Chapter 10

Preventing Infection at Mucosal Surfaces

Most infectious diseases suffered by humans are caused by pathogens much smaller than a human cell. For these microbes, the human body constitutes a vast resource-rich environment in which to live and reproduce. In facing such threats, the body deploys a variety of defense mechanisms that have accumulated over hundreds of millions of years of invertebrate and vertebrate evolution. In considering mechanisms of innate immunity in Chapters 2 and 3 and of adaptive immunity in Chapters 4–11, we principally used the example of a bacterial pathogen that enters the body through a skin wound, causing an innate immune response in the infected tissue that then leads to an adaptive immune response in the draining lymph node. The merits of this example are that it is simple and involves a tissue for which we have all observed the effects of wounds, infection, and inflammation. Until recently, these were the only responses studied by most immunologists, who usually administered their experimental antigens by subcutaneous injection. But in the real world, only a fraction of human infections are caused by pathogens that enter the body's tissues by passage through the skin. Many more infections, including all of those caused by viruses, make their entry by passage through one of the mucosal surfaces. Although the immune response to infection of mucosal tissue has strategies and principles in common with those directed at infections of skin and connective tissue, there are important differences, both in the cells and molecules involved, as well as the ways in which they are used. Appreciation of the extent of these differences has led to the concept that the human immune system actually consists of two semi-autonomous parts: the systemic immune system, which defends against pathogens penetrating the skin, and the mucosal immune system, which defends against pathogens breaching mucosal surfaces. This chapter focuses on mucosal immunity and how it differs from systemic immunity.

10-1 The communication functions of mucosal surfaces render them vulnerable to infection

Mucosal surfaces or the mucosae (singular mucosa) are found throughout much of the body, except the limbs, but they are predominantly out of sight. Continually bathing the mucosae is a layer of the thick, viscous fluid called mucus, which is secreted by the mucosae and gives them their name. Mucus contains glycoproteins, proteoglycans, peptides, and enzymes that protect the epithelial cells from damage and help to limit infection. Mucosal epithelia line the gastrointestinal, respiratory, and urogenital tracts, and are also present in the exocrine glands associated with these organs: the pancreas, the conjunctivae and lachrymal glands of the eye, the salivary glands, and the mammary...
glands of the lactating breast (Figure 10.1). These tissues are all sites of communication, where material and information are passed between the body and its environment. Because of their physiological functions of gas exchange (lungs), food absorption (gut), sensory activity (eyes, nose, mouth, and throat), and reproduction (uterus, vagina, and breast), the mucosal surfaces are by necessity dynamic, thin, permeable barriers to the interior of the body. These properties make the mucosal tissues particularly vulnerable to subversion and breach by pathogens. This fragility, combined with the vital functions of mucosae, has driven the evolution of specialized mechanisms for their defense.

The combined area of a person’s mucosal surfaces is vastly greater than that of the skin: the small intestine alone has a surface area 200 times that of the skin. Reflecting this difference, three-quarters of the body’s lymphocytes and plasma cells are to be found in secondary lymphoid tissues serving mucosal surfaces. A similar proportion of all the antibodies made by the body is secreted at mucosal services as the dimeric form of IgA, also known as secretory IgA or S IgA (see Chapter 9). A distinctive feature of the gut mucosa is its constant contact with the large populations of commensal microorganisms that inhabit the lumen of the gut and constitute the gut microbiota. Other major contents of the gut are the proteins, carbohydrates, lipids, and nucleic acids derived from the plants and animals that contribute to our diet. In this situation, the major challenge is to make immune responses that eliminate pathogenic microorganisms and restrict the growth and location of commensal microorganisms, but do not interfere with our food and nutrition. As most research on mucosal immunity has been on the gut, this will provide our principal example of a mucosal tissue, but first we will examine the constituents and properties of the mucus.
10-2 **Mucins are gigantic glycoproteins that endow the mucus with the properties to protect epithelial surfaces**

In every mucosal tissue, a layer of epithelial cells joined by tight junctions separates the outside environment from the inside of the body. The epithelial layer provides a formidable barrier that prevents commensal and pathogenic organisms from gaining access to the internal issues. Adding to this defense is the mucus, which prevents microorganisms and other environmental material, such as smoke and smog particles, from gaining access to the epithelium. The molecular basis for the viscosity and protective properties of mucus is a family of glycoproteins called mucins that are secreted by the epithelium. These proteins are huge, their polypeptide chains reaching lengths of more than 10,000 amino acids, but they are constructed from simple sequence motifs repeated many times over. The motifs are rich in serine and threonine residues that are glycosylated with short, negatively charged glycans. This carbohydrate comprises more than 70% of the weight of the mucin glycoprotein. The extensive glycosylation forces the mucin polypeptides into extended conformations. Globular domains at the ends of the polypeptides contain cysteine residues that make disulfide bonds between the stretched-out polypeptides, forming polymers and molecular networks that reach sizes greater than 1 million daltons (1 MDa) (Figure 10.2). The intertwining of these gigantic proteins is what makes mucus viscous, so that it physically impedes the movement of microorganisms and particles. The extensive glycosylation of mucins causes mucus to be heavily hydrated and thus able to protect epithelial surfaces by retaining water and preventing dehydration. A major constituent of the mucin glycans is sialic acid, which gives mucins a polyanionic surface. Through this they can bind the positively charged soluble effector molecules of innate immunity, such as defensins and other antimicrobial peptides, and of adaptive immunity, notably secretory IgA. Bacteria negotiating their way through mucus can thus be trapped by IgA and killed by defensins. Mucosal epithelia are dynamic tissues in which the epithelial cell layer turns over every 2 days or so, and mucus with its content of microorganisms is continuously being expelled from the body.

The viscoelastic properties of mucus vary with the mucosal tissue and its state of health or disease. This is achieved by varying the mucin polypeptides that are incorporated into the mucus and the extent of their cross-linking. In the human genome, seven genes encode secreted mucin polypeptides and are expressed in different mucosal tissues; an additional 13 genes encode mucin molecules that are membrane glycoproteins (Figure 10.3). These are expressed on the surface of epithelial cells and are not cross-linked like the secreted mucins. Although not so well characterized as the secretory mucins, these membrane mucins are believed to form a mucus-like environment at the epithelial cell surface that has similar protective properties. Because they are so much bigger than other components of the plasma membrane, the membrane mucins stand out from the cell surface, giving them the potential to trap and kill approaching microorganisms before they can interact with other components at the surface.

10-3 **Commensal microorganisms assist the gut in digesting food and maintaining health**

The gastrointestinal tract extends from the mouth to the anus and is about 9 meters in length in an adult human being (Figure 10.4). Its physiological purpose is to take in food and process it into nutrients that are absorbed by the body and into waste that is eliminated from the body. Alimentation means giving nourishment; hence the older alternative name of alimentary canal for the
gastrointestinal tract. Segments of the gastrointestinal tract serve different specialized functions and are populated to different extents by commensal bacteria. In the mouth, food is physically broken down by chewing in an environment populated by more than 750 species of bacteria. In the stomach, acid and enzymes are used to chemically degrade the masticated food in an environment that is relatively unfriendly for microbes. Here, the main function of the mucus is to protect and buffer the epithelium from the corrosive effects of hydrochloric acid secreted by the stomach. Enzymatic degradation continues the digestive process in the small intestine (the duodenum, jejunum, and ileum), which is the major site for the absorption of nutrients. In the large intestine (the colon), waste is stored, compacted, and periodically eliminated. The cecum is a pouch-like structure that connects the small and large intestines.

As food travels along the gastrointestinal tract and becomes increasingly degraded, it passes through environments with increasing numbers of resident bacteria. Starting in the stomach at 1000 bacteria per milliliter of gut contents, numbers increase to $10^5$ to $10^8$ per milliliter in the small intestine and

<table>
<thead>
<tr>
<th>Mucin polypeptide</th>
<th>Gene location (chromosome)</th>
<th>Mode of action</th>
<th>Tissues where expressed</th>
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<tr>
<td>MUC2</td>
<td>11</td>
<td>Secreted</td>
<td>Small intestine, colon</td>
</tr>
<tr>
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<td>Secreted</td>
<td>Airways, stomach</td>
</tr>
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<td>Secreted</td>
<td>Airways, salivary glands</td>
</tr>
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<td>Stomach, small intestine, gall bladder</td>
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<td>12</td>
<td>Secreted</td>
<td>Airways</td>
</tr>
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<td>12</td>
<td>Secreted</td>
<td>Salivary glands, trachea</td>
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<td>Secreted</td>
<td>Salivary glands</td>
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<td>1</td>
<td>Secreted and membrane-bound</td>
<td>Fallopian tubes</td>
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<td>19</td>
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<td>Membrane-bound</td>
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<td>MUC13</td>
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</tr>
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<tr>
<td>MUC18</td>
<td>4</td>
<td>Membrane-bound</td>
<td>Lung, breast</td>
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Figure 10.3 Mucosal tissues differ in the mucins they produce. In the human genome are genes encoding 20 mucin polypeptides. Six of these encode secreted mucins, 12 encode membrane-bound mucins and 1 encodes both secreted and membrane-bound mucins. Shown are the mucosal tissues in which the mucins are expressed and the chromosomal location of their genes.

Figure 10.4 The human gastrointestinal tract.
reach $10^{12}$ per milliliter in the colon. Digestion is a highly dynamic process in which the flow from stomach to anus is driven by peristalsis in the intestines. The growth of the populations of resident commensal organisms is equally dynamic, and to contain this population at a manageable size, vast numbers of commensals are forced out of the human body each day.

Commensal microorganisms have co-evolved with their human hosts in a symbiotic relationship, which benefits the host in various ways (Figure 10.5). Bacteria provide metabolic building blocks that are essential for human health but cannot be made by human cells. One example is the menaquinone precursors used to make vitamin K, a cofactor in the synthesis of blood-clotting factors. Bacteria also increase the efficiency with which humans digest certain foods, by providing enzymes that convert plant fibers, which are indigestible by human enzymes, into energy-rich metabolites. Other microbial enzymes render toxic substances present in food or secreted by pathogens into innocuous derivatives. The presence of large, healthy populations of commensal microorganisms also prevents the emergence and proliferation of pathogenic variants by depriving them of food and space. In fact, the normal development of the gut lymphoid tissues depends on the presence of a healthy gut microbiota, compelling evidence for the symbiotic co-evolution of commensal species and the human immune system.

Most bacterial infections of gut tissue are caused by commensals, but relatively few bacterial groups are involved. Many potential pathogens belong to the facultatively anaerobic, Gram-negative phylum Proteobacteria, which includes *Salmonella*, *Shigella*, *Helicobacter*, and *Escherichia*. Pathogenic variants of these normally harmless bacteria arise as new genetic variants acquire properties called virulence factors that enable them to leave the gut lumen, breach the gut epithelium, and invade the underlying lamina propria.

A common childhood viral infection of the epithelial lining of the small intestine is caused by rotavirus, a double-stranded RNA virus. The infection causes an acute diarrhea, during which large numbers of stable and infectious virus particles are shed in the feces. Worldwide, 500,000 children die each year from rotavirus infection. In addition to bacteria and viruses, a spectrum of parasitic diseases are caused by helminth worms, as well as protozoans and other microorganisms that inhabit the gastrointestinal tract.
The gastrointestinal tract is invested with distinctive secondary lymphoid tissues

To provide prompt defense against infection, secondary lymphoid tissues and immune-system cells are spread throughout the gut and other mucosal tissues. The *gut-associated lymphoid tissues* (GALT) comprise two functionally distinct compartments. The lymphoid tissue directly beneath the mucosal epithelium is called the *inductive compartment*, because this is where interactions between antigen, dendritic cells, and lymphocytes induce adaptive immune responses. The underlying connective tissue, called the *lamina propria*, comprises the *effector compartment*, because this is where effector cells, including plasma cells, effector T cells, macrophages, mast cells, and eosinophils reside. Although not technically a part of the gut-associated lymphoid tissue, the *mesenteric lymph nodes*, the largest lymph nodes in the body, are dedicated to defending the gut. They form a chain within the mesentery, the membrane of connective tissue that holds the gut in place. Although the gut-associated lymphoid tissues come in a variety of sizes and forms, the microanatomy and organization of their inductive compartments into B-cell and T-cell zones are generally similar to those of other secondary lymphoid tissues. The secondary lymphoid tissues within the gut mucosa continuously sample and monitor the contents of the gut lumen, allowing adaptive immune responses to be quickly made against the gut microbiota and implemented locally before any prospective pathogen can invade the gut tissue. In contrast, a mesenteric lymph node can respond to infection only after the pathogen has invaded gut tissue and is then brought to the node in the draining lymph. This latter mechanism is like that used to respond to infections in the rest of the body, where adaptive immune responses are made in secondary lymphoid organs that are often distant from the site of infection.

At the back of the mouth and guarding the entrance to the gut and the airways are the *palatine tonsils*, *adenoids*, and *lingual tonsils*. These large aggregates of secondary lymphoid tissue are covered by a layer of squamous epithelium and form a ring known as Waldeyer’s ring (Figure 10.6). In early childhood, when pathogens are being experienced for the first time and the mouth provides a conduit for all manner of extraneous material that is not food, the tonsils and adenoids can become painfully swollen because of recurrent infection. In the not-so-distant past, this condition was routinely treated by surgically removing the lymphoid organs, a procedure causing loss of immune capacity as reflected in the poorer secretory IgA response of such children, including the author of this book, to oral polio vaccination.

The small intestine is the major site of nutrient absorption, and its surface is deeply folded into finger-like projections called *villi* (singular *villus*), which greatly increase the surface area available for absorption. It is the part of the gut most heavily invested with lymphoid tissue. Characteristic secondary lymphoid organs of the small intestine are the Peyer’s patches, which integrate into the intestinal wall and have a distinctive appearance, forming dome-like aggregates of lymphocytes that bulge into the intestinal lumen (Figure 10.7). The patches vary in size and contain between 5 and 200 B-cell follicles with germinal centers, interspersed with T-cell areas that also include dendritic cells. The small intestine also contains numerous *isolated lymphoid follicles*, each composed of a single follicle and consisting mostly of B cells. Isolated lymphoid follicles, but not Peyer’s patches, are also a feature of the large intestine. A distinctive secondary lymphoid organ of the large intestine is the appendix (see Figure 10.2). It consists of a blind-ended tube about 10 cm in length and 0.5 cm in diameter that is attached to the cecum. It is packed with lymphoid follicles, and appendicitis results when it is overrun by infection. The only treatment for appendicitis is surgical removal of the appendix, to prevent it from bursting and causing life-threatening peritonitis—infection of the peritoneum, the membrane lining the abdominal cavity.
During early childhood, the human body and its immune system grow and mature in the context of the body’s microbiota and the common pathogens in the environment. Like most other parts of the body, if the immune system is not used regularly it becomes impaired. This is well illustrated by laboratory mice that are born and raised under ‘germ-free’ (gnotobiotic) conditions. In comparison with control mice that have a normal gut microbiota, the gnotobiotic mice have stunted immune systems—with smaller secondary lymphoid tissues, lower levels of serum immunoglobulin, and a generally reduced capacity to make immune responses (Figure 10.8).

10-5 Inflammation of mucosal tissues is associated with causation not cure of disease

The systemic immune response to infection in non-mucosal tissues involves the activation of tissue macrophages, which by secreting inflammatory
cytokines create a state of inflammation in the infected tissue. Neutrophils, NK cells, and other effector cells of innate immunity are recruited from the blood to the infected tissue, and dendritic cells migrate out of the infected tissue to the draining secondary lymphoid tissue to initiate adaptive immunity. Emerging from the adaptive immune response are effector T cells and pathogen-specific antibodies that travel to the infected tissue, where they work in conjunction with innate immunity to eliminate the pathogen and terminate the infection. Afterward, in the recovery phase, inflammation and immunity are suppressed, the damaged tissue is repaired, and both pathogens and effector cells of the immune system become excluded from the now healthy tissue. In effect, short violent episodes of localized and intense inflammation are the price paid to quash the sporadic infections of non-mucosal tissues (Figure 10.9, upper panels).

**Figure 10.9** The systemic and mucosal immune systems use different strategies for coping with infections. Compared here are the immune responses made to infecting bacteria by the systemic immune system (upper panels) and the mucosal immune system (lower panels). As the systemic immune system cannot anticipate infection, it is necessary for macrophages to be activated by the invading bacteria and then to secrete cytokines that recruit effector cells to the infected tissue. This creates a state of inflammation in which the bacteria are killed, but at a cost to the structural integrity of the tissue. Infection is followed by an extensive period for repair and recovery of the damaged tissue (upper panels). In contrast, the mucosal immune system anticipates potential infections by continually making adaptive immune responses against the gut microbiota, which places secretory IgA in the gut lumen and the lamina propria, and effector cells in the lamina propria and the epithelium. When bacteria invade the gut tissue, effector molecules and cells are ready and waiting to contain the infection. In the absence of inflammation, a further adaptive immune response to the invading organism is made in the draining mesenteric lymphoid which augments that in the local lymphoid tissue. Little damage is done to the tissue, and repair occurs as part of the normal process by which gut epithelial cells are frequently turned over and replaced (lower panels).
In contrast to non-mucosal tissues, which interact only occasionally with the microbial world, the mucosal tissues have close and continuous contact with numerous and diverse commensal microorganisms, all of which are a potential source of pathogens. For the gut, any significant breach of the epithelial layer could lead to a massive influx of bacteria and infection of the type that occurs in peritonitis (see Section 10-4). To avoid this, the mucosal immune system adopts two complementary strategies. First, rather than being reactive like systemic immunity, the mucosal immune response is proactive and is constantly making adaptive immune responses against the microorganisms populating the gut. The result is that healthy gut tissue is populated with effector T cells and B cells that stand guard and are poised to respond to any invader from the gut lumen (Figure 10.9, lower panels). The advantage of a proactive strategy is that infections can be stopped earlier and with greater force than is possible in non-mucosal tissues.

The second strategy of the mucosal immune system is to be sparing in the activation of inflammation, because the molecular and cellular weapons of the inflammatory response inevitably cause damage to the tissues where they work, which for mucosal tissues, and particularly the gut, is more likely to exacerbate the infection than clear it up. Inflammation in the gut is the cause of a variety of chronic human diseases.

Of several strategies used to prevent inflammation in mucosal tissues, one is the use of regulatory T cells (CD4 T_{reg}) to turn off inflammatory T cells. IL-10 is a cytokine secreted by T_{reg} that suppresses inflammation by turning off the synthesis of inflammatory cytokines. Rare immunodeficient patients who lack a functional receptor for IL-10 suffer from a chronic inflammatory disease of the gut mucosa that resembles the more prevalent Crohn’s disease and is mediated by inflammatory T_{H1} and T_{H17} subsets of CD4 T cells. Another inflammatory condition, celiac disease, is caused by an immune response in the gut lymphoid tissue that damages the intestinal epithelium and reduces the capacity of those affected to absorb nutrients from their food. This condition can arrest the growth and development of children, and in adults causes unpleasant symptoms including diarrhea and stomach pains and general ill health. Celiac disease is caused by an adaptive immune response to the proteins of gluten, a major component of grains such as wheat, barley, and rye, which are dietary staples for some human populations. Proving this cause-and-effect relationship, the symptoms of celiac disease disappear when patients adopt a strict gluten-free diet, but quickly come back if they consume gluten again. In healthy gut tissue a compromise is made between the competing demands of nutrition and defense. In celiac patients the truce is broken when a staple food is mistakenly perceived as a dangerous pathogen, which ‘infects’ the gut with every square meal.

The qualitatively different responses of the mucosal and systemic immune systems to microorganisms correlates with their developmental origin. During fetal development, the mesenteric lymph nodes and Peyer’s patches differentiate independently of the spleen and the lymph nodes that supply systemic immunity. The distinctive development of the secondary lymphoid tissues of mucosal and systemic immunity occurs under the guidance of different sets of chemokines and receptors for cytokines in the tumor necrosis factor (TNF) family. The differences between the gut-associated lymphoid tissues and the systemic lymphoid organs are thus imprinted early on in life.

10-6 Intestinal epithelial cells contribute to innate immune responses in the gut

Intestinal epithelial cells are very active in the uptake of nutrients and other materials from the gut lumen. They also have Toll-like receptors on their apical and basolateral surfaces, for example TLR5, which recognizes flagellin, the
**Figure 10.10 Epithelial cells contribute to the defense of mucosal tissue.** As well as providing a barrier between the gut tissue and the contents of the gut lumen, the epithelial cells are also first responders to invading microorganisms. Epithelial cell receptors detect the invader and initiate the innate immune response by secreting cytokines and chemokines that recruit neutrophils and monocytes from the blood.

protein from which bacterial flagella are constructed. Toll-like receptors on the apical surface allow the cells to sense bacteria that overcome the defenses of the mucus and reach the epithelium; those on the basolateral surface sense invading bacteria that penetrate the epithelium. The cytoplasm of epithelial cells contains NOD1 and NOD2 receptors, which detect components of bacterial cell walls (see Section 3-5). Signals generated from NOD and Toll-like receptors lead to activation of NFκB and formation of the inflammasome by NOD-like receptor P3 (NLRP3). These events lead to the production and secretion of antimicrobial peptides, chemokines, and cytokines such as IL-1 and IL-6 by the epithelial cells (Figure 10.10). The defensins kill the bacteria, whereas the chemokines attract neutrophils (via the chemokine CXCL8), monocytes (via CCL3), eosinophils (via CCL4), T cells (via CCL5), and immature dendritic cells (via CCL20) from the blood.

In this way, epithelial cells respond to incipient infection with a quick and localized inflammatory response that is usually sufficient to eliminate the infection without causing lasting damage. If not, then an adaptive immune response is initiated in the draining mesenteric lymph node. Because gut epithelial cells turn over every 2 days, their inflammatory responses are tightly controlled and will only persist in the presence of infection.

**10-7 Intestinal macrophages eliminate pathogens without creating a state of inflammation**

In gut-associated lymphoid tissues the lamina propria is populated with intestinal macrophages that provide a first line of defense against microbial invasion. Although intestinal macrophages are proficient at phagocytosis and the elimination of microorganisms and apoptotic dying cells, they cannot perform other functions that characterize blood monocytes and macrophages present in non-mucosal tissues. These functions are those associated with the initiation and maintenance of a state of inflammation (Figure 10.11). Intestinal macrophages do not respond to infection by secreting inflammatory cytokines. Neither do they give a respiratory burst in response to inflammatory cytokines made by other cells. Although intestinal macrophages express MHC class II molecules, they lack B7 co-stimulators and also the capacity to make the cytokines needed to activate and expand naive T cells: IL-1, IL-10, IL-12, IL-21, IL-22, and IL-23. In short, the intestinal macrophage is not a professional antigen-presenting cell and cannot initiate adaptive immune responses. Neither are intestinal macrophages the instigators of inflammation like their counterparts in non-mucosal tissues, but they can fully perform their role of recognizing microorganisms and killing them in an environment free of inflammation. Because of these qualities, some immunologists describe the intestinal macrophages as ‘inflammation-anergic’ macrophages.

Intestinal macrophages live only for a few months, so their population is constantly being replenished through the recruitment of monocytes from the blood. These then differentiate into intestinal macrophages in the lamina propria. When the monocytes arrive at the intestines, they have all the inflammatory properties associated with macrophages in non-mucosal tissues. Under the influence of transforming growth factor (TGF)-β and other cytokines made
by intestinal epithelium, stromal cells, and mast cells, the monocytes differentiate into intestinal macrophages by losing their inflammatory potential.

One way in which the inflammatory response of intestinal macrophages becomes attenuated is by preventing the expression of a subset of the cell-surface receptors and adhesion molecules that are used by macrophages in systemic immunity to generate inflammation. These include Fc receptors for IgA (CD89) and IgG (CD16, CD32, and CD64), the bacterial LPS receptor (CD14), complement receptors CR3 and CR4, the IL-2 and IL-3 receptors, and LFA-1. Another method of preventing inflammatory responses is modification of the signals sent by the cell-surface receptors of intestinal macrophages, for example TLR1 and TLR3–TLR9. This is achieved in various ways that all reach the same endpoint, the failure to activate NFκB, the master regulator of the inflammatory response (see Section 3-3). As a result of the selective disarming of the inflammatory response of monocytes when they become intestinal macrophages, the homeostatic environment in the healthy gut is one that is resistant to inflammation and the tissue disruption it inevitably causes. There is logic to this strategy, because damaged tissue provides the opportunity for invasion by the horde of microbes living just the other side of the gut epithelium.

10-8 M cells constantly transport microbes and antigens from the gut lumen to gut-associated lymphoid tissue

Whereas healthy skin is impermeable to microorganisms, healthy gut epithelium actively monitors the contents of the gut lumen. Absorption of nutrients by the small intestine is the function of the enterocytes in the epithelium of the villi. To aid in this task, the luminal face of an enterocyte (the surface facing into the gut lumen) is folded into numerous projections called microvilli—also called a ‘brush border’ from its appearance in the microscope. Interspersed between the enterocytes are goblet cells, which secrete mucus, and in the crypts between the villi are Paneth cells, which secrete defensins, lysozyme, and other antimicrobial factors. The villous epithelium is thus well defended against microbial invasion. By contrast, the follicle-associated epithelium that overlies lymphoid tissues in the small intestine is poorly defended. Goblet and Paneth cells are absent, and the enterocytes have a different phenotype, characterized by a reduced secretion of antimicrobial digestive enzymes such as alkaline phosphatase, and the possession of a thick glycolcalyx on the brush border that shields the luminal cell surface from microorganisms and particles. These properties preserve approaching microorganisms intact and funnel them toward uniquely specialized cells of the follicle-associated epithelium called microfold cells (M cells). Their name comes from the widely spaced folds on the M cell’s luminal surface, which lacks the brush border of an enterocyte (Figure 10.12). Strategically positioned over Peyer’s patches and lymphoid follicles, M cells provide portals through which microorganisms and their antigens are transported from the gut lumen to the secondary lymphoid tissue by passage through the M cell in membrane vesicles.

The luminal (apical) surface of the M cell, with its characteristic folds, has adhesive properties that facilitate the endocytosis of microorganisms and
particles. The surface also carries a variety of cell-surface receptors and adhesion molecules that recognize microbial antigens. On binding to cell-surface receptors, microorganisms and their antigens are internalized in endocytic vesicles that cross the M cell to fuse with the plasma membrane on the basolateral side. This process, called transcytosis, operates through several different mechanisms, which are used according to the size and physicochemical properties of the cargo. The distance traveled is short (1–2 μm), and the journey takes as little as 15 minutes because of the extensive invagination of the basolateral plasma membrane of the M cell to form the characteristic intraepithelial pocket. The pocket provides a local environment in the mucosal lymphoid tissue where the transported antigens and microorganisms can encounter dendritic cells, T cells, and B cells (Figure 10.13). Subsequent events in the secondary lymphoid tissue parallel those occurring in the systemic immune response.

10-9 Gut dendritic cells respond differently to food, commensal microorganisms, and pathogens

In the Peyer's patch, the dendritic cells that acquire antigens from M cells are present in the region of the subepithelial dome. These dendritic cells express CCR6, the receptor for the chemokine CCL20 produced by the follicle-associated epithelial cells. When taking up and processing antigens, these dendritic cells secrete IL-10, which prevents any production of inflammatory cytokines by the T cells that the dendritic cells activate.

In general, when soluble proteins and other macromolecules enter the body orally via the mouth and the alimentary canal they do not stimulate an antibody response. Thus the normal situation is that we do not make antibodies against the numerous degradation products of food that leave the stomach and pass leisurely through the intestines. This is called oral tolerance. In the healthy gut, potential antigens from food are transported through M cells and are taken up by a subset of CD103-expressing dendritic cells in the lamina propria that travel to the mesenteric lymph nodes. There the dendritic cells present the antigens to antigen-specific T cells and drive their differentiation into FoxP3-expressing regulatory T cells. These cells actively suppress the immune response to food antigens. Food antigens that are present at high concentrations on the dendritic cell surface can also induce antigen-specific T cells to become anergic.

Commensal microorganisms are only beneficial to the human host if they live and multiply in the lumen of the gut. Any commensal organism that breaches the epithelial barrier is a potential pathogen, and is treated as such. To limit the size of the populations of commensal organisms in the gut lumen and to prevent them from infecting the tissues, specific IgA antibodies are made against the commensal species and these are constantly secreted into the gut lumen. In the healthy gut, small numbers of each commensal species enter the gut-associated lymphoid tissue. Dendritic cells take up the microbes and present their peptide antigens on MHC class II molecules to naive antigen-specific CD4 T cells. On activation and differentiation into helper CD4 T cells, these helper T cells form conjugate pairs with antigen-specific B cells that have also taken up the microbes and are presenting their antigens on MHC class II molecules (see Figure 9.7, p. 237). This union drives the B cells to differentiate into

Figure 10.12 Microfold cells have characteristic membrane ruffles. This scanning electron micrograph of intestinal epithelium has a microfold or M cell in the center. It appears as a sunken area of the epithelium that has characteristic microfolds or ruffles on the surface. M cells capture microorganisms from the gut lumen and deliver them to Peyer's patches and the lymphoid follicles that underlie the M cells on the basolateral side of the epithelium. Magnification × 23,000.

Figure 10.13 Uptake and transport of antigens by M cells. Adaptive immune responses in the gut are initiated and maintained by M cells that sample the gut's contents and deliver this material to the intra-epithelial pockets on the basolateral side of the M cell. Here, dendritic cells and B cells take up antigen and stimulate the proliferation and differentiation of antigen-specific T cells and B cells.
plasma cells, which first secrete pentameric IgM and then switch the heavy-chain isotype to make secreted, dimeric IgA. By this mechanism the immune system is able to monitor the constitution of the gut microbiota and ensure that specific IgA is made against all its constituents. In episodes of change in the gut microbiota, such as occur after a course of antibiotic drugs, the synthesis of IgA will respond so that antibodies are made against new colonizing species but not against the species whose populations were exterminated by the drugs.

Although the delivery system of the M cells allows careful monitoring of the gut microbiota, it has the disadvantage of offering pathogenic agents, to which IgA has not been made, access to the tissues underlying the gut epithelium. The rapidity of M-cell transcytosis means that bacteria can survive the journey and establish an infection. For example, invasive species of *Shigella* exploit M cells to infect the colonic mucosa, causing widespread tissue damage. Poliovirus, which enters the human body by the oral route, binds to the CD155 molecule on M cells and is delivered to Peyer’s patches, where it establishes local infections before spreading systemically.

The presence of infection within and around the gut-associated lymphoid tissue leads to dendritic cells carrying the pathogen and its antigens to the draining mesenteric lymph nodes, where an adaptive immune response is made. In the presence of infection, dendritic cells in the lamina propria and outside the organized lymphoid tissues become more mobile and capture pathogens independently of M cells. They move into the epithelial wall or send processes through it that capture microbes and antigens without disturbing the integrity of the epithelial barrier (Figure 10.14). Having obtained a cargo of antigens, the dendritic cells move into the T-cell area of the gut-associated lymphoid tissue, or travel in the draining lymph to the T-cell area of a mesenteric lymph node, to stimulate antigen-specific T cells.

Through this perpetual sampling of the gut lumen’s contents, T cells specific for pathogenic microorganisms, commensal microorganisms, and food antigens are stimulated to become effector cells. Activated helper T cells then activate B cells to become plasma cells, as described in Chapter 9. These plasma cells secrete dimeric IgA specific for pathogens, commensals, and food antigens.

10-10 *Activation of B cells and T cells in one mucosal tissue commits them to defending all mucosal tissues*

On completing their development in the primary lymphoid organs, naïve B cells and T cells enter the bloodstream to recirculate between blood, secondary lymphoid tissue, and lymph. Before encountering a specific antigen, these...
naive lymphocytes can enter the secondary lymphoid tissues of both the systemic and the mucosal compartments of the immune system. Like the spleen and other lymph nodes, the Peyer’s patches and mesenteric lymph nodes release chemokines CCL21 and CCL19, which bind to the chemokine receptor CCR7 expressed by naive B cells and T cells. This induces the naive lymphocytes to leave the blood at high endothelial venules and enter the secondary lymphoid tissue.

If specific antigen is not encountered in the Peyer’s patch or mesenteric lymph node, the naive cells leave in the efferent lymph to continue recirculation. Lymphocytes that find their specific antigen are retained in the lymphoid tissue. Here, dendritic cells that are presenting specific antigens activate the naive T cells, causing them to proliferate and differentiate into effector T cells. This activation requires retinoic acid, a derivative of vitamin A that is made by the dendritic cells of mucosal tissue. The effector cells include helper CD4 T<sub>FH</sub> cells, which activate naive antigen-specific B cells to become effector B cells.

After their activation in a Peyer’s patch, effector B and T cells leave in the lymph and travel via the mesenteric lymph nodes to the thoracic duct and the blood (Figure 10.15). Cells activated in a mesenteric lymph node leave in the efferent

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**Figure 10.15 Lymphocytes activated in mucosal tissues return to those tissues as effector cells.** Pathogens from the intestinal lumen enter a Peyer’s patch through an M cell and are taken up and processed by dendritic cells. Naive T cells (green) and B cells (yellow) enter the Peyer’s patch from the blood at a high endothelial venule (HEV). The naive lymphocytes are activated by antigen, whereupon they divide and differentiate into effector cells (blue). The effector cells leave the Peyer’s patch in the lymph, and after passing through mesenteric lymph nodes they reach the blood, by which they travel back to the mucosal tissue where they were first activated. The effector cells leave the blood and enter the lamina propria and the epithelium, where they perform their functions: killing and cytokine secretion for effector T cells, and secreting IgA for plasma cells.
lymph and similarly reach the blood. During differentiation, these lymphocytes lose expression of CCR7 and the cell-adhesion molecule L-selectin, and this prevents them from entering the secondary lymphoid tissues of the systemic immune system. The mucosa-derived effector cells express adhesion molecules and receptors that allow them to leave the blood at mucosa-associated lymphoid tissues (Figure 10.16). These molecules include integrin α4β7, which binds specifically to the mucosal vascular addressin MAdCAM-1 on endothelial cells of blood vessels in the gut wall, and CCR9, the receptor for chemokine CCL25 secreted by cells in the lamina propria (see Figure 10.16). The homing mechanisms are not specific to the particular mucosal tissue in which the effector cells were activated, but allow the effector B cells and T cells to enter and function in any mucosal tissue. For example, naive B and T cells activated in the gut-associated lymphoid tissue can thus enter and function in lymphoid tissue associated with the respiratory tract and vice versa. The benefit of this unifying arrangement is that the experience obtained by defeating infection in one mucosal tissue is used to improve the defenses of them all.

10-11 A variety of effector lymphocytes guard healthy mucosal tissue in the absence of infection

To avoid acute inflammatory responses of the type used to activate the systemic immune responses, the mucosal tissues are populated at all times with antigen-activated effector cells. This situation contrasts strongly with other tissues, which admit effector cells only when infected. Many of the effector cells in mucosal tissues were stimulated by antigens of commensal species, which likely account for most gut infections. Other effector lymphocytes arise from primary immune responses against pathogens, such as viruses, that are not normal inhabitants of the gut. The majority of the effector cells are T cells, there being more T cells in the gut-associated lymphoid tissue than in the rest of the body. The effector B cells are almost all plasma cells secreting either pentameric IgM or dimeric IgA, and they are mainly confined to the Peyer’s patches. The T cells are heterogeneous and comprise both γδ T cells and αβ T cells, with CD8 T cells predominating in the epithelium and CD4 T cells in the lamina propria (Figure 10.17). In addition to CD4 T cells, the lamina propria also contains CD8 T cells and plasma cells—as well as dendritic cells and the occasional eosinophil or mast cell (see Figure 10.17). Neutrophils are rare in the healthy intestine, but they rapidly populate sites of inflammation and disease.
Integrated into the epithelial layer of the small intestine is a distinctive type of CD8 T cell called the \textit{intraepithelial lymphocyte}. On average, there is about one intraepithelial lymphocyte for every 7–10 epithelial cells (see Figure 10.17). Intraepithelial lymphocytes have already been activated by antigen and contain intracellular granules like those of CD8 cytotoxic T cells. The intraepithelial lymphocytes include both $\alpha$:$\beta$ CD8 T cells or $\gamma$:$\delta$ CD8 T cells. They express T-cell receptors with a limited range of antigen specificities, indicating that they were activated by a limited number of antigens, and they have a distinctive combination of chemokine receptors and adhesion molecules that enables them to occupy their unique position within the intestinal epithelium. Like other gut-homing T cells intraepithelial lymphocytes express the chemokine receptor CCR9, but instead of $\alpha_4$:$\beta_7$ integrin, they express the $\alpha_E$:$\beta_7$ integrin, which attaches the T cell to E-cadherin on the surface of epithelial cells (see Figure 10.16, right panel). This adhesive interaction enables intraepithelial lymphocytes to intercalate within the layer of intestinal epithelial cells while maintaining the epithelium’s barrier function.

10-12 \textbf{B cells activated in mucosal tissues give rise to plasma cells secreting IgM and IgA at mucosal surfaces}

The mucosal surfaces of an adult human have a combined area of around 400 m$^2$. Defending these tissues is a coating of protective antibody that consists of secreted pentameric IgM and dimeric IgA and needs constant replenishment. Maintaining the antibody supply are 60 billion ($6 \times 10^{10}$) mucosal plasma cells, comprising 80% of the body’s plasma cells.

In the Peyer’s patches and mesenteric lymph nodes defending the gut, activation of naive B cells by antigen and antigen-specific T$_{FH}$ cells gives rise to an initial wave of effector B cells that leaves the lymphoid tissue in the efferent lymph en route to the bloodstream. In the blood the effector B cells travel to gut-associated lymphoid tissue, which they enter. This is achieved by the combined interactions of integrin $\alpha_4$:$\beta_7$ on the B cells with MAdCAM-1 on the intestinal vascular endothelium, and B-cell CCR9 binding to chemokine CCL25 emanating from the intestinal epithelial cells. Some B cells activated in
gut-associated lymphoid tissue return to their tissue of origin, but most take up residence in other areas of the gut and in different mucosal tissues. This strategy enables all the mucosal tissues to benefit from the antibody produced in one of them. Effector B cells settle in the lamina propria, where they complete their differentiation into plasma cells that make pentameric IgM and secrete it into the subepithelial space. Here the J chain of the IgM molecule binds to the poly-Ig receptor expressed by immature epithelial cells, also called stem cells, located at the base of intestinal crypts (see Figure 2.18, p. 42). By transcytosis, the poly-Ig receptor carries the antibody from the basal side to the luminal side of the cell, where the IgM is released and bound by the mucus. This transport mechanism for secretory IgM is the same as that used for secretory IgA (see Figure 9.18, p. 248).

Only some of the antigen-activated B cells differentiate into plasma cells secreting IgM. The others remain in the B-cell area of the gut-associated lymphoid tissue, where they undergo affinity maturation and isotype switching. The switch is usually to the IgA isotype, the dominant class of immunoglobulin in mucosal secretions. Switching to IgA is orchestrated by TGF-β and uses the same genetic mechanisms as those described in Chapter 4 for isotype switching and somatic hypermutation in the spleen and lymph nodes (see Sections 4-14 and 4-15). Several other soluble factors enhance switching to the IgA isotype. These include inducible nitric oxide synthase (iNOS), which is produced by dendritic cells and induces increased expression of the B cells’ TGF-β receptor, the vitamin A derivative retinoic acid, IL-4, IL-10, B-cell-activating factor (BAFF), and a proliferation-inducing ligand (APRIL). Both APRIL and BAFF are made by dendritic cells in gut lymphoid tissue and, in combination with IL-4, strongly bias isotype switching toward IgA.

Under the influence of these factors, effector B cells are programmed to make dimeric IgA that has higher affinities for antigen than the IgM antibodies made by the first wave of plasma cells. The isotype-switched cells constitute a second wave of effector B cells that, like the first, travel to the lamina propria of mucosal tissues throughout the body and differentiate into plasma cells. Plasma cells of the second wave make dimeric IgA that is secreted and transported across the mucosal epithelium by the poly-Ig receptor. Comparison of the sequences of IgA secreted by plasma cells of systemic and mucosal immunity shows a more extensive somatic hypermutation in the variable regions of mucosal IgA than in systemic IgA.

Dimeric IgA is the dominant immunoglobulin in tears, saliva, milk, and intestinal fluid. By contrast, IgG predominates in secretions of the nose, lower respiratory tract, and both the female and male urinogenital tracts. Monomeric IgG is actively transported into external secretions by the Fc receptor FcRn (Figure 10.18). IgE is transported across mucosal epithelial cells by the FceRI receptor (CD23) and is present in small concentrations in saliva, the gut, and the respiratory tract. To some extent, all antibody isotypes are present in the secretions at mucosal surfaces, and the amounts increase at sites of inflammation and infection where the barrier function of the mucosal epithelium has been damaged.

10-13 **Secretory IgM and IgA protect mucosal surfaces from microbial invasion**

M cells are continually sampling the gut contents, which activate B cells to make IgM and IgA antibodies specific for the gut microbiota. Transcytosis delivers the antibodies to the mucus layer on the luminal face of the gut epithelium. The antibodies are retained in the mucus by its inherent viscosity and by forming disulfide bonds with the mucin molecules (Figure 10.19). In keeping with the non-inflammatory environment in mucosal tissues, complement components are absent from mucosal secretions. Thus, unlike their systemic

![Figure 10.18 Transport of IgG from blood to mucosal secretions.](image-url)
counterparts, the secretory immunoglobulins do not fix complement as a means of neutralizing pathogens, but instead they coat the microbial surface in ways that impede microbial invasion and proliferation. In approaching the gut epithelium, bacteria are slowed down by the mucus and exposed to the antibodies and antimicrobial peptides it contains. If the bacterium is of a species that has already been sampled by M cells and has stimulated an immune response, it is bound by antibody, prevented from reaching the gut epithelium, and killed by the antimicrobial peptides. Bacteria that reach the epithelium and gain access, via M cells or another route, to the lamina propria can be opsonized with antibody and targeted for phagocytosis by resident macrophages.

Some secretory antibodies are specific for the surface components that bacteria and viruses use to bind to epithelial cells and either infect them or exploit them to gain access to the underlying tissues. By binding to these surface molecules, the antibodies prevent the invasion and infection of gut tissue by such bacteria and viruses. Cholera, caused by the bacterium *Vibrio cholerae*, is a
life-threatening disease in which a toxin secreted by the bacterium perturbs the intestinal epithelium, causing chronic diarrhea and severe dehydration. To have these effects the cholera toxin must bind to the epithelial cells and be endocytosed. The toxin can be neutralized by specific, high affinity IgA that binds the toxin and covers up the site with which it binds to gut epithelial cells (Figure 10.20).

Secretory IgA has little capacity or opportunity to activate complement or act as an opsin, and it cannot induce a state of inflammation. Instead it has evolved to be a non-inflammatory immunoglobulin that limits the access of pathogens, commensals, and food products to mucosal surfaces in a manner that avoids unnecessary damage to these delicate and vital tissues. Antibodies specific for commensal bacteria are well represented in the IgA secreted into the gut. By restricting commensal organisms to the lumen of the gut, and limiting the size of their populations, these antibodies have a crucial role in maintaining the symbiotic relationship of the microbiota with its human host.

10-14 Two subclasses of IgA have complementary properties for controlling microbial populations

There are two subclasses of IgA—IgA1 and IgA2—that are both made as a systemic monomeric IgA and a secretory dimeric IgA. As we saw for the IgG subclasses (see Figure 4.33, p. 106), the two IgA subclasses differ mainly in the hinge region, which is twice as long in IgA1 (26 amino acids) than in IgA2 (13 amino acids) (Figure 10.21). The longer hinge in IgA1 makes it more flexible than IgA2 in binding to pathogens and thus more able to use multiple antigen-binding sites to bind to the same pathogen and deliver it to a phagocyte. The drawback to the longer IgA1 hinge is its greater susceptibility to proteolytic cleavage than the shorter IgA2 hinge. Major bacterial pathogens, including *Streptococcus pneumoniae*, *Neisseria meningitidis*, and *Haemophilus influenzae*, have evolved specific proteases that cleave the IgA1 hinge, thereby disconnecting the Fc and Fab regions. This prevents the antibody from targeting the bacteria to phagocyte-mediated destruction. Exactly the opposite effect can sometimes occur: bacteria coated with Fab fragments of IgA1 become more able to adhere to mucosal epithelium, penetrate the physical barrier, and gain access to the lamina propria to launch an infection.

In situations where IgA1 is ineffective because of the presence of specific proteases, the synthesis of IgA2 helps to control bacterial infection. Although the IgA2 hinge is less flexible, it is highly protected by covalently linked carbohydrate, and bacteria have so far failed to evolve a protease that can cleave IgA2. In the blood, lymphatics, and extracellular fluid of the connective tissue, where bacterial populations are small and the IgA1-specific proteases pose less of a threat, most of the IgA made (93%) is of the IgA1 isotype. In contrast, in the colon, where bacteria are present at the highest density and IgA1-specific proteases are ubiquitous, the majority of IgA made (60%) is of the IgA2 isotype (Figure 10.22).

The switch to IgA secretion normally goes from IgM to IgA1, but in the presence of the TNF-family cytokine APRIL, the isotype switches from IgM to IgA2. In the colon the epithelial cells make APRIL, which drives the switch to the IgA2 isotype in the resident B cells. In general, the synthesis of IgA2 is higher in
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mucosal lymphoid tissues than in systemic lymphoid tissues, but the proportions of plasma cells making IgA1 and IgA2 also vary considerably between the different mucosal tissues (see Figure 10.22). The tissues most heavily populated with microorganisms—the large and small intestines, the mouth (supplied with IgA by the salivary glands), and the lactating breast (exposed to the heavily contaminated oral cavity of the suckling infant)—are those more focused on making IgA2. These differences show that the various mucosal tissues are not immunologically equivalent and that they face different challenges in balancing their burden of commensal and pathogenic microorganisms that make IgA1-specific proteases.

10-15 People lacking IgA are able to survive, reproduce, and generally remain healthy

Expert mucosal immunologists consider that IgA is probably the best-understood and most widely accepted mediator of mucosal immunity. Given the

![Figure 10.21 IgA1 and IgA2 have hinges of different lengths. Models of the three-dimensional structures of IgA1 (panel a) and IgA2 (panel b) are shown in the top panels, and diagrams corresponding to these models are shown in the middle panels: IgA1 (panel c) and IgA2 (panel d). Panel e shows the organization of a dimer of IgA1, with the attached J chain. Panel f shows a molecule of secreted IgA2, which contains both the J chain and the secretory piece of the poly-Ig receptor.]

<table>
<thead>
<tr>
<th>Tissue</th>
<th>IgA1 %</th>
<th>IgA2 %</th>
<th>IgA1/A2 ratio</th>
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</thead>
<tbody>
<tr>
<td>Spleen, peripheral lymph nodes, tonsils</td>
<td>93</td>
<td>7</td>
<td>13.3</td>
</tr>
<tr>
<td>Nasal mucosa</td>
<td>93</td>
<td>7</td>
<td>13.3</td>
</tr>
<tr>
<td>Bronchial mucosa</td>
<td>75</td>
<td>25</td>
<td>3.0</td>
</tr>
<tr>
<td>Lachrymal glands (tear ducts)</td>
<td>80</td>
<td>20</td>
<td>4.0</td>
</tr>
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<td>Salivary glands</td>
<td>64</td>
<td>36</td>
<td>1.8</td>
</tr>
<tr>
<td>Mammary glands</td>
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<td>40</td>
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<tr>
<td>Gastric mucosa (stomach)</td>
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<td>17</td>
<td>4.9</td>
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<tr>
<td>Duodenum–jejunum (upper small intestine)</td>
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<td>29</td>
<td>2.4</td>
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<tr>
<td>Ileum (lower small intestine)</td>
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<td>Colon (large intestine)</td>
<td>36</td>
<td>64</td>
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![Figure 10.22 IgA1 and IgA2 are differentially expressed in mucosal tissues. Shown here are the relative proportions of plasma cells making IgA1 and IgA2 in each tissue. The number of plasma cells correlates directly with the amount of antibody made and secreted. Data courtesy of Per Brandtzaeg.]
importance of mucosal immunity for human health, it is therefore surprising that many seemingly healthy people make little or no IgA. Because other immunoglobulin isotypes are not affected, this condition is called selective IgA deficiency. It occurs throughout the world but at frequencies that vary over two orders of magnitude (Figure 10.23). The cause of the IgA deficiency seems to be defective isotype switching from IgM to IgA. The condition has a genetic basis, as seen from the case of an infant with aplastic anemia and normal IgA who was treated with a bone marrow transplant from his HLA-identical, but IgA-deficient, sister. Upon reconstitution of his immune system by his sister’s hematopoietic stem cells, the boy became IgA deficient but was otherwise healthy and no longer dependent upon medication. So why is IgA so dispensable?

As infants, IgA-deficient individuals need not make IgA because they can get it from their mothers, provided that the mother is not IgA-deficient. In nursing mothers, plasma cells derived from B cells activated in the gut, lungs, and other mucosae home to the lactating mammary gland to contribute their secretory IgA to the breast milk. The milk therefore contains all the different IgA antibodies the mother has recently made in responding to commensal microorganisms, infecting pathogens, and food antigens. On suckling, the infant’s gut receives a portfolio of maternal IgA that provides protection against the gut microbiota and locally endemic pathogens. Until very recently in human history, mothers would have breastfed their children for 3–7 years after birth. In this most vulnerable period of life, most IgA-deficient children would have been protected by maternal IgA, which could explain how deleterious gene variants contributing to IgA deficiency have survived. The trend in present-day populations has been to shorten the period of breastfeeding. Although this is expected to increase the vulnerability of infants to infectious disease, it is mitigated by modern improvements in hygiene, nutrition, and vaccination that reduce the risks of infection.

Because they cannot switch isotype from IgM to IgA, plasma cells making other isotypes are more abundant in IgA-deficient people (Figure 10.24). For mucosal immunity, IgM is particularly important, because it has the J chain that interacts with the poly-Ig receptor and so can be secreted at mucosal surfaces, like IgA. Moreover, IgM always precedes IgA as the first secretory antibody in the adaptive responses of mucosal immunity. Increased secretion of pentameric IgM probably compensates for the absence of secretory IgA, at least in the relatively parasite-free environment of developed countries. Increased transport of IgG from lamina propria to the gut mucosa by FcRn could further augment defenses. IgA-deficient individuals are susceptible to bacterial infections of the lungs, and to intestinal infection by *Giardia lamblia*, a protozoan parasite. Thus the health and vigor of people with IgA deficiency today might in part reflect reduced pressure on the mucosal immune systems of human populations in modern industrialized societies. These people generally eat cooked, highly processed food, and are not infested with helminth worms and the other intestinal parasites that were prevalent in the past and
IgA deficiency is a clinically heterogeneous condition, and its epidemiology and segregation in families are complicated and remain unpredictable. The data cannot be explained by defects in a single gene, and indicate that IgA deficiency is caused by combinations of variants (alleles) of genes on different chromosomes, and that these combinations differ between human populations. As well as TGF-β and its receptor, retinoic acid, IL-4, IL-10, BAFF, and APRIL are all implicated in switching immunoglobulin isotype from IgM to IgA (see Section 10-12), and mutations in their genes are candidates for contributing to selective IgA deficiency.

10-16 T H 2-mediated immunity protects against helminth infections

Helminths are parasitic worms that live and reproduce in the intestines. They comprise three groups—nematodes, trematodes, and cestodes—that can all cause chronic and debilitating disease (Figure 10.25) by competing with the host for nutrients and causing local damage to the intestinal epithelium and blood vessels. With the exception of people in developed countries, virtually all humans are burdened with helminth infections. Because helminths are never commensal organisms but always pathogens, there are worldwide medical programs aimed at de-worming the entire human population. For similar reasons, the immune system has evolved a variety of mechanisms for the containment and elimination of helminth infections. The most effective immune response to a helminth depends on the particular parasite’s life cycle. Some attach to the luminal side of the intestinal epithelium, others enter and colonize the epithelial cells, and yet others invade beyond the intestine and spend part of their life cycle in another tissue, such as the liver, lungs, or muscle.

In order to survive and flourish in the intestines, helminths must at all times avoid being cast into the flowing fluid of the gut lumen by the constant turnover and renewal of the enterocytes. Conversely, in countering helminth infections, the purpose of the immune system is to drive the worms into the gut lumen, from which they can be expelled in the feces. This can only be achieved by mounting an adaptive immune response that is dominated by T H2 CD4 T cells and involves the production of the T H2-associated cytokines IL-4, IL-9, IL-13, IL-25, and IL-33. Most counterproductive is an inflammatory response

<table>
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<th>Common name</th>
<th>Roundworms</th>
<th>Flukes</th>
<th>Tapeworms</th>
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<tbody>
<tr>
<td>Scientific name</td>
<td>Nematodes</td>
<td>Trematodes</td>
<td>Cestodes</td>
</tr>
<tr>
<td>Diseases caused</td>
<td>Ascaris*, Dracunculiasis (guinea worm disease), Elephantiasis (lymphatic filariasis), Enterobiasis* (pinworm), Hookworm* Onchocerciasis (river blindness), Trichinosis*</td>
<td>Schistosomiasis, Fasciolopsiasis*</td>
<td>Tapeworm infection*</td>
</tr>
</tbody>
</table>

Figure 10.25 Helminths are major human pathogens that parasitize the intestines. The helminths comprise four major groups, three of which include human pathogens. *Caused by parasites that live in the gut lumen.
dominated by $\text{T}_{\text{H}1}$ cells and the production of interferon-$\gamma$ (IFN-$\gamma$). This not only fails to eliminate the parasite but also exacerbates the infection and the likelihood of severe, persistent, and crippling disease. One deleterious effect of IFN-$\gamma$, for example, is to decrease the turnover rate of the epithelial cells, thereby making the intestinal environment more stable one for the parasite.

Orchestrating the innate immune response to the invading helminth are the intestinal epithelial cells in the affected area of tissue, which detect the pathogen with their NOD and Toll-like receptors that then activate NFkB. When initiating a $\text{T}_{\text{H}2}$ response, the endothelial cells secrete IL-33, described as a $\text{T}_{\text{H}2}$ accelerator, and thymic stromal lymphopoietin (TSLP). These cytokines influence the local dendritic cells, which have taken up helminth antigens, to travel to the draining mesenteric lymph node and stimulate antigen-specific T cells to differentiate into CD4 $\text{T}_{\text{H}2}$ cells. This also produces CD4 $\text{T}_{\text{FH}}$ cells that engage antigen-specific B cells and make them switch to the IgE isotype. A strong antigen-specific IgE response is one of several features that characterize an effective anti-parasite response (Figure 10.26).

An abundance of mast cells in the helminth-infected tissue is another feature of a protective $\text{T}_{\text{H}2}$ response. IL-3 and IL-9 secreted by CD4 $\text{T}_{\text{H}2}$ cells recruit mast-cell precursors from the blood into the infected tissue, where they become fully differentiated mucosal mast cells. The high-affinity Fc$\varepsilon$RI receptor on mast cells binds IgE tightly in the absence of antigen. If helminth antigens then bind to the IgE and cross-link two Fc$\varepsilon$RI molecules, the mast cells become activated to release the contents of their preformed granules, which are full of highly active inflammatory mediators such as histamine. In the gut, these mediators induce the muscle spasms and watery feces that characterize diarrhea, a condition that thoroughly disrupts the environment in which the parasites live, and can evict them first into the gut lumen and then force them rapidly out of the body (see Section 9-13). CD4 $\text{T}_{\text{H}2}$ cells also secrete IL-5, which is the major cytokine controlling eosinophil development and function. During a helminth infection, the IL-5 increases the numbers of eosinophils in the blood and in the infected gut tissue. Like mast cells, eosinophils express Fc$\varepsilon$RI, which can bind parasite-specific IgE. This can then be cross-linked by the antigens on the worm’s surface to activate the eosinophil. The antibody acts to tether the parasite to the surface of the eosinophil so that degranulation of the activated eosinophil will release the granules’ toxic molecules, such as major basic protein (MBP), directly onto the worm’s surface, where they can injure or kill the parasite. Under attack in these different ways, the parasite is less likely to survive for long in the gut epithelium.

IL-13 is a cytokine secreted by CD4 $\text{T}_{\text{H}2}$ cells that influences the dynamics of the intestinal epithelium. Hyperplasia in the stem cells of the crypt increases the production of goblet cells, which in turn increases the production of mucus. This makes it more likely that the worms will become enmeshed in mucus and more easily shed from the epithelium and flushed from the body. Increasing the production of enterocytes has the effect of increasing enterocyte turnover rate but not their abundance. The resultant halving of the enterocyte life-span perturbs the pathogens’ environment, increasing the likelihood that the worms will be dislodged and shed into the gut lumen. Atrophy of the villi, reduced absorption of nutrients, and loss of weight by the host accompanies the $\text{T}_{\text{H}2}$-mediated immune response. This could represent a temporary channeling of resources into the immune response and away from other physiological functions and from the parasite.

Although the adaptive B-cell and T-cell responses are specific to the helminth causing the infection, there is little selectivity in the effector functions used. The immune response to helminth infections does not adapt to the differences distinguishing the life cycles of different species. The key difference in determining the fate of an infecting helminth and its human host is whether the
Naive CD4 T cells activated during helminth infection differentiate to become TH1 or TH2 effector cells.

**TH2-cell effector functions**

- TH2 cells produce IL-13 which increases production of epithelial cells in the infected tissue.
- TH2 cells produce IL-5 which recruits eosinophils to the infected tissue and activates them.
- TH2 cells drive B cells to produce parasite-specific IgE.
- TH2 cells produce IL-3 and IL-9 which recruit mast cells to the infected tissue.

**TH1-cell effector functions**

- TH1 cells secrete interferon-γ and activate macrophages.
- TH1 cells activate B cells to produce IgG.

**PROTECTIVE EFFECTS**

- Increased number of goblet cells produce more mucus.
- Increased production of enterocytes increases their rate of turnover in the epithelium.
- Activated eosinophils acquire granules containing MBP and parasite-specific IgE bound to FcR1. Degranulation kills helminths.
- Parasyte-specific IgE circulates in blood.
- Mast cells bind parasite-specific IgE. Degranulation causes muscle spasms and diarrhea that expel helminths.
- Interferon-γ facilitates parasite infection. The inflammatory response further disrupts infected tissue.
- IgG antibodies are not effective against helminths.

**HOST DAMAGE**

- Degranulation kills helminths.
- Interferon-γ facilitates parasite infection. The inflammatory response further disrupts infected tissue.
- IgG antibodies are not effective against helminths.

**Figure 10.27** Human responses to helminth infection can either confer protection or cause chronic parasitic disease. CD4 T-cell responses to intestinal helminths usually polarize, becoming either a protective TH2 response (first four panels) or a pathological TH1 response (last two panels). TH2 responses lead to killing and expulsion of the parasite, whereas TH1 responses lead to persistent infection and chronic debilitating diseases of varying severity. MBP, major basic protein.

The mucosal surfaces of the body cover vital organs that communicate material and information between the human body and its internal environment. Because of these functions, the mucosal surfaces form a more fragile barrier than the skin and are more vulnerable to infection. The possibility of infection is increased even more by the greater area of the mucosal surfaces compared with the skin, and by the large, diverse populations of commensal microorganisms that inhabit mucosal surfaces, particularly those of the gut. Consequently, adaptive immune response becomes mainly TH2 or mainly TH1 in nature. A TH2 response kills and eliminates the parasite to the benefit of the host, whereas a TH1 response benefits the parasite at the expense of the health of the host (Figure 10.27).

**Summary to Chapter 10**

The mucosal surfaces of the body cover vital organs that communicate material and information between the human body and its internal environment. Because of these functions, the mucosal surfaces form a more fragile barrier than the skin and are more vulnerable to infection. The possibility of infection is increased even more by the greater area of the mucosal surfaces compared with the skin, and by the large, diverse populations of commensal microorganisms that inhabit mucosal surfaces, particularly those of the gut. Consequently,
some 75% of the immune system’s resources are dedicated to defending the mucosae. The mechanisms and character of adaptive immunity in mucosal tissue, as exemplified by the gut, differ in several important respects from adaptive immunity in other tissues (Figure 10.28).

Secondary lymphoid tissues, which are directly incorporated into the gut wall, continuously sample the gut’s luminal contents and stimulate adaptive immune responses against pathogens, commensal organisms, and food. The effector T cells that are generated populate the epithelium and lamina propria of the gut, and the plasma cells produce dimeric IgA that is transcytosed to the lumen, where it coats the mucosal surface. In the healthy gut there is a chronic adaptive immune response that is not inflammatory in nature. This response, in combination with the mechanisms of innate immunity, ensures that microorganisms are confined to the lumen of the gut and prevented from breaching the mucosal barrier. Helminth worms are pathogens that inhabit the intestines and are controlled by adaptive immune responses made in the mesenteric lymph nodes and orchestrated by CD4 T_{H} cells. This response uses parasite-specific IgE to facilitate eosinophil-mediated killing of the worms and mast-cell mediated ejection of them from the body of the human host.

In conclusion, the strategy of the mucosal immune system is to avoid inflammation by being proactive and constantly making adaptive immune responses against potential pathogens before they cause infections. This approach contrasts with that of the systemic immune system, which avoids making an adaptive immune response unless it is absolutely necessary and then relies on inflammation to orchestrate that response.
Questions

10–1  Mucosae exist in all of the following anatomical locations except _____.
   a. lactating breasts
   b. urogenital tract
   c. the limbs
   d. gastrointestinal tract
   e. salivary glands
   f. lacrimal glands
   g. the respiratory tract
   h. the pancreas.

10–2  All of the following are characteristics of some or all mucosal surfaces except _____. (Select all that apply.)
   a. the secretion of viscous fluid called mucus
   b. reproductive activities
   c. absorption of nutrients
   d. participation in gas exchange
   e. participation in sensory activities
   f. collectively constitute approximately 25% of the body’s immune activities
   g. use of tight junctions to join epithelial layers
   h. tissue regenerates about every 20–30 days.

10–3  Match the term in column A with its description in column B.

<table>
<thead>
<tr>
<th>Column A</th>
<th>Column B</th>
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<tbody>
<tr>
<td>a. mucosae</td>
<td>1. constitute the gut microbiota</td>
</tr>
<tr>
<td>b. cecum</td>
<td>2. epithelial surfaces distributed throughout the body</td>
</tr>
<tr>
<td>c. systemic immune system</td>
<td>3. protective epithelial glycoproteins</td>
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<tr>
<td>d. mucins</td>
<td>4. located between the small and large intestines</td>
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<td>e. commensal microorganisms</td>
<td>5. defends against pathogens that breach the skin</td>
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10–4  The main function of the mucus in the stomach is to _____.
   a. trap and kill ingested microorganisms
   b. enzymatically degrade complex nutrients
   c. protect epithelial cells from the acidicified environment
   d. protect the microbiota from corrosive gastric juices
   e. delay the digestive process to maximize absorption.

10–5  ____ arises from an adaptive immune response to the proteins of gluten.
   a. Cholera
   b. Celiac disease
   c. Selective IgA deficiency
   d. Crohn’s disease.

10–6  The “M” used to name M cells of the gastrointestinal tract derives from _____.
   a. mesenteric
   b. microfold
   c. monocytes
   d. mucosa
   e. mast cells.

10–7  Match the term in column A with its description in column B.

<table>
<thead>
<tr>
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<tbody>
<tr>
<td>a. lamina propria</td>
<td>1. situated at the entrance of the gut and airway</td>
</tr>
<tr>
<td>b. Peyer’s patch</td>
<td>2. a chain of lymph nodes in connective tissue of the gastrointestinal tract</td>
</tr>
<tr>
<td>c. Waldeyer’s ring</td>
<td>3. dome-like bulging aggregates of lymphocytes that extend into the lumen of the gut</td>
</tr>
<tr>
<td>d. M cells</td>
<td>4. transport antigen to pockets on the basolateral side of the gut epithelium</td>
</tr>
<tr>
<td>e. mesenteric lymph nodes</td>
<td>5. CDB T cells with limited range of antigen specificities</td>
</tr>
<tr>
<td>f. intraepithelial lymphocytes</td>
<td>6. connective tissue beneath the gut epithelium</td>
</tr>
</tbody>
</table>

10–8  Identify two ways in which the immune responses in gut mucosal tissues contrast with those initiated in systemic non-mucosal tissues.

10–9  Which of the following statements regarding intraepithelial lymphocytes is false? (Select all that apply.)
   a. They comprise approximately 10% of the cells in the mucosal epithelium.
   b. They are composed of both CD4 and CD8 T cells.
   c. They are separated from the lamina propria by a basement membrane.
   d. They are activated effector T cells with a narrow range of antigen specificities.
   e. They do not include NK cells.
   f. They express the α4β7 integrin that binds to E-cadherin on epithelial surfaces.

10–10  An important distinction between macrophages that populate the lamina propria of the gut and the macrophages that populate the skin is that the former _____.
   a. cannot phagocytose and kill bacterial pathogens
   b. do not present antigens to T cells
   c. do not possess signaling receptors needed for production of inflammatory cytokines
   d. express much higher levels of TLRs
   e. are very rare in the gut mucosa.

10–11  Whereas ____ is the predominant immunoglobulin in intestinal fluid, ____ is the dominant immunoglobulin in the urogenital tract.
   a. dimeric IgA; IgE
   b. IgE; dimeric IgA
   c. dimeric IgA; pentameric IgM
   d. dimeric IgA; IgG
   e. pentameric IgM; monomeric IgA.

10–12  Which of the following pairs is mismatched?
   a. NOD1: a cytoplasmic receptor of intestinal epithelium
   b. NLRP3: assists in the formation of an inflammasome
   c. intestinal macrophages: professional antigen-presenting cells
d. TLR-5: detects flagellin on apical and basolateral epithelial surfaces
e. neutrophils: attracted by CXCL8.

10–13 A T lymphocyte activated in the GALT will subsequently home to all of the following except _____. (Select all that apply.)
   a. mucosal lymphoid tissues of lactating mammary glands
   b. the spleen
   c. mucosal lymphoid tissues of the respiratory tract
   d. systemic lymph nodes
   e. mucosal lymphoid tissues of the gastrointestinal tract
   f. lymphoid tissues of the skin.

10–14 Identify which of the following immune responses is pivotal to the killing and elimination of helminths.
   a. killing by cytotoxic T cells in the lamina propria
   b. TH1-induced inflammation
   c. TH2-associated cytokines
   d. phagocytosis by intestinal macrophages
   e. systemic immune responses
   f. B-cell secretion of IgG.

10–15 Richard Brennan began penicillamine therapy after he was diagnosed with Wilson’s disease (manifested by copper accumulation in the tissues) at age 10 years. Ten months after beginning this treatment he began to experience multiple sinus infections, and one episode of pneumonia. Recently he came to the emergency room with acute diarrhea, vomiting, fever, and foul-smelling intestinal gas. Stool samples revealed the presence of trophozoites of Giardia. Blood tests showed normal levels of B and T cells and normal IgM and IgG concentrations, but markedly decreased IgA at 6 mg/dl (normal range 40–400 mg/dl). Richard was treated for his giardiasis with metronidazole. His selective IgA deficiency was associated with penicillamine, shown previously to be a complication in some patients with Wilson’s disease. His IgA levels returned to normal when penicillamine was discontinued. This is an example of a drug-induced transient form of IgA deficiency. Which of the following antibodies that uses the same transport receptor as dimeric IgA would have been present in the lumen of the gastrointestinal tract and mucosal secretions of Richard while he was taking penicillamine?
   a. IgD
   b. IgM
   c. IgG
   d. IgE
   e. none of the above.
Young boy with measles.