CHAPTER 1

Overview of the epithelial cell

Bacteria must overcome multiple obstacles in order to achieve successful pathogenesis. In many cases, this requires bypassing the first line of host defense: the barrier provided by epithelial surfaces of the integument and the gastrointestinal, respiratory, and urinary tracts. To overcome these barriers, pathogenic organisms frequently initiate mechanisms that exploit essential cellular processes of the epithelium. These cellular processes are therefore critical to our understanding of bacterial pathogenesis. Their description is the goal of this chapter.

GASTROINTESTINAL TRACT

The gastrointestinal epithelium forms a critical interface between the internal milieu and the lumen. The latter should be considered the external environment, since the gut is essentially a tube running through the body that communicates with the external environment at each end. Thus, like the skin, the barrier formed by the gastrointestinal tract is critical in preventing noxious luminal contents from accessing the internal tissues. In contrast to the skin, the gastrointestinal tract must also support digestion and active vectorial transport of nutrients, electrolytes, and water. The barrier formed by the gastrointestinal epithelium must therefore be highly regulated and selectively permeable. Consistent with this, barrier permeability and epithelial transport function vary at individual sites within the gastrointestinal tract according to regional differences in the specific nutrients and ions transported.

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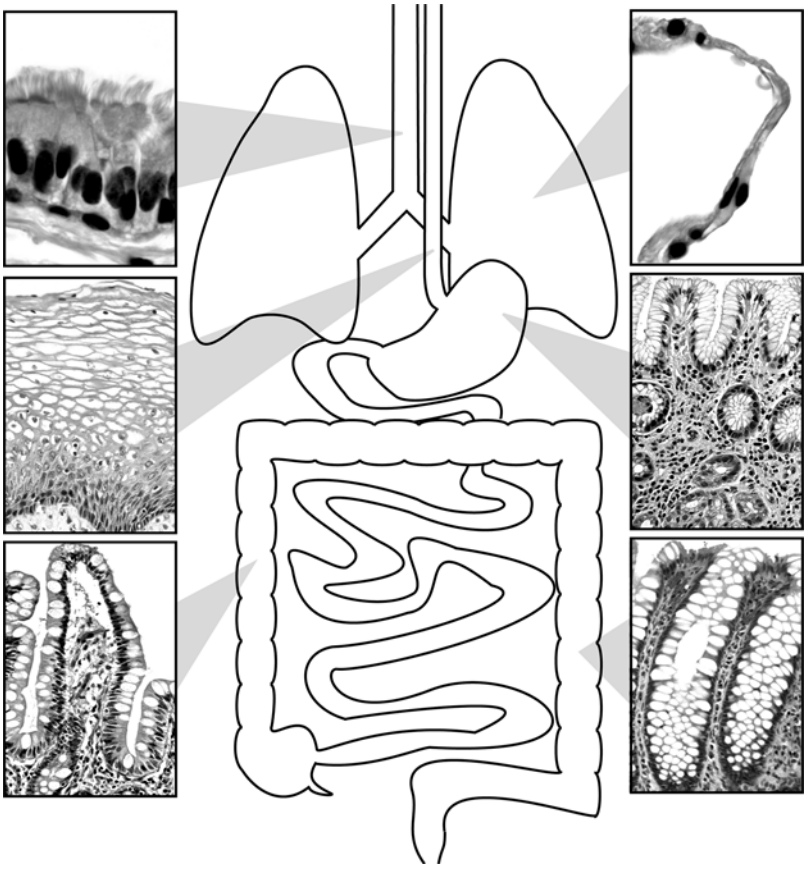

Figure 1.1 The human respiratory and gastrointestinal tracts. Selected histological sections are displayed. Counterclockwise from the top right are epithelium from lung alveolus, gastric fundus, colon, ileum, esophagus, and bronchus. See text for detailed descriptions.

The oral cavity and esophagus are lined by stratified squamous epithelium (Figure 1.1), much like the skin. Keratinization, a normal feature of the skin, is abnormal in the esophagus and typically occurs only as a response to injury or in association with neoplasia. The esophageal squamous epithelium can be divided into three layers. The basal layer houses the stem cells and their progeny, which, in the normal state, extend up to three cell layers above the basement membrane. Normally this represents 10–15% of overall epithelial height, but it can increase dramatically during active inflammation, as often accompanies esophageal infection. Above the basal layer are the post-mitotic

prickle and functional layers. Squamous cells become progressively more glycogenated and flattened as they pass through these layers on their way to the luminal surface. A variety of specialized cell types, including Langerhans cells, which process and present antigens, and endocrine cells, are intermixed with the squamous epithelium. Modified salivary glands are present within the esophageal submucosa. These salivary glands secrete mucus for lubrication, growth factors to augment epithelial cell growth and to aid in repair, and, in the distal esophagus, bicarbonate to neutralize gastric acid. The esophageal squamous mucosa ends at a sharply demarcated site, the Z-line, which marks the transition to the columnar epithelium of the stomach.

The stomach is a distensible saccular portion of the gastrointestinal tract in which food is mixed with gastric acid and digestive enzymes. This mixture, termed chyme, exits the gastric antrum through the pylorus at a controlled rate. The cardia is the small section of the stomach just distal to the Z-line. Like the cardia, the mucasa of the antrum and pylorus, which are the distal portions of the stomach, have long gastric pits and relatively short glands lined by foveolar, or mucus-producing epithelium. This epithelium is preferentially colonized by *Helicobacter pylori*. Thus, the antrum is most frequently affected and the optimal site of diagnostic biopsy for *H. pylori* infection. The fundus, which is a dome-shaped region projecting upward and to the left of the gastroesophageal junction, and the body of the stomach are lined by acid-producing, or oxyntic, mucosa with short pits and an expanded glandular compartment (Figure 1.1). The superficial areas of oxyntic mucosa are lined by foveolar cells that secrete neutral mucins, but the deep pits and glands also contain parietal, chief, and endocrine cells. Parietal cells secrete acid as well as intrinsic factor, which is necessary for the absorption of vitamin B12 in the terminal ileum, while chief cells secrete pepsinogen. These areas are typically colonized by *H. pylori* only after loss of parietal cells and resultant increases in gastric pH. Chronic gastritis involving any portion of the stomach can also lead to metaplasia, with the appearance of small intestinal-like mucin-secreting goblet cells. Unlike gastric foveolar mucus cells, these cells express acid mucins, have a well-developed brush border, and are not readily colonized by *H. pylori*.

Despite its name, the small intestine is the longest portion of the gastrointestinal tract, extending for a length of approximately 6 m in adults. The small intestine is divided into three functionally distinct sections: duodenum, jejunum, and ileum. The primary function of each of these sections is to absorb luminal contents, although the specific molecules absorbed differ by region. For example, although 90% of absorption occurs within the first 100 cm of the small intestine, the jejunum is the principal site of absorption

for Na^+ cotransported monosaccharides and amino acids. The ileum is essential to the enterohepatic recirculation of bile salts and the absorption of certain vitamins, including B12. Moreover, the terminal ileum contains abundant lymphoid tissue, termed Peyer's patches. These lymphoid follicles are a favored site of *Yersina* infection. Since the Peyer's patches actively sample luminal antigens, including those provided by bacteria, the terminal ileum is an effective location for this function; luminal bacterial load averages 1 000 000 bacteria per 1 ml in the distal ileum as opposed to 100 bacterial organisms per 1 ml in the duodenum and jejunum.

Small-intestinal nutrient absorption is facilitated by tissue architecture (Figure 1.1). The surface area of the small intestine is increased greatly by gross mucosal folds, smaller finger-like protrusions, or villi, and a dense microvillus brush border. These combine to provide an overall luminal surface area that is approximately 600-fold greater than the corresponding serosal surface area. Thus, the loss of villi and mucosal flattening that accompany some infections and generalized bacterial overgrowth syndromes can result in significant malabsorption due to loss of absorptive surface area.

In addition to regional specialization along the length of the small intestine, there is significant specialization from the regenerative basally located crypt to the tip of the villus. The epithelial stem cells reside in the crypt, where they proliferate and give rise to specialized enteroendocrine cells and Paneth cells, which remain in the crypt. The stem cells also differentiate into goblet cells, M-cells, and undifferentiated crypt cells. The goblet cell is named for its large mucin vacuole, which compresses the cytoplasm into a shape reminiscent of a wine goblet. Goblet cells are present throughout the crypt–villus axis (Figure 1.1). M-cells, or follicle-associated epithelial cells, are most prominent in the distal ileum overlying Peyer's patches, but they can be found throughout the intestines. M-cells are specialized for the bulk endocytic sampling of luminal contents and are found in the flattened dome epithelium overlying lymphoid follicles. The most prominent feature of the M-cell is its shape, described as having microfolds, from which the 'M' is derived. The basal membrane of the M-cell is retracted from the basement membrane to form a cleft into which lymphocytes and macrophages migrate. Apically derived endocytic vesicles are rapidly released into this cleft by exocytosis, with some luminal particles arriving in the cleft space within 10 min of apical endocytosis. This highly efficient transport pathway has also been exploited as a route into and across the epithelium by some infectious organisms.

The undifferentiated crypt cells remain mitotically active while they are in the crypt and, thus, are somewhat intermediate between stem cells and terminally differentiated cells. Despite their name, undifferentiated crypt cells

possess significant functional differentiation and effectively transport Cl^- into the intestinal lumen. This creates an osmotic gradient that draws water into the lumen. The latter is essential for normal homeostasis and is present in an exaggerated form in many diarrheal diseases, including cholera. As these undifferentiated crypt cells migrate towards the villus, they lose their ability to divide and modify their gene-expression profile to become specialized for absorption. This includes expression of absorptive nutrient and ion transporters, brush-border digestive enzymes, and kinases unique to villus epithelium (Clayburgh, Rosen *et al.*, 2004a; Llor *et al.*, 1999). Ultimately, these absorptive cells become senescent and are shed from the villus tip. This occurs via an orderly process that allows the epithelium to maintain barrier function despite cell shedding (Madara 1990; Rosenblatt *et al.*, 2001).

The colon, which begins at the ileocecal valve and continues to the rectum, is approximately 1 m long in adults. The main functions of the colon are absorption of water and electrolytes and storage of waste products. Transport is accomplished by crypt and surface epithelial cells, which are functionally similar to those in the small-intestinal crypt and villus, although villi are not present (Figure 1.1). Bacterial load in the colonic lumen can be quite high, with typical yields of 10^{11} organisms per 1 ml. These commensal organisms typically coexist peacefully with the host and may serve beneficial roles (Abreu *et al.*, 2005; Fukata *et al.*, 2005; Neish *et al.*, 2000; Rakoff-Nahoum *et al.*, 2004). The presence of these commensal organisms may also prevent overgrowth of pathogenic organisms. For example, incomplete sterilization of the lumen by antibiotic treatment may allow overgrowth of *Clostridium difficile*, which release toxins that glucosylate and inactivate rho (Just *et al.*, 1995). This results in a severe and sometimes fatal colitis characterized by dense adherent pseudomembranes composed of purulent material.

RESPIRATORY TRACT

The proximal larynx is lined by stratified squamous epithelium. Although this is similar to that present in the esophagus, glycogenation is less prominent in the laryngeal epithelium. This gives way to pseudostratified columnar epithelium, although patches of squamous epithelium continue to be present distally. The columnar epithelium can vary in thickness from only a few cells to many cells. Small round reserve cells characterize the basal layer, while the most superficial layers are covered by ciliated epithelial cells and variable numbers of mucin-producing goblet cells. Together, these surface cells provide an important line of defense, as the cilia beat continually and propel a thin layer of mucin with entrapped foreign material upward and out of the

airway. This mucociliary clearance is critical, as demonstrated by patients with Kartagener syndrome, in whom ciliary immotility results in recurrent infection (Eliasson *et al.*, 1977).

The larynx leads to the trachea and bronchi. These large-bore airways are encircled by cartilaginous rings, which prevent their collapse. The trachea and bronchi are lined by ciliated columnar epithelium similar to that seen in the distal larynx. *Corynebacterium diptherial* can colonize these sites and release a toxin that adenosine diphosphate (ADP)-ribosylates elongation factor 2, resulting in epithelial cell death. The mucosal necrosis and sloughing that follow result in accumulation of mucus, cellular debris, and inflammatory cells that obstruct the airways and kill by asphyxiation.

As the bronchi branch into smaller bronchioles, the pseudostratified epithelium becomes simpler, assuming a single-layer cuboidal morphology lined by ciliated and non-ciliated epithelial cells. The latter, termed Clara cells, secrete surfactant and serve as progenitor cells in repair after injury. The bronchioles terminate in the functional unit of the lung, the alveolus, where gas exchange takes place. Alveoli are lined by a thin layer of type 1 squamous and type 2 cuboidal pneumocytes (Figure 1.1). The terminally differentiated type 1 cells cover over 90% of the alveolar surface and provide a thin separation between the airspace and blood that allows for efficient gas exchange. Type 2 cells synthesize and secrete surfactant and also serve as alveolar stem cells, giving rise to additional type 2 cells as well as type 1 cells. In the presence of infection, for example with *Streptococcus pneumoniae*, the most common cause of community-acquired acute pneumonia, the alveoli may become filled with fluid and inflammatory cells, including neutrophils and macrophages. Infection spreads rapidly within contiguous alveoli, leading to consolidation of large areas of lung parenchyma and loss of the surface area necessary for gas exchange.

URINARY TRACT

The renal collecting ducts empty into the renal pelvis. The latter is lined by urothelium, which is histologically similar in the renal pelvis, ureters, and bladder. This epithelium is also termed transitional, as its appearance is intermediate between that of non-keratinizing stratified squamous epithelium, as seen in the esophagus, and pseudostratified columnar epithelium, like that of the bronchi. The thickness of the urothelium varies significantly, from only two to three cells thick in the renal pelvis to seven or more cells in the contracted bladder. As the bladder distends, the urothelium thins to two to three cells that are elongated parallel to the basement membrane. Like other

stratified epithelia, proliferation occurs in the basal zone, which is populated by small cuboidal cells. The surface is comprised of terminally differentiated cells. In the bladder, these surface cells are termed umbrella cells due to their large ellipsoid shape. Like deeper cells, the umbrella cells become flattened and difficult to appreciate in the distended bladder. They are often absent in urothelial carcinoma, making their presence a somewhat reassuring, although non-diagnostic, feature in bladder biopsies. Bacterial infections of the bladder and urethra include *Escherichia*, *Proteus*, *Klebsiella*, and *Enterobacter* and are more common in women than men due to the shorter length of the female urethra. Such infections can extend proximally in a retrograde fashion, to involve ureters, renal pelvis, and renal parenchyma in ascending pyelonephritis.

VECTORIAL TRANSPORT

Despite the differences in specialized cellular functions and morphology, all of these tissues are lined by a polarized epithelium that provides protection from the environment. Therefore, for the purpose of understanding cellular architecture, the structure of these polarized epithelia can be generalized. At the cellular level, polarization allows for asymmetric morphology as well as distinct molecular composition and function of membrane domains. The free surface, which faces the lumen, is referred to as apical, while the surfaces in contact with the basement membrane and adjacent cells are referred to as basal and lateral, respectively. The basal and lateral membrane domains typically function as a single unit, termed the basolateral domain. Specific proteins and lipids can be targeted to apical and basolateral membrane domains. For example, Na^+ nutrient cotransporters are recruited to the apical membrane in transporting epithelia (Harris *et al.*, 1992). Similarly, the GM1 ganglioside used as a receptor for cholera toxin is also primarily apical (Lencer *et al.*, 1995; Wolf *et al.*, 1998). Other proteins, such as β1 integrins and interperson gamma (IFNγ) and tumor necrosis factor alpha (TNFα) receptors, predominantly localize to the basolateral surface in polarized epithelia (Boll *et al.*, 1991; Madara and Stafford, 1989; Moller *et al.*, 1994). Finally, some proteins, such as the polymeric immunoglobulin receptor, have more complex trafficking patterns. The polymeric immunoglobulin receptor is trafficked to the basolateral membrane first, where it picks up immunoglobulin cargo; then the receptor is endocytosed and moved to the apical membrane, where it releases the cargo (Casanova *et al.*, 1990, 1991; Mostov *et al.*, 1986).

In each case, the appropriately polarized distribution of proteins and lipids is necessary for proper epithelial function. For example, as noted

above, Na^+ nutrient cotransporter proteins are apically targeted in intestinal and renal tubular epithelium (Harris *et al.*, 1992; Turner *et al.*, 1996; Wright *et al.*, 1997). In the case of glucose absorption, glucose is transported into the cell against its concentration gradient by SGLT1, the apical intestinal Na^+/glucose cotransporter using Na^+ cotransport and the Na^+ gradient, from high outside the cell to low inside the cell, as the driving force (Hediger *et al.*, 1987). This Na^+ gradient is maintained by the Na^+/K^+ ATPase. The Na^+/K^+ ATPase is localized to the basolateral membrane along with the facilitated glucose transporter GLUT2 (Dominguez *et al.*, 1992; Miyamoto *et al.*, 1992). Thus, after SGLT1 transports Na^+ and glucose across the apical membrane, glucose and Na^+ exit the cytoplasm by crossing the basolateral membrane. This allows for directional net absorption and generates an ionic gradient that draws in water from the lumen. Similar mechanisms move water and solutes across the alveoli (Nielson and Lewis, 1990). Clearly, none of this would be possible in the absence of polarized membrane domains.

Membrane polarization is also essential for secretion. The cystic fibrosis transmembrane conductance regulator (CFTR) is a transporter responsible for apical cyclic adenosine monophosphate (cAMP)-activated anion conductance (Anderson *et al.*, 1991; Kartner *et al.*, 1991; Tabcharani *et al.*, 1991). Cystic fibrosis is linked to mutations in CFTR, and these mutations are responsible for the poorly hydrated viscous luminal accumulations that cause intestinal, pancreatic, and airway obstruction (Gregory *et al.*, 1990; Riordan *et al.*, 1989). In normal subjects without CFTR mutations, cholera toxin activates CFTR by elevating intracellular cAMP levels (Breuer *et al.*, 1992). This results in net transport of Cl^- ions into the intestinal lumen, followed by massive fluid accumulation (Field, 2003). Thus, cholera takes advantage of normal cellular processes to cause massive diarrhea, which spreads infection in areas with poor sanitation.

MEMBRANE TRAFFIC

In addition to transporters that allow solutes to cross apical and basolateral membrane domains, epithelia also utilize vesicular routes in order to traffic materials and to respond to extracellular stimuli. The trafficking of immunoglobulin A (IgA) from basolateral to apical surfaces by polymeric immunoglobulin receptor is a particularly well characterized example of such transport. As noted above, the polymeric immunoglobulin receptor is initially trafficked to the basolateral membrane. Here, it binds polymeric immunoglobulin, most prominently IgA (Casanova *et al.*, 1990, 1991; Mostov *et al.*, 1986). The binding of IgA triggers internalization of the complex and

trafficking of the endocytic cargo to the apical surface. A proteolytic cleavage event at this surface results in release of IgA at the apical, or luminal, surface along with a portion of the receptor, termed the secretory component (Aroeti *et al.*, 1993; Casanova *et al.*, 1991; Song *et al.*, 1994). Although this finely tuned process plays an important role in mucosal immune protection, it has apparently not been able to escape the attention of *Streptococcus pneumoniae*. These bacteria exploit the presence of uncleaved polymeric immunoglobulin receptor at the apical membrane to bind to the secretory component portion of the receptor and invade host cells (Zhang *et al.*, 2000). The binding of *S. pneumoniae* to polymeric immunoglobulin receptor drives transcytosis in reverse, from apical to basolateral, thereby effectively transporting bacteria across the epithelium (Brock *et al.*, 2002). This pathway is clinically relevant, as the pattern of polymeric immunoglobulin receptor expression, greater in nasopharyngeal than distal respiratory epithelium, likely also explains the more frequent colonization of upper airways by *S. pneumoniae* (Zhang *et al.*, 2000).

In general, endocytosis in epithelial and other cells occurs by two main pathways, which are mediated by clathrin and caveolin. Clathrin-mediated endocytosis is best characterized in processes of macromolecule and integral membrane protein internalization. In this pathway, surface receptors bind cargo and initiate a signaling cascade. Receptor-mediated endocytosis of low-density lipoprotein (LDL) and transferrin are classic examples of endocytosis by clathrin-coated vesicles (Anderson *et al.*, 1978; Hewlett *et al.*, 1994). The clathrin coat is readily recognized by electron microscopy as a regular lattice of clathrin molecules that covers the cytoplasmic face of the membrane at sites of internalization (Pearse, 1976). Like other cellular processes, bacterial pathogens have evolved means of using clathrin-mediated endocytosis to their own advantage. For example, Shiga and Shiga-like toxins enter the cell through clathrin-mediated endocytosis (Sandvig and Van Deurs, 1996, 2000). These toxins ultimately cross intracellular membranes to block ribosomal activity, resulting in inhibition of protein synthesis and cell death (Obrig *et al.*, 1987; Reisbig *et al.*, 1981).

The second major endocytic pathway occurs through caveolae, literally little caves, which were initially described morphologically (Palade and Bruns, 1968). Extensive work has defined a cholesterol- and glycosphingolipid-rich microenvironments at these sites that often also contain proteins of the caveolin family (Dupree *et al.*, 1993; Lisanti *et al.*, 1993; Sargiacomo *et al.*, 1993). Although the biophysical structure of these lipid domains remains controversial, data do show that a variety of receptors and signaling molecules are concentrated in caveolae (Penela *et al.*, 2003). Thus, in addition to internalization

of macromolecules such as micronutrients, chemokines, and hormones, caveolae have been proposed to be platforms for signal transduction (Penela *et al.*, 2003). Like clathrin-mediated endocytosis, bacterial pathogens have developed means of co-opting caveolin-mediated endocytosis. For example, cholera toxin binds to ganglioside GM1, which is concentrated in caveolae. This allows the toxin to enter the cell through caveolin-mediated endocytosis (Duncan *et al.*, 2002; Lencer *et al.*, 1995). Similarly, entry of *Chlamydia trachomatis* is associated with caveolin-mediated endocytosis (Duncan *et al.*, 2002; Norkin *et al.*, 2001).

CYTOSKELETON

The cytoskeleton serves as a structural framework for epithelial shape, supports membrane traffic, and plays an essential role in development and maintenance of polarity (Figure 1.2). In addition to actin, tubulin, and intermediate filaments, the epithelial cytoskeleton is composed of a complex array of accessory proteins that regulate filament assembly and movement. The intermediate filaments form a stable fiber system that surrounds the nucleus and extends to the plasma membrane (Brown *et al.*, 1983). In polarized epithelial cells, microtubules provide an important scaffold for cell shape. The microtubule organizing center, located near the nucleus, coordinates nucleation of microtubule polymerization in a defined polarized fashion (Murphy and Stearns, 1996). This polarity allows for directional transport of vesicles to and from specific membranes. In addition to roles in regulating placement of organelles and vesicular targeting, microtubules can also be used to enhance bacterial invasion. For example, *Campylobacter jejuni* have been shown to invade intestinal epithelial cells in a microtubule-dependent, actin-independent manner (Kopecko *et al.*, 2001; Oelschlaeger *et al.*, 1993). In ciliated respiratory epithelial cells, the core of each cilium is a precisely organized ring of microtubules. Dynein, a microtubule-based motor protein, causes the cilia to flex, resulting in the ciliary beating that is necessary for mucociliary clearance (Camner *et al.*, 1975). Patients with primary ciliary dyskinesia, including many with Kartagener syndrome, have mutations that shorten dynein arms, resulting in immotile cilia (Camner *et al.*, 1975). As discussed above, these patients are at increased risk for respiratory infection. Affected males also tend to be sterile, due to inefficiencies of sperm motility, another microtubule and dynein-dependent process.

In contrast to microtubules, actin filaments or microfilaments are found in close association with the plasma membrane of polarized epithelial cells. These filaments are present at apical, lateral, and basal membranes, but they

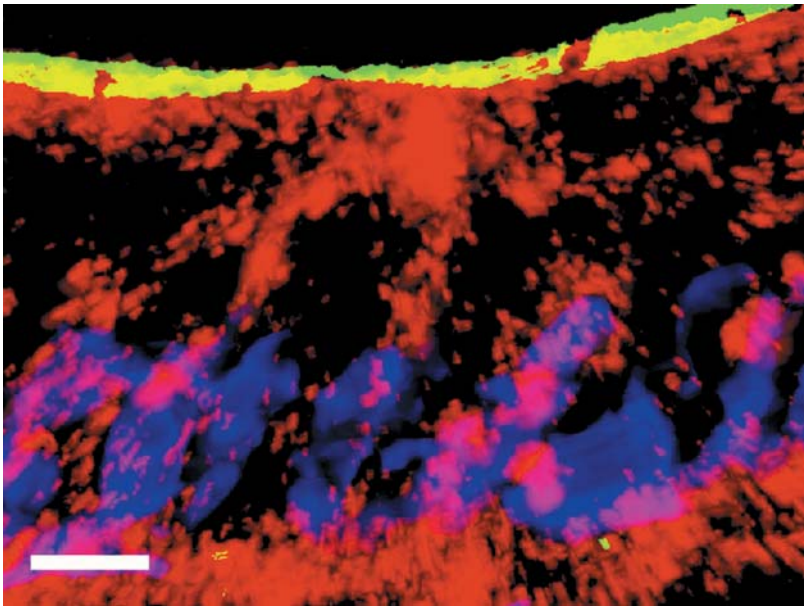

Figure 1.2 Three-dimensional reconstruction of human jejunal epithelium stained for tubulin (red), f-actin (green), and nuclei (blue). Note the prominent actin ring (green) concentrated at the level of the apical junctional complex. Actin is also present within the microvilli and along basolateral membranes but cannot be appreciated in this image due to the high concentration of actin at the perijunctional ring. Microtubules (red) extend throughout the cell and play a significant role in vesicular trafficking. Bar = 5 μm. See also Color plate xx.

are particularly concentrated at sites of cell–cell or cell–substrate contact. In intestinal epithelia, actin cores also form the substructure of microvilli. Moreover, a contractile circumferential actin belt encircles each epithelial cell at the level of the tight and adherens junctions. Contraction of this actomyosin belt can be regulated during normal tissue function and can be disrupted in disease. Microfilaments also play important roles in endocytosis, and the small GTPases of the rho family that regulate actin are frequent targets of bacterial pathogens. For example, *Salmonella* use the type III secretion system to inject a toxin that serves as a guanine exchange factor for cdc42 and rac (Hardt *et al.*, 1998; Lu and Walker, 2001; Parrello *et al.*, 2000). This induces actin-dependent membrane ruffling, leading ultimately to bacterial internalization. As discussed above, *Clostridium difficile* toxins glucosylate and inactivate rho (Hippenstiel *et al.*, 1997; Just *et al.*, 1994, 1995). This key regulator of actin structure is also targeted by *Clostridium botulinum* C3 toxin, which inactivates

rho via ribosylation (Aktories *et al.*, 1992; Narumiya *et al.*, 1990; Wilde *et al.*, 2000).

INTERCELLULAR JUNCTIONS

Many cells function effectively as individual units. Examples include red blood cells, immune cells, and fibroblasts, which at most make occasional transient junctions with other cells. In contrast, epithelia are ineffectual as independent cells. Their function requires that they form an intact surface with a sustained network of junctions joining each cell to its neighbors and, for the basal cell layer, the matrix of the basement membrane. In the absence of these junctions, epithelia would be unable to form a barrier. Thus, efforts to achieve vectorial transport would be unsuccessful, as any transport could easily be negated by diffusion in the reverse direction. Additionally, the mere presence of a barrier is an essential component of epithelial structure. These barriers provide protection from external hazards, including airborne toxins, luminal bacteria, and noxious chemicals. Thus, as one might guess, bacteria have evolved to exploit, evade, and disrupt intercellular junctions.

Interactions between individual cells and the basement membrane are typically mediated by integrins. This family of proteins can interact with many matrix proteins, including laminin and collagen, as well as other cell types by generating a diverse array of molecules with differing alpha- and beta-chain compositions (Albelda *et al.*, 1994; Fuchs *et al.*, 1997; Giancotti and Ruoslahti 1999; Humphries 1990; Luscinskas and Lawler 1994). β1 integrins link the extracellular matrix to the intracellular actin cytoskeleton and can also serve as receptors for *Yersinia enterocolitica* and *Yersinia pseudotuberculosis* invasion (Isberg and Leong, 1990). In this case, since β1 integrins are localized to basolateral membrane domains, some disruption of the apical junctional complex must occur before *Yersinia* can access β1 integrins. Adjacent cells are also bound to one another by desmosomes, which are linked to intermediate filaments, thus providing a semi-rigid framework, and, in many cases, by gap junctions, which facilitate intercellular signaling by allowing diffusion of small molecules between the cytosol of adjacent cells.

Despite the importance of these structures to epithelial function, the components of the apical junctional complex may well be the most critical. This has not escaped the attention of pathogens, such as *Porphyromonas gingivalis* and *Bacteroides fragilis*, that attack the apical junctional complex by secreting proteases that degrade critical components of the complex (Katz *et al.*, 2000; Koshy *et al.*, 1996; Wu *et al.*, 1998).

Assembly and placement of the apical junctional complex are linked closely to the establishment of epithelial polarity. As implied by the name, the apical junctional complex is located at the most apical region of the lateral membrane. The complex consists of the tight junctions, which define the border between apical and basolateral membrane domains, and the adherens junctions, which are located just basal to the tight junctions. The principal proteins of the adherens junction mediate Ca^{+2}-dependent homotypic intercellular adhesions and are termed cadherins, a contraction of Ca^{+2}-dependent and adherens junction (Hatta and Takeichi, 1986). Several distinct cadherins can be expressed, and this differential expression forms the basis of self-recognition by different embryonic cell types (Takeichi, 1988). Epithelia express E-cadherin. The cytoplasmic tail of E-cadherin interacts directly with cytoplasmic scaffolding and signaling proteins, including β-catenin, and indirectly with the actin cytoskeleton (Itoh *et al.*, 1997; McCrea *et al.*, 1991; Ozawa *et al.*, 1990; Yonemura *et al.*, 1995). Thus, adherens junctions perform essential roles in signal transduction in addition to their function in maintenance of intercellular contact. Cadherins can also be used as landmarks by bacteria. For example, *Listeria monocytogenes* use E-cadherin to specifically target human epithelial cells (Mengaud, Lecuit *et al.*, 1996; Mengaud, Ohayon *et al.*, 1996). The bacterial protein internalin binds a specific sequence in E-cadherin that differs in other species (Lecuit *et al.*, 1999). This binding triggers lipid raft-mediated internalization of the E-cadherin–*Listeria* complex, thus facilitating bacterial invasion (Seveau *et al.*, 2004).

Although adherens junctions are thought to provide much of the strength that supports intercellular junctions, they are quite porous and do not form a significant barrier to paracellular diffusion. Thus, sealing of the paracellular space falls to the tight junction. This intercellular seal of variable permeability has been described as a "gate," allowing differential passage of water, ions, and other molecules (Diamond, 1977; Gumbiner, 1987). As noted above, tight junctions also physically separate apical and basolateral membranes, forming a "fence" that prevents diffusion of protein and lipid components of the plasma membrane, thereby maintaining distinct apical and basolateral membrane domains (Diamond, 1977; Gumbiner, 1987).

Although many tight-junction proteins have now been identified, their precise roles in assembly and maintenance of tight-junction structure are poorly understood. At least three classes of transmembrane proteins have been described (Furuse *et al.*, 1998). These include the claudin family, whose members appear to define the charge-selective permeability of tight junctions in different tissues, as demonstrated elegantly by in vitro (Van Itallie *et al.*, 2003) and in vivo (Simon *et al.*, 1999) studies. Specific claudin isoforms are

targeted by *Clostridium perfringens* enterotoxin, which binds to and targets claudins 3 and 4 for degradation, resulting in loss of barrier function in epithelia that express these claudin isoforms (Sonoda *et al.*, 1999).

In contrast to claudins, the role of another tetramembrane-spanning protein, occludin, is less well defined. Numerous in vitro studies have suggested that occludin plays an important role in tight-junction function, including the observation that peptides derived from the second extracellular loop can disrupt epithelial barrier function (Nusrat *et al.*, 2005; Wong and Gumbiner, 1997). However, this and other in vitro observations suggesting the functional importance of occludin are challenged by the report that occludin knockout mice do not have readily apparent barrier defects (Saitou *et al.*, 2000). Nonetheless, several in vivo analyses suggest that occludin internalization is a reliable marker of tight junction disruption by EPE and other inflammatory stimuli (Shifflett *et al.*, 2005). The precise function of occludin remains to be determined.

The third major family of described transmembrane tight junction proteins, junctional adhesion molecules, can localize to tight junctions as well as desmosomes. At these sites they appear to play a host of roles, including mediating transepithelial inflammatory cell migration (Zen *et al.*, 2004) and resealing of tight junctions after injury (Liu *et al.*, 2000). Finally, a long list of peripheral membrane proteins, including ZO-1, ZO-2, ZO-3, MUPP-1, and cingulin, have been described (Citi *et al.*, 1988; Haskins *et al.*, 1998; Jesaitis and Goodenough 1994; Stevenson *et al.*, 1986). These are able to maintain numerous binding interactions with one another and other proteins, leading to complexity of the tight-junction structure.

BARRIER REGULATION

The structure of the tight junction is critically dependent on an intact ring of actin and myosin, the perijunctional actomyosin ring, at the site of the apical junctional complex. Mere depolymerization of this actomyosin ring, as induced by toxin-mediated rho inactivation, is sufficient to cause massive disruption of barrier function (Hecht *et al.*, 1988; Nusrat *et al.*, 1995). Less massive regulation of tight-junction permeability also occurs in response to physiological stimuli. For example, activation of Na^+-nutrient cotransporters causes increases in paracellular permeability that allow paracellular amplification of transcellular nutrient and water absorption at appropriate times, e.g. after a nutrient-rich meal (Madara and Pappenheimer, 1987). In vivo and in vitro studies have shown that initiation of Na^+/glucose cotransport results in activation of a signaling cascade that leads to myosin light chain kinase

(MLCK)-mediated myosin II regulatory light chain (MLC) phosphorylation, perijunctional actomyosin ring contraction, and increased paracellular permeability (Berglund *et al.*, 2001; Turner *et al.*, 1997). The distal portion of this pathway, that following MLCK activation, is also used by enteropathogenic and enterohemorrhagic *Escherichia coli* (EPEC) (Philpott *et al.*, 1998; Spitz *et al.*, 1995; Yuhan *et al.*, 1997). Like initiation of Na^+/glucose cotransport (Turner *et al.*, 1997), EPEC infection results in MLC phosphorylation and increased paracellular permeability (Figure 1.3). In each case, MLCK inhibitors are able to partially or completely prevent increases in paracellular permeability, suggesting an essential role for MLCK in this process (Turner *et al.*, 1997; Yuhan *et al.*, 1997; Zolotarevsky *et al.*, 2002). Although the specific bacterial effectors responsible for EPEC-induced MLC phosphorylation have not been defined, it is clear that the presence of an intact type III secretion system is required, raising the possibility that the effector is injected directly into the epithelial cytoplasm (Simonovic *et al.*, 2000). In the case of EPEC, it also appears likely that upstream activation of protein kinase C zeta PKC-ζ contributes to MLCK activation (Tomson *et al.*, 2004).

Advances in our understanding of the biology of and, potentially, developing therapy for these barrier defects have been limited by the lack of effective and specific inhibitors of MLCK. This deficiency has now been overcome by the development of stable peptide analogs of a highly specific membrane-permeant MLCK inhibitor, termed PIK (Owens *et al.*, 2005; Zolotarevsky *et al.*, 2002). This inhibitor has been used to specifically probe the role of MLCK in intestinal epithelial barrier dysfunction. As noted above, epithelial barrier dysfunction is seen in a variety of infectious diseases of the intestines. As expected, PIK prevents much of the loss of barrier function that accompanies EPEC infection in vitro (Zolotarevsky *et al.*, 2002).

Epithelial barrier function is also compromised in inflammatory diseases such as Crohn's disease (Clayburgh, Shen *et al.*, 2004). In this case, it is thought that barrier disruption is mediated by local release of T helper type 1 (Th1) cytokines, including IFNγ and TNFα. Indeed, mucosal levels of these cytokines are elevated during the active phase of inflammatory bowel disease. Consistent with a role in barrier dysfunction, these cytokines are able to disrupt barrier function in cultured epithelial monolayers, and in vivo antagonism of IFNγ or TNFα can diminish disease severity and restore barrier function (Brown *et al.*, 1999; Ferrier *et al.*, 2003; Musch *et al.*, 2002; Suenaert *et al.*, 2002). To define the mechanisms of this cytokine-dependent barrier dysfunction, MLC phosphorylation was assessed in cultured monolayers after treatment with IFNγ and TNFα. Marked increases in MLC phosphorylation accompanied functional barrier loss (Zolotarevsky *et al.*, 2002). The MLCK

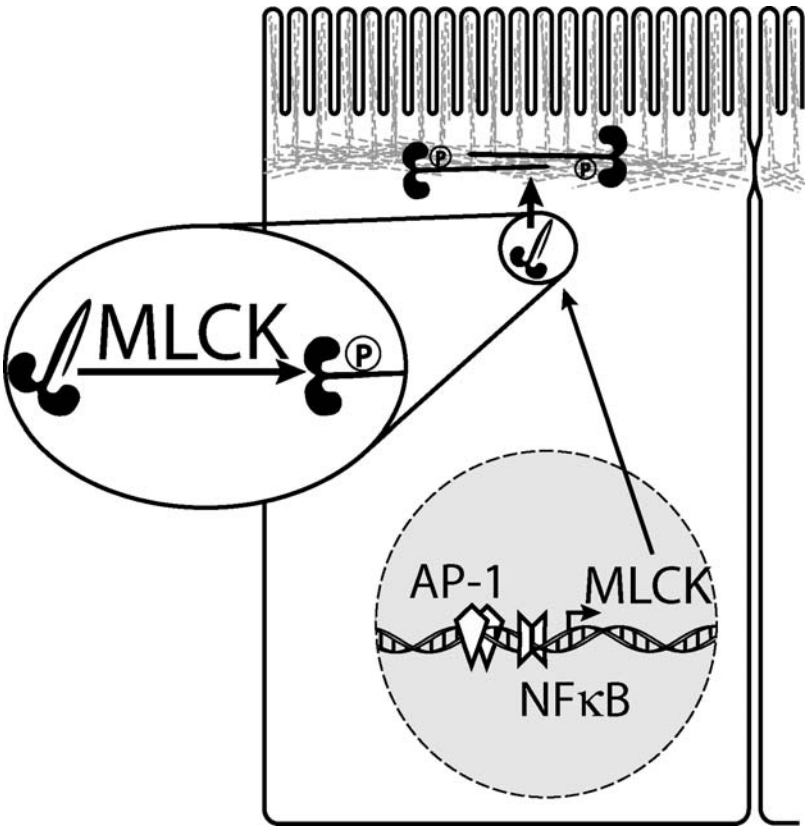

Figure 1.3 Model for epithelial barrier dysfunction secondary to myosin light chain kinase (MLCK) upregulation. Extracellular stimuli trigger a signaling pathway that activates activating protein 1 (AP-1) and/or nuclear factor kappa B (NFκB) transcription factors. These increase transcription of MLCK mRNA, thereby increasing the cytoplasmic pool of MLCK protein. MLCK induces myosin II regulatory light chain (MLC) phosphorylation and a conformational change in myosin. This activates myosin, which leads to contraction of the actomyosin ring and decreased tight-junction barrier function. Although the involvement of this pathway in cytokine-induced barrier dysfunction has been demonstrated, the role of MLCK transcriptional activation in bacterial pathogenesis has not been reported.

inhibitor PIK reduced MLC phosphorylation and normalized barrier function (Zolotarevsky *et al.*, 2002). Thus, MLC phosphorylation is necessary for this in vitro cytokine-dependent barrier dysfunction. Other studies suggest that this may also be the mechanism of acute in vivo cytokine-dependent barrier loss (Clayburgh *et al.*, 2005).

To better understand the mechanisms by which IFNγ and TNFα cause increases in MLC phosphorylation, expression of MLCK, the responsible kinase, was assessed following cytokine treatment. The data show that in cultured epithelial monolayers, MLCK protein expression is upregulated by cytokine treatment and that this correlates with elevated MLC phosphorylation and barrier dysfunction (Ma *et al.*, 2005; Wang *et al.*, 2005). Both IFNγ and TNFα are required for this upregulation of MLCK expression, but the role of IFNγ seems to be in priming the cells to respond to TNFα (Wang *et al.*, 2005). In the absence of IFNγ priming, TNFα is unable to induce changes in barrier function, MLC phosphorylation, or MLCK expression (Wang *et al.*, 2005). Some authors have suggested that these effects might be mediated by nuclear factor kappa B (NFκB), a canonical signaling pathway activated by TNF-α (Ma *et al.*, 2004, 2005). However, other data demonstrate that inhibition of NFκB does not reduce cytokine-dependent barrier dysfunction, MLC phosphorylation, or MLCK upregulation (Wang *et al.*, 2005). The latter observations are consistent with in vitro and in vivo studies suggesting a protective role for epithelial NFκB following cytokine exposure (Chen *et al.*, 2003; Soler *et al.*, 1999).

To better define the mechanisms by which these cytokines and other stimuli, including pathogens, regulate MLCK expression, 4 kilobase pairs (κB) of sequence upstream of the MLCK transcriptional start site was cloned (Graham *et al.*, 2004). When fused to a reporter gene and transfected into intestinal epithelial cells, this construct responded to IFNγ and TNFα like endogenous MLCK (Graham *et al.*, 2004). In silico sequence analysis identified numerous possible transcription factor binding sites, including four putative activating protein 1 (AP-1) binding sites and two putative NFκB binding sites. Several of these sites were confirmed to be functional protein binding sites based on electrophoretic mobility shift assay (Graham *et al.*, 2005). Cotransfection with constitutively active AP-1 and NFκB showed that activation of either transcription factor pathway was able to upregulate MLCK transcription (Graham *et al.*, 2005). Mutational analysis confirmed that these AP-1 and NFκB sites were indeed necessary for MLCK transcriptional activation (Figure 1.3). Further analysis showed clearly that in these differentiated epithelial monolayers, AP-1 activation is responsible for the observed increase in MLCK transcription after cytokine exposure (Graham *et al.*, 2005). Together, these data indicate that AP-1 induces MLCK upregulation in response to IFNγ and TNFα, but they also suggest that other stimuli, including infection by *H. pylori* or EPEC, may upregulate MLCK expression via NFκB activation (Graham *et al.*, 2005; Keates *et al.*, 1997; Munzenmaier *et al.*, 1997; Savkovic *et al.*, 1997; Sharma *et al.*, 1998). Thus, although this has

not yet been demonstrated, it may be another example of bacterial exploitation of host regulatory mechanisms.

CONCLUSIONS

Epithelia perform many specialized functions that are essential to homeostasis as well as host defense. In many cases, these are exploited effectively by bacteria in their efforts to achieve infection and overcome the epithelial barrier.

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