24

Microbial Symbioses with Humans

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Frozen in Time: The Iceman Microbiome

Humans and their microbial associates—collectively called the *human microbiome*—have coevolved for millennia. As we will see in this chapter, the human microbiome influences a person's health, disease, and predisposition to disease. Among our intimate microbial associates, the pathogenic bacterium *Helicobacter pylori* is known to have developed a close relationship with humans in the distant past and to have coevolved with humans. *H. pylori* colonizes the stomachs of about half the human race. Although this bacterium generally does not cause overt disease, it is a major risk factor for the development of ulcers and stomach cancer. Moreover, because *H. pylori* is transmitted primarily by contact within families, the distribution of genetic variants of this bacterium may yield clues to past human migrations.

Unraveling the details of the *H. pylori* ancestry is complicated by the ability of different strains of this bacterium to recombine their genetic information. Because the DNA of various strains has mixed over long periods, the reconstruction of population movement inferred from genome sequences of modern *H. pylori* strains is incomplete. One of the biggest unanswered questions was the origin of strains now common among modern Europeans, which appear to be hybrids of strains originating in Asia and Africa. Unfortunately, the sequence data did not point to a reliable time interval in which that mingling of human populations occurred—an important period of human migration that was estimated to have occurred 10,000–50,000 years ago.

This estimate has now been greatly refined following the remarkable discovery of a well-preserved 5300-year-old European Copper Age mummy frozen in the Italian Alps. Using the newest methods for DNA sequencing, it was possible to reconstruct the genome of *H. pylori* preserved in the stomach of the "Iceman" (see photo), the corpse discovered when melting ice revealed the human remains on the side of a mountain. The Iceman *H. pylori* genome sequence turned out to be an almost pure representative of the Asian population, which means this *H. pylori* strain was present in Europe before hybridization of African and Asian strains produced the modern European variant. Thus, by employing historical biogeography, we now know this important period of human migration was much more recent than previously thought.

Source: Maixner, F., et al. 2016. The 5300-year-old *Helicobacter pylori* genome of the Iceman. *Science 351:* 162–165.

Are we one, or are we many? This somewhat philosophical question has been asked about the functional significance of microorganisms colonizing our bodies. In their totality, this massive assemblage of microorganisms is called the **human microbiome**. A **microbiome** can be defined as a functional collection of different microbes in a particular environmental system. The term **microbiota**, by contrast, is generally used in reference to the types of organisms present in an environmental habitat. The human microbiome is comprised of different microbiota that colonize different habitats of the body. For example, the microbiota colonizing the skin is different from that of the gut, but they are all part of the human microbiome.

With this chapter we begin a new unit focused on microbe–human interactions and the immune system. The vast majority of microbes in and on the human body contribute in one way or another to the health of the individual; relatively few cause harm. In this chapter we explore the human microbiome with a focus on the composition of the **normal microbiota** of various body systems and the growing appreciation for how these important microbes maintain health and occasionally trigger disease.

I • Structure and Function of the Healthy Adult Human Microbiome

We begin with an overview of the human microbiome and then consider the microbiota present in specific regions of the body.

24.1 Overview of the Human Microbiome

The sites of the human body inhabited by microorganisms include the mouth, nasal cavities, throat, stomach, intestines, urogenital tracts, and skin (Figure 24.1). It is estimated that the microbes in the human microbiome number between 10^{13} and 10^{14} cells, which is roughly the same to ten times the total number of human cells in a single person. Together, the human body as the **host** and its associated microbes are increasingly recognized to constitute a *host–microbiome supraorganism*. For example, the gut microbial community in the healthy human was once considered to consist of microorganisms that were merely commensals, but we now know that this community is critical to development of the immune system, overall health later in life, and predisposition to disease.

Future Benefits of Knowing the Human Microbiome

The clinical benefits of knowing a person's microbiome seem promising and include the development of biomarkers for predicting predisposition to specific diseases, the design of therapies targeting selected microbial species in particular body sites, personalized drug therapies, and tailor-made probiotics (Section 24.11). However, a caveat must also be introduced. It should be emphasized that we are only at the early stages of resolving the many interactions between our body and the microbes we host. Sorting out the nature and activities of the human microbiome is an extremely complex problem because not only is a person affected by his or her microbiota but that microbiota is also affected by the person's activities, health, and diet. Thus, cause and effect relationships are often not immediately obvious and can sometimes be difficult to sort out.

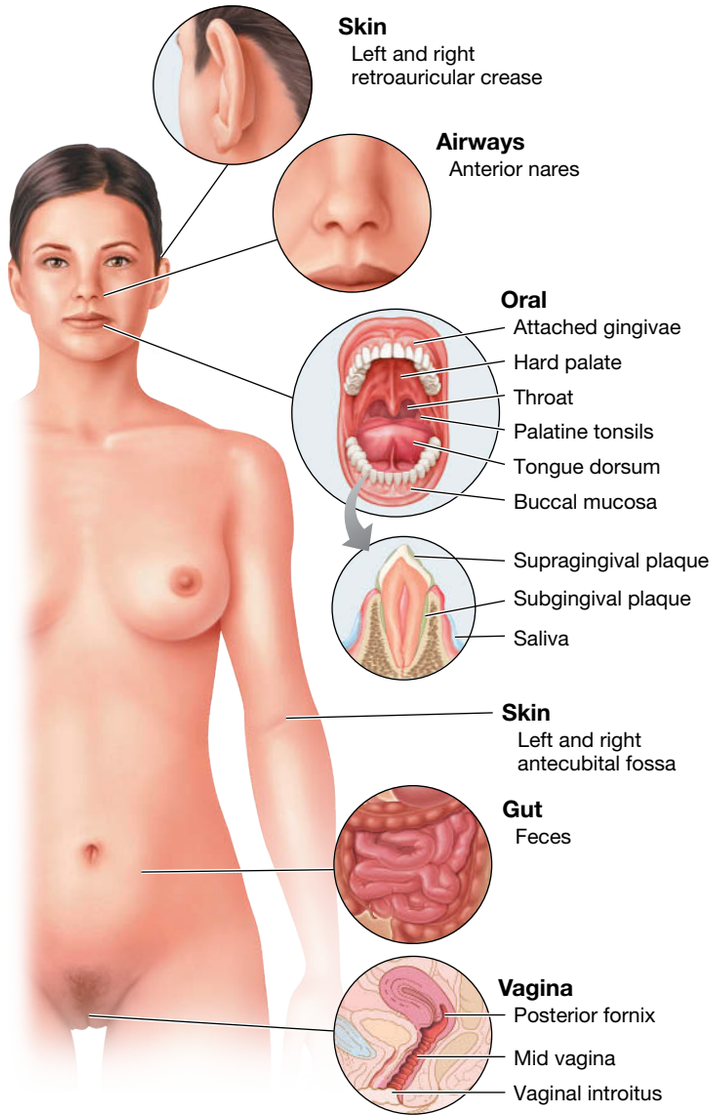


Figure 24.1 Microbial habitats of the human body. Primary body sites characterized in ongoing studies of the human microbiome (see Table 24.1).

Microbiome studies to date have revealed an incredible diversity of microbial life in and on the human body, and have signaled several likely connections between the microbial composition at a body site and health status. However, at this point microbial associations with health or disease states are for the most part only correlations, and a causal relationship between microbiome and the health status of the host has been well established in only a few instances.

Experimental Protocols and Body Target Sites

Because the vast majority of microorganisms cannot yet be readily cultured or enumerated using growth-dependent approaches,

it was the development of advanced nucleic acid sequencing methods (Chapters 9 and 19) that first offered the means to initiate a robust census of microbial diversity and abundance patterns in humans. The megasequencing era has also allowed microbiologists to compare the microbiomes of different individuals and to begin to resolve the dynamic relationship between the microbiome and host history, genetics, health status, and diet.

The focus on molecular sequences in microbiome studies does not diminish the importance of cultivation in the study of the human microbiome. Many hundreds of microorganisms have been isolated from the human body. The physiological and molecular characterization of those isolates has provided and will continue to provide essential understanding of their functional significance in human health and disease. Now that sequence-based surveys show that many human-associated microorganisms have not been isolated, there is also concerted effort to bring those microorganisms into culture. In addition, the development of appropriate culture conditions for isolation is being guided by metagenomic sequencing (Sections 9.8 and 19.8), which provides insights into the nutritional requirements of the uncultured microorganisms. Thus, the fusion of molecular and culture-based analyses will be critical to a full understanding of the functional significance of the human microbiome, as highlighted throughout this chapter.

The microbial diversity of the human microbiome has been mapped for the most part by surveys using 16S rRNA gene sequencing (Sections 13.7 and 19.6) and selected metagenomic analyses (Sections 9.8 and 19.8) as major tools in several national and international microbiome research efforts (**Table 24.1**). Initial studies examined the microbial diversity in hundreds of healthy volunteers, collecting 1518 total samples from various body sites (Figure 24.1) and analyzing them for their microbial species composition. These culture-independent assessments of microbial diversity typically use the definition of a microbial species given earlier in this book (in which 97% or greater 16S rRNA gene sequence identity defines a single species cluster, Section 13.8). However, some studies have used different species definitions, and for this reason, along with the fact that different methodologies (for example, different DNA isolation methods) have been used, the various analyses are not all directly comparable and should be considered only an approximate characterization of these complex microbial systems. Nevertheless, collectively, these studies have shown on the one hand that the microbial diversity between individuals is so great that no one microbial species is present in greatest abundance in all individuals, but that on the other hand, particular microbial groups typically dominate. Similarities in microbial diversity between individuals are more evident at higher bacterial taxonomic levels (such as phyla) and when certain genes are linked to particular body sites.

The general microbial composition of the four major human body sites (skin, oral, urogenital, and gastrointestinal), as defined by higher taxonomic levels, is shown in **Figure 24.2**. The sections to follow will examine each of these sites at a higher taxonomic resolution to more fully describe the huge diversity of microbes inhabiting the human body. Later sections address the functional significance of the microbiome and how it might be rationally modified for health benefit. These ongoing studies—including relationships to disease, ethnicity, and diet—are coordinated under the International Human Genome Consortium. Some of the major questions posed by these integrated projects include the following: (1) Do individuals share a core human microbiome? (2) Is there a correlation between the composition of microbiota colonizing a body site and host genotype? (3) Do differences in the human microbiome correlate with differences in human health? (4) Are differences in the relative abundance of specific bacterial populations important to either health or disease?

We start with a focus on the human gut, the human body site most heavily colonized by microbes.

TABLE 24.1 Major human microbiome research programs

Research program	*Participating countries*	*Programmatic objectives*
MetaGenoPolis	France	Demonstrate the impact of the human gut microbiota on health and disease using metagenomics technology
International Human Microbiome Standards	European Commission	Optimize methods for the assessment of the effects of the gut microbiome on human health through the standardization of procedures and protocols
Korean Twin Cohort Project	Korea	Characterize microbiota associated with epithelial tissue in a twin cohort study group, with the goal of identifying targets for early disease diagnosis and prevention
NIH Human Microbiome Project (HMP)	USA	Characterize the microbes that live in and on the human body, and assess the ability to demonstrate correlations of changes of the human microbiome with health
Canadian Human Microbiome Initiative	Canada	Characterize the microorganisms colonizing the human body. Evaluate their relationship to health and examine compositional changes associated with chronic disease
NIH Jumpstart Program	USA	Generate the complete genome sequences of 200 bacterial strains isolated from the human body; recruit donors for securing samples from five body regions, and perform 16S rRNA and metagenomic sequence analysis of the sampled body regions
Integrative Human Microbiome Project	USA	Crowdsourcing model to secure fecal samples for 16S rRNA sequence analysis

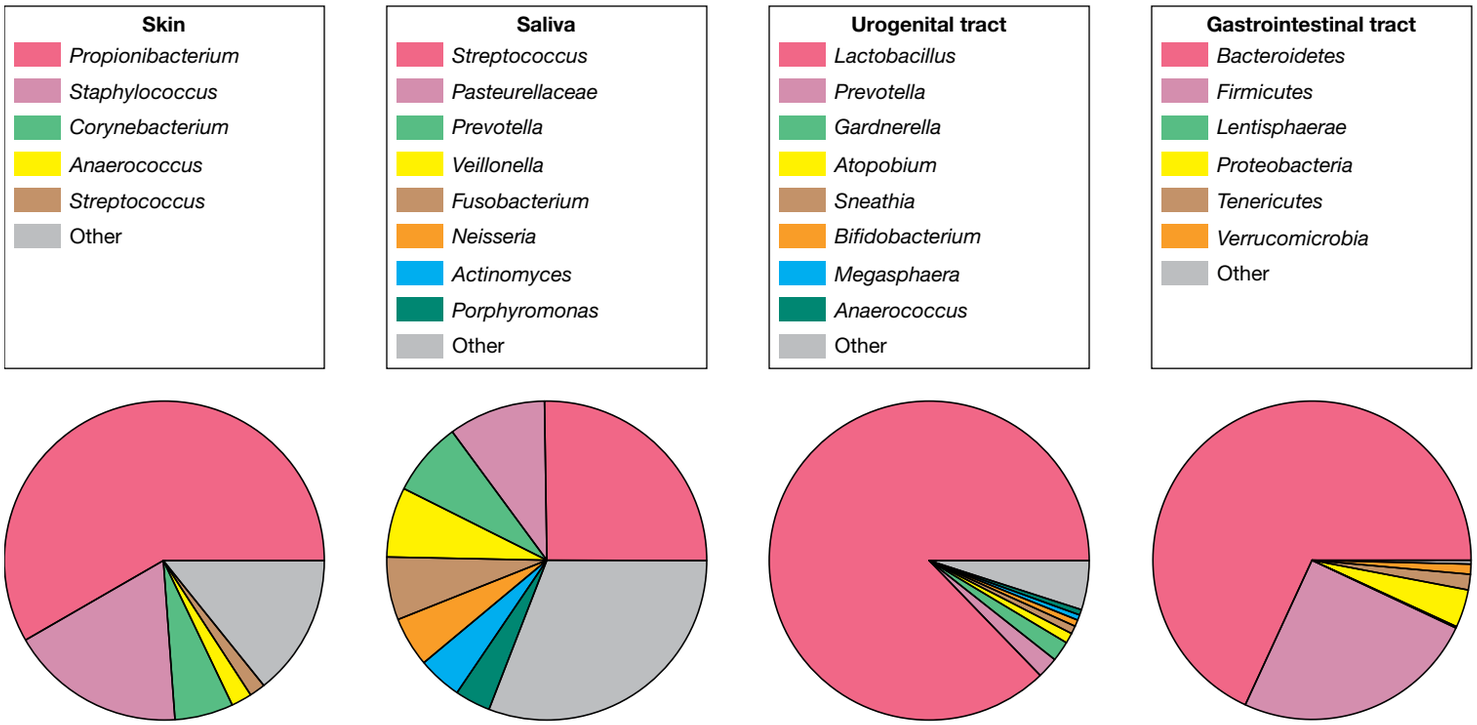


Figure 24.2 Overview of major microbial populations in the body sites sampled by human microbiome projects. Diversity among body sites was evaluated by 16S rRNA gene sequence analysis (Section 13.7). Note that each body site tends to be dominated by one population type: skin by *Propionibacterium* species; oral by *Streptococcus* species; urogenital by *Lactobacillus* species; and gastrointestinal by *Bacteroides* species.

MINIQUIZ

- Which major body sites are heavily colonized by microbes?
- What methods have been used to assess the human microbiome?
- Why might knowing our microbiome and how it functions be useful?

24.2 Gastrointestinal Microbiota

In the previous chapter we reviewed how gut architecture differs among herbivores, carnivores, and omnivores (Section 23.12). Here we examine the microorganisms throughout the entire gastrointestinal tract as well as their functions and special properties. The guts of herbivores include, in addition to the stomach, other compartments needed to foster microbial fermentation of large amounts of ingested plant material. In contrast, *monogastric* mammals, such as omnivorous humans, have only the stomach positioned before the intestines. The human gastrointestinal tract consists of the stomach, small intestine, and large intestine (**Figure 24.3**), and is responsible for the digestion of food and the absorption of nutrients; many important nutrients are also produced by the indigenous microbiota.

Starting with the stomach, the human digestive tract is a long folded tube of nutrients mixed with microbes, primarily species of *Bacteria*. The host and its gut microorganisms share the easily digestible nutrients. The nutrients are moved through this tube, encountering ever-changing microbial communities and abundance (Figure 24.3). The gastrointestinal tract has about 400 m^2 of surface area and is home to a total of about 10^{13} microbial cells. In the human duodenum, ingested food passed down from the stomach is blended with bile, bicarbonate, and digestive enzymes. About 1–4 h after ingestion, food reaches the gut (the large intestine, with near-neutral pH) and total bacterial numbers have increased from about 10^4/g (in the stomach) to 10^8/g (in the small intestine) to 10^{11}–10^{12} per g (in the large intestine)(Figure 24.3).

The Stomach and Small Intestine

The stomach was long thought to be either sterile or only minimally populated until the discovery in 1983 of *Helicobacter pylori* colonizing the stomachs of about 50% of the world's human population. This discovery prompted a closer inspection of the microbiology of the human stomach through both cultivation and 16S rRNA sequence analyses. The stomach is now recognized as the home of a vibrant bacterial community with hundreds of phylotypes distributed between the gastric lumen (pH 1–2) and the mucus layer of the wall (pH 6–7). Although low pH prevents bacterial overgrowth, the healthy human stomach holds a core microbiome that is distinct from the transient passage of ingested oral populations and is dominated by *Bacteroidetes* (*Prevotella*), *Firmicutes* (*Streptococcus, Veillonella, Lactobacillus*), *Actinobacteria* (*Rothia, Propionibacterium*), *Fusobacteria*, and *Proteobacteria* (*Haemophilus, Methylobacterium*).

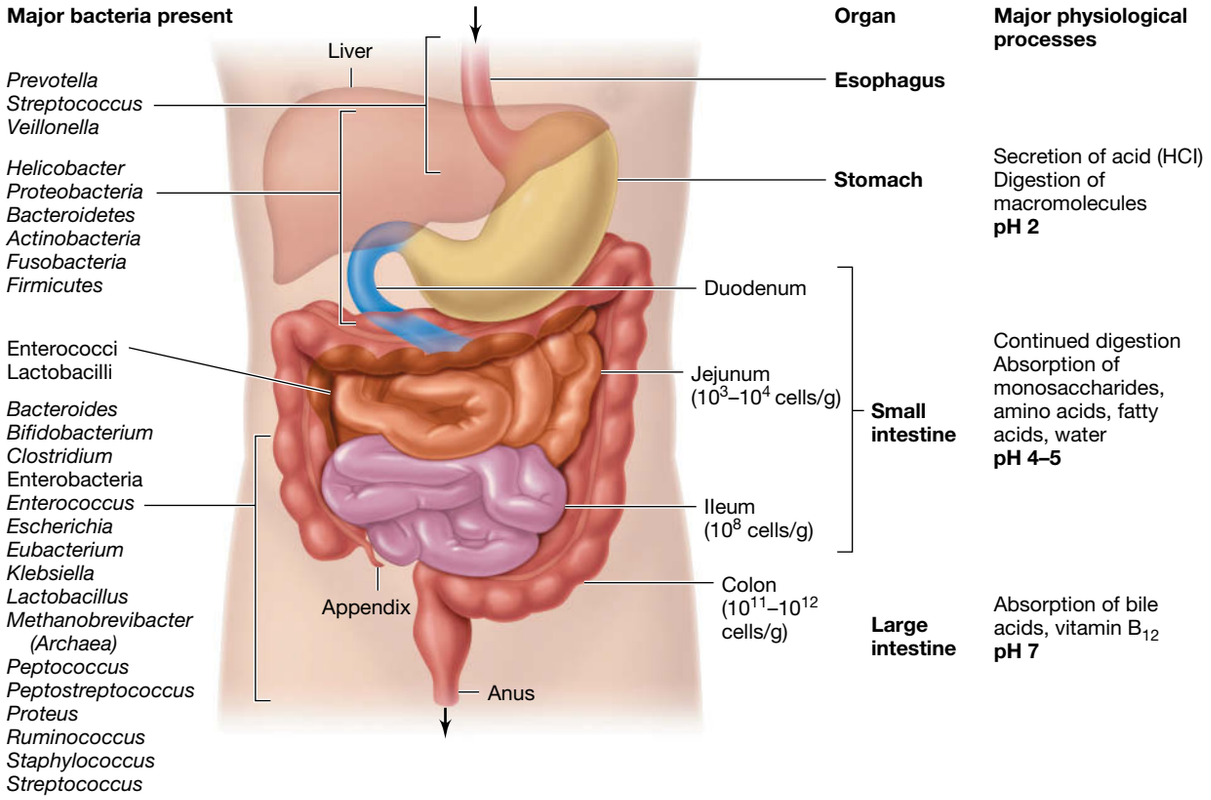
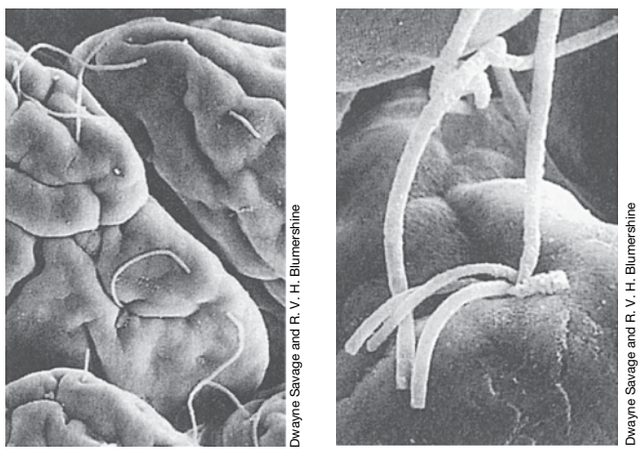


Figure 24.3 The human gastrointestinal tract and major members of its microbiota. These taxa are representative of microorganisms found in healthy adults. Not every individual harbors all of these microorganisms.

Firmicutes, Bacteroidetes, and *Actinobacteria* dominate gastric fluid samples, whereas *Firmicutes* and *Proteobacteria* are more abundant in gastric mucosal samples. When present, *H. pylori* accounts for the vast majority of stomach microbial biomass. This pathogenic proteobacterium is typically transmitted orally between family members and can persist for decades in the gastric mucosa (see page 765). About 20% of *H. pylori*-infected individuals will suffer from upper gastrointestinal tract symptoms during their lifetime (Section 30.10). However, even when infection is asymptomatic, chronic inflammation is considered a major risk factor for the development of ulcers and gastric malignancies. As such, *H. pylori* was recognized as a "definite carcinogen" (group 1) by the World Health Organization in 1994.

Distal to the stomach, the intestinal tract consists of the small intestine and the large intestine, each of which is divided into different anatomical segments and supports a characteristic microbiota (Figure 24.3). The small intestine has three distinct environments in the *duodenum* and the *ileum*, which are connected by the *jejunum*. The duodenum, adjacent to the stomach, is fairly acidic and its normal microbiota resembles that of the stomach. From the duodenum to the ileum, the pH gradually becomes less acidic and bacterial numbers increase. In the lower ileum, cell numbers of 10^5–10^7/gram of intestinal contents are common, even though the environment becomes progressively more anoxic. Fusiform (spindle-shaped) anaerobic bacteria are typically present, attached to the intestinal wall at one end (Figure 24.4). Whereas the colonic microbiota discussed in the following section is largely driven by the efficient degradation of complex indigestible carbohydrates, the microbiota residing in the small intestine must compete with the host for rapid uptake and conversion of small carbohydrates, and consists of species adapted to rapid changes in overall nutrient availability.



(a) (b)

Figure 24.4 Microbiota in the small intestine. Scanning electron micrographs of the microbial community on epithelial cells in the mouse ileum. *(a)* An overview at low magnification shows long, filamentous fusiform bacteria on epithelial cells in the mouse ileum. *(b)* Higher magnification shows several filaments attached at a single depression. The attachment is at the end of the filaments. Individual cells are 10–15 µm long.

The Large Intestine

The ileum empties into the *cecum*, the pouch that is considered the beginning of the large intestine. The *colon* makes up the rest of the large intestine. In the colon, *Bacteria* are present in enormous numbers and large numbers of *Archaea* (primarily methanogens) can be present, too. The colon is essentially an in vivo fermentation vessel, with the microbiota using nutrients derived from the digestion of food (Figure 24.3). Facultative aerobes such as *Escherichia coli* are present but in smaller numbers than other bacteria. The facultative aerobes consume any remaining oxygen, rendering the large intestine strictly anoxic. Anoxia promotes growth of obligate anaerobes such as *Clostridium* and *Bacteroides* species.

The total number of obligate anaerobes in the colon is enormous. Bacteria are by far the dominant population—cell counts of 10^{10} to 10^{11} bacterial cells/gram in distal gut and fecal contents are normal—with *Bacteroidetes* and gram-positive *Bacteria* accounting for greater than 99% of all prokaryotic cells. *Archaea* comprise a small fraction of the gut microbiota and are represented by 0.05–1% of total gene counts in gut content metagenomes (Sections 9.8 and 19.8); these *Archaea* consist only of methanogens. The hydrogenotrophic *Methanobrevibacter smithii* is the most abundant and often the exclusive archaeal population, with the methanol-reducing *Methanosphaera stadtmanae* occasionally present but in much lower abundance. Eukaryotic microbes are also only a minor part of the human gut community (<1%) and are represented primarily by the yeasts *Candida albicans* and *Candida rugosa*, which are considered part of the normal gut microbiota. Protists are absent in the gastrointestinal tract of healthy humans, but they can cause gastrointestinal infections if ingested in contaminated food or water (Chapter 32). **Figure 24.5** gives a molecular snapshot of bacterial diversity in the human colon as determined by 16S rRNA gene sequence analysis of feces. At this finer taxonomic resolution, it becomes possible to see the dominance of two major families of *Firmicutes* (*Lachnospiraceae* and *Ruminococcaceae*) at this body site (Figure 24.5). Species of these two families of anaerobic bacteria are important in the digestion of polysaccharide polymers in plant fiber, such as cellulose and pectin; these are depolymerized and the sugars fermented in the large intestine.

During the passage of food through the gastrointestinal tract, water is absorbed from the digested material, which gradually becomes more concentrated and is converted to feces. Bacteria compose about one-third of the weight of fecal matter. Organisms living in the lumen of the large intestine are continuously displaced downward by the flow of material, and bacteria that are lost are continuously replaced by new growth, similar to a continuous culture system (Section 5.4). The time needed for passage of material through the human gastrointestinal tract is about 24 h, and the growth rate of bacteria in the lumen is one to two doublings per day.

A person sheds a total of about 10^{13} bacterial cells each day in feces. Most of those organisms are restricted to the lumen of the large intestine (**Figure 24.6**). Production of **mucin** (a thick liquid secretion containing water-soluble proteins and glycoproteins) by *goblet cells* (a specialized class of epithelial cells) in the intestinal epithelium forms a protective layer (inner mucus layer) immediately adjacent to the intestinal epithelium. This inner mucus layer, unlike the outer mucus layer, is rarely colonized by bacteria (Figure 24.6). Goblet cells also produce various antimicrobial peptides that help prevent microbial contact with the underlying epithelium.

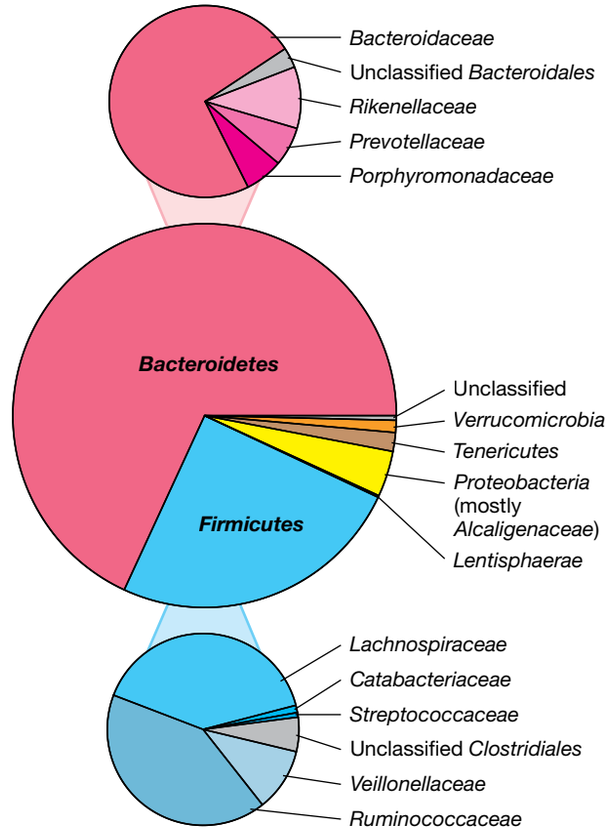


Figure 24.5 Bacterial diversity of feces. The results are pooled analyses of approximately 1 million sequences (from 184 samples) of the V1–V3 region of the 16S ribosomal RNA gene (Figure 13.15). Members of the *Bacteroidetes* and *Firmicutes* dominate the normal microbiota of the large intestine. Many of these groups are covered in Chapters 15 and 16 (*Bacteria*). Data assembled and analyzed by Nícolas Pinel.

Summary of the Gut Microbiota: The Two Major Components

The human gut microbial community is composed of only a few major phyla and shows a species composition distinct from that of any free-living microbial communities. Although we may think of *Escherichia coli* as a significant gut bacterium, the entire phylum *Gammaproteobacteria* (to which *E. coli* belongs; Section 16.3) makes up <1% of all gut bacteria. The vast majority (~98%) of all human gut phylotypes fall into one of three major bacterial phyla: *Firmicutes, Bacteroidetes*, and *Proteobacteria* (Figure 24.5). *Bacteroidetes* and *Firmicutes* dominate but can vary dramatically in relative abundance among individuals. Surveys of the human gut have shown the gut composition to vary from >90% *Bacteroidetes* to >90% *Firmicutes*, and the ratio of these two groups in a given individual may influence aspects of their health, such as leanness versus obesity (Section 24.8).

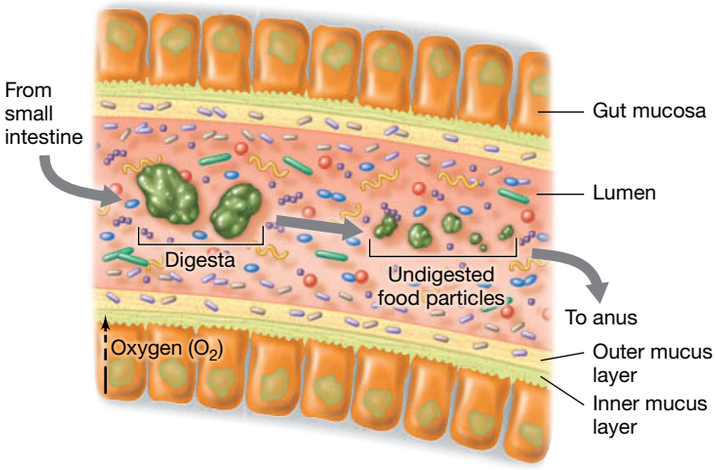


Figure 24.6 Different microenvironments in the large intestine. The inner mucin layer, produced by and contacting the gut mucosa, is partly oxygenated but generally free of bacteria. The sparsely populated outer mucus layer is adjacent to the heavily colonized anoxic lumen, which receives undigested food particles from the small intestine.

In contrast to the rather limited *phylum-level* diversity, the *species* diversity in the mammalian gut is enormous. For instance, a single study of diversity in human fecal samples (based on the analysis of millions of 16S rRNA sequences from a large sample of people) identified between 3500 and 35,000 bacterial "species." Which end of this large range is more accurate depends mainly on whether the 16S rRNA similarity threshold for defining a species (↔ Section 13.8) is set at the 97% sequence identity cutoff or is set more stringently (~98–99% identity), as some microbiologists have proposed. By the less stringent criteria, *any one individual* harbors a total of about 160 species in their large intestine. *Archaea* (represented by a phylotype closely related to the methanogen *Methanobrevibacter smithii*), yeasts, fungi, and protists are either absent or compose only a minor part of the human gut community (compare this with the rumen, ↔ Section 23.13).

Gut Enterotypes

Not surprisingly, comparative studies have also shown that humans share more bacterial genera with each other than with other species of mammals. This suggests that the mammalian gut microbiota may be "fine-tuned" to each mammalian species. Interestingly, however, although there is high variability from person to person in gut community composition, any particular individual's community is relatively stable over long periods. Also, ongoing metagenomic sequencing studies hint at the existence of a limited number of distinct, well-balanced general types of gut microbial communities that may reflect early life experiences, such as breast-feeding versus formula feeding.

Three general gut communities (called *enterotypes)* have been described, differing primarily by the enrichment of one microbial group in each enterotype. Enterotype 1 is enriched in *Bacteroides*, enterotype 2 is enriched in *Prevotella*, and enterotype 3 is enriched in *Ruminococcus*. The association of an individual with a particular enterotype seems to transcend national borders, nutrition, and ethnicity. Moreover, metabolic pathway reconstructions (based on annotation of metagenomic gene sequences, Chapter 9) suggest that each enterotype is functionally distinct; for example, they differ in their capacity for vitamin production. These results also suggest that enterotype influences an individual's response to diet and drug therapy and may contribute to health or disease status in general. As a note of caution, other analyses of these data suggest that enterotypes may not be fully distinct but instead parts of a continuous variation in population structure. Nevertheless, a better understanding of the strengths and weaknesses of each enterotype could introduce exciting new concepts and practices into the field of clinical medicine (for example, fecal transplants, Section 24.10).

Products of Intestinal Microbiota and "Educating" the Immune System

Some products of gut microbial metabolism are relatively simple compounds, such as the volatile fatty acids generated by microbial fermentation of plant material, as also occurs in the rumen of herbivores (↔ Figure 23.39). Other products generated by the activities of fermentative bacteria and methanogens include H_2, CO_2, and CH_4 (similar to fermentation products of the rumen) and several other substances and nutrients (**Table 24.2**). Gut microbes also produce vitamins B_{12} and K. These essential vitamins are not synthesized by humans (and vitamin B_{12} is not present in plants) but are made by the gut microbiota and absorbed from the colon. In addition, steroids, produced in the liver and released into the intestine from the gallbladder as bile acids, are modified in the intestine by the microbiota; the modified bioactive steroid compounds are then absorbed from the gut. Gut microbes also function in amino acid metabolism (Table 24.2). Humans are unable to make 9 of the 20 amino acids; these "essential" amino acids—such as lysine—can be obtained in our diet but are also produced and secreted by certain gut microbes.

Many other microbial metabolites or transformation products that can be generated in the gut have significant influence on host physiology. These include post-translationally modified peptides

TABLE 24.2 Biochemical/metabolic contributions of intestinal microorganisms

Process	*Product or enzyme*
Vitamin synthesis	Thiamine, riboflavin, pyridoxine, B_{12}, K
Amino acid synthesis[a]	Asparagine, glutamate, methionine, tryptophan, lysine, and others
Gas production	CO_2, CH_4, H_2
Odor production	H_2S, NH_3, amines, indole, skatole, butyric acid
Organic acid production	Acetic, propionic, butyric acids
Glycosidase reactions	β-Glucuronidase, β-galactosidase, β-glucosidase, α-glucosidase, α-galactosidase
Steroid metabolism (bile acids)	Esterified, dehydroxylated, oxidized, or reduced steroids

[a]Capacity for amino acid biosynthesis inferred from the identification of biochemical pathways encoded in gut metagenomic sequences (↔ Sections 9.8 and 19.8).

such as the lantibiotics and bacteriocins (Table 24.3), substances that function to help secure colonization of the producing organism by inhibiting organisms closely related to the producer. Gut bacteria can also synthesize high levels (>100 mg/day in some cases) of metabolites derived from the reduction of amino acids. These include substances such as *tryptamine*, a tryptophan metabolite thought to function as a biogenic neurotransmitter that signals the enteric nervous system, and *4-ethylphenylsulfate*, shown to affect behavior in mice (Table 24.3). These examples suggest that an animal's microbiome and its nervous system—called the *gut–brain axis*—are likely connected (see Explore the Microbial World, "The Gut–Brain Axis").

It is known that the immune system does not properly develop in the absence of microbial stimulation and that early life exposure to a variety of microorganisms is essential for developing tolerance to beneficial microorganisms (our normal microbiota) and recognizing pathogens as foreign. For example, the consequences of excessive hygiene in an infant's development may be a poorly trained immune system that is more likely to attack beneficial organisms with an inflammatory response. Such an immune status is thought to promote autoimmune conditions such as allergies, asthma, and inflammatory bowel diseases later in life.

Much of our understanding of how the immune system is trained to accept the normal microbiota comes from the study of mice (Section 24.6). For example, a key factor contributing to the successful colonization of *Bacteroides fragilis*, part of the normal microbiota of the mouse gut, is production of a "symbiosis factor" called *polysaccharide A* (Table 24.3). Polysaccharide A is a diffusible oligosaccharide derived from the *B. fragilis* outer membrane that in some way signals the host's immune system to promote the tolerance needed for successful colonization by this bacterium. Besides promoting its own colonization, *B. fragilis* has also been shown to protect mice from colitis caused by a pathogenic bacterium, *Helicobacter hepaticus*. In experimental animals colonized with *B. fragilis* mutants unable to express polysaccharide A, *H. hepaticus* colonizes the gut and elicits inflammatory bowel disease, unlike the results for a control group of normal animals.

These are just a few examples of the complex "dialogue" that takes place between the host and its normal gut microbiota early in life, a dialogue that is critical to the health of the host. Developing a truly mechanistic understanding of these events will be essential to promoting health and preventing diseases related to imbalances in the microbiome of humans. The identification of common and variably distributed biologically active compounds produced by the human microbiota is an emerging research area of great significance. Indeed, this information will be critical to predicting health outcomes and developing appropriate therapies for pathologies now attributed to imbalances or other abnormalities in the human microbiome.

MINIQUIZ

- How does the general metabolism of microorganisms colonizing the small and large intestines differ and why?
- What is an enterotype?

24.3 Oral Cavity and Airways

Mucous membranes throughout the body support the growth of a normal microbiota that prevents infection by pathogenic microorganisms. Mucous membranes consist of epithelial cells, tightly packed cells that interface with the external environment, and are found throughout the body, lining the urogenital, respiratory, and gastrointestinal tracts (Figure 24.6). Mucous membranes secrete *mucin*, forming the mucus layer that retains moisture and inhibits microbial attachment; invaders are usually swept away by physical processes like swallowing or sneezing, but some microorganisms adhere to the epithelial surface and colonize. Here we discuss two mucosal environments and their resident microbes. In Chapter 25 we explore the mechanism of specific attachment of microbes to mucous membranes leading to growth and development of the normal microbiota or, alternatively, to microbial disease.

Oral Microbes

Saliva contains microbial nutrients, but it is not a good microbial growth medium because the nutrients are present in low concentration and saliva contains antibacterial substances. These include *lysozyme*, an enzyme that cleaves glycosidic linkages in peptidoglycan of the bacterial cell wall, weakening the wall and causing cell lysis (↬ Section 2.4). Another enzyme, *lactoperoxidase*, found in both milk and saliva, kills bacteria by a reaction that generates singlet oxygen (a toxic form of oxygen, ↬ Section 5.14). Despite

TABLE 24.3 Small bioactive molecules produced by bacteria in the large intestine

Class	Compound	Example producer	Activity
RiPP[a] (lantibiotic)	Ruminococcin A	*Ruminococcus gnavus*	Antibiotic
RiPP[a] (bacteriocin)	Ruminococcin C	*Ruminococcus gnavus*	Antibiotic
Amino acid metabolite	Indolepropionic acid	*Clostridium sporogenes*	Protective anti-oxidant
Amino acid metabolite	4-Ethylphenylsulfate	Undefined	Neuromodulatory
Amino acid metabolite	Tryptamine	*Ruminococcus gnavus*	Neurotransmitter
Volatile fatty acid	Propionic acid	*Bacteroides* spp.	Immunomodulatory[b]
Oligosaccharide	Polysaccharide A	*B. fragilis*	Immunomodulatory[b]

[a]Ribosomally synthesized and post-translationally modified peptides.
[b]These small molecules promote colonization by normal microbiota.

THE GUT–BRAIN AXIS

Interactions between the gut microbiota and the host brain and general nervous system (called the *gut–brain axis*) have been gaining attention because of possible contributions to behavioral disorders. We know that there is a clear relationship between gut dysbiosis and pathologies such as inflammatory bowel disease and obesity (Section 24.8). However, gut dysbiosis is also associated with autism, a general term for a spectrum of behavioral disorders (together called *autism spectrum disorder*) that can emerge in the first 36 months of life, causing substantial impairments in social interaction and communication and marked by repetitive behaviors and unusual interests.

Human autism has been attributed to a combination of genetic and environmental factors. An environmental risk factor for a child developing autism and other behavioral disorders is maternal immune activation (MIA), which involves elevated levels of inflammatory factors in the blood, placenta, and amniotic fluid during pregnancy and can be caused, for example, by viral infection. Although the relationship between MIA during pregnancy and impaired development of the fetal central nervous system is complex, mouse models suggest that some features of autism can be alleviated by correcting associated defects in the gut microbial community.

The offspring of mice in which MIA has been induced display autism-like behaviors, such as communication defects and anxiety (**Figure 1*a, b***). Those behavioral changes are associated with a loss of intestinal integrity, a shift in the composition of gut clostridial and bacteroidal populations reminiscent of changes observed in autistic humans, and a 46-fold increase in serum levels of the chemical *4-ethylphenylsulfate* (**Figure 1*d***). This compound is made from the amino acid tyrosine by certain gut microbiota. When administered alone, it induces anxiety-like behavior in normal mice.[1] However, a remarkable finding is the ability to ameliorate defects in communication, anxiety, 4-ethylphenylsulfate levels, and gut permeability by feeding the affected mice a human gut commensal bacterium, either *Bacteroides fragilis* or *Bacteroides thetaiotaomicron* (**Figure 1*c***). It is thought that these organisms displace the 4-ethylphenylsulfate producers and return gut chemistry to normal.

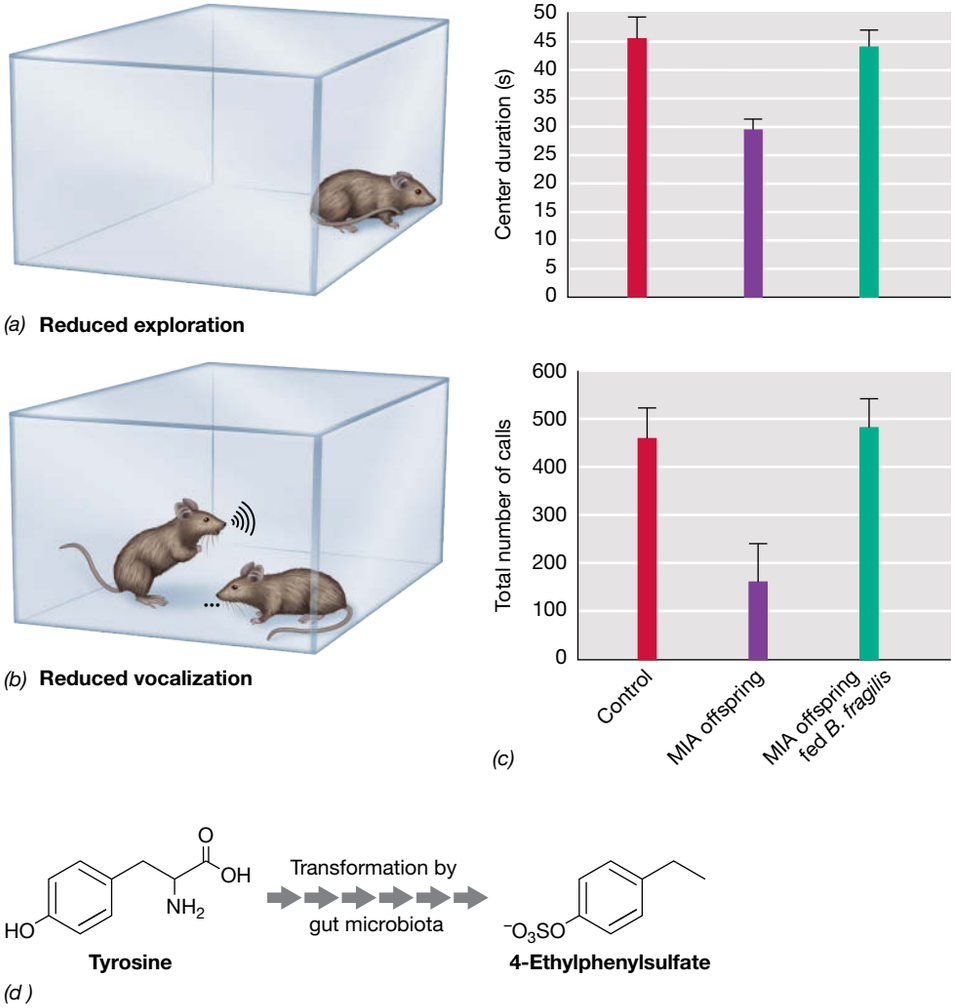


Figure 1 Influence of gut microbiota on behavior. Mice offspring of mothers with maternal immune activation (MIA) show autistic-like behavior, marked by *(a)* a fear of exploring the center of the test field and *(b)* reduced vocalization. *(c)* Feeding the affected offspring the human commensal bacterium *Bacteroides fragilis* ameliorates these behavioral abnormalities. *(d)* Certain gut microbiota can convert the amino acid tryptophan into 4-ethylphenylsulfate, the neuroactive compound thought to trigger the mouse autistic-like behaviors shown in *a* and *b*. Modified from Hsiao, E.Y., et al. 2013. *Cell 155:* 1451–1463.

These studies offer an example of the importance of the gut microbiota–brain connection in behavior and point to the possibility of using a rational modification of the gut community, such as might be possible with targeted probiotic therapy (Section 24.11), to alleviate symptoms in human neurodevelopmental disorders such as autism. Furthermore, amelioration of aberrant behavior by reducing serum levels of the gut microbiota–derived metabolite 4-ethylphenylsulfate to normal levels suggests that other neurodevelopmental illnesses may also be linked to the accumulation of microbial metabolites in serum by an unbalanced gut microbiota. However, one must be cautious about overinterpreting this study because the science thus far is only at the early stages of associating the gut microbiota with behavioral disorders.

[1]Hsiao, E.Y., S.W. McBride, S. Hsien, et al. 2013. Microbiota modulate behavioral and physiological abnormalities associated with neurodevelopmental disorders. *Cell 155:* 1451–1463.

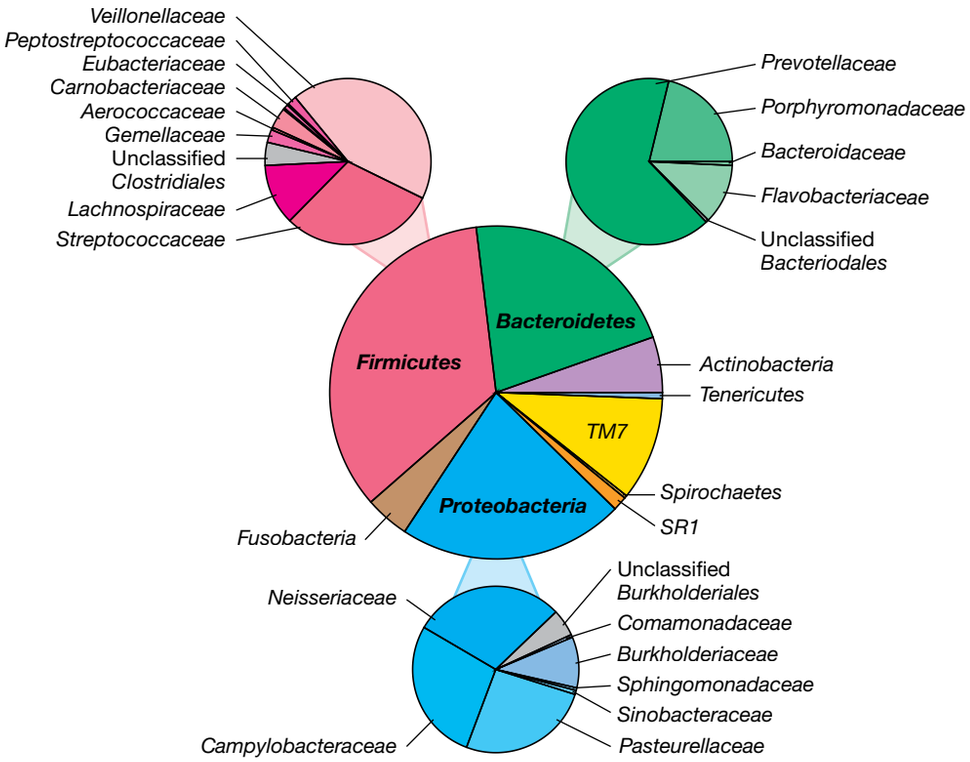


Figure 24.7 Bacterial diversity of saliva. The results are pooled analyses of approximately 750,000 sequences (from 159 samples) of the V1–V3 region of the 16S ribosomal RNA gene (Figure 13.15). Note the lower fraction of anaerobes affiliated with *Fusobacteria* and *Spirochaetes* relative to the distribution of taxa observed in subgingival plaque (Figure 24.9). Many of these groups are covered in Chapters 15 and 16 (*Bacteria*). Data assembled and analyzed by Nícolas Pinel.

the activity of these antibacterial substances, food particles and cell debris provide high concentrations of nutrients near surfaces such as the teeth and gums, creating favorable conditions for extensive local microbial growth and sometimes contributing to tissue damage and disease.

The oral cavity provides a variety of microbial habitats, each colonized by species that grow primarily as biofilms (Sections 7.9 and 20.4). The microbiota found in saliva consists of microorganisms shedding from multiple sites within the oral cavity and provides an overview of oral microbial diversity (**Figure 24.7**). The oral microbiome is essentially as diverse as the gut, but humans share greater proportions of common taxa for the mouth than for the gut. Abundant bacterial genera in the oral cavity include *Streptococcus, Haemophilus, Veillonella, Actinomyces*, and *Fusobacterium* (see page 793).

As for all microbial communities reexamined by molecular methods, 16S rRNA-based sequence surveys of the oral cavity have shown that the culture-based methods of the past have provided a very incomplete census of microbial diversity. At least 750 species of aerobic and anaerobic microbes, including a minor representation of methanogenic *Archaea* and yeast, are known to reside in the oral cavity, distributed among teeth, tissue surfaces, and saliva. Most of these microorganisms have facultatively aerobic metabolisms, but some, such as *Bacteroidetes*, are obligately anaerobic and some have strictly aerobic metabolisms, such as the *Neisseria, Acinetobacter*, and *Moraxella* genera in the *Proteobacteria* phylum. The most abundant genera in the oral cavity are *Firmicutes; Veillonella parvula*, an obligate anaerobe, is the most abundant single species and *Streptococcus* is the most abundant genus in the mouth, comprising about 25% of cells found in some individuals. The related *Firmicutes* genera *Abiotrophia* (a member of *Aerococcaceae*), *Gemella* (a member of *Gemellaceae*), and *Granulicatella* (a member of *Carnobacteriaceae*) are also common; species from these genera were among the 10 taxa most frequently detected. Other genera are present in much lower numbers, with only 17 taxa each contributing more than 1% of the oral microbiome. As is the case for the skin microbiota (Section 24.5), not all bacterial taxa are present or similarly distributed in all individuals.

Oral Microenvironments and Their Microbiota

Bacteria found in the mouth during the first year of life (when teeth are absent) are predominantly aerotolerant anaerobes such as streptococci and lactobacilli, and a few aerobes. When the teeth appear, the newly created surfaces are rapidly colonized by anaerobes that are specifically adapted to growth in biofilms on the surfaces of the teeth and in the gingival crevices (**Figure 24.8**). The primary colonizers of clean tooth surfaces are species of *Streptococcus*; obligate anaerobes such as *Veillonella* and *Fusobacterium* colonize habitats below the gum line. Most of these organisms

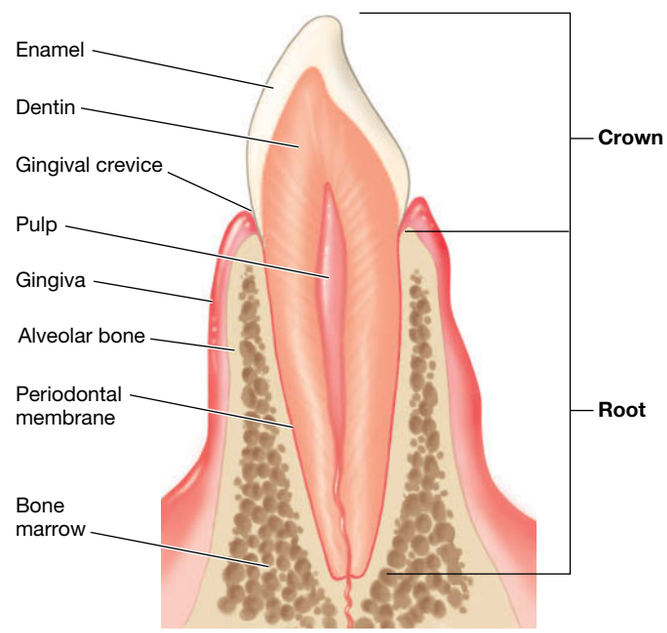


Figure 24.8 Section through a tooth. The diagram shows the tooth architecture and the surrounding tissues that anchor the tooth in the gum.

contribute to the health of the host by keeping pathogenic bacteria in check and preventing them from adhering to mucosal surfaces. Tooth decay, gum inflammation, and periodontal disease are among the most visible manifestations of a breakdown in these generally stable mutualisms. We discuss the microorganisms associated with the hard tooth surface and their contribution to the formation of dental plaque and dental caries in Section 25.2 (Figures 25.7 and 25.8).

There is significant site variation in the diversity and specificity of colonization by different oral bacterial species. For example, there are differences in abundant microbial taxa associated with subgingival plaque (**Figure 24.9**) compared with that found in saliva (Figure 24.7). Different species of *Corynebacterium* demonstrate significant site specificity. For instance, *Corynebacterium matruchotii* is almost exclusively found in the supragingival plaque, whereas *Corynebacterium argentoratense* occurs mostly in the saliva. *Lautropia mirabilis* selectively colonizes the supragingival plaque, whereas the spirochete *Treponema socranskii* is found mostly in subgingival plaque, presumably because this site provides the low-oxygen environment needed for microaerobic growth of this bacterium. The distribution of *Firmicutes, Proteobacteria*, and *Bacteroidetes* is similar between the oropharynx (see Figure 24.10) and saliva. The hard palate (roof of the mouth) harbors a much lower diversity of microbiota than does the gingival plaque, probably as a result of constant shedding of epithelial cells and the shear forces associated with chewing and swallowing.

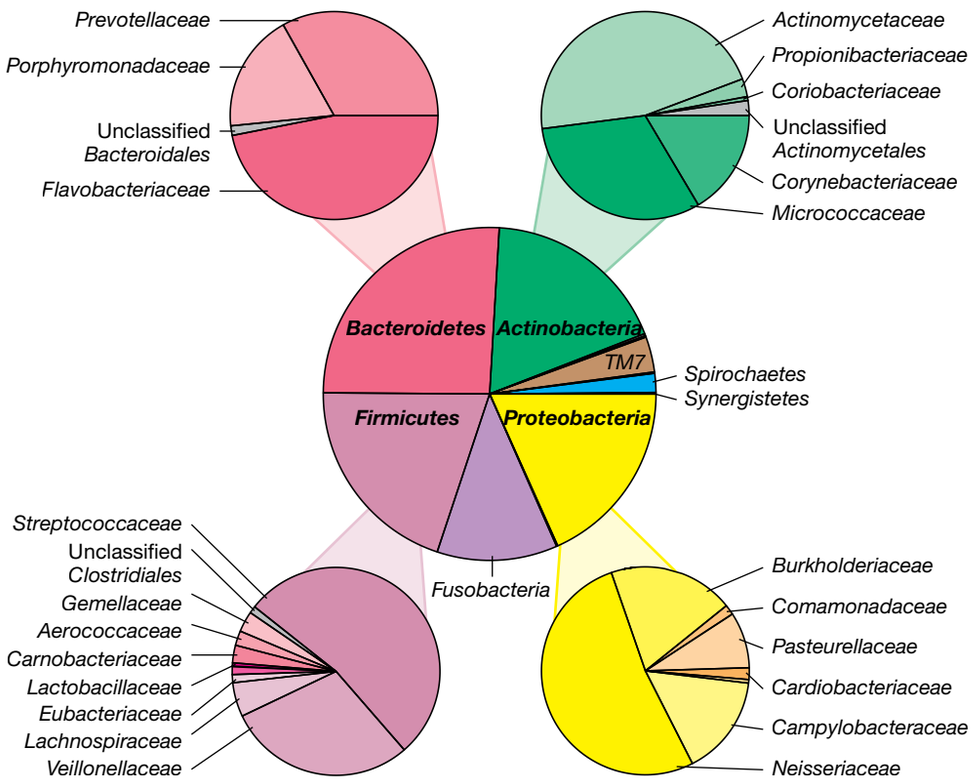


Figure 24.9 Bacterial diversity of subgingival plaque. The results are pooled analyses of approximately one million sequences (from 183 samples) of the V1–V3 region of the 16S ribosomal RNA gene (Figure 13.15). Compare the fractional distribution of different bacterial taxa with that observed in saliva (Figure 24.7), noting the higher representation of anaerobic *Fusobacteria* and *Spirochaete* populations in the oxygen-limited gingival crevice. Many of these groups are covered in Chapters 15 and 16 (*Bacteria*). Data assembled and analyzed by Nícolas Pinel.

Microenvironments of the Respiratory Tract

The anatomy of the respiratory tract is shown in **Figure 24.10** and Figure 24.1. In the **upper respiratory tract** (including the throat/tonsils, nasopharynx, oral cavity, oropharynx, and larynx), microbes live in areas bathed with secretions from the mucous membranes. Bacteria continually enter the upper respiratory tract from the air during breathing, but most are trapped in the mucus of the nasal and oral passages and expelled with nasal secretions, or swallowed and then killed in the stomach. However, a few microbes colonize respiratory mucosal surfaces in all individuals; those most commonly present are species of staphylococci, streptococci, diphtheroid bacilli, and gram-negative cocci.

Occasionally, potential pathogens such as *Staphylococcus aureus* and *Streptococcus pneumoniae* are part of the normal microbiota in the nasopharynx of healthy individuals. These individuals are *carriers* of the pathogens but do not normally develop disease, presumably because the other resident microorganisms compete successfully for nutritional and metabolic resources and limit pathogen attachment, colonization, or activities. The innate

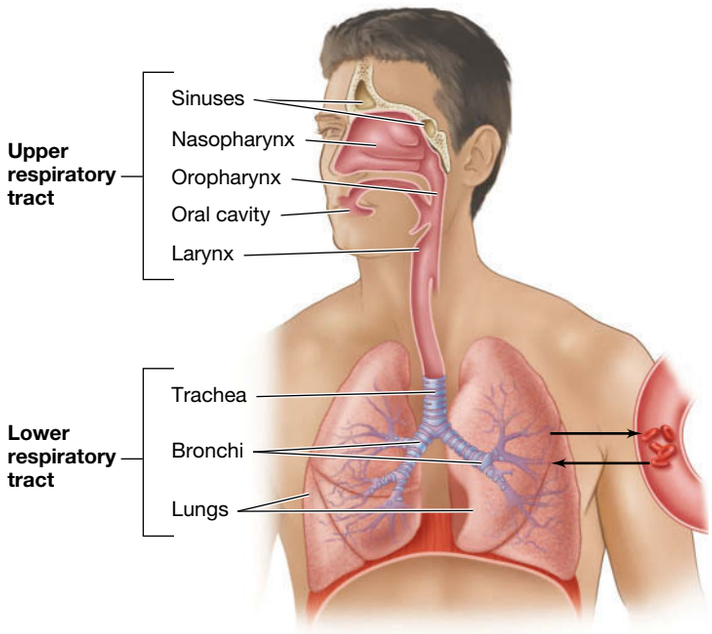


Figure 24.10 The respiratory tract. In healthy individuals the upper respiratory tract has a large variety and number of microorganisms. By contrast, the lower respiratory tract in a healthy person has few if any microorganisms.

immune system (Chapter 26) and components of the adaptive immune system such as secreted antibodies (Chapter 27) are particularly active at mucosal surfaces and inhibit growth and invasion by potential pathogens.

The **lower respiratory tract** (trachea, bronchi, and lungs, Figure 24.10) has no resident microbiota in healthy adults, despite the large number of organisms potentially able to reach this region during normal breathing. Dust particles, which are fairly large, settle in the upper respiratory tract. As the air passes into the lower respiratory tract, the flow rate decreases, and organisms settle onto the walls of the respiratory passages. The walls of the entire respiratory tract are lined with ciliated epithelial cells, and the cilia, beating upward, push bacteria and other particulate matter toward the upper respiratory tract where they are then expelled in saliva and nasal secretions or are swallowed. Only particles smaller than about 10 µm in diameter reach the lungs. Nevertheless, these include some pathogenic microbes, most notably certain bacteria or viruses that cause pneumonia (inflammation of the lungs, Sections 30.1, 30.2, and 30.8).

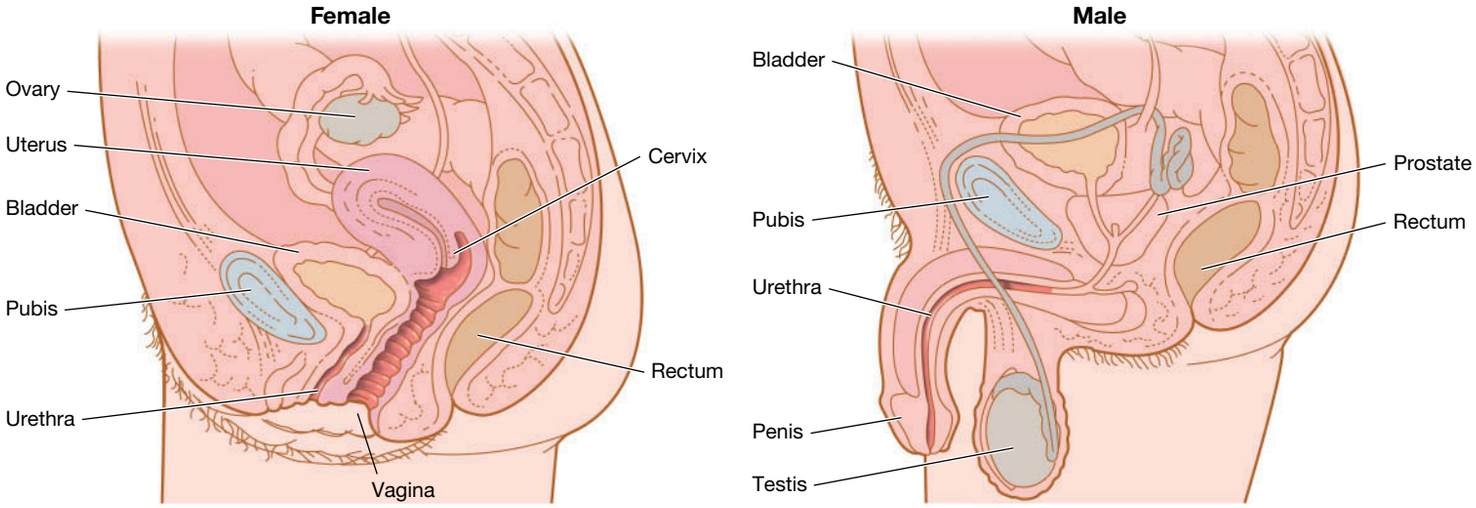
MINIQUIZ

- Compare the microbial microenvironments in the oral cavity in newborns and adults.
- Identify the major microbes that predominate in the adult oral cavity by taxa and metabolic requirements.
- Why is the lower respiratory tract typically microbe-free?

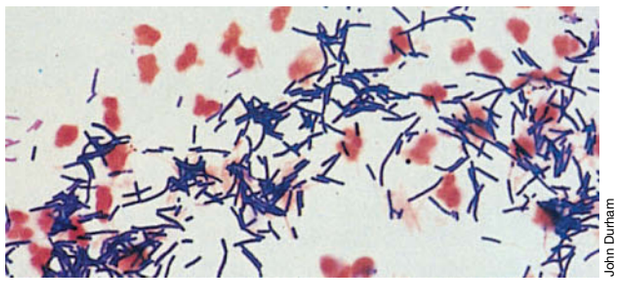
24.4 Urogenital Tracts and Their Microbes

In the urogenital tracts of healthy adults (**Figure 24.11**), the kidneys and bladder are sterile; however, epithelial cells lining the distal urethra are colonized by facultatively aerobic gram-negative *Bacteria*. Potential pathogens such as *Escherichia coli* and *Proteus mirabilis*, normally present in small numbers in the body or in the local environment, can multiply in the urethra and cause disease if conditions such as changes in pH occur. Such organisms are a frequent cause of urinary tract infections, especially in females. *Proteus* can be especially notorious as a urinary tract pathogen. This bacterium is a strong urease producer; it generates ammonia from urea and uses the ammonia as a nitrogen source. However, ammonia also causes urine pH to become quite alkaline, and this can trigger other urinary tract conditions such as the formation of kidney stones.

The vagina of the adult female is weakly acidic (pH~5) and contains significant amounts of glycogen. *Lactobacillus acidophilus*, a resident organism in the vagina, ferments the polysaccharide glycogen, producing lactic acid that maintains a local acidic environment (Figure 24.11*b*). Other organisms, such as species of the yeasts *Torulopsis* and *Candida*, various streptococci, and *E. coli*, may also be present. Before puberty, *L. acidophilus* is absent, the female vagina is neutral in pH and does not produce glycogen, and the microbiota consists predominantly of staphylococci, streptococci, diphtheroids, and *E. coli*. After menopause, glycogen production ceases, the pH rises, and the microbiota again resembles that found before puberty.



(a)



John Durham

(b)

Figure 24.11 Microbial growth in the urogenital tracts. *(a)* The urogenital tracts of the human female and male, showing regions (red) where microorganisms often grow. The upper regions of the urogenital tracts of both males and females are sterile in healthy individuals. *(b)* Gram stain of *Lactobacillus acidophilus*, the predominant organism in the vagina of women between the onset of puberty and the end of menopause. The individual gram-positive rods are 3–4 µm long. Species of *Lactobacillus* are anaerobic bacteria that ferment glucose and other sugars primarily to lactic acid as a fermentation product (Section 16.6).

Culture-independent 16S rRNA sequence analyses have confirmed earlier culture-based observations that the vaginal microbial community is less complex at the genus level than the oral or gut communities, that a healthy vaginal microenvironment is dominated by lactobacilli (Figures 24.1 and 24.11*b*), and that *vaginosis* (major changes in the balance of microbes in the vagina) is characterized by increased bacterial diversity, elevated pH, and a vaginal discharge. But even in the healthy adult female, vaginal microbiota are more diverse than suggested by culture alone (**Figure 24.12**). For example, one molecular study identified 112 genera of *Bacteria* in the vagina. These analyses have also shown that multiple community types constitute a normal vaginal microbiome, but that these normal states can vary in their stability (see Figure 24.22). There appear to be at least five types of "normal vaginal communities" containing different compositions of *Lactobacillus* spp. Four types are defined by dominance by one of *L. crispatus, L. iners, L. reuteri,* or *L. jensenii* (see Figure 24.22), while the fifth is a more heterogeneous type characterized by higher overall diversity and a greater proportion of other strict anaerobes relative to the lactobacilli. Although all vaginal community types are associated with an acidic pH, pH varies with community type. The *L. crispatus* type shows the lowest average pH (~4.0), whereas the heterogeneous type shows the highest average pH (~5.3).

Thus, unlike the picture of microbial diversity we have seen in other body sites or products (Figures 24.2, 24.5, 24.7, and 24.9), the microbiota in the vagina of the healthy female is dominated by lactobacilli (Figure 24.12). In contrast to the vagina, studies of the penis microbiota are fewer, but the general picture shows that the bacterial diversity of the penis is typical of those in the vagina, the patterns being especially so in sexual partners. However, the microbiota on the circumcised versus the uncircumcised penis can be quite different, and bacterial abundance on the uncircumcised penis is typically much greater as well.

MINIQUIZ

- What is the importance of vaginal *Lactobacillus* in healthy adult women?
- What variable feature of the vagina is most closely associated with different community compositions of *Lactobacillus* species?

24.5 The Skin and Its Microbes

The skin is a complex human organ functioning primarily to prevent loss of moisture and restrict the entry of pathogens. An average adult human has about two square meters (2 m^2) of skin surface that varies greatly in chemical composition and moisture content. Skin also provides an environment for part of the human microbiome. The skin microbiota consists of a rich community of microorganisms that associates intimately with the host's hormonal, nervous, and immunological systems.

There are approximately 1 million resident bacteria per square centimeter of skin, for a total of about 10^{10} skin microorganisms covering the average adult. Although these numbers are much lower than the oral and gut communities, molecular analyses have shown that the skin harbors a diverse microbial community of bacteria and fungi (primarily yeast) that vary significantly with location on the body as a function of the diversity of habitats. These habitats consist of microenvironments of varying temperature, pH, moisture, sebum content (sebum is the oily secretions of the sebaceous glands), and surface characteristics. One distinct set of microenvironments includes moist skin areas such as the inside of the nostril, the armpit, and the umbilicus. Moist skin is separated by only a few centimeters from dry microenvironments such as the forearms and the palms of the hands. A third microenvironment consists of areas with high concentrations of sebaceous glands such as those by the side of

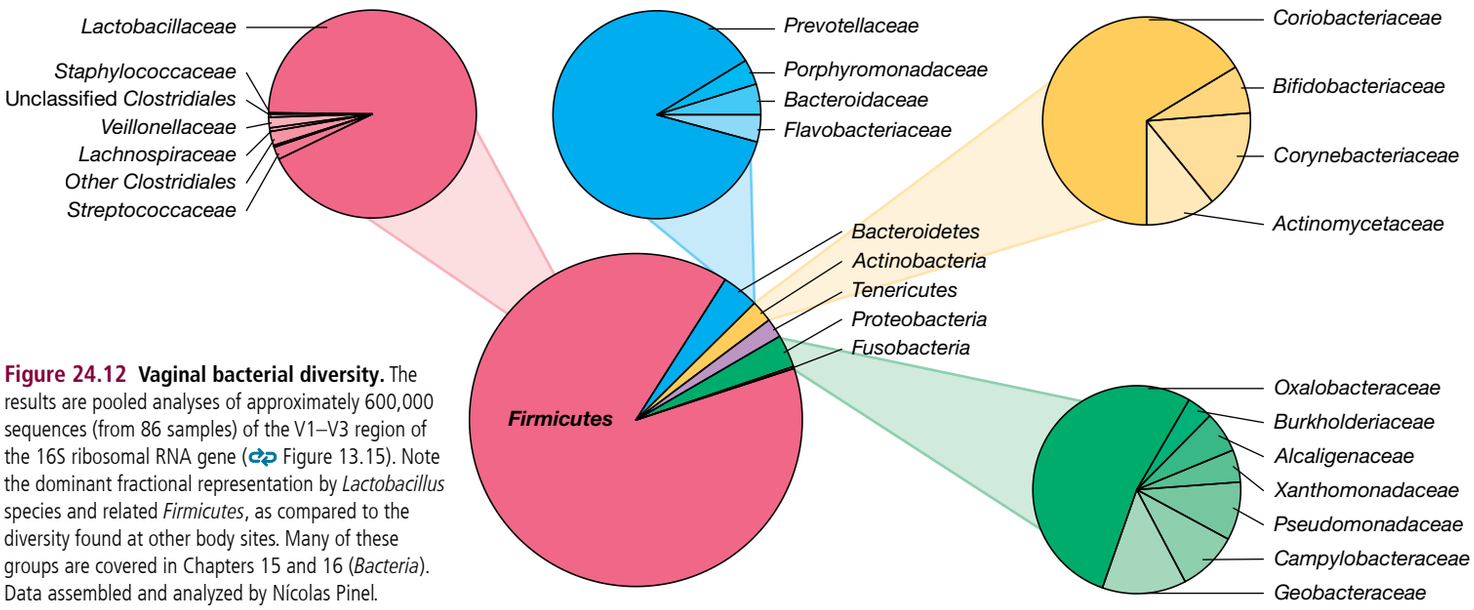


Figure 24.12 Vaginal bacterial diversity. The results are pooled analyses of approximately 600,000 sequences (from 86 samples) of the V1–V3 region of the 16S ribosomal RNA gene (Figure 13.15). Note the dominant fractional representation by *Lactobacillus* species and related *Firmicutes*, as compared to the diversity found at other body sites. Many of these groups are covered in Chapters 15 and 16 (*Bacteria*). Data assembled and analyzed by Nícolas Pinel.

the nose, the back of the scalp, and the upper chest and back. In addition to these site-specific differences, sweat is high in salt and other antimicrobial substances such as free fatty acids and antimicrobial peptides and thus plays a role in controlling diversity.

Microbial Diversity of Skin Microenvironments

A 16S rRNA sequencing comparison of 20 diverse skin sites categorized as moist, dry, or oily revealed tremendous diversity and variation among sites and individuals, but also showed some common patterns (**Figure 24.13*a***). Collectively, nearly 20 bacterial phyla were detected, but most phylotypes affiliated with one of four groups: *Actinobacteria* (~52%), *Firmicutes* (~24%), *Proteobacteria* (~16%), and *Bacteroidetes* (~6%). Over 200 different genera were identified, but species of three genera, *Corynebacterium* and *Propionibacterium* (both *Actinobacteria*) and *Staphylococcus* (*Firmicutes*) typically dominated the observed phylotypes (Figure 24.13*b*).

Each skin microenvironment showed a unique microbiota. Moist sites are dominated by corynebacteria and staphylococci, while drier sites support a mixed population dominated by *Betaproteobacteria*, corynebacteria, and *Flavobacteriales*. Species of *Propionibacterium* predominate in sebaceous areas (Figure 24.13*b*). For example, colonization of the follicular sebaceous gland system by *Propionibacterium acnes* is promoted by its ability to hydrolyze triglycerides present in sebum, resulting in release of free fatty acids that promote adherence of this bacterium, which sometimes causes disease (acne, Section 24.9).

A higher-resolution molecular diversity study of 400 different body sites of one male and one female subject revealed 850 distinct species (using 97% 16S rRNA sequence identity as the species cutoff). The results of a high-resolution analysis of a single dry skin site (the inside of the elbow) are shown in **Figure 24.14**. As shown by the more general studies (Figure 24.13*a*), the most common microbial phyla were *Actinobacteria, Firmicutes, Proteobacteria*, and *Bacteroidetes*, but in this work, the breakdown of each phylum into family level taxa shows a significant hidden diversity within each major group. Although staphylococci, propionibacteria, and *Betaproteobacteria* dominate, many other groups are also present (Figure 24.14).

The abundance of specific bacterial taxa can also be visualized in a "heat map" diagram (↔ Section 9.11 and Figure 9.31) showing the major locations of different taxa on the skin. An example is shown in **Figure 24.15** where it can be seen that *Propionibacterium* tends to localize on sebaceous regions (head, face, upper back, and upper chest), whereas species of *Staphylococcus* and *Corynebacterium* are more prevalent on less exposed regions, such as the groin, under arm, and toe web—areas higher in temperature and moisture content (Figure 24.15*a–c*).

Other Aspects of the Skin Microbiome

Eukaryotic microbes and *Archaea* are also present on the skin. The yeast *Malassezia* is the most common fungus found on the skin, and at least five different species of this yeast are typically present on the skin of healthy individuals. In an individual with a weakened immune system, for example someone who has HIV/AIDS or whose normal microbiota has been compromised, *Candida* and other potentially pathogenic fungi can also colonize the skin and cause serious (even fatal) infections. Fungal pathogens are discussed in Chapter 33. A remarkable finding emerging from 16S rRNA gene surveys of skin is that ammonia-oxidizing *Archaea* (↔ Section 17.5) can comprise as much as 4% of the skin microbiota in some individuals, presumably sustained by ammonia present in the sweat of more physically active individuals.

Environmental and host factors influence the composition of the normal skin microbiota. For example, the *weather* may cause an increase in skin temperature and moisture, which increases

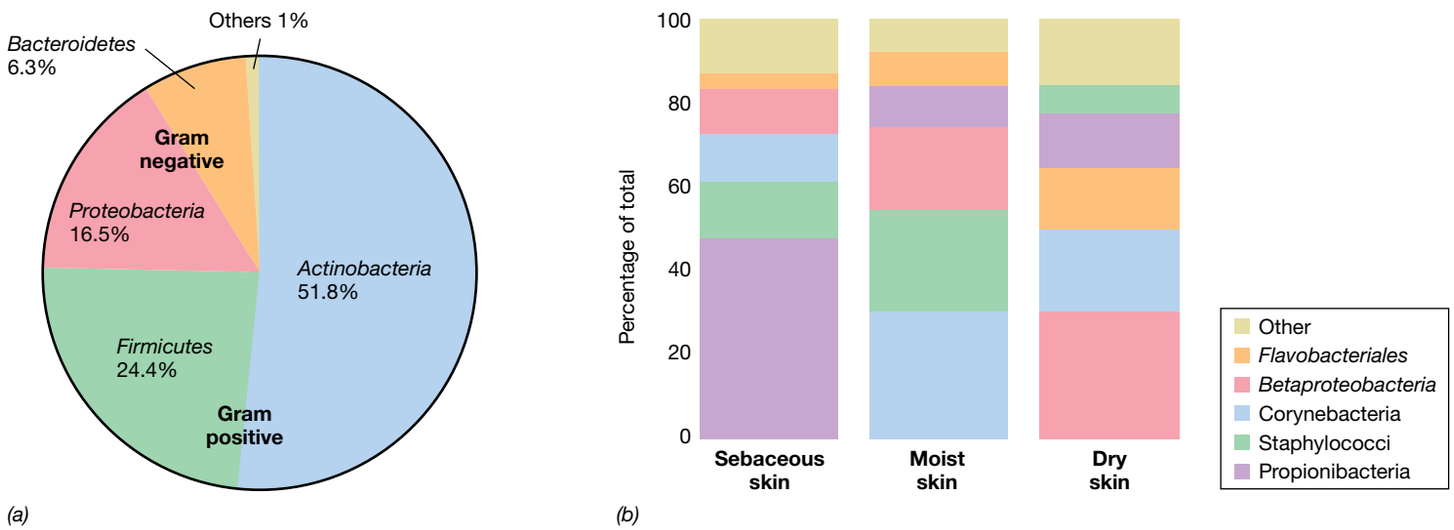


Figure 24.13 Normal skin microbiota. *(a)* Analysis of the skin microbiome from 10 healthy human volunteers detected 19 bacterial phyla. Four phyla were predominant. *(b)* Composite populations of *Bacteria* from the same volunteers, divided according to sebaceous, moist, and dry skin microenvironments. Data are adapted from Grice et al., 2009, *Science 324:* 1190.

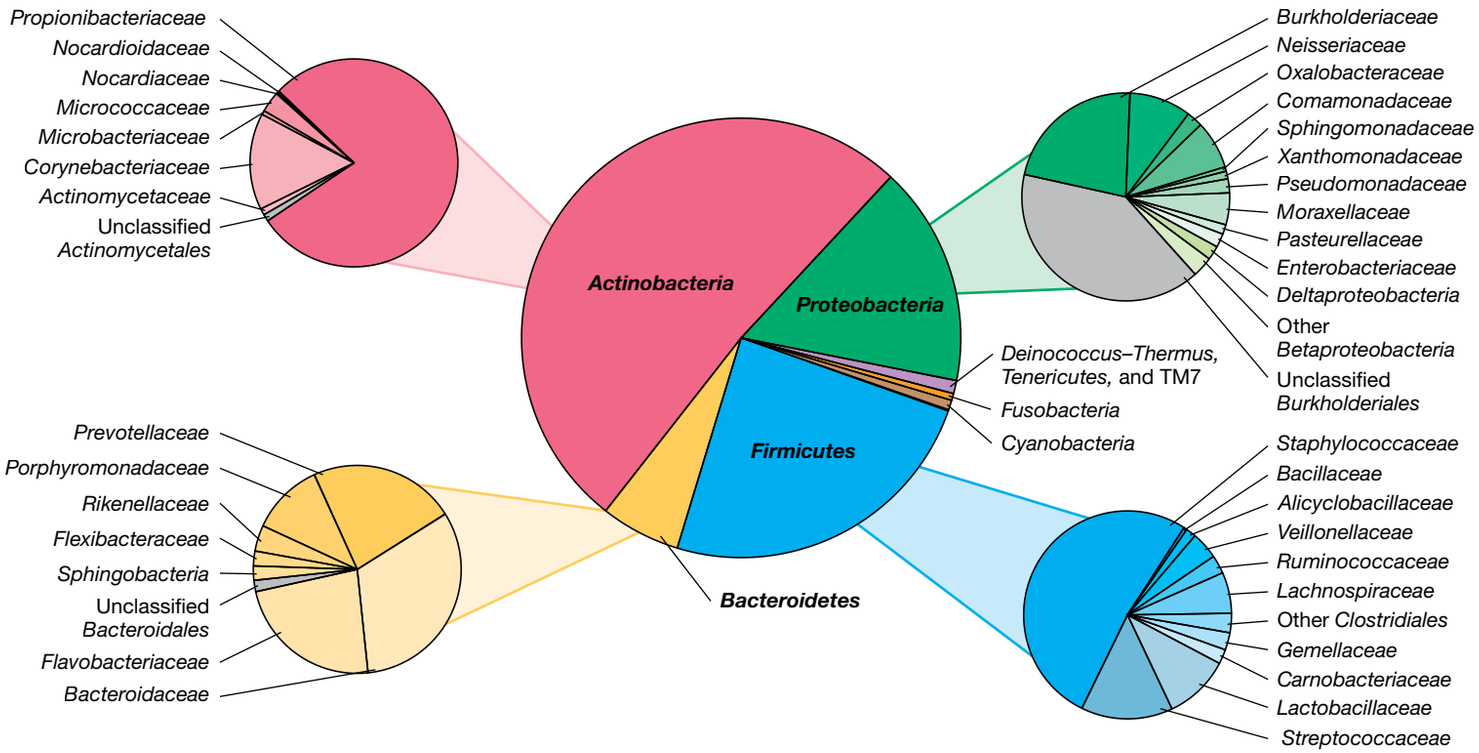
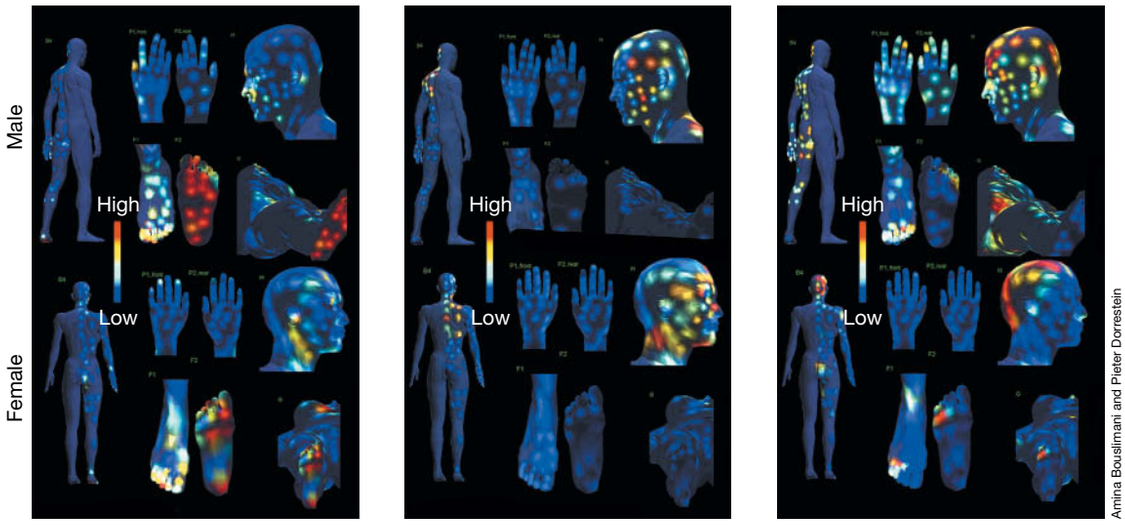


Figure 24.14 Skin bacterial diversity at the inside of the elbow (antecubital fossa; see Figure 24.1). Note that *Propionibacterium* species comprise the vast majority of the *Actinobacteria*, and that *Staphyloccocus* species dominate skin-associated *Firmicutes*. The results are pooled analyses of approximately 80,000 sequences (from 123 samples) of the V1–V3 region of the 16S ribosomal RNA gene (Figure 13.15). Many of these groups are covered in Chapters 15 and 16 (*Bacteria*). Data assembled and analyzed by Nícolas Pinel.

the abundance of the skin microbiota. The *age* of the host also has an effect; young children have a more varied skin microbiota and carry more potentially pathogenic gram-negative *Bacteria* than do adults. *Personal hygiene* also greatly influences the resident skin microbiota; individuals with poor hygiene typically have higher microbial population densities on their skin. And finally, many microorganisms that would otherwise colonize skin cannot survive there simply because of its low moisture content and presence of antimicrobial fatty acids. Thus, the skin is a natural barrier to microbial colonization (Figure 26.2)



(a) **Staphylococcus** (b) **Propionibacterium** (c) **Corynebacterium**

Amina Bouslimani and Pieter Dorrestein

Figure 24.15 Distributions of *Staphylococcus*, *Propionibacterium*, and *Corynebacterium* on the human skin. Heat maps of microbial species distributions at 400 different body sites inferred from 16S rRNA gene sequences from the skin of male (top) and female (bottom) subjects (in scale bar, red indicates higher relative abundance and blue lower abundance). *Propionibacterium* species tend to localize on sebaceous regions (head, face, upper back, and upper chest); *Corynebacterium* are most common on the head, groin, and toes; and *Staphylococcus* are most abundant on the foot.

while at the same time supporting a diverse array of normal microbiota.

As yet, there have been no significant longitudinal studies of early colonization and succession of the skin microbiome as has been conducted for the gut microbiome (Section 24.7). However, it is well recognized that there is a major transition of the skin microbiome associated with sexual maturation. The skin of young children is dominated by *Streptococcus* spp., *Betaproteobacteria*, and *Gammaproteobacteria*. By contrast, these taxa are largely absent from the skin of postadolescent young adults; in this group the skin is characteristically dominated by species of *Propionibacterium* and *Corynebacterium*, as we have seen (Figures 24.13 and 24.15). And finally, encounters with man's best friend can contribute to the human skin microbiota. Studies have shown that adult dog owners have more skin microbes in common with their own dogs than with other dogs. This demonstrates that close and regular contact between two distinctly different species of animal can result in a major sharing of their microbiomes. In contrast to the skin microbiota, microbes in the mouths and guts of canines differ quite distinctly from those of their owners.

MINIQUIZ

- Compare the populations of microorganisms in the three major skin microenvironments.
- Describe the properties of microorganisms that grow well on the skin.

II • From Birth to Death: Development of the Human Microbiome

At birth, a baby is exposed to both maternal microorganisms and microbes present in the local environment. These early encounters determine the composition of microorganisms that first colonize different body sites. In this part of the chapter we focus primarily on colonization of the gut, the body site harboring the largest part of the human microbiome, and consider factors that govern early colonization and subsequent successional events of a community now recognized to be critical to general health and the education of the immune system.

24.6 Human Study Groups and Animal Models

Establishing relationships between the composition of a person's microbiome and its contribution to health and disease has been a difficult exercise because of complications surrounding sampling, the uncertainty about the contribution of the host's genetic background, and the limitations of controlling diet and other contributing lifestyle factors. Nevertheless, several human study groups and animal model experiments have revealed some of the basic principles and given microbiome studies a starting point.

Human Microbiome Study Groups

Most functional understanding of the human microbiome has been based on surveys of selected study groups (Table 24.1). For example, one of the most ambitious early studies was the Human Microbiome Project (HMP) funded by the U.S. National Institutes of Health. This study collected samples from 242 individuals (all American medical students in good health), sampling different body sites (15 to 18 sites depending on the sex) at one to three time points, and then evaluated bacterial diversity based on 16S rRNA gene sequencing and limited metagenomic analyses (Figures 24.1 and 24.2).

The objective of the HMP was to develop baseline information about what constituted a "healthy" microbiome. Although the HMP generated a huge amount of data, the study group represented only a small fraction of human diversity. This limitation was clearly revealed in the Global Gut Project, which examined three distinct populations (U.S. citizens, Malawians, and a group of indigenous peoples from Venezuela) and different age groups within those populations. The gut microbiomes of the two non-U.S. populations were distinct from those of the U.S. individuals, showing that the HMP greatly underestimated the potential for variation in gut microbiomes across nationalities. These studies also suffered from a lack of appropriate metadata; for example, detailed information about dietary habits (vegetarian, vegan, omnivorous) and how much fiber or protein was ingested daily. Because there is still very limited understanding of the influence of specific environmental variables such as lifestyle, diet, gender, and genetics on microbiome structure and function, new and ongoing studies (e.g., the American Gut Project) are obtaining these valuable metadata at the time of sampling. The overarching goal is to develop robust correlations between the microbiome, host genetics, diet, lifestyle, health, and pathologies thought to have a microbial connection, in particular, heart disease, cancer, stroke, diabetes, and obesity.

The Mouse Model

Even if appropriate metadata are available, a major limitation of the human microbiome studies to date is establishing causality, something that is only possible with highly controlled animal studies. Hence the mouse has become the major model animal system for linking cause and effect in the gut microbiome.

Although the mouse and human digestive system have some significant differences (**Figure 24.16**), the mouse (and to a more limited extent, the rat) has been the workhorse of experimental microbiome studies. Compared to humans, mice have a relatively larger colon and cecum, which are needed to extract nutrients derived primarily from the fermentation of plant materials. For example, the average ratio of the length of the murine small intestine to its colon is about 2.5 whereas in humans it is about 7. In mice, fermentation of plant material is

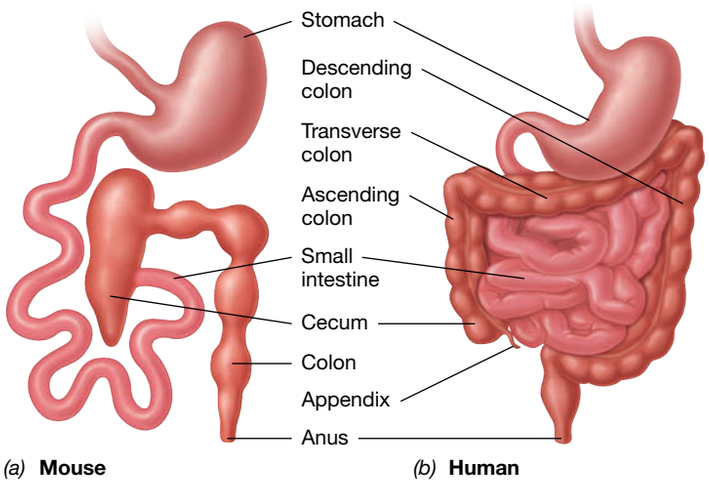


Figure 24.16 Anatomy of the mouse and human intestinal tracts. The mouse *(a)* and human *(b)* intestinal tracts have significant structural differences that are associated with differences in the composition of the microbiota of mice and humans despite similarities in their physiologies.

compartmentalized in the cecum, while in humans this fermentation takes place in the colon. Nevertheless, mice have several experimental advantages, including the availability of well-defined genetic lines, low maintenance costs, and short life cycle. This has allowed researchers to explore the importance of the host genetic background through selective gene knockouts, the manipulation of gut microbiota composition using germ-free mice (raised free of all microbes), tests of the influence of a strictly controlled diet, assessing the consequences of antibiotic treatment, and exploring the transfer of physiological traits through fecal transplantation. These studies have clearly associated the composition of the mouse microbiome to different pathologies, including obesity and inflammatory bowel disease, as we will discuss in Section 24.8.

Unfortunately, because of anatomical differences (Figure 24.16), mouse studies are not directly applicable to humans, and these anatomical differences may in part account for observed differences in the relative abundance of dominant bacterial genera in mouse and human guts. For example, the genera *Prevotella, Faecalibacterium*, and *Ruminococcus* are in high abundance in the human gut, whereas the genera *Lactobacillus, Alistipes*, and *Turicibacter* are more abundant in the mouse gut. Thus, although the mouse model gives us much experimental latitude not available in gut microbiome studies of humans, actual results from the two systems are not directly comparable. However, despite these differences in bacterial composition, experimenters have glimpsed much useful information from the study of animal models that has accelerated our understanding of the human gut microbiome (for example, see Section 24.8 and Figure 24.20).

We now follow development of the human gut microbiota from birth through adulthood, comparing and contrasting the major organisms seen in healthy humans. An overview of the mature and highly stable gut microbiota was presented in Figures 24.3 and 24.5.

MINIQUIZ

- In hindsight, which aspects of the HMP were well controlled for and which were not?
- Why has it been so difficult to associate human disease, or health, with changes in the gut microbial community?
- What are some of the major differences between the mouse and human gastrointestinal systems?

24.7 Colonization, Succession, and Stability of the Gut Microbiota

Colonization of an initially sterile gut begins immediately after birth; a succession of microbial populations replaces each other in turn until a relatively stable, adult microbial community is established. The source of early colonizers is not clear, although some species are clearly transmitted from mother to infant through the birth canal. The infant gut community is dominated by bifidobacteria—fermentative anaerobes of the bacterial class *Actinobacteria* (Section 16.10)—and does not reach an adultlike composition until about age 3. There are also major changes in the gut community in the aged. Indeed, recent studies have correlated frailty in the elderly with two major microbial factors: (1) an overall decrease in gut bacterial diversity, and (2) reduced abundance of *Firmicutes* and increased abundance of *Bacteroides*.

Microbial Activities in the First Year of Life

During the first year of life the newborn's relatively simple community evolves into a more complex and adultlike composition. The early microbial colonizers are an important source of amino acids and vitamins. Microbial genes encoding the synthesis of vitamins K_2 (menaquinone), B_6 (pyridoxal), and B_7 (biotin) are elevated in the newborn's microbiota. As the gut microbiome matures, there is a greater prevalence of microbial genes encoding synthesis of the vitamins thiamine (B_1), pantothenate (B_5), and cobalamin (B_{12}). The presence of bacterial genera such as *Enterococcus* and *Escherichia*, both facultative microbes, in the newborn gut is also indicative of a more aerobic state of the early gut system and a greater role for the citric acid cycle and respiration in microbial energy production in the neonate.

Major factors controlling the early assembly of the gut microbiome following birth is whether birth was vaginal or by cesarean section (C-section) and whether initial nutrition came from breast milk or from formula. A vaginally delivered infant is colonized by a gut microbiota similar to that of the mother, suggesting direct transfer from mother to neonate during passage through the birth canal and subsequent intimate contact with the mother. In contrast, the gut microbiota of a child delivered by C-section is significantly different from that of the mother. In an analysis of approximately 100 newborns, of the 187 taxonomically annotated 16S rRNA gene sequence types present in vaginally delivered newborns, 135 (72%) were found in their own mothers, including species of *Escherichia, Bifidobacterium, Enterococcus, Bacteroides*, and *Bilophila*. By contrast, only 41% of the species matched those of the mother in the gut of the C-section neonate. The C-section neonate microbiome tends to be enriched in groups such as

Enterobacter hormaechei, Haemophilus parainfluenzae, Staphylococcus saprophyticus, S. aureus, Streptococcus australis, and *Veillonella dispar*, indicating that skin and oral microbes, as well as environmental populations, were the first colonizers. *Bacteroides* species were either less prevalent or totally missing in the infants delivered by C-section.

In 4-month-old infants delivered vaginally, the gut microbiome is defined by *Bifidobacterium, Lactobacillus, Collinsella, Granulicatella*, and *Veillonella*, reflecting reduced oxygen availability and increased production and utilization of lactose associated with a diet comprised primarily of milk. These populations are enriched in genes for carbohydrate uptake, and genes encoding lactose-specific transporters are most abundant in the 4-month-old infant. At 4 months there is also a clear difference between children who have received exclusively breast milk compared with those given formula milk. Breast-fed infants have increased levels of taxa commonly used as probiotics (Section 24.11), including *Lactobacillus johnsonii, L. gasseri, L. paracasei, L. casei*, and *Bifidobacterium longum*. The enrichment of *Bifidobacterium* species, in particular *B. longum*, is related to the composition of human milk. Breast milk contains a complex mixture of unusual oligosaccharides that most gut microbes and humans are unable to digest (**Figure 24.17**). However, these sugars are metabolized by *B. longum* growing in the infant's gut. In addition, since the structures of human milk oligosaccharides mimic carbohydrates lining the infant gut, they also function to suppress infection by pathogenic bacteria by blocking the receptors on the pathogens' cells required for attachment.

The abundance of *B. longum* in breast-fed infants also leads to the production of short-chain fatty acids; this creates an environment favoring the growth of commensal normal microbiota important for "educating" the immune system. In contrast, 4-month-old formula-fed infants tend to have elevated numbers of *Clostridium difficile*—a potentially serious pathogen—*Granulicatella adiacens, Citrobacter* spp., *Enterobacter cloacae*, and *Bilophila wadsworthia* instead of large numbers of *B. longum*. The gut of children that remain on breast milk at 12 months continues to be dominated by *Bifidobacterium* and *Lactobacillus* as well as genera of

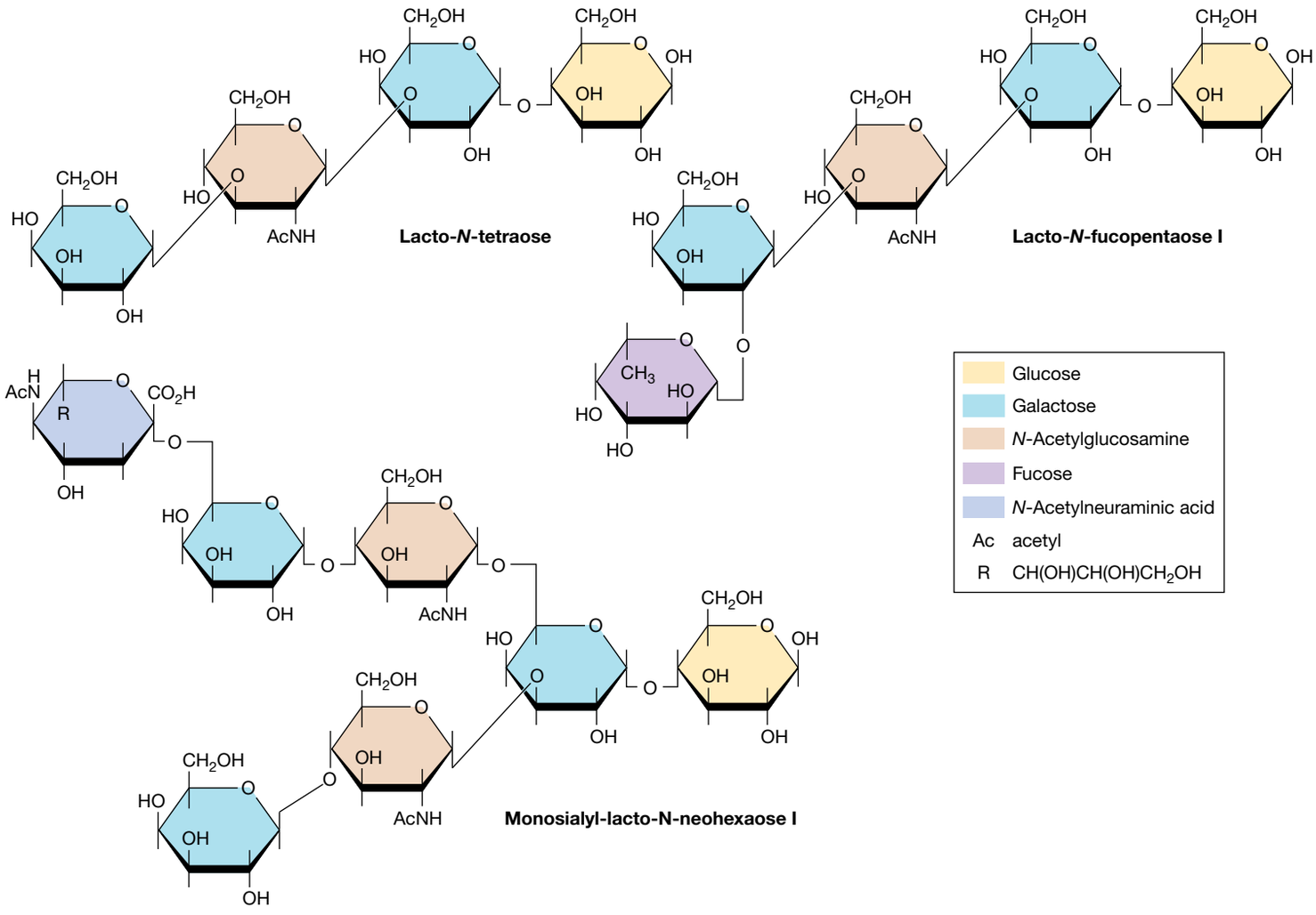


Figure 24.17 Examples of oligosaccharides found in human breast milk. These short polysaccharides selectively nourish and enrich for desirable microbial populations, such as *Bifidobacterium* and *Lactobacillus* species, in the infant's developing gut microbial community.

bacteria typical of a less mature (younger) gut community (e.g., *Collinsella, Megasphaera*, and *Veillonella*). Thus, in addition to providing nutrition to the newborn, mother's milk selects for a specific group of earlier colonizing normal microbiota important to the overall health of the child and proper development of its immune system.

The difference in the gut microbiomes of babies born vaginally or by C-section is significantly less by 12 months of age, but the C-section gut microbial community at 12 months remains distinctive in being more heterogeneous in composition than that of infants born vaginally. Termination of breast-feeding results in a shift of the community toward a more adultlike composition, as reflected by enrichment in *Bacteroides, Bilophila, Roseburia, Clostridium, Anaerostipes*, and *Eikenella*. Many of these later-arriving genera are efficient degraders of dietary fibers and complex carbohydrates, consistent with a transition to more solid foods following the cessation of breast-feeding; they also produce short-chain fatty acids. The increased abundance of *Bacteroides thetaiotaomicron*, a bacterium encoding a diverse set of glycan-degrading enzymes, is specifically associated with the increase in pectin in solid foods. But it is the cessation of breast-feeding rather than the introduction of solid foods, per se, that actually triggers the development of an adultlike gut microbiota. The time of development of a fully adultlike gut microbial community varies among study groups, but the gut microbiome of children generally reaches an adultlike composition when they are 1–3 years old.

Stability of the Adult Microbiome and Transitions with Age

As stated earlier, the 16S rRNA sequence-based census of microbial diversity in adult human fecal material (Figure 24.5) has identified between 3500 and 35,000 microbial species, depending on whether a 97% or more stringent 98–99% identity threshold is used to define a species (Section 24.2). The number of species in any given individual is much lower, fewer than 200 unique species. However, an individual's gut microbiota is relatively stable through time. Longitudinal studies over a 5-year period combining metagenomic and 16S rRNA sequence analyses to achieve precise resolution have shown that 70% of an individual's unique species persist over a one-year sampling period, and that samples taken 4 years later show only a few additional changes in species composition.

The most stable species are typically affiliated with the *Bacteroidetes* and *Actinobacteria*. Although *Actinobacteria*, such as *Bifidobacterium* spp., comprise a very small part of the adult gut microbiota compared to the *Bacteroidetes*, specific species tend to associate with individuals over long periods of time. Species of *Firmicutes* and *Proteobacteria* appear to be significantly less stable members of the gut community. Studies extrapolating gut stability over longer time periods suggest that the majority of species comprising an individual's gut microbiome constitute a stable core that persists for his or her entire adult life. These studies have also shown that species of an individual's core gut microbiome tend to be shared among family members, and likely were acquired very early in life. Thus, the early colonizers—including microorganisms acquired from parents or siblings—may in large part determine the metabolic and immunological character of the adult microbiome. In other words, early life experiences that govern or influence microbial colonization may be an important factor in adult health and predisposition to disease.

As we age, so does our microbiome. The most well-established change is the age-related alteration in the relative proportions of *Firmicutes* and *Bacteroidetes*, with an increasing proportion of *Bacteroidetes* seen in the elderly as compared with the higher proportions of *Firmicutes* in young adults. Also observed with age are significant decreases in bifidobacteria and certain clostridial species. There is also the observation that old age–related inability to perform routine daily activities, a measure of frailty, is correlated with decreased diversity in the gut microbiota. Consistent with this observation, elderly individuals living at home or in supportive communities have a more diverse gut community compared with those living in residential care facilities, such as nursing homes. Thus, maintaining a diverse gut microbiota is likely one of many links to health for the aged and is possibly a positive effector of longevity.

With this overview of changes in the human microbiome with time, we move on to consider some startling discoveries about human diseases linked to unfavorable changes in the human gut microbiota. We then end this chapter by looking at some therapies for dealing with these problems.

MINIQUIZ

- What factors contribute to early colonization of the newborn's gut community?
- How do microorganisms in the infant and adult gut community contribute differently to vitamin and amino acid requirements?
- What factor(s) are most important in the transition from an immature to a mature gut microbial community?

III • Disorders Attributed to the Human Microbiome

Alteration of the structure and activity of the human microbiome is associated with a variety of pathologies, including obesity, type 2 (non-insulin-dependent) diabetes, asthma, atopic dermatitis, liver disease, colorectal cancer, kidney stones, psoriasis, tooth decay (caries), and periodontitis. As we learn more about the relationship between the human microbiome and health and disease, therapeutic intervention may well be possible. This might include promoting the growth of protective beneficial bacteria, inhibiting the growth of specific microbes (or specific assemblages of microbes) that compromise health, fecal transplants to introduce a desirable gut microbiota, and developing appropriate behavioral interventions, such as diet modification.

24.8 Disorders Attributed to the Gut Microbiota

Two well-studied examples of links between the gut microbiota and clinical disease are inflammatory bowel disease and obesity. Both conditions show strong links to alterations in the gut microbiota.

Inflammatory Bowel Disease

The gut microbiota plays important roles in shaping the immune system during infancy, during which time the commensal microbiota and their products interact with immune cells to initiate and maintain host tolerance. The failure to develop tolerance to the normal microbiota early in life is associated with different immune-mediated diseases, including allergies and chronic inflammation of the gut such as *inflammatory bowel disease* (*IBD*).

It is widely accepted that IBD is not caused by a specific pathogenic microbe but rather an imbalance between the immune system and the normal gut microbiota. The observation that antibiotic use in early life increases the risk of IBD points to the importance of the development of a normal gut microbiota in "educating" the immune system to differentiate between the normal microbiota and invading pathogens. For example, relative to healthy children, children with IBD have higher levels of species of *Veillonella, Prevotella, Lactobacillus*, and *Parasporobacterium* and lower levels of species of *Bifidobacterium* and *Verrucomicrobium*. This type of disruption of the homeostasis between the gut microbiota and the host is called **dysbiosis**.

There is also some evidence that once developed, IBD is transmissible. Fostering or co-caging healthy mice with IBD-predisposed mice was sufficient to cause IBD development in the healthy mice and was correlated with the transfer of the enteric bacterial species *Klebsiella pneumoniae* and *Proteus mirabilis* from the IBD mice to the healthy mice. Metagenomic analyses of healthy subjects and patients with IBD shows that the gut microbiota of IBD patients shares fewer genes in common with healthy subjects, relative to the number of genes shared among healthy subjects. The microbial community of IBD patients also tends to have significantly reduced functional capacity, as reflected by a reduction in the number of nonredundant (functionally unique) genes relative to subjects that do not have IBD (**Figure 24.18**).

However, as for the relationship between the gut microbiome and obesity, to be considered next, the causes of IBD and its possible transmission are not well understood. IBD may follow the disruption of mucosal barrier integrity (a condition called *leaky gut*) by a gut pathogen or toxic insult, permitting commensal bacteria to interact with and activate the adaptive immune system (Chapter 27); this stimulates the proliferation and differentiation of T cells into commensal-specific effector cells that can persist in the intestine long after resolution of the infection. For example, the IBD syndromes of *Crohn's disease* and *ulcerative colitis* are known to be associated with a T cell response to intestinal commensal bacteria.

There is also a strong correlation between IBD and diet. With the typical Western diet rich in animal protein, carbohydrates are first fermented in the upper colon (Figure 24.3). As digesta move further along the colon, protein and amino acids are then fermented, generating potentially harmful metabolites such as ammonia, phenols, amines, and H_2S that have been implicated in promoting IBD as well as playing a role in colon cancer. Animal studies have shown that these compounds can promote the development of a leaky gut and gut inflammation. In contrast, a diet rich in plant-based foods (high fiber) appears to inhibit development of these pathologies, highlighting the importance of diet in maintaining a primarily carbohydrate-based fermentation in the healthy gut. Finally, IBD does not always affect identical twins; this observation argues against a strong genetic connection to IBD and supports the hypothesis that the environment and diet are the major triggers of this intestinal condition.

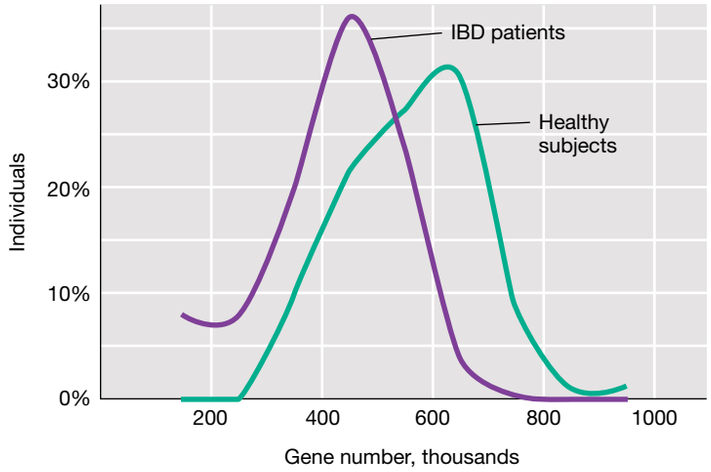


Figure 24.18 Reduced functional capacity of the gut microbiome of patients with inflammatory bowel disease. Metagenomic analysis of human gut microbiota in healthy subjects and patients with inflammatory bowel disease (IBD) revealed a tendency toward fewer nonredundant bacterial genes in patients with IBD.

The Role of the Gut Microbiota in Obesity: Mouse Models

Obesity is a significant health risk that contributes to secondary health issues such as high blood pressure, cardiovascular disease, and diabetes. Gut microorganisms likely play a part in human obesity, although the mechanisms are unclear. However, relatively minor changes in gut energy metabolism can have significant long-term effects on the accumulation of body fat. A small but persistent difference (+12 kcal/day) would result in a greater than 0.45 kg (~1 pound) gain in fat per year. This is the average weight increase experienced by people in the United States from ages 25 to 55.

Initial evidence linking the gut microbiota to host fat accumulation came from studies using germ-free mice. In these experiments, normal mice had 40% more total body fat than those raised under germ-free conditions, although both mouse populations were fed the same amount and type of food. After germ-free mice were inoculated with cecal material from a normal mouse, they developed a gut microbiota and their total body fat increased, although there had been no changes in food intake or energy expenditure. Studies of experimental colonization of germ-free mice with individual microbial species or microbial communities have demonstrated that colonization triggers the expression of *host* genes for glucose uptake and lipid absorption and transport in the ileum. This also indicates that there may be a link between gut microbial composition and the ability of the host to harvest energy from its diet, ultimately contributing to obesity.

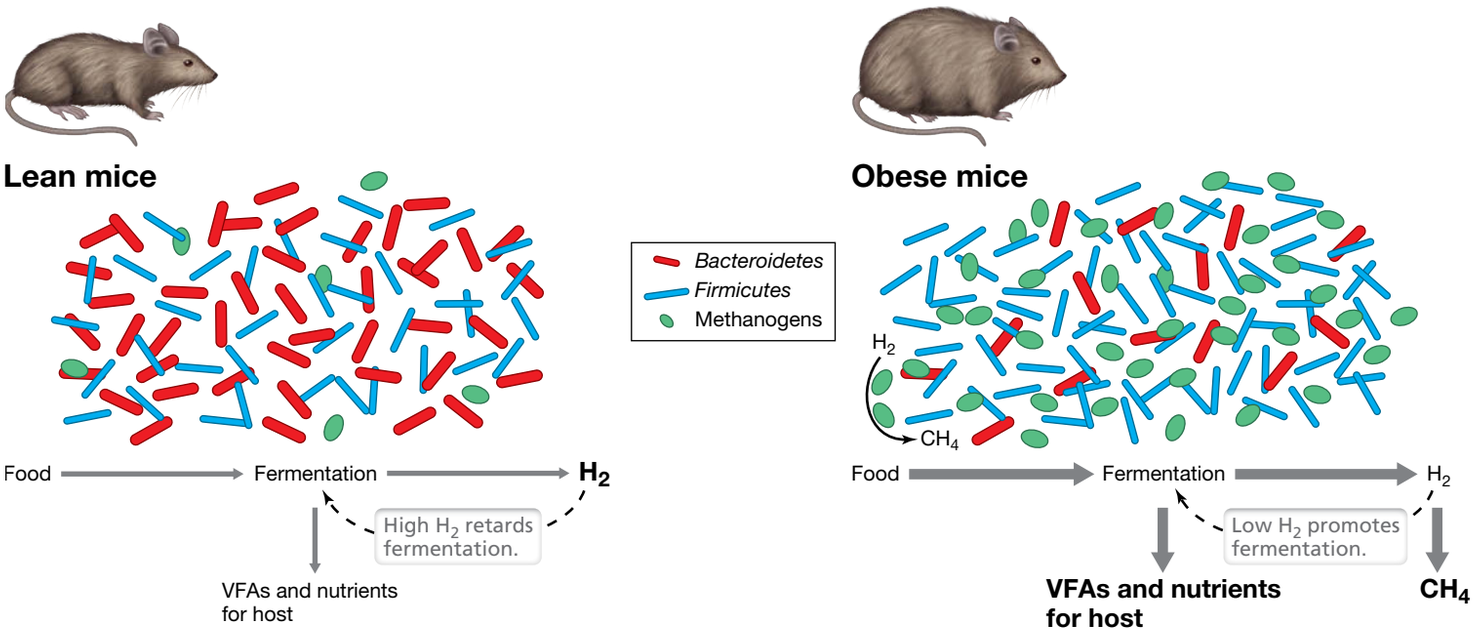


Figure 24.19 Differences in gut microbial communities between lean and obese mice. Obese mice have more methanogens, a 50% reduction in *Bacteroidetes*, and a proportional phylum-wide increase in *Firmicutes*. Nutrient production from fermentation is higher in obese mice due to removal of H_2 by methanogens.

One of the main activities of the intestinal microbiota is to break down and ferment dietary fibers into **volatile fatty acids (VFAs)**, including acetate, propionate, and butyrate. The host absorbs these acids, and humans obtain about 10% of their daily energy requirements from them. Mice that are genetically obese have microbial gut communities that differ from those of normal mice, with 50% fewer *Bacteroidetes*, a proportional increase in *Firmicutes*, and a greater number of methanogenic *Archaea* (Figure 24.19). Methanogens are thought to increase the efficiency of microbial conversion of fermentable substrates by consuming molecular hydrogen (H_2), as mentioned for fermentation in the rumen (↬ Section 23.13). The working hypothesis is that H_2 removal stimulates fermentation, making more fermentation products available for absorption by the host and thus contributing to obesity.

The importance of the gut community to a predisposition to obesity in mice has also been shown by *fecal transplant studies*, transplanting a small sample of the gut contents from a set of paired human twins (one obese and one lean) into germ-free mice (see Section 24.10 for more coverage of fecal transplants). Although the recipient mice were fed identical high-fiber diets, the mice receiving fecal contents from the obese twin gained significantly more weight than the mice receiving fecal contents from the lean twin (Figure 24.20). This is direct experimental evidence that a lean or obese body type can be altered by changes in the gut microbiota, even when the microbiota originates from a different species. Put another way, specific but widely distributed gut microbes may exist that can in some way control an animal's metabolism to yield a lean or obese body type.

The Gut Microbiota and Human Obesity

Animal model inferences have been more difficult to demonstrate with human subjects, since strict control of diet and host genotype is not feasible and gut microbiota manipulation is more difficult to achieve. Nevertheless, studies of humans, while not strictly confirming the *Bacteroidetes–Firmicutes* relationship established in mice, have shown that obese humans are more likely to harbor species of *Prevotella* (a genus of *Bacteroidetes*) and methanogenic *Archaea* than are lean humans, suggesting that the mouse model (Figures 24.19 and 24.20) is likely applicable to humans. In humans, the methanogens are proposed to remove H_2 produced by *Prevotella*, facilitating fermentation by *Prevotella* and increasing nutrients to the host. This model is also supported by the study of germ-free mice colonized with *Bacteroides thetaiotaomicron* (having a metabolism similar to *Prevotella*) and the methanogen *Methanobrevibacter smithii*. Relative to controls containing just one of these species, co-colonized mice have a higher number of total gut bacteria, higher acetate levels in the intestinal lumen and blood, and greater body fat.

This relatively simple explanation for the cause of obesity is insufficient to fully explain microbial contributions to obesity. For example, the gut microbiota in lean mice produce greater amounts of the fatty acids propionate and butyrate and actually digest *more* of the plant fiber than do the microbiota of obese mice. Similarly, although a fiber-rich diet increases the amount of material available for fermentation in the human large intestine, such a diet also reduces the risk of obesity. This may in part be a consequence of volatile fatty acids binding to free fatty acid receptors in the gut and triggering the production of hormones associated with feelings of a full stomach (satiety). In humans, obesity is also associated with a variety of metabolic complications, including low-grade inflammation, hypertension, glucose intolerance, and diabetes, and these factors have not been adequately addressed in the simple microbiota models of obesity proposed to date.

An influence of host physiology on the gut community is also suggested by changes during pregnancy in humans. The period

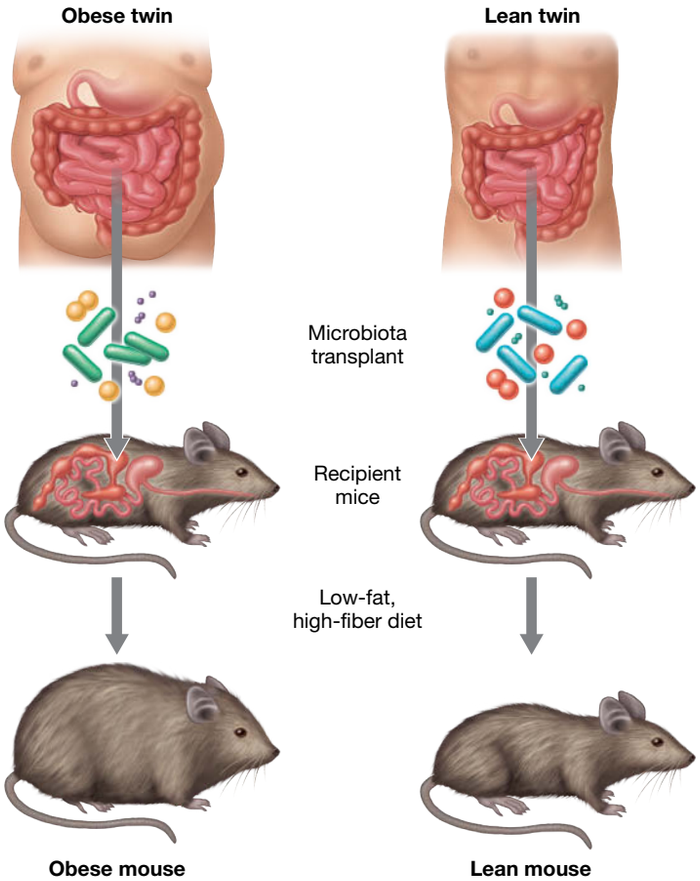


Figure 24.20 Transfer of an obese condition by fecal transplant. Transplanting fecal material from the gut contents of a paired identical human twin study group (one twin was obese and the other lean) to germ-free mice showed that the obese twin microbiota made the mouse obese. Conversely, transfer of gut contents from the lean twin did not contribute to an obese phenotype. Adapted from Ridaura, V.K., et al. *Science 341:* DOI:10.1126/science.1241214.

between the first and third trimesters of pregnancy is associated with a decrease in gut microbial diversity and enrichment in the gut community of species of *Proteobacteria* and *Actinobacteria*. These changes are associated with the increased body fat and insulin insensitivity that develop later in gestation. A simple interpretation of these findings is that a pregnant woman's body in some way manipulates her gut microbiome as part of its preparations for a greater demand on stored energy reserves. If true, this once again underscores the complex interplay between the gut microbiota and multiple host variables (genetic, physiological, behavioral, and environmental) that may be contributing factors in the development of human obesity and associated metabolic disorders.

MINIQUIZ

- What is dysbiosis? How might this condition lead to inflammatory bowel disease?
- What is the mechanism by which higher numbers of gut *Firmicutes* are thought to be linked to obesity?
- Why are the gut microbiota results obtained in mice more difficult to confirm through human studies?

24.9 Disorders Attributed to the Oral, Skin, and Vaginal Microbiota

Imbalances in the normal microbiota have been linked to human health issues other than those affecting the gut. These include in particular diseases of the oral cavity, skin, and vagina, and we consider some of these important disease centers here.

Dental Caries and Periodontitis

Dental caries and periodontal diseases are among the most common chronic human maladies. We consider dental caries in Chapter 25, where we examine how bacteria attach to solid surfaces. The mechanisms by which oral bacteria stick to the teeth and gums has been used as a major model system for bacterial attachment to solid surfaces. The attached cells eventually form a diverse microbial community in *dental plaque* and this leads to dental disease from the generation of lactic acid from fermentation (Section 25.2 and Figures 25.7 and 25.8). Although *Streptococcus mutans* is the major pathogen associated with caries, colonization of tooth surfaces (Figure 24.21) with organisms other than *S. mutans* can also lead to

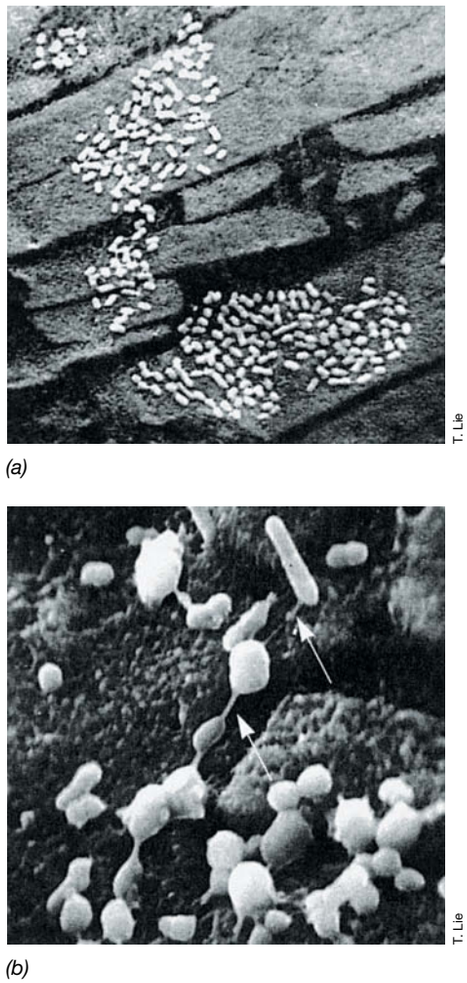


Figure 24.21 Colonization of tooth surfaces. *(a)* The colonies are growing on a model tooth surface inserted into the mouth for 6 h. *(b)* Higher magnification of the preparation in part *a*. Note the diverse morphology of the organisms present and the slime layer (arrows) holding the organisms together.

caries. In the absence of *S. mutans*, other acidogenic and acid-tolerant species can initiate caries.

Species of the bacterial genera *Streptococcus, Granulicatella,* and *Actinomyces* are typically elevated in children who develop severe early dental caries, whereas caries-free children have a higher relative abundance of *Aestuariimicrobium*. In older children, caries is associated with decreasing bacterial diversity and increasing prevalence of *Porphyromonas* and *Prevotella* species. Similarly, the dental plaque of healthy subjects is more complex than those with caries. Chronic periodontitis (inflammation and loss of tissue and bone that supports the teeth) is also associated with decreased microbial diversity, although no single bacterial phylotype seems to be directly associated with disease.

Collectively, these observations suggest that no single pathogen leads to the formation of dental caries or periodontal disease, but rather that community-wide changes in microbial composition trigger the diseases. Periodontal disease is particularly serious because it is thought to contribute to several debilitating systemic conditions, including cardiovascular disease, diabetes, pneumonia, and arthritis. Although the etiology of these systemic pathologies is unclear, they appear to be associated at least in part with dysbiosis of the oral cavity similar to that observed in dysbiosis of the gut microbiota in inflammatory bowel disease (Section 24.8).

Acne Vulgaris

The interrelationship of species of the bacterial genus *Propionibacterium* with the innate and adaptive arms of immunity (Chapters 26 and 27, respectively) has been examined extensively because of the association of this bacterium with *acne vulgaris*, usually just called *acne*, a cutaneous inflammatory disease of the skin that affects more than 85% of adolescents worldwide. However, the suspected causative agent of acne, *P. acnes*, is also a member—and a dominant member—of the normal cutaneous microbiota (Figures 24.2, and 24.13–24.15) and thus is also present in individuals without inflammatory disease.

Although *Propionibacterium* species have the capacity to elicit an inflammatory response, this alone is insufficient to demonstrate a causal association with disease. A better understanding of the cause(s) of acne will likely emerge from a better understanding of variation among strains of *P. acnes*. Notably, recent experiments using metagenomic and multilocus sequence typing (Section 13.9) suggest that specific strains of *P. acnes* may trigger the acne condition, whereas others are associated with skin health. Because of the scarcity of nutrients available for the skin microbiota, even minor differences in nutritional requirements between strains may favor the development of one or another *P. acnes* strain. If true, this might lead to therapies for acne using probiotics or prebiotics (see Section 24.11) that foster the growth of harmless strains. For example, a lotion could be applied to the skin that contains a nonpathogenic strain or a nutrient selective for nonpathogenic strains as a means of protecting the skin from colonization by pathogenic strains.

Vaginal Conditions

Dysbiosis of the normal vaginal microbiota (Section 24.4 and Figure 24.12) is associated with either an inflammatory infection (*vaginitis*) or a less severe clinical form (*vaginosis*) that may be asymptomatic or associated with malodor and discharges. Common types of vaginitis result from the overgrowth of species of the yeast *Candida* (candidiasis) or from infection by the sexually transmitted protozoan *Trichomonas vaginalis*, both discussed in Chapter 33. Diagnosis of the less highly inflammatory vaginosis in a clinical research lab is commonly based on a microscopic scoring of a Gram-stained vaginal smear (*Nugent score*). This ten-point scoring system scores a vaginal swab as normal (0–3), intermediate (4–7), or vaginosis (7–10) based on a *decrease* in gram-positive rods (presumptive lactobacilli, see Figure 24.11*b*) and an *increase* in two morphotypes of gram-variable rods as compared to the normal range of relative abundances.

Culture-independent molecular methods (Chapter 19) are now increasingly being employed in place of the Nugent system to more precisely define vaginal communities associated with health, disease, and predisposition to disease, including neonatal infections, miscarriage, pre-term birth, and increased susceptibility to HIV and sexually transmitted infections. Longitudinal studies of individual women over multiple months have revealed that the vaginal microbial communities of some women are highly dynamic, changing in composition over relatively short time periods, whereas the communities of others are relatively stable (**Figure 24.22**).

Most vaginal microbial communities appear to be stable, displaying *resilience* (the capacity to return to the pre-disturbance community structure following disruption, such as menstruation) or *resistance* (the capacity to resist community disruption in response to disturbance). For example, a low pH–tolerant, *Lactobacillus crispatus*–dominated vaginal community is relatively stable

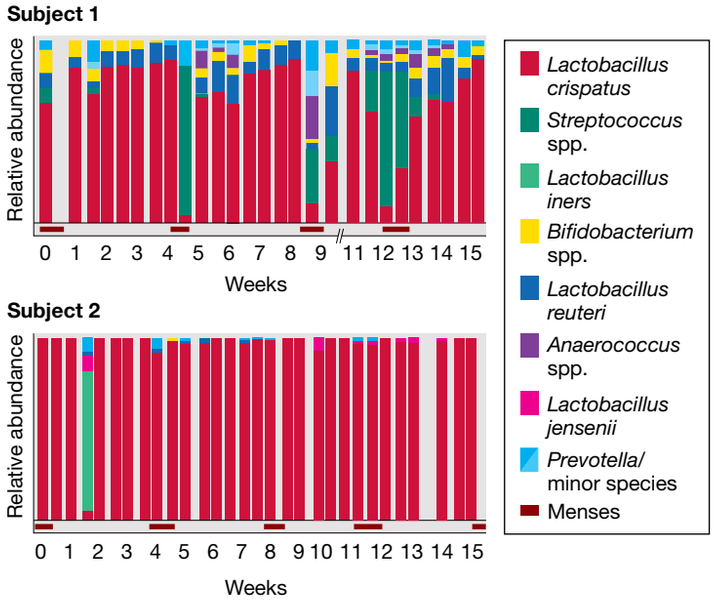


Figure 24.22 Resilience of the normal vaginal microbiota in two subjects. Both vaginal communities were dominated by *Lactobacillus crispatus* and demonstrated resilience to perturbation, most notably the disruption associated with menstruation (indicated by horizontal red bars) in subject 1. In addition to the major population types identified in these figures, populations detected in minor amounts at different times included the following: for subject 1, species of *Alloscardovia, Escherichia, Peptostreptococcus, Finegoldia, Prevotella*, and *Anaerococcus*; and for subject 2, *L. jensenii, Staphylococcus, L. gasseri, Corynebacterium, Clostridium*, and *L. vaginalis*.

(Figure 24.22), showing either resilience (subject 1) or resistance (subject 2). By contrast, other vaginal communities are more prone to converting to a vaginosis form. For example, the healthy *L. crispatus*–dominated community can convert to a healthy *L. iners*–dominated community before returning to *L. crispatus* (Figure 24.22*b*). However, although both the *L. crispatus* and *L. iners* communities are associated with vaginal health, an *L. iners*–dominated community is more likely to transition to vaginosis. Vaginal bacterial diversity also waxes and wanes over shorter time periods, such as a woman's menstrual cycle. Higher bacterial diversity is observed during days of menstruation than in intervening days, with diversity following a cycle inversely related to that of the estrogen hormone estradiol.

As for most ongoing human microbiome research, molecular microbial community analyses of the vagina have revealed possible connections between the microbial diversity of the vagina, vaginal health, and the susceptibility to disease. But clear and direct links have not yet emerged. We do not yet fully understand how the vaginal community is established and maintained or how vaginal dysbiosis develops and resolves. However, if some community types are associated with clinical symptoms or are associated with higher risk for vaginal disorders, then early intervention may be important to maintaining women's health and the health of a mother and baby in particular.

MINIQUIZ

- What observations indicate that dental carries are not due solely to *Streptococcus mutans*?
- Why might it be possible to have high abundance of *Propionibacterium acnes* without developing acne vulgaris?
- What are some clinical advantages of a community-structure-based evaluation of vaginal health?

IV • Modulation of the Human Microbiome

One important basic-science goal of human microbiome studies is to understand how the microbial composition and activity of human-associated microbes promotes health or predisposes the body to a variety of health disorders. An important practical goal is to then use this knowledge to improve the health and fitness of humans. Although at this point we have only a sketchy understanding of the relationships between microbes and humans, there is a strong indication that in at least some cases significant health benefits are associated with altering the human microbiome.

24.10 Antibiotics and the Human Microbiome

Antibiotics are naturally produced antimicrobial substances whose efficacy varies against different bacteria (Chapter 28). However, when an antibiotic is taken orally it kills or inhibits to at least some extent the normal microbiota as well as the targeted pathogen(s). Antibiotic treatment can lead to a significant loss of the gut normal microbiota (Section 24.2). When antibiotic therapy ends, the normal intestinal microbiota is usually, but not always, reestablished spontaneously in adults.

Use of antibiotics during the first few months of life is a particular problem for the developing normal microbiota. This disruption can affect normal development of the immune system and predispose the infant to later autoimmune disorders, such as IBD and allergies (Section 24.8). Recent research has also shown that early disruption of the gut microbiota influences host energy metabolism. For example, antibiotic exposure during the first 6 months of life is associated with increased weight gain in infants between 10 and 38 months of age compared to those not receiving antibiotics. With growing concerns about the frequency of childhood obesity leading to adult obesity along with the growing number of childhood autoimmune disorders, it is clearly important to avoid, if possible, disruption of the normal development of the human gut microbiota early in life.

Clostridium difficile Infections

Sometimes antibiotic-resistant opportunistic pathogens become established in young children or the elderly following treatment with antibiotics that disrupts the normal microbiota. A particularly problematic complication of antibiotic therapy is infection with toxigenic *Clostridium difficile* and the inability to resolve infections with repeated follow-on administrations of antibiotics (**Figure 24.23**). There is a strong association between antimicrobial therapy and the subsequent development of *C. difficile* infection, and the risk of infection is greater if *C. difficile* is resistant to the antimicrobial agents used in therapy.

Clostridium difficile was first described in 1935 as part of the normal intestinal microbiota of healthy neonates. Its role in diarrhea was first described in 1978, and the marked increase in hospital-acquired diarrhea since 2003 is attributed in part to the emergence of extremely virulent and toxigenic *C. difficile* strains. In those infected, symptoms vary from a mild diarrhea to severe abdominal pain and fever. The most severe complications are inflammatory lesions and bowel perforation; as a result of these, septic shock (Section 25.2) and death are possible.

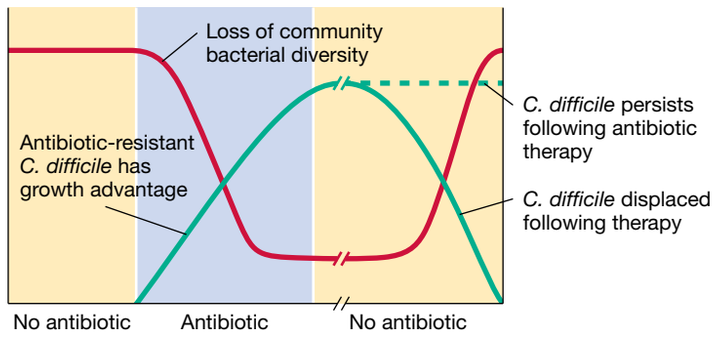


Figure 24.23 Antibiotic treatment increases the risk of *Clostridium difficile* infection. During antibiotic therapy the displacement of sensitive gut populations results in a significant loss of community diversity, allowing antibiotic-resistant populations such as toxigenic *C. difficile* to increase in abundance. When antibiotic therapy ends, *C. difficile* may be displaced (solid green line) by reestablishment of a normal gut microbiota, or alternatively, it may persist (dotted line), causing intestinal disease.

Although elderly hospitalized patients receiving antibiotics are still the main risk group, there is increasing incidence of *C. difficile* infection in younger populations having no previous contact either with a hospital environment or antibiotics. Until recently, there was no effective means to treat patients that were nonresponsive to antibiotic treatment. If medical management with intravenous fluids and antibiotics was not effective, then surgical removal of the colon was a last resort life-saving measure. However, the prognosis for recovery from a *C. difficile* infection today has been dramatically improved by a novel nondrug and nonsurgical therapy: the infusion of fecal material from the gut contents of a healthy donor into the gut of a *C. difficile*–infected patient—more commonly called a **fecal transplant**. In most cases such treatment has been shown to restore a healthy colon in patients suffering recurring *C. difficile* infections. We examine this procedure now.

Fecal Transplants

A changing paradigm in the treatment of inflammatory bowel disease and *C. difficile* infections is the use of a fecal transplant. The goal of a fecal transplant is to reintroduce normal microbiota into the gut of someone with an intestinal disease of bacterial origin and have the transplanted microbial community become established and exclude the disease-causing organisms. Although it has taken the medical community some time to adopt this therapy, the dramatic improvement in the rate of resolution from *C. difficile* infection by fecal transplant from a healthy donor has prompted widespread adoption of this procedure.

As we have seen, once established, *C. difficile* is a particularly difficult pathogen to eradicate (Figure 24.23). However, the percentage of otherwise nonresponsive patients cured of *C. difficile* infection without relapse is nearly 90% with fecal transplant therapy versus only about 25% with standard antibiotic treatment. It has also been shown that fecal transplants can alleviate some forms of *metabolic syndrome*, a condition characterized by elevated blood pressure and glucose levels, excess belly fat, and abnormal cholesterol levels, that is a frequent precursor of type 2 (insulin-nonresponsive) diabetes. Metabolic syndrome patients receiving a fecal transplant from a lean donor had significantly increased insulin sensitivity, and also showed increased fecal levels of butyrate, greater overall gut microbial diversity, and increased abundance of gut bacteria related to the butyrate-producing bacterium *Roseburia intestinalis*, thought to be a beneficial microbe.

Fecal transplants require that the donor be healthy and free of infection. And, because feces are considered a body fluid, screening and testing of the donor is essential. Potential donors must complete a screening questionnaire similar to that required for donating blood, and any prospective donors who have risk factors for HIV (AIDS) or hepatitis infections or any history of gastrointestinal disease, autoimmune disease, or cancer are screened out. Prospective fecal donors must also undergo blood tests for a suite of pathogens and their feces are tested for fecal bacterial pathogens and parasites. If everything checks out, samples of their feces are frozen in small vials and then thawed and used when needed. Fecal transplant recipients typically receive the transplant via a saline solution during colonoscopy to ensure that the transplanted feces actually reach the upper colon.

Although a fecal transplant may seem somewhat crude and an essentially uncontrolled or nonprecise therapy, studies are accumulating that show that the appropriate manipulation of the gut microbiota can have significant health benefits and that gut microbiota transplants can be maintained for long periods if not indefinitely. More generally, the success of fecal transplants has shown that manipulation of the human microbiome for improved health is feasible and that with greater understanding of these complex communities, more targeted intervention may be possible, as we consider in the next section.

MINIQUIZ

- Why do healthy adults usually not contract *Clostridium difficile* infections?
- Why is it unsafe for a fecal transplant recipient to receive feces from an unscreened donor?

24.11 Probiotics and Prebiotics

We close this chapter with a brief discussion of how ingestion of living microbial cultures (*probiotics*) or particular nutrients (*prebiotics*), generally derived from plants, could be used to treat disease and promote health by modulating the gut microbial community.

Probiotics

The United Nations Food and Agriculture Organization and the World Health Organization define a **probiotic** as a "live microorganism which, when administered in adequate amounts, confer a health benefit on the host." Species of *Bifidobacterium* and *Lactobacillus* bacteria are the most commonly discussed probiotics. They are usually delivered to the gastrointestinal system by ingestion of a fermented milk product, such as yogurt (**Figure 24.24**), with the hope of suppressing gut or urogenital disturbances.

Most physicians and scientists agree that ingested probiotic foodstuffs probably have limited therapeutic value. Nevertheless, human microbiome research has spurred a renaissance in probiotics research and investment and specifically for the development of effective and more targeted probiotics. The remarkable success of fecal transplants (Section 24.10) points to the therapeutic

Deborah O. Jung and John Martinko

Figure 24.24 Examples of some probiotic foods and supplements widely available worldwide. Some probiotic products also contain prebiotics (specific polysaccharides obtained from various plants).

potential of rational modification of the gut microbiome. However, although fecal transplants have proven very effective, there is minimal understanding of the mechanisms behind their successes and also why they do not work in a limited number of cases.

Molecular analyses of *C. difficile* infections have highlighted the importance of a mechanistic understanding for the development of effective probiotic therapies. Resistance to *C. difficile* infection following antibiotic therapy (Figure 24.23) in both mice and humans is correlated with the presence of a second *Clostridium* species, *C. scindens*. Administration of this species to mice infected with *C. difficile* has been shown to be an effective probiotic therapy; suppression has been linked to the ability of *C. scindens* to convert cholic acid (found in bile) into deoxycholic acid, a growth inhibitor of *C. difficile*. In similar studies, recovery from diarrhea caused by *Vibrio cholerae* (cholera, Section 32.3) has been associated with an increased abundance of the bacterium *Ruminococcus obeum*. This bacterium produces a quorum-sensing autoinducer (AI-2)(Section 6.8) that suppresses expression of *V. cholerae* colonization factors and effectively prevents the cholera bacterium from establishing an infection. In both of these cases this level of understanding is expected to foster the development of more ecologically based, specifically targeted, and scientifically proven probiotic therapies than can be hoped for from general and nutritionally based approaches (Figure 24.24).

Prebiotics

Probiotics should not be confused with *prebiotics*. The prebiotic approach to developing a healthy normal microbiota promotes the ingestion of certain plant nutrients as microbial growth stimulants with the idea that they will nurture particular bacterial species in the gut known to be associated with a healthy colon. Prebiotics are typically carbohydrates that are indigestible by the body but are excellent carbon and energy sources for certain fermentative gut bacteria. Fructooligosaccharides, polymers of fructose present in many vegetables, are thought to stimulate desirable gut microbes, and the complex polysaccharide *inulin* is widely promoted commercially as a major prebiotic.

Some probiotic formulations (such as certain yogurts) contain prebiotics as well. As has occurred with probiotics, dramatic health benefits have been claimed in some prebiotic studies. But at present, clear benefits of prebiotics for restoring or maintaining a healthy colonic microbiota await carefully controlled and quantitative scientific studies.

MINIQUIZ

- What is a probiotic? How does it differ from a prebiotic?
- Why do you think fecal transplants have stimulated renewed research into the development of probiotic and prebiotic therapies?

Chapter Review

I • Structure and Function of the Healthy Adult Human Microbiome

24.1 The human body is colonized by a diverse assemblage of microorganisms in numbers that equal or exceed the total number of cells in the human body. Different body sites provide distinctive habitats that influence the types of microorganisms inhabiting each site. These microbiota together constitute the human microbiome, now recognized to be intimately associated with human health and disease.

Q Why is understanding the nature of the human microbiome such a complex process?

24.2 The human gastrointestinal tract is composed of many segments whose nutritional and pH characteristics differ dramatically and which support quite distinct bacterial populations.

Q How do microbial diversity and abundance vary along the length of the gastrointestinal tract?

24.3 Distinctive groups of microorganisms colonize the mucous membranes and hard surfaces of the mouth, and are constantly shed in saliva. The different microhabitats of the mouth (hard and soft surfaces, gingival crevice) sustain a high diversity of both aerobic and anaerobic populations.

Q Why does saliva not serve as a good microbial growth medium?

24.4 With the exception of the vagina and distal urethra, the male and female urogenital tracts are sterile. The healthy vaginal microbial community is dominated by *Lactobacillus* species, which suppress the growth of other populations by lowering the vaginal pH to about 5 by fermenting glycogen to lactic acid.

Q What factor most affects the vaginal microbial community at the onset of puberty?

24.5 The skin is a complex human organ covering about 2 square meters of the body and hosting about 10^{10} microorganisms in the healthy adult. Microbial community composition varies among the diversity of skin sites, generally categorized as moist, dry, or oily. The most common skin microorganisms are bacteria of the phyla *Actinobacteria* and *Firmicutes*.

Q What characteristic of *Propionibacterium* species accounts for their colonization of the sebaceous gland system?

II • From Birth to Death: Development of the Human Microbiome

24.6 Our current understanding of relationships between the human microbiome and health status is derived from complementary studies of select human study groups and experimental manipulation of animal models, in particular the mouse. Human studies are limited by incomplete control of lifestyle and genetic factors influencing the microbiome. Animal studies are limited by differences from humans in microbiology, anatomy, and physiology.

Q What are the major anatomical differences between mouse and human gastrointestinal systems, and how might those differences influence microbial composition?

24.7 The newborn gut microbial community evolves into a more complex adultlike community over the first 2–3 years of life. Initial colonization is influenced by vaginal or C-section delivery and by feeding of breast milk or formula. The unique oligosaccharides in human breast milk suppress pathogen colonization and select for beneficial microorganisms such as *Bifidobacterium* species. Changes in the gut microbial community with age have been associated with frailty in the elderly.

Q When a child is transitioned from breast milk to solid food, what are the associated changes in the gut microbial community and what physiological differences do those changes reflect?

III • Disorders Attributed to the Human Microbiome

24.8 The two major disorders associated with changes in the human gut microbial community are inflammatory bowel disease and obesity. Inflammatory bowel disease is associated with dysbiosis, a breakdown in the normal interactions between the human host and its intestinal microbiota reflected by reduced diversity of microbial genes in the diseased state. Obesity is directly associated with the microbial community composition of the gut, which influences host energy recovery, but obesity is likely not determined by a single contributing microbial or host factor.

Q In what way are methanogenic *Archaea* implicated in obesity?

24.9 Dental caries and periodontitis are diseases of the mouth caused by the combined activities of multiple microbial species, not a single pathogen. Strain variation among *Propionibacterium* species is associated with ability to cause acne, a common cutaneous inflammatory disease of adolescents. The vaginal community is dominated by *Lactobacillus* species that limit colonization by other organisms through lowering pH by production of lactic acid. Although generally very resilient to perturbation, the vaginal community transitions to a more diverse community during menstruation and during incidents of vaginitis or vaginosis.

Q Why might a therapy based on colonization of the skin by selected *Propionibacterium* strains be effective in preventing the development of acne?

IV • Modulation of the Human Microbiome

24.10 Antibiotic therapy may predispose an individual to infection by *Clostridium difficile*, a toxigenic bacterium that causes severe intestinal disease, by reducing competition from the normal microbiota of the gut. Infections that cannot be resolved by standard or repeated antibiotic therapy have been shown to respond to fecal transplants, which introduce fecal material from a healthy donor into the gut of the infected individual.

Q Why has the success of fecal transplants in treating *C. difficile* infection encouraged the development of ecologically based therapies for other disorders associated with the human microbiome?

24.11 Probiotics are live microorganisms which, when administered in adequate amounts, confer a health benefit on the host. Although the probiotic health benefits of *Lactobacillus* species are commonly promoted, there is little clinical evidence of their efficacy. Nonetheless, new understanding of the mechanisms by which organisms such as *Clostridium scindens* suppress pathogen colonization show that targeted probiotics can likely be developed to prevent or treat diseases, such as that caused by *C. difficile* infection. Prebiotics are nutritional supplements thought to promote the growth of beneficial gut microbes.

Q What is the mechanism by which *C. scindens* suppresses colonization by *C. difficile*?

Application Question

1. You are told that you must be placed on a high dose of a broad-spectrum antibiotic to treat a serious infection. You are concerned that this therapy will seriously disrupt your intestinal microbiota, possibly leading to intestinal disease such as caused by *Clostridium difficile*. In advance of the treatment, what might you do to ensure that your normal intestinal microbiota is restored after extended antibiotic therapy? Discuss two ways this restoration could be accomplished, one that would return your exact microbiota and one that would return some representative species.

Chapter Glossary

Dysbiosis an alteration or imbalance of an individual's microbiome relative to the normal, healthy state, primarily observed in the microbiota of the digestive tract or the skin

Fecal transplant the transfer of microbiota from the colon of one individual into the colon of another

Host an organism that can harbor pathogenic or beneficial (micro) organisms

Human microbiome the total microbial content in and on the human body

Lower respiratory tract the trachea, bronchi, and lungs

Microbiome a functional collection of different microbes in an environmental system such as the human body

Microbiota the types of organisms present in an environmental habitat, such as the human skin or the human gastrointestinal tract

Mucin a secretion from specialized epithelial cells containing water-soluble glycoproteins and proteins, forming the mucus that retains moisture and impedes microbial invasion on mucosal surfaces

Normal microbiota microorganisms that are usually found associated with healthy body tissue

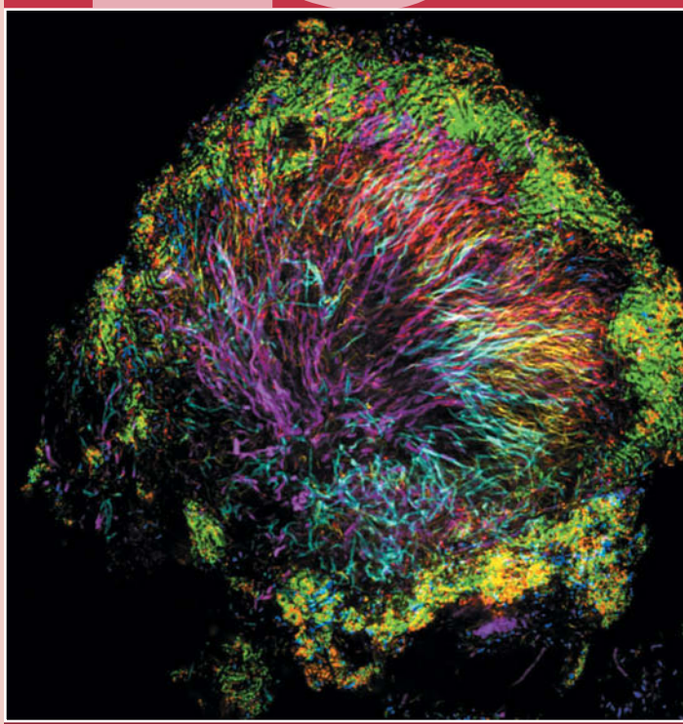
Probiotic a live microorganism that, when administered in adequate amounts, confers a health benefit on the host

Upper respiratory tract the nasopharynx, oral cavity, and throat

Volatile fatty acids (VFAs) the major fatty acids (acetate, propionate, and butyrate) produced during fermentation in the large intestine of monogastric animals and the rumen or cecum of herbivores

25

Microbial Infection and Pathogenesis



microbiologynow

The Microbial Community That Thrives on Your Teeth

Few people have such superb oral hygiene that they lack dental plaque, the microbial biofilm that forms on and between teeth and along or below the gumline. If not removed regularly, dental plaque invariably leads to dental caries (cavities), the condition in which portions of tooth enamel and dentin break down from the onslaught of bacterial activities. Dental plaque and dental caries develop from the natural tendency of oral bacteria such as *Streptococcus mutans* and its close relative *S. sobrinus* to attach firmly to the teeth and gums and ferment sucrose (table sugar) to lactic acid, which attacks the teeth and slowly rots them away.

Until recently, dental plaque was thought to consist largely of the aforementioned streptococci. Both species could easily be isolated from dental plaque and both light and electron microscopy typically showed large numbers of cocci in chains, a hallmark of the genus *Streptococcus*. But a recent molecular ecology study of the microbial diversity of dental plaque revealed that this material is composed of more than just streptococci and develops in a precisely structured way.

The photo here is a light micrograph of a section through human dental plaque stained by fluorescence in situ hybridization (FISH). Different oligonucleotides, each specific for a different major phylum of *Bacteria* and containing a distinct fluorescent dye, were allowed to hybridize to the ribosomal RNA in cells in the plaque and then observed by fluorescence microscopy. Surprisingly, instead of seeing primarily streptococci, the researchers saw a diverse and highly organized microbial community. The micrograph shows streptococci (stained green) located primarily at the periphery of the plaque beyond several other bacteria that combine to form a scaffold emerging from the tooth surface. These include *Corynebacterium* (purple), *Capnocytophaga* (red), *Fusobacterium* (yellow), *Leptotrichia* (blue-green), and *Haemophilus* (orange), among others. A major conclusion that emerged from this study was that the scaffolding microbes likely function to position the streptococci out into the oral cavity where sucrose should be more available.

New views of old problems often reveal surprising results. In the case of dental plaque, FISH technology has revealed a whole new microbial world in a habitat previously thought to be dominated by only two species of well-characterized bacteria.

Source: Mark Welch, J.L., et al. 2016. Biogeography of a human oral microbiome at the micron scale. *Proc. Natl. Acad. Sci. (USA) 113*: doi: 10.1073/pnas.1522149113.

I • Human–Microbial Interactions

Humans are exposed to microorganisms of all sorts in their environment. Whether one is walking outdoors, sitting indoors, or participating in any type of physical activity, environmental microbes and humans interact. The human body is also a natural home to enormous numbers of microorganisms, as we saw in Chapter 24. Most of these are harmless, and only a very small percentage cause disease. However, those that do, along with pathogens that are not part of the normal human microbiota, possess specific traits that underlie their pathogenic lifestyles. A major focus of this chapter will be a consideration of these specific traits and how they trigger the diseased state.

25.1 Microbial Adherence

In the world of infectious diseases, the term **infection** is used to imply the growth of microorganisms on or in the host, whereas the term **disease** is reserved for actual tissue damage or injury that impairs host function. If a **pathogen** gains access to the specific tissues it infects, disease will occur only if it first adheres to those tissues, multiplies to yield many cells or viral particles, and then proceeds to damage tissues (or the entire organism) by the release of toxic or invasive substances (Figure 25.1). Adherence is the first step, and although adherence is *required* to initiate disease, it is not *sufficient* to initiate disease because the host has many innate defenses that can thwart infection; we consider these in Chapter 26.

Adherence Molecules

Pathogens typically adhere to epithelial cells through specific interactions between molecules on the pathogen and molecules on the host tissues. In addition, pathogens may adhere to each other, forming biofilms (Sections 5.1 and 20.4), with the biofilm itself adhering to specific tissues. In medical microbiology, **adherence** is the enhanced ability of a microorganism to attach to a cell or a surface. Pathogens gain access to host tissues by way of a *portal of entry* of one sort or another. These include mucous membranes, the skin surface, or under mucous membranes or the skin during penetration of these sites from puncture wounds, insect bites, cuts, or other abrasions. The portal of entry may be critical for the establishment of an infection because a pathogen that gains access to incompatible tissues is typically ineffective. For example, if cells of the bacterium *Streptococcus pneumoniae* are swallowed, they will be killed by the strong acidity of the stomach, whereas if the same cells reach the respiratory tract, they could trigger a fatal case of pneumonia.

Receptor molecules coating the surfaces of both the pathogen and cells of its host are often critical for adhering the pathogen to host tissues. Specific receptors can be important for the binding of any type of pathogenic microbe including bacteria, viruses, and parasites (Figure 25.2). Pathogen receptors have evolved to bind specifically to complementary molecules on the host cell cytoplasmic membrane, and the complementary nature of the pathogen and host cell receptors alerts the pathogen that it has arrived on a suitable infection site. Receptors on the pathogen surface are called **adhesins** and are composed of glycoprotein or lipoprotein covalently bound to the outer layer of the cell (Figure 25.2*a*). Host cell receptors are typically glycoproteins or complex membrane lipids such as gangliosides or globosides (sphingolipids containing sugars and other molecules).

Adherence Structures: Capsules, Fimbriae, Pili, and Flagella

Some adhesins form part of an outer cell surface structure that may or may not be covalently linked to components of the cell wall. For example, some notable pathogenic bacteria form a **capsule**. In *Bacillus anthracis* (the bacterium that causes anthrax), the capsule is composed of polypeptide containing only the amino acid D-glutamate. The capsule of *B. anthracis* can be seen in cells by light microscopy, and the encapsulated cells form smooth slimy colonies when grown on agar plates (Figure 25.3). The electron microscope can also clearly reveal bacterial capsules (Figure 25.3*c*). The capsule surface contains specific receptors that facilitate adherence to host tissues, but the inherently sticky nature of the capsule itself also assists in the overall attachment process. Although capsules are important for adherence of some pathogens to host tissues, many important pathogens, such as *Vibrio cholerae*, the causative agent of the disease cholera (Figure 25.2*a*), lack them.

Besides adherence, capsules are important for protecting pathogenic bacteria from host defenses. For example, the only known virulence factor for *Streptococcus pneumoniae* (bacterial pneumonia) is its polysaccharide capsule (Figure 25.4). Encapsulated strains of *S. pneumoniae* grow voraciously in the lungs where they initiate

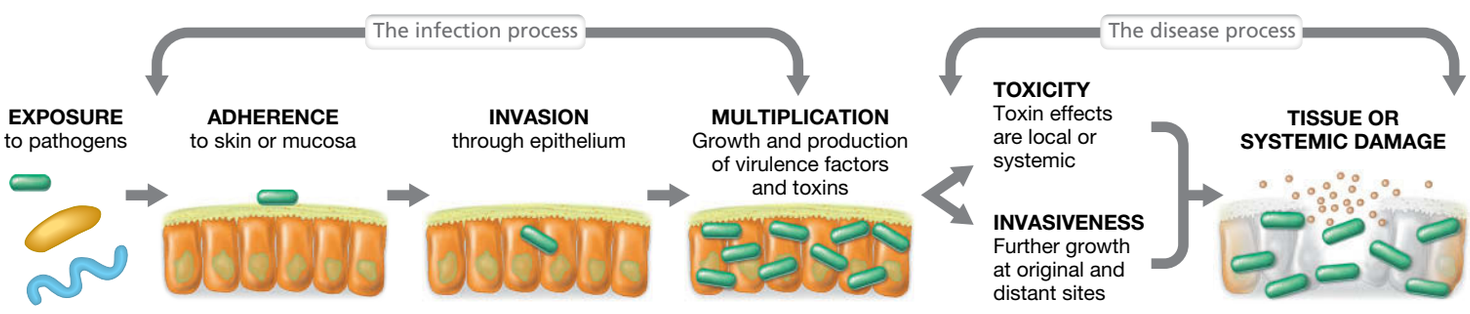


Figure 25.1 Microbial pathogenesis. Following exposure to a pathogenic microbe, a series of events leads to infection and a further series of events results in disease.

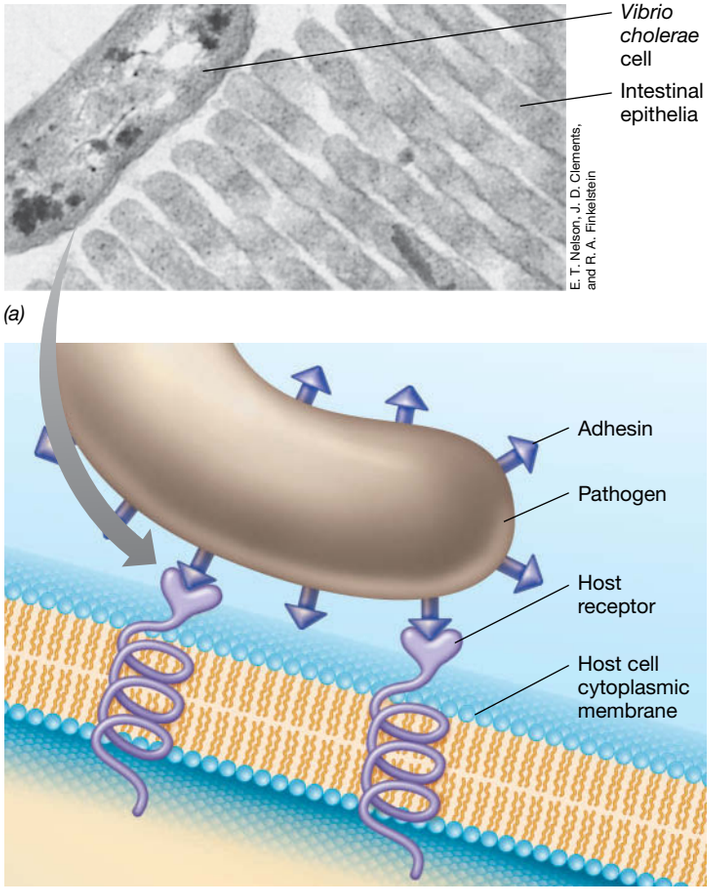


Figure 25.2 Adherence of pathogens to tissues via receptor molecules on the cell surface. *(a)* Transmission electron micrograph of a thin section of the gram-negative bacterium *Vibrio cholerae* adhering to the brush border of microvilli in the intestine. *(b)* A bacterial pathogen attaches specifically to host tissues by way of complementary receptors on the bacterial and host cell surfaces.

host responses that interfere with lung function, cause extensive host damage, and can cause death (Section 30.2). By contrast, nonencapsulated strains of *S. pneumoniae* are quickly and efficiently ingested and destroyed by phagocytes, white blood cells that ingest and kill bacteria by a process called *phagocytosis* (Sections 26.5–26.7).

Many pathogens selectively adhere to particular types of cells through cell surface structures other than adhesins, capsules, or slime layers. For example, *Neisseria gonorrhoeae*, the pathogen that causes the sexually transmitted disease gonorrhea (Section 30.13), adheres specifically to mucosal epithelial cells in the genitourinary tract, eye, rectum, and throat; by contrast, other tissues are not infected. *N. gonorrhoeae* has a cell surface protein called Opa (*o*pacity *a*ssociated *p*rotein) that binds specifically to a host protein found only on the surface of epithelial cells of these body regions, allowing adherence of the pathogen to host cells. Likewise, influenza virus targets upper respiratory tract mucosal cells and attaches specifically to these and later to lung epithelial cells by way of the protein hemagglutinin present on the virus surface (Sections 10.9 and 30.8).

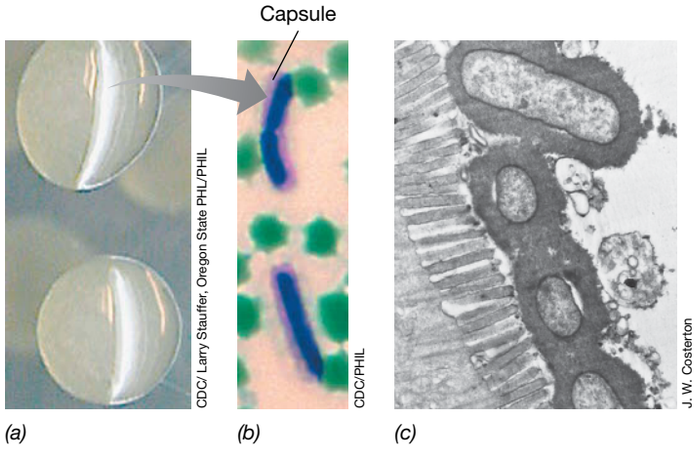


Figure 25.3 The bacterial capsule as a facilitator of pathogen attachment. *(a) Bacillus anthracis* growing on an agar plate. The mucoid colonies of encapsulated cells are about 0.5 cm in diameter. *(b)* Light micrograph of cells of *B. anthracis* growing in horse blood and stained to show the capsule (pink). Cells are about 1 μm in diameter. *(c)* Cells of enteropathogenic *Escherichia coli* attached to the brush border of intestinal microvilli by way of a distinct capsule. The *E. coli* cells are about 0.5 μm in diameter.

Fimbriae and pili are bacterial cell surface protein structures (Section 2.7) that function in attachment (**Figure 25.5**). For instance, along with Opa, the pili of *Neisseria gonorrhoeae* play a key role in attachment to urogenital epithelia, and fimbriated strains of *Escherichia coli* are more frequent causes of urinary tract infections than strains lacking fimbriae. Among the best-characterized fimbriae are the *type I fimbriae* of enteric bacteria (*Escherichia, Klebsiella, Salmonella*, and *Shigella*), which are uniformly distributed on the surface of cells (Figure 25.5). Pili are typically longer and fewer in number than fimbriae, and in addition to attachment, some pili function in the bacterial genetic transfer process of conjugation (Section 11.8). Both pili and fimbriae function by specifically binding to host cell surface glycoproteins, thereby

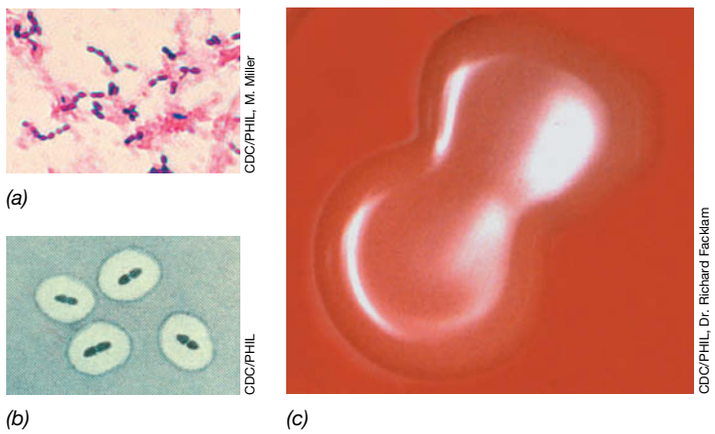


Figure 25.4 Capsules and colonies in *Streptococcus pneumoniae*. *(a)* Gram stain of *S. pneumoniae* cells; capsules are not visible. *(b) S. pneumoniae* treated with anticapsular antibodies (Quellung reaction) that make the capsule visible. *(c)* Colonies of encapsulated *S. pneumoniae* cells grown on blood agar show a mucoid morphology with a sunken center. The colonies are about 2–3 mm in diameter and a single cell of *S. pneumoniae* is about 0.75 μm in diameter.

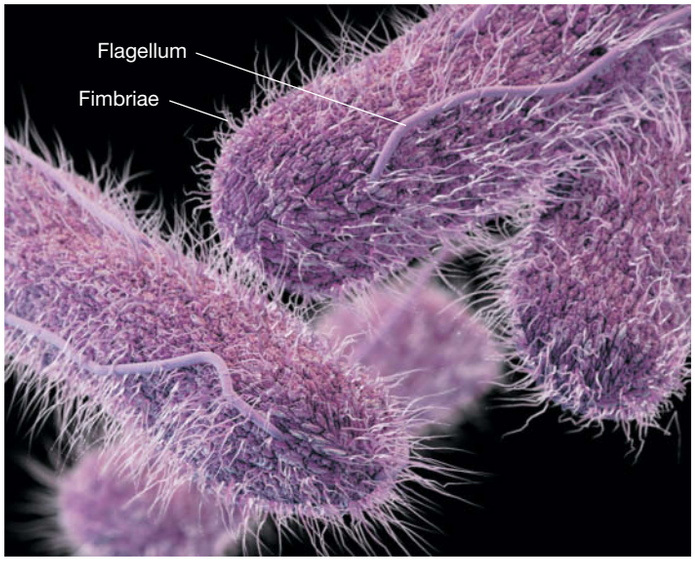


Figure 25.5 Fimbriae. Computer-generated image of a scanning electron micrograph of cells of *Salmonella enterica* (*typhi*) showing the numerous thin fimbriae and the much thicker peritrichously arranged flagella. A single cell is about 1 μm in diameter.

initiating adherence. Flagella may also facilitate adherence of bacterial cells to host cells (Figure 25.5) although their role is thought to be less important than that of fimbriae and pili.

MINIQUIZ

- What event is required but not sufficient to cause an infectious disease?
- Describe the molecules or structures that facilitate pathogen adherence to host tissues.

25.2 Colonization and Invasion

If a single pathogenic virus or cell attaches to its specific host tissue, it alone is insufficient to cause disease; the pathogen must establish residence there and multiply. **Colonization**, the growth of a microorganism after it has gained access to host tissues, begins at birth as a newborn is naturally exposed to a suite of harmless (and in many cases necessary) bacteria and viruses that will be the infant's initial normal microbiota (Chapter 24).

The human body is rich in organic nutrients and provides conditions of controlled pH, osmotic pressure, and temperature that are favorable for the growth of microorganisms. However, each body region such as the skin, respiratory, gastrointestinal, and genitourinary tracts differs chemically and physically from others, and thus provides a selective environment for the growth of certain microbes and not others. The result is that pathogens show rather rigid tissue specificities (Table 26.1), and this reality is often helpful in the diagnosis of microbial infections.

Colonization typically begins at sites in the **mucous membranes** (Figure 25.6). Mucous membranes consist of *epithelial cells*, tightly packed cells that interface with the external environment.

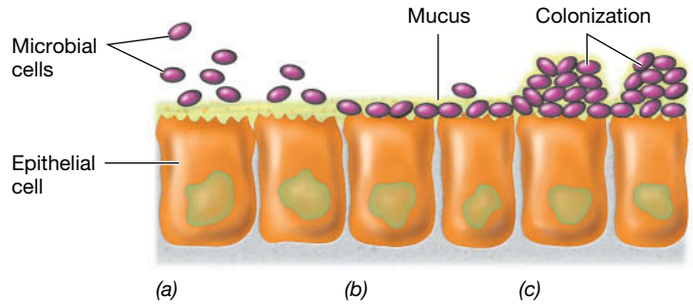


Figure 25.6 Bacterial interactions with mucous membranes. *(a)* Loose association. *(b)* Adhesion. *(c)* Colonization.

They are found throughout the body, lining the urogenital, respiratory, and gastrointestinal tracts. The epithelial cells in mucous membranes secrete **mucus**, a thick liquid secretion that contains water-soluble proteins and glycoproteins. Mucus retains moisture and naturally inhibits microbial attachment because most microbes are swept away by physical processes like swallowing or sneezing. Nevertheless, some microbes—both pathogens and nonpathogens—adhere to the epithelial surface and colonize. If these attached microbes are pathogens, it sets the stage for infection, invasion, and disease (Figure 25.1).

Growth of the Microbial Community: An Example from Human Dental Caries

Infection requires growth of the pathogen after it has attached to and colonized a surface (Figures 25.1 and 25.6), and the actual disease process may not be the result of a single type of microbe but of a community of interacting microorganisms. An excellent example of this is found in the oral microbial disease **dental caries** (tooth decay), where attachment and infection have been well studied as models of these key events in the disease process.

Even on a freshly cleaned tooth surface, acidic glycoproteins from the saliva form a thin organic film several micrometers thick; this film provides an attachment site for bacterial cells, and oral streptococci quickly colonize it. These include in particular the two *Streptococcus* species most often implicated in tooth decay, *S. sobrinus* and *S. mutans*. Both of these organisms produce a capsule (Section 25.1). The *S. sobrinus* capsule contains adhesins (Figure 25.2*a*) specific for host salivary glycoproteins (Figure 25.7*a, b*), whereas *S. mutans* resides in crevices and small fissures where it relies on dextran—a strongly adhesive polysaccharide—that it produces to secure cells to the tooth and gum surface (Figure 25.7*c, d*). Both *S. sobrinus* and *S. mutans* are lactic acid bacteria (Section 16.6) that ferment glucose to lactic acid, the agent that destroys tooth enamel. However, the trigger for decay activities is *sucrose* (table sugar), since it is sucrose that allows these species to produce the thick capsules necessary for attachment and colonization.

Extensive bacterial growth of these oral streptococci results in a thick biofilm called **dental plaque** (Figure 25.7). Using phylogenetic probes, it has been possible to more readily explore the microbial diversity of dental plaque, and it is clear that the two *Streptococcus* species are not the entire story. Many other gram-positive and gram-negative *Bacteria* are present in plaque, including species of *Corynebacterium, Porphyromonas, Leptotrichia,*

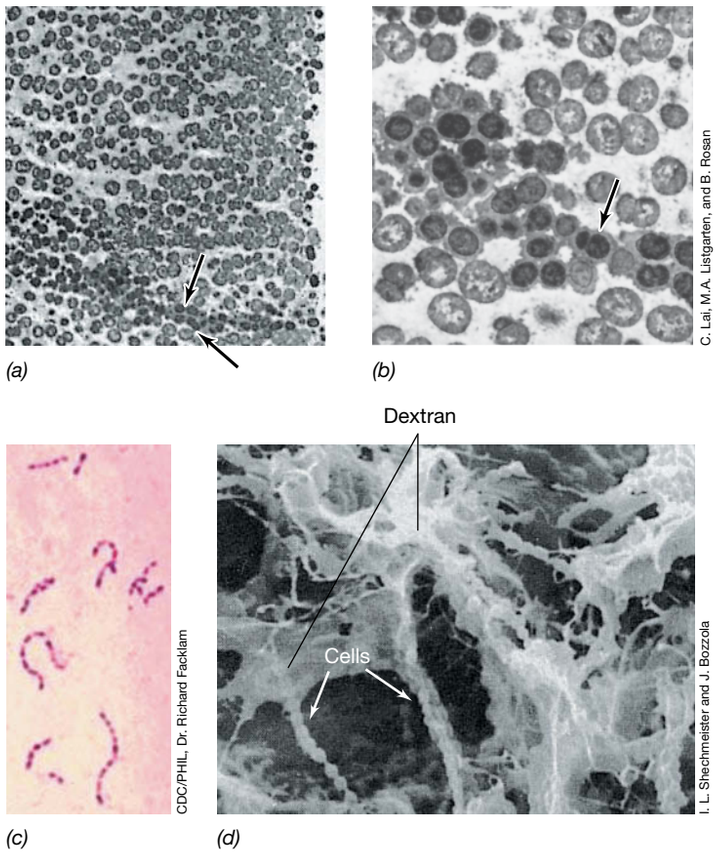


Figure 25.7 Cariogenic *Streptococcus* spp. and dental plaque. *(a)* The low-magnification micrograph shows predominantly streptococcal cell morphology embedded in dental plaque. The species *Streptococcus sobrinus* (arrows) appears darker from a specific staining technique. *(b)* Higher-magnification micrograph showing a region in the plaque with *S. sobrinus* cells (dark, arrow). Note the extensive capsule surrounding the *S. sobrinus* cells. *(c)* Light micrograph of a *Streptococcus mutans* culture showing the characteristic cell chains of streptococci. *(d)* Scanning electron micrograph of the sticky dextran material that holds cells together in filaments. Individual cells of both *S. sobrinus* and *S. mutans* are about 1 μm in diameter.

Neisseria, the filamentous anaerobe *Fusobacterium*, and many others (**Figure 25.8**). Moreover, the different species likely play specific structural and functional roles in mature dental plaque. This can be seen in FISH-stained (Section 19.5) sections of plaque, where filamentous streamers of cells of *Corynebacterium* attached to a thin biofilm on the tooth surface anchor cells of *Streptococcus* and other bacteria a short distance away from the tooth surface (Figure 25.8). Such an arrangement probably allows *Streptococcus* cells to extend out from the tooth surface into regions of the oral cavity where saliva, sugars, and other nutrients are more abundant.

Dental plaque is thus a complex mixed-culture biofilm composed of several different genera of *Bacteria* and their accumulated products. A few *Archaea* are also present in dental plaque, primarily methanogenic species such as *Methanobrevibacter oralis*. As dental plaque accumulates, the microbiota produce locally high concentrations of lactic acid that decalcifies tooth enamel, resulting in dental caries. Tooth enamel is strongly calcified tissue, and the ability of microbes to invade this tissue plays a major role in the extent of dental caries and related more serious oral pathologies, including periodontal conditions (disease in the tooth-supporting gum and bone tissues).

Invasion and Systemic Infection

In the case of dental caries, the bacterial infection primarily resides on the tooth and gum surfaces. By contrast, in most infectious diseases, the pathogen must invade past the tissue surface in order to promote disease. **Invasion** is the ability of a pathogen to enter into host cells or tissues, spread, and cause disease. Some pathogens remain localized after initial entry, multiplying and invading at a single focus of infection such as the boil that may arise from *Staphylococcus* skin infections (Section 30.9). However, sometimes the pathogens enter the bloodstream, from where they can travel to distant parts of the body. Depending on the pathogen and the overall health of an individual and that individual's immune system, the presence of bacteria in the blood can have mild or highly severe consequences.

The mere presence of bacteria in the blood is called **bacteremia**; this condition is typically self-limiting as the bacterial cells do not grow in the bloodstream and thus the immune system quickly removes them. The symptoms of bacteremia may be mild or none. By contrast, in **septicemia** bacteria multiply in the bloodstream and the organism spreads systemically from an initial focus and

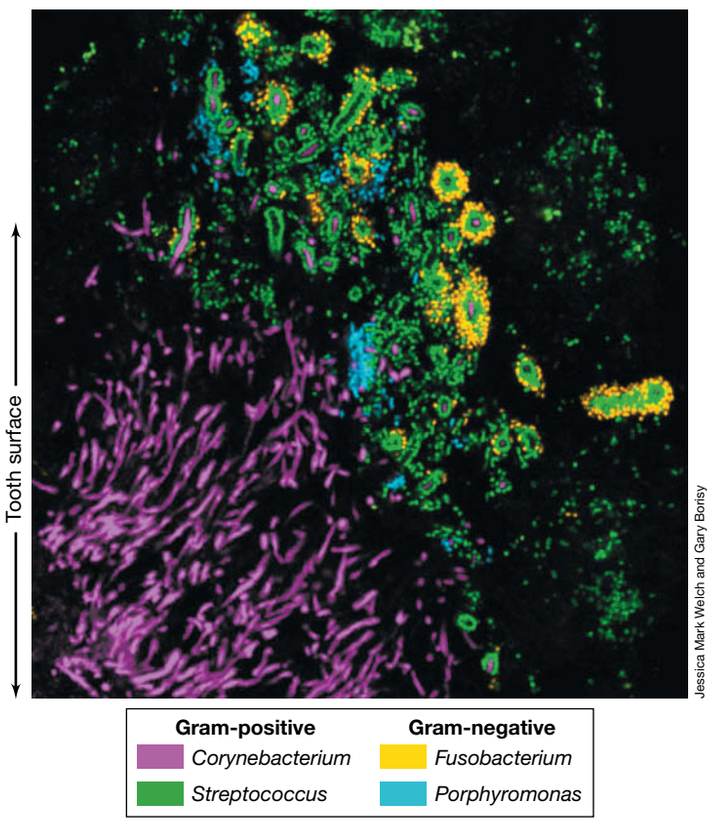


Figure 25.8 Bacterial diversity of dental plaque. Confocal micrograph of a FISH-stained (Section 19.5) section through human dental plaque using a suite of phylogenetic probes containing different fluorescent tags. Color matching to specific groups is shown below the photo. The tooth surface would be on the left with the filamentous *Corynebacterium* species (red) containing attached streptococci flaring out from an attachment site on the tooth surface.

produces toxins or other poisonous substances. Septicemia usually begins as an infection in a specific organ such as the intestine, kidney, or lung, and then spreads rapidly throughout the body from there. Septicemia is typically associated with major symptoms and may lead to massive inflammation, culminating in septic shock (sepsis) and death. *Viremia* is the term used to describe viruses present in the bloodstream, and measles, a highly infectious disease in those not vaccinated (Section 30.6), is a good example of a systemic viremia.

A pathogen that causes disease in a given host can trigger mild or severe outcomes depending on its inherent capacity to elicit disease. We consider the principles that govern these capacities now with a focus on the important infectious disease concept called *virulence*.

MINIQUIZ

- At what body sites do pathogens typically attach and colonize?
- Distinguish between infection and disease.
- Which is the more serious condition, bacteremia or septicemia, and why?

25.3 Pathogenicity, Virulence, and Attenuation

Unique properties of each pathogen contribute to its **pathogenicity**, the overall ability to cause disease. The measure of pathogenicity is called **virulence**, the relative ability of a pathogen to cause disease. Pathogenicity and virulence are not uniform properties of a given pathogen and can differ dramatically between different strains of the same bacterial species or virus. Highly virulent strains of a given pathogen tend to emerge every so often and when they do, they often trigger a rapid, widespread, and particularly severe course of disease (epidemic or pandemic, Chapter 29). The virulence of a given pathogen depends on a number of factors including its relative abilities to adhere, colonize, and invade (Figure 25.1), and its arsenal of virulence factors.

Virulence

Virulence is the net outcome of host–pathogen interactions, a dynamic relationship between the two organisms influenced by ever-changing conditions in the pathogen, the host, and the environment. Host damage in an infectious disease is mediated by **virulence factors**, toxic or destructive substances produced by the pathogen that directly or indirectly enhance invasiveness and host damage by facilitating and promoting infection. The second part of this chapter is devoted to major virulence factors.

Virulence is a quantifiable entity, especially if a pathogen is lethal and an experimental animal model is available. For example, the LD_{50} (LD stands for "lethal dose") is defined as the number of cells of a pathogen (or virions, for a viral pathogen) that kills 50% of the animals in a test group. Highly virulent pathogens frequently show little difference in the number of cells required to kill 100% of a test group of animals as compared with the LD_{50}. To illustrate this, recall the foundational work of the British microbiologist Frederick Griffith (Section 1.12). Griffith worked with the gram-positive bacterium *Streptococcus pneumoniae* and discovered that strains of *S. pneumoniae* that contained a capsule ("smooth" strains because they formed smooth colonies on plates) were highly virulent for mice, whereas mutant derivatives lacking a capsule ("rough" strains) were not. The *S. pneumoniae* capsule is the primary virulence factor of this bacterium because it helps the bacterium evade immune surveillance. Griffith's key discovery was that the smooth phenotype could be transferred to rough cells by treating rough cells with an extract from smooth cells (Figure 1.34). This was the first experimental example of transformation, a bacterial genetic transfer process (Section 11.6), and the active principle in the extract was later shown (by other scientists) to be DNA.

Griffith's choice of experimental organism was fortuitous because *S. pneumoniae* is both readily transformable and highly virulent for mice. Only a few cells of an encapsulated strain of *S. pneumoniae* can establish a fatal infection and kill all mice in a test population. As a result, the LD_{50} for *S. pneumoniae* in mice is not proportional to the number of cells delivered (**Figure 25.9**). By contrast, the number of cells of a less virulent pathogen such as the gram-negative enteric bacterium *Salmonella enterica* (*typhimurium*) necessary to kill all of the mice in the test population is about 10,000-fold greater than the highly virulent *S. pneumoniae* cells, and the LD_{50} is proportionally related to the number of cells of the pathogen cells injected into the mice (Figure 25.9).

There are many examples of highly virulent human pathogens, especially among viruses. For example, some strains of the influenza virus are so highly virulent that only a few virions can initiate disease even though mortality rates are typically low. Ebola virus is

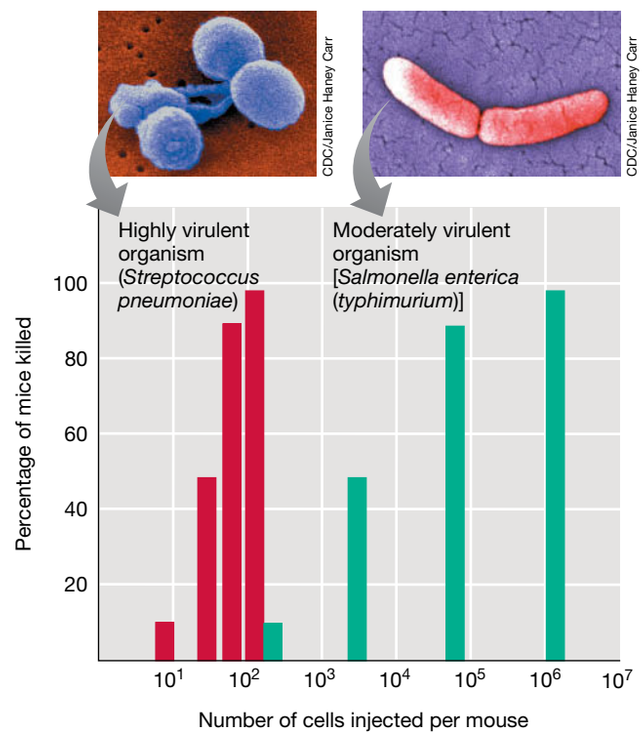


Figure 25.9 Microbial virulence. Differences in microbial virulence demonstrated by the number of cells of *Streptococcus pneumoniae* (red bars) and *Salmonella enterica* (*typhimurium*) (green bars) required to kill mice. Colorized scanning electron micrographs of each bacterium are shown above their respective graph.

also highly virulent, and the tiny inoculum necessary to initiate disease often results in a fatal infection. By contrast, the bacterial pathogen *Vibrio cholerae* (which causes cholera) is not especially virulent, as a large inoculum of this intestinal pathogen is necessary to initiate disease (Section 32.3).

Attenuation

The virulence of a pathogen can change. **Attenuation** is the decrease or loss of virulence of a pathogen. When pathogens are kept in laboratory culture rather than isolated from diseased animals, their virulence often decreases, or may be completely lost. Strains that have either a reduced virulence or are no longer virulent are said to be *attenuated*. Attenuation is thought to occur because nonvirulent or weakly virulent mutants grow faster than virulent strains in laboratory media where virulence has no selective advantage. After successive transfers in fresh media, such mutants are therefore selectively favored. However, if an attenuated culture is reinoculated into an animal, the organism may regain its original virulence, especially with continued in vivo passage as more-virulent strains are naturally selected. In some cases, though, the loss of virulence is permanent. For example, if a deletion mutation led to a major modification of a required receptor molecule (Figure 25.2*b*) or to the inability to produce a key virulence factor, such as the production of a toxin or invasive enzyme, then the mutant strain would be permanently attenuated.

Attenuated strains of various pathogens are valuable to clinical medicine because they are often used for the production of vaccines, especially viral vaccines. For example, vaccines for measles, mumps, and rubella, and rabies vaccines for animals other than humans, employ attenuated strains of each virus. Although attenuated viruses are "live" in the sense that, unlike "killed" strains, they could in principle become once again active and replicate, properly attenuated virus vaccines (those free of any unattenuated virions) typically show greater efficacy and generate an overall stronger immune response than do killed virus vaccines.

MINIQUIZ

- What are virulence factors? How can the LD_{50} test be used to define virulence of a pathogen?
- What circumstances can contribute to attenuation of a pathogen?

25.4 Genetics of Virulence and the Compromised Host

The virulence of a bacterial pathogen and the eventual outcome of an infectious disease are the net result of genetic and physiological features of both the pathogen and the host. In the case of the host, a pathogen may infect a healthy, well-rested young adult or an individual compromised by a physiological condition (old age, hospitalization, immune suppression); an ongoing infectious disease, for example, acquired immunodeficiency syndrome (AIDS) caused by the human immunodeficiency virus (HIV); or a genetic disease (for example, cystic fibrosis). The outcome of infection—health or disease—may be very different in these different individuals, even if they are infected by the same strain of a viral or bacterial pathogen.

The virulence of a pathogen may be encoded by firmly entrenched chromosomal genes or by highly mobile genetic elements. For example, the gram-negative bacterium *Bordetella pertussis*, the causative agent of whooping cough (pertussis, Section 30.3), makes several toxins including pertussis toxin, a potent AB-type exotoxin (Section 25.6); collectively, these toxins trigger the symptoms of whooping cough. Other species of *Bordetella* do not make pertussis toxin, and the chromosomal gene encoding pertussis toxin does not readily move from *B. pertussis* to other species. But in contrast to *B. pertussis*, some bacterial pathogens routinely exchange genes encoding virulence factors with different bacterial species or even genera, and thus highly related versions of their most potent weapons may appear in several different pathogens. *Salmonella* is a well-studied example of the genetic transfer of virulence factors, and we focus on this bacterium now.

Virulence in *Salmonella*: Pathogenicity Islands and Plasmids

Salmonella species infect humans, leading to various gastrointestinal illnesses (Section 32.10). *Salmonella* species encode a large number of virulence factors that are important in disease. These include type I fimbriae (Section 25.1) to facilitate attachment of cells to gastrointestinal tissues; several different classes of exotoxins (Section 25.6); antiphagocytic proteins that block engulfment of bacterial cells by host phagocytes; proteins that promote survival if the bacterium does get phagocytosed; siderophores, organic molecules that bind iron tightly and, in pathogenic bacteria, allow the bacteria to outcompete host sequestration systems for iron; and endotoxin (Section 25.8). With the exception of endotoxin, many of these virulence factors are encoded by genes present on mobile DNA rather than on the cell's chromosome (**Figure 25.10**).

Several genes that encode these virulence factors in *Salmonella* and related gram-negative pathogens such as pathogenic strains of *Escherichia coli* (Section 32.11) are found clustered together on the chromosome as *pathogenicity islands* (Section 9.7). *Salmonella* pathogenicity island 1 (SPI1) is a cluster of genes that encode over 10 distinct proteins that promote virulence and invasion. One of these is *invH*, a gene encoding a surface adhesion protein (Section 25.1). Several *inv* genes encode proteins important for trafficking of virulence factors. For example, the InvJ regulator protein controls assembly of structural proteins InvG, PrgH, PrgI, PrgJ, and PrgK that form a type III secretion system called the *injectisome*, an organelle in the bacterial envelope that allows for the direct transfer of virulence proteins into host cells through a needle-like assembly (Section 4.13 and Figures 4.42 and 4.43).

A second *Salmonella* pathogenicity island, SPI2, contains genes that are responsible for causing more systemic than localized disease and resistance to host defenses. In addition, several plasmid-encoded virulence factors such as antibiotic resistance genes encoded on R plasmids (Section 4.2) can spread between *Salmonella* species and to other genera of enteric bacteria. Pathogenicity islands and R plasmids allow for the facile and rapid transfer of virulence factors. It is thus not uncommon for genes encoding factors in one pathogen to be very similar if not

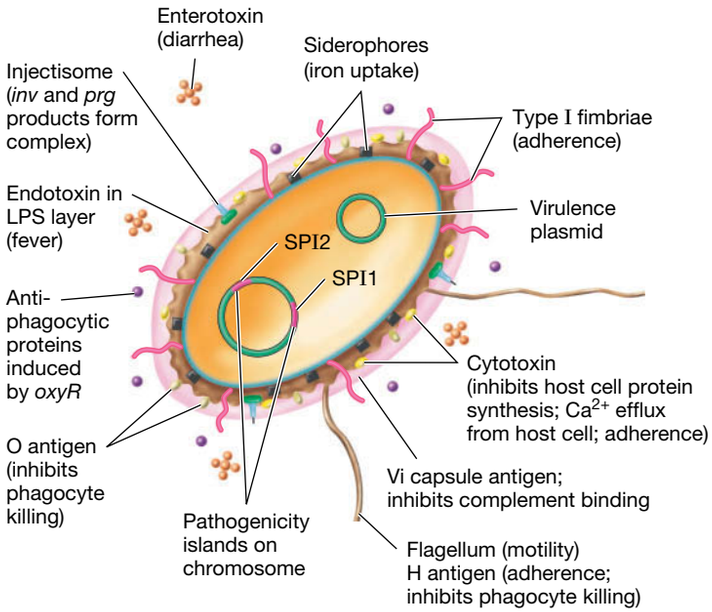


Figure 25.10 Virulence factors in *Salmonella*. Factors important for virulence and the development of pathogenesis in this gram-negative enteric pathogen are shown. Genes encoding many of the factors reside on the pathogenicity islands or plasmids.

identical to those in another because of transfer of parts or all of the islands or plasmids between species by horizontal gene exchange (Chapter 11).

In the well-known opportunistic pathogen (see next subsection) *Pseudomonas aeruginosa*, pathogenicity islands can also contain genes encoding antibiotic resistance. As for *Salmonella*, many cases of multiple antibiotic resistance in *P. aeruginosa* are linked to plasmids. However, in some strains of *P. aeruginosa*, genomic islands containing transposable elements (↔ Section 9.6) are present. By transposition these islands have "captured" multiple antibiotic resistance genes and can then disseminate them to other organisms; these islands are present in *P. aeruginosa* in place of or in addition to resistance plasmids. However, regardless of how they are encoded, these strains have become resistant to virtually all of the clinically useful antibiotics that have traditionally been used to control *P. aeruginosa* infections. This is a particularly serious problem for the compromised host and in the hospital environment, and we consider these issues now.

The Compromised Host

Some individuals are just more susceptible to infection than others for reasons that have little to do with the virulence of the pathogen. These so-called *compromised hosts* are individuals in which one or more resistance mechanisms are inactive and in whom the probability of infection is therefore increased. Many hospital patients with noninfectious diseases (for example, cancer and heart disease) acquire microbial infections more readily because they are compromised hosts. Such *healthcare-associated infections* (also called *nosocomial infections*) affect up to 2 million individuals each year in the United States, with a nearly 5% mortality rate. Invasive medical procedures such as catheterization, hypodermic injection, spinal puncture, biopsy, and surgery may unintentionally introduce microorganisms into the patient. The stress of surgery and the anti-inflammatory drugs given to reduce pain and swelling can also reduce host resistance (↔ Section 28.2).

Some factors can compromise host resistance outside the hospital including lifestyle choices that affect major organs of the body, such as intravenous drug usage, tobacco, excessive alcohol, and the like, or genetic diseases that eliminate parts of the immune system. People that are physically compromised for any of a number of reasons may be more susceptible to infections, not only because they are physically weakened but also because their living conditions and lifestyle choices may put them in more continual contact with infectious agents. For example, infection with the human immunodeficiency virus (HIV) predisposes an individual to infections from **opportunistic pathogens**, microbes that cause disease only in the absence of normal host resistance. HIV causes AIDS by destroying a specific class of immune cell, the CD4 T lymphocytes (↔ Figure 30.44), which are key to an effective immune response. The reduction in CD4 T cells reduces immunity, and an opportunistic pathogen, one that does not cause disease in a healthy, uninfected host, can then cause serious disease or even death. Individuals with immunodeficiencies from underlying genetic causes rather than infection are also more susceptible to opportunistic infections because part of their immune system is either nonfunctional or suboptimal.

The outcome of an infectious disease thus depends on several factors, both host and pathogen related. Two individuals exposed to the same pathogen in the same way may well show different outcomes. But once an infection has proceeded to the actual stage of disease, the symptoms that appear are due to products of the pathogens, and we turn our attention to these now.

MINIQUIZ

- What major virulence factors are produced by *Salmonella*?
- What is an opportunistic pathogen? What steps can a person take to help avoid opportunistic infections?
- What is a nosocomial infection?

II • Enzymes and Toxins of Pathogenesis

Bacterial pathogens damage host tissues (or the entire host) in two major ways: (1) by secreting tissue-destructing enzymes and (2) by secreting or shedding toxins that target specific host tissues or the entire host. In contrast to bacterial pathogens, most viral pathogens damage host tissues by lysing cells directly, although some viruses are nonlytic and instead introduce genes into host cells that may eventually harm the host (↔ Section 8.8).

We turn our focus now to the enzymes and toxins of well-studied pathogenic bacteria, contrasting their efficacy and modes of action. Some of these virulence factors cause only minor disease symptoms, whereas others are some of the most poisonous substances known.

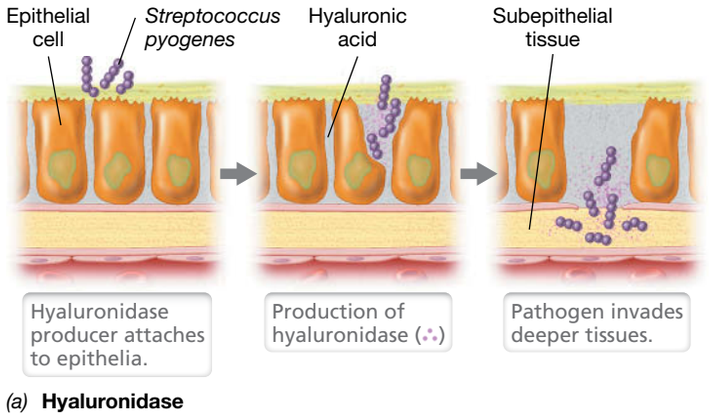
25.5 Enzymes as Virulence Factors

Following adherence, colonization, and infection by a pathogen, invasiveness requires the breakdown of host tissues and access to nutrients released from host cells. In many classical bacterial pathogens, this is accomplished through the activity of *enzymes* that attack and destroy cells in one type of tissue or another (Table 25.1).

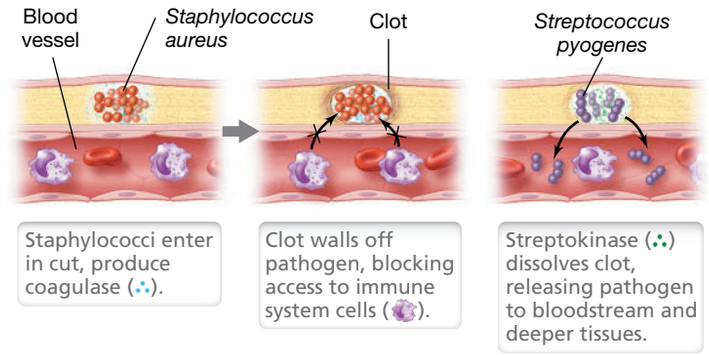
Tissue-Destroying Enzymes

Many virulence factors are enzymes. For example, streptococci, staphylococci, and certain clostridia produce *hyaluronidase* (Table 25.1), an enzyme that promotes spreading of organisms in tissues by breaking down the polysaccharide hyaluronic acid. Among other functions, hyaluronic acid is a component of the extracellular matrix and functions as a type of "intercellular cement" in animal tissues, helping to maintain the organization of individual cells into tissues; the activity of hyaluronidase causes host cells to slough apart, allowing pathogens at an initial colonization site to spread between host cells to attack subsurface tissues (Figure 25.11*a*). Similarly, the clostridia that cause gas gangrene produce *collagenase*, an enzyme that destroys collagen (a major protein of connective tissues in muscle and other body tissues); collagenase enables these bacteria to gain access to deeper host tissues and spread through the body. Recall that clostridia are anaerobes, and colonizing deeper tissues allows them to reach less oxic conditions and provides a ready source of nutrients from destroyed tissues (gangrene clostridia are typically proteolytic species, Sections 16.8 and 31.9). Many pathogenic streptococci and staphylococci also produce proteases, nucleases, and lipases that degrade host proteins, nucleic acids, and lipids, respectively (Table 25.1).

Two virulence factors are enzymes that affect fibrin, the insoluble blood protein that triggers blood clots, but the activities of the enzymes yield opposing results (Figure 25.11*b*). Blood clotting is triggered by tissue injury and functions not only to stop blood loss but also, in the case of a bacterial infection, to isolate the pathogen, limiting the infection to a local region. Some pathogens counter this host protective mechanism by producing fibrinolytic enzymes, such as *streptokinase* produced by *Streptococcus pyogenes*. This bacterium is often associated with pus-forming wounds and secretes streptokinase to dissolve fibrin clots and make further invasion possible (Table 25.1, Figure 25.11*b*). Streptokinase specifically activates the host to produce plasmin, an enzyme that degrades fibrin blood clots. Because of this powerful activity, streptokinase also has a medically beneficial function. The protein is marketed as a pharmaceutical and administered intravenously to dissolve clots for conditions in which blood clots are blocking normal blood flow, such as from heart attacks, deep vein thromboses, or embolisms.



(a) **Hyaluronidase**



(b) **Coagulase and streptokinase**

Figure 25.11 Activity of some enzyme virulence factors. *(a)* Hyaluronidase. *(b)* Coagulase and streptokinase. Among other bacterial pathogens, hyaluronidase and streptokinase are typical of virulent strains of *Streptococcus pyogenes* and coagulase is typical of virulent strains of *Staphylococcus aureus*.

TABLE 25.1 Enzyme virulence factors of some well-known gram-positive bacterial pathogens

Organism	*Disease*	*Enzyme*[a]	*Enzyme activity*
Staphylococcus aureus	Pus-forming infections	Coagulase	Induces fibrin clotting; allows bacterial cells to remain at site of infection (prevents access to pathogens by cells of the immune response)
		Nuclease; lipase	Break down nucleic acids or lipids
Streptococcus pyogenes	Pus-forming infections; scarlet fever; strep throat	Hyaluronidase	Dissolves hyaluronic acid in connective tissues; allows bacterial cells to spread (enhances pathogen invasion)
		Streptokinase	Dissolves fibrin clots; allows bacterial cells to spread
Clostridium perfringens	Gas gangrene; food poisoning	Collagenase	Breaks down collagen (a protein), allowing the bacterium to spread to other tissues
		Protease	Breaks down proteins

[a]The activities of coagulase, hyaluronidase, and streptokinase are depicted in Figure 25.11.

In contrast to the fibrin-*destroying* activity of streptokinase, some pathogens produce enzymes that actually *promote* the formation of fibrin clots. These clots protect the pathogen from host responses. For example, *coagulase* (Table 25.1), produced by virulent *Staphylococcus aureus*, converts fibrinogen to fibrin, resulting in the clotting of blood and the formation of fibrin surrounding the *S. aureus* cells; this blanket of fibrin protects the *S. aureus* cells from attack by cells of the host's immune system (Figure 25.11*b*). The fibrin matrix produced as a result of coagulase activity may also account for the localized nature of many staphylococcal infections, as is typically seen in boils and pimples (Section 30.9). Coagulase-positive *S. aureus* strains are typically more virulent than coagulase-negative strains, a likely reflection of the former's ability to evade innate immune responses such as phagocytosis (Chapter 26) and continue growth and tissue destruction for a longer period.

Enzyme Activities at the Host's Mucosal Surface

Host mucosal surfaces are bathed in immune substances including enzymes such as lysozyme, an enzyme that cleaves the peptidoglycan of bacterial cells and promotes their osmotic lysis (Section 2.4). Virulence factors produced by the gram-positive bacterium *Enterococcus faecalis*—a major cause of bacteremia, surgical wound infections, and urinary tract infections—attack the protective role of lysozyme by altering the structure of the bacterium's peptidoglycan such that lysozyme can no longer recognize its substrate.

Antibodies are also present on mucosal surfaces, in particular a class of antibody called *IgA* (Section 27.3). These "secretory antibodies," as they are called, help prevent pathogen adherence to host tissues (Section 25.1). However, certain pathogenic bacteria counter this protective role by producing enzymes that specifically cleave IgA (IgAases), rendering this host defense useless; *Neisseria* species such as *N. gonorrhoeae* (gonorrhea) and *N. meningitidis* (meningitis) are particularly notorious in this regard.

We thus see that pathogens can produce enzymes both as offensive weapons—to destroy host tissues—and as defensive weapons—to destroy or inactivate offensive weapons of the host. Both strategies accomplish a similar objective: The invasiveness of the pathogen is increased and this allows it to ultimately extract more resources from its host.

MINIQUIZ

- Identify host factors that limit or accelerate infection of a microorganism at selected local sites.
- How do streptokinase and coagulase promote bacterial infection and invasion?
- What is an IgAase and why would a bacterial pathogen produce one?

25.6 AB-Type Exotoxins

Toxicity is the ability of an organism to cause disease by means of a toxin that inhibits host cell function or kills host cells (or the host itself). **Exotoxins** are toxic *proteins* released from the pathogen as it grows. These toxins travel from a site of infection and cause damage at distant sites. Some exotoxins are **enterotoxins**, toxic proteins whose site of action is the small intestine, generally causing secretion of fluid into the intestinal lumen, resulting in vomiting and diarrhea. Exotoxins fall into three categories in terms of their mechanism: *AB toxins*, *cytolytic toxins,* and *superantigen toxins*. In this section we consider the AB toxins and in Section 25.7 we focus on cytolytic and superantigen toxins.

As the name implies, AB toxins consist of two subunits, A and B. The B component binds to a host cell surface molecule, facilitating the transfer of the A subunit across the cytoplasmic membrane, where it damages the cell. Some of the best-known exotoxins are AB toxins, including those expressed in the diseases diphtheria, tetanus, botulism, and cholera (Table 25.2).

Diphtheria Exotoxin: Blockage of Protein Synthesis

The diphtheria toxin produced by the aerobic gram-positive bacterium *Corynebacterium diphtheriae* is an AB toxin and an important virulence factor of the pathogen (Section 30.3). Diphtheria toxin inhibits protein synthesis in eukaryotic cells. Rats and mice are relatively resistant to diphtheria toxin, whereas humans are very susceptible, with only a single molecule of toxin sufficient to

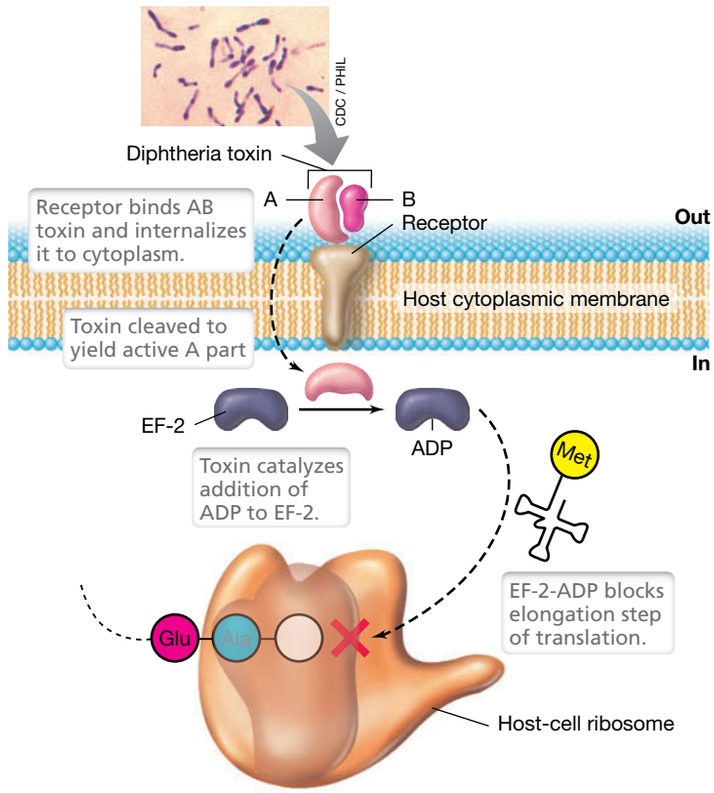


Figure 25.12 The activity of diphtheria toxin. Diphtheria toxin, an AB toxin produced by cells of *Corynebacterium diphtheriae*, binds to the host cytoplasmic membrane by way of its B subunit. Cleavage of the toxin allows the A subunit to enter the cell where it catalyzes ADP-ribosylation of elongation factor 2 (EF-2), a key factor in translation. The modified elongation factor no longer binds to the ribosome, resulting in the cessation of protein synthesis and host cell death. Inset photo: Light micrograph of Gram-stained cells of *Corynebacterium diphtheriae*. A single cell is about 0.75 μm in diameter.

TABLE 25.2 Some classic exotoxins and cytotoxins produced by human bacterial pathogens

Organism	*Disease*	*Toxin*[a]	*Activity*[b]
Bacillus anthracis	Anthrax	Lethal factor Edema factor Protective antigen (AB)	Combine to cause cell death
Bordetella pertussis	Whooping cough	Pertussis toxin (AB)	Blocks G protein function; kills cells
Clostridium botulinum	Botulism	Botulinum toxin (AB)	Causes flaccid paralysis
Clostridium tetani	Tetanus	Tetanospasmin (AB)	Causes rigid paralysis
Clostridium perfringens	Gas gangrene Food poisoning	α, β, γ, δ toxins (AB) Enterotoxin (CT)	Hemolysis, lecithin destruction Alters intestinal tract permeability
Corynebacterium diphtheriae	Diphtheria	Diphtheria toxin (AB)	Inhibits eukaryotic protein synthesis
Escherichia coli (enterotoxigenic strains only)	Gastroenteritis	Shiga-like (*E. coli*) (AB)	Inhibits protein synthesis, induces bloody diarrhea
Pseudomonas aeruginosa	Burn and certain wound and ear infections; cystic fibrosis lung infections	Exotoxin A (AB)	Inhibits eukaryotic protein synthesis
Salmonella sp.	Gastroenteritis	Enterotoxin (AB) Cytotoxin (CT)	Lyses cells; inhibits protein synthesis Induces fluid loss from intestine
Shigella dysenteriae	Gastroenteritis	Shiga toxin (AB)	Bloody diarrhea and hemolytic uremic syndrome
Staphylococcus aureus	Pyogenic (pus-forming) wounds; food poisoning, toxic shock	α, β, γ, δ toxins (CT) Toxic shock toxin (SA) Enterotoxins A–E (SA)	Hemolysis, leukolysis, cell death Systemic shock Vomiting, diarrhea, systemic shock
Streptococcus pyogenes	Pyogenic infections; strep throat; scarlet fever	Streptolysis O, S (CT) Erythrogenic toxin (SA)	Hemolysis Causes scarlet fever
Vibrio cholerae	Cholera	Cholera (AB)	Induces fluid loss from intestine

[a]AB, AB toxin; CT, cytotoxin; SA, superantigen.
[b]See Figures 25.11–25.16 for the mode of action of some of these toxins.

kill a cell. Diphtheria has a significant mortality rate, especially in the young, and death ensues from the destruction of tissues in vital organs such as the heart and liver from the blockage of protein synthesis by diphtheria toxin.

Cells of *C. diphtheriae* secrete diphtheria toxin as a single polypeptide. One component of the toxin, subunit B, specifically binds to a host cell receptor protein on eukaryotic cells, the heparin-binding epidermal growth factor (**Figure 25.12**). After binding, proteolytic cleavage between subunit B and the remaining portion of the protein, subunit A, allows subunit A to move across the host cytoplasmic membrane into the cytoplasm. Here subunit A disrupts protein synthesis by blocking transfer of an amino acid from tRNA to growing polypeptide chains. Diphtheria toxin specifically inactivates elongation factor 2 (EF-2), a protein that functions in growth of the polypeptide chain, by catalyzing the attachment of adenosine diphosphate (ADP) ribose from NAD^+. Following ADP-ribosylation, the activity of the modified EF-2 decreases dramatically and protein synthesis stops (Figure 25.12).

Diphtheria toxin is not encoded by the bacterium but instead by a viral gene called *tox* present in the genome of the lysogenic bacteriophage β. Lysogenic phages are those whose genomes have become integrated into their host's chromosome (↪ Section 8.7). Toxigenic, pathogenic strains of *C. diphtheriae* are infected with phage β and hence produce the toxin. Nontoxigenic, nonpathogenic strains of *C. diphtheriae* can be converted to pathogenic strains by infection with phage β, a process called *phage conversion* (↪ Section 11.7).

Exotoxin A of *Pseudomonas aeruginosa* functions similarly to diphtheria toxin, also modifying EF-2 by ADP-ribosylation. The enterotoxin produced by *Shigella dysenteriae*, called *Shiga toxin*, and the Shiga-like toxin produced by enteropathogenic *E. coli* O157:H7 (↪ Section 32.11) are also AB toxins (Table 25.2). Shiga and Shiga-like toxins target cells of the small intestine near where the pathogen colonized, shutting down protein synthesis. This leads to cell death, bloody diarrhea, and hemolytic uremic syndrome, a kidney disease that can trigger kidney failure, especially in children.

Neurological Exotoxins: Botulinum and Tetanus Toxins

Clostridium botulinum and *Clostridium tetani* are endospore-forming bacteria commonly found in soil and which cause the serious and potentially fatal diseases botulism and tetanus, respectively; both diseases are caused by the secretion of highly poisonous AB exotoxins that function as *neurotoxins* (↪ Sections 31.9 and 32.9). Neither

C. botulinum nor *C. tetani* is highly invasive, and therefore pathogenicity is almost exclusively due to neurotoxicity. Botulinum toxin and tetanus toxin both block the release of neurotransmitters that control muscle activities, but the mode of action and disease symptoms are quite distinct (**Figures 25.13** and **25.14**).

C. botulinum sometimes grows directly in the intestine, causing infant or wound botulism. Most frequently, however, botulism results from cells of *C. botulinum* growing and producing toxin in improperly preserved foods, such as home-canned vegetables (see page 973). Thus, infection and growth of the pathogen in the body are unnecessary. Botulinum toxins, the most potent biological substances known, are composed of seven related AB toxins. One nanogram (10^{-9} g) of botulinum toxin is sufficient to kill a guinea pig. Of the seven distinct botulinum toxins known, at least two are encoded on lysogenic bacteriophages specific for *C. botulinum*. The major botulinum toxin is a protein that forms complexes with nontoxic botulinum proteins to yield a bioactive protein complex. The complex then binds tightly to presynaptic membranes on the termini of the stimulatory motor neurons at the neuromuscular junction, blocking the release of acetylcholine, a neurotransmitter. Normal transmission of a nerve impulse to a muscle cell requires acetylcholine interaction with a muscle receptor; the binding of botulinum toxin poisons the neuron, preventing it from sending the excitatory acetylcholine signal to the muscle (Figure 25.13). This prevents muscle contraction and is recognized as *flaccid paralysis* in a botulism victim. This can lead to death by suffocation if the diaphragm muscles are severely affected.

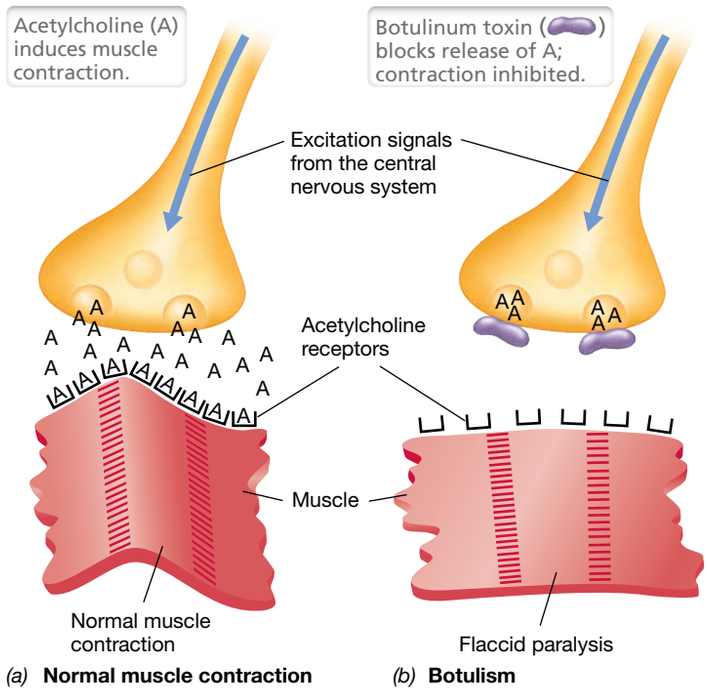


Figure 25.13 The activity of botulinum toxin. *(a)* Upon stimulation of peripheral and cranial nerves, acetylcholine (A) is normally released from vesicles at the neural side of the motor end plate. Acetylcholine then binds to specific receptors on the muscle, inducing contraction. *(b)* Botulinum toxin acts at the motor end plate to prevent release of acetylcholine from vesicles, resulting in a lack of stimulus to the muscle fibers, irreversible relaxation of the muscles, and flaccid paralysis.

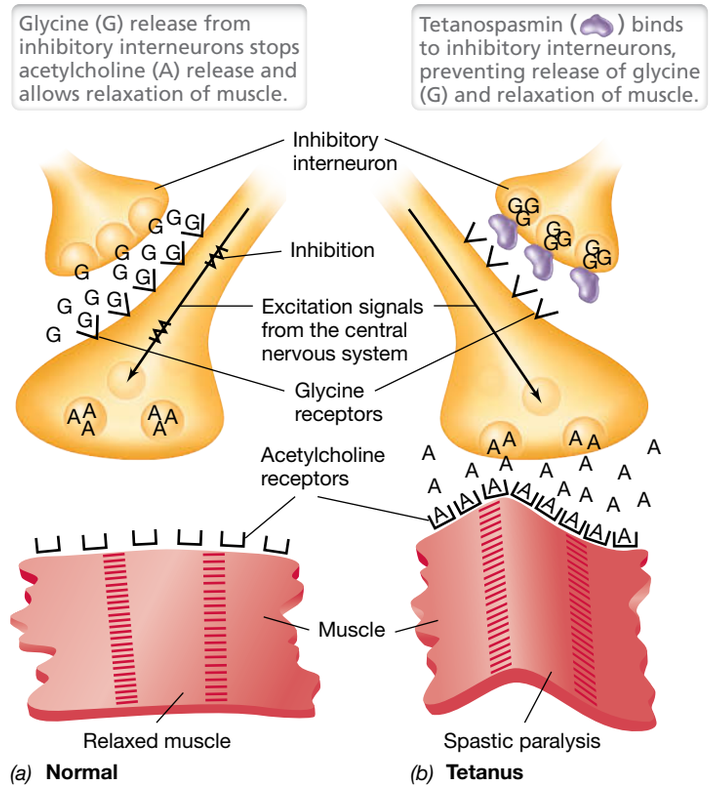


Figure 25.14 The activity of tetanus toxin. *(a)* Muscle relaxation is normally induced by glycine (G) release from inhibitory interneurons. Glycine acts on the motor neurons to block excitation and release of acetylcholine (A) at the motor end plate. *(b)* Tetanus toxin binds to the interneuron to prevent release of glycine from vesicles, resulting in a lack of inhibitory signals to the motor neurons, constant release of acetylcholine to the muscle fibers, irreversible contraction of the muscles, and spastic paralysis. For the purpose of illustration, the inhibitory interneuron is shown near the motor end plate, but it is actually in the spinal cord.

In contrast to *C. botulinum, C. tetani* grows in the body in deep wounds that become anoxic, such as punctures. *C. tetani* cells rarely leave the wound where they were first introduced, growing relatively slowly at the wound site. On contact with the nervous system, tetanus toxin, called *tetanospasmin*, is transported through the motor neurons to the spinal cord, where it binds specifically to ganglioside lipids at the termini of the inhibitory interneurons. The inhibitory interneurons normally work by releasing an inhibitory neurotransmitter, typically the amino acid glycine, which binds to receptors on the motor neurons. Glycine from the inhibitory interneurons then stops the release of acetylcholine by the motor neurons and inhibits muscle contraction, allowing relaxation of the muscle fibers. However, if tetanus toxin blocks glycine release, the motor neurons cannot be inhibited, resulting in the continual release of acetylcholine and uncontrolled contraction of the muscle fibers. Thus, in contrast to botulinum toxin, which prevents muscle *contraction* (Figure 25.13), tetanus toxin prevents muscle *relaxation* (Figure 25.14).

The outcome in a case of tetanus is a twitching, *spastic paralysis*—the hallmark of tetanus (Section 31.9 and Figure 31.33*b*)—as affected muscles are constantly contracting. If the muscles of the mouth are involved, the prolonged contractions restrict the

mouth's movement, resulting in a condition called *lockjaw*. If the diaphragm is affected, its prolonged contraction may result in death due to asphyxiation.

Cholera Enterotoxin: Intestinal Distress

Cholera enterotoxin is an AB-type exotoxin produced by *Vibrio cholerae*, the causative agent of the waterborne disease cholera (Section 32.3). Cholera is characterized by massive fluid loss from the intestines, resulting in severe diarrhea, life-threatening dehydration, and electrolyte depletion (**Figure 25.15**). Cholera starts by ingestion of *V. cholerae* cells from food or water contaminated with human feces. The organism travels to the small intestine, where it colonizes and secretes cholera toxin. In the small intestine, the B subunit of cholera toxin, consisting of five identical monomers, binds specifically to GM1 ganglioside, a complex glycolipid found in the cytoplasmic membrane of intestinal epithelial cells (Figure 25.15).

The B subunit targets cholera toxin specifically to receptors in the intestinal epithelium but has no toxicity itself; toxicity is a function of the A subunit, which crosses the cytoplasmic membrane and activates adenylate cyclase, the enzyme that converts ATP to cyclic adenosine monophosphate (cAMP). This molecule is a cyclic nucleotide (Figure 6.13) that mediates several regulatory systems in cells, including ionic balance. The increased cAMP induced by cholera enterotoxin blocks Na^+ uptake by small intestine epithelial cells and induces secretion of chloride and bicarbonate (HCO_3^-) into the intestinal lumen. This change in ion concentrations leads to the secretion of large amounts of water; the rate of water loss into the small intestine is greater than the possible reabsorption of water by the large intestine, resulting in a large net fluid loss and watery diarrhea. If untreated, cholera victims can die within hours of the major onset. However, if lost fluids are replaced with an oral rehydration solution (the main treatment for cholera), the effects of cholera toxin can be neutralized and a cholera victim can return to normal in just a few days.

A few other enterotoxins, most notably the Shiga-like toxin of enterotoxigenic strains of *Escherichia coli* (Section 32.11) and *Shigella* and *Salmonella* enterotoxins are also of the AB type (Table 25.2), and all of these function by inhibiting protein synthesis. This leads to major bouts of diarrhea, typically bloody and foul smelling, and severe dehydration. In addition, some cytolytic enterotoxins are known and the powerful enterotoxins of *Staphylococcus aureus* (Table 25.2) are of the superantigen type.

MINIQUIZ

- What key features are shared by all AB exotoxins?
- Are bacterial growth and infection in the host necessary for the production of toxins? Explain and cite examples for your answer.
- Why do botulism and tetanus show such opposing symptoms?

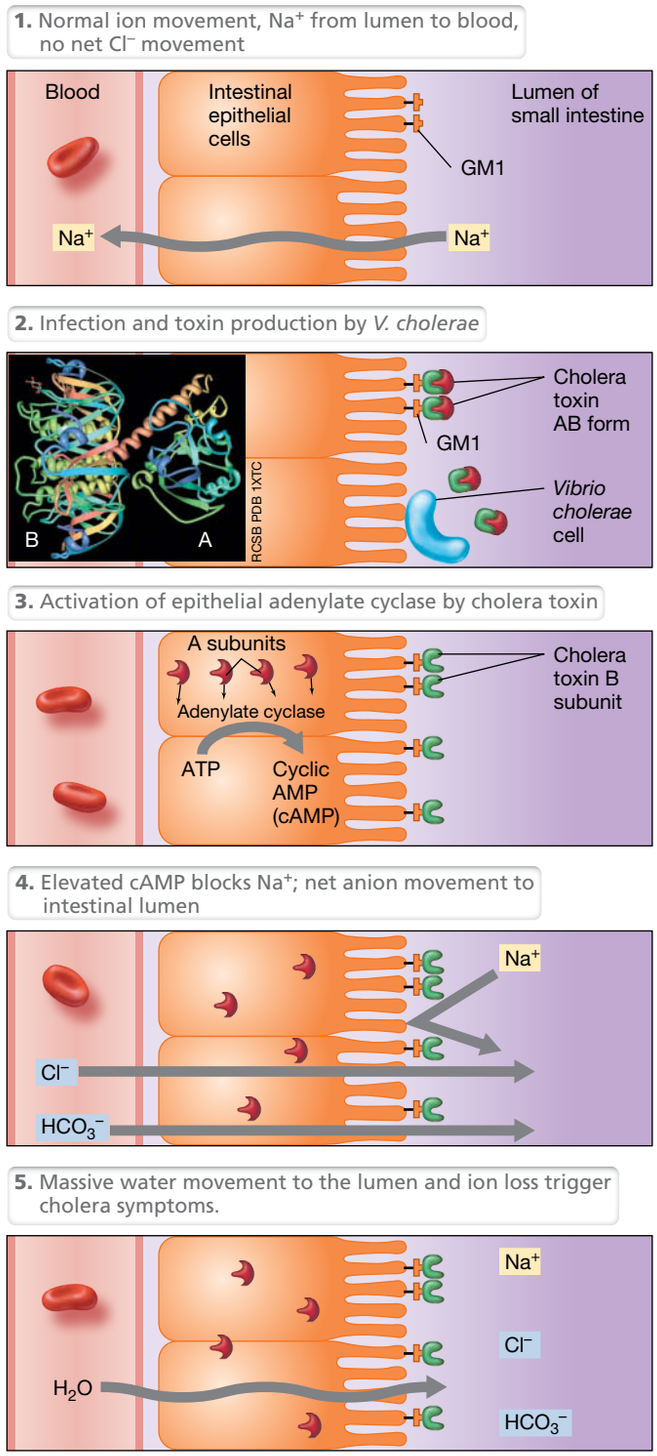


Figure 25.15 The activity of cholera enterotoxin. Cholera toxin is an AB enterotoxin that activates a second-messenger pathway, disrupting normal ion flow in the intestine, resulting in potentially life-threatening diarrhea. The thumbnail photo of the three-dimensional structure shows a side view of the toxin, with the separate cell-binding B subunit and the enzymatically active A subunit.

25.7 Cytolytic and Superantigen Exotoxins

The pathogenesis of exotoxins such as the cytolytic and superantigen toxins differs from those of the classical AB toxins. Cytolytic and superantigen exotoxins function by destroying host cells, such as from the activity of hemolysins, or by triggering a massive immune response, such as in the case of toxic shock exotoxin, the cause of toxic shock syndrome. As for other exotoxins, however,

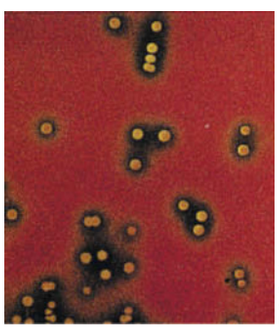
these proteins are produced by the pathogen to increase its invasiveness and release host cell resources that can benefit the pathogen.

Cytolytic Exotoxins

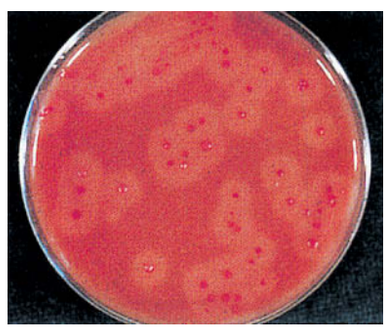
Cytotoxins (also called cytolytic exotoxins) are soluble proteins secreted by a variety of pathogens (Table 25.2). Cytotoxins damage the host cytoplasmic membrane, causing cell lysis and death. Because the lytic activity of these toxins is most easily observed in assays that use red blood cells (erythrocytes), the toxins are often called *hemolysins* (Table 25.2). However, hemolysins also lyse cells other than erythrocytes. The production of hemolysins can be demonstrated by streaking the pathogen on a blood agar plate (a rich medium containing 5% sterile blood). During growth of the colonies, hemolysin is released and lyses the surrounding red blood cells, releasing hemoglobin and creating a clear area, called a zone of *hemolysis*, around the growing colonies (**Figure 25.16*a***).

Some hemolysins attack the phospholipid of the host cytoplasmic membrane. Because the phospholipid lecithin (phosphatidylcholine) is often used as a substrate, these enzymes are called *lecithinases* or *phospholipases*. An example is the α-toxin of *Clostridium perfringens*, a lecithinase that dissolves membrane lipids, resulting in cell lysis (Table 25.2, Figure 23.16*b*). Because the cytoplasmic membranes of all organisms contain phospholipids (Section 2.3), phospholipases can destroy bacterial as well as animal cell cytoplasmic membranes. In the case of *C. perfringens*, a major cause of gas gangrene, the activity of its lecithinase helps destroy tissues and release proteins that are fermented by the bacterium in energy metabolism. Since *C. perfringens* lecithinase is secreted immediately after synthesis and is unable to reenter the cell, the enzyme does not affect the phospholipids in *C. perfringens* cells.

Some hemolysins, however, are not phospholipases. Streptolysin O, a hemolysin produced by streptococci, affects the sterols of the host cytoplasmic membrane. *Leukocidins* lyse white blood cells and thereby decrease the host immune response. Staphylococcal α-toxin (**Figure 25.17** and Table 25.2) kills nucleated cells and lyses erythrocytes. To do this, the seven α-toxin subunits first bind to the phospholipid bilayer. The subunits then combine to form into nonlytic heptamers, now associated with the membrane. Each heptamer then undergoes conformational changes to produce a membrane-spanning pore that releases cytoplasmic contents and allows the influx of extracellular materials, thus killing the cell (Figure 25.17).



(a)



(b)

Figure 25.16 Hemolysis. *(a)* Zones of hemolysis around colonies of *Streptococcus pyogenes* growing on a blood agar plate. *(b)* Activity of lecithinase, a phospholipase, around colonies of *Clostridium perfringens* growing on an agar medium containing egg yolk, a source of lecithin. Lecithinase dissolves the cytoplasmic membranes of red blood cells, producing cloudy zones of hemolysis around each colony.

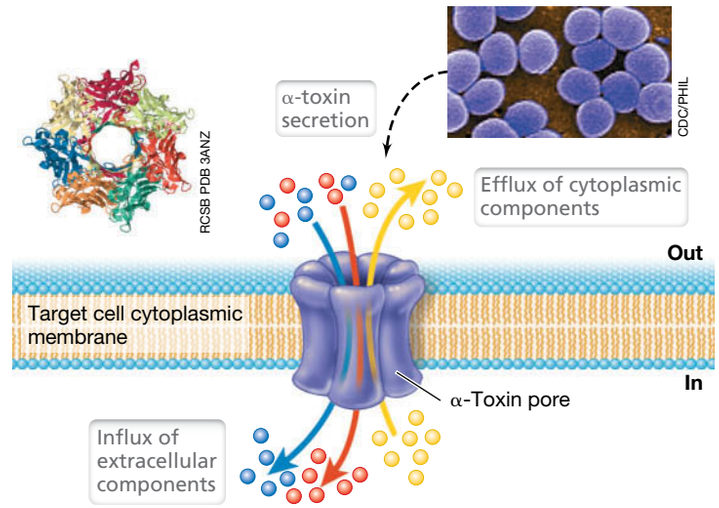


Figure 25.17 Staphylococcal α-toxin. Staphylococcal α-toxin is a pore-forming cytotoxin that is produced by growing *Staphylococcus aureus* cells. Released as monomers, seven identical protein subunits oligomerize in the cytoplasmic membrane of target cells. The oligomer forms a pore, releasing the contents of the cell. In red blood cells, hemolysis occurs, visually indicating cell lysis. The inset photo on the top left shows the structure of α-toxin looking down through the pore. Each of the seven identical subunits is shown in a different color. The inset photo on the top right is a scanning electron micrograph of *S. aureus* cells.

Superantigen Exotoxins

The gram-positive bacteria *Staphylococcus aureus* and *Streptococcus pyogenes* are major producers of exotoxin superantigens. We consider the mode of action of superantigens in Section 27.10 in the context of adaptive immunity and focus here only on some basic disease symptoms.

Superantigen poisoning can be triggered by certain types of food poisoning, in particular that caused by the enterotoxins of *S. aureus*, and also by pyrogenic fever (fever induced from an internal source, typically a small immune system protein called a cytokine, or from an external substance such as endotoxin; see Section 25.8) and toxic shock syndrome. Reactions to superantigen poisoning are severe and can even be fatal in some individuals, particularly those whose immune systems and overall health are weakened from cancer, drug treatments, HIV infection, or old age. Toxic shock syndrome (TSS) is the classic example of the systemic effects of a toxic superantigen and results from exposure to any of a series of exotoxins secreted during infection by certain strains of *S. aureus* or *S. pyogenes* (Sections 30.9 and 30.2, respectively).

S. aureus TSS commonly originates as a result of a localized rather than a generalized infection. By contrast, *S. pyogenes* TSS is typically the result of a systemic infection where bacteremia or septicemia (Section 25.2) is present and tissue damage including extensive tissue necrosis occurs (Figure 30.10); as a result, mortality rates from *S. pyogenes* TSS are considerably higher than from *S. aureus* TSS. In both cases, however, the symptoms of TSS are triggered when the immune system recognizes the superantigen

toxin but then, rather than just activating a small subset of T lymphocytes (key cells in the adaptive immune response, Chapter 27) as usually occurs in the adaptive immune response, activates a large proportion of the entire T lymphocyte pool. It is the structure of the superantigen itself that leads to this overblown immune response, a result of which is widespread inflammation that leads to hypotension (low blood pressure), intestinal disruption, organ failure, and eventually systemic shock.

In a clinical diagnosis, either staphylococcal or streptococcal TSS is suspected when an individual presents with a fever of 39°C or greater, systolic blood pressure of <90 mm Hg, and functional disruption of three or more organ systems, most often gastrointestinal, kidney, and liver. In severe cases of TSS, intensive care hospitalization may be needed with intravenous administration of antibiotics.

MINIQUIZ

- Give an example of a cytolytic exotoxin and a superantigen exotoxin, as well as the bacteria that produce each.
- How can activity of a hemolytic exotoxin be detected?

25.8 Endotoxins

Endotoxins are the toxic lipopolysaccharides found in the cell walls of most gram-negative *Bacteria.* Endotoxins are not proteins but are structural components of the gram-negative outer membrane (↻ Section 2.5). In contrast to exotoxins, which are the secreted products of living cells, endotoxins are cell bound and released in toxic amounts only when the cells lyse. The basic properties of exotoxins and endotoxins are compared in Table 25.3.

Endotoxin Structure and Biology

A major component of the gram-negative cell outer membrane is lipopolysaccharide (LPS) (↻ Figures 2.13 and 2.14). LPS consists of three covalently linked subunits: the membrane-distal O-specific polysaccharide, a membrane-proximal core polysaccharide, and lipid A—a phosphoglycolipid and the membrane-anchoring portion of LPS (Figure 25.18). The lipid A portion of LPS is responsible for toxicity, whereas the polysaccharide fraction by itself is nontoxic. The polysaccharide functions to make the entire LPS complex soluble and immunogenic, and thus both the lipid and polysaccharide fractions must be delivered as a unit for toxicity to occur.

Endotoxins have been well studied in the bacteria *Escherichia, Shigella*, and especially in *Salmonella*, where they are another of the many virulence factors that contribute to pathogenesis (Figure 25.10). The biosynthesis of the toxic component of endotoxin, lipid A, is known and is a highly conserved process among gram-negative bacteria. Nevertheless, not all lipid A is structurally the same, as the molecule can be modified using enzymes that catalyze postsynthesis modifications that control the presence, absence, or number of phosphate groups, and the chemistry and number of fatty acid side chains (Figure 25.18). These subtle but important alterations affect the properties of the LPS molecule and are a virulence strategy that certain pathogens have evolved to either evade recognition by the host immune system or increase the toxicity of the molecule. However, some lipid A alterations affect toxicity in a negative way. For example, the phosphate groups (Figure 25.18) are essential for binding lipid A to animal cell receptors, and although phosphate-free lipid A can evade immune surveillance, its toxicity is greatly reduced.

Endotoxins cause a variety of physiological effects. Fever is an almost universal result of endotoxin exposure because endotoxin stimulates host cells to release cytokines, soluble proteins secreted by certain cells of the immune system that function as *endogenous pyrogens*, proteins that affect the temperature-controlling center of the brain, causing fever. Cytokines released as a result of endotoxin exposure can also cause diarrhea, increased heart rate, a rapid decrease in the numbers of lymphocytes and platelets, and

TABLE 25.3 Properties of exotoxins and endotoxins

Property	*Exotoxins*	*Endotoxins*
Chemistry	Proteins, secreted by certain gram-positive or gram-negative *Bacteria;* generally heat-labile	Lipopolysaccharide–lipoprotein complexes, released on cell lysis as part of the outer membrane of gram-negative *Bacteria;* extremely heat-stable
Mode of action; symptoms	Specific; usually binds to specific cell receptors or structures; either cytotoxin, enterotoxin, or neurotoxin with defined, specific action on cells or tissues	General; fever, diarrhea, vomiting
Toxicity	Often highly toxic in picogram to microgram quantities, sometimes fatal	Moderately toxic in tens to hundreds of microgram amounts, rarely fatal
Immune response	Highly immunogenic; stimulate the production of neutralizing antibody (antitoxin)	Relatively poor immunogen; immune response not sufficient to neutralize toxin
Toxoid potential[a]	Heat or chemical treatment may destroy toxicity, but treated toxin (toxoid) remains immunogenic	None
Fever potential	Nonpyrogenic; does not produce fever in the host	Pyrogenic; often induces fever in the host
Genetic origin	Often encoded on extrachromosomal elements or lysogenic bacteriophages	Encoded by chromosomal genes

[a]A toxoid is a modified toxin that is no longer toxic but can still elicit an immune response against the toxin (↻ Section 28.9).

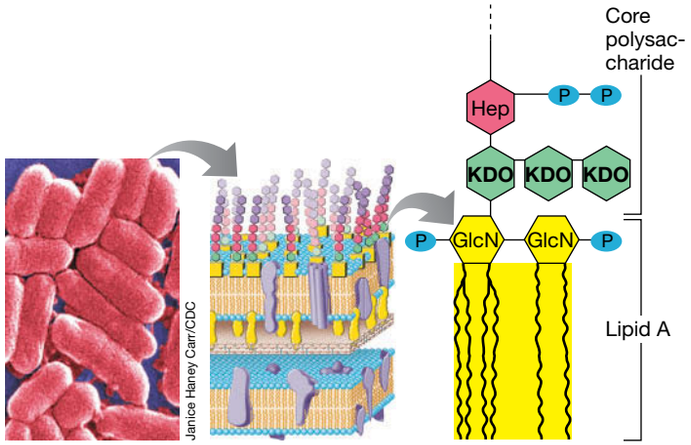


Figure 25.18 Endotoxin. Left to right: Scanning electron micrograph of cells of the gram-negative bacterium *Escherichia coli*; structure of the gram-negative cell wall including the lipopolysaccharide (LPS) outer membrane; detailed structure of lipid A, the toxic portion of LPS, along with part of the core polysaccharide of LPS.

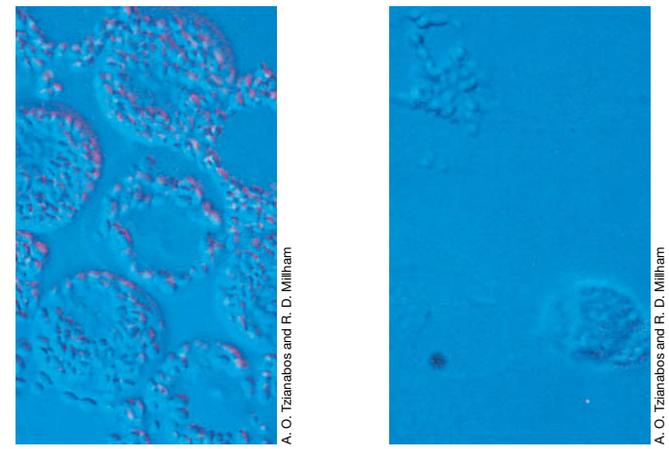


Figure 25.19 *Limulus* amoebocyte assay for endotoxin. *(a)* Normal amoebocytes from the horseshoe crab *Limulus polyphemus*. *(b)* Amoebocytes following exposure to bacterial lipopolysaccharide (LPS). LPS induces degranulation and lysis of the cells.

generalized inflammation (Section 26.8). Other physiological consequences of endotoxin exposure include activation of the complement cascade (complement is a series of immune system proteins, Section 26.9), which also triggers inflammation, and activation of the blood coagulation cascade, which can lead to blood clots and reduced blood flow. Large doses of endotoxin can cause death from hemorrhagic shock and kidney failure.

Although significant virulence factors, endotoxins are generally less toxic than most exotoxins and rarely cause symptoms that can lead to death in an otherwise healthy individual if exposure is from a gastrointestinal source (Table 25.3). For instance, in mice the LD_{50} (see Figure 25.9) for endotoxin is 200–400 *micro*grams per animal, whereas the LD_{50} for botulinum exotoxin is about 25 *pico*grams, about 10 million times less. By contrast, intravenous administration of endotoxin, for example from a heavily contaminated intravenous solution, could have fatal consequences. We see how such faulty solutions can be identified now.

Limulus Amoebocyte Lysate Assay for Endotoxin

Because endotoxins induce fever and can trigger other, more serious symptoms, pharmaceuticals such as injectable antibiotics and intravenous solutions must be free of endotoxin. An endotoxin assay of very high sensitivity and specificity in widespread use employs lysates of amoebocytes from the horseshoe crab *Limulus polyphemus* (amoebocytes are mobile cells in the blood and fluids of invertebrates that are analogous to the white blood cells of vertebrates). Endotoxin specifically causes lysis of the amoebocytes (Figure 25.19). In the *Limulus* amoebocyte lysate (LAL) assay, *Limulus* amoebocyte extracts are mixed with the solution to be tested. If endotoxin is present, the amoebocyte extract forms a gel and precipitates, causing a change in turbidity. This reaction is measured quantitatively with a spectrophotometer and can detect as little as 10 picograms of LPS in a 1-ml sample.

The LAL assay is used to detect endotoxin in clinical samples such as serum or cerebrospinal fluid. A positive test is presumptive evidence for infection by gram-negative bacteria. Drinking water, water used for formulation of injectable drugs, and injectable aqueous solutions are routinely tested using the LAL assay to identify and eliminate endotoxin contamination from gram-negative organisms. A commercially available assay uses horseshoe crab factor C made by recombinant DNA techniques (factor C is the key protein activated by endotoxin in the LAL assay). Rather than relying on amoebocytes collected from harvested horseshoe crabs, the recombinant protein is just as sensitive but less expensive, allows for a more standardized assay protocol, and is totally free of animal products.

MINIQUIZ

- What part of the *Escherichia coli* cell contains endotoxin? Why do gram-positive bacteria not produce endotoxins?
- Why is it necessary to test for endotoxin in water used for injectable drug preparations?

Chapter Review

I • Human–Microbial Interactions

25.1 If a pathogen gains access to the specific tissues it infects, disease will only occur if it first adheres to those tissues. Although adherence is required to initiate disease, it is not sufficient to initiate disease; colonization, invasion, and production of toxic substances are also required.

Q What are adhesins and what are they typically composed of?

25.2 Each body region differs chemically and physically from others and thus provides a variety of selective environments for the growth of certain microbes but not others. Colonization of tissues by a pathogenic microbe followed by growth to form populations sufficient to trigger a biological effect is necessary before disease symptoms appear.

Q Where are mucous membranes found in the body and what do they consist of? How do they prevent pathogen invasions in the human body?

25.3 The pathogenicity of a pathogen is a function of its virulence, its relative ability to cause disease. Virulence is a quantitative measure that can be assayed in terms of the number of cells (or virions, if a viral pathogen) required to infect or kill 50% of a given population—the LD_{50}. Attenuated pathogens are strains with diminished virulence and are quite useful for preparing vaccines.

Q What virulence factor, present in *Streptococcus pneumoniae* but absent from *Salmonella enterica*, makes *S. pneumoniae* so highly virulent for mice?

25.4 The genetics and physiology of both the pathogen and the host affect the outcome of an infectious disease. In *Salmonella* species, chromosomal islands or conjugative plasmids are present that encode several virulence factors; these mobile genetic elements can quickly spread a suite of virulence factors to other gram-negative bacteria. Susceptibility to an infectious disease is increased in a compromised host.

Q What factors might diminish the ability of a host to fight off an infectious disease?

II • Enzymes and Toxins of Pathogenesis

25.5 The virulence of a pathogen depends on the number and kinds of virulence factors it produces. Some pathogenic bacteria produce enzymes that function to either destroy host tissues or disarm host defenses. The activity of these enzymes releases nutrients to support growth of the pathogen and facilitate further invasiveness.

Q Give two reasons why a pathogen might benefit from the secretion of an enzyme that destroys host tissue integrity.

25.6 Exotoxins are toxic proteins and major virulence factors. Each exotoxin affects a specific host cell function. Enterotoxins are exotoxins that affect the small intestine. The clostridial botulinum and tetanus exotoxins are among the most poisonous substances known.

Q Assuming a person was poisoned with either botulinum toxin or tetanus toxin, how could the person's physical state signal the type of poisoning that had occurred?

25.7 Cytotoxins and superantigens are toxic proteins that lyse host cells and trigger a massive immune response against host tissues, respectively. Hemolysis on a blood agar plate is a classic cytotoxic effect, whereas toxic shock syndrome, a potentially fatal condition, is one result of superantigen activity.

Q Distinguish between the mechanism of cytotoxins and AB toxins, and provide one example of each.

25.8 Endotoxins are lipopolysaccharides derived from the outer membrane of gram-negative bacteria. Both the lipid and the polysaccharide components of endotoxin are necessary for toxicity. Symptoms of endotoxin poisoning include fever and intestinal distress.

Q Identify the structural features, origins, and major effects of endotoxins.

Application Questions

1. Coagulase is a virulence factor for *Staphylococcus aureus* that acts by causing clot formation at the site of *S. aureus* growth. Streptokinase is a virulence factor for *Streptococcus pyogenes* that acts by dissolving clots at the site of *S. pyogenes* growth. Reconcile these opposing strategies for enhancing pathogenicity.
2. Although mutants incapable of producing exotoxins are relatively easy to isolate, mutants incapable of producing endotoxins are much harder to isolate. From what you know of the structure and function of these types of toxins, explain the differences in mutant recovery.

Chapter Glossary

Adherence the enhanced ability of a microorganism to attach to a cell or surface

Adhesins glycoproteins or lipoproteins covalently bound to the outer layer of the pathogen that function in attachment to host tissues

Attenuation a decrease or loss of virulence

Bacteremia the presence of bacteria in the blood

Capsule a dense, well-defined polysaccharide or protein layer closely surrounding a cell

Colonization the growth of a microorganism after it has gained access to host tissues

Dental caries tooth decay resulting from bacterial infection

Dental plaque a bacterial biofilm consisting of a matrix of extracellular polymers and salivary products, found on the teeth

Disease an injury to a host organism, caused by a pathogen or other factor, that affects host function

Endotoxin the lipopolysaccharide portion of the cell envelope of most gram-negative *Bacteria*, which is a toxin when solubilized

Enterotoxin a protein released extracellularly by a microorganism as it grows that produces immediate damage to the small intestine of the host

Exotoxin a protein released extracellularly by a microorganism as it grows that produces immediate host cell damage

Infection an event during which a microorganism not a member of the local microbiota is established and grows in a host, regardless of whether the host is harmed

Invasion the ability of a pathogen to enter into host cells or tissues, spread, and cause disease

Mucous membrane layer of mucus-covered epithelial cells that interacts with the external environment

Mucus a liquid secretion that contains water-soluble glycoproteins and proteins that retain moisture and aid in resistance to microbial invasion on mucosal surfaces

Opportunistic pathogen an organism that causes disease only in the absence of normal host resistance

Pathogen an organism, usually a microorganism, that grows in or on a host and causes disease

Pathogenicity the ability of a pathogen to cause disease

Septicemia a bloodborne systemic infection

Toxicity the ability of an organism to cause disease by means of a preformed toxin that inhibits host cell function or kills host cells

Virulence the relative ability of a pathogen to cause disease

Virulence factors substances or strategies of a pathogen that indirectly or directly enhance invasiveness and host damage by facilitating and promoting infection

26

Innate Immunity: Broadly Specific Host Defenses

microbiologynow

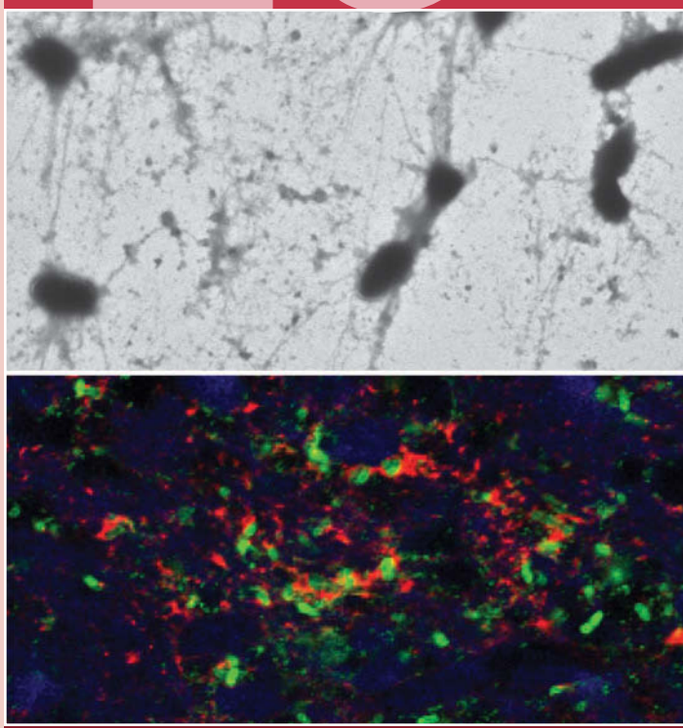
Rehabilitating a Much-Maligned Peptide: Amyloid-β

One of the hallmarks of Alzheimer's disease is the presence of amyloid plaques in the brain tissue of patients. The plaques, which develop from the aggregation of amyloid-β protein (Aβ) into insoluble fibril-like complexes, interfere with cognitive brain function and have traditionally been closely linked to Alzheimer's disease pathology. However, recent work casts Aβ in a more positive light by showing that it functions as a natural antimicrobial that protects the brain from infection and is potentially an important part of the innate immune system, the body's first-line defense against invading pathogens.

Deciphering the natural role of Aβ in the body has required a shift of prevailing thought. Despite the fact that the amino acid sequence of Aβ is highly conserved among nearly all vertebrates, it has traditionally been viewed as a functionless and even detrimental peptide that can hasten neurodegenerative disease. However, it is now evident that Aβ most certainly does have a function, and its normal role is actually protective rather than deleterious. A major clue to the true function of this protein was uncovered when scientists discovered similarities between Aβ and various ancient antimicrobial peptides, including their tendency to bind and agglutinate (clump) pathogens by aggregating into a network of fibrils (top photo). These activities suggested that Aβ might be a previously unrecognized but key component of the innate immune response, especially in the central nervous system where Aβ is abundant and efficacy of the adaptive (antibody- and cell-mediated) immune response is limited.

To examine the role of Aβ in living systems, scientists used mouse, nematode (*Caenorhabditis elegans*), and cell culture models to show that host survival following introduction of a pathogen (*Salmonella* bacteria; bottom photo, green) was enhanced by the activity of Aβ. The bacteria became entrapped in the growing mesh of Aβ fibrils (bottom photo, red), which prevented microbial adhesion to host cells and enabled more efficient pathogen destruction by immune mechanisms. Thus, the antimicrobial activity of Aβ is dependent upon its deposition and propagation of a dense fibril network. This raises the intriguing possibility that the onset of Alzheimer's-associated amyloid plaques may be precipitated by chronic microbial infection of the brain, resulting in cerebral inflammation and an overabundance of Aβ.

Source: Kumar, D.K.V., et al. 2016. Amyloid-β peptide protects against microbial infection in mouse and worm models of Alzheimer's disease. *Sci. Transl. Med. 8:* doi: 10.1126/scitranslmed.aaf1059.



We began this unit by considering the associations of microbes with humans, and in the last chapter we discussed pathogenicity, virulence, and risk factors for infection. In this and the next chapter, we focus on mechanisms used by vertebrates to resist pathogens and the diseases they cause, the science of *immunology*. In this chapter, we present concepts of **innate immunity**, inborn host defenses against a *broad range* of pathogens. In Chapter 27 we discuss **adaptive immunity**, the essential second tier of the immune system that targets *specific* pathogens to minimize their harmful effects.

I • Fundamentals of Host Defense

We begin with an overview of innate and adaptive immunity and follow this with a consideration of the human body's significant natural barriers to invasion by pathogens.

26.1 Basic Properties of the Immune System

Immunity is the ability of an organism to resist infection. The human immune system employs a two-pronged defense against invading pathogens. *Innate immunity*, the first of these interconnected defensive mechanisms, is the built-in capacity of the immune system of multicellular organisms to target common pathogens regardless of their identity. By contrast, *adaptive immunity* is triggered by exposure to specific pathogens that cannot be eliminated from the body by innate mechanisms alone. Each adaptive immune response is specifically targeted to a particular type of invading pathogen, such as a single strain of virus. Figure 26.1 compares and contrasts the fundamental elements of each of these indispensible branches of the immune system.

Principles of Innate Immunity

Innate immunity is a noninducible, preexisting ability of the body to recognize and destroy a broad range of pathogens or their

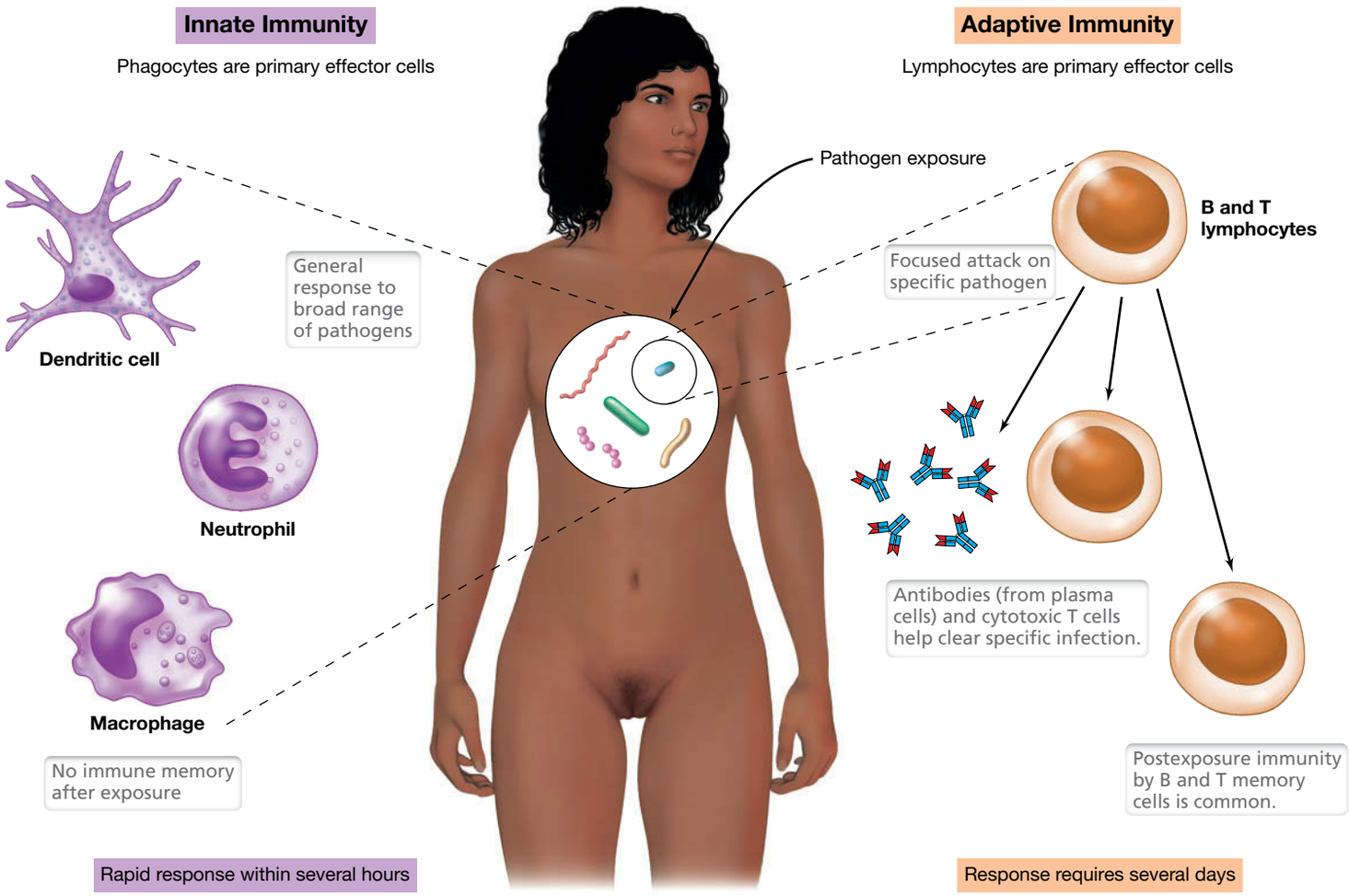


Figure 26.1 Overview of the two-pronged immune response. Principal characteristics of innate and adaptive immunity are depicted. Note the cell types that participate in each form of immunity and compare with their functional descriptions shown in Figure 26.4.

products. Innate immune mechanisms do not require previous exposure to a pathogen or its products for activation. Eukaryotes have functionally similar pathogen recognition mechanisms that lead to rapid and effective host defense. For example, pathogen recognition in the insect *Drosophila* (fruit fly) is carried out in much the same way as it is in humans, using immune cells that clearly show structural and evolutionary homology.

In addition to a variety of physical and chemical barriers to infection that we will describe in Section 26.2, innate immunity is largely dependent on the activity of **phagocytes**, cells of several types that can ingest, kill, and digest microbial pathogens. Innate immune responses develop quickly, within a few hours of exposure to a pathogen. Structural features common to a variety of bacterial pathogens, such as flagella and certain constituents of the cell wall, interact with universal receptors on phagocytes. This interaction activates genes in the phagocyte whose gene products lead to pathogen destruction.

Principles of Adaptive Immunity

Some pathogens are so virulent that innate responses are not completely effective. When this is the case, the innate response activates the adaptive immune response to deal with these infections. Adaptive immunity is the *acquired* ability to recognize and destroy a specific pathogen or its products. Adaptive responses show **specificity** because they are directed at unique pathogen surface molecules called **antigens**, which often define a particular strain of pathogen or type of foreign material. Phagocytes ingest and process antigen molecules and present them to immune cells called **lymphocytes**, essential components of the adaptive response. The presented antigens bind specific receptors on the surface of the lymphocyte, triggering genes that promote lymphocyte multiplication and production of antigen-specific proteins called **antibodies (immunoglobulins)** that interact with the pathogen and mark it for destruction.

Unlike the comparatively rapid innate response, a protective adaptive response usually takes several days to develop, and the strength of the adaptive response increases as the numbers of antigen-reactive lymphocytes increase. Although adaptive immune responses are typically slower than innate response mechanisms, they are highly specific and often result in **immune memory**, the ability to quickly produce specific immune cells or antibodies after subsequent exposure to a previously encountered antigen.

With a broad overview of inborn and acquired immune responses in place, we now consider the many natural barriers to infection that exist in the healthy human body. It is only after one or more of these barriers has been breached that pathogen invasion can occur.

MINIQUIZ

- What major class of immune cells mediates an innate immune response? What additional type of immune cells is required for an adaptive immune response?
- What term is used to describe the unique molecules found on the surface of different pathogens?

26.2 Barriers to Pathogen Invasion

The best defense against infectious diseases for the human body is to prevent pathogens from colonizing in the first place. Humans have a host of innate resistance factors that inhibit pathogen colonization and thereby prevent the onset of infectious diseases. These include a variety of physical and chemical barriers to microbial infection. In addition, the condition of the host and the composition of microorganisms that normally inhabit the host are factors that are often the tipping point between health and disease.

Natural Host Resistance

Several resistance factors common to vertebrate hosts inhibit infection by most pathogens in a nonspecific way (**Figure 26.2**). For example, the normal microbiota associated with the human body are critically important for resisting pathogen infection, especially on the skin and in the gut (Chapter 24). Pathogens do not easily infect tissues on which normal microbiota are well established because the harmless microbes limit available nutrients and sites for infection by the pathogens. Although the *competitive exclusion* of invading pathogens by resident microbes is technically not a component of the host's immune system, the nonpathogenic normal microbiota found in or on the body play a major role in preventing disease through this principle. It is not uncommon for disruptions to the composition of the normal microbiota, such as that which may occur when antibiotic drug treatments nonspecifically kill harmless and even beneficial microbes in the body, to trigger the onset of disease by opportunistic pathogens.

The ability of a particular pathogen to cause disease in an animal is highly variable. For instance, certain animal species, including raccoons and skunks, are much more susceptible to the rabies virus than others, such as the opossum, which only rarely develops the disease. Anthrax infects many species of animals, causing disease symptoms varying from fatal blood poisoning in cattle to the mild pustules of human cutaneous anthrax. Introduction of the same pathogen by other routes, however, may challenge the resistance of the host. For example, anthrax causes a localized infection when acquired through the skin but a lethal, systemic infection when acquired through the mucous membranes of the lungs (Sections 29.9 and 31.8). Another barrier to infection is the fact that diseases of endothermic ("warm-blooded") animals, such as birds and mammals, are rarely transmitted to ectothermic ("cold-blooded") animals, such as amphibians and reptiles, and vice versa. Presumably, the anatomical and metabolic features of one group are not compatible with pathogens that infect the other group.

In addition to these factors, the general condition of the host plays a role in the balance between health and disease. Infectious diseases are typically more common in the very young and the very old, as well as those suffering from nutritional or stress-related problems. For example, the intestinal microbiota matures over time and thus infants under one year of age often contract diarrhea caused by bacterial or viral pathogens more readily than do adults. Conversely, the immune system of older adults may be

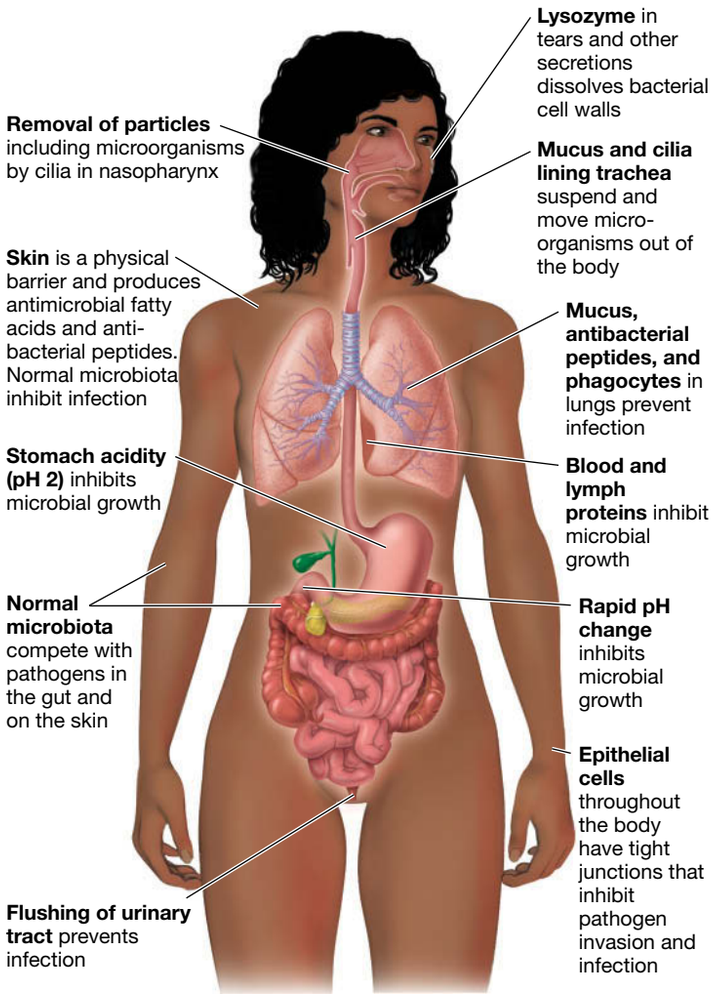


Figure 26.2 Barriers to infection in the human body. These barriers provide natural resistance to colonization and infection by pathogens.

weakened or compromised from medical procedures or drug therapy, leading to a higher susceptibility to infectious disease. Other issues that affect both the young and the old—smoking, poor diet, intravenous drug use, excessive alcohol consumption, chronic lack of sleep—can all play a role in whether a pathogen can infect a host and cause disease.

Infection Site and Tissue Specificity

Most pathogens must adhere and infect at the site of exposure to initiate infection. Even if pathogens adhere to an exposure site, the organisms cannot colonize the host if the site is not compatible with the pathogen's nutritional and metabolic needs. For example, if cells of *Clostridium tetani* (cause of the disease tetanus) were ingested, tetanus would not normally result because the pathogen would be either killed by the acidity of the stomach or unable to compete with the well-developed normal intestinal microbiota. If, on the other hand, *C. tetani* cells or endospores were introduced into a puncture wound, the organism would grow and produce tetanus toxin in the anoxic zones created by local tissue death. Conversely, enteric bacteria, such as *Salmonella* and *Shigella*, do not normally cause wound infections but can infect the intestinal tract and cause gastrointestinal illness.

In some cases, pathogens interact exclusively with members of a few closely related host species because the hosts share tissue-specific receptors, important in establishing infection (Section 25.2). For example, human immunodeficiency virus (HIV, the causative agent of AIDS) infects only humans and their closest primate relatives, including the great apes. This is because the HIV-binding cell surface proteins CXCR4, present on human T lymphocytes, and CCR5, present on human macrophages (T cells and macrophages are cells of the immune system), are also expressed in great apes. Other animals, even most other primates, lack CXCR4 and CCR5 and are therefore not susceptible to HIV infection. Many other pathogens exhibit tissue specificity to such a degree that they infect only a single species. For example, a rhinovirus that infects nasal epithelial cells in humans, causing symptoms of the common cold, would generally not infect and cause similar symptoms in a nonhuman host. Table 26.1 presents some other examples of tissue specificity in pathogens.

Physical and Chemical Barriers to Infection

The structural integrity of tissue surfaces poses a barrier to penetration by microorganisms (Section 25.2). The tight junctions between epithelial cells in all body tissues inhibit invasion and infection (Figure 26.2). In the skin and mucosal tissues, potential pathogens must first adhere to tissue surfaces and then grow at these sites before traveling elsewhere in the body. Mucosal surfaces are coated with a layer of protective mucus that traps microorganisms, pollen, and other foreign agents. Epithelial

TABLE 26.1 Tissue specificity in infectious disease

Disease	*Tissue infected*	*Pathogen*
Acquired immunodeficiency syndrome (AIDS)	T-helper lymphocytes	Human immunodeficiency virus (HIV)
Botulism	Motor end plate	*Clostridium botulinum*
Cholera	Small intestine epithelium	*Vibrio cholerae*
Dental caries	Oral epithelium	*Streptococcus mutans, S. sobrinus, S. mitis*
Diphtheria	Throat epithelium	*Corynebacterium diphtheriae*
Gonorrhea	Mucosal epithelium	*Neisseria gonorrhoeae*
Influenza	Respiratory epithelium	Influenza A and influenza B virus
Malaria	Blood (erythrocytes)	*Plasmodium* spp.
Pyelonephritis	Kidney medulla	*Proteus* spp.
Spontaneous abortion (cattle)	Placenta	*Brucella abortus*
Tetanus	Inhibitory interneuron	*Clostridium tetani*

cells underlying the mucus layer have cilia on their surfaces that carry out coordinated movements to expel suspended pathogens and keep them from adhering to tissues. This combination of mucus and ciliary motion plays a key role in removing inhaled microorganisms from the mucosal surfaces of the trachea and bronchial tubes, thus preventing microbial colonization of the lungs.

Sebaceous glands in the skin secrete fatty acids and lactic acid, lowering the acidity of the skin to pH 5 and inhibiting colonization by many pathogenic bacteria (blood and most internal organs are about pH 7.4). Potential pathogens ingested in food or water must survive the strong acidity (pH 2) and digestive enzymes, such as pepsin, in the stomach (Figure 26.2). Although the pH returns to neutral in the lower intestinal tract, pathogens that survive passage through the stomach must then compete with the abundant resident microbiota present in the small and large intestines (Figure 24.3). In addition to the established normal microbiota that exist at many potential infection sites, resistance to infection and invasion is enhanced by antibacterial substances called *defensins*, produced in the skin, lungs, and gut. Finally, the lumen of the kidney, the eye, the respiratory system, and the cervical mucosa are constantly bathed with tears, mucus, or other secretions that contain *lysozyme*, an enzyme that can kill bacteria by digesting the cell wall.

We have seen how the human body is naturally protected by a host of physical barriers, chemicals, and secretions, all of which combine to naturally suppress pathogen invasion and infection. Beyond this first line of defense, however, the immune system becomes activated, beginning with innate responses. We consider the tools of the innate immune response—the cells and organs of the immune system—now.

MINIQUIZ

- Describe host tissue specificity for pathogens.
- Identify physical and chemical barriers to pathogens. How might these barriers be compromised?
- What other factors may control the outcome of an infectious disease?

II • Cells and Organs of the Immune System

The immune response results from the activities of a wide variety of specialized cells that circulate throughout the body, primarily through the blood and *lymph*, a fluid similar to blood but which lacks red blood cells. Blood and lymph interact directly or indirectly with every major organ system.

26.3 The Blood and Lymphatic Systems

Circulatory systems in the human body include separate vasculature for blood and lymph. It is through circulation of these fluids that cells and proteins of the immune system are transported to the various tissues and organs of the body. All cells found in the blood and lymph are derived from **stem cells** in the bone marrow. These cells are continuously dividing and differentiating to supply the body with both erythrocytes (red blood cells) and **leukocytes** (white blood cells).

Blood and Lymph Circulation

Blood is pumped by the heart through arteries and capillaries throughout the body and is returned through the veins (**Figure 26.3*a, b***). In the capillary beds, leukocytes and solutes pass to and from the blood into the lymphatic system. Lymph drains from extravascular tissues into lymphatic capillaries (lymph ducts) and then into **lymph nodes** throughout the lymphatic system (Figure 26.3*c*, *d*). Lymph nodes contain lymphocytes and phagocytes arranged to encounter microorganisms and antigens as they travel through the lymphatic circulation. **Mucosa-associated lymphoid tissue (MALT)** is another part of the lymphatic system that interacts with antigens and microorganisms that enter the body through mucous membranes, including those of the gut, the genitourinary tract, and bronchial tissues. MALT also contains phagocytes and lymphocytes. Lymph fluid with antibodies and immune cells empties into the blood circulatory system via the thoracic lymph duct (Figure 26.3*a*).

The *spleen* is an important organ in immunity and consists of *red pulp*—rich in red blood cells—and *white pulp*, which consists of organized lymphocytes and phagocytes arranged to filter blood in a manner similar to lymph nodes and MALT in the lymphatic system. Collectively, the lymph nodes, MALT, and spleen are called **secondary lymphoid organs** (Figure 26.3). The secondary lymphoid organs are the sites where antigens interact with antigen-presenting phagocytes and lymphocytes to trigger the adaptive immune response.

Hematopoietic Stem Cells, Blood, and Lymph

Hematopoietic stem cells are precursor cells found in bone marrow that can differentiate into any blood cell (see Figure 26.4). Stem cells grow in the bone marrow where they differentiate into a variety of cell types under the influence of soluble cytokines and chemokines, proteins that influence many aspects of immune cell differentiation (Section 26.5). The differentiated cells then travel through the blood and lymph to other parts of the body.

Blood consists of cellular and noncellular components, including many cells and molecules active in the immune response (**Table 26.2**). The most numerous cells in human blood are *erythrocytes*, small, nonnucleated cells that carry oxygen from the lungs to the tissues. About 0.1% of the cells in blood, however, are white blood cells—leukocytes—of which there are many different types (Table 26.2). All phagocytes of the innate immune system are leukocytes, as are lymphocytes, the cells active in the adaptive response.

Blood is composed of suspended cells and **plasma**, a liquid that contains proteins and other solutes. Outside the body, blood or plasma quickly forms an insoluble fibrin clot, remaining liquid only when an anticoagulant such as potassium citrate or heparin is added. When blood clots, the insoluble proteins trap the cells in a large, insoluble mass. The remaining fluid, called **serum**, lacks both cells and clotting proteins. Serum does, however, contain a

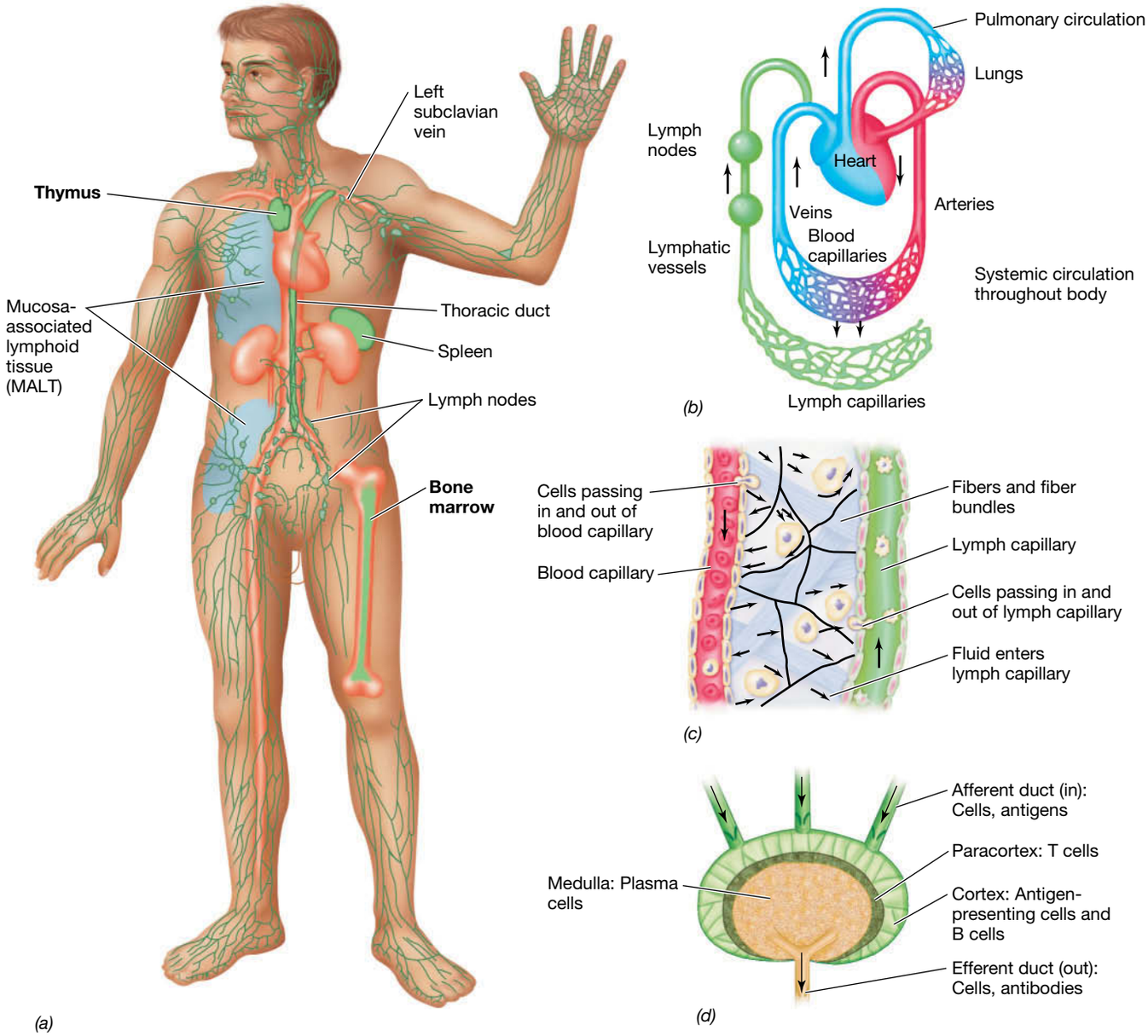


Figure 26.3 The blood and lymphatic systems. *(a)* Blood and lymph circulation in the body. Major blood vessels and associated organs are shown in red. Major lymphatic organs and vessels are shown in green. The primary lymphoid organs are the bone marrow and thymus. The secondary lymphoid organs are the lymph nodes, spleen, and MALT. *(b)* Connections between the lymphatic and blood systems. Blood flows from the veins to the heart, to the lungs, and then through the arteries to capillaries in tissues. Exchange of solutes and cells occurs between blood and lymphatic capillaries. Lymph drains from the thoracic duct into the left subclavian vein of the blood circulatory system. *(c)* The exchange of cells between the blood and lymphatic systems is shown in detail. Both blood and lymphatic capillaries are closed vessels, but cells pass from blood capillaries to lymphatic capillaries and back by the process of extravasation. *(d)* A secondary lymphoid organ, the lymph node, showing the major anatomical areas and the immune cells concentrated in each area. The anatomy of the MALT and the spleen is analogous to that of the lymph nodes.

high concentration of other proteins, in particular antibodies, key players in the adaptive immune response.

MINIQUIZ

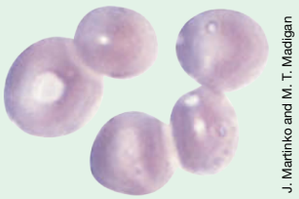
- Describe the circulation of a leukocyte from the blood to the lymph and back to the blood.
- What soluble molecules determine whether a particular stem cell will become a phagocyte, lymphocyte, or erythrocyte?

26.4 Leukocyte Production and Diversity

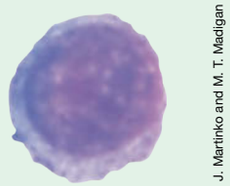
Several distinct leukocytes participate in innate and adaptive immunity (Table 26.2). From its hematopoietic stem cell precursor, a leukocyte will differentiate and mature through either the *myeloid* lineage or the *lymphoid* lineage (Figure 26.4). Leukocytes move throughout the body and, through a process called *extravasation* (also called *diapedesis*), pass from blood to interstitial spaces. They are then collected with lymph into lymphatic

TABLE 26.2 Major cell types found in normal human blood

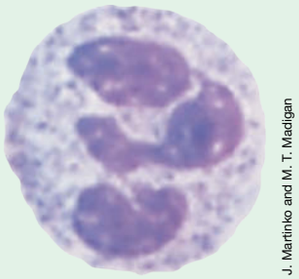
Cell type	*Cells per milliliter*
Erythrocytes	$4.2–6.2 \times 10^9$
Leukocytes[a]	$4.5–11 \times 10^6$
Lymphocytes	$1.0–4.8 \times 10^6$
Granulocytes and monocytes	Up to 7.0×10^6



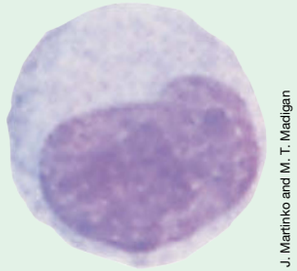
(a) **Red blood cells (erythrocytes)**



(b) **Lymphocyte**



(c) **Neutrophil (a granulocyte)**



(d) **Monocyte**

[a]Leukocytes include all nucleated blood cells. They include lymphocytes and cells derived from myeloid stem cells, the monocytes and granulocytes, such as neutrophils.

vessels, where they are eventually returned to the blood circulatory system (Figure 26.3*c*).

Myeloid Cells—Monocytes and Granulocytes

Myeloid cells, active in innate immunity, are derived from myeloid precursor cells. Mature myeloid cells develop from one of two lineages: **monocytes** or **granulocytes** (Figure 26.4 and Table 26.2). Immature monocyte precursor cells circulate in the blood for several days before moving into other tissues and differentiating into specialized phagocytic cells called **antigen-presenting cells (APCs)**. These cells engulf, process, and present antigens to lymphocytes to initiate an adaptive immune response (Chapter 27). Phagocytic APCs derived from monocytes include **macrophages** and **dendritic cells** (Figure 26.4).

Macrophages are often the first defensive cells that interact with a pathogen and are abundant in many tissues and organs, especially the spleen, lymph nodes, and MALT, where they constitute up to 15% of total cells. Because they ingest and destroy most pathogens and foreign molecules that invade the body, macrophages are essential to the innate response. Dendritic cells are found throughout the body tissues and are especially abundant along epithelial linings, including the skin and mucous membranes. When dendritic cells ingest antigen, they migrate to nearby lymph nodes, where they are extremely efficient at presenting antigen to T lymphocytes. Thus, dendritic cells are an important link between innate and adaptive immunity. The specialized antigen-presenting properties of macrophages and dendritic cells will be examined in Section 27.5.

Granulocytes are the second lineage of cells derived from myeloid precursors. Granulocytes contain cytoplasmic inclusions, or granules, that are visible in stained microscopic preparations. These granules contain toxins or enzymes that are released to destroy target cells. One granulocyte, the **neutrophil**, also called a *polymorphonuclear leukocyte* (*PMN*), is an abundant, highly motile phagocyte that responds rapidly to pathogen challenge, an activity that is central to innate immunity. Neutrophils are found predominantly in the bloodstream and bone marrow, from where they migrate to sites of active infection. A second type of granulocyte, called a **mast cell**, functions to initiate an inflammatory response by releasing its granule contents, a process called *degranulation*. Mast cell degranulation is responsible for certain types of allergic reactions (Section 27.9).

Lymphocytes

Lymphocytes are specialized leukocytes derived from lymphoid precursor cells (Figure 26.4 and Table 26.2). There are three types of lymphocytes: *B cells* (B lymphocytes), *T cells* (T lymphocytes), and *natural killer cells* (Figure 26.4). Mature B and T cells circulate through the blood and lymph system but are concentrated in the lymph nodes and spleen where they interact with antigens.

B cells originate and mature in the *bone marrow*. Like dendritic cells and macrophages, B cells are specialized APCs, but in addition to this, they are the precursors of antibody-producing **plasma cells**. Antibodies (immunoglobulins) are soluble proteins that interact with specific antigens and are produced by B cells and plasma cells. **T cells**, which interact with antigens presented by APCs, begin their development in the bone marrow but migrate to the *thymus* to mature (Figure 26.3*a* and see Figure 27.2). In mammals, the bone marrow and thymus are called **primary lymphoid organs** because they are the sites where lymphoid stem cells mature into functional, antigen-reactive lymphocytes (Figure 26.3*a*). We detail the role of B and T lymphocytes in the adaptive immune response in Chapter 27.

Like B and T cells, natural killer (NK) cells (Figure 26.4) are derived from lymphoid precursors. However, unlike B and T lymphocytes, NK cells function primarily in innate immunity. NK cells rid the body of virus-infected and tumor cells using a mechanism that distinguishes healthy versus compromised cells based on the presence (or absence) of specific surface molecules (Section 26.10).

MINIQUIZ

- How does the development of B, T, and NK cells differ from that of dendritic cells and macrophages?
- Distinguish between the primary lymphoid organs and the secondary lymphoid organs. What is the relationship between the components of these two groups?

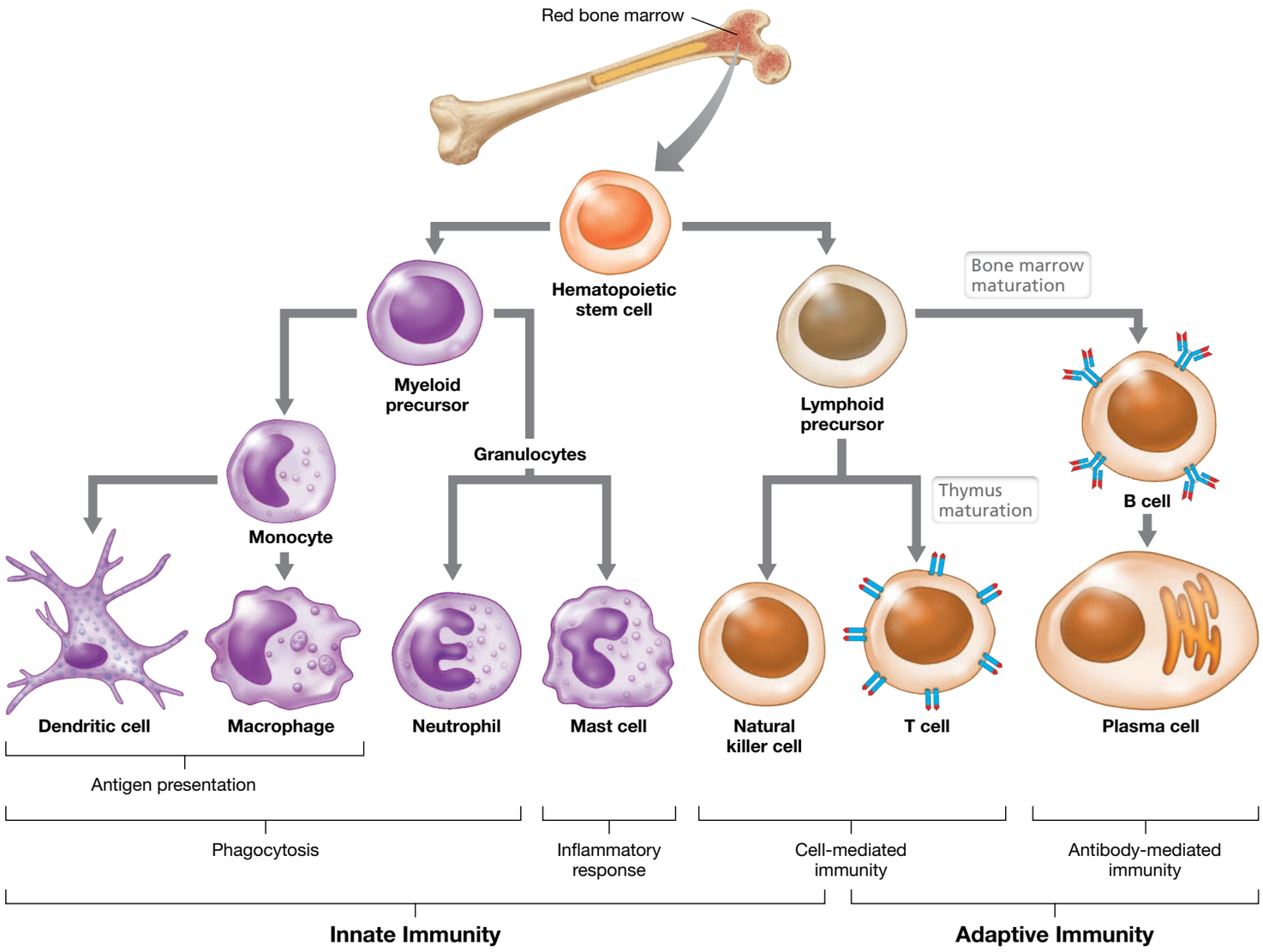


Figure 26.4 Lineage and diversity of immune response cells. Immune cells develop from hematopoietic stem cells in the bone marrow into either myeloid precursors or lymphoid precursors. These precursors, in turn, differentiate into mature cells that have various immune functions. (Erythrocytes also develop from myeloid precursors.)

III • Phagocyte Response Mechanisms

Innate immunity is primarily driven by the activities of phagocytes (Section 26.1). These cells recognize common structural features found on and in pathogens. Interactions with pathogens activate genes in the phagocytes that control the transcription, translation, and expression of proteins that destroy the pathogens.

Innate immunity develops immediately when a phagocyte contacts a pathogen, but it is not always effective enough to prevent dangerous infections. To counter these, certain phagocytes also activate adaptive immunity by processing and presenting antigens to receptors on lymphocytes (Chapter 27). However, because an adaptive response to a specific pathogen takes several days to develop, the ability of phagocytes to quickly respond to microbial invasion is indispensible for controlling infection.

26.5 Pathogen Challenge and Phagocyte Recruitment

Successful pathogens can breach physical and chemical host barriers (Figure 26.2), leading to host infection. When this happens, the immune system is mobilized to protect the host from further damage. Innate immunity is the first line of defense and is critical for host protection for about four days after an infection begins. Phagocytes engulf and destroy pathogens, often initiating complex host-mediated inflammatory reactions when they do (Section 26.8).

Microbial Invasion

The initial inoculum of a pathogen is usually insufficient to cause host damage, even if a pathogen gains access to tissues. For the pathogen to be successful, it must attach, multiply, and colonize the tissue (↩ Figure 25.1), and these events require that the

pathogen find appropriate nutrients and environmental conditions for growth.

Following colonization, a pathogen must usually invade tissues to initiate disease. **Invasion** is the ability of a pathogen to enter into host cells or tissues, multiply, spread, and cause disease. In most cases, microbial infections begin at breaks or wounds in the skin or on the mucous membranes of the respiratory, digestive, or genitourinary tract, surfaces that are normally microbial barriers (Figure 26.2). In some cases, growth may also begin on intact mucosal surfaces, especially if the composition of the normal microbiota has been altered or eliminated, for example by antibiotic therapy. Tissue damage caused by invasive pathogens or injury triggers the recruitment of large numbers of phagocytes and other immune cells to the site of infection.

Tissue Damage and Chemokine Release

When microbial invasion causes trauma to host tissues, resident phagocytes and damaged host cells release **cytokines**, a family of soluble proteins that function as chemical mediators to allow communication between different cells of the body (Figure 26.5*a*). Cytokines bind specific receptors on cells and induce a particular response from them, usually by activating a signaling pathway that controls transcription and protein synthesis. **Chemokines** are an important subclass of cytokines that have the specific role of recruiting immune cells to sites of injury.

Resident macrophages, which are found in all of the body's organ systems, are stimulated by the presence of invading pathogens to secrete chemokines that establish a gradient of *chemoattractants* (Figure 26.5*b*). These molecules bind receptors on other immune cells, especially neutrophils and T cells, and recruit them to the site of infection. This triggers both a neutrophil-mediated inflammatory response and, later, an adaptive immune response facilitated by lymphocytes against the specific invading agent or its toxic products.

Localized inflammation allows neutrophils, the most numerous circulating phagocytes, to migrate quickly along the chemotactic gradient to the site of infection (Figure 26.5*b*). Once there, chemokine-mediated binding reactions promote neutrophil adhesion to the inner wall of the blood capillary, a process called *margination*. The halted neutrophils then undergo extravasation, the movement of leukocytes from the bloodstream to surrounding infected tissues by squeezing between endothelial cells lining the capillaries (Figure 26.5*b*). Higher than normal numbers of neutrophils in the blood or at a site of inflammation indicate an active response to a current infection.

Neutrophils and other phagocytes that encounter pathogens in damaged areas must be able to recognize, capture, and destroy pathogens to clear infections and restore body tissues to a healthy state (Figure 26.5*c*). We move on to explore the molecular mechanisms that facilitate these cellular interactions in the next section.

MINIQUIZ

- Although technically not part of the immune system, nonpathogenic normal microbiota play a major role in preventing disease. Describe this role.
- Describe the mechanisms by which circulating phagocytic cells are recruited to a site of infection.

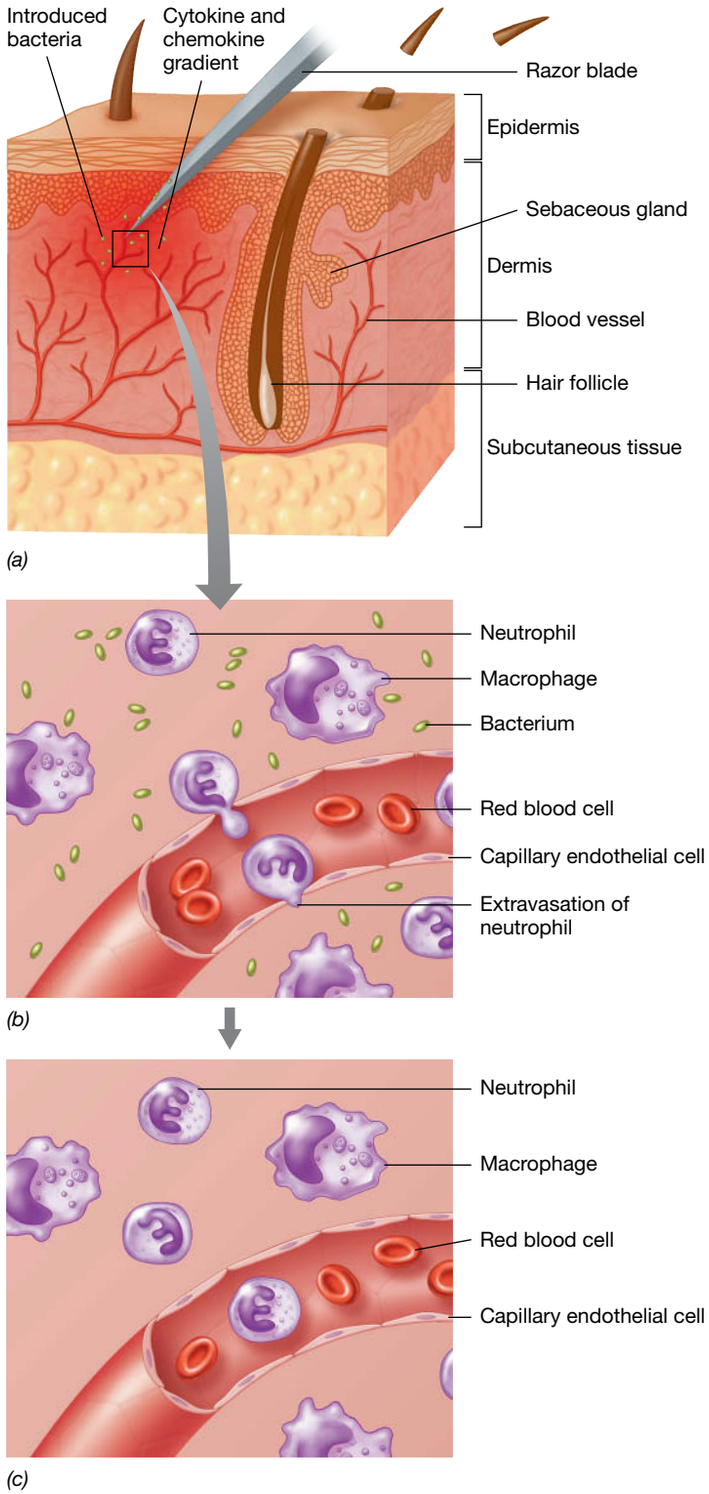


Figure 26.5 Microbial invasion and the innate immune response. (*a*) Tissue damage, such as that caused by a cut from a razor, can lead to invasion by microorganisms and the release of cytokines and chemokines from damaged cells. (*b*) Phagocytes are recruited to the site of infection by the chemokine gradient, squeezing out of dilated capillaries via extravasation (diapedesis). (*c*) Invading microorganisms are cleared by phagocytosis, and the tissue is restored to health. See Figure 26.4 for functional descriptions of some of the cells and components shown here.

26.6 Pathogen Recognition and Phagocyte Signal Transduction

The first type of immune cell to be activated in the innate response is typically a phagocyte, whose primary function is to engulf and destroy pathogens. Phagocytes include neutrophils and monocytes, which are found primarily in the blood, and macrophages and dendritic cells, which occur primarily in the body tissues (Figure 26.4). But how do these cells distinguish pathogens and other foreign agents from the myriad other cells in the body? Moreover, once invaders are recognized, how do phagocytes capture and destroy them? We address these topics now.

Pathogen-Associated Molecular Patterns

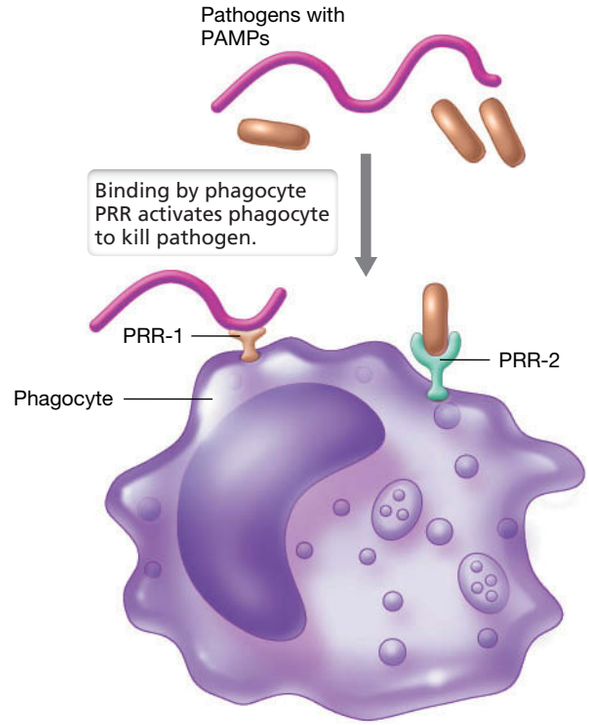
The macromolecules inside and on the surface of pathogens display **pathogen-associated molecular patterns (PAMPs)**, repeating structural subunits common to broadly related groups of infectious agents. PAMPs may include polysaccharides, proteins, nucleic acids, or even lipids. For example, a common PAMP is the lipopolysaccharide (LPS) common to all gram-negative bacterial outer membranes (Section 2.5). Other PAMPs include bacterial flagellin, the double-stranded RNA (dsRNA) of certain viruses, and the lipoteichoic acids of gram-positive bacteria (Section 2.4). Like all PAMPs, these molecules are found on various pathogens but are absent from host cells. Therefore, PAMPs serve as markers by which phagocytes can identify pathogenic microbes, even if the pathogens have not been encountered previously.

Pattern Recognition Receptors

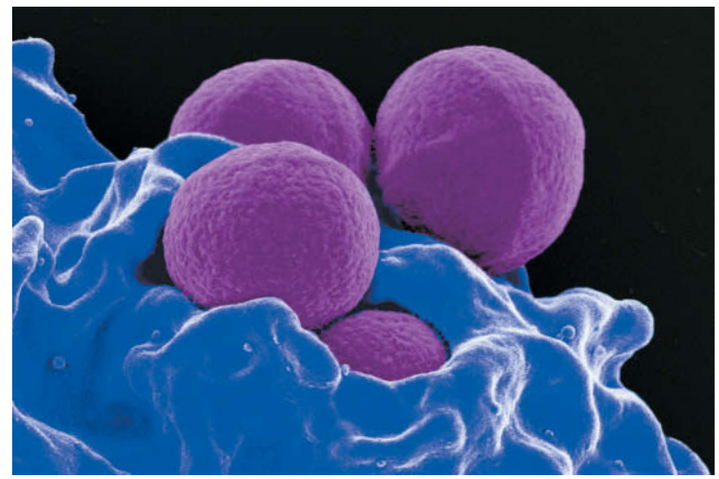
Phagocytes have a pathogen-recognition system that triggers a timely and appropriate response, leading to recognition, containment, and destruction of pathogens. Phagocytes can interact quickly and effectively with pathogens because phagocyte surfaces contain numerous **pattern recognition receptors (PRRs)**, soluble or membrane-bound proteins that recognize and bind PAMPs (**Figure 26.6*a***). The interaction of a PAMP with a PRR activates the phagocyte to ingest and destroy the targeted pathogen by phagocytosis (Figure 26.6*b* and Section 26.7).

PRRs were first observed in phagocytes of *Drosophila*, the fruit fly, where they are called *Toll receptors* (see Explore the Microbial World, "*Drosophila* Toll Receptors—An Ancient Response to Infections"). Structural, functional, and evolutionary homologs of the Toll receptors, called **Toll-like receptors (TLRs)**, are widely expressed on mammalian innate immune cells. TLRs are found associated with membranes on the surface of or in intracellular vesicles of all types of phagocytes. At least nine TLRs in humans interact with a variety of cell surface and soluble PAMPs from viruses, bacteria, and fungi (**Table 26.3**).

Each TLR on a human phagocyte recognizes and interacts with a specific PAMP. For example, TLR-2, a PRR on human phagocytes, interacts with peptidoglycan, a PAMP present in the cell wall of nearly all bacteria (**Figure 26.7**). This PAMP–PRR interaction activates the phagocytes, which then target gram-positive pathogens with exposed peptidoglycan. Access to the peptidoglycan of gram-negative bacterial cell walls is blocked by the surface lipopolysaccharides. However, another PRR found on phagocytes, TLR-4,



(a)



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(b)

Figure 26.6 Pathogen recognition by phagocytes. (*a*) Phagocytes interact with pathogens by way of preformed pattern recognition receptors (PRRs) that bind to pathogen-associated molecular patterns (PAMPs). Binding of a PAMP by a phagocyte PRR stimulates the phagocyte to destroy the pathogen and activate other phagocytes. (*b*) Colorized scanning electron micrograph of a neutrophil (blue) phagocytosing several cells of methicillin-resistant *Staphylococcus aureus* (MRSA; pink). For more on MRSA, see Sections 28.12 and 30.9.

interacts with the endotoxic LPS of gram-negative bacteria, including that from all pathogenic strains of *Salmonella* spp., *Escherichia coli*, and *Shigella* spp. (Table 26.3 and see Figure 26.8).

Several soluble host molecules function similarly to these phagocyte-associated PRRs. The *NOD-like receptors* (*NLRs*) are a family of soluble PRRs found in the cytoplasm that have a nucleotide-binding domain (NOD). NOD1 and NOD2 interact

DROSOPHILA TOLL RECEPTORS—AN ANCIENT RESPONSE TO INFECTIONS

Invertebrates and plants lack adaptive immunity but have a well-developed innate immune response to a wide variety of pathogens. Virtually all multicellular organisms respond by recognizing molecules found on the pathogenic cell or virus. These molecules contain conserved, repetitive structures called pathogen-associated molecular patterns (PAMPs) that include such things as the lipopolysaccharide (LPS) and flagellin of gram-negative bacteria, the peptidoglycan of gram-positive bacteria, and the double-stranded RNA unique to certain viruses, among others. By recognizing features found in many pathogens, the innate immune mechanism provides protection against a broad range of common pathogenic agents.

Responses to pathogens by the fruit fly, *Drosophila melanogaster* (**Figure 1**), have provided insight into innate immune mechanisms in many other groups of organisms. Several proteins required for fruit fly development are also important receptors for recognizing invading bacteria. These proteins function in their immune response role as pattern recognition receptors (PRRs) that interact with PAMPs on the macromolecules produced by the pathogen. The best example of a PRR is *Drosophila* Toll, a transmembrane protein essential for dorsoventral axis formation, as well as the innate immune response of the fly.

Toll immune signaling is initiated by the interaction of a pathogen or its components with the Toll protein displayed on the surface of phagocytes. *Drosophila* Toll, however, does not interact directly with the pathogen. Signal transduction events start with the binding of a PAMP, such as the LPS of gram-negative bacteria, by one or more accessory proteins (Figure 26.8 shows the analogous TLR-4 system in humans). The LPS–accessory protein complex then binds to Toll. The membrane-integrated Toll protein initiates a signal transduction cascade, activating a nuclear transcription factor and inducing transcription of several genes that encode antimicrobial peptides. Toll-associated transcription factors induce expression of antimicrobial peptides including drosomycin, active against fungi; diptericin, active against gram-negative bacteria; and defensin, active against gram-positive bacteria. These peptides, produced in the liver-like fat body of *Drosophila*, are released into the fly's circulatory system where they interact with the target organism and cause cell lysis.

Structurally, the Toll proteins are related to lectins, a group of proteins found in all multicellular organisms, including invertebrates and plants. Lectins interact specifically with certain oligosaccharide monomers. In humans, Toll-like receptors (TLRs) react with a wide variety of PAMPs. As with *Drosophila* Toll, human TLR-4 provides innate immunity against gram-negative bacteria through indirect interactions with LPS, initiating a kinase signal cascade and activating nuclear transcription factor NFκB that activates transcription of cytokines and other phagocyte proteins key to the host's innate immune response (Figure 26.8).

Jarmo Holopainen

Figure 1 *Drosophila melanogaster*, the common fruit fly. The Toll protein, a homolog of the Toll-like receptors of higher vertebrates, was first discovered in the fruit fly.

Drosophila Toll is an evolutionary, structural, and functional relative of the Toll-like receptors present in vertebrates, including humans. Toll and its homologs are evolutionarily ancient, highly conserved components of the innate immune system in animals and have even been found in plants. The absence of a highly specific adaptive immune response in invertebrates points to this more specific system as appearing later in the course of evolution, possibly as a mechanism to counter disease threats that could not be controlled by the innate response alone.

with peptidoglycan components of gram-negative and gram-positive bacterial cell walls, respectively, stimulating production of antimicrobial peptides and inflammatory cytokines (Table 26.3). NOD-like receptor pyrin 3 (NLRP3) interacts with other proteins to form a structure called an *inflammasome*. The cytoplasmic inflammasome senses cellular stress indicators, such as the loss of potassium ions (K^+) from damaged cells, and triggers the production of proinflammatory cytokines, initiating inflammation. Later in this chapter we discuss the soluble PRRs in the context of their ability to activate proteins that enhance phagocytosis and destruction of pathogens (Section 26.9).

Signal Transduction in Phagocytes

Interaction of a PAMP with a PRR triggers transmembrane *signal transduction*, initiating gene transcription and translation of host-response proteins in a fashion similar to the two-component regulatory systems previously discussed in *Bacteria* and *Archaea* (Section 6.6). Signal transduction initiated by PAMP–PRR interaction results in enhanced phagocytosis, killing of pathogens, inflammation, and tissue healing.

For example, the binding of LPS (a PAMP derived from the cell wall of degraded gram-negative bacteria) to TLR-4 (a PRR on the surface of phagocytes) typically activates a signal transduction pathway (**Figure 26.8**). TLR-4 then binds proteins in the cytosol of the phagocyte, starting a cascade of reactions that activates transcription factors such as NFκB (nuclear factor kappa B), a protein that binds to specific regulatory sites on DNA, initiating transcription of downstream genes. Many of the NFκB-regulated genes encode host-response proteins, such as the cytokines that activate cells and initiate inflammation (Section 26.5).

TABLE 26.3 Receptors and targets in the innate immune response

Pattern recognition receptors (PRRs)	*Pathogen-associated molecular patterns (PAMPs) and targets*	*Result of interaction*
Soluble extracellular PRRs[a]		
Mannose-binding lectin (soluble)	Mannose-containing components of microbial cell surface, as in gram-negative bacteria	Complement activation
C-reactive protein (soluble)	Components of gram-positive cell walls	
Plasma membrane–associated PRRs		
TLR-1 (Toll-like receptor 1)	Lipoproteins in mycobacteria	Signal transduction, phagocyte activation, and inflammation[c]
TLR-2	Peptidoglycan on gram-positive bacteria; zymosan in fungi	
TLR-4	LPS (lipopolysaccharide) in gram-negative bacteria	
TLR-5	Flagellin in bacteria	
TLR-6	Lipoproteins in mycobacteria; zymosan in fungi	
Endosomal membrane–associated PRRs[b]		
TLR-3	Double-stranded viral RNA	Signal transduction, phagocyte activation, and inflammation
TLR-7, TLR-8	Single-stranded viral RNA	
TLR-9	Unmethylated CpG oligonucleotides in bacteria	
Cytoplasmic PRRs: NLRs (NOD-like receptors)		
NOD1	Peptidoglycan on gram-negative bacteria	Stimulate production of antimicrobial peptides and proinflammatory cytokines
NOD2	Peptidoglycan on gram-positive bacteria	
NLRP3	Inflammasome component	Triggers release of proinflammatory cytokines, increasing inflammation

[a]The extracellular soluble PRRs are produced by liver cells in response to inflammatory cytokines.
[b]TLR-3, -7, -8, and -9 are found in intracellular organelle membranes such as in lysosomes. A 10th Toll-like receptor, TLR-10, has unknown ligand specificity and function.
[c]Toll-like receptors initiate phagocyte activation via signal transduction.

TLR-4 is an integral protein having external, transmembrane, and cytoplasmic domains. The external domain of TLR-4 binds LPS complexed with a cell surface protein called CD14 (Figure 26.8). Binding of the CD14–LPS complex by the TLR-4 external domain causes a conformational change in TLR-4 that allows the cytoplasmic domain to interact with an adaptor protein called MyD88, which then binds a protein tyrosine kinase (PTK) called IRAK4. PTKs transfer energy-rich phosphates from ATP to target-protein tyrosines that are exposed when binding alters conformation. Binding of MyD88 by IRAK4 initiates a *kinase cascade* that triggers successive ATP-mediated phosphorylation of TRAF6, IκK (inhibitor of kappa kinase), and IκB (inhibitor of kappa B) proteins. Phosphorylation of IκB causes it to dissociate from, and thereby activate, NFκB. Activated NFκB can then diffuse across the nuclear membrane, bind to NFκB-binding motifs on DNA, and initiate transcription of downstream genes.

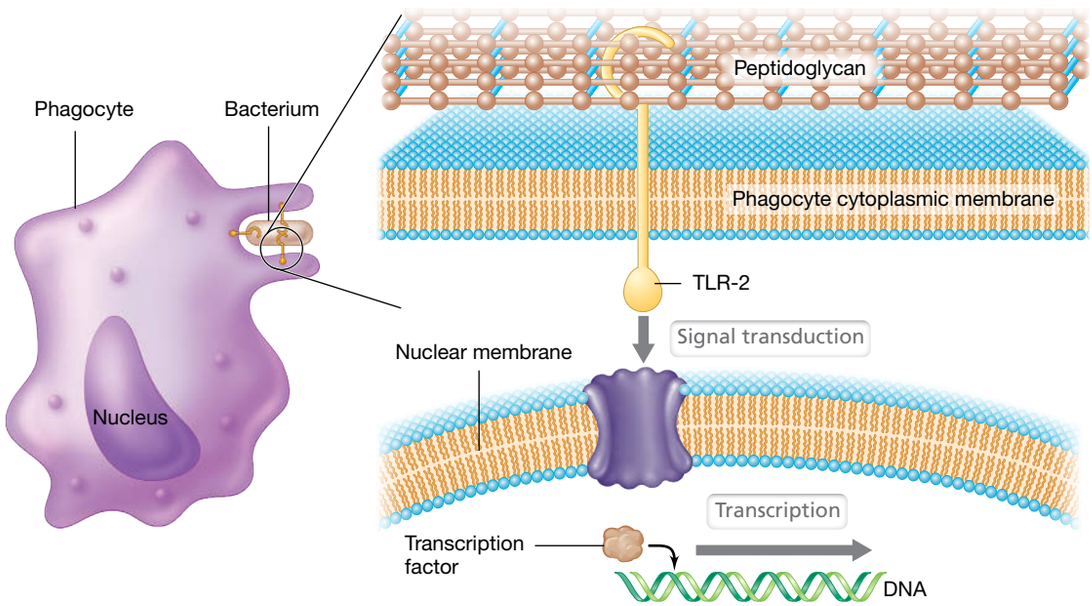


Figure 26.7 A Toll-like receptor. Membrane-spanning TLR-2 interacts with peptidoglycan from gram-positive pathogens. This interaction stimulates signal transduction, activating transcription factors in the nucleus. The result is transcription of genes encoding proteins that induce inflammation and other phagocyte activities. All Toll-like receptors have analogous mechanisms for activating innate immunity.

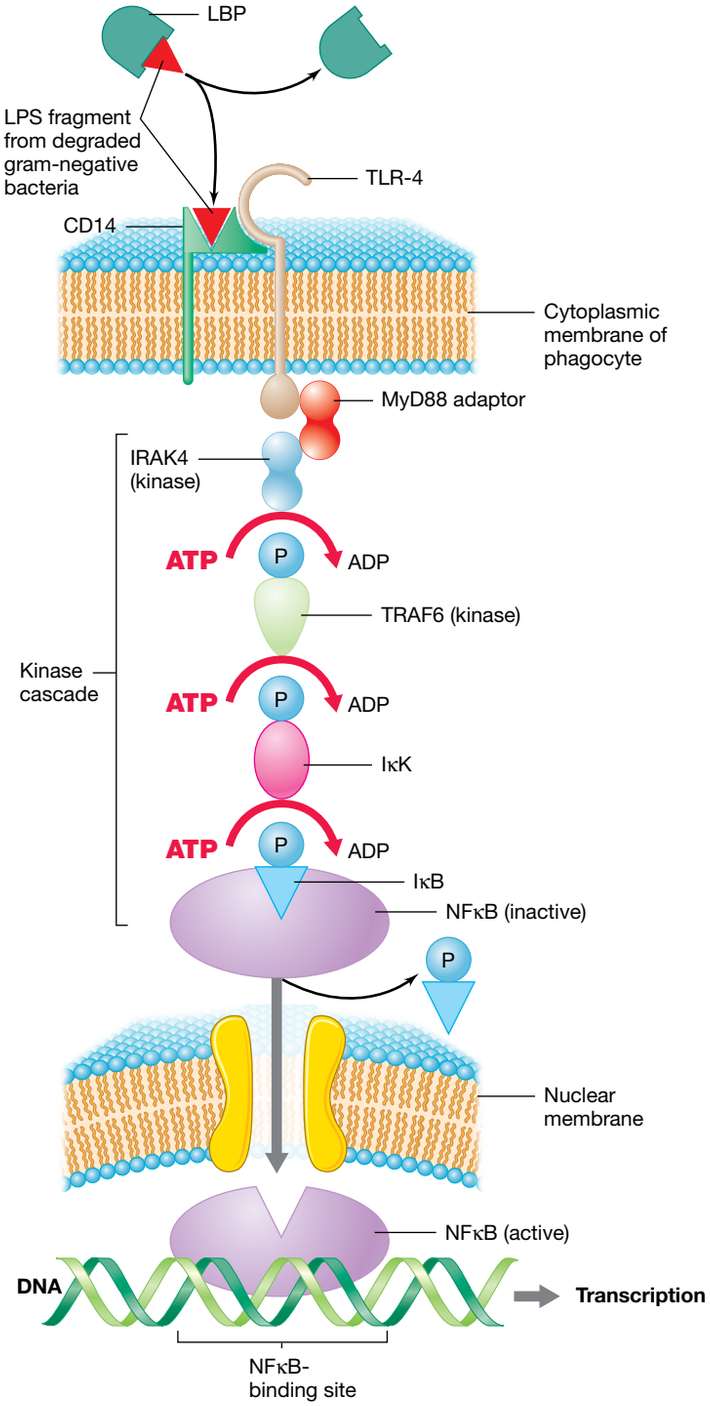


Figure 26.8 Signal transduction in innate immunity. Signal transduction is the transmission of a molecular signal across a cytoplasmic membrane by way of chemical modifications—typically involving a series of proteins in a signaling cascade—that results in a response, such as differential gene expression. In innate immunity, signal transduction is initiated when LPS, a PAMP, is bound by LBP (lipopolysaccharide-binding protein), which then transfers LPS to CD14 on the surface of a phagocyte. The LPS–CD14 complex then binds to the transmembrane TLR-4 receptor. The binding of TLR-4 initiates a series of reactions involving adaptor proteins and kinases, resulting in activation of the transcription factor NFκB. NFκB then diffuses across the nuclear membrane, binds to DNA, and initiates transcription of genes encoding proteins essential for innate immunity.

As Figure 26.8 shows, signal transduction pathways initiate activation of transcription through ligand–receptor binding on the surface of the phagocytic cell. The ligand–receptor interaction outside the cell induces the binding, recruitment, and concentration of the adaptor proteins and kinase enzymes inside the cell. A single kinase can phosphorylate many signal cascade proteins, thus amplifying the effect of a single ligand–receptor interaction. Signal transduction leading to activation of shared transcription factors and protein synthesis is also the mechanism by which lymphocytes are activated in adaptive immunity, as we discuss in Chapter 27.

MINIQUIZ

- Identify a PAMP shared by a group of microorganisms. Then, identify the cell types that use PRRs to provide innate immunity to pathogens.
- Outline the general features of a signal transduction pathway starting with binding of a PAMP by a membrane-associated PRR.

26.7 Phagocytosis and Phagocyte Inhibition

When phagocytes encounter infectious agents or their harmful products, the activation of signal transduction pathways (Figure 26.8) triggers genetic responses in the phagocyte that direct the containment and removal of the threat—**phagocytosis** (Figure 26.9). While these mechanisms effectively protect the body from most infectious exposures, they are not foolproof; many pathogens deploy effective defenses against phagocytes in attempts to thwart the innate immune response.

Phagocytosis and the Phagolysosome

Most phagocytes contain multiple membrane-bound inclusions called *lysosomes*, cytoplasmic vacuoles containing bactericidal substances, such as toxic oxygen compounds, lysozyme, proteases, phosphatases, nucleases, and lipases. Through the molecular mechanisms we have just discussed, phagocytes identify and engage pathogens on surfaces, such as blood vessel walls or fibrin clots, before initiating phagocytosis (Figure 26.9). Activation of the phagocyte through signal transduction causes the phagocyte membrane to envelop and engulf pathogens, eventually pinching off inwardly to form a *phagosome*. The **phagosome**, a vacuole containing the engulfed pathogen, moves into the cytoplasm and fuses with a lysosome to form a *phagolysosome* (Figure 26.10). The toxic chemicals and enzymes within the phagolysosome combine to kill and digest the engulfed microbial cell.

Genes that control the production of oxygen compounds toxic to pathogens are highly transcribed in activated phagocytes. These toxic compounds include hydrogen peroxide (H_2O_2), superoxide anions (O_2^-), hydroxyl radicals (OH·), singlet oxygen ($^1O_2$), hypochlorous acid (HOCl), and nitric oxide (NO) (Figure 26.10) (Section 5.14). Phagocytic cells use these toxic oxygen compounds to kill ingested bacterial cells by

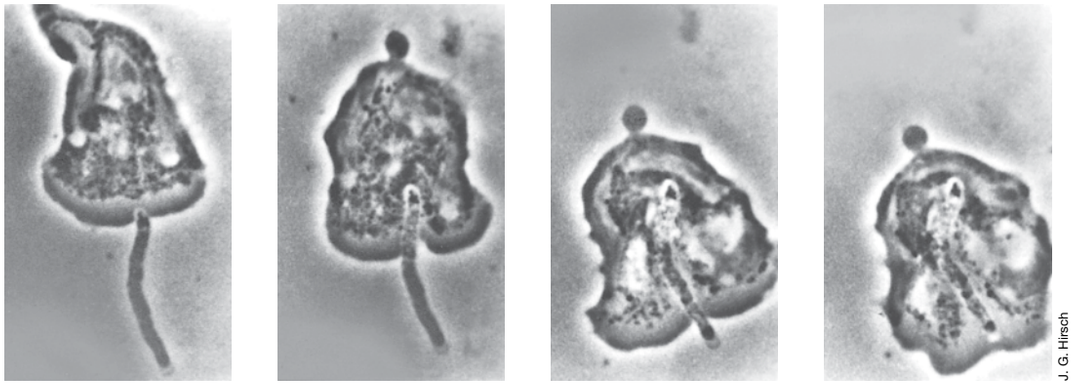


Figure 26.9 Phagocytosis. Time-lapse phase-contrast micrographs of the phagocytosis and digestion of a chain of *Bacillus megaterium* cells by a human macrophage. The bacterial chain is about 20 μm long.

oxidizing key cellular constituents. The lethal oxidative reactions are contained within the phagolysosome, and this prevents damage to the phagocyte itself.

Inhibiting Phagocytes

Some pathogens have mechanisms for neutralizing toxic phagocyte products, for killing the phagocytes, or for avoiding phagocytosis. For example, several species of *Mycobacterium* produce pigmented compounds called carotenoids that neutralize singlet oxygen and prevent pathogen killing. In addition, *Mycobacterium tuberculosis*, the bacterium that causes tuberculosis, grows and persists within phagocytic cells (Section 30.4). Cells of *M. tuberculosis* use their cell wall glycolipids (Section 16.11) to absorb hydroxyl radicals and superoxide anions, the most lethal toxic oxygen species produced by phagocytes (Figure 26.10).

Some intracellular pathogens produce phagocyte-killing proteins called *leukocidins*. In such cases, the pathogen is ingested as usual, but the leukocidin kills the phagocyte, releasing the pathogen unharmed. Dead phagocytes make up much of the material of *pus*, and organisms such as *Streptococcus pyogenes* (scarlet and rheumatic fevers) and *Staphylococcus aureus* (skin infections) are major leukocidin producers and *pyogenic* (pus-forming) pathogens. Localized infections by pyogenic bacteria thus form boils or abscesses.

Another important pathogen defense against phagocytosis is the bacterial capsule (Section 2.7). Because the capsule prevents necessary molecular interactions between the surface of the phagocyte and that of the bacterial cell, encapsulated bacteria are often highly resistant to phagocytosis. For example, fewer than ten cells of an encapsulated strain of *Streptococcus pneumoniae* can kill a mouse within a few days (Figure 25.9), whereas strains lacking a capsule are harmless. Surface components other than capsules can also inhibit phagocytosis. For instance, pathogenic *S. pyogenes* produces M protein, a substance that alters the surface of the pathogen and inhibits phagocytosis.

Soluble PRRs and other host molecules such as antibodies (Chapter 27) can interact with capsules and other pathogen surface molecules, thereby reversing the protective effect of bacterial defense mechanisms and enhancing phagocytosis. As an example, the effective vaccine directed against *Streptococcus pneumoniae*, the agent of bacterial pneumonia, uses capsule polysaccharides to induce protective antibodies (Section 28.9). Thus, the battle between pathogen and the host innate immune system is a dynamic one, where both sides deploy multiple weapons in attempts to thwart the success of the other.

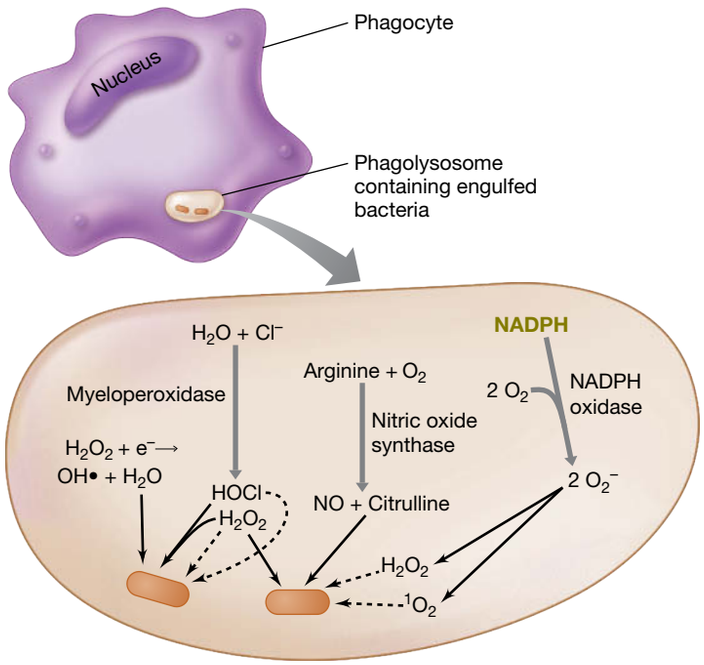


Figure 26.10 Activity of phagocyte enzymes in generating toxic oxygen compounds. These compounds include hydrogen peroxide (H_2O_2), the hydroxyl radical (OH•), hypochlorous acid (HOCl), the superoxide anion (O_2^-), singlet oxygen ($^1O_2$), and nitric oxide (NO). Formation of these toxic compounds requires a substantial increase in the uptake and utilization of molecular oxygen, O_2. This increase in oxygen uptake and consumption by activated phagocytes is called the *respiratory burst*.

MINIQUIZ

- Identify the mechanism used by phagocytes to induce pathogen killing.
- Describe several reasons why phagocytes are not always effective at removing pathogens from the body.

IV • Other Innate Host Defenses

In addition to the physical and chemical barriers to pathogen invasion and the direct destruction of pathogens by activated phagocytes, mammalian immune systems have other inborn

mechanisms that help counter infection by pathogens. Although unpleasant for the host, inflammation and fever can be effective host defense strategies for controlling microbial growth in the body and eliminating pathogens. These mechanisms, along with a consideration of the complement system and the activities of special lymphocytes called *natural killer cells*, round out our discussion of the innate immune response.

26.8 Inflammation and Fever

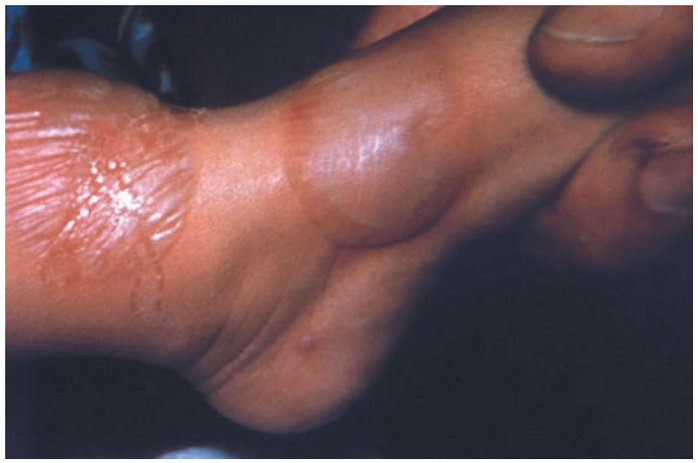
Inflammation is a nonspecific reaction to noxious stimuli, such as toxins and pathogens. Inflammation is characterized by redness (erythema), swelling (edema), pain, and heat, usually localized at the site of infection (Figure 26.11 and Figure 27.27). The mediators of inflammation are a group of cell activator and chemoattractant molecules, including cytokines and chemokines. Various cells, including those damaged by injury, produce these activators. The most important chemokines and cytokines are called *proinflammatory* because of their inflammation-inducing capacity, and they are produced in high concentrations by phagocytes and lymphocytes during pathogen challenge.

Both innate and adaptive immune responses to infection can cause inflammation, and both responses trigger the release of molecules that recruit and activate effector cells, such as neutrophils. Although it is a generalized immune response, inflammation plays a crucial role to isolate and limit tissue damage by initiating the destruction of pathogen invaders and the removal of damaged cells. As we will soon discuss, however, the inflammatory response may also inadvertently result in considerable damage to healthy host tissue.

Inflammatory Cells and Local Inflammation

Immune-mediated inflammation is an acute condition that begins at the site of pathogen entry into the body. The innate PRRs on macrophages and other tissue cells at the site of infection engage the pathogen PAMPs (Figure 26.6). This activates local cells to produce and release mediators including cytokines and chemokines that interact with receptors on other cells, such as neutrophils (Figure 26.4). For example, local tissue macrophages that are activated by PAMP–PRR interaction secrete a chemokine called CXCL8. This molecule activates neutrophils to migrate along the chemokine gradient toward the source of the CXCL8, where they begin to ingest and kill the pathogen. The neutrophils, in turn, secrete even more CXCL8, attracting more neutrophils and amplifying the response, eventually destroying the pathogens (Figure 26.11*b*).

The chemokine and cytokine mediators released by injured cells and phagocytes contribute to inflammation. For example, macrophages and other cells at the site of infection produce proinflammatory cytokines including interleukin-1 (IL-1), IL-6, and tumor necrosis factor α (TNF-α). These cytokines increase vascular permeability, causing the swelling (edema), reddening (erythema), and local heating associated with inflammation (Figure 26.12*a*). Although edema stimulates local neurons, causing pain, the pressure associated with swelling also serves to force fluids away from blood vessels and into the lymphatic system, simultaneously helping to strengthen the immune response and prevent the spread of pathogens to the bloodstream. This condition, called *bacteremia*, could trigger the much more serious *septicemia* (Section 25.2).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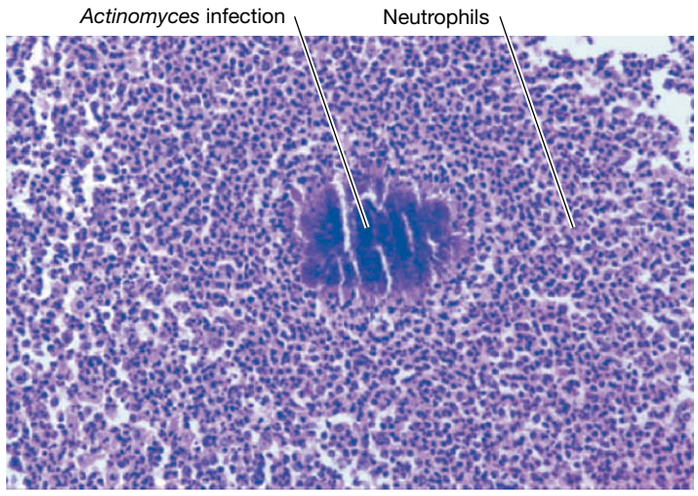


James V. Little

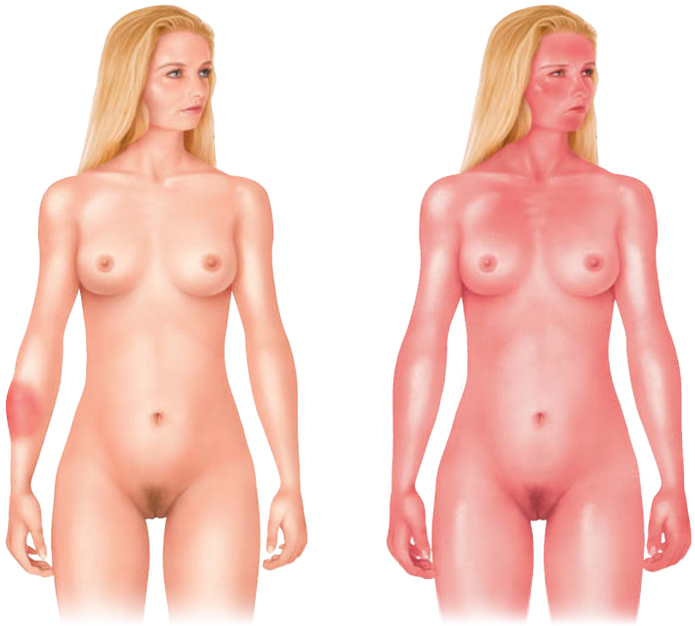
(b)

Figure 26.11 Inflammation. *(a)* Photograph of a child's foot showing swelling due to infection with vaccinia virus; fluid accumulation results from the inflammatory response. *(b)* Photomicrograph showing infection by *Actinomyces*, a filamentous bacterium. The stained cells surrounding the dark mass of bacteria in the center are neutrophils (Figure 26.4), indicating acute inflammation.

The usual outcome of the inflammatory response is a rapid localization and destruction of the pathogen by macrophages and recruited neutrophils. As the pathogens are destroyed, inflammatory cells are no longer stimulated, and as a result, their numbers at the infection site are diminished. As cytokine production decreases, the attraction of phagocytes to affected tissues lessens, and inflammation subsides.

Fever

Certain cytokines released during an inflammatory response induce **fever**, a condition of elevated body temperature. For example, the proinflammatory cytokines IL-1, IL-6, and TNF-α are *endogenous pyrogens*. These substances stimulate the hypothalamus, the temperature-controlling center of the brain, to produce *prostaglandins*, chemical signals that raise body temperature and cause fever. These same cytokines released in small amounts at local sites of infection induce localized heating, which increases blood flow and promotes healing. The release of endogenous



(a) Local infection leads to inflammation in a small part of the body, followed by healing.

(b) Systemic infection leads to inflammation and disease throughout the body.

Figure 26.12 Local and systemic inflammation. *(a)* Local infection, mediated by proinflammatory cytokines from local macrophages, results in inflammation that subsides as the infection is cleared. *(b)* Systemic infection causes systemic release of proinflammatory cytokines, resulting in widespread systemic inflammatory symptoms including severe edema, fever, and septic shock, even if the infection is controlled.

pyrogens is a physiological response to the presence of *exogenous pyrogens*, components of pathogens that induce fever, such as the lipid A endotoxin of gram-negative bacteria LPS (Section 2.5).

Although uncomfortable for the host, fever is an important component of the innate immune response to some infections. The rise from normal human body temperature, about 37°C (98.6°F), to fever temperature, usually 38–40°C (100.4–104°F), is a beneficial response to infection because higher body temperatures inhibit growth of most pathogens. Slower growth limits multiplication of the invading pathogen, thereby minimizing tissue damage and easing the workload on other immune cells, especially phagocytes. Elevated body temperatures also increase the production of *transferrins*, molecules that bind and sequester iron in blood and lymph, thus depriving pathogens of this important nutrient (Sections 3.1 and 25.4).

While low to moderate fever is an important component of the innate immune response, a continuous or uncontrolled rise in body temperature (>40°C) is a rare but life-threatening condition that may accompany certain disease conditions and requires immediate medical intervention. This usually includes the administration of antipyretic (fever-reducing) medications, such as acetaminophen or ibuprofen, which counteract the effect of endogenous pyrogens on the hypothalamus.

Systemic Inflammation and Septic Shock

In some cases, the inflammatory response fails to localize the pathogens, and the reaction spreads throughout the body. Uncontrolled *systemic inflammation* can be more dangerous than the original infection, with inflammatory cells and mediators contributing to body-wide inflammation. An inflammatory response that spreads inflammatory cells and mediators through the entire circulatory and lymphatic systems can lead to *septic shock*, a life-threatening condition.

Although there are potentially many causes of pathogen-induced septic shock, one example is systemic infection by enteric bacteria, such as *Salmonella* species or *Escherichia coli*, which can be introduced into the peritoneal cavity or the bloodstream by a ruptured or leaking bowel. The primary infection is often cleared by the activity of phagocytes and antibiotic treatment. However, the endotoxic outer membrane LPS from these gram-negative bacteria interacts with a PRR on phagocytes, stimulating production of proinflammatory cytokines that are released into the circulation. These cytokines induce systemic responses that parallel the localized inflammatory response but on a much larger scale that affects many organ systems, ultimately leading to a body-wide inflammatory event (Figure 26.12*b*). The result is a massive efflux of fluids from the central vascular tissue causing a loss of systemic blood pressure and the influx of fluids from vascular tissues into extravascular spaces. Septic shock causes death in up to 30% of affected individuals.

MINIQUIZ

- Identify the molecular mediators of inflammation and fever and define their individual roles.
- Identify the major symptoms of localized inflammation and of septic shock.

26.9 The Complement System

The **complement system**, or simply *complement*, is a group of sequentially interacting proteins, many with enzymatic activity, that functions to boost the efficiency of both innate and adaptive immune responses for the destruction of pathogens. Complement proteins are produced in the liver and found throughout the body, and their activities can be triggered by innate or adaptive mechanisms. The major outcomes of activating the complement system are enhanced phagocytosis, inflammation, and lysis of invading cells.

The individual proteins of complement are designated C1, C2, C3, and so on. Complement proteins exist in an inactive conformation until they are enzymatically split to assume their active forms. The splitting of C3 to its products, C3a and C3b, is the key event in activating complement. At least three different mechanisms lead to this outcome, and we consider each of these now.

Classical Complement Activation

The *classical pathway* of complement activation is initiated when complement proteins, attracted by bound antibodies, attach to pathogen surfaces. The antibodies are said to *fix* (bind) the ever-present complement proteins, and thus, classical complement activation depends upon the adaptive immune response. The complement proteins react in a defined sequence, or *cascade*, with activation of one complement protein leading to activation of the next, and so on.

The key steps of classical activation of complement, shown in Figure 26.13*a*, start with binding of antibody to antigen (initiation), followed by binding of C1 components (C1q, C1r, and C1s) to the

antibody–antigen complex. This complex recruits and splits C2 into its fragments, C2a and C2b, and C4 into its fragments, C4a and C4b. C2a and C4b interact and are deposited at an adjacent membrane site. The resulting C2a-C4b complex functions as a *C3 convertase*, an enzyme that cleaves C3 to C3a and C3b. C3b then binds to the convertase, forming a complex that cleaves C5 into C5a and C5b. The liberated C5b then binds C6 and C7 and inserts into the membrane of the target cell. The C5b–7 complex recruits C8 and C9, forming a large C5b–9 unit called the *membrane attack complex* (*MAC*). The MAC forms a pore through the cytoplasmic membrane of the pathogenic cell, allowing extracellular fluids to rush in and lyse the cell (Figure 26.13*a*). Dozens to hundreds of MACs may perforate a single bacterial cell at the point of lysis.

When activated by specific antibody, MAC formation lyses many gram-negative bacteria. However, gram-positive bacteria, such as *Streptococcus* species, are not usually lysed by complement because their thick cell walls make the cytoplasmic membrane less accessible to MAC proteins. Gram-positive bacteria can, however, be destroyed through *opsonization*.

Opsonization is the coating of pathogens with antimicrobial host proteins, such as antibodies or C3b, resulting in enhanced phagocytosis of target cells (Figure 26.14*a*). Opsonization neutralizes pathogens and makes them much more likely to be identified, engulfed, and destroyed by phagocytes. This is because most phagocytes, including neutrophils and macrophages, have antibody receptors (FcR) and C3b receptors (C3R) on their surfaces, which bind antibody and C3b complement protein, respectively. Normal phagocytic processes are enhanced about 10-fold by antibody–FcR interactions and amplified another 10-fold by C3b–C3R interactions.

By-products of complement activation include chemoattractants called *anaphylatoxins*; these molecules cause inflammatory reactions at the site of complement deposition. For example, when C3 is cleaved to C3a and C3b, C3b opsonizes the target cell as described above; meanwhile, release of soluble C3a attracts and activates phagocytes, increasing phagocytosis. In addition, both C3a and the C5a cleavage product are able to bind receptors on the surface of mast cells, causing them to degranulate and release large amounts of proinflammatory histamine (Figure 26.14*c*).

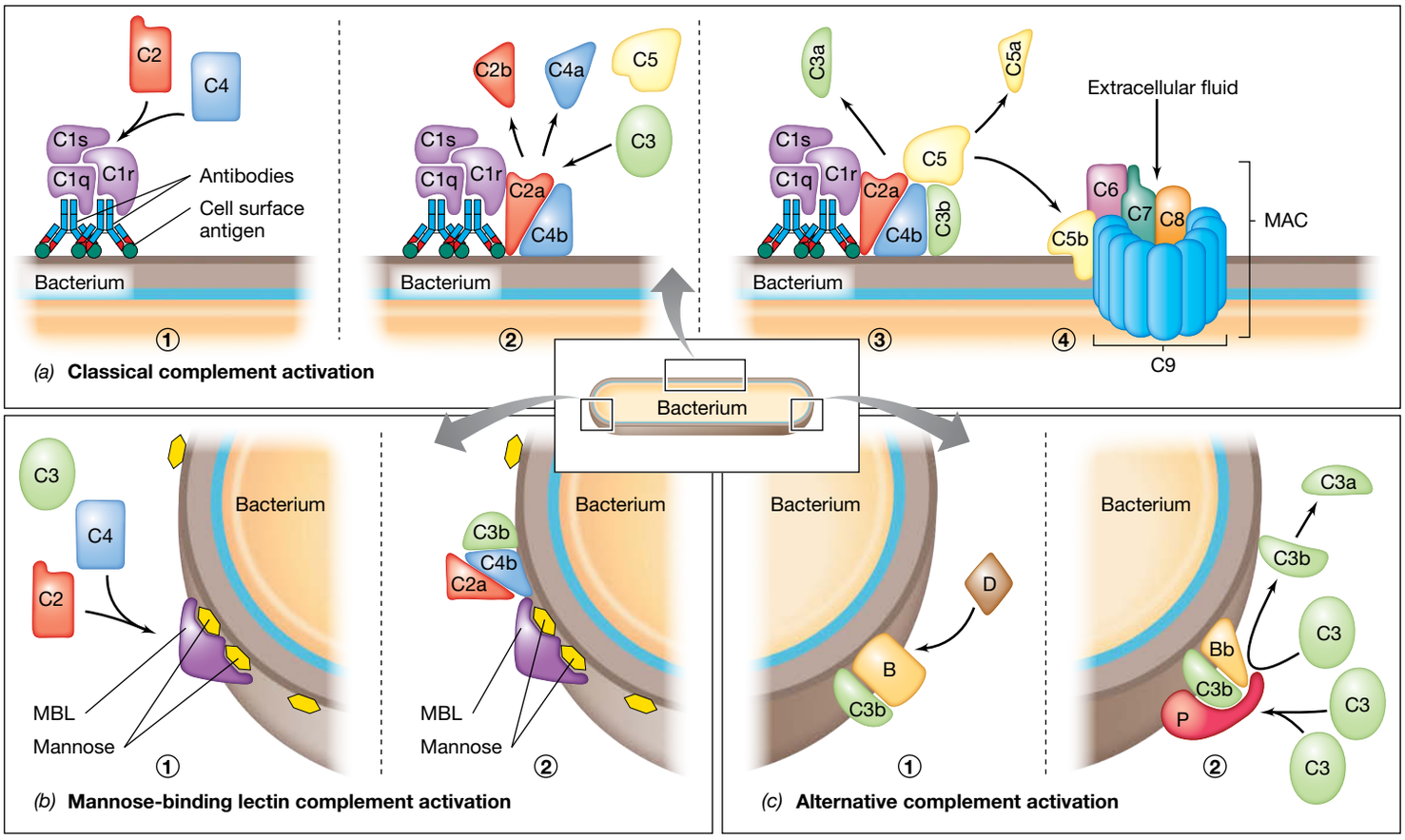


Figure 26.13 Complement proteins and complement activation in the immune response. *(a)* The sequence, orientation, and activity of the components of the classical complement pathway as they interact to lyse a cell. ① Binding of the antibody and the C1 protein complex (C1q, C1r, and C1s). ② The C2a-C4b complex is a C3 convertase that splits C3. ③ The C2a-C4b-C3b complex cleaves C5, and C5b then binds an adjacent membrane site. ④ Sequential binding of C6, C7, C8, and C9 to C5b produces a pore, the membrane attack complex (MAC), in the membrane. *(b)* The mannose-binding lectin (MBL) pathway. ① MBL binds to mannose on the bacterial membrane and recruits C2 and C4. ② MBL anchors formation of C2a-C4b-C3b. This complex activates C5, as in step 3 of part *a*, and initiates formation of the MAC (step 4 in *a*). *(c)* The alternative pathway. ① C3b bound to the cell binds protein B, which is cleaved by protein D. ② The resulting C3b-Bb complex is stabilized on the membrane by factor P (properdin) and then acts on C3 in the blood, causing more C3b to bind to the membrane. Bound C3b-Bb-P then activates C5, as in step 3 of the classical activation pathway above, and initiates formation of the MAC (step 4 in *a*).

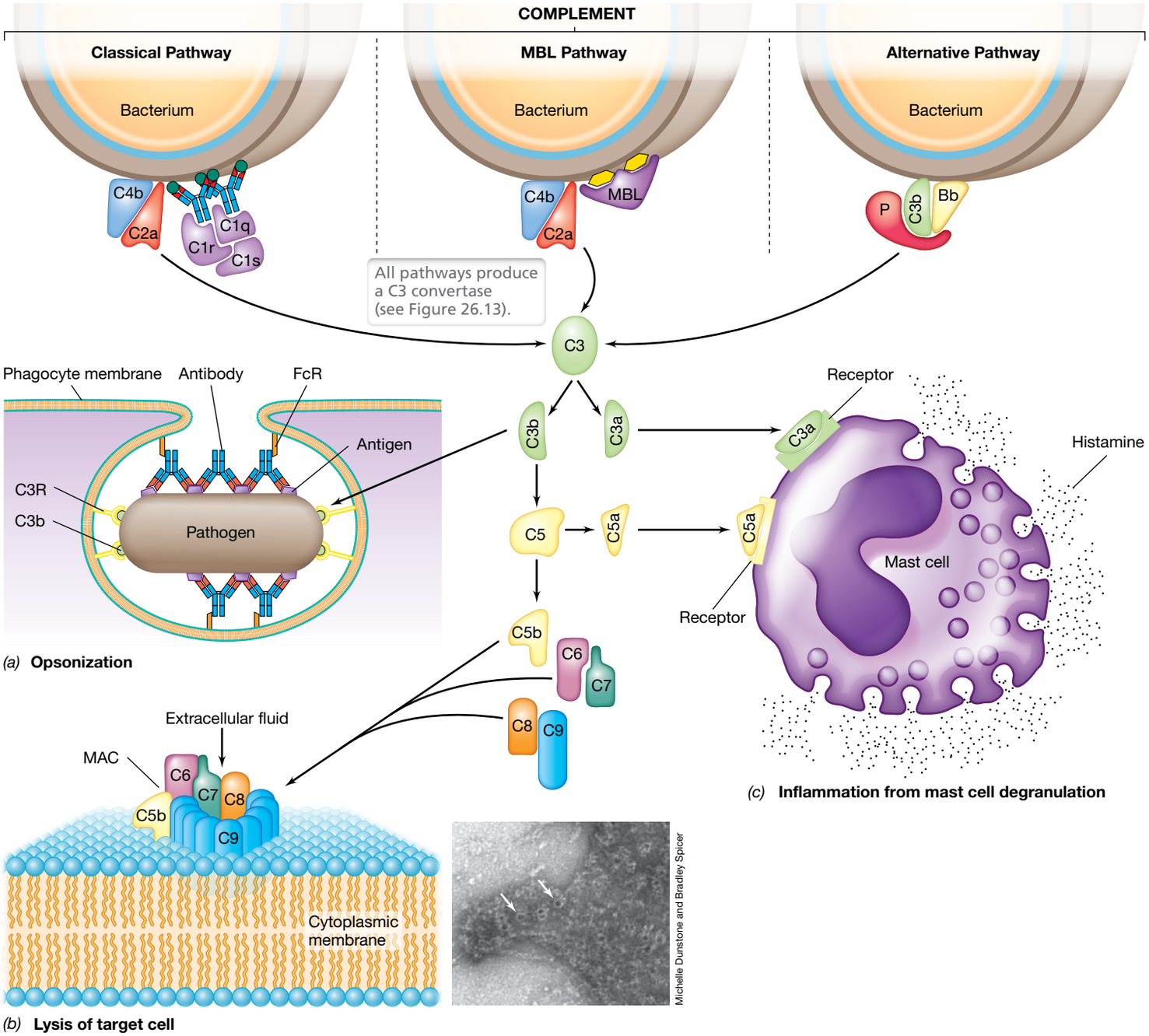


Figure 26.14 Complement proteins and the outcomes of complement activation. Each complement activation pathway leads to the production of a C3 convertase that cleaves C3 to its active products, C3a and C3b. These initiate three possible outcomes: opsonization, lysis by membrane attack complex (MAC) formation, and inflammation. *(a)* Pathogens coated with C3b (green) or with specific antibodies become opsonized, allowing for enhanced recognition by phagocytes through C3R and FcR proteins. *(b)* Transmembrane pores (arrows) formed by MAC components C5b through C9 cause lysis of target cells. The transmission electron micrograph is a negative stain image showing human MACs attacking a foreign (rabbit) erythrocyte. *(c)* During complement-activated inflammation, liberated C3a and C5a bind receptor proteins on mast cells, causing the mast cells to degranulate and release proinflammatory histamine.

Mannose-Binding Lectin and Alternative Pathways

In addition to the classical activation pathway, the *mannose-binding lectin pathway* and the *alternative pathway* can activate complement (Figure 26.13*b* and *c*). These pathways depend on recognition of shared pathogen components and are an important part of the innate immune response, especially in the initiation of inflammation.

The *mannose-binding lectin* (*MBL*) *pathway* depends on the activity of a serum MBL protein. MBL is a soluble PRR (Section 26.6) that binds to mannose-containing polysaccharides found only on bacterial cell surfaces (Figure 26.13*b*). The MBL–polysaccharide complex is similar to the C1 complex of the classical pathway in that it fixes C2a and C4b, again producing C3 convertase and binding C3b to C2a-C4b. As before, this complex catalyzes

formation of the C5–9 MAC and leads to lysis or opsonization of the bacterial cell.

The *alternative pathway* is a nonspecific complement activation mechanism that uses many of the classical complement pathway components, as well as several unique serum proteins. Together they induce opsonization and activate the C5–9 MAC. The first step in alternative pathway activation is the binding of C3b to LPS on the bacterial cell surface (C3b is produced by spontaneous cleavage of C3, which occurs at low levels in the blood and tissues) (Figure 26.13*c*). C3b on the membrane can then bind the alternative pathway serum protein factor B, which is cleaved by factor D to yield soluble Ba and Bb. The C3b-Bb complex may then be bound by factor P (properdin) to form C3b-Bb-P, another C3 convertase. C3b-Bb-P then attracts and cleaves additional C3, depositing more C3b on the membrane and initiating the remaining steps of the complement cascade (Figure 26.14).

Both the alternative pathway and the MBL pathway nonspecifically target bacterial invaders and lead to activation of MACs and opsonization via formation of stable C3 convertases. MBL, factors B, D, and P, and classical complement proteins are part of the innate immune response, but unlike the classical pathway, neither the alternative pathway nor the MBL pathway requires prior antigen exposure or the presence of antibodies for activation.

MINIQUIZ

- In what ways does the classical pathway of complement activation differ from the mannose-binding lectin and alternative pathways?
- What is opsonization, and how does opsonization help fight bacterial infection?
- Why are the mannose-binding lectin and alternative pathways considered part of the innate immune system?

26.10 Innate Defenses against Viruses

In addition to opsonization and the activity of phagocytes, the immune system has other innate defenses that are especially important for controlling and eliminating viral infections. These include *natural killer cells* (Figure 26.4) and *interferons*. Natural killer cells are lymphocyte-like cells that recognize and kill compromised (unhealthy) cells, such as those infected with intracellular pathogens, in particular viruses. Whereas natural killer cells help rid the body of already infected cells, interferons, small proteins of the cytokine family, help healthy cells ward off viral infection. We consider both of these innate defenses here.

Natural Killer Cells

Natural killer cells (NK cells) are cytotoxic lymphocytes that are distinct from T cells and B cells (Figure 26.4). The role of NK cells is to seek out and destroy compromised cells, such as cells infected with intracellular pathogens (such as viruses) or cancer cells. When an NK cell engages an infected or otherwise diseased cell, granules in the cytoplasm of the NK cell migrate to the contact site and release their contents. These granules contain *perforin* and proteases called *granzymes*. Perforin binds the membrane of the target cell and forms a pore through which granzymes enter the target cell (**Figure 26.15**). Granzymes are cytotoxins that cause *apoptosis*, or programmed cell death, characterized by death and degradation of the target cell from within. During the killing process, NK cells remain unaffected and their membranes are not damaged by perforin. Also during this process, NK cell numbers do not increase nor do NK cells exhibit immune memory after interaction with target cells. With this in mind, how do NK cells recognize compromised host cells that should be destroyed?

Most cells in the body contain a suite of surface proteins called the **major histocompatibility complex (MHC)** (Chapter 27).

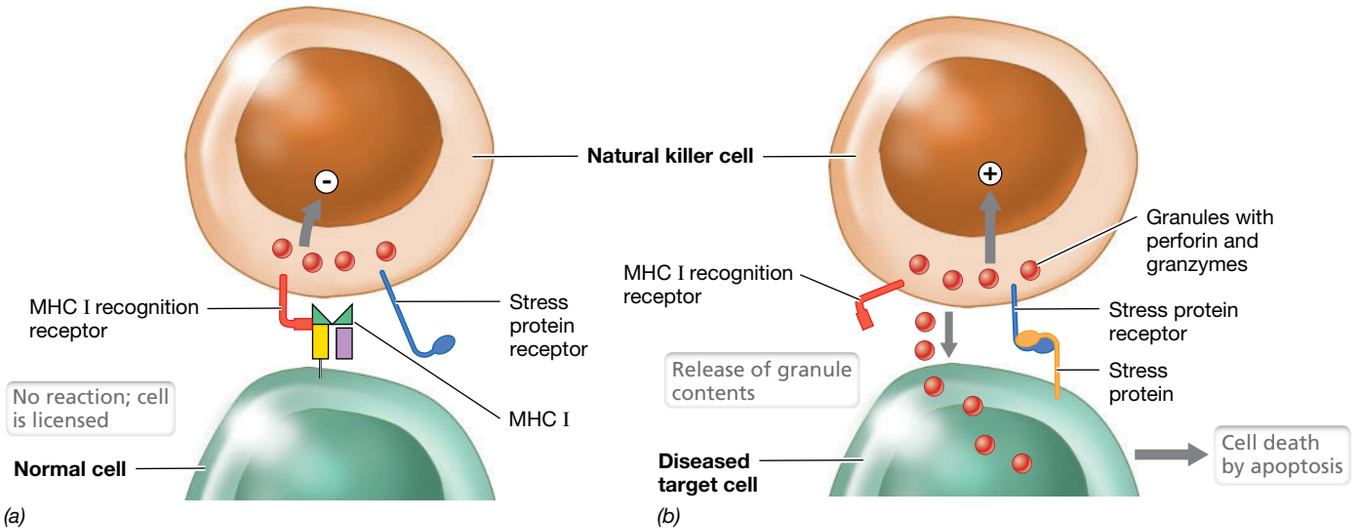


Figure 26.15 Natural killer cells. Natural killer (NK) cells have two receptors: One interacts with MHC I on healthy cells; the second one interacts with cell stress proteins, found only on tumor cells or pathogen-infected cells. *(a)* MHC I recognition licenses the healthy cells, preventing the NK cell from releasing its contents. *(b)* Pathogen-infected cells or tumor cells express stress proteins and often reduce MHC I expression. Especially in the absence of MHC I recognition, the NK cell interacts with the stress protein and releases perforin and granzymes, inducing apoptosis and killing the diseased cell.

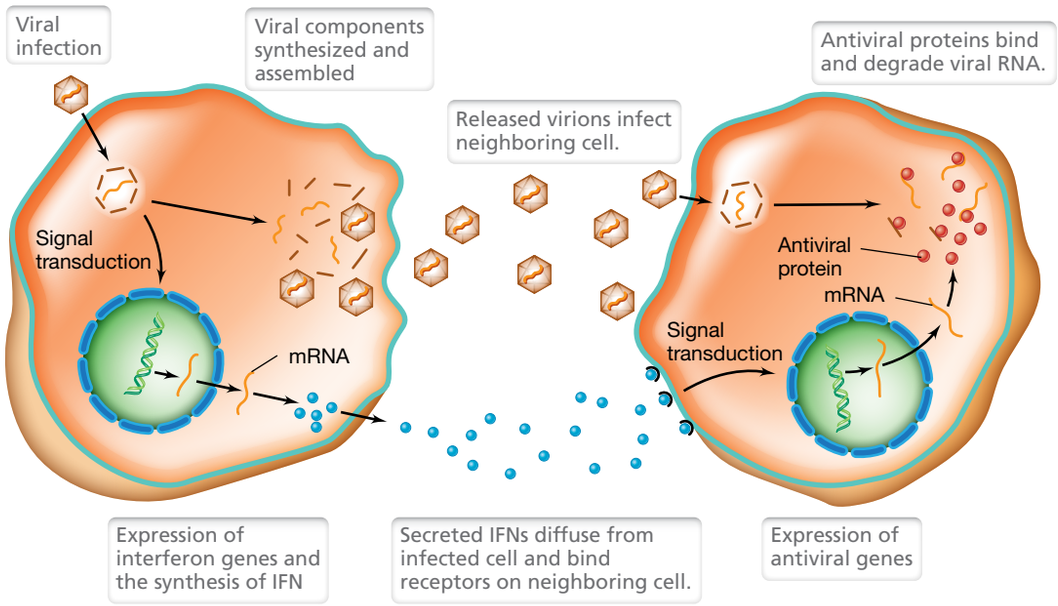


Figure 26.16 Antiviral activity of interferons. Host cells secrete interferons, a type of cytokine, in response to viral infection. The interferons bind to uninfected cells, triggering a signal transduction pathway that leads to the synthesis of proteins that bind viral nucleic acids and interfere with viral replication.

There are two classes of MHC proteins—*class I* and *class II*—and their primary function is to present antigen to various immune cells to trigger an immune response. MHC II proteins are expressed on antigen-presenting cells (APCs) only, which include B cells, macrophages, and dendritic cells (Figure 26.4). By contrast, MHC I proteins are found on the surfaces of all nucleated cells. In uninfected cells, which contain no pathogens or foreign antigens, MHC I proteins bind and present *self peptides*, protein fragments derived from the normal degradation of self proteins during growth, to the external environment. In cells that have been infected by viruses or other intracellular pathogens, MHC I proteins display peptides derived from the infectious agent. This serves as a signal to a special type of T lymphocyte called a *T-cytotoxic cell* to destroy the infected cell.

Because MHC I proteins function to interrupt multiplication of the pathogen by signaling the infected state of the host cell, many viral genomes encode proteins that suppress expression of MHC I in host cells. Without MHC I proteins on their surfaces—a condition that is also common in cancer cells—T-cytotoxic cells cannot identify and eliminate diseased host cells. This, then, is where NK cells play a major role; NK cells recognize and destroy compromised host cells that have reduced expression of MHC I on their surfaces.

NK cells recognize and destroy pathogen-infected or tumor cells by using a two-receptor system. The molecular targets of NK cells are MHC I proteins on the surface of other cells (Figure 26.15*a*). As NK cells circulate and interact with other cells in the body, they use special MHC I receptors on their surfaces to recognize MHC I proteins on normal, healthy cells. Binding of the MHC I recognition receptors on NK cells to MHC I on other cells deactivates the NK cell, turning off the perforin and granzyme killing mechanisms. In addition to a deficiency of MHC I proteins, pathogen-infected or tumor cells frequently express *stress proteins* on their surfaces; NK cells have complementary receptors for many of these stress proteins. Especially in the absence of the MHC I interactions, the stress receptors on NK cells engage stress proteins on target cells. This interaction triggers the release of perforin and granzymes from the NK cell (Figure 26.15*b*). In this way, pathogen-infected or tumor cells that exhibit stress proteins and no longer express the MHC I proteins of healthy cells are removed from the body.

Interferons

Interferons, in particular IFN-α and IFN-β, are small proteins in the cytokine family that prevent viral replication by stimulating the production of antiviral proteins in uninfected cells (**Figure 26.16**). Host cells produce and secrete interferons in response to certain viral infections or exposure to inactivated viruses or viral nucleic acids. Interferons are produced in large amounts by cells infected with viruses of low virulence, but highly virulent viruses inhibit host protein synthesis before interferon can be produced, significantly reducing interferon production. The presence of double-stranded RNA (dsRNA) also induces interferon synthesis. In nature, dsRNA exists only in certain RNA viruses, such as rhinoviruses (one of many common cold viruses) (Section 30.7); the viral dsRNA stimulates the animal cell to synthesize and release interferon.

Interferon activities are *host*-specific rather than *virus*-specific. That is, interferon produced by a species activates receptors only on cells from the same species. As a result, interferon produced by cells of an animal in response to, for example, a rhinovirus, could also inhibit multiplication of, for example, influenza viruses in cells of the same species. However, the interferon would have no effect on the multiplication of viruses, including the original rhinovirus, in other species. We examine the potential use of interferons as chemotherapeutic or prophylactic treatments in Chapter 28.

MINIQUIZ

- Identify and compare the targets and the recognition mechanisms used by T-cytotoxic cells and NK cells.
- Under what conditions are interferons produced, and how do they limit the transmission of viruses from one host cell to another?

Chapter Review

I • Fundamentals of Host Defense

26.1 Innate immunity is an inborn protective response to infection characterized in part by recognition and elimination of common pathogens, primarily through the activity of phagocytes. Adaptive immunity is the acquired ability of the immune system to eliminate specific pathogens from the body via lymphocyte-mediated responses, including the production of antibodies that bind foreign antigens on pathogens or their products.

Q List two different types of phagocytes. How do T cells and B cells differ in their functions? From where in the human body do all of these cells originate and which require maturation before they are functional?

26.2 The human body possesses numerous protective defenses against infectious agents. Natural host resistance to infection includes physical barriers to infection posed by the skin and mucosa, as well as chemical barriers to infection including acidic secretions, defensins, and lysozyme. The specificity of pathogens for particular tissues limits which hosts and tissues might be susceptible to infection.

Q How does the human normal microbiota play a role in preventing disease?

II • Cells and Organs of the Immune System

26.3 Cells involved in innate and adaptive immunity originate from hematopoietic stem cells in bone marrow. The blood and lymph systems circulate cells and proteins that are important components of the immune response. Diverse leukocytes participate in immune responses in all parts of the body.

Q Describe the significance of bone marrow, blood, and lymph to cells and proteins associated with the immune system.

26.4 Leukocytes are differentiated white blood cells derived from either myeloid or lymphoid precursor cells. Cells of myeloid lineage include monocytes and granulocytes. Monocytes include macrophages and dendritic cells, specialized phagocytes that function as antigen-presenting cells (APCs) to initiate an adaptive immune response. Granulocytes include neutrophils, which are also phagocytes but not APCs, and mast cells, which are important inducers of the inflammatory response but may also cause allergic reactions. Lymphocytes include B and T cells, which facilitate adaptive immunity, and natural killer cells, which play a key role in destroying virus-infected and cancerous host cells.

Q What is the origin of the phagocytes and lymphocytes active in the immune response? Track the maturation of B cells and T cells.

III • Phagocyte Response Mechanisms

26.5 Pathogens may colonize host tissues when appropriate nutrients and growth conditions are present, such as on mucosal surfaces, especially where the composition of the normal microbiota has been altered. Innate responses to microbial invasion and tissue damage are initiated by the release of chemokines, which recruit phagocytes and other immune cells to sites of infection.

Q Describe a scenario in which microorganisms invade body tissues. What factors allow for the migration of phagocytes to sites of infection?

26.6 Innate recognition of common pathogens occurs through pathogen-associated molecular patterns (PAMPs). Phagocytes recognize PAMPs through preformed pattern recognition receptors (PRRs). The recognition and interaction process stimulates phagocytes to destroy the pathogens through a signal transduction mechanism that induces phagocytosis of the infectious agent.

Q Identify some PAMPs that are recognized by PRRs. Which cells express PRRs? How do PRRs associate with PAMPs to promote innate immunity?

26.7 Phagocytosis is the engulfing of infectious particles by phagocytes. Engulfed pathogens are bathed in toxic oxygen compounds inside the phagolysosome, killing and degrading them. However, some pathogens have developed various defense mechanisms to avoid or inhibit phagocytes, including secretion of leukocidins, the presence of a capsule, and biosynthesis of carotenoid pigments, which combat oxidative stress.

Q Explain how phagocytes kill microorganisms, with particular attention to oxygen-dependent mechanisms. Then identify at least three properties of pathogens that inhibit the effectiveness of phagocytes.

IV • Other Innate Host Defenses

26.8 Fever and inflammation, characterized by pain, swelling (edema), redness (erythema), and heat, are normal and generally beneficial outcomes that result from activation of nonspecific immune response effectors. However, uncontrolled systemic inflammation, called septic shock, can lead to serious illness or death.

Q Identify the cells that initiate inflammation and the cells that are activated by inflammatory signals.

26.9 The complement system is composed of soluble proteins that catalyze bacterial opsonization and cell lysis. Complement is triggered by antibody interactions or by interactions with nonspecific activators, such as mannose-binding lectin. Complement activation may be a product of either innate or adaptive immunity. Complement may enhance phagocytosis, cause target cell lysis, or induce an inflammatory response.

Q Describe the complement system. Is the order of protein interactions important? Why or why not? Identify the components of the mannose-binding lectin pathway for complement activation. Identify the components of the alternative pathway for complement activation. How do these complement activation pathways differ from the classical pathway?

26.10 Through an antigen-independent mechanism, natural killer cells respond to both the presence of stress proteins and the absence of MHC I on the surface of virus-infected cells or tumor cells, using perforin and granzymes to kill the compromised target cells. Interferons are cytokines produced by virus-infected cells that limit the spread of infection by stimulating the expression of antiviral proteins in uninfected cells.

Q What is the activation signal for NK cells? How does this differ from the activity of T-cytotoxic lymphocytes?

Application Questions

1. Describe the relative importance of innate immunity compared to adaptive immunity. Is one more important than the other? Can we survive in a normal environment without immunity?
2. Describe the potential problems that would arise if a person had an acquired inability to phagocytose pathogens. Could the person survive in a normal environment such as a college campus? What defects in the phagocyte might cause lack of phagocytosis? Explain.
3. Inflammation is the hallmark of an active immune response. Explain how inflammation is triggered by innate immune mechanisms. Why does inflammation subside as an infection is controlled?
4. Do you agree with the following statement? Complement is a critical component of antibody-mediated defense. Explain your answer. What might happen to persons who lack complement component C3? C5? Factor B (alternative pathway)? Mannose-binding lectin (MBL)?

Chapter Glossary

Adaptive immunity the acquired ability to recognize and destroy a particular pathogen or its products, dependent on previous exposure to the pathogen or its products

Antibody a soluble protein produced by B cells and plasma cells that interacts with antigen; also called immunoglobulin

Antigen a molecule that interacts with specific components of the immune system

Antigen-presenting cell (APC) a macrophage, dendritic cell, or B cell that takes up and processes antigen and presents it to T-helper cells

B cell a lymphocyte that has immunoglobulin surface receptors, produces immunoglobulin, and may present antigens to T cells

Chemokine a soluble protein that recruits immune cells to an injury site; a type of cytokine

Complement system a series of proteins that react sequentially with antibody–antigen complexes, mannose-binding lectin, or alternative activation pathway proteins to amplify or potentiate target cell destruction

Cytokine a soluble protein produced by a leukocyte or damaged body cell; modulates an immune response

Dendritic cell a phagocytic antigen-presenting cell found in various body tissues; transports antigen to secondary lymphoid organs

Fever an increase in body temperature resulting from infection or the presence of toxins in the body

Granulocyte a leukocyte derived from a myeloid precursor that contains cytoplasmic granules consisting of toxins or enzymes that are released to destroy target cells.

Immunity the ability of an organism to resist infection

Immunoglobulin a soluble protein produced by B cells and plasma cells that interacts with antigen; also called antibody

Inflammation a nonspecific reaction to noxious stimuli such as toxins and pathogens, characterized by redness (erythema), swelling (edema), pain, and heat (fever), usually localized at the site of infection

Innate immunity the noninducible ability to recognize and destroy an individual pathogen or its products that does not rely on previous exposure to a pathogen or its products

Interferons cytokine proteins produced by virus-infected cells that induce signal transduction in nearby cells, resulting in transcription of antiviral genes and expression of antiviral proteins

Invasion the ability of a pathogen to enter into host cells or tissues, spread, and cause disease

Leukocyte a nucleated cell in blood; also called a white blood cell

Lymph nodes organs that contain lymphocytes and phagocytes arranged to encounter microorganisms and antigens as they travel through the lymphatic circulation

Lymphocytes a subset of nucleated cells in blood involved in the adaptive immune response

Macrophage a large leukocyte found in tissues that has phagocytic and antigen-presenting capabilities

Major histocompatibility complex (MHC) a genetic region that encodes several proteins important for antigen processing and presentation. MHC I proteins are expressed on all cells. MHC II proteins are expressed only on antigen-presenting cells

Mast cell tissue cells adjoining blood vessels throughout the body that contain granules with inflammatory mediators

Memory (immune memory) the ability to rapidly produce large quantities of specific immune cells or antibodies after subsequent exposure to a previously encountered antigen

Monocyte Circulating phagocyte that contains many lysosomes and can differentiate into a macrophage or dendritic cell

Mucosa-associated lymphoid tissue (MALT) a part of the lymphatic system that interacts with antigens and microorganisms that enter the body through mucous membranes, including those of the gut, the genitourinary tract, and bronchial tissues

Natural killer (NK) cell a specialized lymphocyte that recognizes and destroys infected host cells or cancer cells in a nonspecific manner

Neutrophil a leukocyte exhibiting phagocytic properties, a granular cytoplasm (granulocyte), and a multilobed nucleus; also called polymorphonuclear leukocyte or PMN

Opsonization the deposition of antibody or complement protein on the surface of a pathogen or other antigen that results in enhanced phagocytosis

Pathogen-associated molecular pattern (PAMP) a repeating structural component of a microorganism or virus recognized by a pattern recognition receptor (PRR)

Pattern recognition receptor (PRR) a protein in a phagocyte membrane that recognizes a pathogen-associated molecular pattern (PAMP)

Phagocyte a cell that engulfs foreign particles, and can ingest, kill, and digest most pathogens

Phagocytosis the process of engulfing and killing foreign particles and cells

Phagosome an intracytoplasmic vacuole containing engulfed materials, especially pathogens or foreign particles

Plasma the liquid portion of the blood containing proteins and other solutes

Plasma cell a differentiated B cell that produces soluble antibodies

Primary lymphoid organ an organ in which antigen-reactive lymphocytes develop and become functional; the bone marrow is the primary lymphoid organ for B cells; the thymus is the primary lymphoid organ for T cells

Secondary lymphoid organ an organ at which antigens interact with antigen-presenting phagocytes and lymphocytes to generate an adaptive immune response; these include lymph nodes, spleen, and mucosa-associated lymphoid tissue

Serum the liquid portion of the blood with clotting proteins removed

Specificity the ability of the immune response to interact with particular antigens

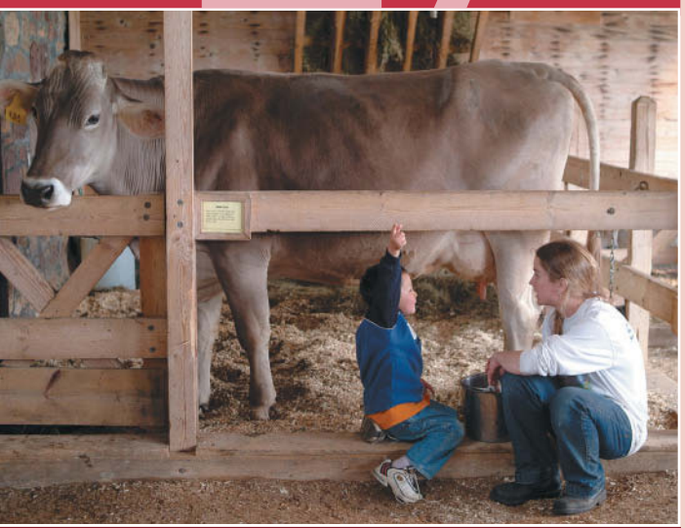
Stem cell a progenitor cell that can develop into other cell types

T cell a lymphocyte that interacts with antigens through a T cell receptor for antigen; T cells are divided into functional subsets including Tc (T-cytotoxic) cells and Th (T-helper) cells. Th cells are further subdivided into Th1 (inflammatory) cells and Th2 cells, which aid B cells in antibody formation

Toll-like receptor (TLR) one of a family of pattern recognition receptors (PRRs) found on phagocytes, structurally and functionally related to Toll receptors in *Drosophila*, that recognize a pathogen-associated molecular pattern (PAMP)

27

Adaptive Immunity: Highly Specific Host Defenses



microbiologynow

Got (Raw) Milk? The Role of Unprocessed Cow's Milk in Protecting against Allergy and Asthma

In recent decades, the onset of allergies and asthma during childhood has become an increasingly common complication that, as we will see in this chapter, stems from hypersensitivity reactions in the adaptive immune response. However, for nearly 20 years now, researchers have been aware of one demographic of young people that has consistently shown resistance to asthma and allergies: kids that grow up on farms. Several hypotheses have been proposed to explain this phenomenon, including early childhood exposure to livestock and their feed (see photo), but while this may contribute to a protective "farm effect," it is the consumption of unprocessed cow's milk that is emerging as the major underlying explanation for the trend.

A recent report documented the correlation between the consumption of raw, unpasteurized cow's milk and the incidence of asthma in over 1100 children from rural regions of five European countries. Researchers found that compared to shop milk purchased at a supermarket, which is pasteurized (heated to 72°C for at least 15 seconds), centrifuged, and homogenized to achieve a uniform product, regular ingestion of unprocessed farm milk was inversely related to the onset of asthma in children, with the asthma-protective effect increasing over time.

To elucidate this farm milk effect, the scientists compared the fatty acid composition of unprocessed farm milk samples to that of shop milk samples. They found that the raw milk contained substantially higher levels of omega-3 (ω-3) fatty acids than shop milk, and this was attributed to both the higher overall fat content of raw milk and its lack of pasteurization, which breaks down heat-labile components of the milk. This finding was important because ω-3 fatty acids are precursors of anti-inflammatory immune mediators that suppress hypersensitivity reactions, including those that trigger allergies and asthma.

Although the implications of this research in asthma prevention are potentially significant, public health officials strongly discourage ingestion of unpasteurized milk, mainly because of the risk of foodborne illnesses, including salmonellosis, listeriosis, Q fever, staphylococcal food poisoning, and gastroenteritis. Interestingly, changing this stance may be neither warranted nor necessary since it may be possible to restore the asthma-protective effect by supplementing industrially processed milk with ω-3 fatty acids. Only time—and more research—will tell!

Source: Brick, T., et al. 2016. ω-3 fatty acids contribute to the asthma-protective effect of unprocessed cow's milk. *J. Allergy Clin. Immunol. 137(6):* 1699–1706.e13 doi:10.1016/j.jaci.2015.10.042.