CHAPTER 18

Epithelia and gastrointestinal function

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Chapter menu

All cavities within the alimentary tract, from the small ducts and acini of the pancreas to the gastric lumen, are lined by sheets of polarized epithelial cells. Common to all of these epithelia is the ability to create selective barriers that separate luminal and tissue spaces. Most epithelia are also able to direct vectorial transport of solutes and solvents. These essential functions are based on the structural polarity of individual cells, the complex organization of membranes, cell–cell and cell–substrate interactions, and interactions with other cell types. This chapter reviews intestinal wall structure and examines how mucosal functions are supported by the organization of the gut and the biological properties of the epithelial barrier and transepithelial transport.

Organization of the gut wall

As exemplified by the small intestine and depicted in Figure 18.1, there are four principal layers in the wall of the gastrointestinal tract: mucosa, submucosa, muscularis propria, and serosa or adventitia. The mucosa consists of the epithelium, an underlying layer of loose connective tissue carrying nerves and vessels (i.e., lamina propria), and a thin layer of smooth muscle (i.e., muscularis mucosae). The mucosa also contains an array of lymphocytes, mast cells, macrophages, and, in disease states, polymorphonuclear leukocytes, all of which are capable of modulating epithelial function.

An underlying layer of fibroconnective tissue called the submucosa, which contains nerves, vessels, and lymphatics, supports the mucosa. The submucosa rests on the muscularis propria, which is composed of two or three layers of smooth muscle and is home to the myenteric plexus (see Chapters 1, 15, and 16). In most instances, gastrointestinal organs are encased by an outermost delicate layer of fibrofatty tissue, the serosa, encircled by a continuous layer of mesothelial cells. In areas where no serosa exists, as in portions of the esophagus and the distal colorectum, fibrofatty tissues interface with the external portion of the muscularis propria. These organs are said to have an adventitial, rather than a serosal, encasement. Microscopic anatomy varies along the length of the gastrointestinal tract. A simple columnar epithelium lines the stomach, small intestine, colon, pancreatobiliary ducts, and exocrine pancreas. In contrast, the oral cavity, esophagus, and anus are lined by a nonkeratinized, stratified squamous epithelium that is capable of withstanding the mechanical stresses of swallowing and defecation but plays no role in transepithelial transport. The three-dimensional structure of epithelia also exhibits significant variation within the gastrointestinal tract, such as the prominent mucosal folds and villi in the small intestine (see Figure 18.1), the lobular organization of the exocrine pancreatic acini (Chapter 7), and the deep epithelial extensions into salivary and Brunner glands in the esophagus and duodenum, respectively. The liver, which lacks a single large lumen, is composed primarily of hepatocytes. These possess only a small apical

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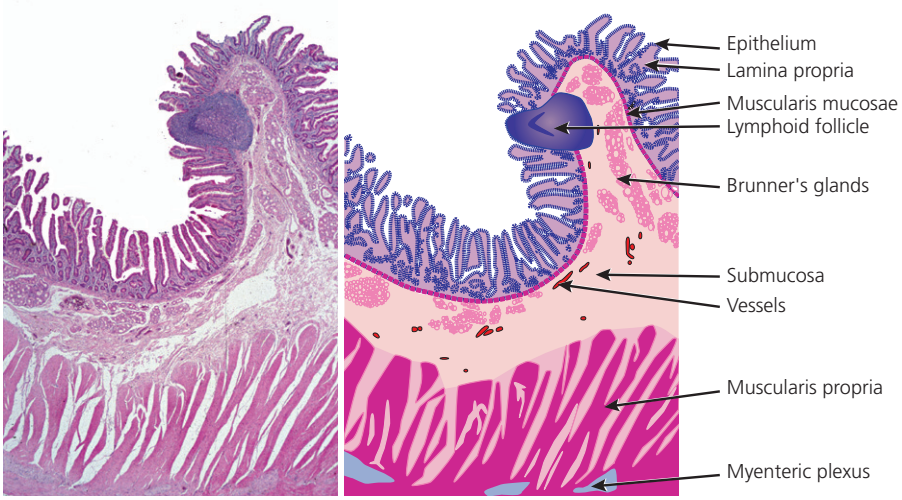

Figure 18.1 Organization of the intestinal wall. This transmural section of normal human duodenum exemplifies many of the structural features that are common throughout the gastrointestinal tract. The corresponding line diagram delineates specific structures.

(canalicular) surface and lack complex deeper cell layers; capillaries and hepatocytes are separated by a thin basement membrane in the space of Disse (see Chapter 10). Despite these complexities of regional specialization, common structural features critical to epithelial functions are present throughout the gastrointestinal tract.

Organization of epithelial cells and sheets

To function properly as a barrier, epithelial cells must assemble into a multicellular sheet. This is a complex task that requires individual cells to establish polarity uniformly; to form intercellular junctions; and to develop stable interactions with the basement membrane (Figure 18.2).

A central function of many gastrointestinal epithelia is the vectorial transport of solutes and solvents. For example, parietal cells are polarized to effect secretion of acid into the lumen (see Chapter 23). Defective polarization of these cells could result in acid secretion into the interstitium and severe tissue damage. Absorptive villous enterocytes of the small intestine are specialized to accomplish vectorial transport of ions, nutrients, and water from the lumen to the interstitium (Figure 18.3) by expressing specific transporters within the apical (luminal), but not basolateral, membrane domain [1–3]. These transporters often rely on a luminal Na^+ concentration that is much higher than the intracellular Na^+ required for cotransport of sugars, amino acids, ions, and bile salts. In general, the absorbed solutes exit the cytosol by way of Na^+-independent facilitated (diffusive) transporters present in basolateral, but not apical, membranes. These include GLUT2, which carries glucose across the basolateral membrane [4]. There are, however, examples of nutrients that are absorbed from the lumen by diffusive transporters, e.g., fructose by GLUT5, and some authors have argued that GLUT2 may also be inserted apically to allow for diffusive glucose absorption [4]. The high extracellular and low intracellular Na^+ concentrations that provide the driving force for apical Na^+-dependent transporters for this system are maintained by the basolateral localization of Na^+,K^+-adenosine triphosphatase (ATPase) that pumps Na^+ out of the cell in exchange for K^+. Thus, the net result of apical Na^+-coupled transport is vectorial transport of both the specific solute and Na^+ from the lumen to the interstitium. The polarized distribution of these three classes of transport proteins – apical Na^+-coupled transporters, basolateral Na^+-independent diffusive transporters, and the basolateral Na^+,K^+-ATPase – is critical for active transepithelial transport. Sufficient luminal Na^+ to drive these processes is also critical for absorption, but dietary Na^+ alone is insufficient to meet the demands of apical transporters. Thus, Na^+ recycling across the tight junction, which, as discussed below, seals the paracellular space, is essential for nutrient absorption [5].

One result of active transcellular solute transport is the deposition of osmotically active molecules (e.g., nutrients and ions) in the subepithelial interstitial space. This provides the driving force for passive paracellular water absorption. Such integration of active transcellular and passive, primarily paracellular, transport partially explains the improved efficacy of oral rehydration solutions supplemented with Na^+ and carbohydrates [6,7]. Although details of these transport processes will be addressed below in further detail, it should be clear that polarized distribution of surface membrane components within individual cells is essential to epithelial function. It is also evident that the entire epithelial sheet must be uniformly polarized, i.e., with all cells polarized in the same orientation, as adjacent cells could otherwise negate one another's contributions and make net transport impossible.

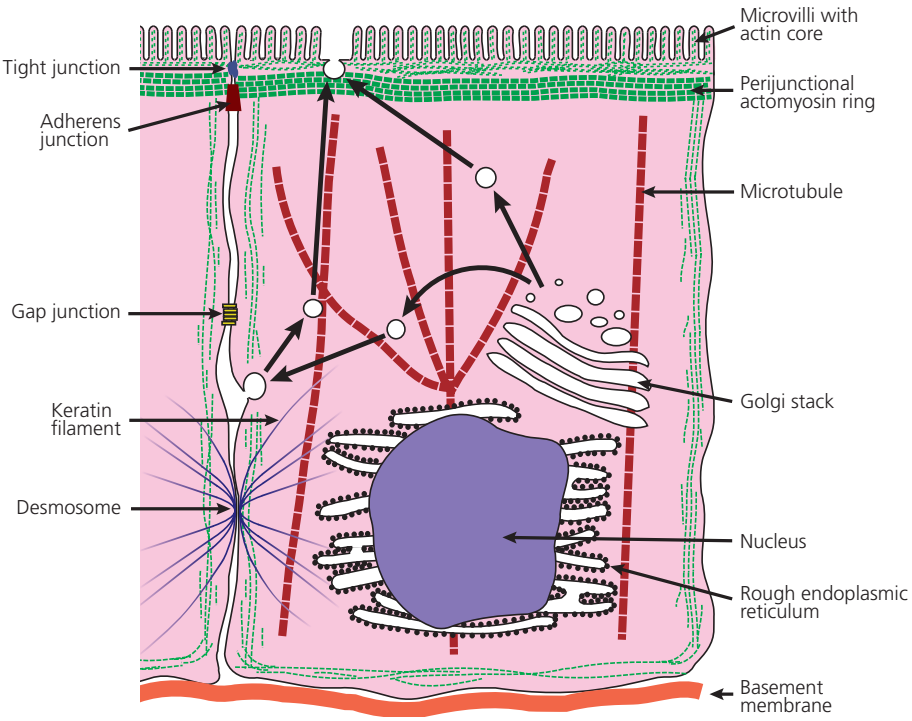

Figure 18.2 Organization of a typical gastrointestinal columnar epithelial cell. The precisely orchestrated architecture of polarized epithelia includes numerous specialized structures, such as cytoskeletal elements, transport vesicles, and intercellular junctions.

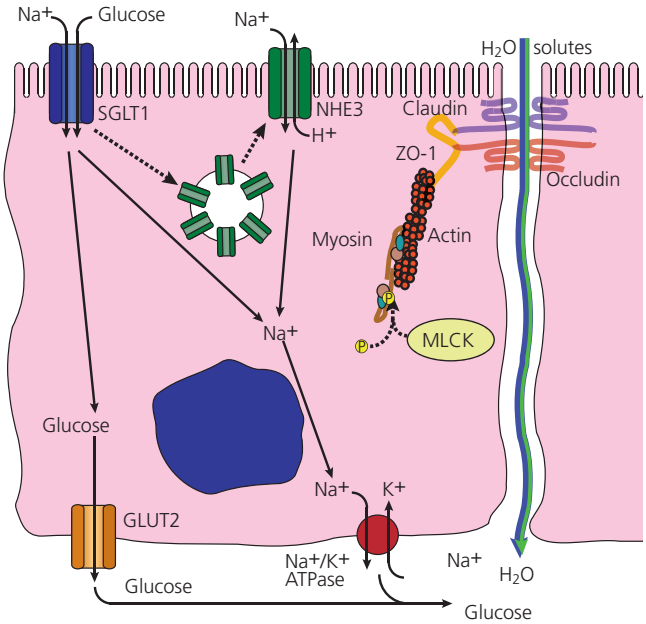

Figure 18.3 Coordination of transcellular and paracellular absorption of water, nutrients, and ions. The polarized delivery of SGLT1, GLUT2, and Na^+,K^+-adenosine triphosphatase (ATPase) to the appropriate apical (SGLT1) and basolateral (GLUT2 and Na^+,K^+-ATPase) membranes is essential for efficient vectorial glucose absorption. SGLT1 activation also stimulates apical NHE3-mediated Na^+ absorption, thereby linking these absorptive processes. Incompletely defined downstream signaling events subsequently activate myosin light chain kinase (MLCK). This, in turn, phosphorylates MLC and increases paracellular permeability, thereby linking transcellular and paracellular absorption. NHE3, Na^+H^+ exchanger.

Initiation of epithelial polarization

The spatial cues that induce and maintain polarization have generally been thought to require contact with the extracellular matrix and adjacent epithelial cells. For example, the interaction between the epithelial Ca^{2+}-dependent adherens junction protein, E-cadherin on adjacent cells is a critical trigger for polarization. E-cadherin is concentrated at the adherens junction, the basal-most aspect of the apical junction complex (see Figure 18.2), where it is coupled to a perijunctional ring of actin and myosin filaments through cytoplasmic linker proteins (e.g., α-actinin and α- and β-catenins) [6,7]. In addition to E-cadherin-mediated and other forms of intercellular adhesion, cellular interactions with matrix components through integrins, heparan sulfate proteoglycans, and other membrane proteins are also important in inducing polarization and maintaining differentiation [8–10].

The discovery of polarity genes encoding Par proteins in *Caenorhabditis elegans* and *Drosophila* facilitated the identification of mammalian homologs which functionally define epithelial apical and basolateral plasma membrane domains [11,12]. Not surprisingly, Par protein mutations have been linked to disease. For example, mutation of LKB1, the mammalian homolog of the *C. elegans* protein Par-4 is associated with Peutz–Jeghers syndrome [13]. LKB1 is a serine/threonine kinase that is activated by the STRAD adapter protein, which may also be mutated in Peutz–Jeghers syndrome [14]. In model systems, STRAD-mediated LKB1 activation can initiate epithelial polarization [15]. While the mechanisms responsible for

this incomplete polarization have not yet been defined, one potential mediator may be adenosine monophosphate (AMP)-activated protein kinase, which is activated by LKB1 and plays an important role in maintaining cellular energy balance [16]. Alternatively, LKB1 loss has also been shown to facilitate inappropriate activation of Wnt pathway signaling [8,9] (see Chapter 2), which may explain Wnt5a upregulation in both *Lkb1*$^{+/-}$ mice and polyps from Peutz–Jeghers patients [10]. These data together with the recent discovery that Wnt5a promotes crypt fission during wound healing [11], may explain the complex, arborizing architecture of Peutz–Jeghers polyps.

Consistent with the idea that aberrant epithelial polarization may contribute to neoplasia, mutations of the E-cadherin and adenomatous polyposis coli (APC) binding partner β-catenin that result in Wnt pathway activation and are common in human colorectal cancers [12–15]. The transcriptional repression of E-cadherin during the epithelial–mesenchymal transition that is typical of invasive cancer [17], strong correlation between the loss of E-cadherin expression and the invasive phenotype of many human gastrointestinal neoplasms [18,19], and germline E-cadherin mutations associated with familial gastric cancer [16] provide further support for the conclusion that polarity defects are linked to neoplasia. The underlying mechanisms by which E-cadherin, APC, and β-catenin mutations contribute to carcinogenesis are discussed further in Chapters 2 and 31.

Structure of intercellular junctions

All polarized epithelia share a common set of intercellular junctions. These include, from the luminal aspect, the tight and adherens junctions, which form continuous circumferential contacts, and, below these, desmosomes and gap junctions, which form macular or spot contacts. Together these junctions maintain polarity, seal the paracellular space, provide intercellular communication, and stabilize the monolayer to preserve overall epithelial integrity.

As noted above, homotypic interactions between E-cadherin proteins on adjacent cells are an initial cue for the generation cell polarity. These are followed by assembly of the apical junctional complex, which is composed of the adherens junction and the tight junction. The latter defines the boundary between apical and basolateral membrane domains. As a result, the tight junction has been suggested to have a "fence" function, whereby it prevents mixing of transmembrane proteins and outer leaflet membrane lipids between apical and basolateral domains [17]. However, it appears that polarized distribution of membrane proteins can still be established and maintained when tight junction assembly is blocked [18]. Thus, at least for proteins, tight junction fence function may have been overstated; a role in the maintenance of polarized lipid distributions remains possible.

When examined by electron microscopy, tight junctions appear as 100–300-nm-deep zones where adjacent cells closely abut (Figure 18.4). Series of punctate fusions or "kisses" between these plasma membranes form a seal between adjacent cells. These fusion sites are arrayed in a linear fashion around the cell and correspond to the net-like series of anastomosing grooves and strands seen in freeze-fracture replicas (Figure 18.4).

(a) (b)

Figure 18.4 Tight junction ultrastructure. Electron microscopic appearance of the tight junction (arrows) of a small intestinal absorptive epithelial cell. The transmission electron micrograph **(a)** shows that the tight junction is a zone of closely apposed cell membranes. Freeze-fracture electron microscopy **(b)** reveals the dense interconnecting network of strands that define the tight junction.

Directly below the tight junction lies the adherens (or intermediate) junction, in which the lateral membranes of adjacent cells lie parallel to each other and are joined via E-cadherin binding [19]. At this site, the perijunctional ring of actin and myosin interacts with E-cadherin through α-actinin, vinculin, and α- and β-catenin [20,21]. This perijunctional actomyosin ring is also essential to the maintenance of the tight junction [22–24]. Directly below the adherens junctions are desmosomes (see Figure 18.2). Distant relatives of the cadherin family, desmogelins and desmocollins, form cell contacts at desmosomes [25]. Desmosomal proteins associate with intermediate filaments and anchor the cytokeratin-based cytoskeleton between neighboring cells, thereby providing resistance to mechanical stress. The spontaneous chronic colitis that develops in mice lacking the principal intestinal epithelial keratin, keratin-8, emphasizes the importance of keratin proteins in epithelial function [26], although human inflammatory bowel disease has not yet been associated with altered keratin expression [27].

To varying degrees, all epithelial cells of the gastrointestinal tract express gap junctions. At the site of the gap junction, the cytoplasm of adjacent cells is in physical continuity through transmembrane channels formed by members of the connexin protein family [28]. Six connexin molecules assemble on each membrane to form a channel and, by adhering across the paracellular space, they create a small conduit that joins the cytoplasm of adjacent cells without allowing exposure to the extracellular space. Signaling molecules up to about 1500 Da (e.g., Ca^{2+}, inositol triphosphate) and small nucleotides can

diffuse freely through gap junctions and are important vehicles for the integration of physiological responses among cells. There are numerous connexin genes in humans and the pattern of expression varies among cell types in order to allow tissue-specific regulation of communication. Gap junctions coordinate epithelial function by allowing sheets of cells to behave as syncytia, for example coordinating exocytosis of zymogen granules from the pancreas (see Chapter 25). Gap junction communication in the liver, working through intracellular Ca^{2+} waves, modifies bile secretion in response to glucagon and vasopressin. Gap junctions are often mutated or their expression altered in gastrointestinal cancers [29,30], which likely contributed to aberrant intercellular communication.

Polarized protein delivery

Plasma membrane proteins and secreted proteins pass through a series of distinct vesicular compartments as they are sorted to the apical or basolateral surface. These proteins share a common site of synthesis on ribosomes bound to the rough endoplasmic reticulum and undergo posttranslational modification (e.g., glycosylation) in the Golgi apparatus. Membrane proteins and secreted proteins are then sorted into distinct vesicles in the trans-Golgi network (see Figure 18.2). In most cells, proteins destined for the basolateral surface are delivered directly to that domain; studies using live cell imaging have suggested that basolateral delivery may even be targeted to specific sites along the lateral membrane [31]. Detailed analysis of basolaterally targeted proteins has shown that specific amino acid sequences located within the cytoplasmic tail are sufficient to direct basolateral delivery [32]. Several of these sequences, including those with conserved tyrosine residues, are sorted by the epithelial adapter protein AP-1B [33,34]. This protein selects cargo destined for the basolateral membrane and coordinates the assembly of the exocytic machinery necessary for fusion of transport vesicles with the plasma membrane. The exocytic machinery, which is also involved in endocytic recycling of apical and basolateral membrane proteins, includes members of the Rab family of small guanosine triphosphate (GTP)-binding proteins and SNARE proteins that target delivery of transport vesicles to specific membrane domains [35].

In contrast to basolateral proteins, apically targeted proteins are transported by both direct and indirect pathways [36,37]. Proteins that traffic directly to the apical membrane include those that associate with glycolipid- and cholesterol-rich membrane domains, such as the brush border hydrolase sucrose-isomaltase, as well as proteins that are targeted independently of these membrane domains, such as lactase–phlorizin hydrolase. Dependence on actin also differentiates these two direct transport pathways, as transport of sucrose–isomaltase occurs along actin tracks and is inhibited by actin depolymerization, whereas lactase–phlorizin hydrolase transport is actin independent. The targeting motifs that direct apical delivery have been more difficult to identify than their basolateral counterparts. Ectodomain glycosylation sites and transmembrane protein domains, including those that allow association with glycolipid- and cholesterol-rich membranes, have been implicated in apical targeting [38].

Direct trafficking to the apical membrane is not used by all proteins or cell types. In hepatocytes, for example, all membrane proteins are first delivered to the basolateral surface. The apically destined proteins are then transcytosed to the apical, or canalicular, surface. A simple example of this type of sorting in hepatocytes as well as intestinal epithelia is provided by the polyimmunoglobulin (IgA) receptor, which binds IgA on the basal surface and is then transcytosed and released as secretory component into bile or the intestinal lumen [39,40]. In addition to specific targeting sequences that direct basolateral and then apical delivery, transcytosis also requires microtubules, which serve as tracks for the movement of transport vesicles from basolateral to apical surfaces. Why apical proteins without specific basolateral functions take this indirect pathway remains unclear. However, this mechanism is useful for the redistribution of apical proteins mistakenly targeted to the basolateral membrane, as well as for the sorting of membrane proteins during the initial stages of epithelial polarization.

Maintenance of membrane domains

Once delivered to the correct plasma membrane domain, proteins can be retained through interactions with actin-based cytoskeletal proteins. For example, the Na^+,K^+-ATPase is stabilized on the basolateral membrane domain by attachment to the cytoskeleton through the linker proteins ankyrin and spectrin. The complex functions of the apical membrane require intricate structures, such as parietal cell secretory canaliculi (see Chapters 23, 24, and 25) and enterocyte microvilli. Assembly and maintenance of these membrane domains depends, in part, on ezrin–radixin–moesin (ERM) proteins, which play a critical role in the organization of membrane domains diverse organisms and cell types [41]. These highly conserved cytoskeletal proteins link membrane proteins to the actin cytoskeleton by way of separate cargo-binding and actin-binding domains. The cargo-binding domain can interact with transmembrane proteins either directly or through accessory proteins, such as NHERF-1, NHERF-2, and PDZK1 [42–45]. These accessory proteins can also anchor protein kinases (e.g., protein kinase A), thus serving as a scaffold for the organization of signaling complexes [46–48]. For example, the cystic fibrosis transmembrane regulator (CFTR) is bound to PDZ domains in NHERF-2. These interactions stabilize CFTR at the apical membrane and tether CFTR to protein kinase A, thereby enhancing the ability of protein kinase A to activate CFTR [46,49]. Ezrin-dependent mechanisms have also been implicated in trafficking of the apical Na^+/H^+ exchanger NHE3 to the brush border following initiation of Na^+/glucose cotransport as well as the rapid delivery of the H^+,K^+-ATPase to the surface of histamine-stimulated parietal cells [45,50,51]. Studies in mice lacking ezrin, the only ERM protein expressed in enterocytes, confirm the central role of this protein in organizing the apical membrane [52]. Enterocytes in

these mice develop only primitive microvilli and fail to correctly target some proteins to the apical membrane. Moreover, the villi are irregularly shaped, with intravillous lumens and fused profiles. Not surprisingly, these mice die in the early postnatal period as a result of intestinal failure.

Organization of the cytoskeleton

The cytoskeleton is considered here in the context of the villous absorptive enterocyte, a cell type that has been a useful model for studies of cytoskeletal structure and function in polarized epithelia. The cytoskeletal organization of columnar epithelia exhibits only relatively minor differences among sites within the gastrointestinal tract. The stabilization of epithelial cell structure first requires support for the tall columnar shape; in the absence of the cytoskeleton, a sphere would have the most thermodynamically favorable properties. Maintenance of cell shape is primarily a function of the actin microfilaments that form a network beneath the entire plasma membrane (see Figure 18.2). Bundles of 20–30 actin filaments also form the submembranous cores responsible for microvillous architecture. Within these cores, individual microfilaments are cross-linked to each other by actin-bundling proteins and to the microvillous membrane by a member of the myosin family, myosin IA. The microvillous actin bundles jut into the apical pole of the cell and associate with a terminal web composed of actin and type II myosin that interfaces with the apical junctional complex. The contractile status of this perijunctional actomyosin ring can be adjusted in response to physiological and pathophysiological stimuli, allowing modulation of epithelial barrier function.

Cables composed of intermediate filaments course through the cells and function as support structural cables. By associating with plasma membranes and desmosomes, these tonofilaments form a network that allows intestinal epithelia to survive despite exposure to the turbulent environment of the gut lumen.

Microtubules also form a unique array in polarized epithelial cells. In contrast to nonpolarized cells in which microtubules radiate from a single microtubule organizing center adjacent to the nucleus, microtubules in polarized epithelia are aligned apicobasally. With the assistance of microtubule-dependent motor proteins, such as kinesins and dyneins, which can transport vesicles along microtubule arrays, membrane-bound structures are trafficked throughout the cell. This network is particularly important in transcytosis, and microtubule disruption markedly slows IgA secretion.

Basement membrane

In addition to its important structural and supportive roles, the basement membrane serves as a source of signals that promote epithelial polarity. All alimentary epithelia reside on a basement membrane that is 20–40 nm deep, consists of a fibrillar network, and rests on an underlying extracellular matrix (see Figure 18.1). The basement membrane in the alimentary tract, similar to basement membranes in other tissues, is composed primarily of laminin, heparan sulfate proteoglycans, and type IV collagen. Minor constituents that may be functionally important include thrombospondin and entactin/nidogen-1.

Laminin exhibits specific binding sites for type IV collagen, heparan sulfate proteoglycans, cell surface laminin receptors, and entactin. Other matrix components also possess binding sites for additional components, adding to the complexity of interactions between the epithelial cell and its surrounding environment. Heparan sulfate proteoglycans, which are the major proteoglycans of the basement membrane, consist of long chains of glycosaminoglycans linked to a protein core. The structure of these massive molecules is often likened to a test-tube brush with the glycosaminoglycan extensions comprising the bristles. Proteoglycans probably organize water within the basement membrane, hydrating this environment through their capacity to bind water, and possibly imparting solute-sieving characteristics under conditions of bulk water flow. Although a controversial concept, the thickened basement membrane present in patients with collagenous colitis may effect impaired water and electrolyte absorption and the resulting watery diarrhea that is the symptomatic hallmark of this condition [53,54].

Type IV collagen is a triple-stranded helical molecule, which, unlike other collagens, does not have its propeptides removed after deposition in the extracellular space and does not cross-link into dense fibrils; instead it assumes a loose, net-like structure by associating with other collagen IV molecules. This mesh-like organization establishes the fundamental structure of the basement membrane.

Basement membrane components can exert significant effects on epithelia, including modulation of proliferation, adhesion, migration, differentiation, and even barrier function. In the intestine, type IV collagen is produced primarily by mesenchymal cells, heparan sulfate proteoglycans by epithelial cells, and laminin by both mesenchymal and epithelial cells. Many basement membrane components bind to integrins, a family of epithelial cell surface molecules that are connected to the actin cytoskeleton through linker proteins. Through such associations, structural elements within the cell are able to connect with, and potentially be modulated by, events occurring within the basement membrane and even more deeply within the extracellular matrix.

Mucosal barriers

Various sites in the alimentary tract must cope with the presence of acid, bile, undigested potentially antigenic proteins, bacterial proteins, and live bacteria. It is not surprising, therefore, that the epithelial barrier consists of numerous components that prevent injury from these varying potential insults. Some of these components are site specific and others are common throughout the gastrointestinal tract. These barriers may be conceptually divided into two major categories: those

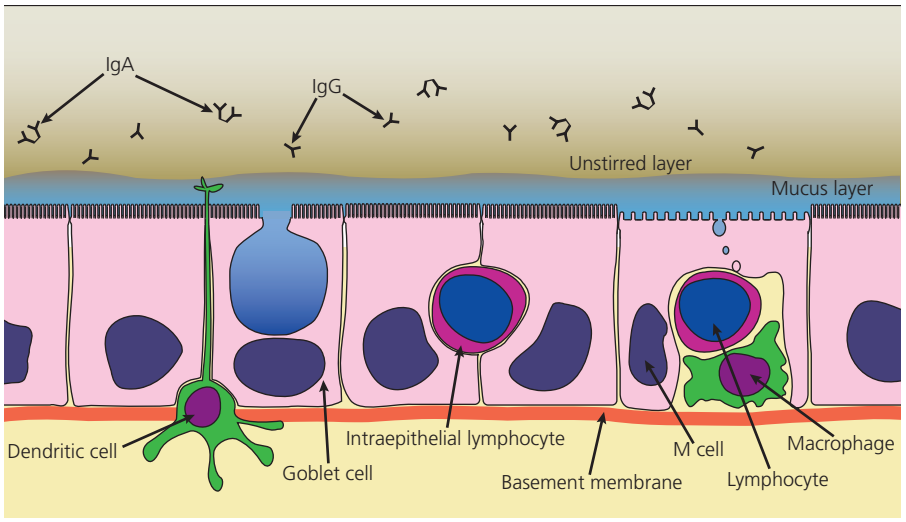

Figure 18.5 Epithelial barriers. The intestinal epithelium is the focal point around which the interaction of luminal material and subepithelial cells, including those of the immune system, is organized. In addition to conducting vectorial transport and maintaining a cellular barrier, epithelia also contribute to host defense by elaborating mucus and transporting immunoglubulins (IgA and IgG).

that are extrinsic to the epithelium (although in some instances produced by the epithelium) and those provided by the physical presence of the epithelium, which we describe as intrinsic barriers.

Extrinsic barriers

Mucus

Nearly all alimentary epithelia are coated with a layer of mucus that protects against bacteria as well as shear forces (Figure 18.5). Most surfaces, including those of the stomach, the intestine, the pancreatobiliary ducts, and the gallbladder, contain specialized cell types that synthesize, package, and secrete mucin. In the esophagus, mucin is derived from saliva as well as small glands that lie under the epithelium and connect to the lumen by way of small ducts. In contrast, foveolar mucus cells and goblet cells release mucin directly onto the luminal surface of the stomach and intestines, respectively.

Despite variations in the composition of mucus throughout the alimentary tract, all mucin molecules are polydispersed glycoproteins (250–20 000 kDa) of which about 80% of the mass is carbohydrate. There are at least eight human mucin-producing genes (*MUC*) genes. *MUC2* is the predominant mucin expressed in the small intestine and colon. Although *MUC2* is not normally present in the esophagus, it can be expressed by metaplastic specialized columnar epithelium, such as that found in Barrett esophagus, and can be used as a tool to diagnose this condition.

Mucins act as a barrier by forming a viscous hydrated gel, i.e., mucus. In the intestine, mucus is organized into a dense inner layer that is firmly attached to the epithelial surface and an overlying loose layer that is much thicker [55]. Luminal bacteria are trapped within the loose mucus layer and are rarely present in the dense inner layer [56]. This may be facilitated by the carbohydrate groups of mucin molecules that can bind to cell walls and thereby inhibit bacterial adhesion to and colonization of epithelial surfaces [57]. This is critical, as the distal gastrointestinal tract is densely colonized by bacteria and viruses. Interactions between the mucosa and luminal microbiome have become the focus of intense study and may contribute to both homeostasis and disease (see Chapter 32).

Exposure of epithelial surfaces to threats such as bacterial toxins and noxious chemicals often results in mucin secretion, further augmenting their protective effects [58]. Mucin release and synthesis can also be regulated in response to pathogens, such as *Helicobacter pylori*, and host-derived inflammatory mediators [59]. As a result, mucin depletion from the goblet cell compartment which produces it may be prominent in many pathologies. Thus, although mucin depletion is often noted in biopsy specimens, it is not specific to any etiology and should be interpreted as a nonspecific indicator of mucosal injury. The essential protective role of mucus is demonstrated by the spontaneous colitis that develops in in *MUC2* null mice [60,61]. Notably, Muc2 expression is also downregulated in human inflammatory bowel disease [62–64].

Unstirred layer

Peristalsis creates a turbulent environment in the gastrointestinal lumen. This turbulence or convective force does not extend to the epithelial surface. The best estimate is that an aqueous layer with a thickness of 300–800 μm lies above the epithelia. This apical microenvironment is still, i.e., unstirred. Convective forces then grow rapidly along with distance from the mucosal surface. Although not fully determined, the physiological impact of the unstirred layer on the immediate environment to which epithelial cells are exposed must be profound.

The presence and volume of the unstirred layer may significantly affect nutrient absorption. For example, if the epithelial transport system can transport a given nutrient more rapidly than the nutrient can diffuse into the unstirred layer, diffusion becomes the rate-limiting step in absorption. In contrast, polymeric nutrients that are broken down into monomers at the brush border (e.g., carbohydrates) may be formed at very high rates within the unstirred layer. As discussed below, these local nutrient concentrations may exceed the capacity of transcellular transport pathways. While it is difficult to measure solute concentrations within the unstirred layer precisely, this is an important consideration when analyzing the biophysics and kinetics of gastrointestinal transport.

Secreted immunoglobulins

Intestinal epithelial surfaces are bathed by secretory IgA and IgG (see Figure 18.5). Secretory IgA is produced as a dimer by lamina propria plasma cells, transcytosed by the polyimmunoglublin receptor, and released into the lumen as a consequence of proteolytic receptor cleavage [40,65,66]. IgG is transcytosed by the neonatal Fc receptor (FcRN), which can function in both basolateral to apical as well as apical to basolateral directions [67]. The latter allows FcRN, which is expressed well beyond the neonatal period, to mediate absorption of antibodies by nursing infants. With the intestinal lumen, secretory IgA and IgG act as immune barriers by binding to luminal threats, including pathogenic bacteria and toxins [68,69]. This adaptive immune barrier is highly specific and dependent on prior antigenic sensitization (see Chapter 17).

Secreted bicarbonate

In contrast to the extrinsic barriers discussed above, some extrinsic barriers have regional variation. One well-described example is the net bicarbonate (HCO_3^-) secretion by epithelia that interface with the acidic luminal environment of the stomach. The proximal duodenum also must protect itself from gastric acid, as pancreatic bicarbonate secretions do not enter the gut lumen until the ampulla of Vater. Epithelial bicarbonate secretion and neutralization is the first line of defense against acid-induced damage (see Chapters 24 and 29). As a result of this secretion, the unstirred layer overlying the gastric epithelium has a pH much closer to neutral than the majority of luminal fluid [70,71]. Intracellular HCO_3^- may also contribute to duodenal epithelial cytoprotection [71,72].

Antimicrobial peptides

Gut epithelial cells produce and secrete peptides with antimicrobial functions. Paneth cells at the base of the crypts in the small intestine and ascending colon are important in this process and release proteins and enzymes with antimicrobial activity, including defensins, lysozyme, and type II phospholipase A_2 [73–75]. The bactericidal activity of most defensins may actually shape the composition of the intestinal microbiome [76]. Thus, the observations that the Crohn's disease-associated gene *NOD2* for is required for expression of some defensins, that patients carrying mutations in the Crohn's disease-associated gene *ATG16L1* have abnormal Paneth cell granules [77], and that defensin expression is altered [78,79] suggest that dysbiosis, i.e., imbalances of microbiome composition (see Chapter 32), may contribute to Crohn's disease pathogenesis [80].

Intrinsic barriers

The intrinsic barrier is formed by the continuous sheet of epithelial cells that lines the entire gastrointestinal tract. This uninterrupted epithelial layer separates luminal material from the subepithelial space.

Epithelial permeability depends on two routes by which material may traverse the barrier: the transcellular and paracellular pathways (see Figure 18.3). The exact physical site where solutes cross the epithelium was a topic of considerable controversy until it was recognized that transport proteins insert into and form channels across lipid bilayers. Similarly, the paracellular pathway was thought to be impermeable and unregulated, a misconception perpetuated well into the 20th century because of the static appearance of intercellular contacts seen in early electron micrographs. Indeed, even the term "tight" junction is a misnomer, as tight junctions form a transcellular barrier that selectively allows paracellular flux of ions, nutrients, and some larger molecules. Present understanding of transcellular and paracellular transport emerged along with the recognition that both are physiologically regulated and vary widely in different tissues. Transepithelial transport of hydrophilic solutes along these two pathways is discussed below. Hydrophobic compounds can cross epithelial cells directly by virtue of their solubility in the lipid bilayer. For example, saturated fatty acids cross jejunal epithelial cell microvillous membranes at rates more than 10^{11}-fold faster than they diffuse in aqueous solution.

Transcellular pathway

The transcellular pathway is highly restrictive to the passive flow of hydrophilic solutes. To traverse an epithelial cell, an ion or other hydrophilic solute must interact with three barriers in series: the apical membrane, the cytosol, and the basolateral membrane (see Figure 18.3). Although the cytosol has the potential to limit transcellular molecular flux, the two plasma membranes are the key barriers that restrict the passive movement of hydrophilic solutes.

The lipid bilayers of the apical and basolateral membranes prevent massive flux of hydrophilic solutes and preserve transmembrane electrochemical gradients; the high resistance to passive ion flow across model lipid bilayers approaches impermeability. Biological membranes, which are composed of lipid bilayers and membrane proteins, are slightly more permeable, but still demonstrate resistances to passive ion flow that are several orders of magnitude greater than those of gastrointestinal epithelial plasma membranes. Integral membrane proteins

such as transporters, pumps, and channels contribute to the relative permeability of alimentary epithelia. These proteins serve a critical need as the transmembrane movement of ions is essential for cellular homeostasis as well as nutrition.

As discussed above, vectorial transport relies on the polarized delivery of transporters, pumps, and channels to the apical and basolateral membranes. The specific example of glucose absorption in the small intestine will be considered here (see Figure 18.3). For detailed discussions of absorption and secretion, see Chapters 23–26. Glucose is actively transported across the apical plasma membrane by the Na^+/glucose cotransporter SGLT1 [1]. The absence of this critical transporter results in glucose–galactose malabsorption, an autosomal recessive disease characterized by the failure to absorb these carbohydrates from the diet [81]. Affected children present with severe diarrhea, dehydration, and failure to thrive. The disease is generally fatal unless glucose and galactose are eliminated from the diet.

The energy source that allows efficient uptake of luminal glucose by SGLT1 is the high extracellular, and low intracellular, Na^+ concentration; two Na^+ ions are absorbed along with each glucose molecule. The apical positioning of SGLT1 ensures that glucose is never secreted into the lumen, as the Na^+ gradient makes this thermodynamically unfavorable. Once within the cytosol, Na^+ and glucose diffuse to the basolateral membrane. Here, Na^+ ions are pumped out of the cell and into the subepithelial and basolateral interstitium by the Na^+,K^+-ATPase, and glucose molecules diffuse across the membrane in a concentration-dependent manner facilitated by the glucose transporter GLUT2. In the absence of luminal nutrients, the basolateral positioning of GLUT2 allows it to also operate in the reverse direction, bringing glucose into the epithelial cell from the subepithelial interstitium. SGLT1 and GLUT2 are specific transporters for sugars; other transporters with similar properties manage the transport of amino acids and other nutrients. It should be apparent that the subepithelial deposition of Na^+ and glucose results in an osmotic gradient that drives water absorption (see Figure 18.3).

Paracellular pathway

The paracellular pathway is a major route of passive solute permeation. Although plasma membranes tend toward high resistance, alimentary epithelia, with the exception of the esophagus, have low net resistances, meaning that they are relatively permeable. Detailed molecular, biophysical, and morphological analyses have shown that the paracellular pathway is responsible for the marked permeability of gastrointestinal epithelia, relative to those in the bladder or skin.

The paracellular pathway consists of the apical intercellular tight junction and the underlying paracellular space. Under most conditions, the tight junction is the rate-limiting step that limits passive movement of hydrophilic solutes through the paracellular space. The permeability of tight junctions to ions and solutes varies between tissues, between sites within tissues (e.g., crypt vs. villus), and in response to physiological and pathophysiological stimuli [24,82–86].

The ion-selective permeability of tight junctions is largely defined by the ensemble of claudin protein family members expressed [86–90]. Some proteins within the 27 member claudin family form charge-selective paracellular pores that allow molecules with radii $<\sim 4$ Å to traverse the tight junction. For example, familial hypomagnesemia, a disease of deficient renal tubular Mg^{+2} reabsorption, is the result of mutations in either claudin-16 or claudin-19, which form a complex that facilitates paracellular Mg^{+2} flux [91–95].

Genetic mutations in specific claudin family members have not yet been described in gastrointestinal disease. However, the patterns of claudin protein expression along the length of the gastrointestinal tract, as well as along the crypt–villus axis, explain, at least in part, variations in paracellular permeability at different sites [96]. Moreover, changes in the specific pattern of claudins expressed by intestinal epithelia may be critical to disease pathogenesis. For example, inflammatory bowel disease is associated with loss of claudin-5 and claudin-8 as well as claudin-2 expression [97–100]. Claudin-2 forms small pores that allow paracellular flux of monovalent cations [86,90,101], primarily Na^+ and water [102], and may therefore be responsible for some of the barrier loss and diarrhea that occurs in patients with inflammatory bowel disease.

Like claudin-2, claudin-15 forms a pore that mediates paracellular Na^+ flux. Both of these claudins are critical to nutrient absorption and homeostasis. In mice, intestinal claudin-2 expression is highest shortly after birth and marked downregulated before weaning [96]. Conversely, there is little expression of claudin-15 until weaning, at which time it increases markedly [96]. Consistent with the low levels of claudin-2 expression throughout most of life, adult mice lacking claudin-2 are healthy and do not have apparent intestinal defects [103,104]. In contrast, mice lacking claudin-15 display marked intestinal hypertrophy [104,105]. A potential explanation for this aberrant intestinal growth becomes clear when one considers that mice lacking both claudin-2 and claudin-15 die in the perinatal period due to malnutrition [104]. This malnutrition can be understood when one recognizes that the many Na^+-dependent intestinal absorptive processes require quantities of luminal Na^+ that exceed those provided by the diet. Trans–tight junction flux of Na^+ into the lumen, which likely occurs in the proximal small intestine, may therefore allow recycling of absorbed Na^+. Failure to recycle Na^+ explains defects in glucose, amino acid, and fat absorption in mice lacking both claudin-2 and claudin-15, as transport of each of these depends on apical Na^+ cotransporters [5,104]. Thus, the intestinal hypertrophy of claudin-15-deficient mice may simply represent an adaptive response to inadequate absorptive function. Claudin-2 and claudin-15 upregulation, which occurs in inflammatory bowel disease, may therefore reflect an effort to compensate for loss of functional absorptive surface area.

Water movement across the epithelial barrier

Despite the obvious importance of fluid transport across gastrointestinal epithelia, controversy remains about the relative importance of the transcellular vs paracellular routes. Potential mechanisms of transcellular water movement include passage through transmembrane channels created by members of the aquaporin protein family [106]. These small integral membrane proteins are well studied in tissues specialized for regulated water and salt transport, such as the collecting duct of the kidney, the parotid gland, and osmoregulatory organs of saltwater animals [107–110]. Although aquaporins are expressed in gastrointestinal epithelia and their expression may be altered in disease [111–113], it appears that they play a limited role in gastrointestinal water transport [114]. Although others have suggested that the apical Na^+/glucose cotransporter SGLT1 may serve as a molecular water pump, carrying a large number of water molecules with each glucose molecule transported [115], it is more likely that the osmotic gradient generated by transcellular transport, along with increases in tight junction permeability that follow SGLT1 activation, explain the water absorption associated with Na^+/glucose cotransport [83,85,116–119]. Together with the observation that increased flux across the paracellular pathway is necessary to support the massive water secretion that accompanies acute immune-mediated diarrhea [84], these data suggest that the paracellular pathway is the major route of water flow across intestinal epithelia [120–122].

Epithelial barrier and disease

Epithelial renewal

Epithelial injury is most readily apparent when gaps within the epithelium such as erosions or ulcerations are present. However, because the gut has a remarkable capacity for repair, many forms of focal acute injury do not result in functionally significant defects. One example is the rapid sealing of defects that occurs routinely during the physiological turnover of gastrointestinal epithelia.(see Chapter 2). These cells are replaced, on average, once or twice each week through coordinated proliferation, migration, apoptosis, and shedding. For example, small intestinal enterocytes arise from the stem cell compartment, i.e., the crypt, migrate upward through the proliferative zone, and undergo an ordered process of differentiation as their phenotype is modified from undifferentiated secretory cell to fully differentiated villous absorptive cell. They are then shed from the villous surface. Membrane proteins, most of which have half-lives considerably shorter than that of the epithelial cells, also turn over. This facilitates the evolution in protein expression that occurs during differentiation. Lipid turnover undoubtedly occurs as well but technical challenges hinder its documentation. Perhaps most remarkable in this continual process of renewal is that the barrier remains intact at sites of epithelial cell detachment [123–127].

Regulation of barrier function by physiological stimuli

Intestinal permeability can be regulated by physiological processes. For example, it is well documented that Na^+/nutrient cotransport results in enhanced permeability of tight junctions to molecules the size of amino acids and glucose [82,83,119, 128,129]. These increases in permeability may represent an increase in the number or open probability of small pores in the upper villus [119], i.e., the site of nutrient transport. In contrast, flux across lower villus and crypt paracellular pathways that allow larger molecules to cross the tight junction is not affected by Na^+/nutrient cotransport [119]. Thus, the localized and size-selective tight junction permeability increases that follow Na^+/nutrient cotransport do not result in greater paracellular flux of larger molecules, such as bacterial products [130]. Na^+/glucose cotransport also activates trafficking of cytoplasmic Na^+H^+ exchanger 3 (NHE3) to the apical, brush border membrane [45,51], which results in further increases in transcellular Na^+ absorption (see Figure 18.3). Together, this transcellular nutrient, e.g., glucose, and Na^+ absorption creates a transepithelial osmotic gradient that drives water absorption from the unstirred layer. As noted above, the unstirred layer lies just above the brush border membranes, which contain digestive enzymes, and is enriched in small nutrients after eating. This fluid therefore acts as a solvent that carries small nutrients through tight junction pores. This mechanism, termed "solvent drag," is most active at high luminal nutrient concentrations, where paracellular absorption may even exceed transcellular absorption [131]. Thus, transcellular absorption activates processes that enhance paracellular absorption [118]. In this manner, paracellular absorption amplifies transcellular absorption. This tight junction regulation can also facilitate paracellular absorption of undegradable compounds, such as short D-amino acid-substituted peptides and creatinine [132,133], and may be of use in oral drug delivery. Finally, it is important to recognize that the combination of events described likely explains the efficacy of of Na^+- and glucose-containing oral rehydration solutions, including those containing starches that are broken down by colonic bacteria [134,135].

Physiological tight junction regulation initiated by Na^+/glucose cotransport requires activation of MLCK [82,85]. Epithelial MLCK is transcribed from the same gene as the smooth muscle MLCK, and these two enzymes have identical catalytic domains [136,137]. However, the epithelial isoform is much larger, as it is transcribed from an upstream promoter that is distinct from the smooth muscle MLCK promoter [138,139]. The signal transduction mechanisms that link Na^+/glucose cotransport to MLCK activation are incompletely defined, but the result is phosphorylation of myosin II regulatory light chain. This initiates contraction of the perijunctional actomyosin ring and induces subtle morphological changes, such as perijunctional actomyosin condensation [83] and undulation of tight junction profiles [140] that increase tight junction permeability [85,140,141]. At a molecular level, MLCK activation enhances

ATP- and actin-dependent exchange of the tight junction scaffolding protein ZO-1 [141]. Thus, Na^+/nutrient cotransport-induced increases in paracellular permeability may be mediated by modulation of the continuous, molecular remodeling of the tight junction protein complex that occurs at steady state [141,142].

Dysregulation of epithelial barrier function

Despite an intact epithelium, tight junction permeability is enhanced in many inflammatory, infectious, ischemic, and immune-mediated intestinal diseases [23,143]. For example, permeability defects that occur in both graft-versus-host disease and human immunodeficiency virus infection are associated with increases in serum lipopolysaccharide absorption from the gut lumen [144–150]. These changes can be reversible, as a gluten-free diet can restore barrier function in celiac disease and diarrhea predominant irritable bowel syndrome [151,152].

Barrier defects are also seen in Crohn's disease. During clinical remission, increased intestinal permeability can be a marker of impending disease reactivation [153,154]. In addition, permeability defects are present in a subset of healthy first-degree relatives of patients with Crohn's disease [155–157]. This has led to speculation that a primary defect in tight junction barrier function may contribute to the pathogenesis of Crohn's disease [156,158,159]. Conversely, inflammation, including that in Crohn's disease, can also cause increased permeability. As an example, treatment of Crohn's disease patients with antibodies that neutralize tumor necrosis factor reduces inflammatory activity and restores barrier function [160].

Tumor necrosis factor acutely reduces barrier function in cultured intestinal epithelial monolayers in vitro and mouse jejunal epithelia in vivo [120,161–164]. This is accomplished by exploitation of the normal physiological regulatory mechanism of myosin II regulatory light chain phosphorylation-induced tight junction regulation [138,161,165,166]. However, unlike the MLCK-dependent, size-selective increase in tight junction permeability that follows Na^+/glucose cotransport, tumor necrosis factor-mediated MLCK activation results in increased paracellular flux of both large and small molecules [84,86,120]. Moreover, tumor necrosis factor triggers endocytosis of the tight junction protein occludin [84,164,167]. This occludin removal is required for tumor necrosis factor-induced barrier loss, as either inhibition of occludin endocytosis or occludin overexpression, both of which preserve the tight junction-associated occludin pools, limit tumor necrosis factor-induced barrier loss [164]. This suggests that tumor necrosis factor activates both MLCK and a second signal that promotes occludin endocytosis and that this explains the differences between size selectivity of Na^+/glucose cotransport- and tumor necrosis factor-induced tight junction regulation. Consistent with this, occludin knockdown enhances paracellular flux of large and small molecules, both in vitro and in vivo [167,168]. Thus, despite being unnecessary for the assembly of intestinal epithelial tight junctions [169,170], occludin is a key regulator of a pathway that allows charge nonselective flux of large macromolecules (Figure 18.6). This route, termed the leak pathway [24,171], is a low capacity conductance path that can be regulated by a variety of pathophysiological stimuli including tumor necrosis factor, IL-1β, and LIGHT, i.e. lymphotoxin-like inducible protein that competes with glycoprotein D for herpes virus entry on T cells [172,173].

In contrast to tumor necrosis factor, the cytokine IL-13 only increases paracellular flux of small cations and uncharged molecules. This size-selective route is referred to as the pore pathway [86]. The increased pore pathway conductance that follows IL-13 exposure is due to increased expression of claudin-2 which, as noted above, creates cation- and size-selective paracellular pores [86]. Claudin-2 expression can also be enhanced by IL-6 and IL-17 [86,98,174,175]. Both of these mechanisms appear to be activated in inflammatory bowel disease, where MLCK expression and activity [176] as well as claudin-2 expression [98,101] are increased while occludin expression is decreased [98].

The divergent effects of IL-13 and tumor necrosis factor on barrier function demonstrate that the immune system can differentially regulate conductance across either the high-capacity, charge- and size-selective pore pathway or the low capacity, relatively nonselective leak pathway [177]. In turn, barrier regulation can regulate mucosal immunity, as even small in vivo increases in intestinal paracellular permeability induce complex immune responses [86,178,179]. When considered as a single system, these observations are consistent with a model where impaired mucosal barrier function can lead to immune activation, cytokine release, and further loss of barrier function that result is a self-amplifying cycle of barrier dysfunction and inappropriate immune activation [23,24]. Thus, compromised barrier function may be a critical event in disease pathogenesis. Alternatively, mucosal immune activation and cytokine release can initiate this vicious cycle.

Contributions of barrier loss to immune-mediated disease

To address the specific impact of MLCK-dependent tight junction barrier regulation on intestinal physiology, transgenic mice expressing an intestinal epithelial-specific constitutively active-MLCK (CA-MLCK) were generated [178]. Augmented intestinal epithelial myosin II regulatory light chain phosphorylation caused modest increases in intestinal epithelial tight junction permeability [140,178]. Notably, the permeability increases observed were quantitatively similar to the defects seen in healthy first-degree relatives of Crohn's disease patients [155,157]. It is, therefore, not surprising that CA-MLCK transgenic mice failed to develop spontaneous disease. Nevertheless, mucosal immune activation, including increased expression of tumor necrosis factor, IL-13, interferon-γ, IL-10, and claudin-2, was observed [86,178]. Further, CA-MLCK transgenic mice developed a more severe colitis with reduced survival, relative

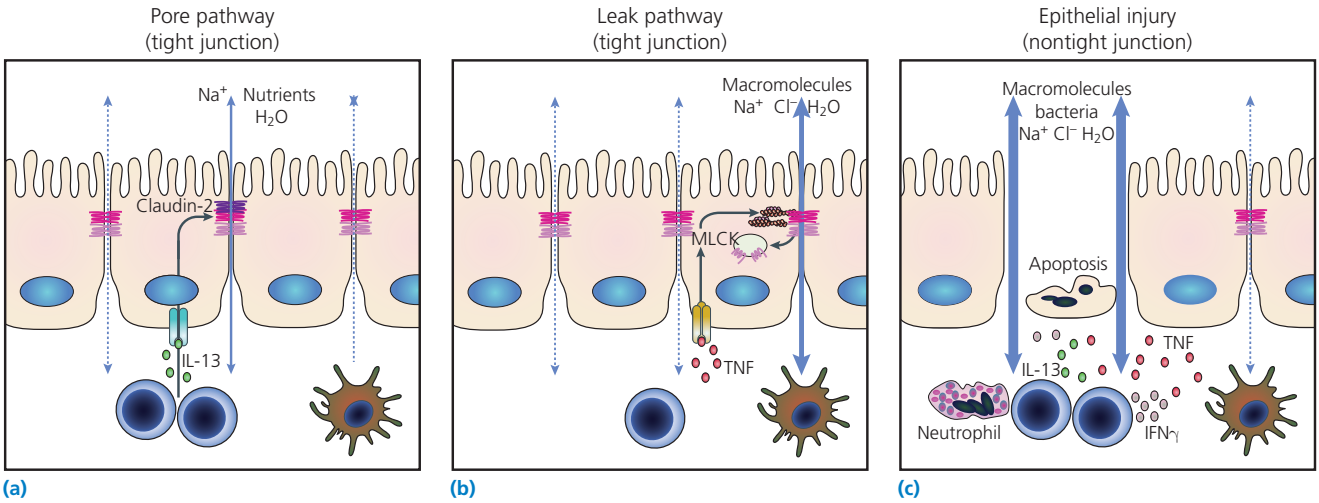

Figure 18.6 Mechanisms of intestinal barrier loss. The intestinal epithelial barrier is established by the monolayer of columnar epithelial cells. Tight junctions form a selectively permeable seal between adjacent cells to restrict paracellular flux. In the context of disease, this epithelial barrier can be modified by three separate processes that differentially impact paracellular flux of solutes, water, and larger materials, e.g., bacterial. Claudin-2 expression **(a)** can be induced experimentally by a variety of cytokines, including interleukin-13 (IL-13; shown), IL-6, and IL-17, and is also upregulated in experimental colitis and human inflammatory bowel disease. Claudin-2 forms a tight junction channel that allows paracellular flux of small monovalent cations, primarily Na^+, small nutrients, e.g., glucose and amino acids, and water. This high-capacity route, termed the pore pathway, is both size and charge selective. Other cytokines, including tumor necrosis factor (TNF), IL-1β, and LIGHT increase trans–tight junction conductance across the leak pathway **(b)**. Myosin light chain kinase (MLCK) can be acutely activated by these cytokines in model systems and is also activated in experimental colitis and human inflammatory bowel disease. Although the carrying capacity is more limited than the pore pathway, the leak pathway allows paracellular flux of much larger molecules. The size selectivity of the leak pathway has not been defined, but some estimates suggest that it can accommodate molecules with radii up to ~60 Å. Finally, damage, including that caused by apoptosis (shown), necroptosis, necrosis, and direct injury, can result in complete elimination of the epithelial barrier **(c)**. This mechanism of barrier loss reflects the absence of tight junctions locally, i.e., at sites of erosions or ulcers. The residual barrier formed by mucus and the basement membrane, if these are present, allows free exchange in a size- and charge-nonselective manner, although bacteria may be trapped within the mucus layer (see text). IFNγ, interferon γ.

to their nontransgenic littermates, in an immune-mediated model of inflammatory bowel disease. Thus, intestinal epithelial tight junction barrier loss can accelerate disease pathogenesis in a susceptible individual. However, barrier loss alone is insufficient to cause disease in otherwise normal subjects. The potential of barrier loss and mucosal immune activation to initiate disease in a susceptible individual may also explain the increased incidence of inflammatory bowel disease and irritable bowel syndrome following acute infectious enterocolitis [180–183].

The model presented above could prompt a therapeutic trial of MLCK inhibitors in inflammatory bowel disease. However, given the identity of epithelial and smooth muscle MLCK catalytic domains, an enzymatic inhibitor is likely to have unacceptable toxicities, including visceral paralysis, e.g., aperistalsis, hypotension, and cardiac arrest [184,185]. In contrast, knockout mice lacking only the epithelial long MLCK isoform have normal smooth muscle MLCK function and are healthy in the absence of exogenous stressors [84,186,187]. These mice were used to investigate the potential of targeted epithelial MLCK inhibition in colitis [186]. Long myosin light chain knockout mice were protected from acute tumor necrosis factor-driven, MLCK-dependent leak pathway barrier loss [84] and were also protected from leak pathway barrier loss, claudin-2 upregulation, and early disease activation in a model of immune-mediated colitis [186]. However, disease ultimately developed in association with epithelial apoptosis that resulted in tight junction-independent barrier loss (see Figure 18.6). Thus, while immune-mediated colitis can be limited by epithelial MLCK inhibition, this can be overcome by epithelial injury. These data suggest that epithelial MLCK inhibition could be helpful as maintenance therapy, if a targeted therapeutic agent were available, but that this approach might be less useful in active disease with ongoing mucosal damage.

Interactions of epithelial and immune cells

Despite the correlation of increased intestinal permeability with disease and the therapeutic potential of barrier restoration, the failure of barrier loss by itself to cause disease remains puzzling. This point has been demonstrated repeatedly by human studies, such as those showing increased intestinal permeability in a subset of healthy first-degree relatives of Crohn's disease patients [155,156,188], as well as a plethora of experimental models, including the transgenic mice described above [178,189] despite nonscientifically based assertions to the contrary [143]. Several groups of investigators therefore asked why intestinal barrier loss is unable to cause disease [24,178,179,190]. The question is

addressed most directly by a study that assessed disease susceptibility in mice following transient epithelial cell damage and barrier loss [179]. This resulted in increased numbers of mucosal regulatory T cells, including populations producing IL-10 or expressing surface latency-associated peptide (LAP), a transforming growth factor-β precursor. Following recovery from the transient insult, these mice were resistant to chemically induced colitis by a mechanism that required, and could be adoptively transferred by, the mucosal LAP-expressing T cells [179]. Similarly, barrier defects induced by CA-MLCK resulted in increased mucosal IL-10 and IgA production. Further, mice lacking junctional adhesion molecule A (JAM-A), which results in supraphysiological barrier defects, possibly as a result of mucosal damage and increased epithelial turnover [191], also displayed increased production of IgA and transforming growth factor-β [190]. As a whole, these data suggest that a healthy immune system is able to regulate inflammatory reactions in order to limit immune activation in response to barrier loss and prevent initiation of the vicious cycle that leads to chronic disease.

IL-10 deficient mice provide a clear example of the interaction between mucosal immune dysregulation and barrier loss. These mice develop barrier defects in a manner that depends on microbial colonization [192]. Either reductions in microbial load or a more direct pharmacological approach to barrier restoration limit severity of the spontaneous colitis that develops in these mice [192–194]. Although the pathogenic mechanisms active in these mice may not be identical to those in patients, they are likely related, as polymorphisms flanking the *IL-10* gene [195] as well as IL-10 receptor mutations are associated with susceptibility to ulcerative colitis [196]. Thus the epithelial barrier is likely to be a critical regulator of interactions between the gut microbiome and the mucosal immune system in both experimental and human disease.

Overall, it is clear that the epithelial barrier and mucosal immune function are interrelated and that, if one is to understand disease, they must be considered as a whole. The data indicate that, in an immunocompetent host, limited barrier defects induce a robust immunoregulatory response that prevents disease. The difference between Crohn's disease patients and their healthy relatives with increased permeability may therefore be in the quality of this immunoregulation and the ability to manage inflammatory responses to barrier loss.

Integration of mucosal function

Gut epithelial function may be modulated by a host of influences from nonepithelial sources, including growth factors, cytokines and chemokines, and neural mediators. Together, these signaling and regulatory networks interact to maintain gastrointestinal mucosal homeostasis. This includes balancing barrier function with the need for transepithelial movement of ions, nutrients, and antigens; rapid repair of mucosal defects; and management of interactions between mucosal immune cells and the microbiome. The precise integration of these properties centers on the epithelium. Maintenance of the epithelial barrier is critical to these functions and requires integrity of cellular plasma membranes and intercellular tight junctions. The mucosal barrier also benefits from epithelial secretions, including mucus and HCO_3^-. Based on the examples provided in this chapter, as well as other chapters in this textbook, it is evident that dysregulation of any of these functions can result in diseases with overlapping clinical presentations.

References are available at www.yamadagastro.com/textbook

Further reading

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