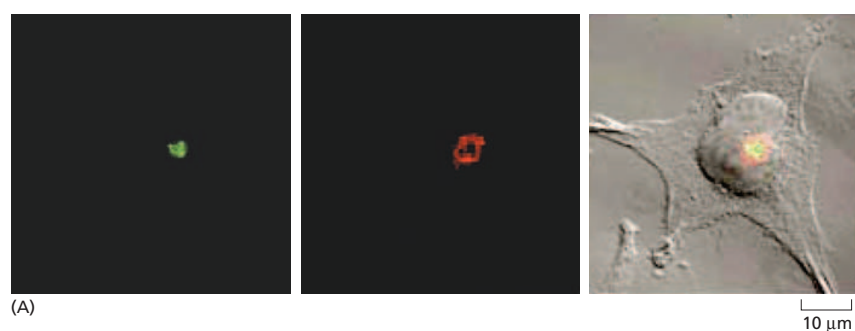
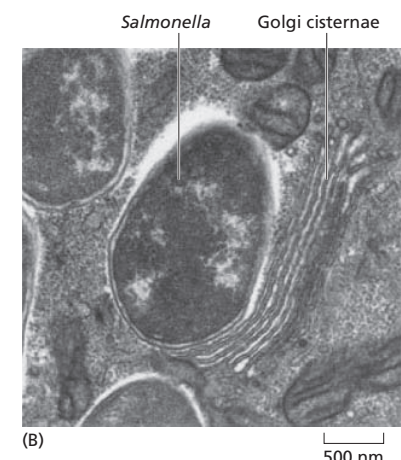


Figure 24-34 Proximity of Golgi stacks to endosomes containing *Salmonella enterica*. (A) In this infected cell, the bacteria are green and the Golgi has been labeled (red) with antibodies against a Golgi-specific structural protein. (B) Electron micrograph showing the very close apposition of a Golgi 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.)



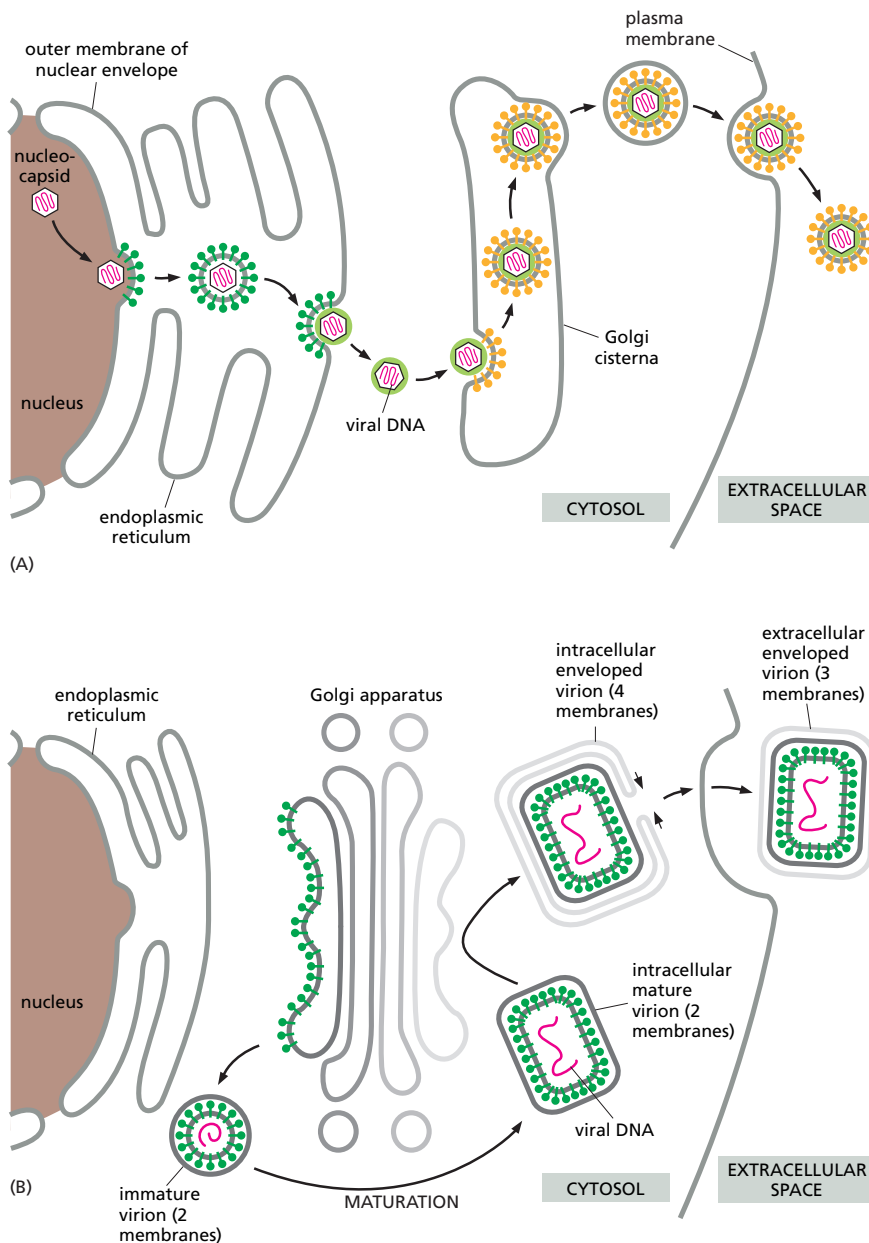


Figure 24–35 Complicated strategies for viral envelope acquisition. (A) Herpes virus nucleocapsids assemble in the nucleus and then bud through the inner nuclear membrane into the space between the inner and outer nuclear membranes, acquiring a membrane coat. The virus particles then apparently lose 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

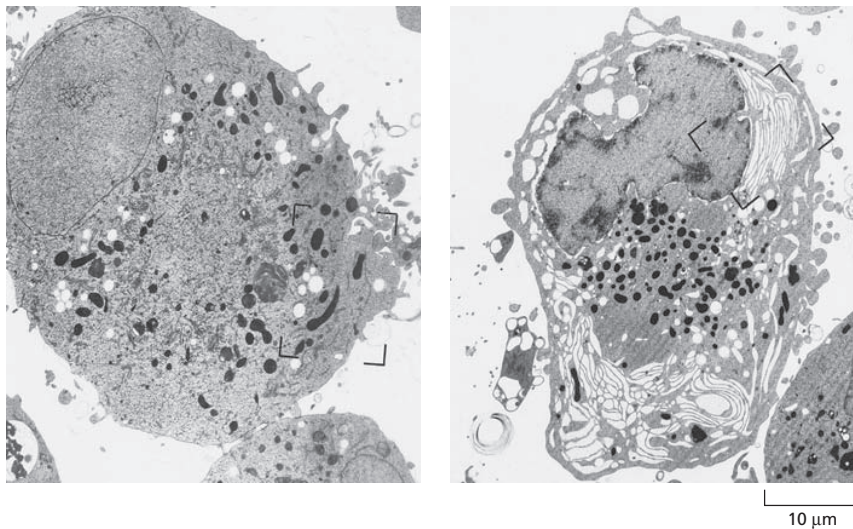


Figure 24-36 Intracellular membrane alterations induced by a poliovirus protein. Poliovirus, like other single-stranded RNA viruses, replicates its RNA genome by using a polymerase that associates with intracellular membranes. Several of the proteins encoded by its genome alter the structure or dynamic 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).

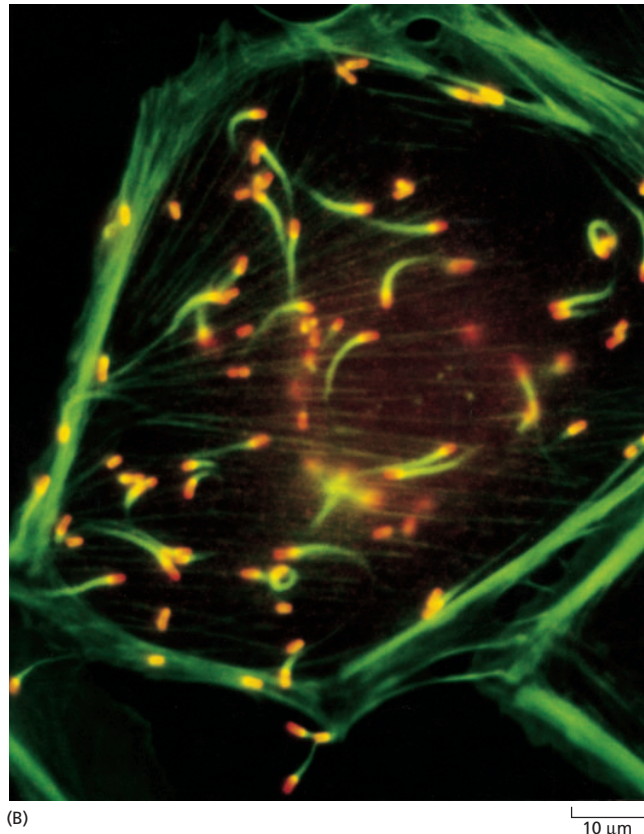
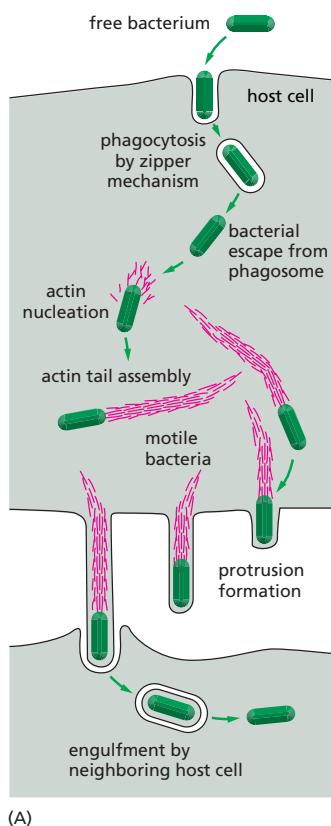


Figure 24-37 The actin-based movement of *Listeria monocytogenes* within and between host cells. <GTAT> (A) These bacteria induce the assembly of actin-rich tails in the host cell cytoplasm, enabling the bacteria to move rapidly. Motile bacteria spread from cell to cell by forming membrane-enclosed protrusions 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.)

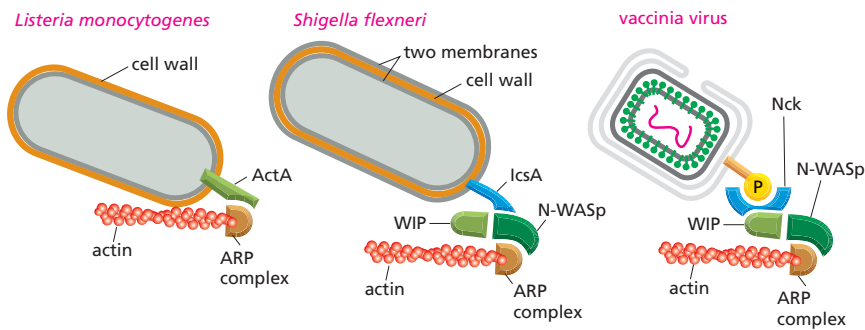


Figure 24-38 Molecular mechanisms for actin nucleation by various pathogens.

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.

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

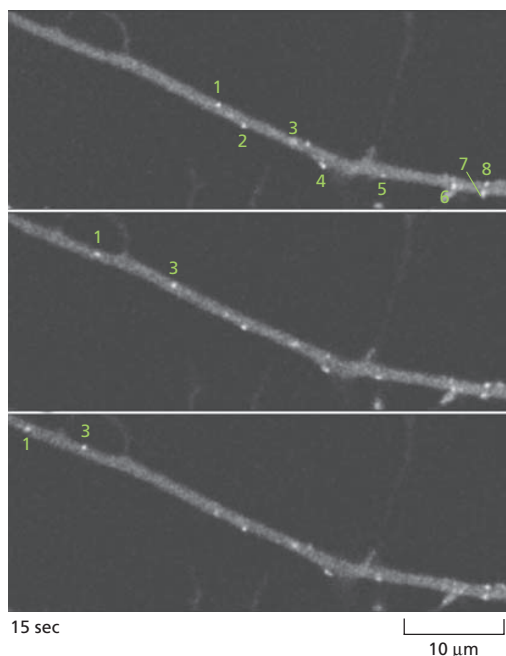
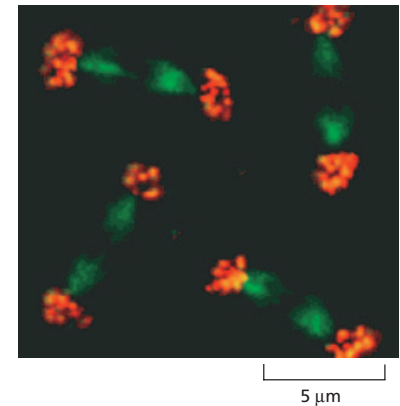


Figure 24-39 Fluorescence micrographs of herpes virus moving in an axon. This nerve cell has been infected with an alpha herpes virus that has been genetically engineered to express green fluorescent protein (GFP) fused to one of its capsid proteins. In this segment of the axon, several viral particles 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

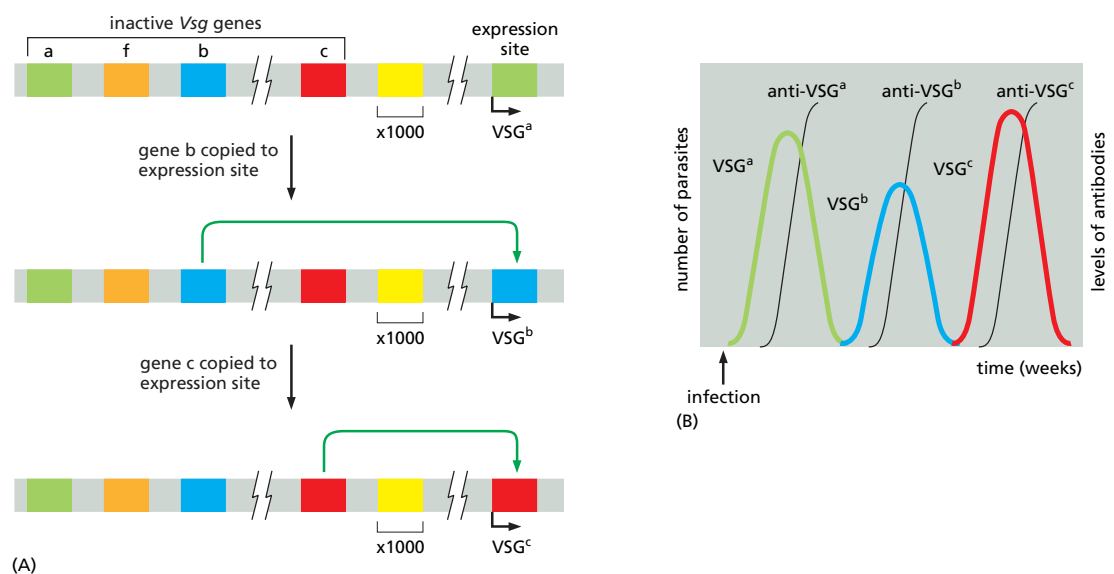
modern antipathogen drugs, both of which destroy pathogens that fail to change, are the source of these selective pressures.

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

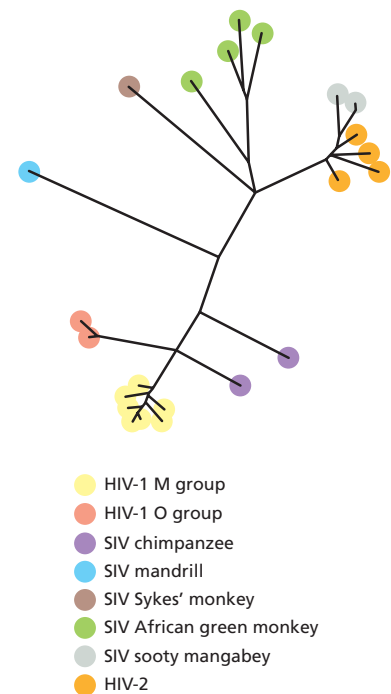


Figure 24-42 Diversification of HIV-1, HIV-2, and related strains of SIV. The genetic distance between any two viral isolates is found by following the shortest path joining them in the tree. HIV-1 is divided into two groups, major (M) and outlier (O). The HIV-1 M group is responsible for the global 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.

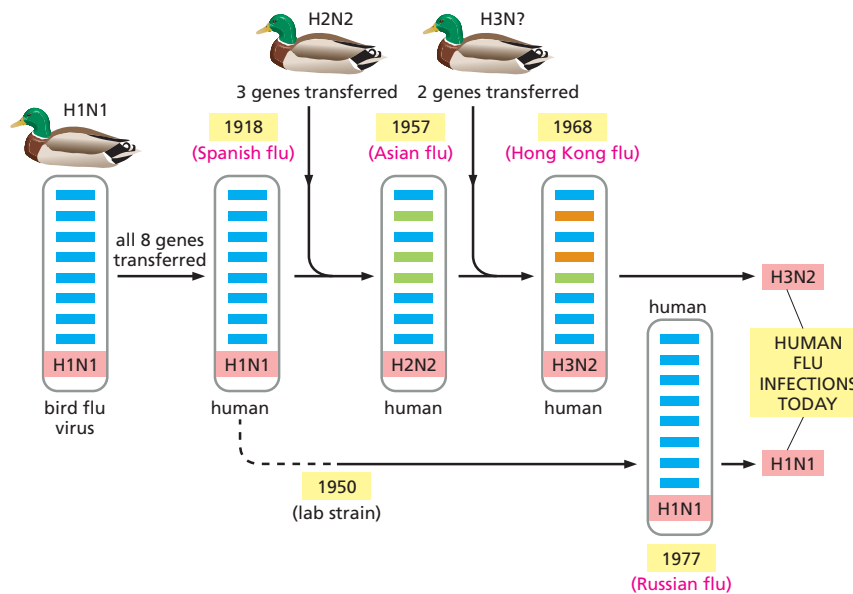


Figure 24-43 Model for the evolution of pandemic strains of influenza virus by recombination. Influenza A virus is a natural pathogen of birds, particularly waterfowl, and is always present in wild bird populations. Before 1900, influenza virus caused sporadic mild disease in humans. In 1918, a particularly 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.

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.

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

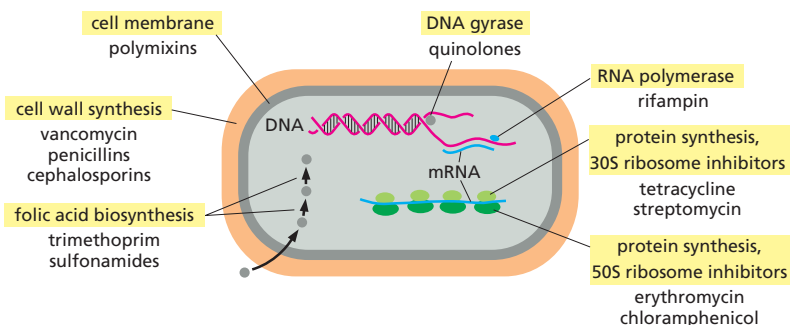


Figure 24-44 Antibiotic targets. Although there are many antibiotics in clinical use, they have a narrow range of targets, which are highlighted in yellow. A few representative antibiotics in each class are listed. Nearly all antibiotics used to treat human infections fall into one of these categories.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

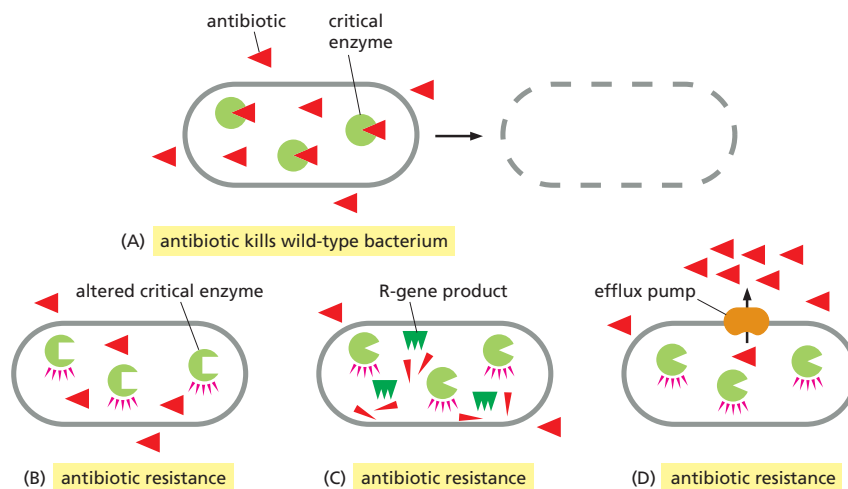


Figure 24-45 Three general mechanisms of antibiotic resistance. (A) Non-resistant bacterial cells bathed in a drug (red triangles) that binds to and inhibits a critical enzyme (light green) will be killed due to enzyme inhibition. (B) Bacteria that have altered the target enzyme so that it no longer 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

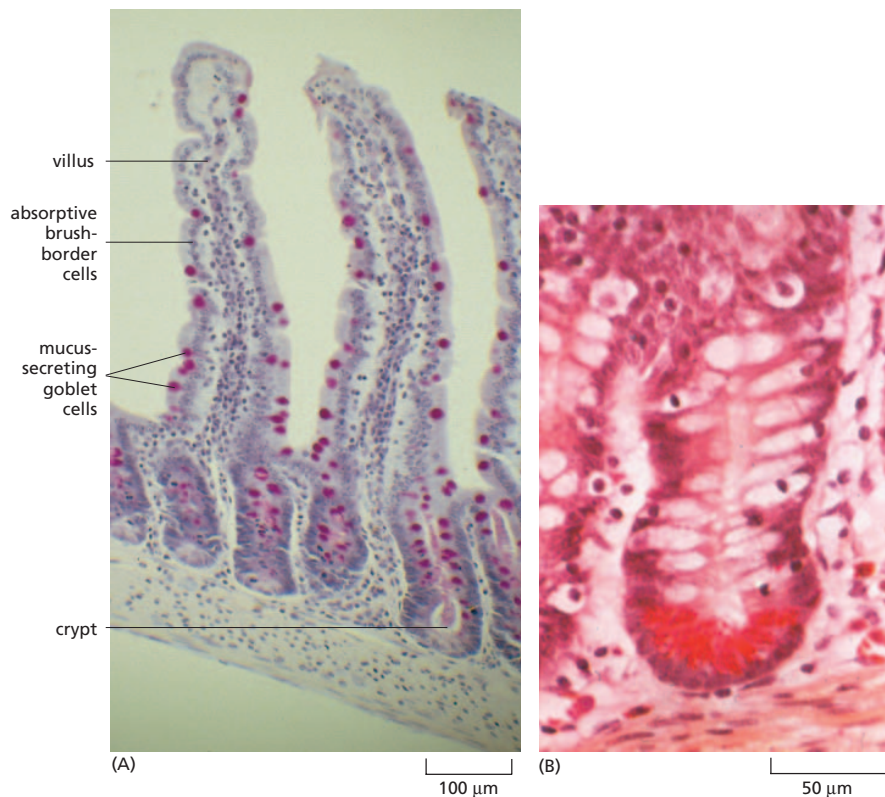


Figure 24-46 Epithelial defenses against microbial invasion. (A) Light micrograph of a cross section through the wall of the human small intestine, showing three villi. Goblet cells secreting mucus are stained *magenta*. The protective mucus layer covers the exposed surfaces of the villi. At the base 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

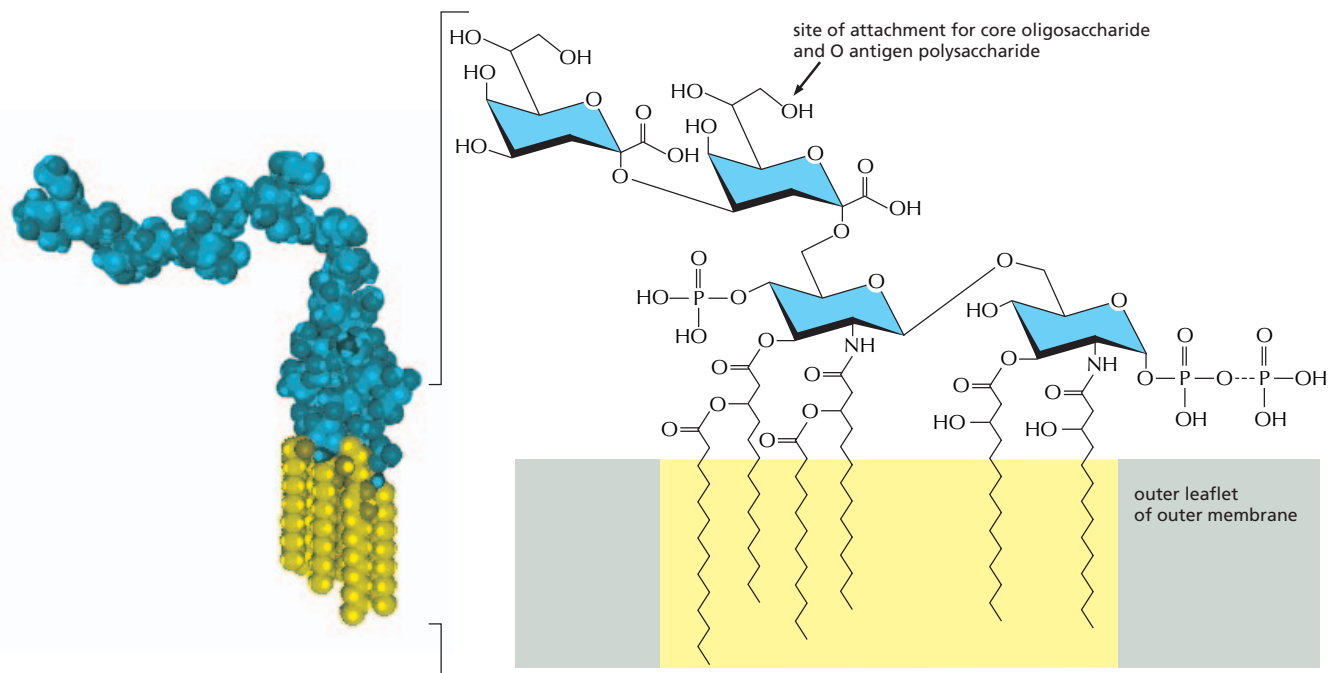


Figure 24-47 Structure of lipopolysaccharide (LPS). On the *left* is the three-dimensional structure of a molecule of LPS, with the fatty acid tails shown in *yellow* and the sugars in *blue*. The molecular structure of the bottom part of LPS is shown on the *right*. The membrane anchor is made up of 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

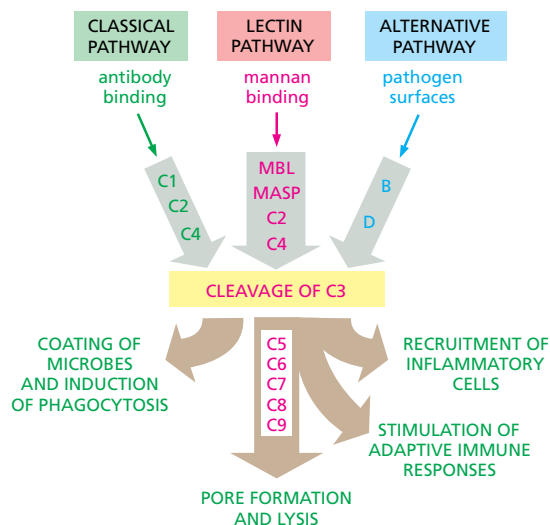


Figure 24–48 The principal stages in complement activation by the classical, lectin, and alternative pathways. In all three pathways, the reactions of complement activation usually take place on the surface of an invading microbe, such as a bacterium, and lead to the cleavage of C3. C1–C9, mannose-binding 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.

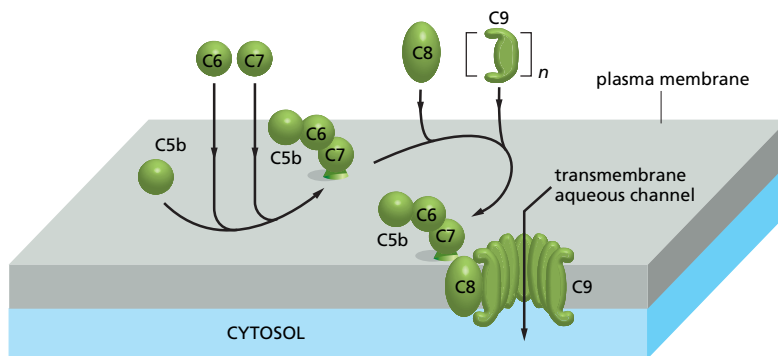


Figure 24–49 Assembly of the late complement components to form a membrane attack complex. When C3b is produced by any of the three complement activation pathways, it is immobilized on a membrane, where it recruits C4b and C2b to form a proteolytic complex. This complex then cleaves the first of the 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.

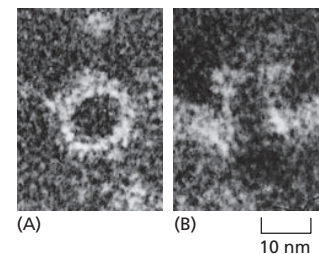


Figure 24–50 Electron micrographs of negatively stained complement lesions in the plasma membrane of a red blood cell. The lesion in (A) is seen *en face*, while that in (B) is seen from the side as an apparent transmembrane channel. The negative stain fills the channels, which therefore look black. 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

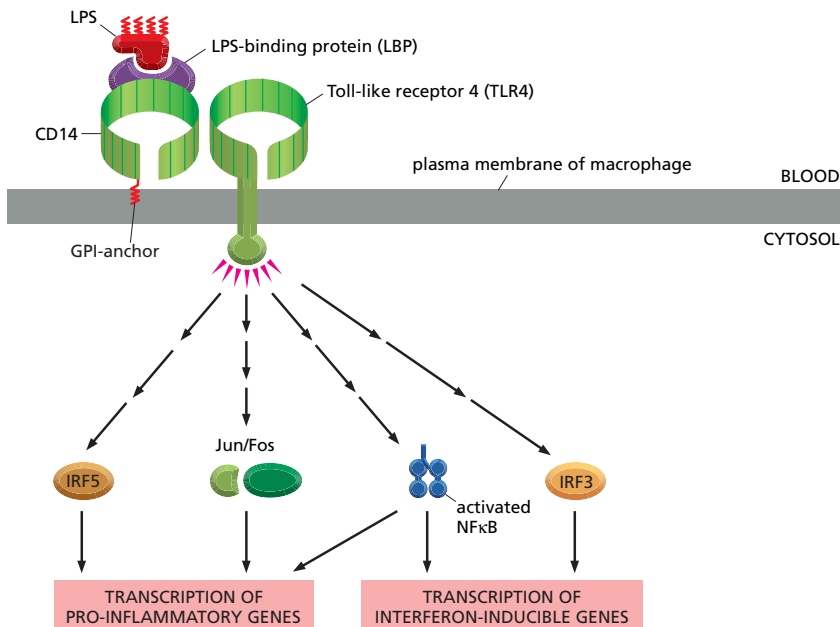


Figure 24–51 The activation of a macrophage by lipopolysaccharide (LPS). LPS binds to LPS-binding protein (LBP) in the blood, and the complex binds to the GPI-anchored protein CD14 on the macrophage surface. The ternary complex then activates Toll-like receptor 4 (TLR4), which activates multiple, downstream, 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

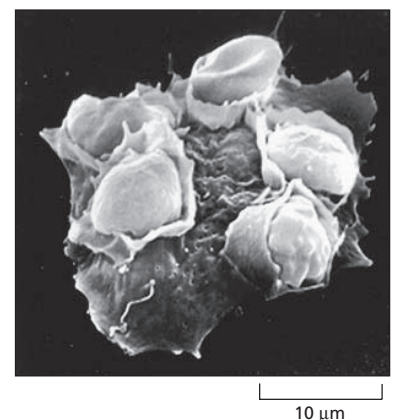


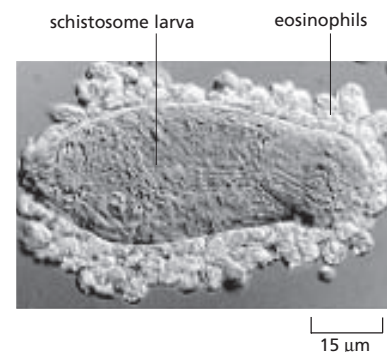
Figure 24-53 Phagocytosis. This scanning electron micrograph shows a macrophage in the midst of ingesting five red blood cells that have been coated with an antibody against a surface glycoprotein. (From E.S. Gold et al., *J. Exp. Med.* 190:1849–1856, 1999. With permission from The Rockefeller University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



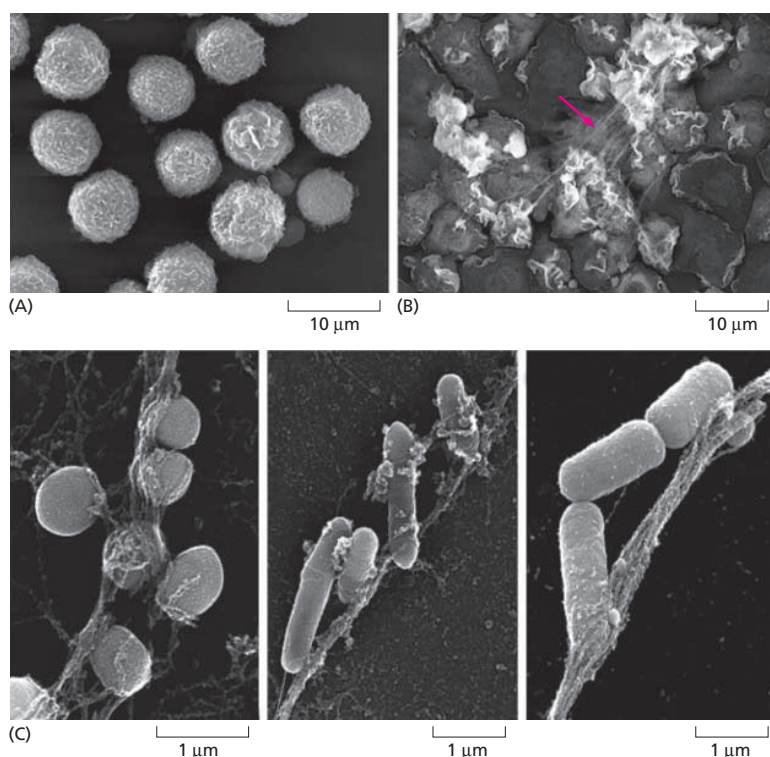


Figure 24-55 Neutrophils eject their chromatin to trap bacteria in a sticky web. (A) Scanning electron micrograph showing resting neutrophils. (B) Activated neutrophils have multiple surface ruffles and are associated with fibrous strands (arrow). The strands contain DNA and histones and appear to be 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.)

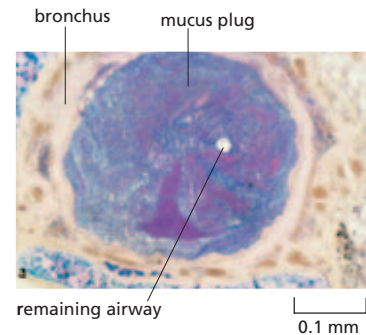
Activated Macrophages Contribute to the Inflammatory Response at Sites of Infection

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

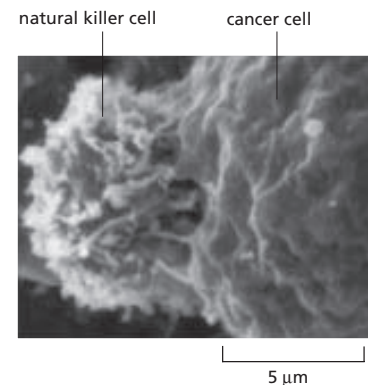


Figure 24–57 A natural killer (NK) cell attacking a cancer cell. The NK cell is the smaller cell on the left. This scanning electron micrograph was taken shortly after the NK cell attached, but before it induced the cancer cell to kill itself. (Courtesy of J.C. Hiserodt, in *Mechanisms of Cytotoxicity 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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25

The Adaptive Immune System

Our **adaptive immune system** saves us from certain death by infection. An infant born with a severely defective adaptive immune system will soon die unless extraordinary measures are taken to isolate it from a host of infectious agents, including bacteria, viruses, fungi, and parasites. All multicellular organisms need to defend themselves against infection by such potentially harmful invaders, collectively called **pathogens**. Invertebrates use relatively simple defense strategies that rely chiefly on protective barriers, toxic molecules, and phagocytic cells that ingest and destroy invading microorganisms (*microbes*) and larger parasites (such as worms). Vertebrates, too, depend on such **innate immune responses** as a first line of defense (discussed in Chapter 24), but they can also mount much more sophisticated defenses, called **adaptive immune responses**. In vertebrates, the innate responses call the adaptive immune responses into play, and both work together to eliminate the pathogens (Figure 25–1).

Whereas the innate immune responses are general defense reactions, the adaptive responses are highly specific to the particular pathogen that induced them, and they provide long-lasting protection. A person who recovers from measles, for example, is protected for life against measles by the adaptive immune system, although not against other common viruses, such as those that cause mumps or chickenpox. In this chapter, we focus on adaptive immune responses, and, unless we indicate otherwise, we use the term “immune responses” to refer to them.

Adaptive immune responses eliminate or destroy invading pathogens and any toxic molecules they produce. Because these responses are destructive, it is important that they are directed only against foreign molecules and not against molecules of the host itself. The adaptive immune system uses multiple mechanisms to avoid damaging responses against self molecules. Occasionally, however, these mechanisms fail, and the system turns against the host, causing *autoimmune diseases*, which can be fatal.

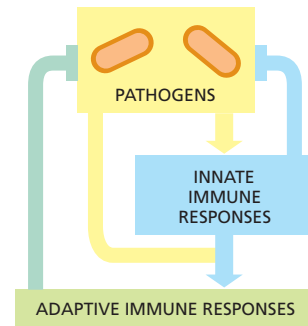
Many harmless foreign molecules enter the body, and it would be pointless and potentially dangerous to mount adaptive immune responses against them. Allergic conditions such as hayfever and allergic asthma are examples of deleterious adaptive immune responses against apparently harmless foreign molecules. An individual normally avoids such inappropriate responses because the innate immune system only calls adaptive immune responses into play when it recognizes conserved patterns of molecules that are specifically expressed by invading pathogens. The innate immune system can even distinguish between different classes of pathogens and recruit the most effective form of adaptive immune response to eliminate them.

Any substance capable of eliciting an adaptive immune response is referred to as an **antigen** (*antibody generator*). Most of what we know about such responses has come from studies in which an experimenter tricks the adaptive immune system of a laboratory animal (usually a mouse) into responding to a harmless foreign molecule, such as a foreign protein. The trick involves injecting the harmless molecule together with immunostimulants (usually microbial in origin) called *adjuvants*, which activate the innate immune system. This trick is called **immunization**. If administered in this way, almost any macromolecule, as long as it is foreign to the recipient, can induce an adaptive immune response

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Figure 25–1 Innate and adaptive immune responses. Innate immune responses are activated directly by pathogens and defend all multicellular organisms against infection. In vertebrates, pathogens, together with the innate immune responses they activate, stimulate adaptive immune responses, which then work together with innate immune responses to help fight the infection.



that is specific to that macromolecule. Remarkably, the adaptive immune system can distinguish between antigens that are very similar—such as between two proteins that differ in only a single amino acid, or between two optical isomers of the same molecule. Thus, the adaptive immune system recognizes the fine molecular details of macromolecules.

Adaptive immune responses are carried out by white blood cells called **lymphocytes**. There are two broad classes of such responses—*antibody responses* and *T-cell-mediated immune responses*—and different classes of lymphocytes, called B cells and T cells, respectively, carry them out. In **antibody responses**, B cells are activated to secrete antibodies, which are proteins called *immunoglobulins*. The antibodies circulate in the bloodstream and permeate the other body fluids, where they bind specifically to the foreign antigen that stimulated their production (Figure 25–2). Binding of antibody inactivates viruses and microbial toxins (such as tetanus toxin or diphtheria toxin) by blocking their ability to bind to receptors on host cells. Antibody binding also marks invading pathogens for destruction, mainly by making it easier for phagocytic cells of the innate immune system to ingest them.

In **T-cell-mediated immune responses**, the second class of adaptive immune responses, activated T cells react directly against a foreign antigen that is presented to them on the surface of a host cell, which is therefore referred to as an *antigen-presenting cell*. Remarkably, T cells can detect microbes hiding inside host cells and either kill the infected cells or help the infected cells or other cells to eliminate the microbes. The T cell, for example, might kill a virus-infected host cell that has viral antigens on its surface, thereby eliminating the infected cell before the virus has had a chance to replicate (see Figure 25–2). In other cases, the T cell produces signal molecules that either activate macrophages to destroy the microbes that they have phagocytosed or help activate B cells to make antibodies against the microbes.

We begin this chapter by discussing the general properties of lymphocytes. We then consider the functional and structural features of antibodies that enable them to recognize and neutralize extracellular microbes and the toxins they make. Next, we discuss how B cells can produce a virtually unlimited number of different antibody molecules. Finally, we consider the special features of T cells and the immune responses they mediate.

LYMPHOCYTES AND THE CELLULAR BASIS OF ADAPTIVE IMMUNITY

Lymphocytes are responsible for the astonishing specificity of adaptive immune responses. They occur in large numbers in the blood and lymph (the colorless fluid in the lymphatic vessels that connect the lymph nodes in the body to each other and to the bloodstream). They are also concentrated in **lymphoid organs**, such as the thymus, lymph nodes (also called lymph glands), spleen, and appendix (Figure 25–3). In this section, we discuss the general properties of lymphocytes that apply to both B cells and T cells.

Lymphocytes Are Required for Adaptive Immunity

There are about 2×10^{12} lymphocytes in the human body, making the immune system comparable in cell mass to the liver or the brain. Despite their abundance, their central role in adaptive immunity was not definitively demonstrated until

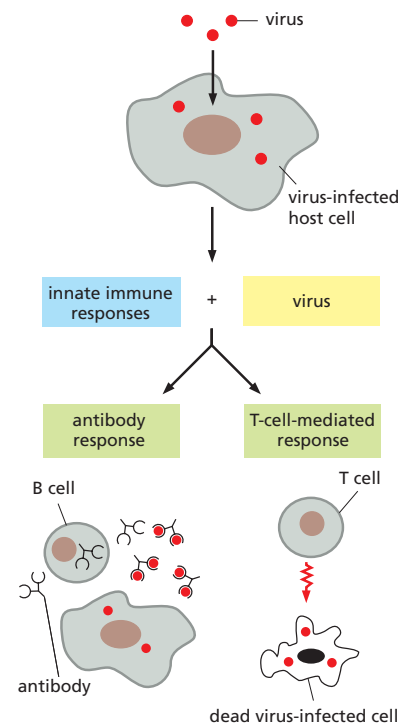


Figure 25–2 The two main classes of adaptive immune responses. Lymphocytes carry out both classes of responses. Here, the lymphocytes are responding to a viral infection. In one class of adaptive response, B cells secrete antibodies that neutralize the virus. In the other, a T-cell-mediated response, T cells kill the virus-infected cells. In both cases, innate immune responses help activate the adaptive immune responses through pathways that are not shown.

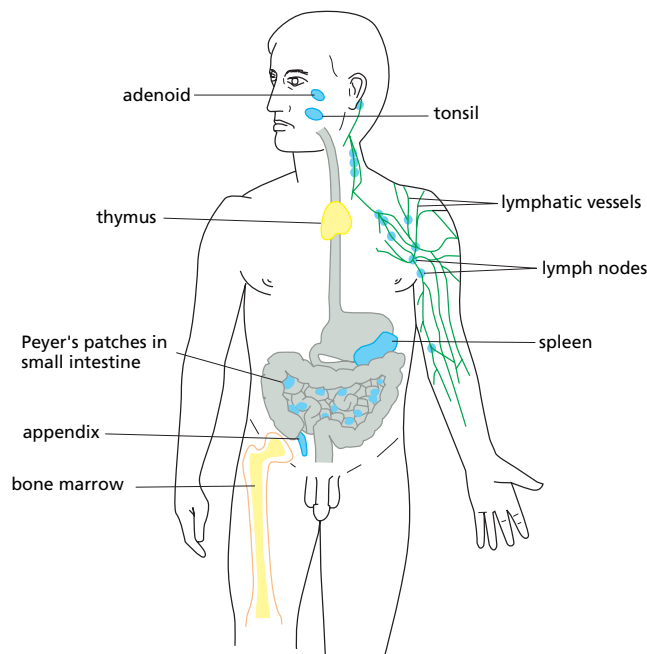


Figure 25–3 Human lymphoid organs. Lymphocytes develop in the thymus and bone marrow (yellow), which are therefore called *central (or primary) lymphoid organs*. The newly formed lymphocytes migrate from these primary organs to *peripheral (or secondary) lymphoid organs*, where they can react with foreign antigen. Only some of the peripheral lymphoid organs (blue) and lymphatic vessels (green) are shown; many lymphocytes, for example, are found in the skin and respiratory tract. As we discuss later, the lymphatic vessels ultimately empty into the bloodstream (not shown).

the late 1950s. The crucial experiments were performed in mice and rats that were heavily irradiated to kill most of their white blood cells, including lymphocytes. This treatment makes the animals unable to mount adaptive immune responses. Then, by transferring various types of cells into the animals it was possible to determine which cells reversed the deficiency. Lymphocytes were the only cell type able to restore the adaptive immune responses of irradiated animals, indicating that they are required for these responses (Figure 25–4).

The Innate and Adaptive Immune Systems Work Together

As mentioned earlier, lymphocytes usually respond to foreign antigens only if the **innate immune system** is first activated. As discussed in Chapter 24, the rapid innate immune responses to an infection depend largely on **pattern recognition receptors** made by cells of the innate immune system. These receptors recognize

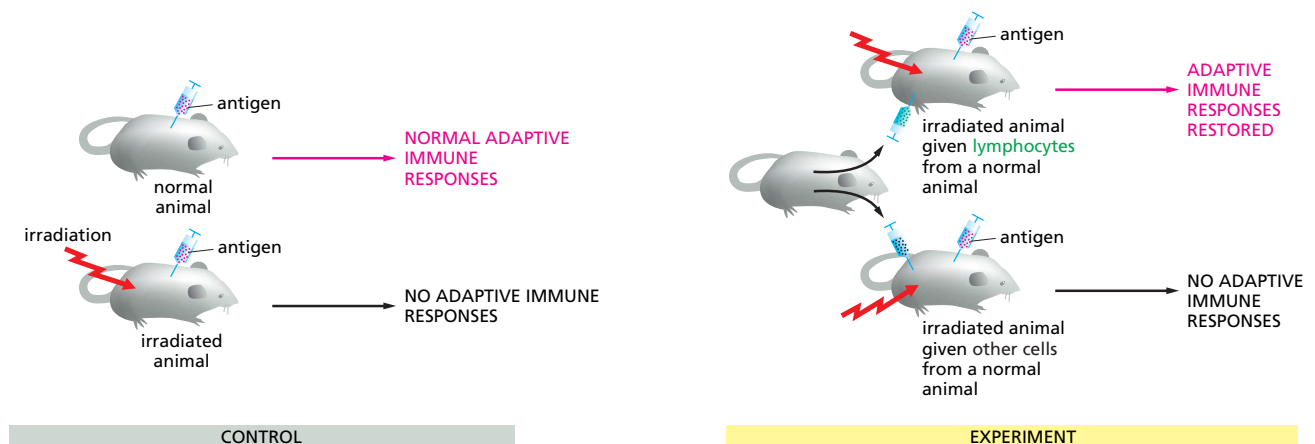
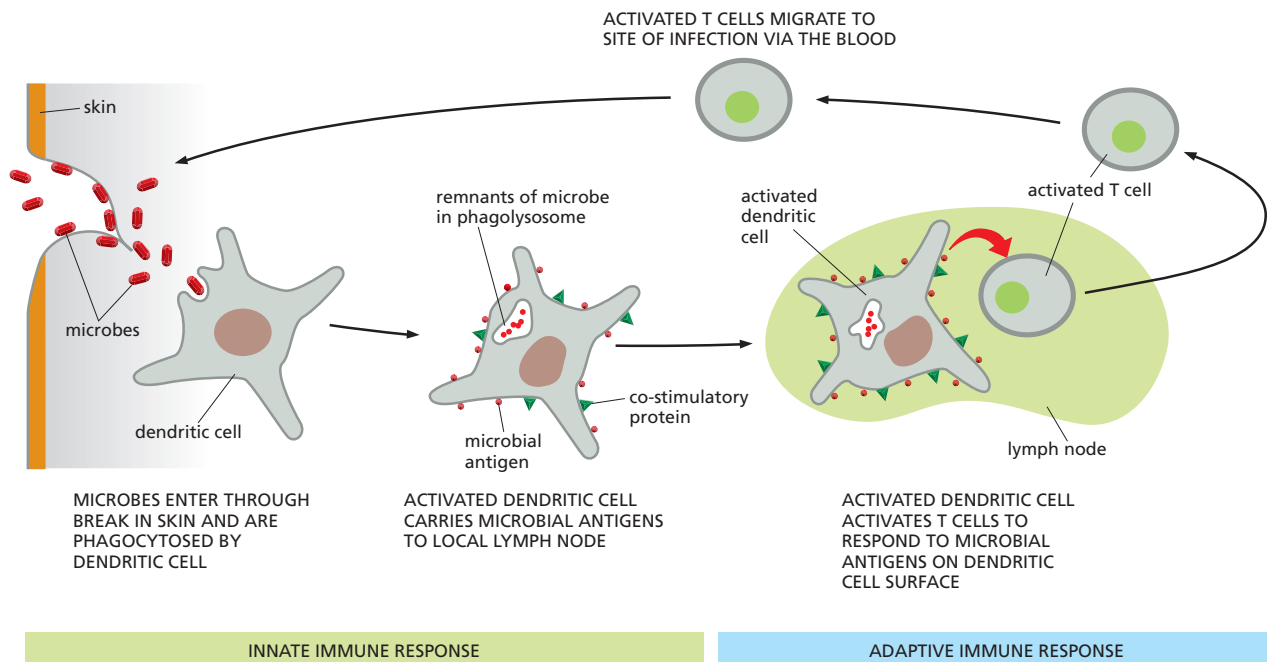


Figure 25–4 A classic experiment showing that lymphocytes are required for adaptive immune responses to foreign antigens. An important requirement of all such cell-transfer experiments is that cells are transferred between animals of the same *inbred strain*. Members of an inbred strain are genetically identical. If lymphocytes are transferred to a genetically different animal that has been irradiated, they react against the “foreign” antigens of the host and can kill the animal. In the experiment shown, the injection of lymphocytes restores both antibody and T-cell-mediated adaptive immune responses, indicating that lymphocytes are required for both types of responses.



microbe-associated molecules that are not present in the host organism, called *microbe-associated immunostimulants*. Because they often occur in repeating patterns, they are also called *pathogen-associated molecular patterns (PAMPs)*. PAMPs include repeated patterns of molecular structure in microbial nucleic acids, lipids, polysaccharides, and proteins.

Some of the pattern recognition receptors are present on the surface of professional phagocytic cells (phagocytes) such as macrophages and neutrophils, where they mediate the uptake of pathogens, which are then delivered to lysosomes for destruction. Others are secreted and bind to the surface of pathogens, marking them for destruction by either phagocytes or a system of blood proteins collectively called the *complement system* (discussed in Chapter 24). Still others, including the *Toll-like receptors (TLRs)* discussed in Chapter 24, activate intracellular signaling pathways that lead to the secretion of extracellular signal molecules that promote inflammation and help activate adaptive immune responses.

The cells of the vertebrate innate immune system that respond to PAMPs and activate adaptive immune responses most efficiently are **dendritic cells**. Present in most tissues, dendritic cells express high levels of TLRs and other pattern recognition receptors, and they function by presenting microbial antigens to T cells in peripheral lymphoid organs. In most cases, they recognize and phagocytose invading microbes or their products or fragments of infected cells at a site of infection and then migrate with their prey to a nearby lymph node; in other cases, they pick up microbes or their products directly in a peripheral lymphoid organ such as the spleen. In either case, the microbial PAMPs activate the dendritic cells so that they, in turn, can directly activate the T cells in peripheral lymphoid organs to respond to the microbial antigens displayed on the dendritic cell surface. Once activated, some of the T cells then migrate to the site of infection, where they help destroy the microbes (**Figure 25–5**). Other activated T cells remain in the lymphoid organ, where they help keep the dendritic cells active, help activate other T cells, and help activate B cells to make antibodies against the microbial antigens.

Thus, innate immune responses are activated mainly at sites of infection (or injury), whereas adaptive immune responses are activated mainly in peripheral lymphoid organs such as lymph nodes and spleen. Both types of responses work together to eliminate invading pathogens and foreign macromolecules.

Figure 25–5 How the innate immune system can help activate the adaptive immune system. Dendritic cells ingest invading microbes or their products at the site of an infection. The microbial PAMPs activate the dendritic cells to express *co-stimulatory proteins* on their surface and to migrate in lymphatic vessels to a nearby lymph node. In the lymph node, the activated dendritic cells activate the small fraction of T cells that express a receptor for the microbial antigens displayed on the dendritic cell surface. These T cells proliferate and some then migrate to the site of infection, where they help eliminate the microbes, by either helping to activate macrophages or killing infected cells (not shown).

B Lymphocytes Develop in the Bone Marrow; T Lymphocytes Develop in the Thymus

T cells and B cells derive their names from the organs in which they develop. T cells develop in the *thymus*, and B cells, in mammals, develop in the *bone marrow* in adults or the liver in fetuses.

Both T and B cells are thought to develop from the same *common lymphoid progenitor cells*. The common lymphoid progenitor cells themselves derive from multipotential *hemopoietic stem cells*, which give rise to all of the blood cells, including red blood cells, white blood cells, and platelets. These stem cells (discussed in Chapter 23) are located primarily in *hemopoietic tissues*—mainly the liver in fetuses and the bone marrow in adults.

T cells develop in the thymus from common lymphoid progenitor cells that migrate there from the hemopoietic tissues via the blood. In most mammals, including humans and mice, B cells develop from common lymphoid progenitor cells in the hemopoietic tissues themselves (Figure 25–6). Because they are sites where lymphocytes develop from precursor cells, the thymus and hemopoietic tissues are referred to as **central (primary) lymphoid organs** (see Figure 25–3).

As we discuss later, most lymphocytes die in the central lymphoid organs soon after they develop, without ever functioning. Others, however, mature and migrate via the blood to the **peripheral (secondary) lymphoid organs**—mainly, the lymph nodes, spleen, and epithelium-associated lymphoid tissues in the gastrointestinal tract, respiratory tract, and skin (see Figure 25–3). It is in these peripheral lymphoid organs that foreign antigens activate T and B cells (see Figure 25–6).

T and B cells become morphologically distinguishable from each other only after they have been activated by antigen. Resting T and B cells look very similar, even in an electron microscope. Both are small, only marginally bigger than red blood cells, and contain little cytoplasm (Figure 25–7A). After activation by an antigen, both proliferate and mature into *effector cells*. Effector B cells secrete antibodies. In their most mature form, called *plasma cells*, they are filled with an extensive rough endoplasmic reticulum that is busily making antibodies (Figure 25–7B). In contrast, effector T cells (Figure 5–7C) contain very little endoplasmic reticulum and do not secrete antibodies; instead, they secrete a variety of signal proteins called **cytokines**, which act as local mediators.

There are three main classes of T cells—cytotoxic T cells, helper T cells, and regulatory (suppressor) T cells. *Cytotoxic T cells* directly kill infected host cells. *Helper T cells* help activate macrophages, dendritic cells, B cells, and cytotoxic T cells by secreting a variety of cytokines and displaying a variety of co-stimulatory proteins on their surface. *Regulatory T cells* are thought to use similar strategies

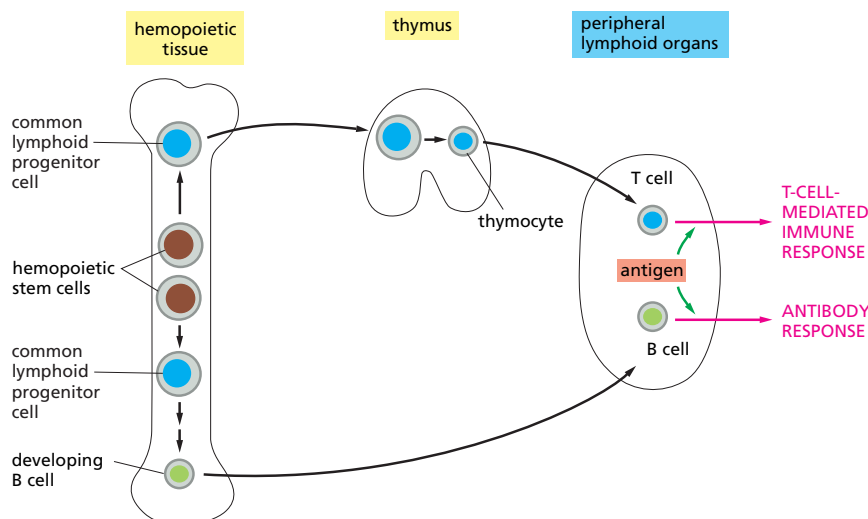
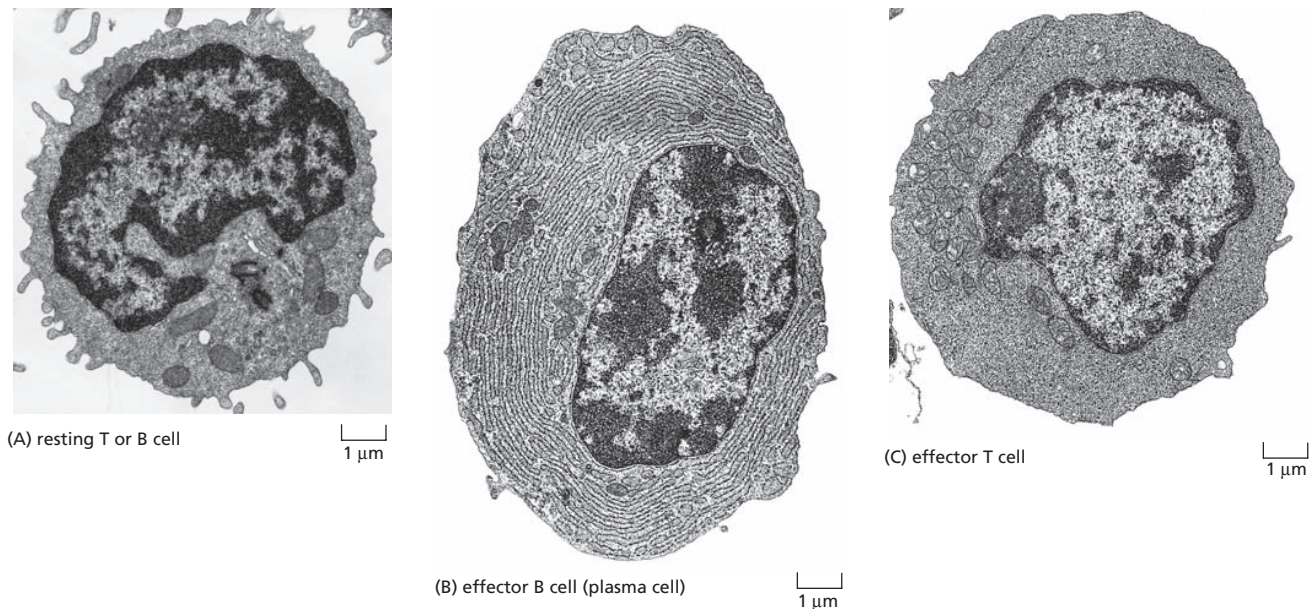


Figure 25–6 The development of T and B cells. The central lymphoid organs, where lymphocytes develop from common lymphoid progenitor cells, are labeled in yellow boxes. The common lymphoid progenitor cells develop from multipotent hemopoietic stem cells in the bone marrow. Some of the common lymphoid progenitor cells develop locally in the bone marrow into immature B cells, while others migrate to the thymus (via the bloodstream) where they develop into thymocytes (developing T cells). T cells and B cells are activated by foreign antigens mainly in peripheral lymphoid organs, such as lymph nodes or the spleen.



to inhibit the function of helper T cells, cytotoxic T cells, and dendritic cells. Thus, whereas B cells can act over long distances by secreting antibodies that are widely distributed by the bloodstream, T cells can migrate to distant sites, but, once there, they act only locally on neighboring cells.

The Adaptive Immune System Works by Clonal Selection

The most remarkable feature of the adaptive immune system is that it can respond to millions of different foreign antigens in a highly specific way. Human B cells, for example, can make more than 10^{12} different antibody molecules that react specifically with the antigen that induced their production. How do B cells produce such a diversity of specific antibodies? The answer began to emerge in the 1950s with the formulation of the **clonal selection theory**. According to this theory, an animal first randomly generates a vast diversity of lymphocytes and then selects for activation those lymphocytes that can react against the foreign antigens that the animal actually encounters. As each lymphocyte develops in a central lymphoid organ, it becomes committed to react with a particular antigen before ever being exposed to the antigen. It expresses this commitment in the form of cell-surface receptor proteins that specifically bind the antigen. When a lymphocyte encounters its antigen in a peripheral lymphoid organ, the binding of the antigen to the receptors activates the lymphocyte, causing it to proliferate, thereby producing many more cells with the same receptor—a process called *clonal expansion* (as cells derived from a common ancestor cell are referred to as a *clone*). The encounter with antigen also causes the cells to differentiate into *effector cells*. An antigen therefore selectively stimulates those cells that express complementary antigen-specific receptors and are thus already committed to respond to it (**Figure 25-8**). This arrangement is what makes adaptive immune responses antigen-specific.

Compelling evidence supports the main tenets of the clonal selection theory. But how can the adaptive immune system produce lymphocytes that collectively display such an enormous diversity of receptors, including ones that recognize synthetic molecules that never occur in nature? We shall see later that, in humans, the antigen-specific receptors on both T and B cells are encoded by genes that are assembled from a series of gene segments by a special form of genetic recombination that occurs early in a lymphocyte's development, before it has encountered antigen. This assembly process generates an enormous diversity of receptors and lymphocytes, thereby enabling the immune system to respond to an almost unlimited variety of antigens.

Figure 25-7 Electron micrographs of resting and effector lymphocytes.

(A) This resting lymphocyte could be either a T cell or a B cell, as these cells are difficult to distinguish morphologically until they have been activated to become effector cells. (B) An effector B cell (a plasma cell). It is filled with an extensive rough endoplasmic reticulum (ER), which is distended with antibody molecules. (C) An effector T cell, which has relatively little rough ER but is filled with free ribosomes. The three cells are shown at the same magnification. (A, courtesy of Dorothy Zucker-Franklin; B, courtesy of Carlo Grossi; A and B, from D. Zucker-Franklin et al., *Atlas of Blood Cells: Function and Pathology*, 2nd ed. Milan, Italy: Edi. Ermes, 1988; C, courtesy of Stefanello de Petris.)

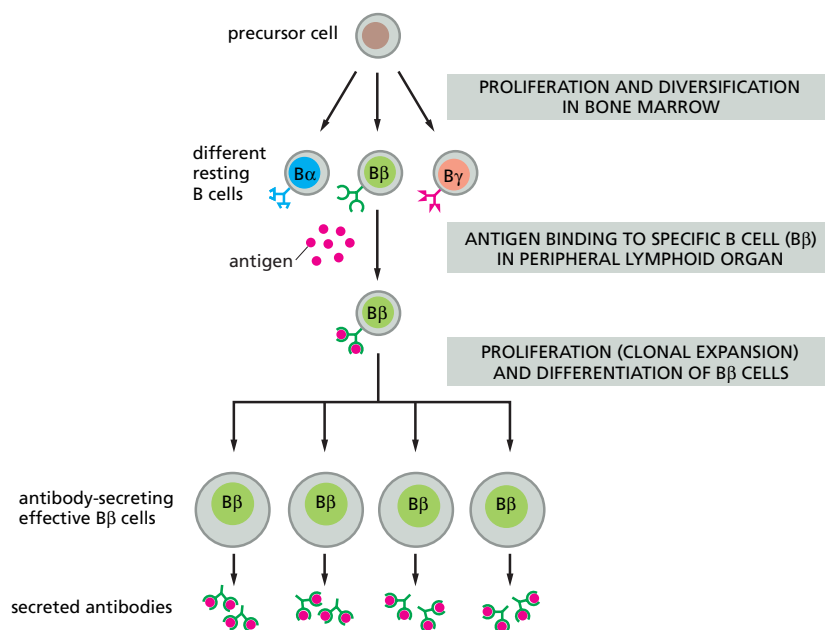


Figure 25–8 The clonal selection theory.

An antigen activates only those lymphocytes that are already committed to respond to it. A cell committed to respond to a particular antigen displays cell-surface receptors that specifically recognize the antigen. The human immune system is thought to consist of many millions of different lymphocyte clones, with cells within a clone expressing the same unique receptor. Before their first encounter with antigen, a clone would usually contain only one or a small number of cells. A particular antigen may activate hundreds of different clones. Although only B cells are shown here, T cells operate in a similar way. Note that the receptors on B cells are antibody molecules and that those on the B cells labeled “B β ” in this diagram bind the same antigen as do the antibodies secreted by the effector “B β ” cells.

Most Antigens Activate Many Different Lymphocyte Clones

Most large molecules, including virtually all proteins and many polysaccharides, can act as antigens. Those parts of an antigen that bind to the antigen-binding site on either an antibody molecule or a lymphocyte receptor are called **antigenic determinants** (or *epitopes*). Most antigens have a variety of antigenic determinants that can stimulate the production of antibodies, specific T cell responses, or both. Some determinants of an antigen produce a greater response than others, so that the reaction to them may dominate the overall response. Such determinants are called *immunodominant*.

Any antigen determinant is likely to activate many lymphocyte clones, each of which produces an antigen-binding site with its own characteristic affinity for the determinant. Even a relatively simple structure, like the *dinitrophenyl* (DNP) group in **Figure 25–9**, can be “looked at” in many ways. When it is coupled to a protein, as shown in the figure, it usually stimulates the production of hundreds of species of anti-DNP antibodies, each made by a different B cell clone. Such responses are said to be *polyclonal*. In *oligoclonal* responses, only a few clones are activated, and in *monoclonal* responses only a single B or T cell clone is activated. Monoclonal antibodies are widely used as tools in biology and medicine, but they have to be produced in a special way (see Figure 8–8), as the responses to most antigens are polyclonal.

Immunological Memory Involves Both Clonal Expansion and Lymphocyte Differentiation

The adaptive immune system, like the nervous system, can remember prior experiences. This is why we develop lifelong immunity to many common infectious diseases after our initial exposure to the pathogen, and it is why vaccination works. The same phenomenon can be demonstrated in experimental animals. If an animal is immunized once with antigen A, an immune response (antibody, T-cell-mediated, or both) appears after several days, rises rapidly and

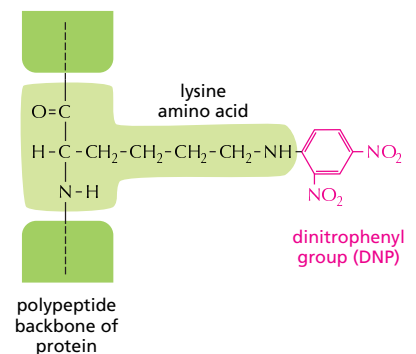


Figure 25–9 The dinitrophenyl (DNP) group. Although it is too small to induce an immune response on its own, when it is coupled covalently to a lysine side chain on a protein, as illustrated, DNP stimulates the production of hundreds of different species of antibodies that all bind specifically to it.

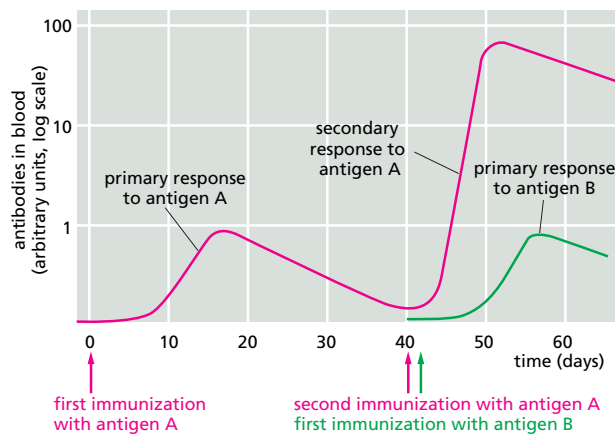


Figure 25–10 Primary and secondary antibody responses. The secondary response induced by a second exposure to antigen A is faster and greater than the primary response and is specific for A, indicating that the adaptive immune system has specifically remembered its previous encounter with antigen A. The same type of immunological memory is observed in T-cell-mediated responses. As we discuss later, the types of antibodies produced in the secondary response are different from those produced in the primary response, and these antibodies bind the antigen more tightly.

exponentially, and then, more gradually, declines. This is the characteristic course of a **primary immune response**, occurring on an animal's first exposure to an antigen. If, after some weeks, months, or even years have elapsed, the animal is immunized again with antigen A, it will usually produce a **secondary immune response** that differs from the primary response: the lag period is shorter, and the response is greater and more efficient. These differences indicate that the animal has “remembered” its first exposure to antigen A. If the animal is given a different antigen (for example, antigen B) instead of a second immunization with antigen A, the response is typical of a primary, and not a secondary, immune response. The secondary response must therefore reflect antigen-specific **immunological memory** for antigen A (Figure 25–10).

The clonal selection theory provides a useful conceptual framework for understanding the cellular basis of immunological memory. In an adult animal, the peripheral lymphoid organs contain a mixture of lymphocytes in at least three stages of maturation: *naïve cells*, *effector cells*, and *memory cells*. When **naïve cells** encounter their antigen for the first time, the antigen stimulates some of them to proliferate and differentiate into **effector cells**, which then carry out an immune response (effector B cells secrete antibody, while effector T cells either kill infected cells or influence the response of other cells). Some of the antigen-stimulated naïve cells multiply and differentiate into **memory cells**, which do not themselves carry out immune responses but are more easily and more quickly induced to become effector cells by a later encounter with the same antigen. When they encounter their antigen, memory cells (like naïve cells), give rise to either effector cells or more memory cells (Figure 25–11).

Thus, the primary response generates immunological memory because of clonal expansion, whereby the proliferation of antigen-stimulated naïve cells creates many memory cells, as well as because these memory cells are able to respond more sensitively, rapidly, and effectively to the same antigen than do naïve cells. And, unlike most effector cells, which die within days or weeks, memory cells can persist for the lifetime of the animal, even in the absence of their specific antigen, thereby providing lifelong immunological memory.

As we discuss later, memory B cells produce antibodies of different classes and of much higher affinity for antigen than those produced by naïve B cells. This is the main reason that secondary antibody responses are much more effective at eliminating pathogens than are primary antibody responses.

Although most effector T and B cells die after an immune response is over, some survive as effector cells and help provide long-term protection against the

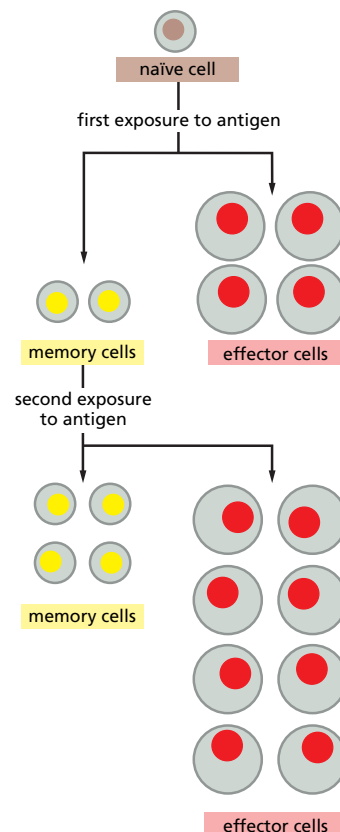


Figure 25–11 A model for the cellular basis of immunological memory. When stimulated by their specific antigen, naïve cells proliferate and differentiate. Most become effector cells, which function and then usually die, while others become memory cells. During a subsequent exposure to the same antigen, the memory cells respond more readily, rapidly, and efficiently than did the naïve cells: they proliferate and give rise to effector cells and to more memory cells. Memory T cells might also develop from effector cells (not shown).

pathogen. A small proportion of the plasma cells produced in a primary B cell response, for example, can survive for many months in the bone marrow, where they continue to secrete their specific antibodies into the bloodstream.

Immunological Tolerance Ensures That Self Antigens Are Not Normally Attacked

As discussed in Chapter 24, cells of the innate immune system use pattern recognition receptors to distinguish pathogens from the normal molecules of the host. The adaptive immune system has a far more difficult recognition task: it must be able to respond specifically to an almost unlimited number of foreign macromolecules, while avoiding responding to the large number of molecules made by the host organism itself. How is this possible? It helps that self molecules do not induce the innate immune reactions required to activate the adaptive immune system. But even when an infection or tissue injury triggers innate reactions, the vast excess of self molecules present normally fail to induce an adaptive immune response. Why not?

One answer is that the adaptive immune system has “learned” not to respond to self antigens. Transplantation experiments provide one line of evidence for this learning process. When tissues are transplanted from one individual to another (and the two individuals are not identical twins), the immune system of the recipient usually recognizes the donor cells as foreign and destroys them. (For reasons we discuss later, the foreign antigens on the donor cells are so powerful that they can stimulate adaptive immune responses in the absence of infection, injury, or an adjuvant.) If, however, we introduce cells from one strain of mouse into a newborn mouse of another strain, some of these cells survive for most of the recipient animal's life, and the recipient will now accept a graft from the original donor, even though it rejects “third-party” grafts. Apparently, nonself antigens can, in particular circumstances, induce the immune system to become specifically unresponsive to them. This antigen-specific unresponsiveness to foreign antigens is known as *acquired immunological tolerance* (Figure 25–12).

The unresponsiveness of an animal's adaptive immune system to its own macromolecules (*natural immunological tolerance*, or **self-tolerance**) is acquired in the same way. Normal mice, for example, cannot make an immune response against one of their own protein components of the complement system called C5 (discussed in Chapter 24). But, mutant mice that lack the gene encoding C5 (but are otherwise genetically identical to the normal mice) can make a strong immune response to this blood protein when immunized with it. Similarly, humans that lack a normal gene that codes for the clotting protein Factor VIII (and therefore bleed excessively) make antibodies against the protein when it is administered to them to control bleeding.

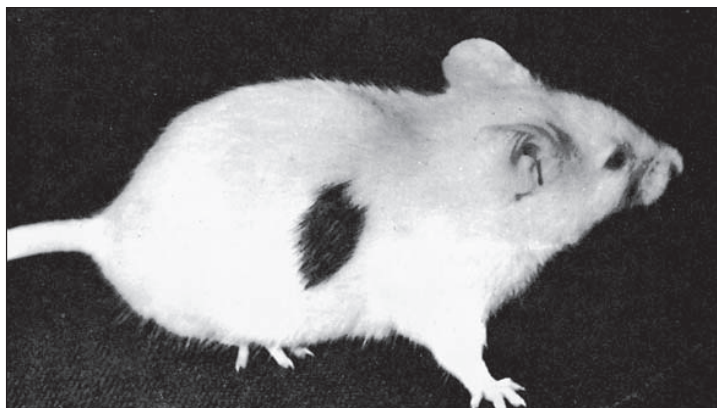
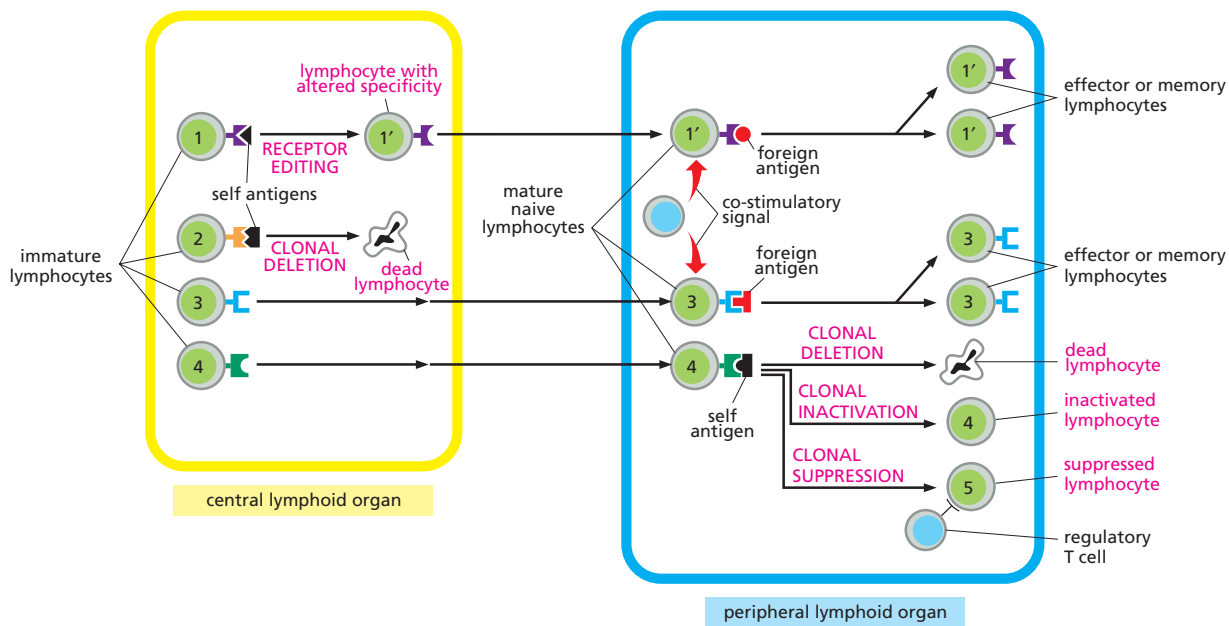


Figure 25–12 Acquired immunological tolerance. The skin graft seen here was transplanted from an adult brown mouse to an adult white mouse. It has survived for many weeks only because the white mouse, at the time of its birth, received an injection of bone marrow cells from the brown mouse and therefore became immunologically tolerant to them. Some of the bone marrow cells (and their progeny) from the brown mouse persist in the adult white mouse and continue to induce tolerance in newly formed lymphocytes that would otherwise react against the brown skin. (Courtesy of Leslie Brent, from I. Roitt, *Essential Immunology*, 6th ed. Oxford, UK: Blackwell Scientific, 1988.)



The natural immunological tolerance for a particular self molecule persists only for as long as the molecule remains present in the body. If a self molecule such as C5 is experimentally removed from an adult mouse, the animal gains the ability to respond to it after a few weeks or months. Thus, the immune system is genetically capable of responding to self molecules but learns not to do so.

Self-tolerance depends on a number of distinct mechanisms:

1. In *receptor editing*, developing lymphocytes that recognize self molecules (*self-reactive lymphocytes*) change their antigen receptors so that they no longer recognize self antigens.
2. In *clonal deletion*, self-reactive lymphocytes die by apoptosis when they bind their self antigen.
3. In *clonal inactivation* (also called clonal anergy), self-reactive lymphocytes become functionally inactivated when they encounter their self antigen.
4. In *clonal suppression*, regulatory T cells suppress the activity of self-reactive lymphocytes.

Some of these mechanisms—especially the first two, clonal deletion and receptor editing—operate in central lymphoid organs when newly formed self-reactive lymphocytes first encounter their self antigens, and they are largely responsible for the process of *central tolerance*. Clonal inactivation and clonal suppression, by contrast, operate mainly when lymphocytes encounter their self antigens in peripheral lymphoid organs, and they are responsible for the process of *peripheral tolerance*. Clonal deletion and clonal inactivation, however, are known to operate both centrally and peripherally (**Figure 25–13**).

Why does the binding of a self antigen lead to tolerance rather than activation? The answer is still not completely known. As we discuss later, to activate a lymphocyte in a peripheral lymphoid organ, the cell must do more than bind its antigen: it must also receive membrane-bound and secreted co-stimulatory signals (the secreted signals are various cytokines). Both types of signals are provided by a helper T cell in the case of a B lymphocyte and by an activated dendritic cell in the case of a T lymphocyte. Because the production of these signals is usually triggered by exposure to a pathogen, a self-reactive lymphocyte normally encounters its self antigen in the absence of such signals. Under these conditions, a B cell interacting with its antigen or a T cell interacting with its antigen on the surface of a nonactivated dendritic cell will not only fail to be activated, it will often be rendered tolerant—being either killed, inactivated, or actively suppressed by a regulatory T cell (see **Figure 25–13**). As we discuss later, in peripheral lymphoid organs, both T cell tolerance and activation usually occur on the surface of a dendritic cell.

Figure 25–13 Mechanisms of immunological tolerance to self antigens. When a self-reactive immature lymphocyte binds its self antigen in the central lymphoid organ where the cell is produced, it may alter its antigen receptor so that it is no longer self-reactive (cell 1). This process is called receptor editing and is thought to occur mainly in developing B cells. Alternatively, the cell may die by apoptosis, a process called clonal deletion (cell 2). Because these two forms of tolerance (shown on the left) occur in central lymphoid organs, they are called *central tolerance*.

When a self-reactive naïve lymphocyte escapes tolerance in the central lymphoid organ and binds its self antigen in a peripheral lymphoid organ (cell 4), it will generally not be activated, because the binding usually occurs in the absence of appropriate co-stimulatory signals; instead, the cell may die by apoptosis (often after a period of proliferation), be inactivated, or be suppressed by regulatory T cells (if the self-reactive lymphocyte is an effector T cell). These forms of tolerance shown on the right are called *peripheral tolerance*.

The tolerance mechanisms sometimes break down, causing T or B cells (or both) to react against the organism's own tissue antigens. *Myasthenia gravis* is an example of such an **autoimmune disease**. Affected individuals make antibodies against the acetylcholine receptors on their own skeletal muscle cells. These antibodies interfere with the normal functioning of the receptors so that the patients become weak and may die because they cannot breathe. Similarly, in *childhood (type 1) diabetes*, immune reactions against insulin-secreting cells in the pancreas kill these cells, leading to severe insulin deficiency.

For the most part, the mechanisms responsible for the breakdown of tolerance to self antigens in autoimmune diseases are unknown. It is thought, however, that activation of the innate immune system by infection or tissue injury may help trigger anti-self responses in individuals with defects in their self-tolerance mechanisms, leading to autoimmunity.

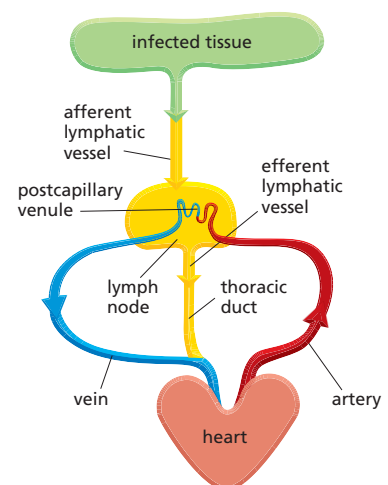
Lymphocytes Continuously Circulate Through Peripheral Lymphoid Organs

Pathogens generally enter the body through an epithelial surface, usually through the skin, gut, or respiratory tract. To induce an adaptive immune response, microbial antigens must travel from these entry points to a peripheral lymphoid organ, such as a lymph node or the spleen, the sites where lymphocytes are activated (see Figure 25–5). The route and destination depend on the site of entry. Lymphatic vessels (see Figure 25–3) carry antigens that enter through the skin or respiratory tract to local lymph nodes; antigens that enter through the gut end up in gut-associated peripheral lymphoid organs such as Peyer's patches; and the spleen filters out antigens that enter the blood. As discussed earlier, in many cases, dendritic cells will carry the antigen from the site of infection to the peripheral lymphoid organ, where they play a crucial part in activating T cells (see Figure 25–5).

But only a tiny fraction of the total lymphocyte population can recognize a particular microbial antigen in a peripheral lymph organ (estimated to be between 1/10,000 and 1/100,000 of each class of lymphocyte). How do these rare cells find an antigen-presenting cell displaying their antigen? The answer is that the lymphocytes continuously circulate between one peripheral lymphoid organ and another via the lymph and blood. In a lymph node, for example, lymphocytes continually leave the bloodstream by squeezing out between specialized endothelial cells lining small veins called *postcapillary venules*. After percolating through the node, they accumulate in small lymphatic vessels that leave the node and connect with other lymphatic vessels that pass through other lymph nodes downstream (see Figure 25–3). Passing into larger and larger vessels, the lymphocytes eventually enter the main lymphatic vessel (the *thoracic duct*), which carries them back into the blood (Figure 25–14).

The continuous recirculation between the blood and lymph ends only if a lymphocyte is activated by its specific antigen in a peripheral lymphoid organ. Now the lymphocyte remains in the peripheral lymphoid organ, where it proliferates and differentiates into either effector cells or memory cells. Many of the effector T cells leave the lymphoid organ via the lymph and migrate through the

Figure 25–14 The path followed by lymphocytes as they continuously circulate between the lymph and blood. The circulation through a lymph node (yellow) is shown here. Microbial antigens are usually carried into the lymph node by dendritic cells (not shown), which enter the node via afferent lymphatic vessels draining an infected tissue (green). T and B cells, by contrast, enter the lymph node via an artery and migrate out of the bloodstream through postcapillary venules. Unless they encounter their antigen, the T and B cells leave the lymph node via efferent lymphatic vessels, which eventually join the thoracic duct. The thoracic duct empties into a large vein carrying blood to the heart to complete the circulation process for T and B cells. A typical circulation cycle for these lymphocytes takes about 12–24 hours.



blood to the site of infection (see Figure 25–5), whereas others stay in the lymphoid organ and help activate B cells or other T cells there. Some effector B cells (plasma cells) remain in the peripheral lymphoid organ and secrete antibodies into the blood for days until they die; others migrate to the bone marrow, where they secrete antibodies into the blood for months or years. The memory T and B cells produced join the recirculating pool of lymphocytes.

Lymphocyte recirculation depends on specific interactions between the lymphocyte cell surface and the surface of the endothelial cells lining the blood vessels in the peripheral lymphoid organs. Many cell types in the blood come into contact with the specialized endothelial cells lining the postcapillary venules in lymph nodes, but only lymphocytes adhere and then migrate out of the bloodstream into the nodes. The lymphocytes initially adhere to the endothelial cells via *homing receptors* that bind to specific ligands (often called *counterreceptors*) on the endothelial cell surface. Lymphocyte migration into lymph nodes depends on a homing receptor protein called *L-selectin*, a member of the selectin family of cell-surface lectins. This protein binds to specific sugar groups on a counterreceptor that is expressed exclusively on the surface of the specialized endothelial cells lining the postcapillary venules in lymph nodes, causing the lymphocytes to adhere weakly to the endothelial cells and to roll slowly along their surface. The rolling continues until another, much stronger adhesion system is called into play by the chemoattractant proteins (called *chemokines*; see below) secreted by endothelial cells. This strong adhesion is mediated by members of the *integrin* family of cell adhesion molecules, which become activated on the lymphocyte surface. Now the lymphocytes stop rolling and crawl out of the blood vessel into the lymph node (Figure 25–15). Both selectins and integrins are discussed in Chapter 19.

Chemokines are a type of cytokine. They are small, secreted, positively charged proteins that have a crucial role in guiding the migrations of various types of cells, including white blood cells. <ACCG> They are all structurally related and bind to the surface of endothelial cells, as well as to negatively charged proteoglycans of the extracellular matrix in organs. By binding to G-protein-coupled chemokine receptors (discussed in Chapter 15) on the surface of specific blood cells, chemokines attract these cells from the bloodstream into an organ, guide them to specific locations within the organ, and then help stop migration. (Unfortunately, the AIDS virus, HIV, also binds to certain chemokine receptors, as well as to the CD4 co-receptor that we discuss later, and thus allows the virus to infect white blood cells.) The T and B cells initially enter the same region of a lymph node, but then different chemokines guide them to separate regions of the node—T cells to the *paracortex* and B cells to *lymphoid follicles* (Figure 25–16).

Unless they encounter their antigen, both T and B cells soon leave the lymph node via efferent lymphatic vessels. If they encounter their antigen, however, they are stimulated to display adhesion receptors that trap the cells in the node; the cells accumulate at the junction between the T cell and B cell areas, where the rare specific T and B cells can interact, leading to their proliferation and differentiation

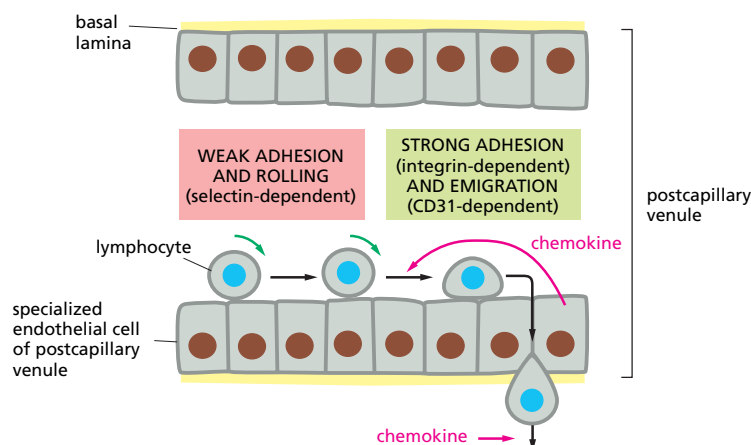


Figure 25–15 Migration of a lymphocyte out of the bloodstream into a lymph node. <CCCC> A circulating lymphocyte adheres weakly to the surface of the specialized endothelial cells lining a postcapillary venule in a lymph node. This initial adhesion is mediated by L-selectin on the lymphocyte surface. The adhesion is sufficiently weak to enable the lymphocyte to roll along the surface of the endothelial cells, pushed along by the flow of blood. Stimulated by chemokines secreted by the endothelial cells (curved red arrow), the lymphocyte rapidly activates a stronger adhesion system, mediated by an integrin. This strong adhesion enables the cell to stop rolling. The lymphocyte then uses an Ig-like cell adhesion protein (CD31) to bind to the junctions between adjacent endothelial cells and migrate out of the venule. CD31 is located both on the surface of the lymphocyte and at the junctions between the endothelial cells. The subsequent migration of the lymphocytes in the lymph node depends on chemokines produced within the node (straight red arrow).

The migration of other white blood cells out of the bloodstream into sites of infection occurs in a similar way.

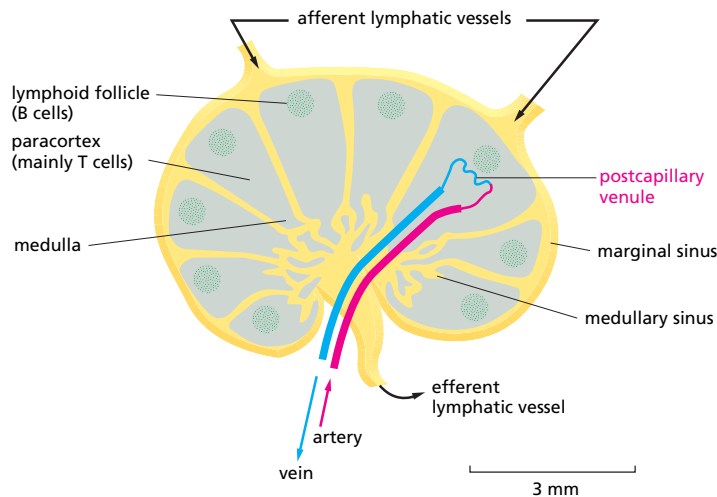


Figure 25–16 A simplified drawing of a human lymph node. B cells are primarily clustered in structures called lymphoid follicles, whereas T cells are found mainly in the paracortex. Chemokines attract both types of lymphocytes into the lymph node from the blood via postcapillary venules (see Figure 25–15). T and B cells then migrate to their respective areas, attracted by different chemokines. If they do not encounter their specific antigen, both T cells and B cells then enter the medullary sinuses and leave the node via the efferent lymphatic vessel. This vessel ultimately empties into the bloodstream, allowing the lymphocytes to begin another cycle of circulation through a peripheral lymphoid organ (see Figure 25–14).

If they encounter their specific antigen, T and B cells are retained in the node and are activated to become effector cells or memory cells; T cells and B cells responding to the same pathogen can interact in and around lymphoid follicles.

into either effector cells or memory cells. Many of the effector cells leave the node, expressing different chemokine receptors that help guide them to their new destinations—T cells to sites of infection and B cells to the bone marrow.

Summary

Innate immune responses are triggered at sites of infection by pathogen-associated molecular patterns (PAMPs), which are recognized by pattern recognition receptors made by cells of the innate immune system. In addition to fighting infection directly, these innate immune responses help activate adaptive immune responses in peripheral lymphoid organs. Unlike innate immune responses, adaptive responses display immunological memory and thereby provide specific and long-lasting protection against the particular pathogen that induced them.

The adaptive immune system is composed of many millions of lymphocyte clones, with the cells in each clone sharing a unique cell-surface receptor that enables them to bind a particular antigen. The binding of antigen to these receptors, however, is usually not sufficient to stimulate a lymphocyte to proliferate and differentiate into an effector cell that can help eliminate the pathogen. Membrane-bound co-stimulatory signals and a variety of secreted signals (cytokines) provided by another specialized cell in a peripheral lymphoid organ are also required. Helper T cells provide such signals for B cells, while dendritic cells usually provide them for T cells. Effector B cells secrete antibodies, which can act over long distances to help eliminate extracellular pathogens and their toxins. Effector T cells, by contrast, act locally to either kill infected host cells or help other cells to eliminate the pathogen. As part of the adaptive immune response, some lymphocytes proliferate and differentiate into memory cells, which are able to respond faster and more efficiently the next time the same pathogen invades. Both B and T cells circulate continuously between one peripheral lymphoid organ and another via the blood and lymph. Only if they encounter their specific foreign antigen in a peripheral lymphoid organ do they stop migrating, proliferate, and differentiate into effector cells or memory cells. Lymphocytes that would react against self molecules either alter their receptors or are eliminated, inactivated, or suppressed by regulatory T cells, so that the adaptive immune system normally avoids attacking the molecules and cells of the host.

B CELLS AND ANTIBODIES

Vertebrates inevitably die of infection if they are unable to make antibodies. Antibodies defend us against infection by binding to viruses and microbial toxins, thereby inactivating them (see Figure 25–2). When antibodies bind to invading pathogens, they also recruit some of the components of the innate immune system, including various types of white blood cells and components of the

complement system (discussed in Chapter 24). The white blood cells and activated complement components work together to attack the invaders.

Synthesized exclusively by B cells, antibodies are produced in billions of forms, each with a different amino acid sequence. Collectively called **immunoglobulins** (abbreviated as **Ig**), they are among the most abundant protein components in the blood, constituting about 20% of the total protein in plasma by weight. Mammals make five classes of antibodies, each of which mediates a characteristic biological response following antigen binding. In this section, we discuss the structure and function of antibodies and how they interact with antigen.

B Cells Make Antibodies as Both Cell-Surface Antigen Receptors and Secreted Proteins

All antibody molecules made by an individual B cell have the same antigen-binding site. The first antibodies made by a newly formed B cell are not secreted but are instead inserted into the plasma membrane, where they serve as receptors for antigen. Each B cell has approximately 10^5 such receptors in its plasma membrane. As we discuss later, each of these receptors is stably associated with a complex of transmembrane proteins that activate intracellular signaling pathways when antigen on the outside of the cell binds to the receptor.

Each B cell clone produces a single species of antibody, with a unique antigen-binding site. When an antigen (with the aid of a helper T cell) activates a naïve or a memory B cell, that B cell proliferates and differentiates into an antibody-secreting effector cell. Such effector cells make and secrete large amounts of soluble (rather than membrane-bound) antibody, which has the same unique antigen-binding site as the cell-surface antibody that served earlier as the antigen receptor (Figure 25–17). Effector B cells can begin secreting antibody while they are still small lymphocytes, but the end stage of their maturation pathway is a large plasma cell (see Figure 25–7B), which continuously secretes antibodies at the astonishing rate of about 5000 molecules per second. Although most plasma cells die after several days, some survive in the bone marrow for months or years and continue to secrete antibodies into the blood, helping to provide long-term protection against the pathogen that stimulated their production.

A Typical Antibody Has Two Identical Antigen-Binding Sites

The simplest antibodies are Y-shaped molecules with two identical antigen-binding sites, one at the tip of each arm of the Y (Figure 25–18). Because of their two antigen-binding sites, they are described as *bivalent*. As long as an antigen has three or more antigenic determinants, bivalent antibody molecules can cross-link it into a large lattice (Figure 25–19) that macrophages can readily phagocytose and degrade. The efficiency of antigen binding and cross-linking is greatly increased by the flexible *hinge region* in most antibodies, which allows the distance between the two antigen-binding sites to vary (Figure 25–20).

The protective effect of antibodies is not due simply to their ability to bind and cross-link antigen. The tail of the Y-shaped molecule mediates many other activities of antibodies. As we discuss later, antibodies with the same antigen-binding sites can have any one of several different tail regions. Each type of tail region gives the antibody different functional properties, such as the ability to activate the complement system, to bind to phagocytic cells, or to cross the placenta from mother to fetus.

An Antibody Molecule Is Composed of Heavy and Light Chains

The basic structural unit of an antibody molecule consists of four polypeptide chains, two identical **light (L) chains** (each containing about 220 amino acids) and two identical **heavy (H) chains** (each usually containing about 440 amino

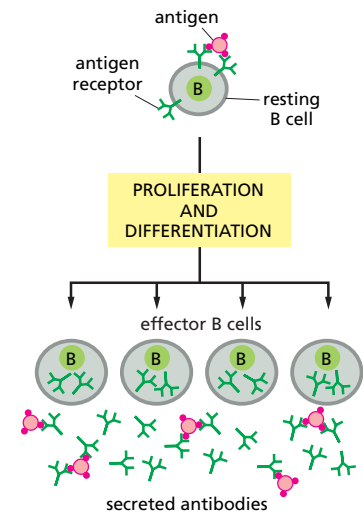


Figure 25–17 The membrane-bound and secreted antibodies made by a B cell clone. When an antigen (aided by a helper T cell—not shown) binds to and thereby activates either a naïve or a memory B cell, the cell proliferates and differentiates into effector cells. The effector cells produce and secrete antibodies with a unique antigen-binding site, which is the same as that of their original membrane-bound antibody that served as their antigen receptors.

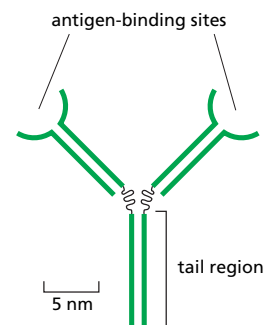


Figure 25–18 A simple representation of an antibody molecule. Note that its two antigen-binding sites are identical.

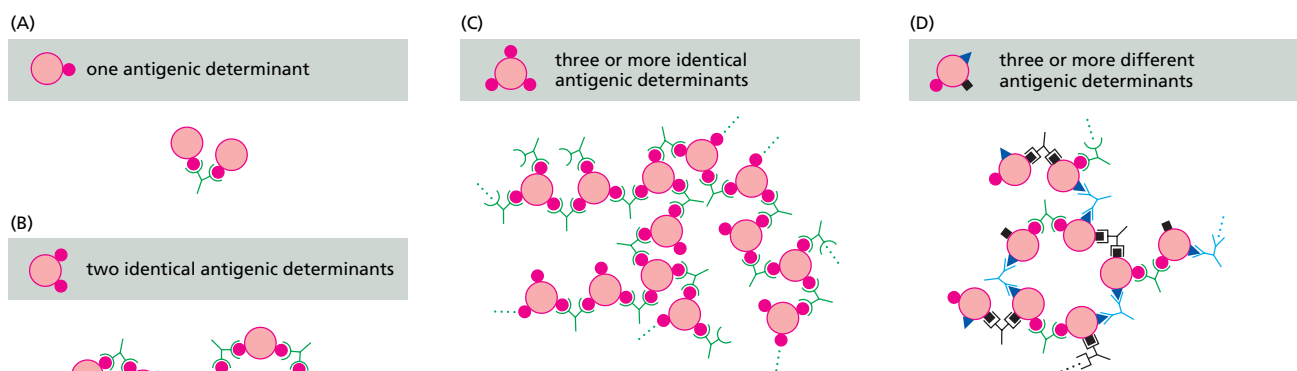


Figure 25-19 Antibody–antigen interactions. Because antibodies have two identical antigen-binding sites, they can cross-link antigens. The types of antibody–antigen complexes that form depend on the number of antigenic determinants on the antigen. (A–C) A single species of antibody (a monoclonal antibody) is shown binding to antigens containing one, two, or three copies of a single type of antigenic determinant. Antigens with two identical antigenic determinants can form small cyclic complexes or linear chains with the antibodies, while antigens with three or more identical antigenic determinants can form large three-dimensional lattices that readily precipitate out of solution. (D) Most antigens have many different antigenic determinants (see Figure 25-29A), and different antibodies that recognize different determinants can cooperate in cross-linking the antigen into large three-dimensional lattices.

acids). A combination of noncovalent and covalent (disulfide) bonds holds the four chains together. The molecule is composed of two identical halves, each with the same antigen-binding site. Both light and heavy chains usually cooperate to form the antigen-binding surface (**Figure 25-21**).

There Are Five Classes of Antibody Heavy Chains, Each with Different Biological Properties

In mammals, there are five *classes* of antibodies, IgA, IgD, IgE, IgG, and IgM, each with its own class of heavy chain— α , δ , ϵ , γ , and μ , respectively. IgA molecules have α chains, IgG molecules have γ chains, and so on. In addition, there are a number of subclasses of IgG and IgA immunoglobulins; for example, there are four human IgG subclasses (IgG1, IgG2, IgG3, and IgG4), having γ_1 , γ_2 , γ_3 , and γ_4 heavy chains, respectively. The various heavy chains give a distinctive conformation to the hinge and tail regions of antibodies, so that each class (and subclass) has characteristic properties of its own.

IgM, which has μ heavy chains, is always the first class of antibody that a developing B cell makes, although many B cells eventually switch to making other classes of antibody when an antigen stimulates them (discussed below). The first cells in the B cell lineage that make Ig are *pro-B cells*, which make only μ chains. They give rise to *pre-B cells*, in which the μ chains associate with so-called *surrogate light chains* (substituting for genuine light chains) and insert into the plasma membrane. Signaling from this pre-B cell receptor is required for the cell to progress to the next stage of development, where it makes bona fide light chains. The light chains combine with the μ chains, replacing the surrogate light chains, to form four-chain IgM molecules (each with two μ chains and two light chains). These molecules then insert into the plasma membrane, where they function as receptors for antigen. At this point, the cell is called an *immature naïve B cell*. After leaving the bone marrow, the cell starts to produce cell-surface **IgD** molecules as well, with the same antigen-binding site as the IgM molecules. It is now called a *mature naïve B cell*. It is this cell that can respond to foreign antigen in peripheral lymphoid organs (**Figure 25-22**).

IgM is not only the first class of antibody to appear on the surface of a developing B cell. It is also the major class secreted into the blood in the early stages of a primary antibody response, on first exposure to an antigen. (Unlike IgM, IgD

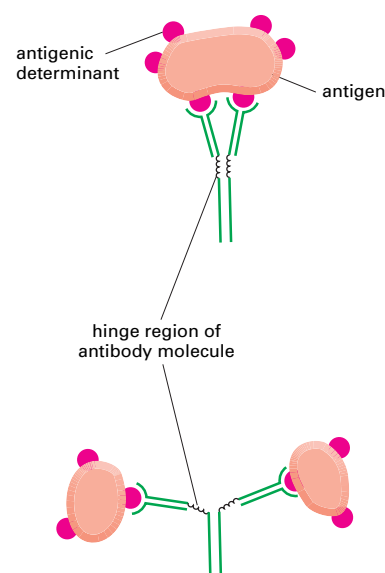


Figure 25-20 The hinge region of an antibody molecule. Because of its flexibility, the hinge region improves the efficiency of antigen binding and cross-linking.

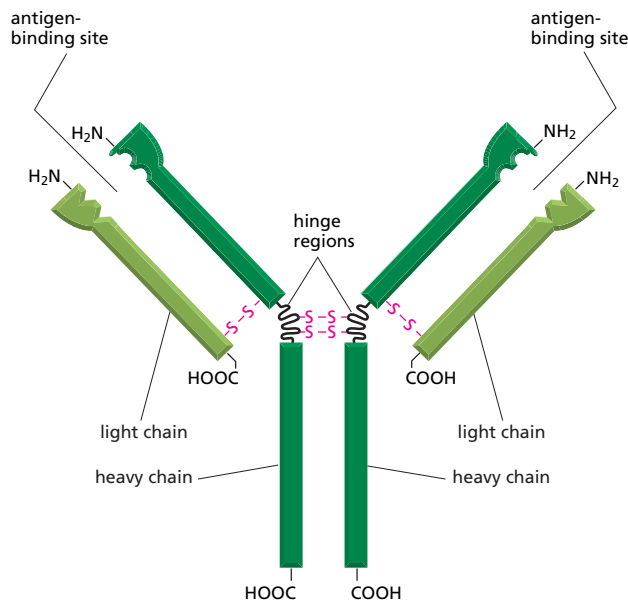


Figure 25-21 A schematic drawing of a bivalent antibody molecule. It is composed of four polypeptide chains—two identical heavy chains and two identical light chains. The two antigen-binding sites are identical, each formed by the N-terminal region of a light chain and the N-terminal region of a heavy chain. The two heavy chains also form both the tail and hinge region of the antibody.

molecules are secreted in only small amounts and seem to function mainly as cell-surface receptors for antigen.) In its secreted form, IgM is a pentamer composed of five four-chain units, giving it a total of 10 antigen-binding sites. Each pentamer contains one copy of another polypeptide chain, called a *J (joining) chain*. The J chain is produced by IgM-secreting cells and is covalently inserted between two adjacent tail regions (Figure 25-23).

When an antigen with multiple identical antigenic determinants (see Figure 25-19) binds to a single secreted pentameric IgM molecule, it alters the structure of the pentamer, allowing it to activate the complement system. As discussed in Chapter 24, when the antigen is on the surface of an invading pathogen, this activation of complement can either mark the pathogen for phagocytosis or kill it directly. As we discuss later, complement activation can also greatly increase the immune response to an antigen: the binding of an activated complement component to an antibody-antigen complex, for example, can increase the ability of the antigen to stimulate a B cell response more than a thousand fold (see Figure 25-71A).

The major class of immunoglobulin in the blood is **IgG**, which is a four-chain monomer (see Figure 25-21) produced in large quantities during secondary antibody responses. Besides activating complement, the tail region of an IgG molecule binds to specific receptors on macrophages and neutrophils.

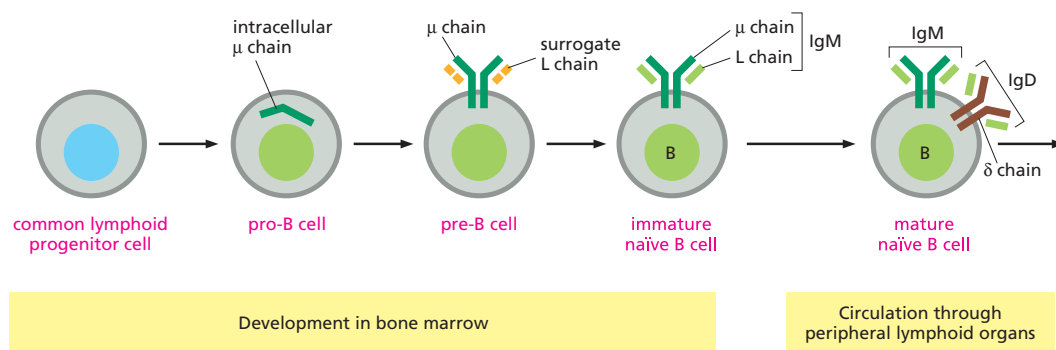


Figure 25-22 The main stages in B cell development. All of the stages shown occur independently of antigen. The pro-B cell makes μ chains, but they remain in the endoplasmic reticulum until surrogate light chains are made. Although not shown, all of the cell-surface Ig molecules are associated with transmembrane proteins that help convey signals to the cell interior (see Figure 25-70). When they are activated by their specific foreign antigen and helper T cells in peripheral lymphoid organs, mature naïve B cells proliferate and differentiate into either antibody-secreting cells or memory cells (not shown).

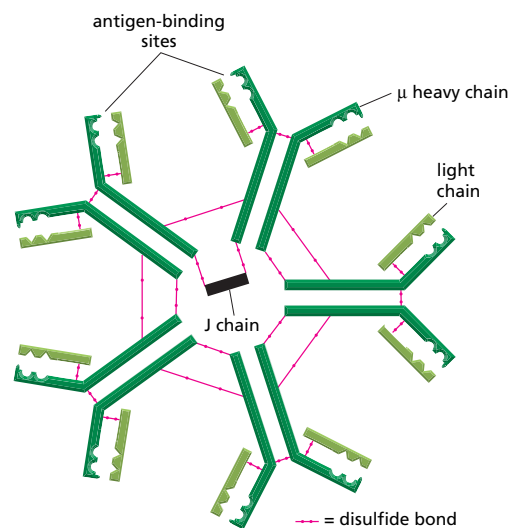


Figure 25–23 A pentameric IgM molecule. Disulfide bonds (red) hold the five four-chain units together. A single J chain, which has a structure similar to that of a single Ig domain (discussed later), is covalently attached by disulfide bonds to the tails of two μ heavy chains. The J chain is required for pentamer formation. The addition of each successive four-chain IgM unit requires a J chain, which is then discarded, except for the last one, which is retained. Note that IgM molecules do not have hinge regions.

Largely by means of such **Fc receptors** (so-named because antibody tails are called *Fc* regions), these phagocytic cells bind, ingest, and destroy infecting microorganisms that have become coated with the IgG antibodies produced in response to the infection (Figure 25–24).

Some IgG subclasses are the only antibodies that can pass from mother to fetus via the placenta. Cells of the placenta that are in contact with maternal blood have Fc receptors that bind these blood-borne IgG molecules and direct their passage to the fetus. The antibody molecules bound to the receptors are first taken into the placental cells by receptor-mediated endocytosis. They are then transported across the cell in vesicles and released by exocytosis into the fetal blood (a process called *transcytosis*—see Figure 25–26). Because other classes of antibodies do not bind to these particular Fc receptors, they cannot pass across the placenta. Later, IgG is secreted into the mother's milk and is then

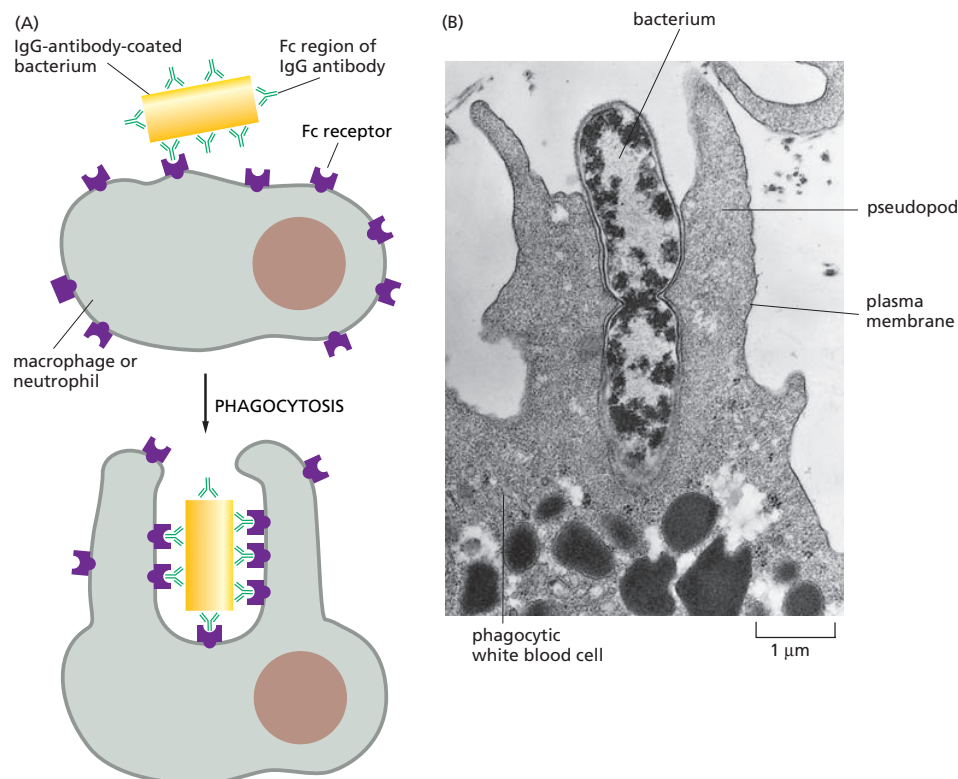


Figure 25–24 Antibody-activated phagocytosis. (A) An IgG-antibody-coated bacterium is efficiently phagocytosed by a macrophage or neutrophil, which has cell-surface receptors that bind the tail (Fc) region of IgG molecules. The binding of the antibody-coated bacterium to these Fc receptors activates the phagocytic process. The tail of an antibody molecule is called an Fc region because, when antibodies are cleaved with the proteolytic enzyme papain, the fragments containing the tail region readily crystallize. (B) Electron micrograph of a neutrophil phagocytosing an IgG-coated bacterium, which is in the process of dividing. The process in which antibody (or complement) coating of a pathogen increases the efficiency with which the pathogen is phagocytosed is called *opsonization*. (B, courtesy of Dorothy F. Bainton, from R.C. Williams, Jr. and H.H. Fudenberg, *Phagocytic Mechanisms in Health and Disease*. New York: Intercontinental Medical Book Corporation, 1971.)

Figure 25–25 A highly schematized diagram of a dimeric IgA molecule found in secretions. In addition to the two IgA monomers, there is a single J chain and an additional polypeptide chain called the *secretory component*, which is derived from the Fc receptor (see Figure 25–26) and is thought to protect the IgA molecules from digestion by proteolytic enzymes in secretions.

taken up from the gut of the neonate into the blood by transcytosis, providing protection for the baby against infection.

IgA is the principal class of antibody in secretions, including saliva, tears, milk, and respiratory and intestinal secretions. IgA is a four-chain monomer in the blood which is assembled into a dimer by the addition of two other polypeptide chains before it is released into secretions (Figure 25–25). It is transported through secretory epithelial cells from the extracellular fluid into the secreted fluid by transcytosis mediated by another type of Fc receptor that is unique to secretory epithelia (Figure 25–26). This Fc receptor can also transport IgM into secretions (but less efficiently), which is probably why individuals with a selective IgA deficiency, the most common form of antibody deficiency, are only mildly affected by the defect.

The tail region of **IgE** molecules, which are four-chain monomers, binds with unusually high affinity ($K_a \sim 10^{10}$ liters/mole) to yet another class of Fc receptors. These receptors are located on the surface of *mast cells* in tissues and of *basophils* in the blood. The IgE molecules bound to them function as passively acquired receptors for antigen. Antigen binding triggers the mast cell or basophil to secrete a variety of cytokines and biologically active amines, especially *histamine* (Figure 25–27). The histamine causes blood vessels to dilate and become leaky, which in turn helps white blood cells, antibodies, and complement components to enter sites where mast cells have been activated. The release of amines from mast cells and basophils is largely responsible for the symptoms of such *allergic* reactions as hay fever, asthma, and hives. In addition, mast cells secrete factors that attract and activate white blood cells called *eosinophils*. Eosinophils also have Fc receptors that bind IgE molecules, and they can kill extracellular parasitic worms, especially if the worms are coated with IgE antibodies.

In addition to the five classes of heavy chains found in antibody molecules, higher vertebrates have two types of light chains, κ and λ , which seem to be functionally indistinguishable. Either type of light chain may be associated with any of the heavy chains. An individual antibody molecule, however, always contains identical light chains and identical heavy chains: an IgG molecule, for instance, may have either κ or λ light chains, but not one of each. As a result, an antibody's antigen-binding sites are always identical. Such symmetry is crucial for the cross-linking function of secreted antibodies (see Figure 25–19).

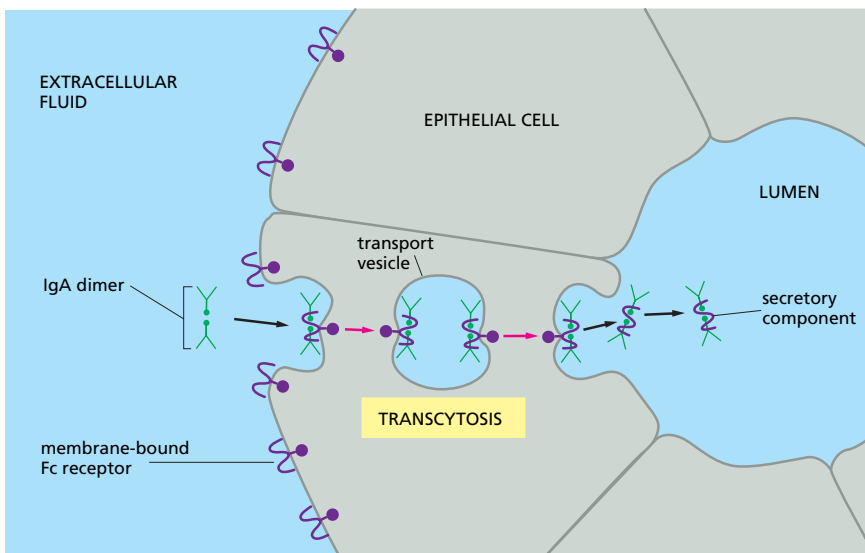
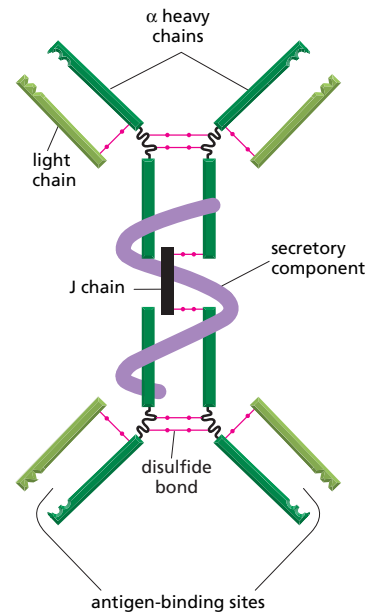


Figure 25–26 The mechanism of transport of a dimeric IgA molecule across an epithelial cell. The IgA molecule, as a J-chain-containing dimer, binds to a transmembrane receptor protein on the nonluminal surface of a secretory epithelial cell. (The J chain has been omitted in this diagram for clarity.) The receptor–IgA complexes are ingested by receptor-mediated endocytosis, transferred across the epithelial cell cytoplasm in vesicles, and secreted into the lumen on the opposite side of the cell by exocytosis. When exposed to the lumen, the part of the Fc receptor protein that is bound to the IgA dimer (the *secretory component*) is cleaved from its transmembrane tail, thereby releasing the antibody in the form shown in Figure 25–25.

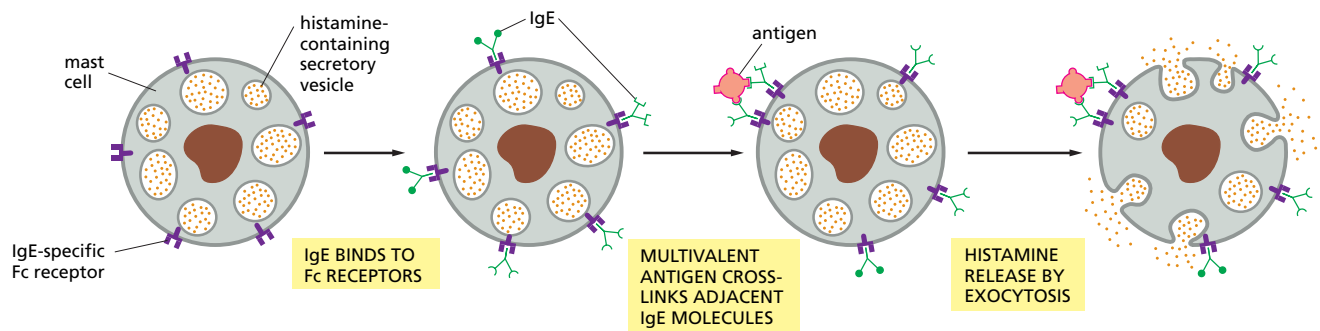


Figure 25–27 The role of IgE in histamine secretion by mast cells. A mast cell (or a basophil) binds IgE molecules after they are secreted by effector B cells. The soluble IgE antibodies bind to Fc receptor proteins on the mast cell surface that specifically recognize the Fc region of these antibodies. The bound IgE molecules serve as cell-surface receptors for antigen. Thus, unlike B cells, each mast cell (and basophil) has a set of cell-surface antibodies with a wide variety of antigen-binding sites. When an antigen molecule binds to these membrane-bound IgE antibodies so as to cross-link them to their neighbors, it signals the mast cell to release its histamine and other local mediators by exocytosis.

All classes of antibody can be made in a membrane-bound form, as well as in a soluble, secreted form. The two forms differ only in the C-terminus of their heavy chain. The heavy chains of membrane-bound antibody molecules have a transmembrane hydrophobic C-terminus, which anchors them in the lipid bilayer of the B cell's plasma membrane. The heavy chains of secreted antibody molecules, by contrast, have instead a hydrophilic C-terminus, which allows them to escape from the cell. The switch in the character of the antibody molecules made occurs because the activation of B cells by antigen (and helper T cells) induces a change in the way in which the H-chain RNA transcripts are made and processed in the nucleus (see Figure 7–99).

The properties of the various classes of antibodies in humans are summarized in [Table 25–1](#).

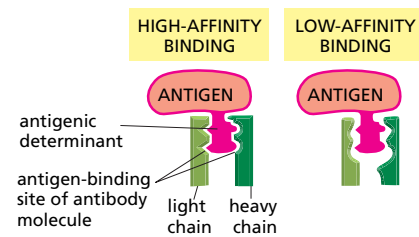
The Strength of an Antibody–Antigen Interaction Depends on Both the Number and the Affinity of the Antigen-Binding Sites

The binding of an antigen to an antibody, like the binding of a substrate to an enzyme, is reversible. The sum of many relatively weak non-covalent forces, including hydrogen bonds, hydrophobic and van der Waals forces, and ionic interactions determine the strength of the interaction. These weak forces are effective only when the antigen molecule is close enough to allow some of its atoms to fit into complementary recesses on the surface of the antibody. The complementary regions of a four-chain antibody unit are its two identical antigen-binding sites; the corresponding region on the antigen is an antigenic determinant ([Figure 25–28](#)). Most antigenic macromolecules have many different antigenic determinants and are said to be *multivalent*; if two or more of the determinants are identical (as in a polymer with a repeating structure), the antigen is said to be *polyvalent* ([Figure 25–29](#)).

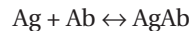
Table 25–1 Properties of the Major Classes of Antibodies in Humans

PROPERTIES	CLASS OF ANTIBODY				
	IgM	IgD	IgG	IgA	IgE
Heavy chains	μ	δ	γ	α	ϵ
Light chains	κ or λ	κ or λ	κ or λ	κ or λ	κ or λ
Number of four-chain units	5	1	1	1 or 2	1
Percentage of total Ig in blood	10	<1	75	15	<1
Activates complement	++++	–	++	–	–
Crosses placenta	–	–	+	–	–
Binds to macrophages and neutrophils	–	–	+	–	–
Binds to mast cells and basophils	–	–	–	–	+

Figure 25–28 Antigen binding to antibody. In this highly schematized diagram, an antigenic determinant on a macromolecule is shown interacting with one of the antigen-binding sites of two different antibody molecules, one of high affinity and one of low affinity. Various weak noncovalent forces hold the antigenic determinant in the binding site, and the site with the better fit to the antigen has a greater affinity. Note that both the light and heavy chains of the antibody molecule usually contribute to the antigen-binding site.



The reversible binding reaction between an antigen with a single antigenic determinant (denoted Ag) and a single antigen-binding site (denoted Ab) can be expressed as



The equilibrium point depends both on the concentrations of Ab and Ag and on the strength of their interaction. Clearly, a larger fraction of Ab will become associated with Ag as the concentration of Ag increases. The strength of the interaction is generally expressed as the **affinity constant** (K_a) (see Figure 3–43), where

$$K_a = [\text{AgAb}] / [\text{Ag}][\text{Ab}]$$

(the square brackets indicate the concentration of each component at equilibrium).

One can determine the affinity constant, also known as the association constant, by measuring the concentration of free Ag required to fill half of the antigen-binding sites on the antibody. When half the sites are filled, $[\text{AgAb}] = [\text{Ab}]$ and $K_a = 1/[\text{Ag}]$. Thus, the reciprocal of the antigen concentration that produces half the maximum binding is equal to the affinity constant of the antibody for the antigen. Common values range from as low as 5×10^4 to as high as 10^{11} liters/mole.

The **affinity** of an antibody for an antigenic determinant describes the strength of binding of a single copy of the antigenic determinant to a single antigen-binding site, and it is independent of the number of antigen-binding sites. When, however, a polyvalent antigen, carrying multiple copies of the same antigenic determinant, combines with a polyvalent IgM antibody (see Figure 25–23), the binding strength is greatly increased because all of the antigen–antibody bonds must be broken simultaneously before the antigen and antibody can dissociate. Even a bivalent IgG molecule can bind at least 100 times more strongly to a polyvalent antigen if both antigen-binding sites are engaged than if only one site is engaged. The total binding strength of a bivalent or polyvalent antibody with a polyvalent antigen is referred to as the **avidity** of the interaction.

If the affinity of the antigen-binding sites in an IgG and an IgM molecule is the same, the IgM molecule (with 10 binding sites) will have a much greater avidity for a polyvalent antigen than an IgG molecule (which has two binding sites). This difference in avidity, often 10^4 -fold or more, is important because antibodies produced early in an immune response usually have much lower affinities than those produced later. Because of its high total avidity, IgM—the major Ig class produced early in primary immune responses—can function effectively even when each of its binding sites has only a low affinity.

So far, we have considered the general structure and function of antibodies. Next, we look at the details of their structure, as revealed by studies of their amino acid sequence and three-dimensional structure.

Antibody Light and Heavy Chains Consist of Constant and Variable Regions

Comparison of the amino acid sequences of different antibody molecules reveals a striking feature with important genetic implications. Both light and heavy chains have a variable sequence at their N-terminal ends but a constant sequence at their C-terminal ends. Consequently, when we compare the amino acid sequences of many different κ chains, the C-terminal halves are the same or show only minor differences, whereas the N-terminal halves all differ. Light chains have a **constant region** about 110 amino acids long and a **variable region**

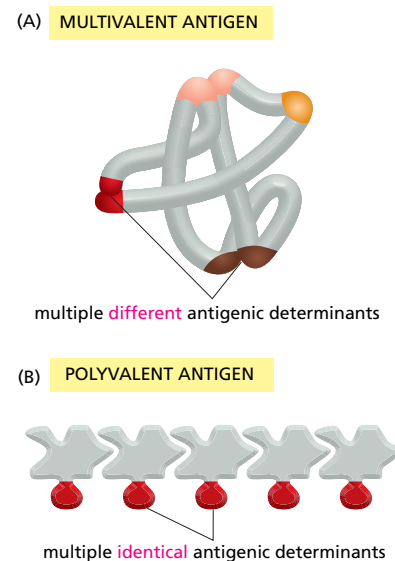


Figure 25–29 Molecules with multiple antigenic determinants. (A) A globular protein is shown with a number of *different* antigenic determinants. Different regions of a polypeptide chain usually come together in the folded structure to form each antigenic determinant on the surface of the protein, as shown for three of the four determinants. (B) A polymeric structure is shown with many *identical* antigenic determinants.

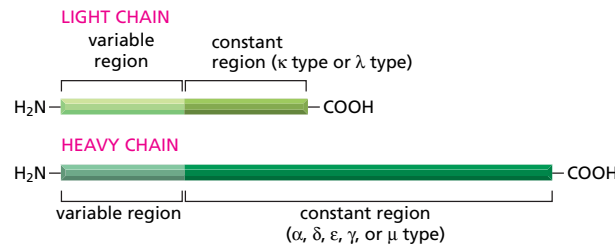


Figure 25–30 Constant and variable regions of immunoglobulin chains. The variable regions of the light and heavy chains form the antigen-binding sites, while the constant regions of the heavy chains determine the other biological properties of an antibody.

of the same size. The variable region of the heavy chains is also about 110 amino acids long, but the constant region is about three or four times longer (330 or 440 amino acids), depending on the class (**Figure 25–30**).

It is the N-terminal ends of the light and heavy chains that come together to form the antigen-binding site, and the variability of their amino acid sequences provides the structural basis for the diversity of antigen-binding sites. The greatest diversity occurs in three small **hypervariable regions** in the variable regions of both light and heavy chains; the remaining parts of the variable region, known as *framework regions*, are relatively constant.

Only about 5–10 amino acids in each hypervariable region form the actual antigen-binding site (**Figure 25–31**). As a result, the size of the antigenic determinant that an antibody recognizes is generally comparably small. It can consist of fewer than 10 amino acids on the surface of a globular protein, for example.

The Light and Heavy Chains Are Composed of Repeating Ig Domains

Both light and heavy chains are made up of repeating segments—each about 110 amino acids long and each containing one intrachain disulfide bond. Each repeating segment folds independently to form a compact functional unit called an **immunoglobulin (Ig) domain**. As shown in **Figure 25–32**, a light chain consists of one variable (V_L) and one constant (C_L) domain (equivalent to the variable and constant regions shown in the top half of **Figure 25–30**). V_L pairs with the variable (V_H) domain of the heavy chain to form the antigen-binding region. C_L pairs with the first constant domain of the heavy chain (C_{H1}), and the remaining constant domains of the heavy chains form the Fc region, which determines the other biological properties of the antibody. Most heavy chains have three constant domains (C_{H1} , C_{H2} , and C_{H3}), but those of IgM and IgE antibodies have four.

The similarity in their domains suggests that antibody chains arose during evolution by a series of gene duplications, beginning with a primordial gene coding for a single 110 amino acid domain of unknown function. Each domain of the constant region of a heavy chain is encoded by a separate coding sequence (exon), which supports this hypothesis (**Figure 25–33**).

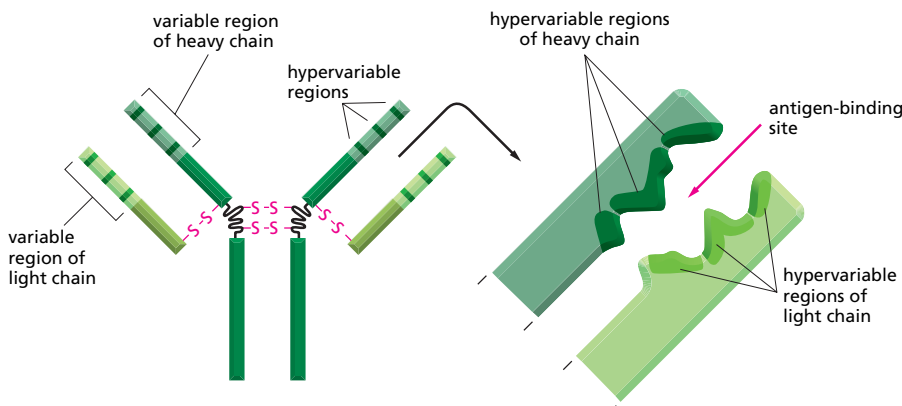


Figure 25–31 Antibody hypervariable regions. Highly schematized drawing of how the three hypervariable regions in each light and heavy chain together form the antigen-binding site of an antibody molecule.

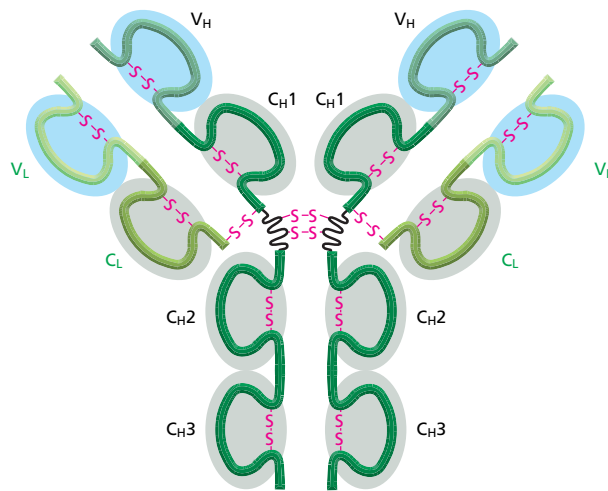


Figure 25–32 Immunoglobulin domains. The light and heavy chains in an antibody molecule are each folded into similar repeating domains. The variable domains (shaded in blue) of the light and heavy chains (V_L and V_H) make up the antigen-binding sites, while the constant domains of the heavy chains (mainly C_H2 and C_H3) determine the other biological properties of the molecule. The heavy chains of IgM and IgE antibodies do not have a hinge region and have an extra constant domain (C_H4). Hydrophobic interactions between domains on adjacent chains help hold the chains together in the antibody molecule: V_L binds to V_H , C_L binds to C_H1 , and so on (see Figure 25–34). All antibodies are glycosylated on their C_H2 domains (not shown); the attached oligosaccharide chains vary from antibody to antibody and influence the biological properties of the antibody.

An Antigen-Binding Site Is Constructed from Hypervariable Loops

Many antibody fragments, as well as some intact antibody molecules, have been studied by x-ray crystallography. From these examples, we can understand the way in which billions of different antigen-binding sites are constructed on a common structural theme.

As illustrated in **Figure 25–34**, each Ig domain has a very similar three-dimensional structure consisting of a sandwich of two β sheets held together by a disulfide bond. As we discuss later, many other proteins on the surface of lymphocytes and other cells, many of which function as cell–cell adhesion molecules (discussed in Chapter 19), contain similar domains and hence are members of a very large *immunoglobulin (Ig) superfamily* of proteins.

The variable domains of antibody molecules are unique in that each has its particular set of three hypervariable regions, which are arranged in three *hypervariable loops* (see Figure 25–34). The hypervariable loops of both the light and heavy variable domains cluster together to form the antigen-binding site. Because the variable domain of an antibody molecule consists of a highly conserved rigid framework, with hypervariable loops attached at one end, changes in only the lengths and amino acid sequences of the hypervariable loops can generate an enormous diversity of antigen-binding sites. The overall three-dimensional structure necessary for antibody function remains constant.

X-ray analyses of crystals of antibody fragments bound to an antigenic determinant reveal exactly how the hypervariable loops of the light and heavy variable domains cooperate to form an antigen-binding surface in particular

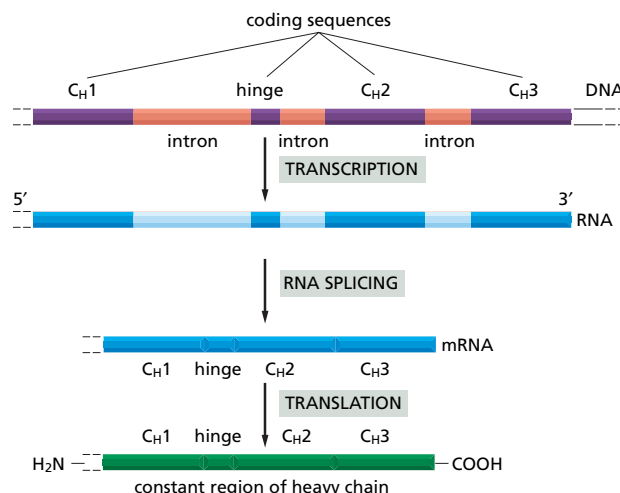


Figure 25–33 The organization of the DNA sequences that encode the constant region of an antibody heavy chain, such as that found in IgG. The coding sequences (exons) for each domain and for the hinge region are separated by noncoding sequences (introns). The intron sequences are removed by splicing the primary RNA transcripts to form mRNA. The presence of introns in the DNA is thought to have facilitated accidental duplications of DNA segments that gave rise to the antibody genes during evolution (discussed in Chapter 4). The DNA and RNA sequences that encode the variable region of the heavy chain are not shown.

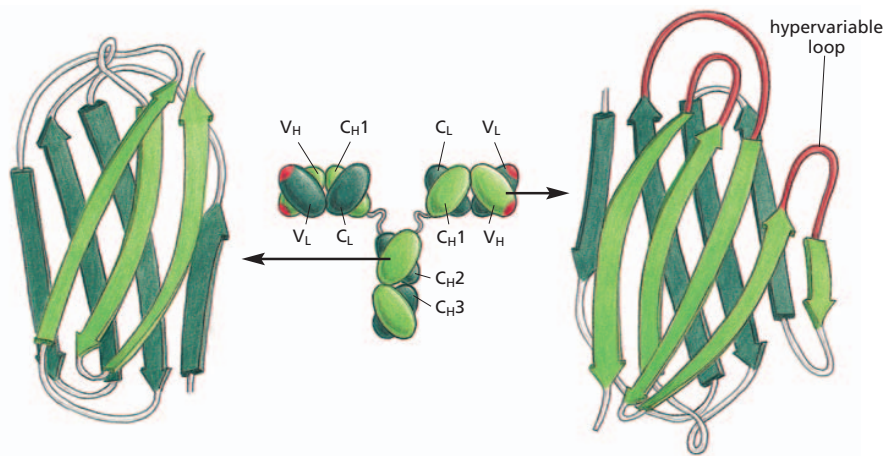


Figure 25-34 The folded structure of an IgG antibody molecule, based on x-ray crystallography studies. <GCG> The structure of the whole protein is shown in the middle, while the structure of a constant domain is shown on the left and that of a variable domain on the right. Both domains consist of two β sheets, which are joined by a disulfide bond (not shown). Note that all the hypervariable regions (red) form loops at the far end of the variable domain, where they come together to form part of the antigen-binding site (see also Figure 3-41).

cases. The dimensions and shape of each different site vary depending on the conformations of the polypeptide chain in the hypervariable loops, which in turn are determined by the sequences of the amino acid side chains in the loops. The shapes of binding sites vary greatly—from pockets, to grooves, to undulating flatter surfaces, and even to protrusions—depending on the antibody (**Figure 25-35**). Smaller ligands tend to bind to deeper pockets, whereas larger ones tend to bind to flatter surfaces. In addition, the binding site can alter its shape after antigen binding to fit the ligand better.

Now that we have discussed the structure and functions of antibodies, we are ready to consider the crucial question that puzzled immunologists for many years—what are the genetic mechanisms that enable each of us to make many billions of different antibody molecules?

Summary

Antibodies defend vertebrates against infection by inactivating viruses and microbial toxins and by recruiting the complement system and various types of white blood cells to kill invading pathogens. A typical antibody molecule is Y-shaped, with two identical antigen-binding sites at the tips of the Y, plus binding sites for complement components and various cell-surface receptors on the tail of the Y.

Each B cell clone makes antibody molecules with a unique antigen-binding site. Initially, during B cell development in the bone marrow, the antibody molecules are inserted into the plasma membrane, where they serve as receptors for antigen. In peripheral lymphoid organs, antigen binding to these receptors, together with co-stimulatory signals provided by helper T cells, activates the B cells to proliferate and differentiate into either memory cells or antibody-secreting effector cells. The effector cells secrete large amounts of antibodies with the same unique antigen-binding site as the membrane-bound antibodies.

A typical antibody molecule is composed of four polypeptide chains, two identical heavy chains and two identical light chains. Parts of both the heavy and light chains usually combine to form the antigen-binding sites. There are five classes of antibodies (IgA, IgD, IgE, IgG, and IgM), each with a distinctive heavy chain (α , δ , ϵ , γ , and μ , respectively). The heavy chains also form the tail (Fc region) of the antibody, which determines what other proteins will bind to the antibody and therefore what biological properties the antibody class has. Either type of light chain (κ or λ) can be associated with any class of heavy chain; this choice has no effect on the properties of the antibody, except for its specificity for antigen.

Each light and heavy chain is composed of a number of Ig domains— β sheet structures constructed from about 110 amino acids. A light chain has one variable (V_L) and one constant (C_L) domain, while a heavy chain has one variable (V_H) and either three or four constant (C_H) domains. The amino acid sequence variation in the variable domains of both light and heavy chains is concentrated in several small hypervariable regions, which protrude as loops at one end of these domains to form the antigen-binding site.

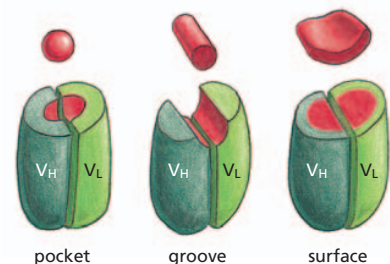


Figure 25-35 The variety of antigen-binding surfaces in antibodies. The hypervariable loops of different V_L and V_H domains can combine to form a large variety of binding surfaces. The antigenic determinants and the antigen-binding site of the antibodies are shown in red. Only one antigen-binding site is shown for each antibody.

THE GENERATION OF ANTIBODY DIVERSITY

Even in the absence of antigen stimulation, a human can probably make more than 10^{12} different antibody molecules—its preimmune, **primary antibody repertoire**. The primary repertoire consists of IgM and IgD antibodies and is apparently large enough to ensure that there will be an antigen-binding site to fit almost any potential antigenic determinant, albeit with low affinity. (The antigen-binding sites of many antibodies can cross-react with a variety of related but different antigenic determinants, making this primary antibody defense force even more formidable.)

After stimulation by antigen (and helper T cells), B cells can switch from making IgM and IgD to making other classes of antibodies—a process called *class switching*. In addition, the affinity of these antibodies for their antigen progressively increases over time—a process called *affinity maturation*. Thus, antigen stimulation generates a **secondary antibody repertoire**, with a greatly increased diversity of both Ig classes and antigen-binding sites.

Antibodies are proteins, and proteins are encoded by genes. Antibody diversity therefore poses a special genetic problem: how can an animal make more antibodies than there are genes in its genome? (The human genome, for example, contains only about 25,000 genes.) This problem is not quite as formidable as it might first appear. Recall that the variable regions of the light and heavy chains of antibodies usually combine to form the antigen-binding site. Thus, an animal with 1000 genes encoding light chains and 1000 genes encoding heavy chains could, in principle, combine their products in 1000×1000 different ways to make 10^6 different antigen-binding sites (although, in reality, not every light chain can combine with every heavy chain to make an antigen-binding site). Nonetheless, unique genetic mechanisms have evolved to enable adaptive immune systems to generate an almost unlimited number of different light and heavy chains in a remarkably economical way.

Not all vertebrates use the same genetic mechanisms to diversify antibodies, and there are even substantial differences in the mechanisms used by different mammals. We discuss the mechanisms used by mice and humans, in which antibody diversity is generated in two steps. First, before antigen stimulation, developing B cells join together separate *gene segments* in DNA in order to create the genes that encode the primary repertoire of low-affinity IgM and IgD antibodies. Second, after antigen stimulation, the assembled antibody-coding genes can undergo two further changes—mutations that can increase the affinity of the antigen-binding site and DNA rearrangements that switch the class of antibody made. Together, these changes produce the secondary repertoire of high-affinity IgG, IgA, and IgE antibodies.

We begin this section by discussing the mechanisms that B cells use to produce the primary antibody repertoire and then discuss the mechanisms that they use to produce the secondary repertoire.

Antibody Genes Are Assembled From Separate Gene Segments During B Cell Development

Mice and humans produce their primary antibody repertoire by joining separate antibody **gene segments** together during B cell development. Each type of antibody chain— κ light chains, λ light chains, and heavy chains—is encoded by a separate locus on a separate chromosome. Each locus contains a large number of gene segments encoding the V region of an antibody chain, and one or more gene segments encoding the C region. During the development of a B cell in the bone marrow (or fetal liver), a complete coding sequence for each of the two antibody chains to be synthesized is assembled by site-specific genetic recombination (discussed in Chapter 5). In addition to bringing together the separate gene segments of the antibody gene, these rearrangements also activate transcription from the gene promoter through changes in the relative positions of the enhancers and silencers acting on the gene. Thus, a complete antibody chain can be synthesized only after the DNA has been rearranged.

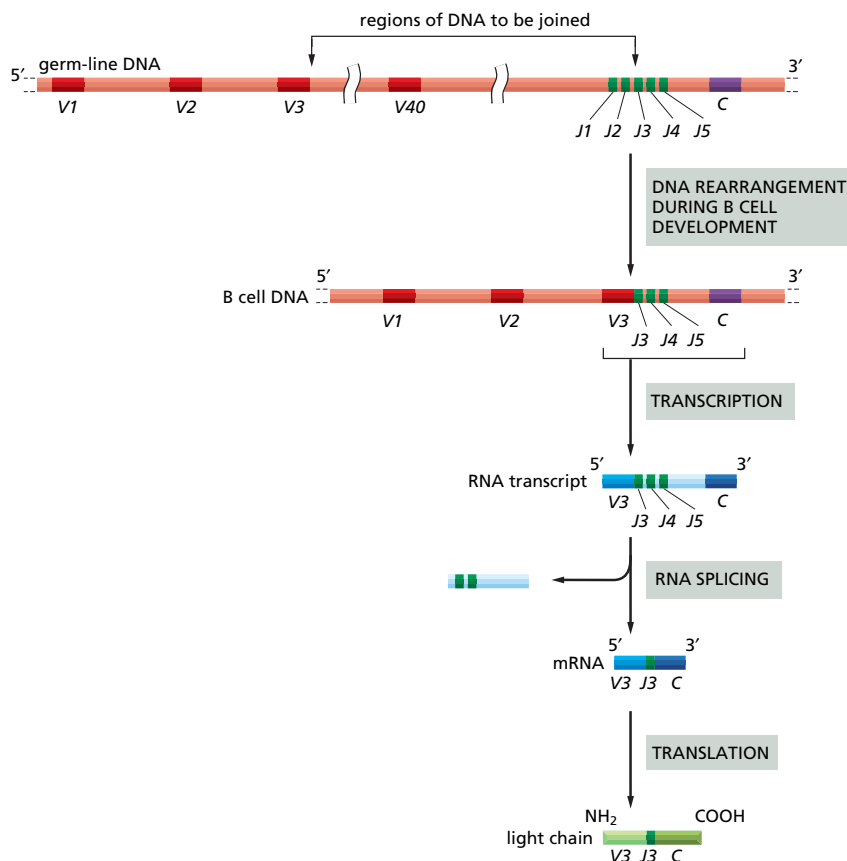


Figure 25–36 The V–J joining process involved in making a human κ light chain. In the “germ-line” DNA (where the antibody genes are not rearranged and are therefore not being expressed), the cluster of five *J* gene segments is separated from the C-region coding sequence by a short intron and from the 40 *V* gene segments by thousands of nucleotide pairs. During the development of a B cell, a randomly chosen *V* gene segment (*V3* in this case) is moved to lie precisely next to one of the *J* gene segments (*J3* in this case). The “extra” *J* gene segments (*J4* and *J5*) and the intron sequence are transcribed (along with the joined *V3* and *J3* gene segments and the C-region coding sequence) and then removed by RNA splicing to generate mRNA molecules with contiguous *V3*, *J3*, and C sequences, as shown. These mRNAs are then translated into κ light chains. A *J* gene segment encodes the C-terminal 15 or so amino acids of the V region, and a short sequence containing the V–*J* segment junction encodes the third hypervariable region of the light chain, which is the most variable part of the V region.

Each light-chain V region is encoded by a DNA sequence assembled from two gene segments—a long **V gene segment** and a short **joining, or J gene segment** (not to be confused with the protein *J chain* (see Figure 25–23), which is encoded elsewhere in the genome). **Figure 25–36** illustrates the sequence of events involved in the production of a human κ light-chain polypeptide from its separate gene segments. Each heavy-chain V region is similarly constructed by combining gene segments, but here an additional **diversity segment, or D gene segment**, is also required (**Figure 25–37**).

The large number of inherited *V*, *J*, and *D* gene segments available for encoding antibody chains contributes substantially to antibody diversity, and the combinatorial joining of these segments (called **combinatorial diversification**) greatly increases this contribution. Any of the 40 *V* segments in the human κ light-chain locus, for example, can be joined to any of the 5 *J* segments (see Figure 25–36), so that this locus can encode at least 200 (40×5) different κ -chain V regions. Similarly, any of the 40 *V* segments in the human heavy-chain locus can be joined to any of the 25 *D* segments and to any of the 6 *J* segments to encode at least 6000 ($40 \times 25 \times 6$) different heavy-chain V regions.

The combinatorial diversification resulting from the assembly of different combinations of inherited *V*, *J*, and *D* gene segments is an important mechanism for diversifying the antigen-binding sites of antibodies. By this mechanism alone, called **V(D)J recombination**, a human can produce 320 different V_L regions (200 κ and 120 λ) and 6000 different V_H regions. In principle, these could then be combined to make about 1.9×10^6 (320×6000) different antigen-binding sites. In addition, as we discuss next, the joining mechanism itself greatly increases this number of possibilities (probably more than 10^8 -fold), making the primary antibody repertoire much larger than the total number of B cells (about 10^{12}) in a human.

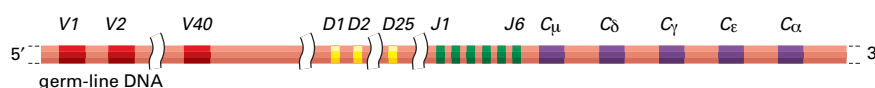


Figure 25–37 The human heavy-chain locus. There are 40 *V* segments, 25 *D* segments, 6 *J* segments, and an ordered cluster of C-region coding sequences, each cluster encoding a different class of heavy chain. The *D* segment (and part of the *J* segment) encodes amino acids in the third hypervariable region, which is the most variable part of the heavy-chain V region. The genetic mechanisms involved in producing a heavy chain are the same as those shown in Figure 25–36 for light chains, except that two DNA rearrangement steps are required instead of one. First a *D* segment joins to a *J* segment, and then a *V* segment joins to the rearranged *DJ* segment. The figure is not drawn to scale and omits detail: for example, the total length of the heavy chain locus is over 2 megabases.

Imprecise Joining of Gene Segments Greatly Increases the Diversity of V Regions

In the process of **V(D)J recombination**, site-specific recombination joins separate antibody gene segments together to form a functional V_L - or V_H -region coding sequence. Conserved *recombination signal sequences* flank each gene segment and serve as recognition sites for the joining process, ensuring that only appropriate gene segments recombine. Thus, for example, a light-chain *V* segment will always join to a *J* segment but not to another *V* segment. An enzyme complex called the *V(D)J recombinase* mediates joining. This complex contains two proteins that are specific to developing lymphocytes, as well as enzymes that help repair damaged DNA in all our cells.

Two closely linked genes called *Rag1* and *Rag2* (*Rag* = recombination activating genes) encode the lymphocyte-specific proteins of the V(D)J recombinase, RAG1 and RAG2. To mediate V(D)J joining, the two proteins come together to form a complex (called **RAG**), which functions as an endonuclease, introducing double-strand breaks precisely between the gene segments to be joined and their flanking recombination signal sequences. RAG then initiates the rejoining process by recruiting enzymes involved in DNA double-strand repair in all cells (**Figure 25–38**). Mice or humans deficient in either of the two *Rag* genes or in nonhomologous end joining are highly susceptible to infection because they are unable to carry out V(D)J recombination and consequently do not have functional B or T cells, a condition called *severe combined immunodeficiency (SCID)*. (As we discuss later, T cells use the same V(D)J recombinase to assemble the gene segments that encode their antigen-specific receptors.)

During the joining of antibody (and T cell receptor) gene segments, as in nonhomologous end-joining (see Figure 5–51A), a variable number of nucleotides are often lost from the ends of the recombining gene segments, and one or more randomly chosen nucleotides may also be inserted. This random loss and gain of nucleotides at joining sites is called **junctional diversification**, and it enormously increases the diversity of V-region coding sequences created by V(D)J recombination, specifically in the third hypervariable region. This

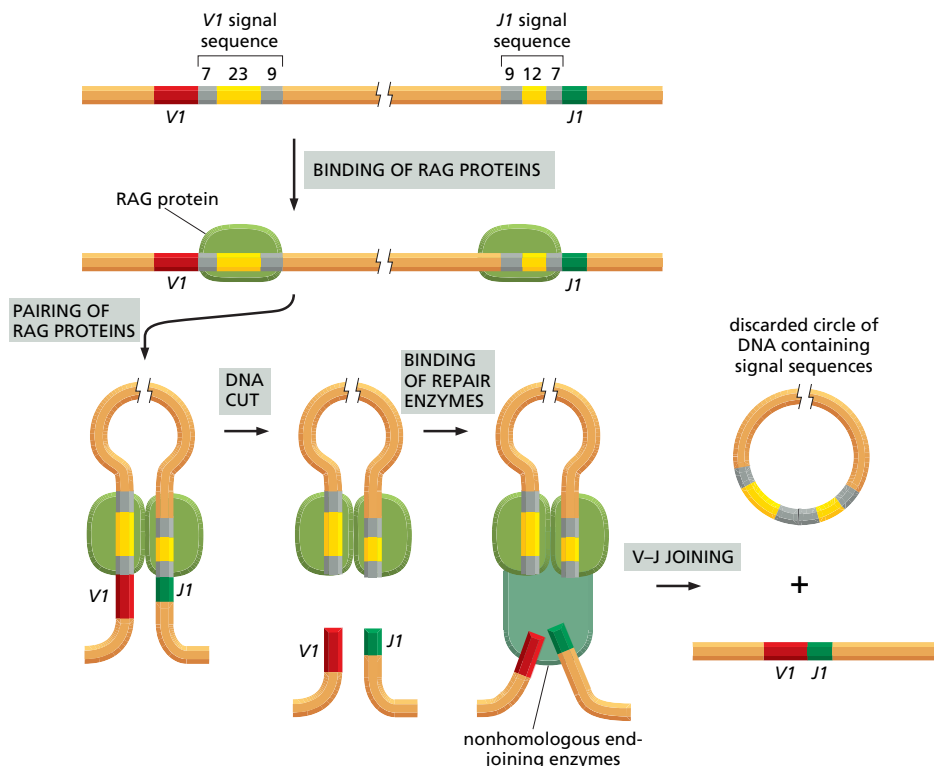


Figure 25–38 The role of recombination signal sequences in RAG-mediated gene segment joining. In the case shown, V1 is joined to J1 in a light-chain locus. Two types of DNA signal sequences are involved in V(D)J recombination, and recombination can only occur between different types: both have the same 7-base-pair (bp) sequence at one end and the same 9-bp sequence at the other end, but in one type the ends are separated by a 12-bp spacer and in the other they are separated by a 23-bp spacer, as shown. When one RAG protein binds to a 12-bp spacer and another to a 23-bp spacer, and the two RAG proteins bind to each other, the two different signal sequences are juxtaposed. The RAG complex then cuts the two signal sequences at their 7-bp ends, and DNA repair enzymes join the cut V1 and J1 segments together. The signal sequences are also joined together and discarded as a small circle of DNA that contains all the DNA originally located between V1 and J1.

The same process and signal sequences are used to join V, D, and J gene segments in a heavy-chain locus. The arrangement of signal sequences and the “12/23 rule” just described ensure that only appropriate gene segments recombine.

increased diversification comes at a price, however. In many cases, it will shift the reading frame to produce a nonfunctional gene. Because roughly two in every three rearrangements are “nonproductive” in this way, many developing B cells never make a functional antibody molecule and consequently die in the bone marrow.

B cells making functional antibody molecules that bind strongly to self antigens in the bone marrow would be dangerous. Such B cells maintain expression of the RAG proteins and can undergo a second round of V(D)J recombination in a light-chain locus (usually a κ locus), thereby changing the specificity of the cell-surface antibody they make—a process referred to as **receptor editing**. To provide a further layer of protection, clonal deletion eliminates those self-reactive B cells that fail to change their specificity (see Figure 25–13).

The Control of V(D)J Recombination Ensures That B Cells Are Monospecific

B cells are *monospecific*. That is, all the antibodies that any one B cell produces have identical antigen-binding sites. This property enables antibodies to cross-link antigens into large aggregates, thereby promoting antigen elimination (see Figure 25–19). It also means that an activated B cell secretes antibodies with the same specificity as that of its membrane-bound antibody receptor, guaranteeing the specificity of antibody responses (see Figure 25–17).

To achieve monospecificity, each B cell must make only one type of V_L region and one type of V_H region. Since B cells, like other somatic cells, are diploid, each cell has six loci encoding antibody chains: two heavy-chain loci (one from each parent) and four light-chain loci (one κ and one λ from each parent). If DNA rearrangements occurred independently in each heavy-chain locus and each light-chain locus, a single B cell could make up to eight different antibodies, each with a different antigen-binding site.

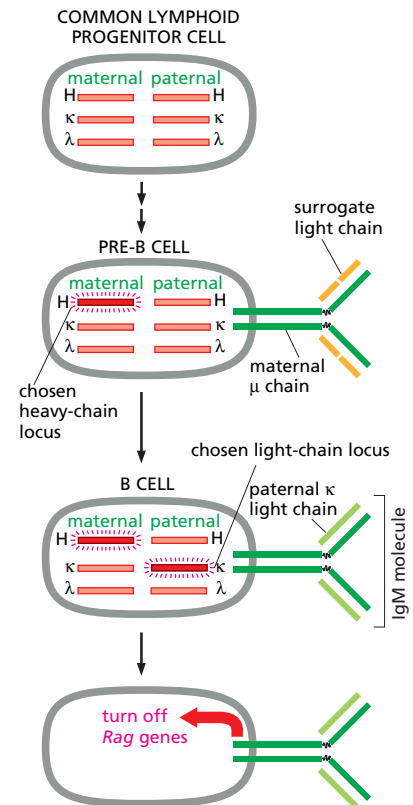
In fact, however, each B cell uses only two of the six antibody loci: one of the two heavy-chain loci and one of the four light-chain loci. Thus, each B cell must choose not only between its κ and λ light-chain loci, but also between its maternal and paternal light-chain and heavy-chain loci. This second choice is called **allelic exclusion**. Allelic exclusion also occurs in the expression of some genes that encode T cell receptors and genes that encode olfactory receptors in the nose (discussed in Chapter 15). However, for most proteins that are encoded by autosomal genes, both the maternal and paternal gene copies in a cell are expressed about equally.

Allelic exclusion and κ versus λ light-chain choice during B cell development depend on negative feedback regulation of the V(D)J recombination process. A functional rearrangement in one antibody locus suppresses rearrangements in all remaining loci that encode the same type of antibody chain (**Figure 25–39**). In B cell clones isolated from transgenic mice expressing a rearranged μ -chain gene, for example, the rearrangement of all of the endogenous heavy-chain genes is usually suppressed. Comparable results have been obtained for light chains. The suppression does not occur if the product of the rearranged gene fails to assemble into a receptor that inserts into the plasma membrane. It has therefore been proposed that either the receptor assembly process itself or extracellular signals that act on the receptor suppress further gene rearrangements.

Although no biological differences between the constant regions of κ and λ light chains have been discovered, there is an advantage in having two separate loci encoding light-chain variable regions. Having two separate loci increases the chance that a pre-B cell that has successfully assembled a V_H -region coding sequence will then successfully assemble a V_L -region coding sequence to become a B cell. This chance is further increased because, before a developing pre-B cell produces ordinary light chains, it makes surrogate light chains (see Figure 25–22), which assemble with μ heavy chains. The resulting receptors are displayed on the cell surface and allow the cell to proliferate, producing large numbers of progeny cells, some of which are likely to succeed in producing bona fide light chains.

Figure 25–39 Selection of antibody loci during B cell development in the bone marrow. To produce antibodies with only one type of antigen-binding site, a developing B cell must use only one L-chain locus and one H-chain locus. Although the choice between maternal and paternal loci is thought to be random, the assembly of V-region coding sequences in a developing B cell proceeds in an orderly sequence, one segment at a time, usually beginning with the H-chain locus. In this locus, *D* segments first join to *J_H* segments on both parental chromosomes; then *V_H* to *DJ_H* joining occurs on one of these chromosomes (not shown). If this rearrangement produces a functional gene, the resulting production of complete μ chains (always the first H chains made) leads to their expression on the cell surface in association with surrogate light chains (see Figure 25–22). The cell now shuts down all further rearrangements of *V_H*-region-encoding gene segments and initiates *V_L* rearrangement. *V_L* rearrangement usually occurs first in a κ locus, and only if that fails does it occur at the other κ locus or at a λ locus. If, at any point, “in-phase” *V_L*-to-*J_L* joining leads to the production of light chains, these combine with preexisting μ chains to form IgM antibody molecules, which insert into the plasma membrane. The IgM cell-surface receptors are thought to enable the newly formed B cell to receive extracellular signals that shut down all further V(D)J recombination, by turning off the expression of the *Rag1* and *Rag2* genes.

If a developing B cell makes a receptor with high affinity for a self antigen, *Rag* gene expression is maintained and the cell undergoes another round of V(D)J recombination in a light-chain locus (called receptor editing—see Figure 25–13), thereby changing the specificity of its receptor (not shown). If a cell fails to assemble both a functional *V_H*-region and a functional *V_L*-region coding sequence, it is unable to make antibody molecules and dies by apoptosis (not shown).



The production of a functional B cell is a complex and highly selective process: in the end, all B cells that fail to produce intact antibody molecules die by apoptosis.

We turn now from the mechanisms responsible for generating the primary antibody repertoire before antigen stimulation to those responsible for generating the secondary antibody repertoire after antigen stimulation. We begin with the remarkable Darwinian-like mechanism responsible for increasing the affinity of the antigen-binding sites of antibodies for their specific antigen.

Antigen-Driven Somatic Hypermutation Fine-Tunes Antibody Responses

As mentioned earlier, with the passage of time after immunization, there is usually a progressive increase in the affinity of the antibodies produced against the immunizing antigen. This phenomenon, known as **affinity maturation**, is due to the accumulation of point mutations in both heavy-chain and light-chain V-region coding sequences. The mutations occur long after the coding regions have been assembled. After B cells have been stimulated by antigen and helper T cells in a peripheral lymphoid organ, some of the activated B cells proliferate rapidly in the lymphoid follicles (see Figure 25–16) and form structures called **germinal centers**. Here, the B cells mutate at the rate of about one mutation per V-region coding sequence per cell generation. Because this is about a million times greater than the spontaneous mutation rate in other genes and occurs in somatic cells rather than germ cells (discussed in Chapter 21), the process is called **somatic hypermutation**.

Very few of the altered antibodies generated by hypermutation will have an increased affinity for the antigen. Because the same antibody genes produce the antigen receptors on the B cell surface, the antigen will stimulate preferentially those few B cells that do make such antibodies with increased affinity for the antigen. Clones of these altered B cells will preferentially survive and proliferate, especially as the amount of antigen decreases to very low levels late in the response. Most other B cells in the germinal center will die by apoptosis. Thus, as a result of repeated cycles of somatic hypermutation, followed by antigen-driven proliferation of selected clones of effector and memory B cells, antibodies of increasingly higher affinity become abundant during an immune response, providing progressively better protection against the pathogen. (In

some mammals, including sheep and cows, a similar somatic hypermutation also plays a major part in diversifying the primary antibody repertoire before B cells encounter their antigen.)

A breakthrough in understanding the molecular mechanism of somatic hypermutation came with the identification of an enzyme that is required for the process. It is called **activation-induced deaminase (AID)** because it is expressed specifically in activated B cells and deaminates cytosine (C) to uracil (U) in transcribed V-region coding DNA. The deamination produces U:G mismatches in the DNA double helix, and the repair of these mismatches produces various types of mutations, depending on the repair pathway used (**Figure 25–40**). Somatic hypermutation affects only actively transcribed V-region coding sequences, possibly because the AID enzyme is specifically loaded onto RNA transcripts (discussed in Chapter 7). AID is also required when activated B cells switch from IgM production to the production of other classes of antibody, as we now discuss.

B Cells Can Switch the Class of Antibody They Make

As discussed earlier, all B cells begin their antibody-synthesizing lives by making IgM molecules and inserting them into the plasma membrane as receptors for antigen. After the B cells leave the bone marrow, but before they interact with antigen, they begin making both IgM and IgD molecules as membrane-bound antigen receptors, both with the same antigen-binding sites (see Figure 25–22). Stimulation by antigen and helper T cells activates many of these cells to become IgM-secreting effector cells, so that IgM antibodies dominate the primary antibody response. Later in the immune response, however, when activated B cells are undergoing somatic hypermutation, the combination of antigen and helper-T-cell-derived cytokines stimulates many of the B cells to switch from making membrane-bound IgM and IgD to making IgG, IgA, or IgE antibodies—a process called **class switching**. Some of these cells become memory cells that express the corresponding class of antibody molecules on their surface, while others become effector cells that secrete the antibodies. The IgG, IgA, and IgE molecules are collectively referred to as *secondary classes* of antibodies, because they are produced only after antigen stimulation, dominate secondary antibody responses, and make up the secondary antibody repertoire. As we saw earlier, each different class of antibody is specialized to attack pathogens in different ways and in different sites.

The constant region of an antibody heavy chain determines the class of the antibody. Thus, the ability of B cells to switch the class of antibody they make without changing the antigen-binding site implies that the same assembled V_H -region coding sequence (which specifies the antigen-binding part of the heavy chain) can sequentially associate with different C_H -coding sequences. This has important functional implications. It means that, in an individual animal, a

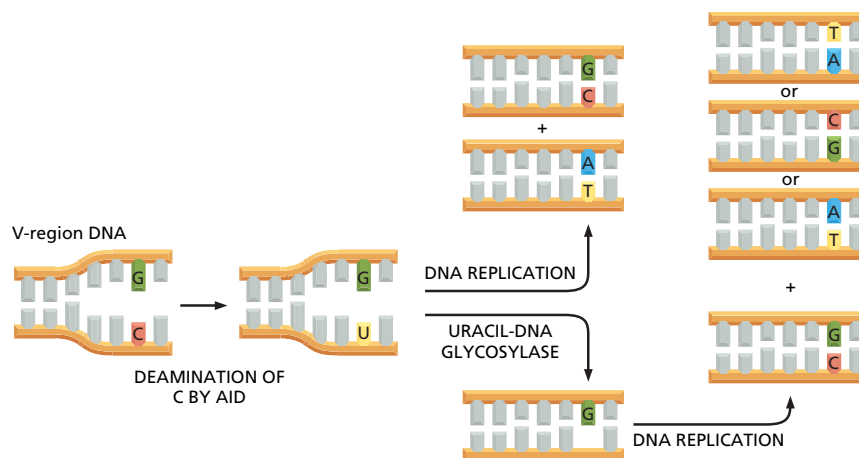


Figure 25–40 Some ways in which AID can cause mutations during somatic hypermutation. AID deaminates some cytosines to uracil in transcribed V-region coding DNA, causing U:G mismatches, which lead to mutations in various ways. Some mutations occur when DNA containing unprocessed U:G mismatches are replicated (see Figure 5–49A). Others occur when the uracil is removed by uracil-DNA glycosylase before the DNA is replicated, as this generates a position on one of the DNA template strands that lacks a base for DNA polymerase to copy. Still others (not shown) occur when the area around the U:G mismatch is excised by the mismatch repair system (discussed in Chapter 5), producing a gap that can be repaired by error-prone DNA polymerases, thereby generating mutations at A:T as well as C:G pairs.

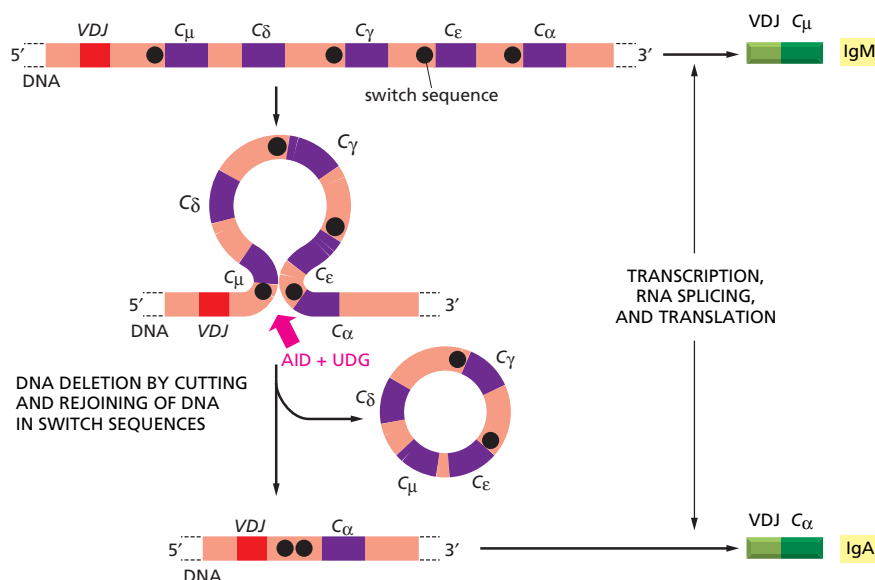


Figure 25–41 An example of the DNA rearrangement that occurs in class switch recombination. A B cell making an IgM antibody from an assembled VDJ DNA sequence is stimulated to switch to making an IgA antibody. In the process, it deletes the DNA between the VDJ sequence and the C α -coding sequence. Specific DNA sequences (*switch sequences*) located upstream of each C H -coding sequence (except C δ) recombine with each other, with the deletion of the intervening DNA. As discussed in the text, the recombination process depends on AID and uracil-DNA glycosylase (UDG), the same enzymes that are involved in somatic hypermutation (see Figure 25–40).

particular antigen-binding site that has been selected by environmental antigens can be distributed among the various classes of antibodies, thereby acquiring the different biological properties of each class.

When a B cell switches from making IgM and IgD to one of the secondary classes of antibody, an irreversible change at the DNA level occurs—a process called **class-switch recombination**. It entails the deletion of all the C H -coding sequences between the assembled VDJ-coding sequence and the particular C H -coding sequence that the cell is destined to express. Class-switch recombination differs from V(D)J recombination in several ways. (1) It happens after antigen stimulation, mainly in germinal centers, and depends on helper T cells. (2) It uses different recombination signal sequences, called *switch sequences*, which are composed of short motifs tandemly repeated over several kilobases. (3) It involves cutting and joining the switch sequences, which are noncoding sequences, and so the coding sequence is unaffected (**Figure 25–41**). (4) Most importantly, the molecular mechanism is different. It depends on AID, which is also involved in somatic hypermutation, rather than on RAG, which is responsible for V(D)J recombination.

The cytokines that activate class switching induce the production of transcription factors that activate transcription from the relevant switch sequences, allowing AID to bind to these sequences. Once bound, AID initiates switch recombination by deaminating some cytosines to uracil in the vicinity of these switch sequences. Excision of these uracils by uracil-DNA glycosylase (see Figure 25–40) is thought to lead somehow to double-strand breaks in the participating switch regions, which are then joined by a form of nonhomologous end-joining (discussed in Chapter 5).

Thus, whereas the primary antibody repertoire in mice and humans is generated by V(D)J joining mediated by RAG, the secondary antibody repertoire is generated by somatic hypermutation and class-switch recombination, both of which are mediated by AID. **Figure 25–42** summarizes the main mechanisms involved in diversifying antibodies that we have discussed in this chapter.

Summary

Antibodies are encoded by three loci on separate chromosomes, each of which produces a different polypeptide chain. One encodes κ light chains, one encodes λ light chains, and one encodes heavy chains. Each antibody locus contains separate gene segments that code for different parts of the variable region of the particular antibody chain. Each light-chain locus contains one or more constant- (C-) region coding sequences and sets of variable (V) and joining (J) gene segments. The heavy-chain locus contains sets of C-region coding sequences and sets of V, diversity (D), and J gene segments.

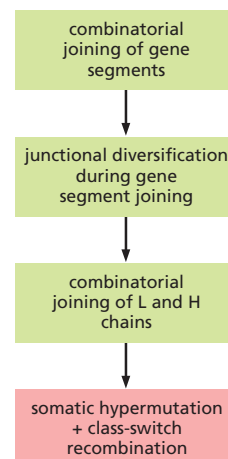


Figure 25–42 The main mechanisms of antibody diversification in mice and humans. Those shaded in green occur during B cell development in the bone marrow (or fetal liver), whereas the mechanisms shaded in red occur when B cells are stimulated by foreign antigen and helper T cells in peripheral lymphoid organs, either late in a primary response or in a secondary response.

During B cell development in the bone marrow (or fetal liver), before antigen stimulation, separate gene segments are brought together by site-specific recombination that depends on the RAG complex. A V_L gene segment recombines with a J_L gene segment to produce a DNA sequence coding for the V region of a light chain, and a V_H gene segment recombines with a D and a J_H gene segment to produce a DNA sequence coding for the V region of a heavy chain. Each of the assembled V-region coding sequences is then co-transcribed with the appropriate C-region sequence to produce an RNA molecule that codes for the complete polypeptide chain. Once a B cell makes functional heavy and light chains that form antigen-binding sites (that do not bind a self antigen with high affinity), it turns off the V(D)J recombination process, thereby ensuring that the cell makes only one species of antigen-binding site.

By randomly combining inherited gene segments that code for V_L and V_H regions during B cell development, humans can make hundreds of different light chains and thousands of different heavy chains. Because the antigen-binding site is formed where the hypervariable loops of the V_L and V_H come together in the final antibody, the heavy and light chains can potentially pair to form antibodies with millions of different antigen-binding sites. The loss and gain of nucleotides at the site of gene-segment joining increases this number enormously. These antibodies, made by RAG-dependent V(D)J recombination before antigen stimulation, are low-affinity IgM and IgD antibodies, and they constitute the primary antibody repertoire.

Antibodies are further diversified following antigen stimulation in peripheral lymphoid organs by the AID- and helper-T-cell-dependent processes of somatic hypermutation and class-switch recombination, which produce the high-affinity IgG, IgA, and IgE antibodies that constitute the secondary antibody repertoire. Class switching allows the same antigen-binding site to be incorporated into antibodies that have different biological properties.

T CELLS AND MHC PROTEINS

Like antibody responses, T-cell-mediated immune responses are exquisitely antigen-specific, and they are at least as important as antibodies in defending vertebrates against infection. Indeed, most adaptive immune responses, including most antibody responses, require helper T cells for their initiation. Most importantly, unlike B cells, T cells can help eliminate pathogens that would otherwise be invisible inside host cells. Much of the rest of this chapter is concerned with how T cells accomplish this feat.

T cell responses differ from B cell responses in at least two crucial ways. First, T cells are activated by foreign antigen to proliferate and differentiate into effector cells only when the antigen is displayed on the surface of *antigen-presenting cells*, usually dendritic cells in peripheral lymphoid organs. T cells require antigen-presenting cells for activation because the form of antigen they recognize is different from that recognized by B cells. Whereas B cells recognize intact protein antigens, for example, T cells recognize fragments of protein antigens that have been partly degraded inside the antigen-presenting cell. Special proteins, called *MHC proteins* (introduced in Chapter 24), bind to the peptide fragments and carry them to the surface of the antigen-presenting cell, where T cells can recognize them.

The second difference is that, once activated, effector T cells act only at short range, either within a secondary lymphoid organ or after they have migrated into a site of infection. Effector B cells, by contrast, secrete antibodies that can act far away. Effector T cells interact directly with another host cell in the body, which they either kill (as in the case of an infected host cell, for example) or signal in some way (as in the case of a B cell or macrophage, for example). We shall refer to such host cells as *target cells*. However, because these target cells must display an antigen bound to an MHC protein on their surface for a T cell to recognize them, they are also antigen-presenting cells.

There are three main classes of T cells—cytotoxic T cells, helper T cells, and regulatory (suppressor) T cells. Effector *cytotoxic T cells* directly kill cells that are infected with a virus or some other intracellular pathogen. Effector *helper T cells*

help stimulate the responses of other cells—mainly macrophages, dendritic cells, B cells, and cytotoxic T cells. Effector *regulatory T cells* suppress the activity of other cells, especially of self-reactive effector T cells.

In this section, we describe these three main classes of T cells and their respective functions. We discuss how they recognize foreign antigens on the surface of antigen-presenting cells and target cells and consider the crucial part played by MHC proteins in the recognition process. Finally, we describe how T cells are selected during their development in the thymus to ensure that only cells with potentially useful receptors survive and mature. We begin by considering the nature of the cell-surface receptors that T cells use to recognize antigen.

T Cell Receptors (TCRs) Are Antibodylike Heterodimers

Because T cell responses depend on direct contact with an antigen-presenting cell or a target cell, **T cell receptors (TCRs)**, unlike antibodies made by B cells, exist only in membrane-bound form and are not secreted. For this reason, TCRs were difficult to isolate, and it was not until the 1980s that researchers identified their molecular structure. TCRs resemble antibodies. They are composed of two disulfide-linked polypeptide chains, each of which contains two Ig-like domains, one variable and one constant (Figure 25–43A). Moreover, the three-dimensional structure of the extracellular part of a TCR has been determined by x-ray diffraction, and it looks very much like one arm of a Y-shaped antibody molecule (Figure 25–43B).

On most T cells, the TCRs have one α chain and one β chain. The genetic loci that encode the α and β chains are located on different chromosomes. Like an antibody heavy-chain locus, the TCR loci contain separate *V*, *D*, and *J* gene segments (or just *V* and *J* gene segments in the case of the α chain locus), which are brought together by site-specific recombination during T cell development in the thymus. With one exception, T cells use the same mechanisms to generate TCR diversity as B cells use to generate antibody diversity. Indeed, they use the same V(D)J recombinase, including the RAG proteins discussed earlier. The mechanism that does not operate in TCR diversification is antigen-driven somatic hypermutation. Thus, the affinity of the receptors tends to be low ($K_a \sim 10^5$ – 10^7 liters/mole), although T cells with the highest affinities are preferentially selected by antigen to persist as memory cells. T cells can partly compensate for their low affinity by increased avidity, which results when multiple TCRs bind simultaneously to multiple membrane-bound ligands (the

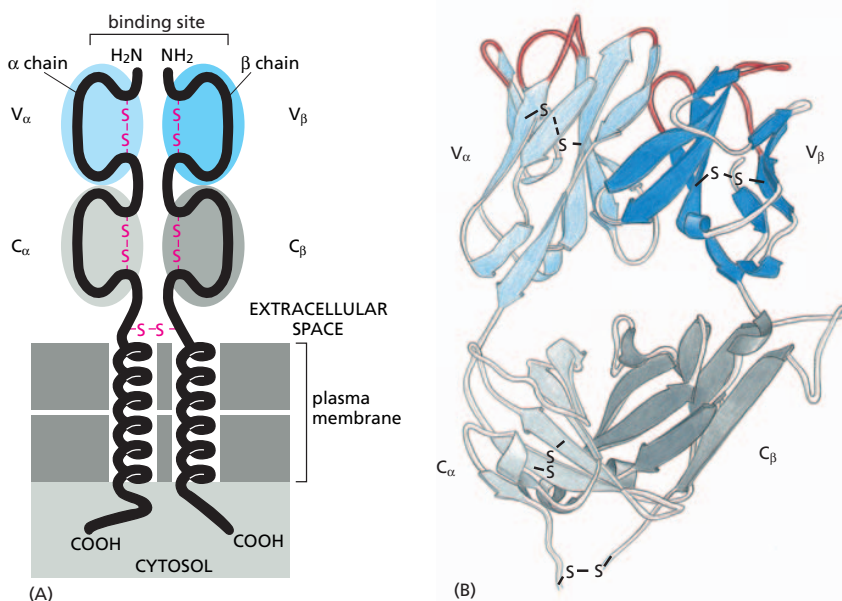


Figure 25–43 A T cell receptor (TCR) heterodimer. (A) Schematic drawing showing that the receptor is composed of an α and a β polypeptide chain. Each chain is about 280 amino acids long and has a large extracellular part that is folded into two Ig-like domains—one variable (V) and one constant (C). The antigen-binding site is formed by a V_α and a V_β domain (shaded in blue). Unlike antibodies, which have two binding sites for antigen, TCRs have only one. The $\alpha\beta$ heterodimer is noncovalently associated with a large set of invariant membrane-bound proteins (not shown), which help activate the T cell when the TCRs bind to antigen. A typical T cell has about 30,000 such receptor complexes on its surface. (B) The three-dimensional structure of the extracellular part of a TCR. The antigen-binding site is formed by the hypervariable loops of both the V_α and V_β domains (red), and it is similar in its overall dimensions and geometry to the antigen-binding site of an antibody molecule. (B, based on K.C. Garcia et al., *Science* 274:209–219, 1996. With permission from AAAS.)

peptide–MHC complexes that we describe later). Moreover, various co-receptors and cell–cell adhesion proteins greatly strengthen the binding of a T cell to an antigen-presenting cell or a target cell.

A minority of T cells, instead of making α and β chains, make a different but related type of receptor heterodimer, composed of γ chains and δ chains. Although these cells normally make up 5–10% of the T cells in human blood, they can be the dominant T cell population in epithelia (in the skin and gut, for example). The functions of these cells are less well understood than those of T cells expressing α/β TCRs, and we will not discuss them further.

As with antigen receptors on B cells, the TCRs are tightly associated in the plasma membrane with a number of invariant membrane-bound proteins that are involved in passing the signal from an antigen-activated receptor to the cell interior (see Figure 25–66). We will discuss these proteins in more detail later. First, we must consider the special ways in which T cells recognize foreign antigen on the surface of an antigen-presenting cell.

Antigen Presentation by Dendritic Cells Can Either Activate or Tolerize T Cells

Naïve cytotoxic or helper T cells must be activated to proliferate and differentiate into effector cells before they can kill or help their target cells, respectively. This activation occurs in peripheral lymphoid organs on the surface of activated **dendritic cells** (Figure 25–44) that display foreign antigen complexed with MHC proteins on their surface, along with co-stimulatory proteins. By contrast, memory T cells can be activated by other types of antigen-presenting cells, including macrophages and B cells, as well as by dendritic cells.

Various kinds of dendritic cells interact with T cells, but they all have a single known function, which is to present antigens that either activate or suppress the T cells. Dendritic cells are located in tissues throughout the body, including the central and peripheral lymphoid organs. Wherever they encounter invading microbes, they endocytose the pathogens or their products. If the encounter is not in a lymphoid organ, the dendritic cells carry the foreign antigens via the lymph to local lymph nodes or gut-associated lymphoid organs. The encounter with a pathogen activates pattern recognition receptors of the dendritic cell, which is thereby induced to mature from an antigen-capturing cell to an activated antigen-presenting cell that can activate T cells (see Figure 25–5). Dendritic cells have to be activated in order to activate naïve T cells, and they can also be activated by tissue injury or by effector helper T cells. Tissue injury is thought to activate dendritic cells by the release of heat shock proteins and uric acid crystals when cells die by necrosis rather than by apoptosis (discussed in Chapter 18).

Activated dendritic cells display three types of protein molecules on their surface that have a role in activating a T cell to become an effector cell or a memory cell (Figure 25–45): (1) *MHC proteins*, which present foreign antigen to the TCR, (2) *co-stimulatory proteins*, which bind to complementary receptors on the T cell surface, and (3) *cell–cell adhesion molecules*, which enable a T cell to bind to the antigen-presenting cell for long enough to become activated, which is usually hours. In addition, activated dendritic cells secrete a variety of cytokines that can influence the type of effector helper T cell that develops (discussed later), as well as where the T cell migrates after it has been stimulated. T cells activated by dendritic cells isolated from gut-associated Peyer's patches (see Figure 25–3), for example, but not by those isolated from lymph nodes, migrate to the small intestine where their antigens are likely to be located.

Nonactivated dendritic cells also have important roles. They help induce self-reactive T cells to become tolerant, both in the thymus and in other organs; such dendritic cells present self antigens in the absence of the co-stimulatory molecules required to activate naïve T cells. They induce tolerance in at least two ways: they can stimulate abortive responses in the T cell that lead to either inactivation or apoptosis, and they can activate regulatory T cells to suppress the activity of another T cell.

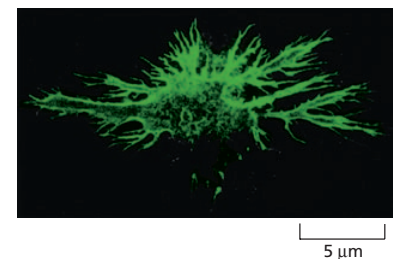
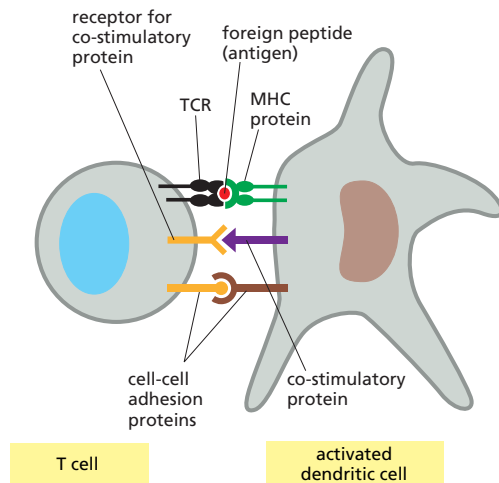


Figure 25–44 Immunofluorescence micrograph of a dendritic cell in culture. These antigen-presenting cells derive their name from their long processes, or “dendrites.” The cell has been labeled with a monoclonal antibody that recognizes a surface antigen on these cells. (Courtesy of David Katz.)



Before discussing the role of MHC proteins in presenting antigen to T cells, we consider the functions of the three major classes of T cells.

Effector Cytotoxic T Cells Induce Infected Target Cells to Kill Themselves

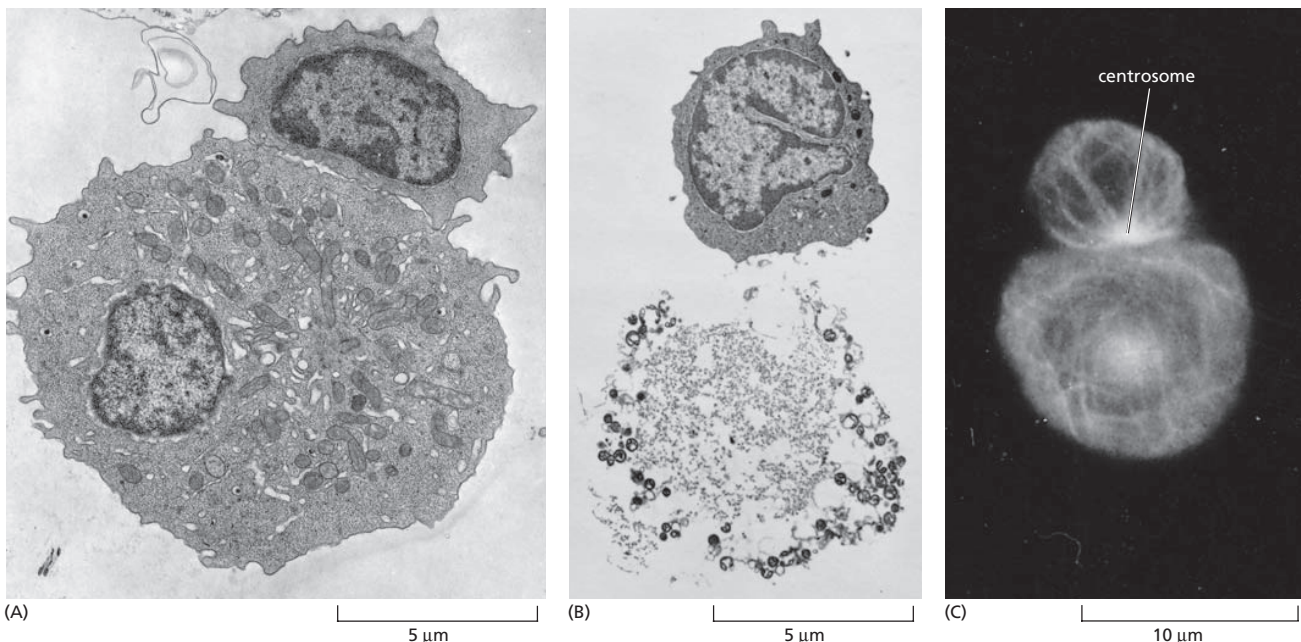
Cytotoxic T cells protect vertebrates against intracellular pathogens such as viruses and some bacteria and parasites that multiply in the host-cell cytoplasm, where they are sheltered from antibody-mediated attack. Cytotoxic T cells do this by killing the infected cell before the microbes can proliferate and escape from the infected cell to infect neighboring cells. As we discuss later, the intracellular microbes can be recognized by T cells because vertebrate cells have mechanisms for displaying fragments of their intracellular proteins on the cell surface, where they are bound to MHC proteins.

Once a cytotoxic T cell has been activated by an infected antigen-presenting cell to become an effector cell, it can kill any target cell harboring the same pathogen. Using its TCR, the effector cytotoxic T cell first recognizes a microbial antigen bound to an MHC protein on the surface of an infected target cell. This causes the T cell to reorganize its cytoskeleton and focus its killing apparatus on the target (**Figure 25–46**). Focus is achieved when the TCRs actively aggregate—

Figure 25–45 Three types of proteins on the surface of an activated dendritic cell involved in activating a T cell. The invariant polypeptide chains that are always stably associated with the T cell receptor (TCR) are not shown.

Figure 25–46 Effector cytotoxic T cells killing target cells in culture. <GTCA>

(A) Electron micrograph showing an effector cytotoxic T cell binding to a target cell. The cytotoxic T cells were obtained from mice immunized with the target cells, which are foreign tumor cells. (B) Electron micrograph showing a cytotoxic T cell and a tumor cell that the T cell has killed. In an animal, as opposed to in a culture dish, the killed target cell would be phagocytosed by neighboring cells long before it disintegrated in the way that it has here. (C) Immuno-fluorescence micrograph of a T cell and tumor cell after staining with anti-tubulin antibodies. Note that the centrosome in the T cell is located at the point of cell–cell contact with the target cell—an immunological synapse. The secretory granules (not visible) in the T cell are initially transported along microtubules to the centrosome, which then moves to the synapse, delivering the granules to where they can release their contents. See also Figure 16–103. (A and B, from D. Zagury et al., *Eur. J. Immunol.* 5:818–822, 1975. With permission from John Wiley & Sons, Inc. C, reproduced from B. Geiger, D. Rosen and G. Berke, *J. Cell Biol.* 95:137–143, 1982. With permission from The Rockefeller University Press.)



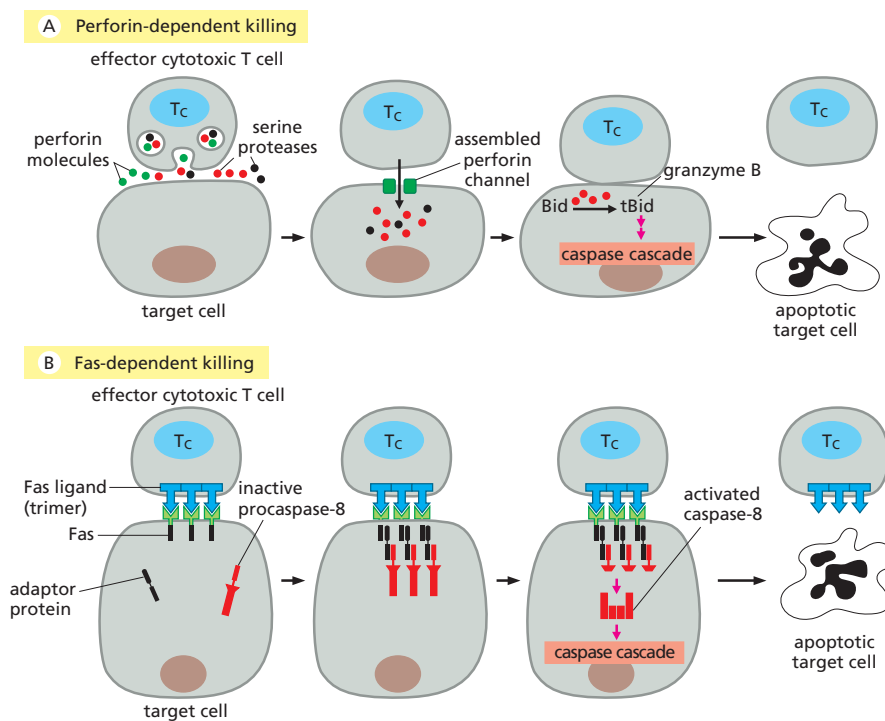


Figure 25-47 Two strategies by which effector cytotoxic T cells kill their target cells. In both cases, the T cell has to contact the target cell to kill it, and a single cytotoxic T cell can kill multiple target cells in sequence. (A) The cytotoxic T cell (T_C) releases perforin and proteolytic enzymes onto the surface of an infected target cell by localized exocytosis. The high concentration of Ca^{2+} in the extracellular fluid causes the perforin to assemble into transmembrane channels in the target cell plasma membrane. The channels are thought to allow the proteolytic enzymes to enter the target cell cytosol. One of the enzymes, granzyme B, cleaves the Bid protein to produce the truncated form tBid, which releases cytochrome *c* from mitochondria to initiate a caspase cascade leading to apoptosis. (B) The homotrimeric Fas ligand on the surface of the cytotoxic T cell binds to and activates the Fas protein on the surface of a target cell. The cytosolic tail of Fas contains a *death domain*, which, when activated, binds to an adaptor protein, which in turn recruits a specific procaspase (procaspase-8). Clustered procaspase-8 molecules become activated and initiate a proteolytic caspase cascade leading to apoptosis (see Figure 18-6).

along with various co-receptors, adhesion molecules, and signaling proteins—at the T cell/target cell interface, forming an *immunological synapse*. A similar synapse forms when an effector helper T cell interacts with its target cell. In this way, effector T cells avoid delivering their signals to neighboring cells.

Once bound to its target cell, an effector cytotoxic T cell can employ one of two strategies to kill the target, both of which operate by inducing the target cell to kill itself by undergoing apoptosis (discussed in Chapter 18). In killing an infected target cell, the cytotoxic T cell usually releases a pore-forming protein called **perforin**, which is homologous to the complement component C9 (see Figure 24-49). The cytotoxic T cell stores perforin in secretory vesicles and releases it by local exocytosis at the point of contact with the target cell. Perforin then polymerizes in the target cell plasma membrane to form transmembrane channels. The secretory vesicles also contain serine proteases, which are thought to enter the target cell cytosol through the perforin channels. One of the proteases, called *granzyme B*, activates a pro-apoptotic Bcl2 protein called *Bid* by producing a truncated form of the protein called *tBid*; tBid then releases cytochrome *c* from mitochondria, triggering a proteolytic caspase cascade that kills the cell by apoptosis (discussed in Chapter 18) (Figure 25-47A). Mice with an inactivated perforin gene cannot generate pathogen-specific cytotoxic T cells, and they show an increased susceptibility to certain viral and intracellular bacterial infections.

In the second killing strategy, the cytotoxic T cell activates a death-inducing caspase cascade in the target cell less directly. A homotrimeric protein on the cytotoxic T cell surface called **Fas ligand** binds to transmembrane receptor proteins on the target cell called **Fas**. The binding alters the Fas proteins so that their clustered cytosolic tails recruit procaspase-8 into a complex via an adaptor protein. The recruited procaspase-8 molecules thereby become activated and initiate a caspase cascade that leads to apoptosis (Figure 25-47B).

Effector Helper T Cells Help Activate Other Cells of the Innate and Adaptive Immune Systems

In contrast to cytotoxic T cells, **helper T cells** are crucial for defense against both extracellular and intracellular pathogens. They help stimulate B cells to make

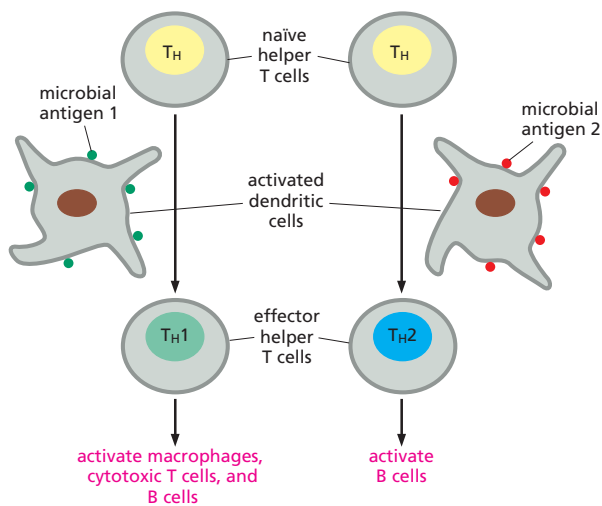


Figure 25–48 Differentiation of naïve helper T cells into either T_H1 or T_H2 effector helper cells in a peripheral lymphoid organ. The nature of the activated dendritic cell and the characteristics of the pathogen that activated it mainly determine which type of effector helper cell develops.

antibodies that help inactivate or eliminate extracellular pathogens and their toxic products. They also activate macrophages to destroy any intracellular pathogens multiplying within the macrophage's phagosomes, and they help activate cytotoxic T cells to kill infected target cells. They can also stimulate a dendritic cell to maintain it in an activated state.

Once an antigen-presenting cell activates a helper T cell to become an effector cell, the helper cell can then help activate other cells. It does this both by secreting a variety of co-stimulatory cytokines and by displaying co-stimulatory proteins on its surface. When activated by its binding to an antigen on a dendritic cell, a naïve helper T cell usually differentiates into either of two distinct types of effector helper cell, called T_H1 and T_H2 . T_H1 cells are mainly involved in immunity to intracellular microbes and help activate macrophages, cytotoxic T cells, and B cells. T_H2 cells are mainly involved in immunity to extracellular pathogens, especially multicellular parasites, and they help activate B cells to make antibodies against the pathogen (Figure 25–48). As we discuss later, the nature of the invading pathogen and the types of innate immune responses it elicits largely determine which type of helper T cell develops. This, in turn, determines the nature of the adaptive immune responses mobilized to fight the invaders.

In some cases, a naïve helper T cell that encounters its antigen in a peripheral lymphoid organ develops into an effector cell that suppresses rather than helps an immune response. Such *regulatory T cells*, however, mostly develop in the thymus, as a distinct class of T cell, as we now discuss.

Regulatory T Cells Suppress the Activity of Other T Cells

Regulatory T cells have been difficult to study and characterize, largely because, until recently, there were no good markers to identify them. Indeed, for many years immunologists questioned whether such cells existed or not. They were originally identified by their ability to suppress the activity of other lymphocytes and were therefore called *suppressor T cells*. When markers became available, they were renamed *regulatory T cells* and shown to suppress the activity of effector helper and cytotoxic T cells and of dendritic cells. Although they make up less than 10% of the T cells in the blood and peripheral lymphoid organs, regulatory T cells play a crucial part in immunological self tolerance by suppressing the activity of self-reactive effector helper and cytotoxic T cells. They also help prevent excessive T cell responses to microbial antigens in chronic infections. In both these ways, they help prevent adaptive immune responses from damaging host tissues.

A breakthrough in understanding regulatory T cells was the discovery that they alone express the transcription factor *Foxp3*, which serves as both an unambiguous marker of these cells and a master controller of their development. When the gene encoding this protein is inactivated in mice or humans, for example, the individuals specifically fail to produce regulatory T cells and

develop an early and fatal autoimmune disease involving multiple organs. It is still uncertain how regulatory T cells suppress the action of effector T cells or dendritic cells, but one pathway is thought to involve the secretion of the inhibitory cytokines *TGF β* and *interleukin 10 (IL10)*.

We now turn to the crucial role of MHC proteins in presenting antigen to T cells.

T Cells Recognize Foreign Peptides Bound to MHC Proteins

As discussed earlier, both cytotoxic T cells and helper T cells are initially activated in peripheral lymphoid organs by recognizing foreign antigen on the surface of an antigen-presenting cell, which is a dendritic cell in the case of a naïve T cell. The antigen is usually in the form of peptide fragments that are generated by the degradation of foreign protein antigens inside the antigen-presenting cell. The recognition process depends on the presence of **MHC proteins** in the antigen-presenting cell. These bind fragments, carry them to the cell surface, and present them there—along with co-stimulatory signals—to the T cells. Once activated, effector T cells then recognize the same peptide–MHC complex on the surface of the target cell they influence, which may be a B cell, a cytotoxic T cell, or an infected macrophage in the case of a helper T cell, or any infected host cell in the case of a cytotoxic T cell; for helper T cells, it may also be the dendritic cell itself.

A large complex of genes called the **major histocompatibility complex (MHC)** encodes MHC proteins. There are two main classes of MHC proteins, and they are both structurally and functionally distinct. *Class I MHC proteins* mainly present foreign peptides to cytotoxic T cells, and *class II MHC proteins* mainly present foreign peptides to helper and regulatory T cells (**Figure 25–49**).

Before examining the mechanisms by which protein antigens are processed for presentation to T cells, we must look more closely at the MHC proteins themselves, which have such a critical role in T cell function.

MHC Proteins Were Identified in Transplantation Reactions Before Their Functions Were Known

MHC proteins were initially identified as the main antigens recognized in **transplantation reactions**. When organ grafts are exchanged between adult individuals, either of the same species (*allografts*) or of different species (*xenografts*), they are usually rejected. In the 1950s, skin grafting experiments between different strains of mice demonstrated that *graft rejection* is an adaptive immune response to the foreign antigens on the surface of the grafted cells. Rejection is mediated mainly by T cells, which react against genetically “foreign” versions of cell-surface proteins called *histocompatibility molecules* (from the Greek word *histos*, meaning “tissue”). The MHC proteins encoded by the clustered genes of the major histocompatibility complex (MHC) are by far the most important of these. MHC proteins are expressed on the cells of all higher vertebrates. They were first demonstrated in mice, where they are called *H-2 antigens* (*histocompatibility-2*

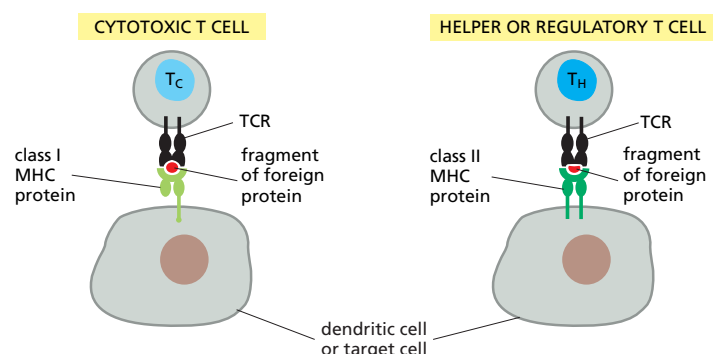


Figure 25–49 Recognition by T cells of foreign peptides bound to MHC proteins. Cytotoxic T cells recognize foreign peptides in association with class I MHC proteins, whereas helper T cells and regulatory T cells recognize foreign peptides in association with class II MHC proteins. In both cases, the T cell recognizes the peptide–MHC complexes on the surface of a dendritic cell or a target cell.

antigens). In humans they are called *HLA antigens* (human-leucocyte-associated antigens) because they were first demonstrated on leucocytes (white blood cells).

Three remarkable properties of MHC proteins baffled immunologists for many years. First, MHC proteins are overwhelmingly the antigens that are recognized in T-cell-mediated transplantation reactions. Second, an unusually large fraction of T cells are able to recognize foreign MHC proteins: whereas fewer than 0.001% of an individual's naïve T cells respond to a typical viral antigen, up to 10% of them respond to the foreign MHC proteins of another individual. Third, some of the genes that code for MHC proteins are the most *polymorphic* known in higher vertebrates. That is, within a species, there are an extraordinarily large number of *alleles* (alternative forms of the same gene) present (in some cases more than 400), without any one allele predominating. As each individual has at least 12 genes encoding MHC proteins (as discussed later), it is very rare for two unrelated individuals to have an identical set of MHC proteins. These differences make it very difficult to match donor and recipient for organ transplantation unless they are closely related.

Of course, a vertebrate does not need to protect itself against invasion by foreign vertebrate cells. So the apparent obsession of its T cells with foreign MHC proteins and the extreme polymorphism of these molecules were a great puzzle. The puzzle was at least partly solved when researchers discovered that (1) MHC proteins bind fragments of foreign proteins and display them on the surface of host cells for T cells to recognize, and (2) T cells respond to foreign MHC proteins in the same way they respond to self MHC proteins that have foreign antigen bound to them.

Class I and Class II MHC Proteins Are Structurally Similar Heterodimers

Class I and class II MHC proteins have very similar overall structures. They are both transmembrane heterodimers with extracellular N-terminal domains that bind antigen for presentation to T cells.

Class I MHC proteins consist of a transmembrane α chain, which is encoded by a class I MHC gene, and a small extracellular protein called β_2 -microglobulin (Figure 25–50A). The β_2 -microglobulin does not span the membrane and is

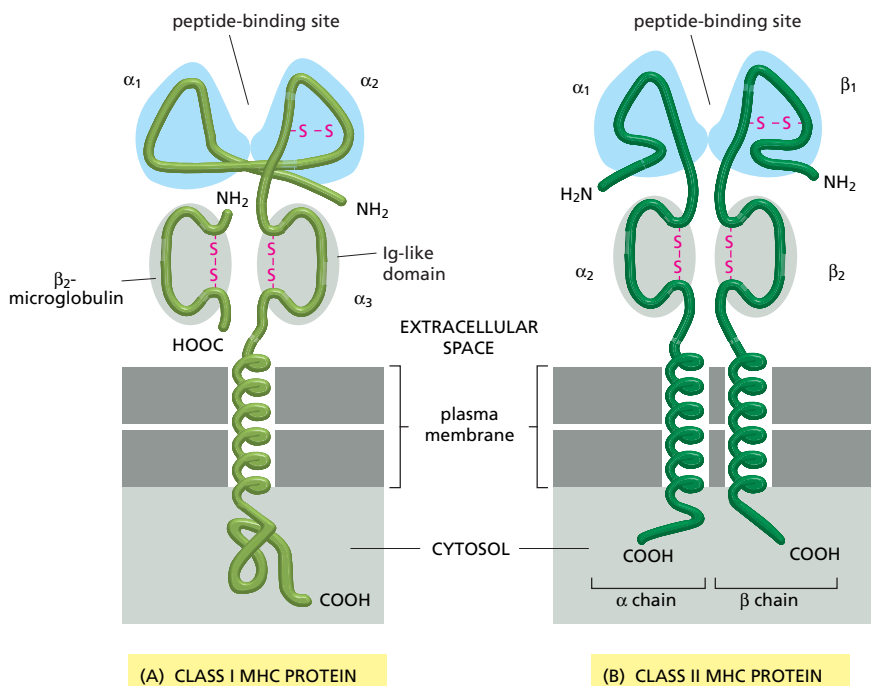


Figure 25–50 Class I and class II MHC proteins. (A) The α chain of the class I molecule has three extracellular domains, α_1 , α_2 , and α_3 , encoded by separate exons. It is noncovalently associated with a smaller polypeptide chain, β_2 -microglobulin, which is not encoded within the MHC. The α_3 domain and β_2 -microglobulin are Ig-like. While β_2 -microglobulin is invariant, the α chain is extremely polymorphic, mainly in the α_1 and α_2 domains. (B) In class II MHC proteins, both chains are polymorphic, mainly in the α_1 and β_1 domains; the α_2 and β_2 domains are Ig-like. Thus, there are striking similarities between class I and class II MHC proteins. In both, the two outermost domains (shaded in blue) are polymorphic and interact to form a groove that binds peptide fragments of foreign proteins and presents them to T cells.

encoded by a gene that does not lie in the MHC gene cluster. The α chain is folded into three extracellular globular domains (α_1 , α_2 , and α_3), and the α_3 domain and the β_2 -microglobulin, which are closest to the membrane, are both similar to an Ig domain. The two N-terminal domains of the α chain, which are farthest from the membrane, contain the polymorphic (variable) amino acids that T cells recognize in transplantation reactions. These domains bind a peptide and present it to cytotoxic T cells.

Like class I MHC proteins, **class II MHC proteins** are heterodimers with two conserved Ig-like domains close to the membrane, and two polymorphic (variable) N-terminal domains farthest from the membrane. In these proteins, however, both chains (α and β) are encoded by genes within the MHC, and both span the membrane (Figure 25–50B). The two polymorphic domains bind a peptide and present it to helper or regulatory T cells.

The presence of Ig-like domains in class I and class II proteins suggests that MHC proteins and antibodies have a common evolutionary history. The locations of the genes that encode class I and class II MHC proteins in humans are shown in **Figure 25–51**, where we illustrate how an individual can make six types of class I MHC proteins and more than six types of class II proteins.

In addition to the classic class I MHC proteins, there are many *class-I-MHC-like proteins*, which form dimers with β_2 -microglobulin. These proteins are encoded by genes outside the MHC and are much less polymorphic than MHC proteins, but some of them present specific microbial antigens, including some lipids and glycolipids, to T cells. Although the functions of most of them are unknown, some have a role in brain development.

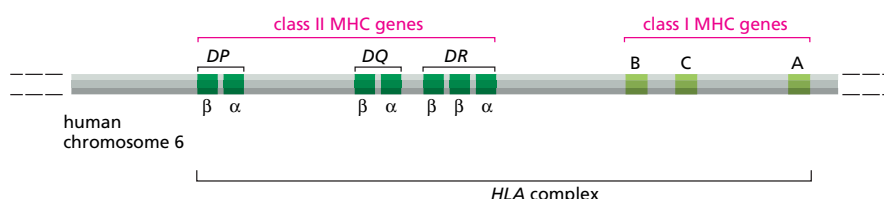
An MHC Protein Binds a Peptide and Interacts with a T Cell Receptor

Any individual can make only a small number of different classic MHC proteins, which together must be able to present peptide fragments from almost any foreign protein to T cells. Thus, unlike an antibody molecule, each MHC protein has to be able to bind a very large number of different peptides. X-ray crystallographic analyses of MHC proteins have revealed the structural basis for this versatility.

As shown in **Figure 25–52A**, a class I MHC protein has a single *peptide-binding site* located at one end of the molecule, facing away from the plasma membrane. This site consists of a deep groove between two long α helices; the groove narrows at both ends so that it is only large enough to accommodate an extended peptide about 8–10 amino acids long. In fact, when a class I MHC protein was first analyzed by x-ray crystallography, this groove contained bound peptides that had co-crystallized with the MHC protein (Figure 25–52B), suggesting that once a peptide binds to this site it does not normally dissociate.

A typical peptide binds in the groove of a class I MHC protein in an extended conformation, with its terminal amino group bound to invariant amino acids of the MHC protein at one end of the groove and its terminal carboxyl group bound to invariant amino acids at the other end of the groove (**Figure 25–53**). Some amino acid side chains of the peptide bind to variable (polymorphic) amino acids of the MHC protein distributed along the groove, while other side chains point outward, in a position to be recognized by TCRs on cytotoxic T cells. Because the invariant amino acids of the MHC protein at the ends of the groove recognize features of the peptide backbone that are common to all peptides, each allelic form of a class I MHC protein can bind a large variety of peptides of

Figure 25–51 Human MHC genes. This simplified schematic drawing shows the location of the genes that encode the transmembrane subunits of class I (light green) and class II (dark green) MHC proteins. The genes shown encode three types of class I proteins (HLA-A, HLA-B, and HLA-C) and three types of class II MHC proteins (HLA-DP, HLA-DQ, and HLA-DR). An individual can therefore make six types of class I MHC proteins (three encoded by maternal genes and three by paternal genes) and more than six types of class II MHC proteins. The number of class II MHC proteins that can be made is greater than six because there are two DR β genes and because maternally encoded and paternally encoded polypeptide chains can sometimes pair.



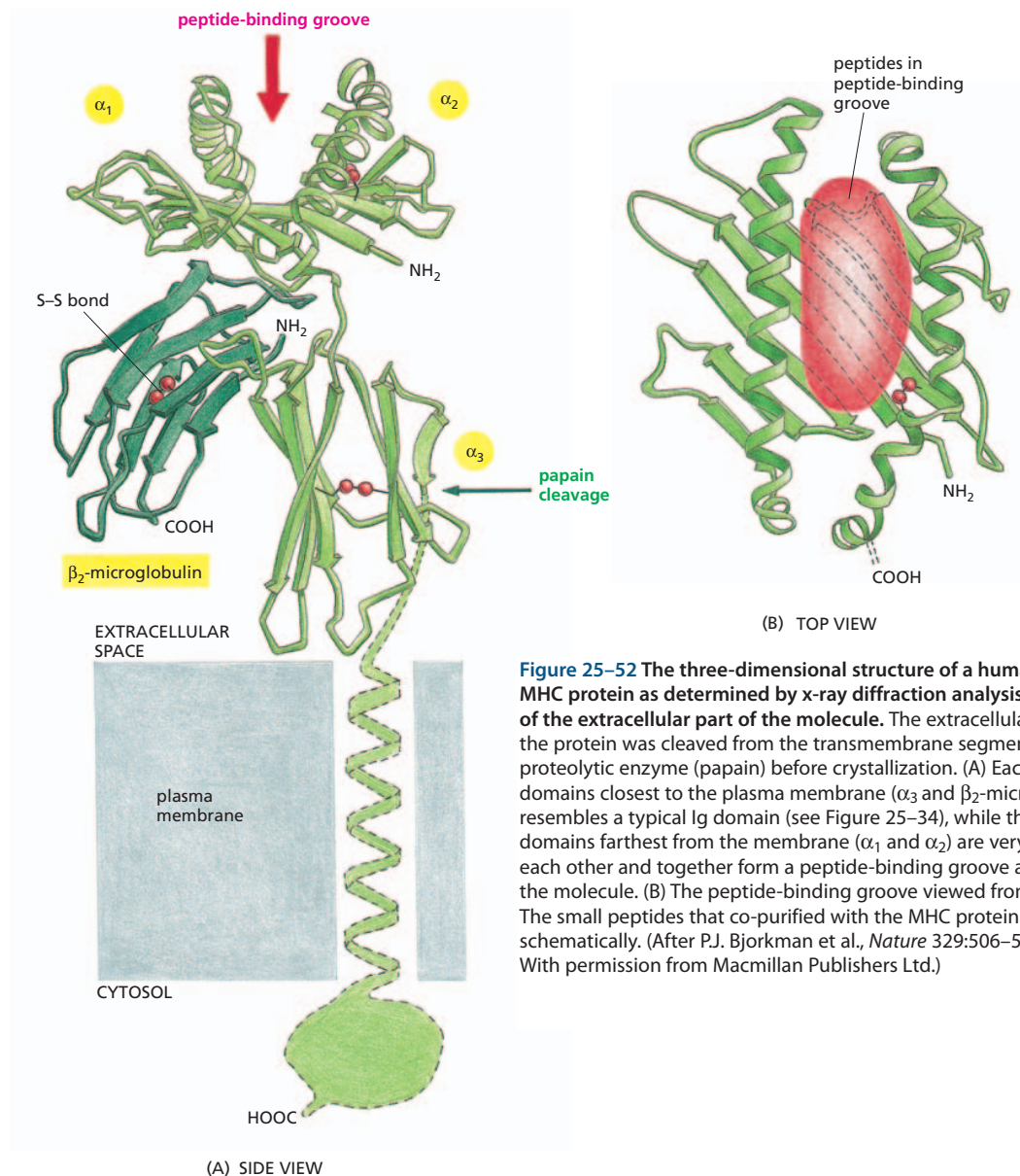
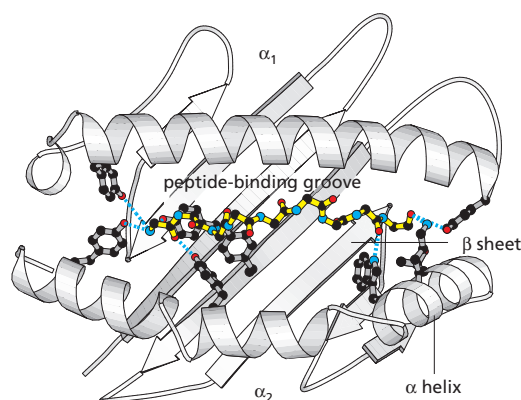


Figure 25-52 The three-dimensional structure of a human class I MHC protein as determined by x-ray diffraction analysis of crystals of the extracellular part of the molecule. The extracellular part of the protein was cleaved from the transmembrane segment by a proteolytic enzyme (papain) before crystallization. (A) Each of the two domains closest to the plasma membrane (α_3 and β_2 -microglobulin) resembles a typical Ig domain (see Figure 25-34), while the two domains farthest from the membrane (α_1 and α_2) are very similar to each other and together form a peptide-binding groove at the top of the molecule. (B) The peptide-binding groove viewed from above. The small peptides that co-purified with the MHC protein are shown schematically. (After P.J. Bjorkman et al., *Nature* 329:506–512, 1987. With permission from Macmillan Publishers Ltd.)

diverse sequence. At the same time, the polymorphic MHC amino acids along the groove, which bind specific side chains of the peptide, ensure that each allelic form binds and presents a distinct characteristic set of peptides. Thus, the six types of class I MHC proteins in an individual can present a broad range of foreign peptides to the cytotoxic T cells, but in each individual they do so in slightly different ways.

The three-dimensional structure of class II MHC proteins is very similar to that of class I proteins, but the antigen-binding groove does not narrow at the ends, so it can accommodate longer peptides, which are usually 12–20 amino acids long. Moreover, the peptide is not bound at its ends but is instead held by interactions with invariant amino acids of the MHC protein distributed along the length of the groove (Figure 25-54). As in the case of class I MHC proteins, side chains of other amino acids in the peptide either bind to polymorphic MHC amino acids along the groove or point upward to be recognized by TCRs on helper or regulatory T cells. A class II MHC groove can accommodate a more heterogeneous set of peptides than can a class I MHC groove. Thus, although an individual makes only a small number of types of class II proteins, each with its



own unique peptide-binding groove, together these proteins can bind and present an enormous variety of foreign peptides to helper T cells, which have a crucial role in almost all adaptive immune responses.

X-ray crystallographic analyses of complexes formed between a soluble TCR and a soluble MHC protein with peptide in its binding groove revealed the way in which the TCR recognizes a peptide–MHC complex. Recombinant DNA technology produced the soluble proteins for these experiments. In each case studied, the TCR fits diagonally across the peptide-binding groove and binds through its V_α and V_β hypervariable loops to both the walls of the groove and the peptide (**Figure 25-55**). Soluble peptide–MHC complexes are now widely used to detect T cells with a particular specificity; they are usually cross-linked into tetramers so that they can bind to four TCRs on the T cell surface with strong avidity.

MHC Proteins Help Direct T Cells to Their Appropriate Targets

Virtually all nucleated vertebrate cells express class I MHC proteins. This is presumably because effector cytotoxic T cells must be able to focus on and kill any cell in the body that becomes infected with an intracellular microbe such as a virus. Class II proteins, by contrast, are normally confined largely to cells that take up foreign antigens from the extracellular fluid and interact with helper T cells. These cells also express class I MHC proteins. They include dendritic cells, which initially activate naïve helper T cells, as well as the targets of effector helper T cells, such as macrophages and B cells.

It is important that effector cytotoxic T cells focus their attack mainly on cells that *make* the foreign antigens (such as viral proteins), while helper T cells focus their help mainly on cells that have taken up foreign antigens from the extracellular fluid. Since the former type of target cell is always a menace, while

Figure 25-53 A peptide bound in the groove of a class I MHC protein. <AAGT>

Schematic drawing of a top view of the groove. A ribbon drawing of the MHC groove is shown in gray; it is formed by the α_1 and α_2 domains of the protein (see Figures 25-50A and 25-52A). The backbone of the bound peptide is shown in yellow, with the carbon atoms in black, oxygen atoms in red, and nitrogen atoms in blue; the amino terminus of the peptide is to the left. Note that the terminal amino and carboxyl groups of the peptide backbone bind via hydrogen and ionic bonds (shown as dotted blue lines) to the side chains of invariant MHC amino acids of the MHC protein towards the ends of the groove (shown in full). Although not illustrated in the drawing, the side chains of some amino acids of the peptide bind to variable (polymorphic) amino acids of the groove, while others point upward and can be recognized by TCRs on a cytotoxic T cell. (Courtesy of Paul Travers.)

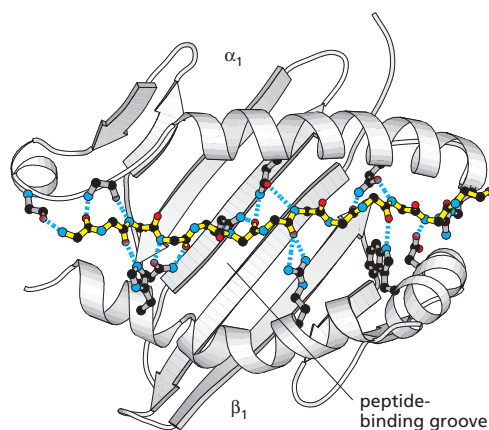


Figure 25-54 A peptide bound in the groove of a class II MHC protein. <GAAA>

Schematic drawing similar to that shown in Figure 25-53. The groove is formed by the amino terminal domains of the α and β chains (α_1 and β_1 —see Figure 25-50B). Note that the peptide extends beyond the ends of the groove and that its backbone binds through hydrogen bonds distributed along the length of the peptide to invariant amino acid side chains in the groove. (Courtesy of Paul Travers.)

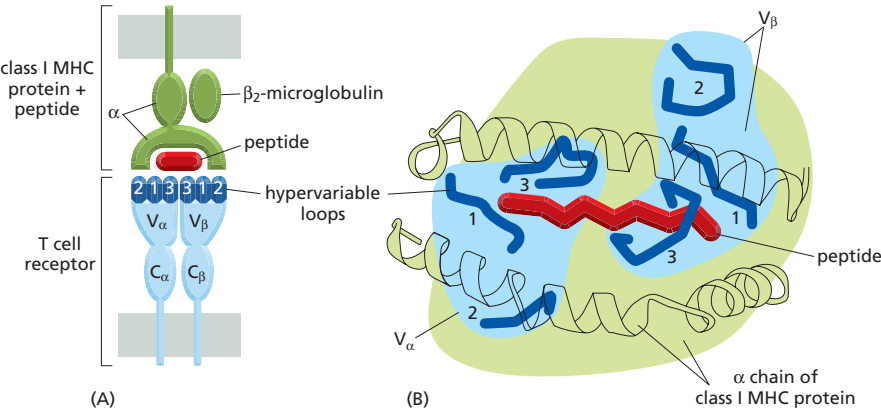


Figure 25–55 The interaction of a T cell receptor with a viral peptide bound to a class I MHC protein. (A) Schematic view of the hypervariable loops of the V α and V β domains of the T cell receptor interacting with the peptide and the walls of the peptide-binding groove of the MHC protein. Note that the third hypervariable loops, which are the most variable, primarily interact with the peptide, whereas the other hypervariable loops primarily bind to the walls of the peptide-binding groove. (B) Drawing of the “footprint” of the V domains (light blue) and hypervariable loops (dark blue) of the receptor over the peptide-binding groove, as determined by x-ray diffraction. The V α domain covers the amino half of the peptide, while the V β domain covers the carboxyl half. Note that the receptor is oriented diagonally across the peptide-binding groove. (B, adapted from D.N. Garboczi et al., *Nature* 384:134–141, 1996. With permission from Macmillan Publishers Ltd.)

the latter type is essential for the body’s adaptive immune defenses, it is vitally important that T cells do not confuse the two target cells and misdirect their cytotoxic and helper functions. Therefore, in addition to the antigen receptor that recognizes a peptide–MHC complex, each of the three major classes of T cells also expresses a *co-receptor* that recognizes a separate, invariant part of the appropriate class of MHC protein. These two co-receptors, called CD4 and CD8, help direct helper (and regulatory) T cells and cytotoxic T cells, respectively, to their appropriate targets. **Table 25–2** reviews the properties of class I and class II MHC proteins.

CD4 and CD8 Co-Receptors Bind to Invariant Parts of MHC Proteins

The affinity of TCRs for peptide–MHC complexes on an antigen-presenting cell or target cell is usually too low by itself to mediate a functional interaction between the two cells. T cells normally require *accessory receptors* to help stabilize the interaction by increasing the overall strength of the cell–cell adhesion. Unlike TCRs or MHC proteins, the accessory receptors do not bind foreign antigens and are invariant.

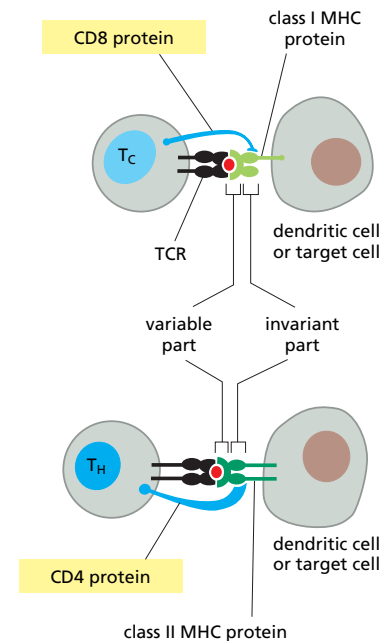
When accessory receptors have a direct role in activating the T cell by generating their own intracellular signals, they are called **co-receptors**. The most important and best understood of the co-receptors on T cells are the CD4 and CD8 proteins, both of which are single-pass transmembrane proteins with extracellular Ig-like domains. Like TCRs, they recognize MHC proteins, but, unlike TCRs, they bind to invariant parts of the protein, far away from the peptide-binding groove. **CD4** is expressed on both helper T cells and regulatory T cells and binds to class II MHC proteins, whereas **CD8** is expressed on cytotoxic T cells and binds to class I MHC proteins (**Figure 25–56**). Thus, CD4 and CD8 contribute to T cell recognition by helping the T cell to focus on particular MHC proteins, and thereby on particular types of target cells: the recognition of class I

Table 25–2 Properties of Human Class I and Class II MHC Proteins

	CLASS I	CLASS II
Genetic loci	HLA-A, HLA-B, HLA-C	DP, DQ, DR
Chain structure	α chain + β_2 -microglobulin	α chain + β chain
Cell distribution	most nucleated cells	dendritic cells, B cells, macrophages, thymus epithelial cells, some others
Presents antigen to	cytotoxic T cells	helper T cells, regulatory T cells
Source of peptide fragments	mainly proteins made in cytoplasm	mainly endocytosed plasma membrane and extracellular proteins
Polymorphic domains	$\alpha_1 + \alpha_2$	$\alpha_1 + \beta_1$
Recognition by co-receptor	CD8	CD4

Figure 25–56 CD4 and CD8 co-receptors on the surface of T cells.

Cytotoxic T cells (T_C) express CD8, which recognizes class I MHC proteins, whereas helper T cells (T_H) and regulatory T cells (not shown) express CD4, which recognizes class II MHC proteins. Note that the co-receptors bind to the same MHC protein that the TCR has engaged, so that they are brought together with TCRs during the antigen recognition process. Whereas the TCR binds to the variable (polymorphic) parts of the MHC protein that form the peptide-binding groove, the co-receptor binds to the invariant part, well away from the groove.



MHC proteins allows cytotoxic T cells to focus on any host cell, while the recognition of class II MHC proteins allows helper T cells to focus on a small subset of cells—most notably dendritic cells, macrophages, and B cells. The cytoplasmic tail of the CD4 and CD8 proteins is associated with a member of the Src family of cytoplasmic tyrosine kinases called *Lck*, which phosphorylates various intracellular proteins on tyrosines and thereby participates in the activation of the T cell (discussed in Chapter 15). Antibodies to CD4 and CD8 are widely used as tools to help distinguish between the main classes of T cells, in both humans and experimental animals: whereas only cytotoxic T cells express CD8, both helper and regulatory T cells express CD4.

Ironically, the AIDS virus (HIV) uses CD4 molecules (as well as chemokine receptors) to enter helper T cells. AIDS patients are susceptible to infection by microbes that are not normally dangerous because HIV depletes helper T cells. As a result, most AIDS patients die of infection within several years of the onset of symptoms, unless they are treated with a combination of powerful anti-HIV drugs. HIV also uses CD4 and chemokine receptors to enter macrophages, which also have both of these receptors on their surface.

Before a T cell can recognize a foreign protein, the protein has to be processed inside an antigen-presenting cell or target cell so that it can be displayed as peptide–MHC complexes on the cell surface. We now consider how a virus-infected antigen-presenting cell or target cell processes viral proteins for presentation to a cytotoxic T cell. We then discuss how ingested foreign proteins are processed for presentation to a helper T cell.

Cytotoxic T Cells Respond to Fragments of Foreign Cytosolic Proteins in Association with Class I MHC Proteins

An experiment performed in the 1970s provided dramatic evidence that class I MHC proteins are involved in the recognition of viral antigens by cytotoxic T cells. Researchers found that effector cytotoxic T cells from a virus-infected mouse could kill cultured cells infected with the same virus only if these target cells expressed some of the same class I MHC proteins as the infected mouse. This experiment demonstrated that the cytotoxic T cells of any individual can recognize a specific foreign antigen on a target cell only when the target cell expresses at least some of the allelic forms of class I MHC proteins expressed by that individual, a phenomenon known as *MHC restriction* (Figure 25–57).

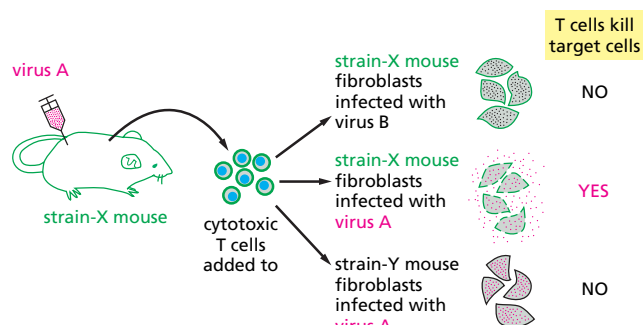
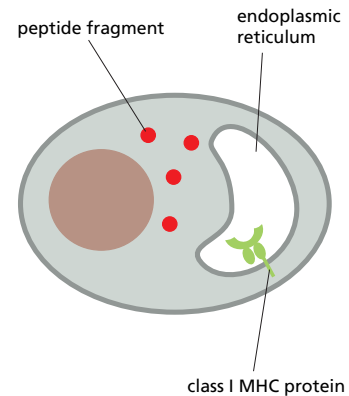


Figure 25–57 The classic experiment showing that an effector cytotoxic T cell recognizes some aspect of the surface of the host target cell in addition to a viral antigen. Mice of strain X are infected with virus A. Seven days later, the spleens of these mice contain effector cytotoxic T cells able to kill virus-infected, strain-X fibroblasts in cell culture. As expected, they kill only fibroblasts infected with virus A and not those infected with virus B. Thus, the cytotoxic T cells are virus-specific. The same T cells, however, are also unable to kill fibroblasts from strain-Y mice infected with the same virus A, indicating that the cytotoxic T cells recognize a genetic difference between the two kinds of fibroblasts and not just the virus. Pinning down the difference required the use of special strains of mice (known as *congenic strains*) that either were genetically identical except for the alleles at their class I MHC loci or were genetically different except for these alleles. In this way, it was found that the killing of infected target cells required that they express at least one of the same class I MHC alleles as expressed by the original infected mouse. This result suggested that class I MHC proteins are somehow necessary to present cell-surface-bound viral antigens to effector cytotoxic T cells and that they do so in a highly specific manner.

Figure 25–58 The peptide-transport problem. How do peptide fragments get from the cytosol, where they are produced, into the ER lumen, where the peptide-binding grooves of class I MHC proteins are located? A special transport process is required.



Subsequent evidence indicated that, in killing a virus-infected cell, a cytotoxic T cell recognizes degraded fragments of internal proteins of the virus that are bound to class I MHC proteins on the infected cell surface. Because a T cell can recognize tiny amounts of foreign antigen (as few as 1–10 peptide–MHC complexes for T cells with the highest affinity receptors), only a tiny fraction of the fragments generated from viral proteins have to bind to class I MHC proteins and get to the cell surface to trigger an effector cytotoxic T cell to attack.

The internal viral proteins are synthesized in the cytosol of the infected cell. As discussed in Chapter 3, proteolytic degradation in the cytosol is mainly mediated by an ATP- and ubiquitin-dependent mechanism that operates in *proteasomes*—large proteolytic enzyme complexes constructed from many different protein subunits. All proteasomes are probably able to generate peptide fragments of appropriate length to fit in the groove of class I MHC proteins, as even bacterial proteasomes can do so. This suggests that the MHC groove evolved to fit peptides of around this length. Nonetheless, some proteasomes are apparently specialized for producing peptides for class I MHC proteins, as they contain two subunits that are encoded by genes located within the MHC chromosomal region.

How do peptides generated in the cytosol make contact with the peptide-binding groove of class I MHC proteins in the lumen of the endoplasmic reticulum (**Figure 25–58**)? The answer initially came through observations of mutant cells in which class I MHC proteins are not expressed at the cell surface but are instead degraded within the cell. The mutant genes in these cells proved to encode subunits of a protein belonging to the family of *ABC transporters*, which we discuss in Chapter 11. This transporter is located in the ER membrane and uses the energy of ATP hydrolysis to pump peptides from the cytosol into the ER lumen. The genes encoding its two subunits are located in the MHC chromosomal region, and if either gene is inactivated by mutation, cells are unable to supply peptides to class I MHC proteins. It is striking that the class I MHC proteins in such mutant cells are degraded inside the cell without reaching the cell surface. This occurs because peptide binding is normally required for the proper folding of these proteins: until it binds a peptide, a class I MHC protein remains in the ER, tethered to an ABC transporter by a chaperone protein; without a peptide bound, the trapped MHC proteins in the mutant cells eventually undergo proteolysis (**Figure 25–59**).

In all cells, peptide fragments come from the cells' own cytosolic and nuclear proteins that are degraded in the processes of normal protein turnover and quality control mechanisms. (Surprisingly, more than 30% of the proteins made by mammalian cells are apparently faulty and are degraded in proteasomes soon after they are synthesized.) These peptides are constantly being pumped into the ER and are carried to the cell surface by class I MHC proteins. They are not antigenic, however, because the cytotoxic T cells that could recognize them have either been eliminated, inactivated, or suppressed by regulatory T cells in the process of self-tolerance (see **Figure 25–13**).

When an antigen activates cytotoxic T cells or T_H1 helper T cells to become effector cells, the effector cells secrete the cytokine **interferon- γ (IFN γ)**, which greatly enhances anti-viral responses. The IFN γ acts on virus-infected host cells in two ways. It blocks viral replication, and it increases the expression of many genes within the MHC chromosomal region. These genes include those that encode class I MHC proteins, the two specialized proteasome subunits, and the two subunits of the peptide transporter located in the ER (**Figure 25–60**). Thus, the machinery in a host cell that is required for presenting viral antigens to cytotoxic T cells is coordinately called into action by IFN γ , creating a positive feedback loop that amplifies the immune response and culminates in the death of the infected cell.

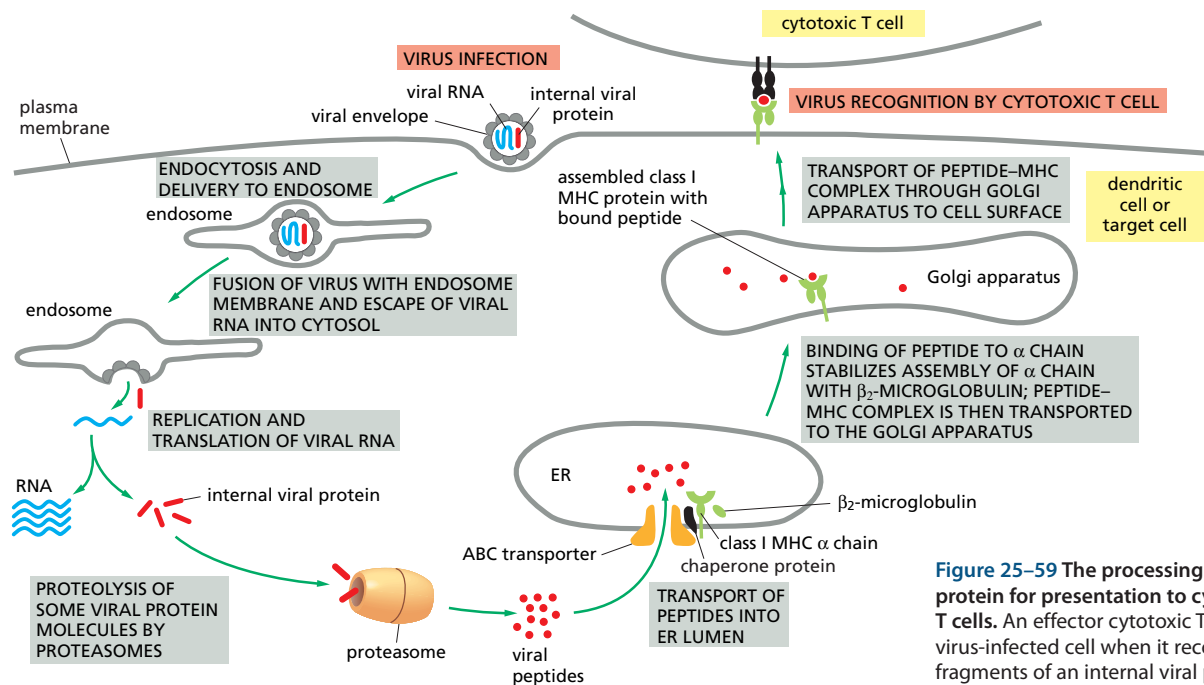


Figure 25–59 The processing of a viral protein for presentation to cytotoxic T cells. An effector cytotoxic T cell kills a virus-infected cell when it recognizes fragments of an internal viral protein bound to class I MHC proteins on the surface of the infected cell. Not all viruses enter the cell in the way that this enveloped RNA virus does, but fragments of internal viral proteins always follow the pathway shown. Only a small proportion of the viral proteins synthesized in the cytosol are degraded and transported to the cell surface, but this is sufficient to attract an attack by a cytotoxic T cell. Several chaperone proteins (only one of which is shown) in the ER lumen aid the folding and assembly of class I MHC proteins. The chaperones bind to the class I MHC α chain and act sequentially. The last one binds the MHC protein to the ABC transporter, as shown. The assembly of class I MHC proteins and their transport to the cell surface requires the binding of peptide.

Helper T Cells Respond to Fragments of Endocytosed Foreign Protein Associated with Class II MHC Proteins

Unlike cytotoxic T cells, helper T cells do not act directly to kill infected cells. Instead, they stimulate macrophages to be more effective in destroying intracellular microorganisms, and they help B cells and cytotoxic T cells to respond to microbial antigens.

Like the viral proteins presented to cytotoxic T cells, the proteins presented to helper T cells on dendritic cells or target cells are degraded fragments of foreign proteins. The fragments are bound to class II MHC proteins in much the same way that virus-derived peptides are bound to class I MHC proteins. But both the source of the peptide fragments presented and the route they take to find the MHC proteins generally differ.

Rather than being derived from foreign protein synthesized in the cytosol of a cell, the foreign peptides presented to helper T cells are derived from endosomes. Some come from extracellular microbes or their products that the antigen-presenting cell has endocytosed and degraded in the acidic environment of its endosomes. Others come from microbes growing within the endocytic compartment of the antigen-presenting cell. In either case, the peptides do not have to be pumped across a membrane because they are generated in a compartment that is topologically equivalent to the extracellular space. Instead of entering the lumen of the ER, where the class II MHC proteins are synthesized and assembled, they bind to preassembled class II heterodimers in an endosomal compartment. When the peptide binds, the class II MHC protein alters its conformation, trapping the

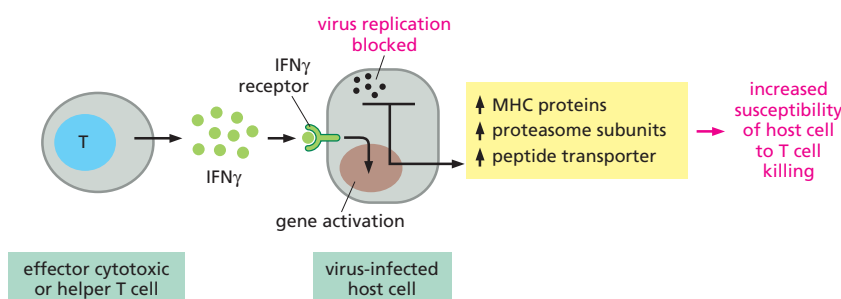


Figure 25–60 Some effects of interferon- γ (IFN γ) on virus-infected cells. The activated IFN γ receptors signal to the nucleus, altering gene transcription, which leads to the effects indicated. The effects shaded in yellow tend to make the infected cell a better target for killing by an effector cytotoxic T cell.

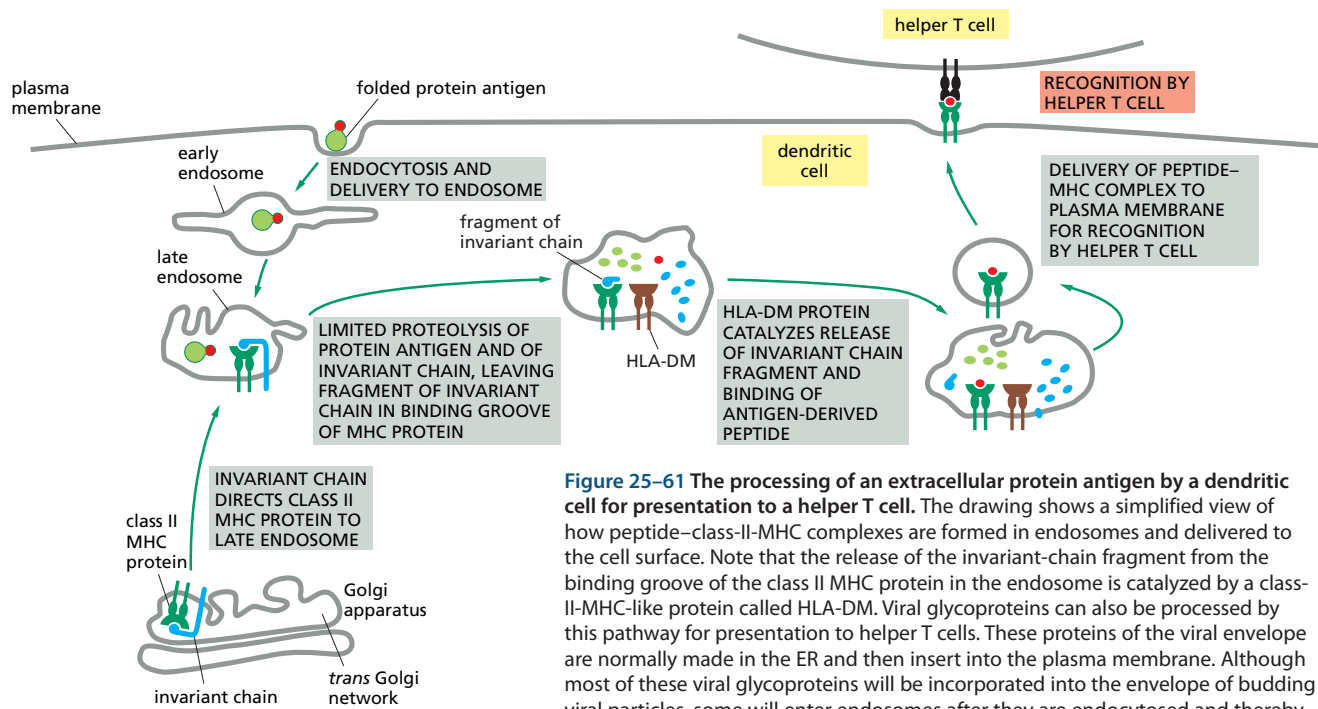


Figure 25–61 The processing of an extracellular protein antigen by a dendritic cell for presentation to a helper T cell. The drawing shows a simplified view of how peptide–class-II-MHC complexes are formed in endosomes and delivered to the cell surface. Note that the release of the invariant-chain fragment from the binding groove of the class II MHC protein in the endosome is catalyzed by a class-II-MHC-like protein called HLA-DM. Viral glycoproteins can also be processed by this pathway for presentation to helper T cells. These proteins of the viral envelope are normally made in the ER and then insert into the plasma membrane. Although most of these viral glycoproteins will be incorporated into the envelope of budding viral particles, some will enter endosomes after they are endocytosed and thereby enter the class II MHC pathway.

peptide in the binding groove and transferring it to the cell surface for presentation to helper T cells.

A newly synthesized class II MHC protein must avoid clogging its binding groove prematurely in the ER lumen with peptides pumped in from the cytosol. A special polypeptide, called the **invariant chain**, ensures this by associating with newly synthesized class II MHC heterodimers in the ER. Part of its polypeptide chain lies within the peptide-binding groove of the MHC protein, thereby blocking the groove from binding peptides in the lumen of the ER. The invariant chain also directs class II MHC proteins from the *trans* Golgi network to a late endosomal compartment. Here, proteases cleave the invariant chain, leaving only a short fragment bound in the peptide-binding groove of the MHC protein. This fragment is then released, freeing the MHC protein to bind peptides derived from endocytosed proteins (**Figure 25–61**). In this way, the functional differences between class I and class II MHC proteins are ensured—the former mainly presenting molecules that come from the cytosol, the latter mainly presenting molecules that come from the endocytic compartment.

This distinction between antigen presentation to cytotoxic T cells and to helper T cells, however, is not absolute. Dendritic cells, for example, need to be able to activate cytotoxic T cells to kill virus-infected cells even when the virus does not infect dendritic cells themselves. To do so, dendritic cells use a process called **cross-presentation**, which begins when they phagocytose fragments of virus-infected cells. They then actively transport viral proteins out of the phagosome into the cytosol, where they are degraded in proteasomes; resulting fragments of the viral proteins are then transported into the ER lumen, where they load onto assembling class I MHC proteins. Cross-presentation in dendritic cells also operates to activate cytotoxic T cells against tumor antigens of cancer cells and against the MHC proteins of foreign organ grafts.

Most of the class I and class II MHC proteins on the surface of a target cell have peptides derived from self proteins in their binding groove. For class I proteins, the fragments mainly derive from degraded cytosolic and nuclear proteins. For class II proteins, they mainly derive from degraded proteins that originate in the plasma membrane or extracellular fluid and are endocytosed. Only a small fraction of the 10^5 or so class II MHC proteins on the surface of an antigen-presenting cell will have foreign peptides bound to them. This is sufficient, however:

even a single copy of such a peptide–MHC complex on a dendritic cell can activate a helper T cell that has a TCR that binds the complex with a high enough affinity.

Potentially Useful T Cells Are Positively Selected in the Thymus

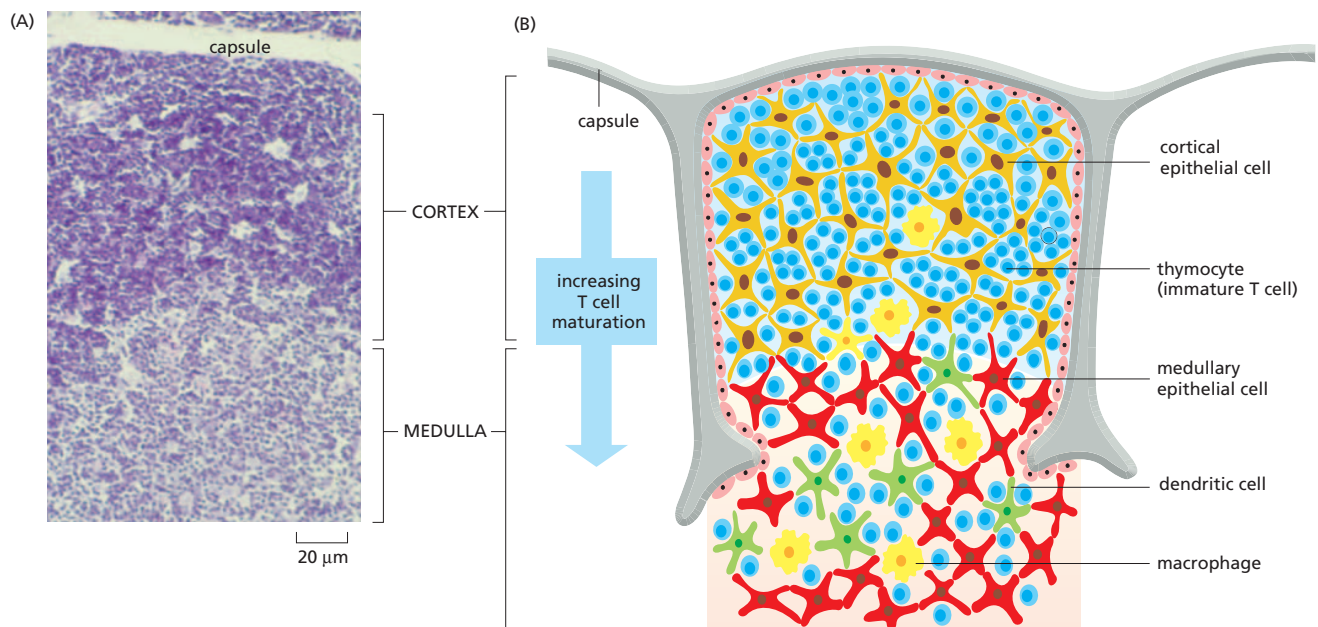
We have seen that T cells recognize antigen in association with self MHC proteins but not in association with foreign MHC proteins (see Figure 25–57): that is, T cells show *MHC restriction*. This restriction results from a process of **positive selection** during T cell development in the thymus. In this process, those immature T cells (*thymocytes*) that will be capable of recognizing foreign peptides presented by self MHC proteins are selected to survive and mature, while most of the remainder, which would be of no use to the animal, undergo apoptosis. Thus, MHC restriction is an acquired property of the immune system that emerges as T cells develop in the thymus.

The most direct way to study the selection process is to follow the fate of a set of developing T cells of known specificity. This can be done by using transgenic mice that express a specific pair of rearranged α and β TCR genes derived from a T cell clone of known antigen and MHC specificity. Such experiments show that the transgenic T cells mature in the thymus and populate the peripheral lymphoid organs only if the transgenic mouse also expresses the same allelic form of MHC protein as is recognized by the transgenic TCR. If the mouse does not express the appropriate MHC protein, the transgenic T cells die in the thymus. Thus, the survival and maturation of a developing T cell depend on a match between its TCR and the MHC proteins expressed in the thymus (which have self peptides derived from the body's own proteins bound to them). Similar experiments using transgenic mice in which MHC expression is confined to specific cell types in the thymus indicate that it is MHC proteins on epithelial cells in the cortex of the thymus that are responsible for this positive selection (Figure 25–62).

After positively selected T cells leave the thymus, their continued survival as naïve T cells depends on their continual stimulation by self-peptide–MHC complexes (and the cytokine *IL7*); this stimulation is enough to promote cell survival but not enough to activate the T cells to proliferate or become effector or memory cells.

As part of the positive selection process in the thymus, developing T cells that express TCRs recognizing class I MHC proteins are selected to become

Figure 25–62 The cellular organization of the human thymus. (A) A light micrograph of a stained section of one thymus lobule showing the outer cortex and inner medulla. (B) A schematic drawing of the lobule showing the cellular composition. The cortex contains immature thymocytes, and the medulla contains mature thymocytes. The thymocytes, macrophages, and dendritic cells develop from cells that migrate in from the bone marrow. The functions of these different regions and cell types will be discussed later, when we consider how developing thymocytes are selected for survival. Because of these selection processes, more than 95% of the thymocytes produced in the thymus die by apoptosis. The dead cells are rapidly phagocytosed and digested by the macrophages. (Adapted from K. Murphy et al., *Janeway's Immunobiology*, 7th ed. New York: Garland Science, 2008.)



cytotoxic cells, while T cells that express TCRs recognizing class II MHC proteins are selected to become either helper cells or regulatory cells. Thus, genetically engineered mice that lack cell-surface class I MHC proteins lack cytotoxic T cells specifically, whereas mice that lack class II MHC proteins lack both helper and regulatory T cells specifically. The development of regulatory T cells depends on special groups of epithelial cells in the thymus medulla called *Has-sall's corpuscles*.

The cells that are undergoing positive selection initially express both CD4 and CD8 co-receptors, and these are required for the selection process: without CD4, helper and regulatory T cells fail to develop, and without CD8, cytotoxic T cells fail to develop. Once they develop, cytotoxic T cells lose CD4, and helper and regulatory T cells lose CD8.

Positive selection still leaves a large problem to be solved. If developing cytotoxic and helper T cells with receptors that recognize self peptides associated with self MHC proteins were to mature in the thymus and migrate to peripheral lymphoid tissues, they might wreak havoc. A second, *negative selection* process in the thymus is required to help avoid this potential disaster.

Most Developing Cytotoxic and Helper T Cells That Could Be Activated by Self-Peptide–MHC Complexes Are Eliminated in the Thymus

As discussed previously, a fundamental feature of the adaptive immune system is that it can learn to distinguish self from nonself and normally does not react against self molecules. An important mechanism in achieving this state of immunological self tolerance is the deletion in the thymus of developing self-reactive cytotoxic and helper T cells—that is, T cells whose TCRs bind strongly enough to the complex of a self peptide and a self MHC protein to become activated. Because, as we discuss later, most B cells require helper T cells to respond to antigen, the elimination of self-reactive helper T cells also helps ensure that any self-reactive B cells that escape from the mechanisms responsible for B cell tolerance induction are harmless (see Figure 25–13).

Before discussing the negative selection process that removes self-reactive T cells in the thymus, it is useful to speculate on the logic behind a two-step system that ends up selecting for the small fraction of developing T cells that express a TCR that binds weakly, but not strongly, to a self MHC protein carrying a self-peptide. As illustrated in **Figure 25–63**, it is thought that the production of a large repertoire of such T cells guarantees that at least a few of the T cells will be able to bind strongly to the complex of a foreign peptide with the same MHC protein, thus triggering an adaptive immune response. However, it is of course not enough for the thymus to select *for* T cells that recognize self MHC proteins; it must also select *against* cytotoxic and helper T cells that could become activated by self MHC proteins complexed with self peptides in peripheral lymphoid

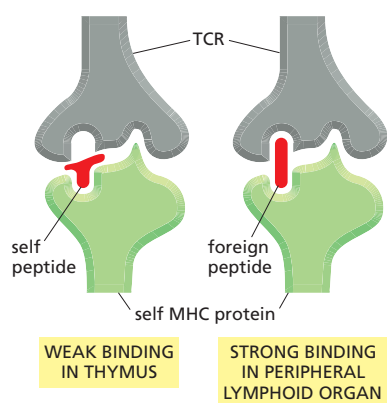


Figure 25–63 Schematic drawing showing how a TCR selected in the thymus because it binds weakly to a self MHC protein complexed with a self peptide can bind strongly to the same MHC protein complexed with a foreign peptide. Because the self peptide complex gives weak binding and the foreign peptide is not present in the thymus, a T cell expressing this TCR in the thymus would be positively selected and also avoid negative selection.

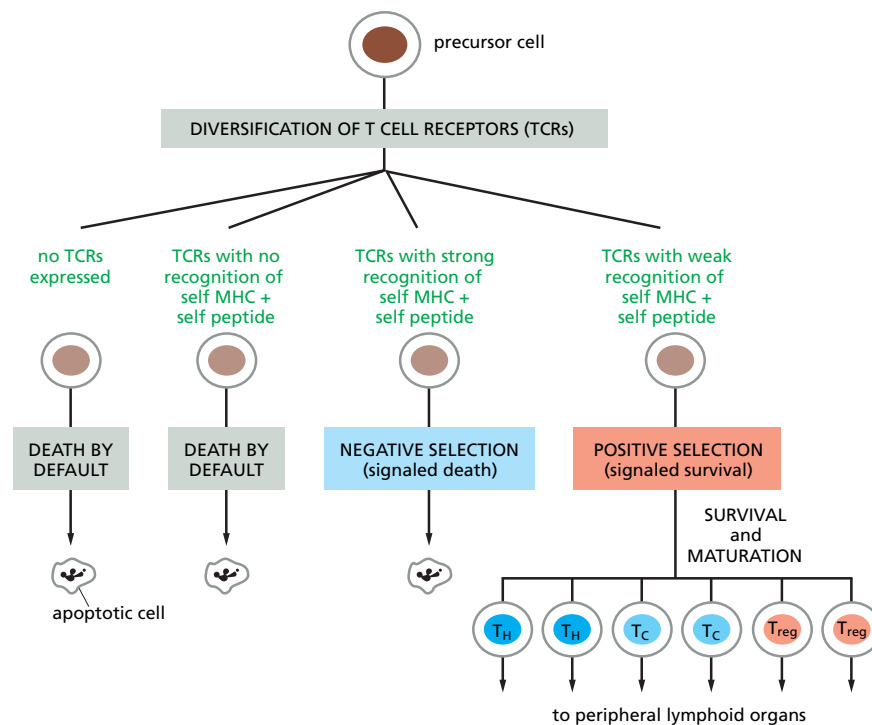


Figure 25–64 The result of positive and negative selection in the thymus. Cells with TCRs that would enable them to respond to foreign peptides in association with self MHC proteins (see Figure 25–63) are positively selected: they survive, mature, and migrate to peripheral lymphoid organs. All of the other cells undergo apoptosis—either because they do not express TCRs that recognize self MHC proteins with self peptides bound or because they recognize such complexes too well and undergo negative selection.

Although not shown, the cells undergoing positive selection initially express both CD4 and CD8 co-receptors. During the process of positive selection, helper T cells (T_H), cytotoxic T cells (T_C), and regulatory T cells (T_{reg}) diverge by a poorly understood mechanism. In this process, helper cells and regulatory cells develop that express CD4 but not CD8 and recognize peptides in association with class II MHC proteins, while cytotoxic cells develop that express CD8 but not CD4 and recognize peptides in association with class I MHC proteins.

organs. The overall goal can be achieved by (1) ensuring the death of cytotoxic and helper T cells that bind *strongly* to the self-peptide–MHC complexes in the thymus while (2) promoting the survival of those that bind weakly and (3) permitting the death of those that do not bind at all. Process 2 constitutes a major part of the positive selection we have just discussed. Process 1 is called **negative selection**, or clonal deletion in the thymus (see Figure 25–13). In both process 1 and process 3, the cells die by apoptosis (Figure 25–64).

The most convincing evidence for negative selection in the thymus derives once again from experiments with transgenic mice. The introduction of TCR transgenes encoding a receptor that recognizes a male-specific peptide antigen, for example, results in large numbers of mature T cells expressing the transgenic receptor in both the thymus and peripheral lymphoid organs of female mice, which do not express the peptide. Very few, however, are found in male mice, in which the cells die in the thymus before they have a chance to mature. Like positive selection, negative selection requires the interaction of a TCR and a CD4 or CD8 co-receptor with an appropriate MHC protein. Unlike positive selection of developing helper and cytotoxic T cells, however, which occurs mainly on the surface of epithelial cells in the thymus cortex, negative selection of these cells occur in the thymus medulla, mainly on the surface of dendritic cells that are descendants of cells that have migrated into the thymus from the bone marrow.

Some Organ-specific Proteins Are Ectopically Expressed in the Thymus Medulla

After the discovery of negative selection of developing T cells in the thymus, immunologists wondered how T cells avoid responses against self proteins that are not present in the thymus. One explanation is that some potentially self-reactive T cells are deleted or functionally inactivated after they leave the thymus. This occurs when the cells recognize self peptides bound to MHC proteins on the surface of dendritic cells that have not been activated by pathogens and therefore do not provide appropriate activating signals. It can also occur via regulatory T cells in the periphery that suppress the activity of some self-reactive effector T cells. These two mechanisms are examples of *peripheral tolerance*,

because, unlike T cell deletion in the thymus (*central tolerance*), they occur after T cells leave the thymus (see Figure 25–13).

Recently, a third explanation has been discovered. A special class of epithelial cells in the thymus medulla ectopically expresses proteins previously thought to be expressed only outside the thymus in specific organs; insulin, for example, which is made by β cells in the pancreas, is also made by a small subset of medullary thymic epithelial cells. The ectopic expression of many of these proteins, including insulin, depends on a nuclear protein called *autimmune regulator* (AIRE), which is specifically expressed in the same medullary thymic epithelial cells. Inactivation of the gene encoding AIRE in mice or humans results in a severe multi-organ autoimmune disease, indicating the importance of AIRE-dependent central tolerance to at least some “organ-specific” self proteins. It remains a mystery how AIRE promotes this ectopic expression of genes in the thymus medulla.

The Function of MHC Proteins Helps Explain Their Polymorphism

The role of MHC proteins in binding foreign peptides and presenting them to T cells helps explain the extensive polymorphism of these proteins. In the evolutionary war between pathogens and the adaptive immune system, pathogens tend to change their antigens to avoid associating with MHC proteins. When a pathogen succeeds, it is able to sweep through a population as an epidemic. In such circumstances, the few individuals that produce a new MHC protein that can associate with an antigen of the altered pathogen have a large selective advantage. In addition, individuals with two different alleles at any given MHC locus (heterozygotes) have a better chance of resisting infection than those with identical alleles at the locus, as they have a greater capacity to present peptides from a wide range of pathogens. Thus, this type of selection will tend to promote and maintain a large diversity of MHC proteins in the population. Support for the idea that infectious diseases have been a driving force for MHC polymorphism has come from studies in West Africa. In this region, individuals with a specific MHC allele have a reduced susceptibility to a severe form of malaria. Although the allele is rare elsewhere, it is found in 25% of the West African population where this form of malaria is common.

If greater MHC diversity means greater resistance to infection, why do we each have so few MHC genes encoding these molecules? Why have we not evolved strategies for increasing the diversity of MHC proteins—by alternative RNA splicing, for example, or by the genetic recombination mechanisms used to diversify antibodies and TCRs? One reason for the restricted diversity of MHC proteins in an individual may be that each time a new MHC protein is added to the repertoire, the T cells with TCRs that bind strongly to self peptides bound to the new MHC protein must be eliminated to maintain self tolerance. The elimination of these T cells would counteract the advantage of adding the new MHC protein. Thus, the number of MHC proteins we express is thought to represent a balance between the advantages of presenting a wide diversity of foreign peptides to T cells against the disadvantages of severely restricting the T cell repertoire during negative selection in the thymus. Computer modeling studies support this explanation.

Summary

There are three main functionally distinct classes of T cells. Cytotoxic T cells kill infected cells directly by inducing them to undergo apoptosis. Helper T cells help activate B cells to make antibody responses, cytotoxic T cells to kill their target cells, dendritic cells to stimulate T cell responses, and macrophages to destroy microorganisms that either invaded the macrophage or were ingested by it. Finally, regulatory T cells suppress the activity of effector T cells and dendritic cells and are crucial for self tolerance.

All T cells express cell-surface, antibodylike receptors (TCRs), which are encoded by genes that are assembled from multiple gene segments during T cell development

in the thymus. TCRs recognize fragments of foreign proteins that are displayed on the surface of host cells in association with MHC proteins. T cells are activated in peripheral lymphoid organs by antigen-presenting cells, which express peptide–MHC complexes, co-stimulatory proteins, and various cell–cell adhesion molecules on their cell surface. The most potent of these antigen-presenting cells are dendritic cells, which are specialized for antigen presentation and are required for the activation of naïve T cells.

Class I and class II MHC proteins have crucial roles in presenting foreign protein antigens to T cells: class I MHC proteins present antigens to cytotoxic T cells, while class II MHC proteins present antigens to helper and regulatory T cells. Whereas class I proteins are expressed on almost all vertebrate cells, class II proteins are normally restricted to those cell types that interact with helper T cells, such as dendritic cells, macrophages, and B lymphocytes.

Both classes of MHC proteins have a single peptide-binding groove, which binds small peptide fragments derived from proteins. Each MHC protein can bind a large set of peptides, which are constantly being produced intracellularly by normal protein degradation processes. However, class I MHC proteins mainly bind fragments produced in the cytosol, while class II MHC proteins mainly bind fragments produced in endocytic compartments. After they have formed inside the target cell, the peptide–MHC complexes are transported to the cell surface. Complexes that contain a peptide derived from a foreign protein are recognized by TCRs, which interact with both the peptide and the walls of the peptide-binding groove of the MHC protein. T cells also express CD4 or CD8 co-receptors, which simultaneously recognize non-polymorphic regions of MHC proteins on the antigen-presenting cell or target cell: helper cells and regulatory cells express CD4, which recognizes class II MHC proteins, while cytotoxic T cells express CD8, which recognizes class I MHC proteins.

A combination of positive and negative selection processes operates during T cell development in the thymus to shape the TCR repertoire. These processes help to ensure that only T cells with potentially useful receptors survive and mature, while all of the others die by apoptosis. First, T cells that can respond to peptides complexed with self MHC proteins are positively selected; subsequently, the T cells in this group that can react strongly with self peptides complexed with self MHC proteins are eliminated. Helper and cytotoxic T cells that leave the thymus with receptors that could react with self antigens are eliminated, functionally inactivated, or actively suppressed when they recognize self antigens on nonactivated dendritic cells.

HELPER T CELLS AND LYMPHOCYTE ACTIVATION

Helper T cells are arguably the most important cells in adaptive immunity, as they are required for almost all adaptive immune responses. They help activate B cells to secrete antibodies, and they help macrophages to destroy ingested pathogens. They also help activate cytotoxic T cells to kill infected target cells, at least partly by stimulating dendritic cells to activate naïve cytotoxic cells more efficiently. As dramatically demonstrated in AIDS patients, without helper T cells we cannot even defend ourselves against many microbes that are normally harmless.

Helper T cells themselves, however, can function only when activated to become effector cells. Naïve helper cells are activated on the surface of dendritic cells, which become activated during the innate immune responses triggered by an infection. The innate responses, mainly via activated dendritic cells, also dictate what kind of effector cell a helper T cell will develop into, and they thereby determine the nature of the adaptive immune responses elicited.

In this final section, we discuss the multiple signals that help activate a T cell and how a helper T cell, once activated to become an effector cell, helps activate other cells. We also consider how innate immune responses determine the nature of adaptive responses by stimulating helper T cells to differentiate into different types of effector cells. Finally, we discuss the probable evolutionary origins of the Ig superfamily of proteins, which includes the MHC proteins, antibodies, and TCRs.

more strongly to its Ig-like ligand on the surface of the dendritic cell. This increased adhesion enables the T cell to remain bound to the antigen-presenting cell long enough to become activated.

This initial signaling through the TCR and associated proteins triggers the active assembly of an **immunological synapse** at the interface between the T cell and the dendritic cell. In these bull's-eye-like structures, TCRs and their associated CD3 subunits, co-receptors, and intracellular signaling proteins are clustered at the center, with cell–cell adhesion proteins forming a peripheral ring. Similar structures form when an effector helper or cytotoxic cell interacts with its target cell. Not all of the TCRs in the synapse are bound to a foreign peptide complexed with an MHC protein; some are bound to a self peptide bound to an MHC protein, and these TCRs also contribute to the activation of the T cell (recall that all T cells are initially positively selected in the thymus for their weak recognition of such self-peptide–MHC complexes).

The combined actions of the various signals just discussed stimulate helper T cells to proliferate and to begin to differentiate into effector cells by a curiously indirect mechanism. The signals cause the T cells to help stimulate their own proliferation and differentiation by inducing the cells to secrete a cytokine called **interleukin-2 (IL2)** and simultaneously to synthesize high-affinity cell-surface receptors that bind it. The binding of IL2 to the IL2 receptors activates intracellular signaling pathways that turn on genes that help the T cells to proliferate and differentiate into effector cells (**Figure 25–67**). Although some T cells do not make IL2, as long as they have been activated by their antigen and therefore express IL2 receptors they can be helped to proliferate and differentiate by IL2 made by neighboring activated T cells. IL2 also plays an important part in the development of regulatory T cells in the thymus, because without it these cells fail to develop.

Dendritic cells are not only important for activating T cells, they are also important for inactivating or eliminating self-reactive T cells. When T cells recognize self-peptide–MHC complexes on the surface of dendritic cells that have not been activated by a pathogen, they are either inactivated, so that they do not respond to the same peptide–MHC complexes even on activated dendritic cells, or they proliferate briefly and then die by apoptosis. Both of these mechanisms—clonal inactivation and clonal deletion—contribute to peripheral self tolerance. Dendritic cells also contribute to peripheral self tolerance by activating regulatory T cells, which then suppress the activity of self-reactive effector T cells, although the details of how dendritic cells selectively activate regulatory T cells are poorly understood (see **Figure 25–13**).

The Activation of T Cells Is Controlled by Negative Feedback

During the multi-step activation of a T cell, the cell starts to express a cell-surface protein called *CTLA4*, which inhibits intracellular signaling. This protein resembles CD28, and, like CD28, it binds to B7 proteins on the surface of the activating dendritic cell (see **Figure 25–67**). CTLA4 binds B7 with much higher affinity than does CD28 and, in doing so, it blocks the activating activity of CD28,

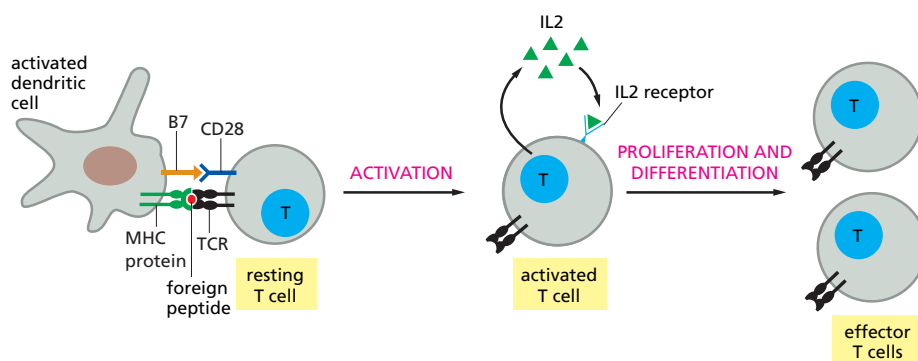


Figure 25–67 The stimulation of T cells by IL2. This model can apply to both helper and cytotoxic T cells, at least in culture. The combination of peptide–MHC complexes and a co-stimulatory B7 protein (either B7-1 or B7-2; also called CD80 and CD86, respectively) on the surface of an activated dendritic cell helps stimulate a resting T cell to make high affinity IL2 receptors and to secrete IL2. The binding of IL2 to its receptors then helps stimulate the T cells to proliferate and differentiate into effector cells. The various proteins associated with the TCRs (see **Figure 25–65**) are not shown.

Table 25–3 Some Accessory Proteins on the Surface of T Cells

PROTEIN*	SUPERFAMILY	EXPRESSED ON	LIGAND ON TARGET CELL	FUNCTIONS
CD3 complex	Ig (except for ζ)	all T cells	—	helps transduce signal when antigen–MHC complexes bind to TCRs; helps transport TCRs to cell surface
CD4	Ig	helper T cells, regulatory T cells	class II MHC	promotes adhesion to dendritic cells and target cells; signals T cell
CD8	Ig	cytotoxic T cells	class I MHC	promotes adhesion to dendritic cells and infected target cells; signals T cell
CD28	Ig	most T cells	B7 proteins (CD80 and CD86)	helps activate T cells
CTLA4	Ig	activated T cells	B7 proteins (CD80 and CD86)	inhibits T cell activation
CD40 ligand	Fas ligand family	effector helper T cells	CD40	co-stimulatory protein that helps activate macrophages, B cells, and dendritic cells

*CD stands for cluster of differentiation, as each of the CD proteins was originally defined as a blood cell “differentiation antigen” recognized by multiple monoclonal antibodies. Their identification depended on large-scale collaborative studies in which hundreds of such antibodies, generated in many laboratories, were compared and found to consist of relatively few groups (or “clusters”), each recognizing a single cell-surface protein. Since these initial studies, however, more than 240 CD proteins have been identified.

thereby providing negative feedback that holds the activation process in check. Thus, mice with a disrupted *Ctla4* gene die from a massive accumulation of activated T cells.

Table 25–3 summarizes some of the co-receptors and other accessory proteins found on the surface of T cells that we have discussed in this chapter.

Most of the T (and B) effector cells produced during an immune response must be eliminated after they have done their job. Although most of the cells die by apoptosis, the extracellular mechanisms responsible for their elimination are poorly understood. One possibility is that, as antigen levels fall and the response subsides, effector T cells are deprived of the antigen and cytokine stimulation that they need to survive, so that only memory cells and some long-lived effector cells survive. The death of effector T cells, however, does not occur solely through a lack of survival signals. In the case of effector cytotoxic T cells, for example, the cytokine *interferon- γ* (*IFN γ*) plays an important part in inducing the cell death; as effector cytotoxic T cells make *IFN γ* (see Figure 25–60), this is another form of negative feedback.

Before considering how effector helper T cells help activate macrophages and B cells, we need to discuss the two main functionally distinct subclasses of effector helper T cells, T_H1 and T_H2 cells, and how they are generated.

The Subclass of Effector Helper T Cell Determines the Nature of the Adaptive Immune Response

When an activated dendritic cell activates a naïve helper T cell in a peripheral lymphoid organ, the T cell usually differentiates into a T_H1 or T_H2 effector helper cell. The outcome depends on the affinity of the TCR for the peptide–MHC complex, on the density of the complex on the dendritic cell surface, and on the nature of the dendritic cell.

The two main subclasses of effector helper T cells can be distinguished by the cytokines they secrete. **T_H1 cells** mainly secrete *IFN γ* and *tumor necrosis factor- α* (*TNF α*), which activate macrophages to kill microbes located within the macrophages’ phagosomes. They also help activate cytotoxic T cells to kill infected cells. Thus, T_H1 cells mainly defend an animal against intracellular microbes. They also, however, stimulate B cells to secrete specific subclasses of IgG antibodies that can coat extracellular microbes and activate complement, thereby helping to eliminate some extracellular microbes as well.

T_H2 cells, by contrast, mainly defend an animal against extracellular pathogens, including microbes and multicellular parasites. They secrete a variety

of cytokines, including *interleukins 4 and 10* (*IL4* and *IL10*), and they help stimulate B cells to make most classes of antibodies, including IgM, IgA, IgE, and some subclasses of IgG. Some of these antibodies bind to mast cells, basophils, and eosinophils; when activated by antigen binding, these cells release local mediators that cause sneezing, coughing, or diarrhea and thereby help expel extracellular microbes and larger parasites from epithelial surfaces of the body.

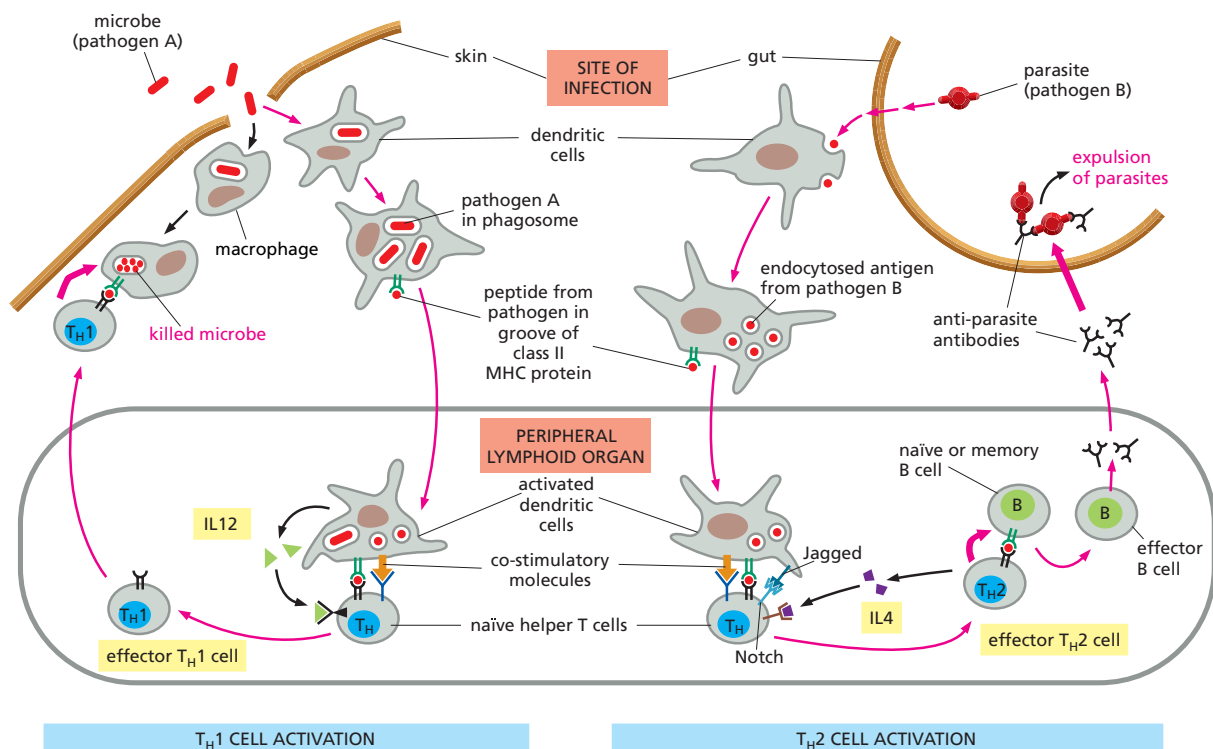
Thus, the decision of naïve helper T cells to differentiate into T_H1 or T_H2 effector cells influences the type of adaptive immune response that an animal mounts against the pathogen—whether it will be dominated by macrophage activation or by antibody production. The specific cytokines produced during the process of helper T cell activation have an important influence on the type of effector helper cell produced. Some intracellular bacteria, for example, stimulate dendritic cells to produce *IL12*, which induces T_H1 development, and thereby macrophage activation. As expected, mice that are deficient in either *IL12* or its receptor are much more susceptible to these bacterial infections than are normal mice. Many parasitic protozoa and worms, by contrast, stimulate a dendritic cell to express the *Jagged protein* on its surface. Jagged is an activating ligand for *Notch receptors* (discussed in Chapters 15 and 22) on the T cell surface, and the resulting Notch signaling helps induce T_H2 development and the production of *IL4*. The T_H2 cells and *IL4* help stimulate antibody production and eosinophil activation, leading to parasite expulsion (Figure 25–68). The *IL4* also generates a positive feedback loop, as it is a potent inducer of T_H2 development.

Once a T_H1 or T_H2 effector cell develops, it inhibits the differentiation of the other type of helper T cell. $IFN\gamma$ produced by T_H1 cells inhibits the development of T_H2 cells, while *IL4* and *IL10* produced by T_H2 cells inhibit the development of T_H1 cells. Thus, as the response proceeds, it reinforces the initial choice through its effect on the response of other T cells nearby.

Individuals infected with *Mycobacterium leprae*, the bacterium that causes leprosy, illustrate the importance of the T_H1/T_H2 decision. This bacterium replicates mainly within macrophages and causes either of two forms of disease, depending mainly on the genetic make-up of the infected individual. In some patients, the *tuberculoid* form of the disease occurs. Here, T_H1 cells develop and stimulate the infected macrophages to kill the bacteria. This produces a local inflammatory response, which damages skin and nerves. The result is a chronic

Figure 25–68 The activation of T_H1 and T_H2 cells. The differentiation of naïve helper T cells into either T_H1 or T_H2 effector cells determines the nature of the subsequent adaptive immune responses. Whether a naïve helper T cell becomes a T_H1 or T_H2 cell depends mainly on the signal proteins present when an activated dendritic cell in a peripheral lymphoid organ stimulates the helper T cell. The types of signal proteins produced depend on the local environment and the nature of the pathogen that activated the dendritic cell at the site of infection. *IL12* secreted by activated dendritic cells promotes T_H1 cell development. By contrast, both the transmembrane Notch ligand Jagged on the surface of activated dendritic cells and the *IL4* made by basophils, mast cells, and T_H2 cells promote T_H2 cell development.

In this figure, the effector T_H1 cell produced in the peripheral lymphoid organ migrates to the site of infection and helps a macrophage kill the microbes it has phagocytosed. The effector T_H2 cell remains in the lymphoid organ and helps activate a B cell to produce antibodies against the parasite. In addition to binding to the parasites, the antibodies bind to Fc receptors on mast cells, basophils, and eosinophils (see Figure 25–27), which then can help expel the parasite from the gut. Although not shown here, T_H1 cells also help activate B cells to make antibodies.



disease that progresses slowly but does not kill the host. In other patients, by contrast, the *lepromatous* form of the disease occurs. Here, T_H2 cells develop and stimulate the production of antibodies. As the antibodies cannot get through the plasma membrane to attack the intracellular bacteria, the bacteria proliferate unchecked and eventually kill the host. For unknown reasons, there is also a general depression of T-cell-mediated immunity to most antigens in the lepromatous form of the disease.

Naïve helper T cells can also develop into a recently described third type of effector cell called *T_H17 cells* because they secrete the pro-inflammatory interleukin *IL17*. T_H17 cells help defend against extracellular pathogens, but they also play an important part in many autoimmune diseases. They develop when some naïve helper T cells are activated by antigen in the presence of $TGF\beta$ and the interleukin *IL6*.

T_H1 Cells Activate Infected Macrophages and Stimulate An Inflammatory Response

Macrophages and dendritic cells ingest pathogens and their products at sites of infection. The dendritic cells become activated and carry microbial antigens to a peripheral lymphoid organ, where they preferentially induce the development of T_H1 cells. The T_H1 cells then migrate to the site of infection to help activate infected macrophages (see Figure 25–68).

T_H1 effector cells use two signals to activate the specific macrophages they recognize. They secrete $IFN\gamma$, which binds to $IFN\gamma$ receptors on the macrophage surface, and they display the co-stimulatory protein CD40 ligand, which binds to CD40 on the macrophage (Figure 25–69). (We see later that CD40 ligand is also used by helper T cells to activate B cells.) Once activated, the macrophage can kill the microbes in their phagosomes: lysosomes can now fuse more readily with the phagosomes, unleashing a hydrolytic attack, and the activated macrophage makes oxygen radicals and nitric oxide, both of which are highly toxic to the microbes (discussed in Chapter 24). Because dendritic cells also express CD40, the T_H1 cells at sites of infection can also help activate them. As a result, the dendritic cells increase their production of class II MHC proteins, co-stimulatory proteins, and various cytokines, especially *IL12*. This makes them more effective at stimulating naïve helper T cells to differentiate into T_H1 effector cells in peripheral lymphoid organs, providing a positive feedback loop that increases the production of T_H1 cells and, thereby, the activation of macrophages.

T_H1 effector cells also stimulate an *inflammatory response* (discussed in Chapter 24) by recruiting more phagocytic cells into the infected site. They do so in three ways:

1. They secrete cytokines that act on the bone marrow to increase the production of monocytes (macrophage precursors that circulate in the blood) and neutrophils.
2. They secrete other cytokines that activate endothelial cells lining local blood vessels to express cell adhesion molecules that cause monocytes and neutrophils in the blood to adhere there.
3. They secrete chemokines that direct the migration of the adherent monocytes and neutrophils out of the bloodstream into the site of infection.

A T_H1 cell can also help activate a cytotoxic T cell in a peripheral lymphoid organ by producing chemokines that attract the cytotoxic cells to the site of the T_H1 -cell–dendritic-cell interaction, while at the same time stimulating the dendritic cell to produce more co-stimulatory proteins. In addition, T_H1 cells can help effector cytotoxic T cells kill virus-infected target cells by secreting $IFN\gamma$, which increases the efficiency with which target cells process viral antigens for presentation to cytotoxic T cells (see Figure 25–60). An effector T_H1 cell can also directly kill some cells itself, including effector lymphocytes: by expressing *Fas ligand* on its surface, it can induce effector T or B cells that express cell-surface *Fas* to undergo apoptosis (see 25–47B).

Both T_H1 and T_H2 cells can also help stimulate B cells to proliferate and differentiate and to switch the class of antibody they make, from IgM and IgD to

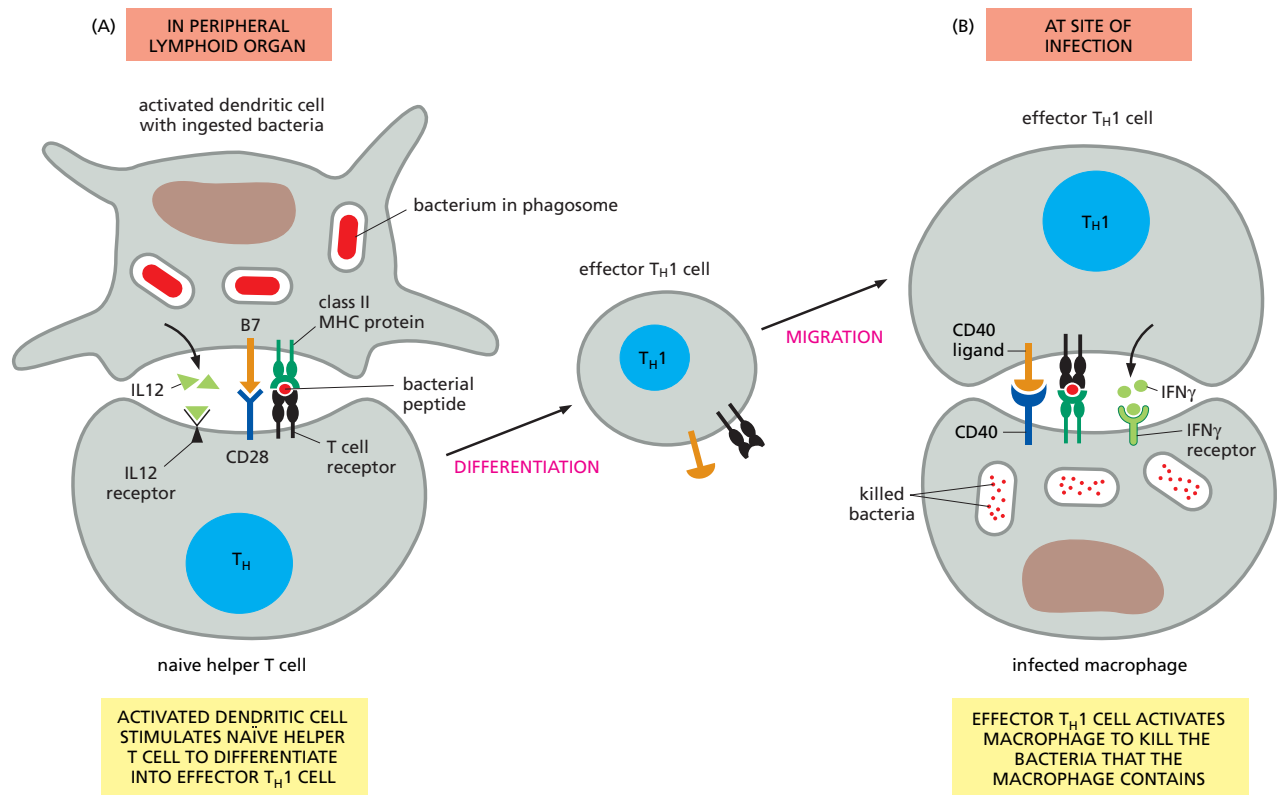


Figure 25–69 The differentiation of T_H1 cells and their activation of macrophages. (A) An activated dendritic cell that has ingested bacteria at a site of infection and migrated to a peripheral lymphoid organ activates a naïve helper T cell to differentiate into a T_H1 effector cell. The dendritic cell uses both cell-surface co-stimulatory proteins such as the B7 proteins and secreted IL12 to induce T_H1 cell differentiation. (B) The activated T_H1 effector cell then migrates from the peripheral lymphoid organ to the infected site, where it helps activate macrophages to kill the bacteria harbored within the macrophages' phagosomes. As indicated, it does this by means of secreted IFN γ and membrane-bound CD40 ligand, which binds to CD40 on the macrophage.

one of the secondary classes of antibody. Before considering how helper T cells do this, we need to discuss the role of the B cell antigen receptor in the activation of B cells.

Antigen Binding to B Cell Receptors (BCRs) Is Only One Step in B Cell Activation

Like T cells, most B cells require multiple extracellular signals to become activated. One signal is provided by antigen binding to the **B cell receptor (BCR)** for antigen, which, as discussed previously, is a membrane-bound antibody molecule. A helper T cell usually provides the other required signals. If a B cell receives the first signal only, it may be eliminated or functionally inactivated, which is one way in which B cells become tolerant to self antigens.

Signaling through the BCR works in much the same way as signaling through the TCR (see Figure 25–66). The receptor is associated with two invariant protein chains, Ig α and Ig β , which help convert antigen binding to BCRs into intracellular signals. When antigen cross-links BCRs on the surface of a B cell, it causes them and their associated invariant chains to cluster into small aggregates. This aggregation leads to the assembly of an intracellular signaling complex at the site of the clustered receptors and to the initiation of a tyrosine phosphorylation cascade (**Figure 25–70**).

A co-receptor complex that binds complement proteins greatly enhances the efficiency of signaling through the BCR and its associated invariant chains.

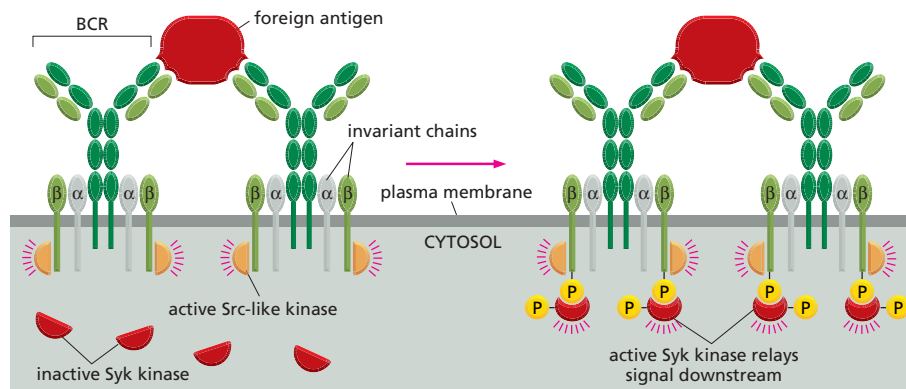


Figure 25-70 Early signaling events in B cells activated by the binding of antigen to BCRs. The antigen cross-links adjacent BCRs, which are transmembrane antibody molecules. The cross-linking causes the BCRs and their associated invariant chains (heterodimers of Ig α and Ig β) to cluster. A Src-like cytoplasmic tyrosine kinase, which can be *Fyn*, *Blk*, or *Lyn*, is associated with the cytosolic tail of Ig β . It joins the cluster and phosphorylates Ig α and Ig β (for simplicity, only the phosphorylation on Ig β is shown). As in the case of TCR activation, the protein tyrosine phosphatase CD45 is also required to activate these Src-like kinases (not shown). The resulting phosphotyrosines on Ig α and Ig β serve as docking sites for another Src-like tyrosine kinase called Syk, which is homologous to ZAP70 in T cells (see Figure 25-66). Like ZAP70, Syk becomes phosphorylated and thereby activated, and it then relays the signal downstream.

If a microbe directly activates the complement system (discussed in Chapter 24), complement proteins are often deposited on the microbe surface, greatly increasing the B cell response to the microbe. Now, when the microbe clusters BCRs on a B cell, the *complement-binding co-receptor complexes* are brought into the cluster, increasing the strength of signaling by activating PI 3-kinase (discussed in Chapter 15) (**Figure 25-71A**). As expected, antibody responses are greatly reduced in mice lacking either one of the required complement components or one of the complement-binding co-receptor subunits on B cells.

Later in the immune response, by contrast, when IgG antibodies are present on the surface of the microbe, a different co-receptor dampens down the B cell response. These are *Fc receptors*, which bind the tails of the IgG antibodies. They recruit both lipid and protein phosphatase enzymes into the signaling complex that decrease the strength of signaling (Figure 25-71B). In this way, the Fc receptors on B cells act as inhibitory co-receptors, just as the CTLA4 proteins do on T cells. Thus, the co-receptors on a T cell or B cell allow the cell to gain additional information about the antigen bound to its receptors and thereby make a more informed decision as to how to respond.

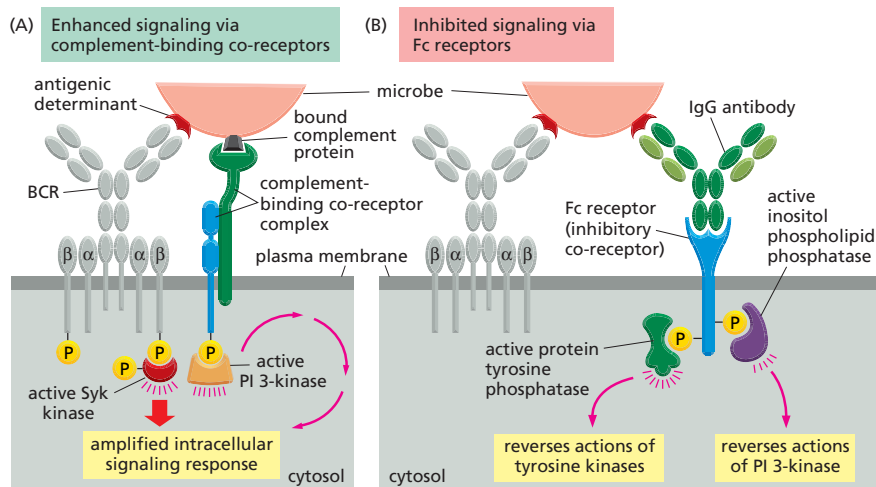


Figure 25-71 The influence of B cell co-receptors on the effectiveness of signaling through BCRs. (A) The binding of microbe–complement complexes to BCRs cross-links the BCRs to complement-binding, co-receptor complexes. The cytosolic tail of one component of the co-receptor complex becomes phosphorylated on tyrosines, which then serve as docking sites for PI 3-kinase. As discussed in Chapter 15, PI 3-kinase is activated to phosphorylate specific inositol phospholipids in the plasma membrane, which then act as docking sites to recruit intracellular signaling proteins (not shown). These signaling proteins act together with the signals generated by the Syk kinase to amplify the response. (B) When IgG antibodies bind to foreign antigen, usually late in a response, the Fc regions of the antibodies bind to Fc receptors on the B cell surface and are thus recruited into the signaling complex. The Fc receptors become phosphorylated on tyrosines; these then serve as docking sites for two types of phosphatase enzymes: (1) an inositol phospholipid phosphatase, which dephosphorylates the inositol phospholipid docking sites in the plasma membrane generated by PI 3-kinase, thereby reversing the activating effects of PI 3-kinase; (2) protein tyrosine phosphatases, which inhibit signaling by the activated tyrosine kinases.

Antigen-Specific Helper T Cells Are Essential for Activating Most B Cells

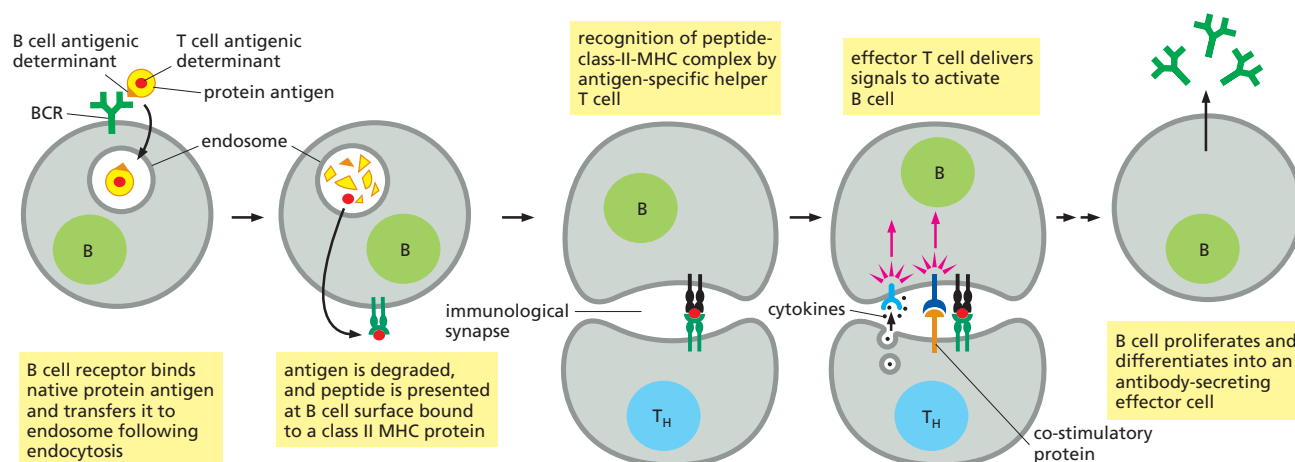
Whereas antigen-presenting cells such as dendritic cells and macrophages are omnivorous and ingest and present antigens on their MHC proteins nonspecifically, a B cell generally presents only peptides derived from an antigen that it specifically recognizes using its BCRs. Thus, BCRs do more than just bind antigen to begin the process of B cell activation; they also play a crucial part in recruiting T cell help. Through endocytosis, they deliver their bound protein antigen to an endosomal compartment, where the antigen is degraded into peptides; many of these peptides are returned to the B cell surface bound to class II MHC proteins (see Figure 25–61). These peptide–class-II-MHC complexes are recognized by antigen-specific helper T cells, which then deliver further signals to the B cell that are required for its proliferation and antibody secretion (Figure 25–72).

How do the antigen-specific T cells required for B cell activation originate? As discussed previously, during primary antibody responses, naïve helper T cells are activated in a peripheral lymphoid organ by binding to a foreign peptide bound to a class II MHC protein on the surface of an activated dendritic cell. The effector helper T cells that result from this activation can then activate a B cell that displays the same complex of foreign peptide and class II MHC protein on its surface. Thus, the helper T cell activates only those B cells with BCRs that specifically recognize the antigen that initially activated the T cell, even though the TCRs and BCRs usually recognize distinct antigenic determinants on the antigen (see Figure 25–72). This requirement for *linked recognition* of antigen by a T cell and a B cell helps avoid autoimmune B cell responses, which would require the simultaneous presence of both helper T cells and B cells that recognize the same self antigen.

In secondary antibody responses, memory B cells themselves can act as antigen-presenting cells and activate helper T cells, as well as being the subsequent targets of the effector helper T cells. The mutually reinforcing actions of helper T cells and B cells lead to an antibody response that is both intense and highly specific.

Once a helper T cell has been activated to become an effector cell and contacts a B cell, the contact initiates an internal rearrangement of the helper cell's cytoplasm. The T cell orients its centrosome and Golgi apparatus toward the B cell, as described previously for an effector cytotoxic T cell contacting its target cell (see Figure 25–46). In this case, however, the orientation is thought to enable the effector helper T cell to direct both membrane-bound and secreted cytokines onto the B cell surface (see Figure 25–72). One crucial membrane-bound signal molecule is CD40 ligand, which we encountered earlier. It is expressed on the surface of effector helper T cells, but not on nonactivated naïve or memory helper T cells, and it is recognized by the CD40 protein on the B cell surface. This interaction between CD40 ligand and CD40 is required for helper T cells to activate B cells to proliferate and differentiate into memory or antibody-

Figure 25–72 The activation of a B cell by a protein antigen and an effector helper T cell. Note that the B cell and T cells recognize different antigenic determinants on the antigen and that the effector helper T cell uses both secreted and membrane-bound co-stimulatory molecules to help activate the B cell.



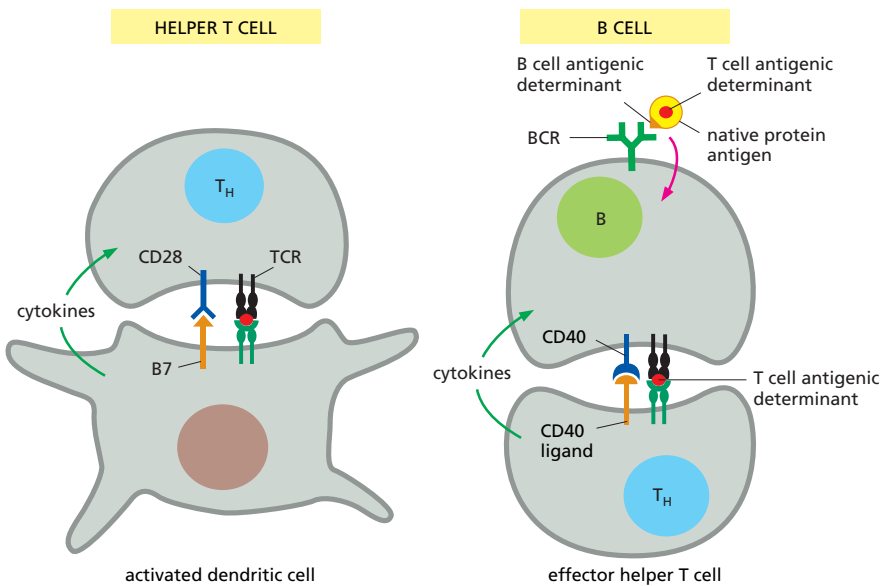


Figure 25–73 Comparison of the signals required to activate a helper T cell and a B cell to the same protein antigen. Note that in both cases secreted and membrane-bound molecules cooperate in the activation process. The *red arrow* indicates the endocytosis of the protein antigen. Although not shown, CD40 ligand is also used by effector helper T cells to increase and maintain the activation of dendritic cells, which express CD40, thereby creating a positive feedback loop. The antigenic determinant recognized by the helper T cell is presented on the surface of both the dendritic cell and the B cell as a peptide fragment of the protein antigen bound to a class II MHC protein. By contrast, the B cell recognizes a different antigenic determinant on the surface of the folded (native) protein.

secreting effector cells. Individuals that lack CD40 ligand are severely immunodeficient. They are susceptible to the same infections that affect AIDS patients whose helper T cells have been destroyed.

Helper T cells also secrete cytokines to help B cells proliferate and differentiate and, in some cases, to switch the class of antibody they produce. The cytokines include the interleukins IL2 and IL4. IL4, for example, is produced by T_H2 cells and collaborates with CD40 ligand in stimulating B cell proliferation and differentiation; it also promotes switching to IgE antibody production. Mice deficient in IL4 production are severely impaired in their ability to make IgE.

Figure 25–73 compares the signals required for T and B cell activation; **Table 25–4** lists some of the cytokines discussed in this chapter.

A Special Class of B Cells Recognize T-cell-independent Antigens

Some antigens can stimulate B cells to proliferate and differentiate into antibody-secreting effector cells without help from T cells. Most of these *T-cell-independent antigens* are microbial polysaccharides that do not activate helper T cells. Some activate B cells directly by providing both the antigen signal and the accessory signals normally provided by helper T cells. Others are large polymers with repeating, identical antigenic determinants (see Figure 25–29B); their multipoint binding to BCRs can generate a strong enough signal on its own to activate the B cell directly, without additional signals.

Table 25–4 Properties of Some Cytokines

CYTOKINE	SOME SOURCES	SOME ACTIONS
IL2	all helper T cells; some cytotoxic T cells	stimulates proliferation and differentiation of activated T cells; required for regulatory T cell development in thymus
IL4	T _H 2 cells, basophils, and mast cells	stimulates B cell proliferation, differentiation, and class switching to IgE and IgG1; promotes T _H 2 and inhibits T _H 1 cell development
IL7	many non-T cells	promotes memory T cell survival
IL10	T _H 2 cells, macrophages, and dendritic cells	inhibits macrophages and T _H 1 cell development
IL12	B cells, macrophages, dendritic cells, and granulocytes	induces T _H 2 cell development and inhibits T _H 1 cell development
IL15	many non-T cells	promotes memory T cell survival
IL17	some effector helper T cells	stimulates inflammatory responses
IFN γ	T _H 1 cells and cytotoxic T cells	activates macrophages; increases MHC expression in many cell types
TGF β	regulatory T cells	suppresses effector T cell activity, dendritic cells, and macrophages
TNF α	T _H 1 cells and macrophages	activates endothelial cells and macrophages

Because T-cell-independent antigens do not activate helper T cells, they fail to induce B cell memory, affinity maturation, or class switching, all of which require help from T cells. They therefore mainly stimulate the production of low-affinity (but high-avidity) IgM antibodies. Most B cells that make antibodies without T cell help belong to a distinct B cell lineage. They are called *B1 cells* to distinguish them from *B2 cells*, which require T cell help. B1 cells seem to be especially important in defense against intestinal pathogens.

Immune Recognition Molecules Belong to the Ancient Ig Superfamily

Most of the proteins that mediate cell–cell recognition or antigen recognition in the immune system contain Ig or Ig-like domains, suggesting that they have a common evolutionary history. Included in this **Ig superfamily** are antibodies, TCRs, MHC proteins, the CD4, CD8, and CD28 co-receptors, the B7 co-stimulatory proteins, and most of the invariant polypeptide chains associated with TCRs and BCRs, as well as the various Fc receptors on lymphocytes and other white blood cells. All of these proteins contain one or more Ig or Ig-like domains. In fact, about 15% of the 250 or so proteins that have been characterized on the surface of white blood cells belong to this superfamily. Many of these molecules are dimers or higher oligomers in which Ig or Ig-like domains of one chain interact with those in another (**Figure 25–74**).

A separate exon usually encodes the amino acids in each Ig-like domain. It seems likely that the entire gene superfamily evolved from a gene coding for a single Ig-like domain—similar to that encoding β_2 -microglobulin (see Figures 25–50A and 25–52) or the Thy-1 protein (see Figure 25–74)—that may have mediated cell–cell interactions. There is evidence that such a primordial gene arose before vertebrates diverged from their invertebrate ancestors about 400 million years ago. New family members presumably arose by exon and gene duplications.

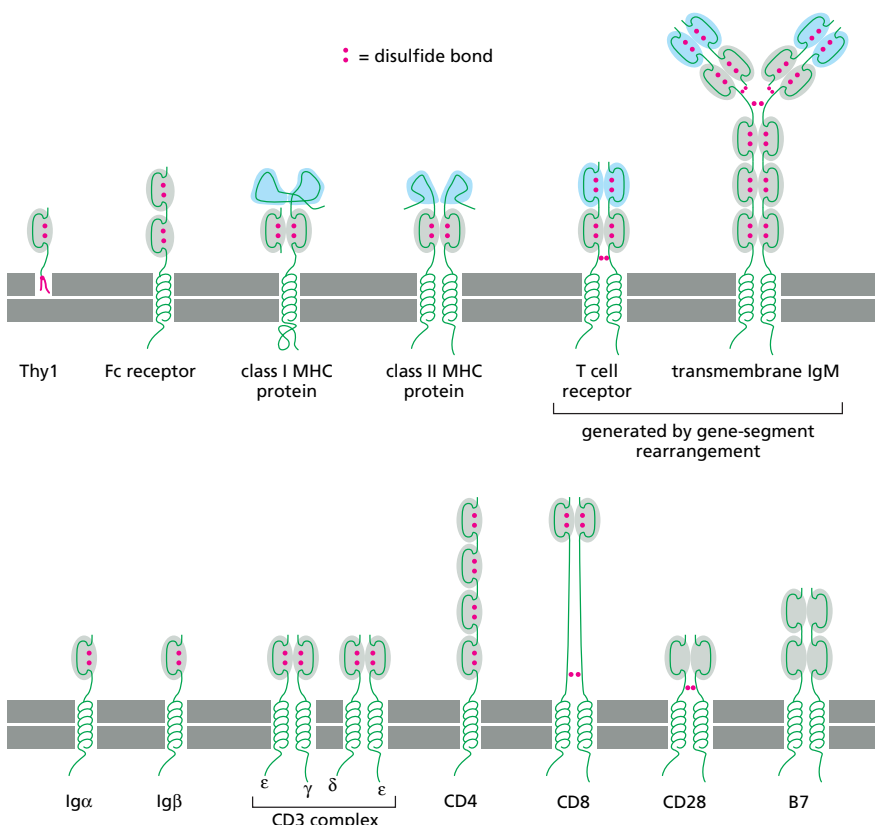


Figure 25–74 Some of the cell-surface proteins discussed in this chapter that belong to the Ig superfamily. The Ig and Ig-like domains are shaded in gray, except for the antigen-binding domains (not all of which are Ig domains), which are shaded in blue. The function of Thy1 is unknown, but it is held in the plasma membrane by a glycosylphosphatidylinositol (GPI) anchor and is widely used to identify T cells in mice. The Ig superfamily also includes many cell-surface proteins involved in cell–cell interactions outside the immune system, such as the neural cell adhesion molecule (NCAM) discussed in Chapter 19 and the receptors for various protein growth factors discussed in Chapter 15 (not shown). There are more than 750 members of the Ig superfamily in humans.

The multiple gene segments that encode antibodies and TCRs may have arisen when a transposable element, or transposon (discussed in Chapter 5), inserted into an exon of a gene encoding an Ig family member in an ancestral lymphocyte-like cell. The transposon may have contained the ancestors of the *Rag* genes, which, as discussed earlier, encode the proteins that initiate V(D)J recombination; the finding that the RAG proteins can act as transposons in a test tube strongly supports this view. Once the transposon had inserted into the exon, the gene could be expressed only if the transposon was excised by the RAG proteins and the two ends of the exon were rejoined, much as occurs when the *V* and *J* gene segments of an Ig light chain gene are assembled (see Figure 25–38). A second insertion of the transposon into the same exon may then have divided the gene into three segments, equivalent to the present-day *V*, *D*, and *J* gene segments. Subsequent duplication of either the individual gene segments or the entire split gene may then have generated the arrangements of gene segments that characterize the adaptive immune systems of present-day vertebrates.

Summary

The production of an effector helper T cell from a naïve helper T cell requires multiple signals from an activated dendritic cell. MHC–peptide complexes on the dendritic cell surface provide one signal, by binding to both TCRs and a CD4 co-receptor on the T cell. Co-stimulatory proteins on the dendritic cell surface, including CD28, and secreted cytokines are the other signals. When naïve helper T cells are initially activated on a dendritic cell, most differentiate into either T_H1 or T_H2 effector cells, depending mainly on the signal proteins in their environment. T_H1 cells activate macrophages, cytotoxic T cells, and B cells, while T_H2 cells activate mainly B cells. In both cases, the effector helper T cells recognize the same complex of foreign peptide and class II MHC protein on the target cell surface as they initially recognized on the dendritic cell that activated them. They activate their target cells by a combination of membrane-bound and secreted co-stimulatory proteins. One membrane-bound signal protein used by both T_H1 and T_H2 cells is CD40 ligand.

Like T cells, B cells require multiple signals for activation. Antigen binding to the B cell antigen receptors (BCRs) provides one signal, while antigen-specific effector helper T cells provide the other signals. The requirement for multiple signals to activate either a T cell or a B cell helps to prevent inappropriate and dangerous activation of lymphocytes, including self-reactive lymphocytes.

Most of the proteins involved in cell–cell recognition and antigen recognition in the immune system, including antibodies, TCRs, and MHC proteins, as well as the various co-receptors discussed in this chapter, belong to the ancient Ig superfamily. This superfamily is thought to have evolved from a primordial gene encoding a single Ig-like domain. The mechanisms for diversifying antibodies and T cell receptors by recombining gene segments may have arisen when a transposon inserted into an exon of a gene encoding an Ig family member.

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