Chapter 12

Epithelial Cells: Structure, Transport, and Barrier Function

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Chapter Outline

INTRODUCTION

Most mucosal surfaces are lined by a single layer of columnar epithelial cells that provide the necessary absorptive and secretory processes required for organ-specific function while also serving as a physical barrier that prevents free passage of particles from the lumen. Such particles can range from inhaled pollutants, toxins, and bacteria in the lung to the complex milieu of nutrients, waste products, microbiota, and microbial products in the intestine. Of all columnar epithelia, barrier function has been the best studied in the intestine, reflecting the recognized importance of the intestinal barrier to health. Thus, the intestine will be used as the primary example throughout this chapter. Although it is certain that differences in epithelial barrier function exist between tissues (e.g., lung, kidney, and intestine), most of the general principles addressed are applicable across organ systems.

STRUCTURAL ORGANIZATION OF THE INTESTINE

Different regions of the gastrointestinal tract exhibit distinct structural characteristics that are unique to the specialized function of the tissue. This chapter will focus on the simple columnar epithelium that lines the small intestine and colon. Anatomically, the small intestine can be divided into the duodenum, jejunum, and ileum, and although the small intestine is the primary site of nutrient absorption, the specific nutrients absorbed differ between these regions. For example, the duodenum is the primary site of absorption for fatty acids and water-soluble vitamins, the jejunum for glucose and amino acids, and the ileum for bile salts and vitamin B_{12}. To maximize nutrient absorption, the small intestine is arranged into mucosal folds (Figure 1(a)) and finger-like villi (Figure 1(b)), which increase surface area 3- and 10-fold, respectively. In contrast, although mucosal

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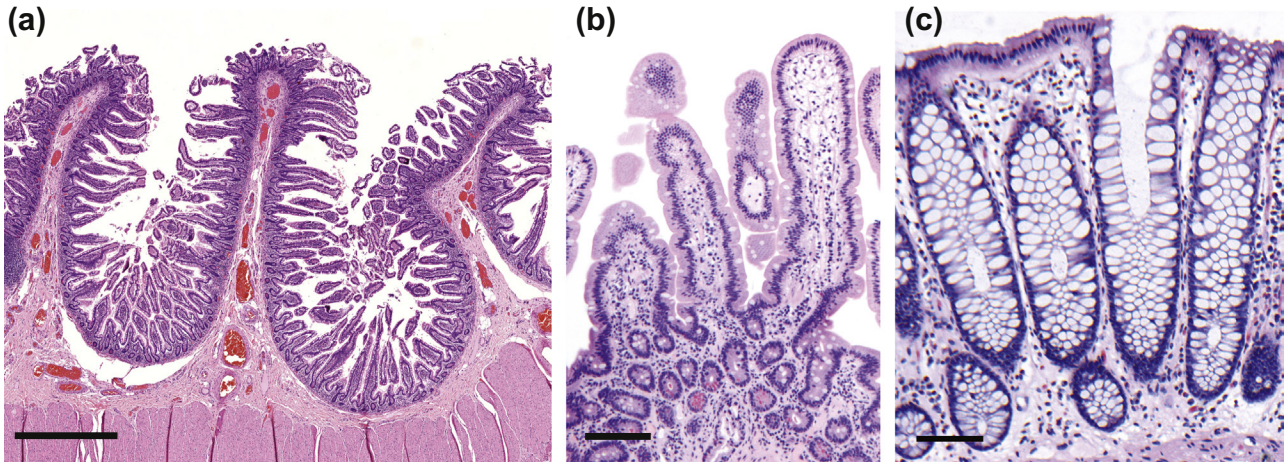

FIGURE 1 Organization of intestinal architecture. Absorption of nutrients and water is maximized by increasing epithelial surface area. This is achieved through the organization of (a) mucosal folds, scale bar = 1 mm and (b) villi in the human small intestine and (c) colonic crypts, scale bar = 100 μm.

folds are present in the colon, the mucosal surface lacks villi (Figure 1(c)).

INTESTINAL EPITHELIAL CELL TYPES

The epithelial cells that cover each small intestinal villus are derived from progenitors (i.e., stem cells) within the crypt (Potten and Loeffler, 1990; Barker et al., 2007). Relatively undifferentiated, secretory epithelial cells exit the crypt base and proliferate within a region termed the *transit amplifying zone*. As they continue to migrate luminally, the epithelial cells lose the ability to proliferate and begin to mature into highly specialized absorptive enterocytes (Figure 2). This differentiation includes assembly of a dense microvillus brush border, which increases the surface area 20-fold, and expression of a new complement of transport and barrier proteins (Figure 3). These cells are ultimately shed into the lumen, which allows for continual epithelial turnover and renewal of the entire epithelium every 3–5 days (Figure 2). This simple anatomic organization has made the small intestine a favorite model system for analyses of stem cell regulation, epithelial differentiation, and programmed cell turnover (van der Flier and Clevers, 2009).

In addition to generating absorptive enterocytes, intestinal epithelial stem cells also give rise to other terminally differentiated cell types, such as enteroendocrine, Paneth, and goblet cells (Cheng et al., 1969; Merzel and Leblond, 1969). Enteroendocrine cells are found in the crypt and throughout the villus. These cells secrete gastrointestinal hormones, such as somatostatin, and can be recognized by their fine basolaterally oriented granules (Figure 4(a)). Enteroendocrine cells are also the cell of origin for gastrointestinal neuroendocrine carcinomas, which are most common in the small intestine.

Paneth cells are found exclusively within the crypt base. The apical cytoplasm of these cells is filled with large

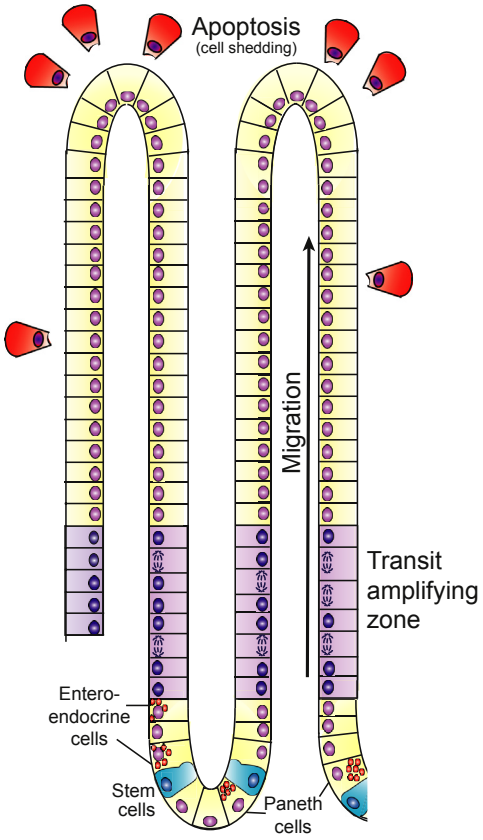

FIGURE 2 The intestinal epithelium undergoes continuous renewal. Stem cells located near the base of the crypt give rise to all four lineages of gastrointestinal cells. As undifferentiated secretory epithelial cells migrate up the crypt–villus axis in the small intestine, proliferation occurs in the transit amplifying zone, after which cells cease dividing and undergo differentiation. Migration continues until cells reach the villus where they undergo apoptosis and are shed into the lumen. Enterocytes along the crypt–villus axis undergo anoikis and are shed into the lumen through an unknown mechanism (Watson et al., 2005a).

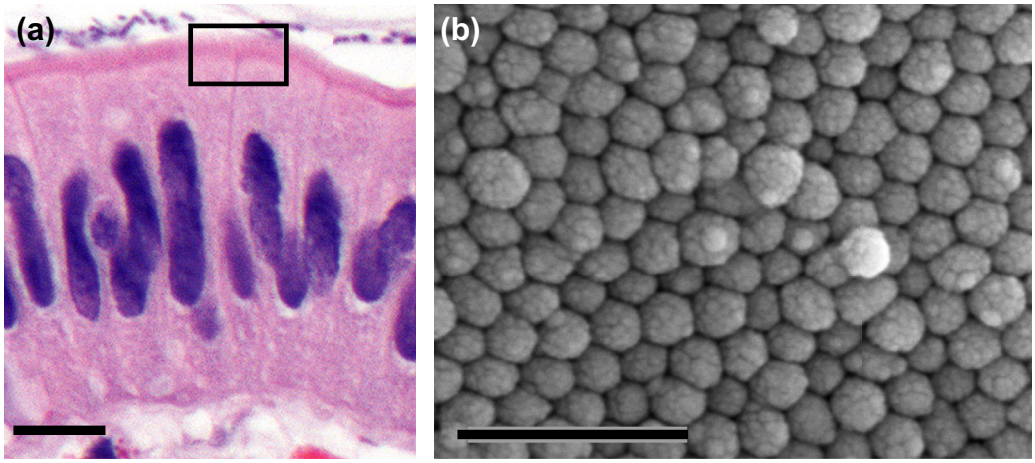

FIGURE 3 Enterocytes are highly specialized absorptive cells with a microvillus brush border. These differentiated epithelial cells are polarized to form an apical and basolateral membrane to promote epithelial transport. (a) The tightly packed microvilli form the brush border (boxed area). Scale bar = 10 μm. (b) Scanning electron micrograph of microvilli. Scale bar = 0.5 μm. *Scanning electron micrograph courtesy of S. Crawley, Tyska Laboratory, Vanderbilt University.*

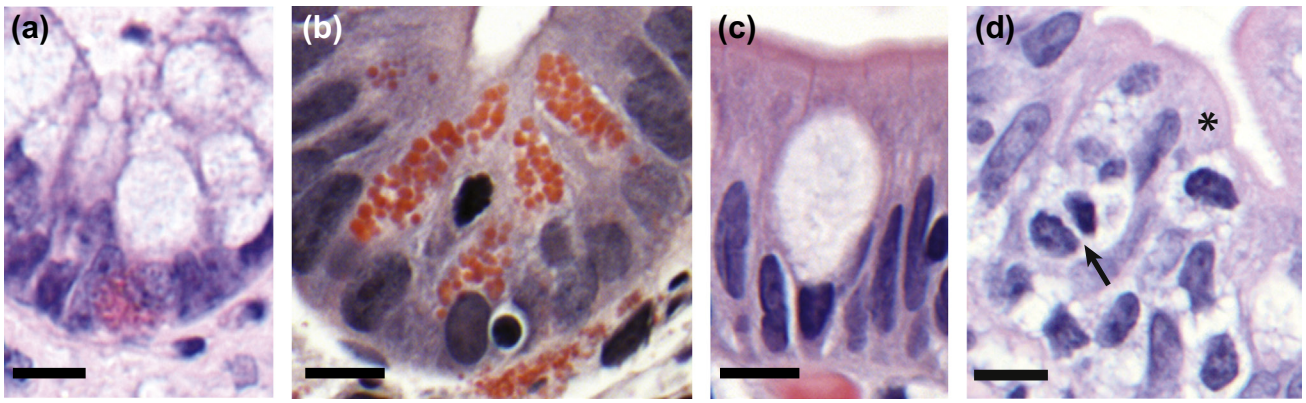

FIGURE 4 Terminally differentiated cells of the intestinal epithelium. In addition to enterocytes, intestinal stem cells give rise to secretory cells and M cells located in Peyer's patches: (a) enteroendocrine cell, (b) Paneth cell, (c) goblet cell, and (d) M cell (asterisk) with a lymphocyte pocket (arrow). Scale bar 10 μm.

secretory granules (Figure 4(b)) containing antimicrobial defensin peptides (see Chapter 16) and other molecules that promote innate defense, such as RegIIIγ (Vaishnava et al., 2011). Therefore, Paneth cells can be considered to be innate immune cells. Although Paneth cells are abundant throughout the small intestine, they are normally found only in the right side of the colon (i.e., proximal to the splenic flexure). However, Paneth cells can be found in the left colon in the context of chronic injury (e.g., inflammatory bowel disease (IBD); Yoshimoto et al., 2013). The significance of this Paneth cell metaplasia is not known, but it may represent an adaptive response to ongoing mucosal damage and microbial contamination.

Goblet cells are located throughout the upper crypt and entire villus and can be recognized by a large apical mucin vacuole (Figure 4(c)). This vacuole displaces the cytoplasm and can impart an appearance reminiscent of a goblet, or chalice. Mucin is released from these cells constitutively, thus giving rise to the layers of mucin that overlie the epithelium throughout the small intestine and colon (see Chapter 14). Goblet cells may also be induced to secrete mucin in response to inflammatory stimuli. It can be difficult to recognize goblet cells after the mucin vacuole has been expelled; therefore, many inflammatory conditions are associated with an apparent loss of goblet cells, termed *mucin depletion*. Such goblet cell loss may also reflect an increased rate of cell turnover and incomplete differentiation in disease.

The intestine contains the largest mass of lymphoid tissue in the body (May et al., 1993). The gut-resident immune cells in the lamina propria are physically separated from the intraepithelial compartment by the basement membrane. One subset of dendritic cells ($CX3CR1^+$) located in the terminal ileum extends processes across the basement membrane and through the epithelial monolayer, where they may sample luminal antigens (see Chapter 26). However,

the primary sites of antigen sampling in the ileum occur in specialized lymphoid structures called Peyer's patches. Epithelial microfold (M) cells, which are present at the surfaces of Peyer's patches and isolated lymphoid follicles, can internalize luminal microorganisms and macromolecules (Figure 4(d)). These antigens may simply be transcytosed, or released at the basolateral surface, or processed and presented to lymphocytes. In this manner, M cells can be characterized as epithelial antigen-presenting cells (see Chapter 28).

EPITHELIAL POLARITY

Epithelial cells are uniquely positioned at the interface where self and non-self meets. In the lung, epithelial cells must separate the airways, and potential harmful materials within them, from the blood stream while allowing the free diffusion of oxygen and carbon dioxide. The intestine is an even more challenging environment, for in addition to preventing luminal toxins, microbiota, and microbial products from accessing deeper tissues, the intestinal epithelium must support vectorial, or directional, transport of nutrients, ions, and water. The development of epithelial polarity requires accurate delivery of specific transport proteins to either the apical surface (i.e., the surface in contact with the lumen) or the basolateral surface, which interfaces with the interstitium. These transporters are not only critical for promoting active transcellular transport, but also establish transepithelial gradients that provide the driving force for passive paracellular transport.

Epithelial polarity depends on several components, including (1) partitioning of the plasma membrane into separate apical and basolateral compartments; (2) appropriate specialization of these membrane domains (e.g., with apical microvilli (in the intestine), lateral desmosomes, and basal focal adhesions); (3) coordinated organization of intracellular compartments according to cell polarization; and (4) processes that ensure delivery of newly synthesized proteins and lipids to the correct plasma membrane domain as well as retrieval of incorrectly trafficked proteins and lipids. Many of these processes have been defined in detail and depend on several protein complexes that begin to segregate and establish separate domains as soon as two cells make contact (Shin et al., 2006; Schluter and Margolis, 2009).

The apical junctional complex is formed by the tight and adherens junctions and is closely associated with a ring of actin and myosin that forms a belt at the level of the epithelial apical junction complex. As discussed below, this perijunctional actomyosin ring is a critical regulator of paracellular barrier function. Since the tight junction is positioned where apical and basolateral domains meet, it was proposed that tight junctions had gate and fence functions, with the former referring to regulation of flux across the paracellular pathway and the latter referring to the ability to prevent mixing of membrane components (Mandel et al., 1993). However, more recent studies demonstrate that an intact tight junction is not required for the polarized distribution of membrane proteins (Umeda et al., 2006).

The adherens junction, which depends on homophilic E-cadherin-mediated intercellular interactions, is also linked to the perijunctional actomyosin ring. Loss of adherens junction function interferes with several cellular functions, including cell–cell and cell-matrix contacts, intercellular communication, and normal cellular polarization and differentiation. Furthermore, in the absence of the adherens junction, tight junction assembly and maintenance is disrupted, leading to profound paracellular barrier defects (Hermiston and Gordon, 1995a; Rajasekaran et al., 1996; Wu et al., 1998). Mice expressing a dominant-negative cadherin protein that disrupts the adherens junction not only demonstrate a loss of barrier integrity but also develop enteritis and epithelial dysplasia (Hermiston and Gordon, 1995b).

THE PARACELLULAR BARRIER

Although each cell within the epithelial monolayer forms a local barrier, the paracellular space between adjacent epithelial cells must also be sealed. This paracellular barrier function is regulated by the tight junction that is located at the most apical aspect of the basolateral membrane (Figure 5(a)). Transmission electron microscopy shows the tight junction as a site of very close apposition of adjacent cell membranes, whereas freeze-fracture electron microscopy shows a more dramatic view dominated by anastomosing strands arranged in series (Figure 5(b) and (c)). These strands are formed by tight junction transmembrane proteins and correspond to tight junction barrier properties. This is evident by the logarithmic relationship between the number of strands and paracellular permeability in different tissues. For example, tight junctions in the urinary bladder epithelium allow very little to cross the paracellular pathway, have transepithelial electrical resistances (TERs) of over $12{,}000\,\Omega\cdot cm^2$, and typically include at least nine strands. In contrast, the more permeable tight junctions of the proximal renal tubule and small intestine have only ~2 and ~5 strands and electrical resistances of 10 and $120\,\Omega\cdot cm^2$, respectively (Claude, 1978; Marcial et al., 1984). The stark differences in the paracellular permeability of tight junctions within different tissues directly reflect organ physiology, but they also have important experimental implications. Under most conditions, changes in TER primarily reflect altered tight junction permeability in "leaky" epithelia (e.g., proximal tubule and small intestine) because the vast majority of transepithelial ion flux is paracellular. In contrast, a

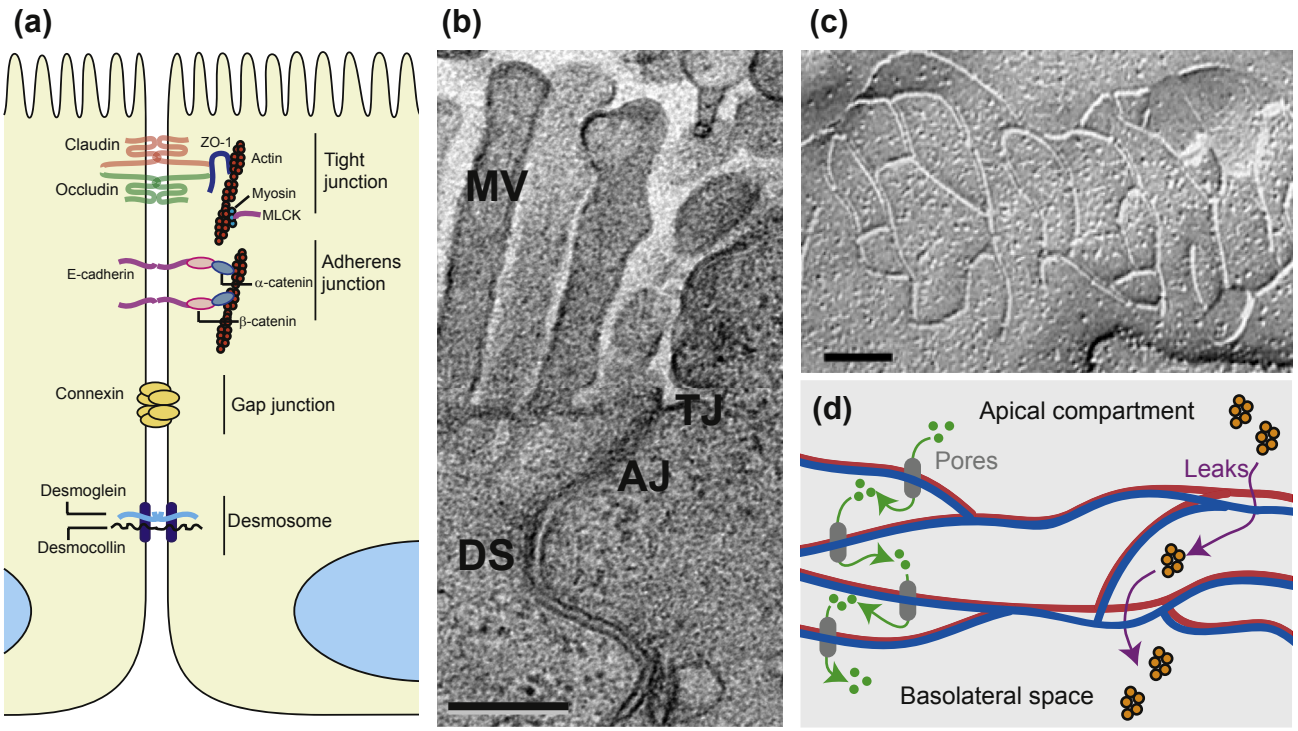

FIGURE 5 Epithelial junctional complexes. (a) Intercellular communication occurs at various junctional complexes between adjacent epithelial cells. The tight junction is made of several anastomosing strands containing transmembrane proteins that form the functional paracellular barrier. (b) Transmission electron micrograph. Scale bar = 250 nm. TJ, tight junction; AJ, adherens junction; DS, desmosome; MV, microvilli. Scale bar = 250 nm. (c) Freeze fracture electron micrograph of tight junction strands. Scale bar = 125 nm. (d) Pore and leak model of *trans*-tight junction paracellular transport.

smaller fraction of transepithelial ion flux is paracellular in "tight" epithelia (e.g., bladder), and TER reflects a combination of transcellular and paracellular ion flux. As discussed in greater detail below, tight junction permeability may also be characterized in terms of the relative permeability of materials of different sizes and charges, or size- and charge-selectivity. These properties vary between tissues and within different regions of individual tissues, such as the villus and crypt in the small intestine (Marcial et al., 1984). Insight into the molecular determinants and regulation of these properties has evolved rapidly over the last decade and will be discussed below.

Distinct Pathways across the Tight Junction Barrier

In the intestine, size selectivity of the tight junction varies along the crypt–villus axis (Fihn et al., 2000; Marcial et al., 1984). The upper portion of the villus allows solutes with radii up to ~6 Å to pass, whereas molecules with radii up to ~10 Å may cross tight junctions within the lower villus (Fihn et al., 2000). Permeability for even larger molecules with radii up to ~60 Å has been observed within the crypt (Fihn et al., 2000). This gradient of size selectivity correlates with the number of tight junction strands along the crypt–villus axis (Marcial et al., 1984).

Paracellular charge and size selectivity can be recapitulated in cell culture models by expressing different tight junction proteins or by treatment with different cytokines. For example, in vitro treatment of cultured intestinal epithelia with interferon-γ (IFNγ) or tumor necrosis factor (TNF) increases paracellular flux of large molecules (Weber et al., 2010; Watson et al., 2005b). Similar changes occur in the small intestine in vivo after acute systemic administration of recombinant TNF (Clayburgh et al., 2006). In contrast, interleukin (IL)-13 only increases paracellular flux of small cations and uncharged molecules in vitro and in vivo (Weber et al., 2010). On the basis of these characteristics of charge and size selectivity, a model was developed describing two distinct routes of *trans*-tight junction paracellular transport—the pore and leak pathways (Figure 5(d); Turner, 2009; Anderson and Van Itallie, 2009). The pore pathway is a high-capacity, charge-selective route that allows flux of a small, charged and uncharged molecules with radii less approximately 4 Å. The leak pathway is a low-capacity pathway that is relatively size and charge nonselective (Shen et al., 2011).

TRANSEPITHELIAL TRANSPORT

Transport across the epithelial monolayer is referred to as *transepithelial transport*. This can occur by transcellular or

paracellular pathways, with the sum of these representing total transepithelial transport.

Transcellular Transport

As noted above, vectorial transport across the single cell layer of the intestinal epithelium is dependent on the expression of specific transporters within the apical or basolateral membrane domains. This requires all cells within the epithelial layer to be polarized in the same direction. Nutrient absorption provides one example of the importance of polarized transport in the intestinal epithelium. Glucose within the intestinal lumen is initially transported across the apical membrane by the sodium glucose cotransporter (SGLT1), the genetic deficiency of which results in congenital glucose-galactose malabsorption (Wright, 1993). The driving force for this absorption is provided by the lumen to cytoplasm Na^+ gradient, in which two Na^+ molecules are absorbed for each glucose molecule transported (Figure 6(a)). Glucose then crosses the basolateral membrane via the facilitated transport protein (GLUT2) to allow bidirectional equilibration of glucose according to the concentration gradient. Na^+ is actively transported across the basolateral membrane by the Na^+/K^+-ATPase, the ultimate energy source for the entire process. If epithelial cells were randomly polarized with SGLT1, with GLUT2 and Na^+/K^+-ATPase delivered to the luminal surface of some cells and the basolateral surface of others, then individual cells would transport glucose in opposing directions. The net result would depend on the relative proportions of cells polarized in each direction and could potentially result in net glucose secretion into the lumen.

In contrast to Na^+, Cl^- ions are secreted into the lumen. In this case, transport across the basolateral membrane is dependent on cotransport by the $Na^+/K^+/Cl^-$ cotransporter 1 (NKCC1, or SLC12A2), primarily expressed in crypt epithelium. This electroneutral transporter moves one Na^+ ion, one K^+ ion, and two Cl^- ions into the cell and relies on the Na^+ gradient created by the basolateral Na^+/K^+-ATPase. This increases the intracellular Cl^- concentration and, when apical anion channels such as the cAMP-dependent cystic fibrosis transmembrane rectifier (CFTR) are opened, it allows Cl^- to flow into the lumen (Figure 6(c)). Less well-characterized calcium-activated apical chloride channels also exist. Water follows the transepithelial Cl^- gradient, and this secretion is necessary for hydration of luminal contents and digestion. Therefore, impaired secretion explains the presence of viscous airway mucus and increased frequency of intestinal obstruction in cystic fibrosis patients, who exhibit reduced CFTR function. Conversely, excessive CFTR activation results in massive water secretion, as occurs in cholera.

Unlike ions and nutrients, transcellular transport of large macromolecules does not depend on transmembrane channels. The well-studied example of this is immunoglobulin (Ig)-A transport, in which the IgA dimer produced by lamina propria plasma cells is bound at the basolateral surface by the polymeric Ig receptor, which directs endocytosis and vesicular trafficking of IgA to the apical surface. Proteolytic cleavage of the receptor allows release of secretory IgA into the lumen, where it can then bind to microbes or toxins to prevent pathogen-epithelial interactions (Kaetzel et al., 2014; Russell et al., 1981). However, data suggest that at some mucosal surfaces, pathogens can co-opt this machinery to transport from the lumen to the lamina propria (Zhang et al., 2000). The transport of IgG back to the lamina propria also occurs

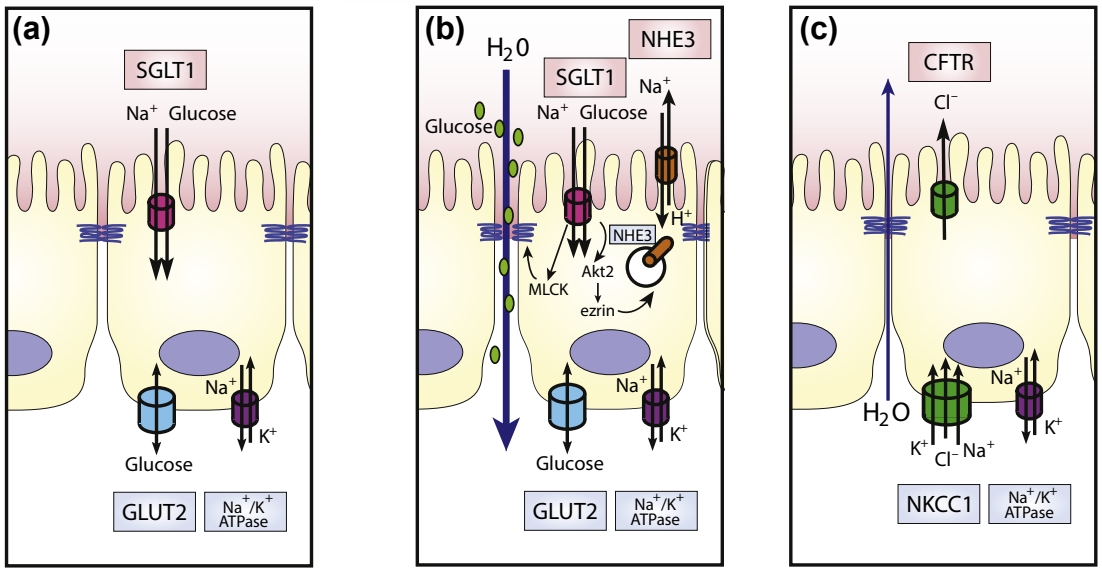

FIGURE 6 Transcellular transport across polarized epithelium. Na^+-glucose cotransport-dependent regulation of (a) transcellular and (b) paracellular glucose and water absorption. (c) Transcellular chloride transport promotes paracellular water secretion in small intestinal and colonic crypts.

via the neonatal Fc receptor, which facilitates absorption of maternal antibodies from breast milk (see Chapter 20).

Paracellular Transport

Paracellular transport refers to the passage of molecules between adjacent epithelial cells. The rate-limiting step in this process is transport across the tight junction. This makes the tight junction the primary determinant of paracellular permeability. However, two important qualifiers must be added to this simple statement. First, an immutable characteristic of paracellular transport is that it is passive; therefore, it depends entirely on local concentration gradients. Second, as discussed in greater detail below, processes independent of tight junction regulation can affect mucosal permeability. The most obvious of these is epithelial damage, including erosion or ulceration. In this case, epithelial cells have been lost and, as a result, neither tight junctions nor a functional epithelial barrier are present. Although this may be self-evident, it is important to keep this in mind when interpreting data because an alarming number of publications incorrectly attribute epithelial damage-associated barrier loss to tight junction regulation.

Regulation of epithelial tight junction permeability plays an important role in physiologic and pathologic transport at many mucosal surfaces. These have been characterized in the greatest detail within intestinal epithelia, which will serve as the primary example throughout the remainder of this chapter.

Physiological Integration of Transcellular and Paracellular Absorption

The first, and still best understood, example of integration between transcellular and paracellular transport is that triggered by intestinal Na^+-glucose cotransport. As described previously, Na^+-glucose cotransport across the apical brush border membrane is mediated by SGLT1. In addition to initiating transcellular nutrient absorption, this activates long myosin light chain kinase (MLCK), the MLCK isoform expressed in epithelial cells. Long MLCK then phosphorylates myosin II regulatory light chain to increase contractile activity of the perijunctional actomyosin ring (Turner et al., 1997; Madara and Pappenheimer, 1987), driving a size-selective increase in paracellular permeability that facilitates paracellular water flux (Turner et al., 1997; Meddings and Westergaard, 1989; Fihn et al., 2000). Na^+-glucose cotransport-dependent barrier regulation may also explain the observation that luminal glucose markedly enhances the response of sensitized animals to oral antigen challenge (Zhang and Castro, 1992).

The enhanced paracellular water flow induced by Na^+-glucose cotransport-dependent tight junction regulation is determined by the osmotic gradient created by transcellular Na^+ and glucose transport. This combination of paracellular water absorption and increased paracellular permeability also allows increased absorption of molecules with radii less than ~4 Å, such as glucose (Turner et al., 1997, 2000; Fihn et al., 2000). These small, nutrient-sized molecules are carried passively as solutes within water (i.e., the solvent); therefore, the process has been termed *solvent drag* (Atisook et al., 1990). In this manner, solvent drag allows for paracellular amplification of transcellular transport and likely explains the inability of excess luminal glucose to saturate intestinal absorption (Meddings and Westergaard, 1989).

In addition to enhancing paracellular permeability, Na^+-glucose cotransport also activates a mitogen-activated protein kinase-dependent signal transduction pathway in which MAPKAPK-2 phosphorylates p38 MAPK, which, in turn, activates Akt2 (Shiue et al., 2005). Akt2-mediated phosphorylation of the cytoskeletal linker protein ezrin then triggers vesicular trafficking of the apical Na^+-H^+ exchanger (NHE3) to the brush border (Zhao et al., 2004; Figure 6(b)). This increases transcellular Na^+ absorption that further enhances paracellular water absorption (Clayburgh et al., 2006; Lin et al., 2010). Thus, in addition to mediating transcellular Na^+-glucose cotransport, SGLT1-mediated transport initiates a series of signaling events that also increase transcellular Na^+ absorption and paracellular water and glucose absorption (Figure 6(b)). These mechanisms likely contribute to the clinical efficacy of Na^+- and carbohydrate-based oral rehydration solutions (Ramakrishna et al., 2000).

For the many Na^+-dependent intestinal absorptive mechanisms to proceed normally, large quantities of luminal Na^+ must be absorbed each day. However, the normal diet does not contain nearly enough Na^+ to support these processes. The recycling of absorbed Na^+ likely occurs in the proximal small intestine, where HCO_3^- undergoes active transcellular secretion and is followed by passive paracellular Na^+ and water and secretion. This paracellular Na^+ transport requires the tight junction proteins claudin-2 or claudin-15 (Wada et al., 2013), which, as discussed in the next section, form *trans*-tight junction Na^+ channels (Amasheh et al., 2002; Colegio et al., 2002). It is interesting to note that intestinal epithelial claudin-2 expression is high at birth but is then markedly reduced, whereas claudin-15 expression is low at birth and only increases later (Holmes et al., 2006). Thus, although adult claudin-2-deficient mice are phenotypically normal, claudin-15-knockout mice display Na^+ deficiency and glucose malabsorption in adulthood (Tamura et al., 2011). Mice lacking claudin-2 and claudin-15 die of malabsorption in the early perinatal period (Wada et al., 2013). This essential physiology may provide insight into the marked claudin-2 upregulation present in many intestinal diseases, as discussed in the next section.

Cytokines Co-Opt Physiological Mechanisms to Modulate Transcellular and Paracellular Transport

Many diseases can be simply explained as defects in normal regulatory processes, and immune-mediated diarrheal disease is no different. For example, in a manner similar

to Na^+-glucose cotransport-dependent barrier regulation, long MLCK-dependent myosin II regulatory light chain phosphorylation is required for acute, TNF-induced tight junction permeability increases as well as diarrhea (Figure 7; Clayburgh et al., 2005; Zolotarevsky et al., 2002). Likewise, IL-1β and lymphotoxin-like inducible protein that competes with glycoprotein D for herpes virus entry mediator on T cells (LIGHT) are also able to drive acute, MLCK-dependent increases in tight junction permeability (Schwarz et al., 2007; Al-Sadi et al., 2010). However, unlike acute, systemic administration of TNF, LIGHT administration does not cause diarrhea (Clayburgh et al., 2006). This difference between LIGHT and TNF can be explained by the observation that, in addition to activating MLCK, TNF triggers protein kinase Cα-dependent endocytosis of the apical NHE3. This inhibits NHE3-dependent transcellular Na^+ absorption and reduces the transepithelial Na^+ gradient, which provides the driving force for paracellular water absorption. In the absence of this gradient, water moves in the opposite direction, or into the lumen. In contrast to TNF, LIGHT does not inhibit NHE3. This allows maintenance of the transepithelial Na^+ gradient under conditions of increased tight junction permeability and explains the slight increase in intestinal water absorption induced by systemic LIGHT administration (Clayburgh et al., 2006). This difference also suggests that the bulk of intestinal transepithelial water absorption occurs by the paracellular route. This is different from transporting epithelia, such as the kidney and salivary gland, where transcellular water transport requires expression of aquaporins, a family of transmembrane water channels.

MOLECULAR ARCHITECTURE OF TIGHT JUNCTIONS

More than 60 integral and peripheral membrane proteins are concentrated at the tight junction (Raleigh et al., 2010; Anderson et al., 2004; Van Itallie and Anderson, 2006; Furuse et al., 1993, 1998a; Ikenouchi et al., 2005; Stevenson et al., 1986). Analyses of the multiple interactions between these proteins led to the hypothesis that extensive protein cross-linking would result in the formation of a large, but static, tight junction complex (Itoh et al., 1999; Fanning et al., 1998, 2007a,b; Nomme et al., 2011; Piontek et al., 2011; Sasaki et al., 2003). However, studies analyzing the mobility of fluorescently tagged tight junction proteins demonstrated that the binding interactions between different tight junction proteins were highly dynamic, even at steady state (Shen et al., 2008). These and other data have resulted in new models of tight junction structure, in which regulation of binding affinities between different tight junction proteins defines paracellular permeability (Shen et al., 2011; Raleigh et al., 2011; Yu et al., 2010; Van Itallie et al., 2012; Piontek et al., 2008).

Claudins as Determinants of Paracellular Ion Flux

One main component of tight junction strands includes members of the claudin family (Furuse et al., 1998a,b; Mineta et al., 2011). Although 27 claudin genes have been identified (Mineta et al., 2011), only a few are expressed in any individual tissue. Claudin expression is modified

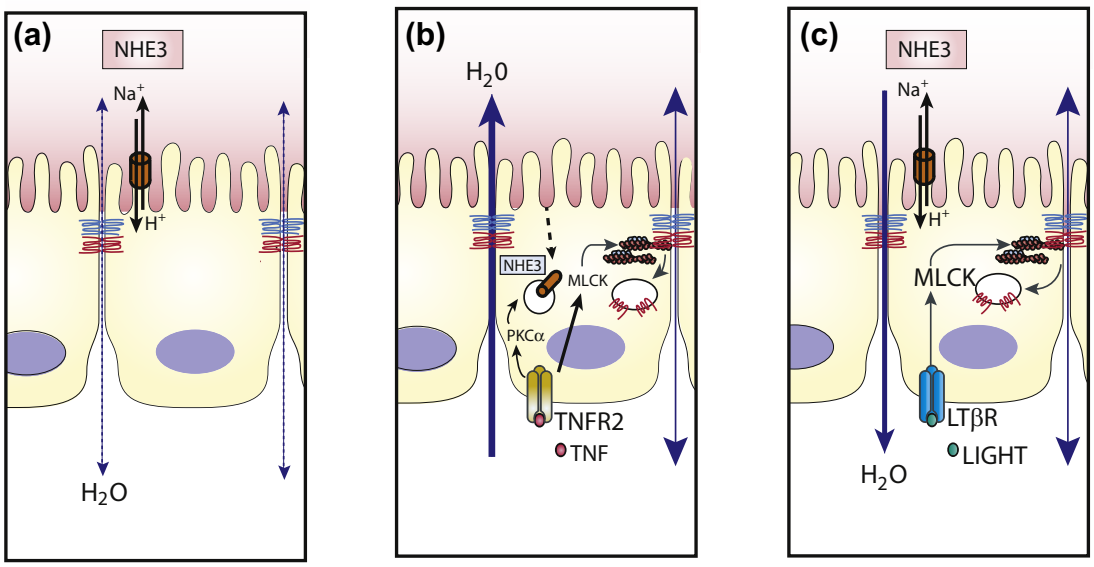

FIGURE 7 Cytokine-induced alterations to epithelial paracellular transport. (a) Normal physiological conditions. (b and c) TNF and LIGHT promote increased permeability through MLCK-induced activation of the leak pathway and occluding internalization; however, TNF also induces protein kinase-Cα-mediated internalization of NHE3, resulting in increased water secretion in the lumen. In contrast, intact NHE3-mediated Na^+ absorption results in increased net water absorption after exposure to LIGHT.

normally during development and in response to pathogenic stimuli (Holmes et al., 2006; Weber et al., 2008, 2010; Zeissig et al., 2007; Heller et al., 2005; Tamagawa et al., 2003; Kitajiri et al., 2004b; Krause et al., 2008). As described below, these changes directly affect paracellular flux of ions and small molecules.

Structurally, claudins have a tetraspanning structure in which the transmembrane regions and extracellular loops are highly conserved. In contrast, the short amino and carboxy terminal cytoplasmic tails are divergent. Despite this divergence, the carboxy terminus of most claudins includes a PDZ-binding motif that interacts with the first PDZ domain of zonula occludens-1 (ZO-1). The extracellular loop closest to the carboxy terminus, the second extracellular loop, is also thought to contribute to interprotein interactions, including those with claudins on adjacent cells (Piehl et al., 2010; Blasig et al., 2006; Krause et al., 2009; Robertson et al., 2010).

The amino terminal first extracellular loop of claudin proteins defines paracellular charge selectivity. The best characterized in this regard is claudin-2, which forms a channel that allows paracellular flux of small cations, primarily Na^+ and K^+ (Angelow and Yu, 2009; Yu et al., 2009). It is also clear that reversal of charge at specific residues within the first extracellular loop can change paracellular permeability from cation to anion selective and vice versa (Colegio et al., 2003, 2002). However, much about the molecular function of claudin proteins remains to be defined. For example, although it is clear that claudins must be present on both adjacent cells, the manner in which they join to form a paracellular pore, or even the stoichiometry of this interaction, remains unknown.

Most studies exploring claudin function have relied on knockout mice or cultured cell lines after transgenic expression or small interfering RNA knockdown of specific wild-type or mutant claudin proteins (Table 1). These experiments may be misleading since they interpret global changes in epithelial resistance or charge selectivity as an indication of individual claudin function. For example, claudin-4 expression is known to reduce paracellular Na^+ conductance, which has led some to speculate that rather than forming channels, claudin-4 forms the tight junction barrier. Although this remains a possibility, it is also possible that claudin-4 reduces paracellular Na^+ conductance by disrupting claudin-2 pore function due to displacement of claudin-2 from the tight junction. Likewise, the phenotypes of claudin-1 and claudin-5 knockout mice, which have defective epidermal and blood–brain barrier function (Furuse et al., 2002; Nitta et al., 2003), respectively, may reflect the important role of claudins in promoting tight junction assembly and the dominance of these particular claudins in those tissues (Table 1). Thus, when interpreting stimulus-induced changes in claudin expression, it is critical to consider the effect on expression of each protein relative to the other claudins present in that tissue, not only changes in expression relative to the control condition. It is important to note that claudin function can be regulated by expression or post-transcription mechanisms; therefore, this caveat holds even greater relevance when only mRNA expression is assessed.

Intestinal epithelial claudin-2 expression can be upregulated in vitro by IL-6, IL-13, and IL-17 (Heller et al., 2005; Weber et al., 2010; Suzuki et al., 2011; Kinugasa et al., 2000),

TABLE 1 Phenotype of Tight Junction Protein Knockout or Transgenic Mice

Tight Junction Protein	Phenotype of Knockout and/or Transgenic Mice
Claudins	CLDN1, CLDN6 Tg: skin barrier defect (Turksen and Troy, 2002; Furuse et al., 2002) CLDN2 KO: subtle renal defects (Muto et al., 2010) CLDN5 KO: blood–brain barrier defect (Nitta et al., 2003) CLDN11 KO: central nervous system myelin defect, loss of endocochlear potential (Gow et al., 1999) CLDN14 KO: phenocopy of human deafness (Kitajiri et al., 2004a; Ben-Yosef et al., 2003) CLDN15 KO: megaintestine (Tamura et al., 2008) CLDN2/CLDN15 KO: death by malnutrition (Wada et al., 2013) CLDN16 KD: phenocopy of familial hypomagnesmia (Mayer et al., 1991; Burton et al., 2007) CLDN19 KO: Schwann cell barrier defect (Miyamoto et al., 2005)
Occludin	KO: Viable with complex phenotype (Saitou et al., 2000), but no intestinal paracellular defect in unchallenged mice (Schulzke et al., 2005). Tg: Partially protected from TNF-induced intestinal barrier loss (Marchiando et al., 2010).
Junctional adhesion molecules (JAMs)	KO: Reduced barrier function, increased intestinal epithelial apoptosis and proliferation (Laukoetter et al., 2007; Vetrano et al., 2008)
Coxsackie adenovirus receptor (CAR)	Embryonic lethal due to cardiac defects (Marano et al., 1993)
Zonula occludens (ZO-1) ZO-2 ZO-3	ZO-1 KO: embryonic lethal E10.5 (Katsuno et al., 2008) ZO-1 Tg: no reported phenotype (Marchiando et al., 2010). ZO-2 KO: embryonic lethal (Xu et al., 2008) ZO-3 KO: no obvious phenotype (Adachi et al., 2006)

and in vivo studies have confirmed these findings using systemically administered IL-13 (Weber et al., 2010). IL-13-induced claudin-2 upregulation enhances paracellular flux of small cations, primarily Na^+, resulting in reduced transepithelial resistance and increased Na^+:Cl^- permeability ratio, without increasing the paracellular flux of larger molecules (Weber et al., 2010). Small interfering RNA-mediated inhibition of claudin-2 after IL-13 treatment completely prevented Na^+:Cl^- permeability ratio increases, thus demonstrating that IL-13 induces a cation-selective increase in pore pathway permeability by upregulating claudin-2. In addition, claudin-2 pores have also been shown to facilitate paracellular water transport (Rosenthal et al., 2010). This may indicate that the increased claudin-2 expression associated with colitis enhances paracellular Na^+ and water flux to promote diarrhea.

Occludin and the Tight Junction-Associated MARVEL Proteins

The first tight junction-associated MARVEL protein (TAMP) identified was occludin (Furuse et al., 1993). Although there is no sequence homology between occludin and claudins, occludin contains a conserved tetraspanning MARVEL (MAL and related proteins for vesicle trafficking and membrane link) domain that is present in proteins concentrated in cholesterol-rich microdomains and involved in membrane apposition (Sanchez-Pulido et al., 2002). MARVEL domains are also found in tricellulin, which is concentrated at tricellular tight junction regions (Ikenouchi et al., 2005), and marvelD3 (Raleigh et al., 2010; Steed et al., 2009). As a group, these proteins make up the TAMP family (Raleigh et al., 2010). The functions of these proteins remain incompletely defined. However, the involvement of tricellulin in human autosomal-recessive hearing loss (Riazuddin et al., 2006; Chishti et al., 2008) and the sterility of occludin knockout male mice (Saitou et al., 2000) suggest that they play essential roles in forming barriers in some tissues. Nevertheless, gut, renal, and pulmonary functions are essentially normal in occludin knockout mice under nonstressed conditions (Saitou et al., 2000; Schulzke et al., 2005). This may reflect a partial compensation of TAMPs for one another; for example, occludin loss causes tricellulin to be redistributed to bicellular tight junction regions (Ikenouchi et al., 2008; Raleigh et al., 2010; Westphal et al., 2010).

It remains possible that the primary function of occludin relates to tight junction regulation rather than assembly. If true, then this would explain the absence of an intestinal phenotype in occludin knockout mice under normal housing conditions (Table 1). In vitro data support this hypothesis (Yu et al., 2005; Al-Sadi et al., 2011; Shen and Turner, 2005; Kuwabara et al., 2001; Huber et al., 2000; Balda et al., 2000), but until recently, in vivo data were lacking. Occludin endocytosis is closely associated with TNF-induced, long MLCK-dependent intestinal barrier loss (Clayburgh et al., 2005). Further analysis of tissue sections and real-time, intravital imaging of transgenic mice expressing enhanced green fluorescent protein occludin demonstrated that TNF-induced occludin internalization requires caveolin-1 (Marchiando et al., 2010). Furthermore, caveolin-1-deficient mice were protected from TNF-induced barrier loss and diarrhea (Marchiando et al., 2010). Intestinal epithelial-specific occludin overexpression also limited acute TNF-induced barrier loss and prevented diarrhea, indicating that occludin is an essential functional regulator of tight junction permeability.

In contrast to IL-13-induced claudin-2 expression, TNF causes a size- and charge-nonselective increase in paracellular permeability (Weber et al., 2010; Clayburgh et al., 2005). Similar increases in macromolecular paracellular permeability can be induced by occludin knockdown in vitro and in vivo (Yu et al., 2005; Al-Sadi et al., 2011). This may explain the observation that TNF cannot induce further barrier loss in occludin knockdown cell lines (Van Itallie et al., 2010).

ZO-1 as a Prototypic Molecular Integrator

The peripheral membrane protein ZO-1 was the first tight junction protein identified (Stevenson et al., 1986). However, dissection of specific ZO-1 contributions to barrier function has begun only recently. During epithelial polarization, ZO-1 is initially recruited to the nascent adherens junction, likely via ZO-1 U5-GuK region-dependent binding to α-catenin (Ando-Akatsuka et al., 1999; Baas et al., 2004). Occludin is then trafficked to these sites of enrichment, likely by direct interaction between the U5-GuK region in the middle of ZO-1 and the occludin coiled-coil ELL domain near the occludin carboxy-terminus (Fanning et al., 1998, 2007a; Li et al., 2005). Claudin proteins are then recruited by a mechanism dependent on interactions between the amino-terminal ZO-1 PDZ1 domain and the carboxy-terminal claudin PDZ-binding motif (Umeda et al., 2006; Itoh et al., 1999). However, details of these processes are emerging slowly, reflecting the complexity of ZO-1 interactions (Fanning and Anderson, 2009). For example, ZO-1 is also able to bind to the related proteins ZO-2 and ZO-3 through PDZ domain 2 (Fanning et al., 2007b), junctional adhesion molecule-A (JAM-A) through PDZ domain 3, F-actin through the actin binding region (ABR) in the C-terminal half of ZO-1 (Fanning et al., 2002), and various other cytoskeletal and regulatory proteins (Fanning and Anderson, 2009). Thus, simple ZO-1 knockdown provides limited information because it eliminates all of these interactions (Umeda et al., 2004, 2006; Fanning et al., 2012; Yamazaki et al., 2008).

Despite all of the protein interactions mediated by ZO-1, knockdown in cultured epithelial cells does not result in a

dramatic loss of transepithelial resistance, possibly because of functional redundancy with ZO-2 and ZO-3 (Umeda et al., 2004, 2006; Fanning et al., 2012; Yamazaki et al., 2008; Yu et al., 2010). However, ZO-1 knockdown does result in increased leak pathway flux (Van Itallie et al., 2009), and there is evidence that ZO-1 binding interactions are critical to tight junction regulation. For example, the ABR stabilizes ZO-1 at the tight junction after MLCK inhibition (Yu et al., 2010). ABR expressed as a soluble protein behaved in a dominant negative fashion to block the effects of MLCK inhibition on ZO-1 stability at the tight junction and barrier function. Thus, MLCK-dependent increases in tight junction permeability partly depend on release of ABR-dependent interactions that anchor ZO-1 at the tight junction.

ZO-1 has also been shown to play a critical role in regulation of the pore pathway by linking occludin to claudin-2 (Figure 8; Raleigh et al., 2011). The kinase CK2 phosphorylates occludin at serine 408, just proximal to the C-terminal occludin coiled-coil ELL domain (Smales et al., 2003). This induces formation of occludin homodimers, which diffuse freely at the tight junction. CK2 inhibition and serine 408 dephosphorylation cause homodimer dissociation. This promotes occludin binding to ZO-1 as well as ZO-1 binding to claudin-2 (Raleigh et al., 2011). Although these occludin:ZO-1:claudin-2 complexes diffuse less freely than occludin homodimers, they are more mobile than claudin-2 complexes. This reduced anchoring at the tight junction interferes with claudin-2 pore function and reduces paracellular Na^+ permeability. Thus, CK2-dependent occludin serine 408 phosphorylation acts as a molecular switch that, via ZO-1, regulates claudin-2 pore function (Raleigh et al., 2011). This information may be useful therapeutically as pharmacological CK2 inhibition, inhibited claudin-2 pore function, and corrected IL-13-induced barrier loss (Raleigh et al., 2011). Phosphorylation and ubiquitination of additional sites in the occludin C-terminal tail can also regulate occludin trafficking, interactions with ZO-1, and tight junction barrier function (Cummins, 2012; Huppert et al., 2010; Suzuki et al., 2009; Elias et al., 2009; Seth et al., 2007; Andreeva et al., 2001; Wong, 1997; Murakami et al., 2009).

JAM-A and Coxsackie Adenovirus Receptor: Ig-Like Tight Junction Proteins

Many other transmembrane proteins are located within the tight junction, including JAM. JAMs are differentially

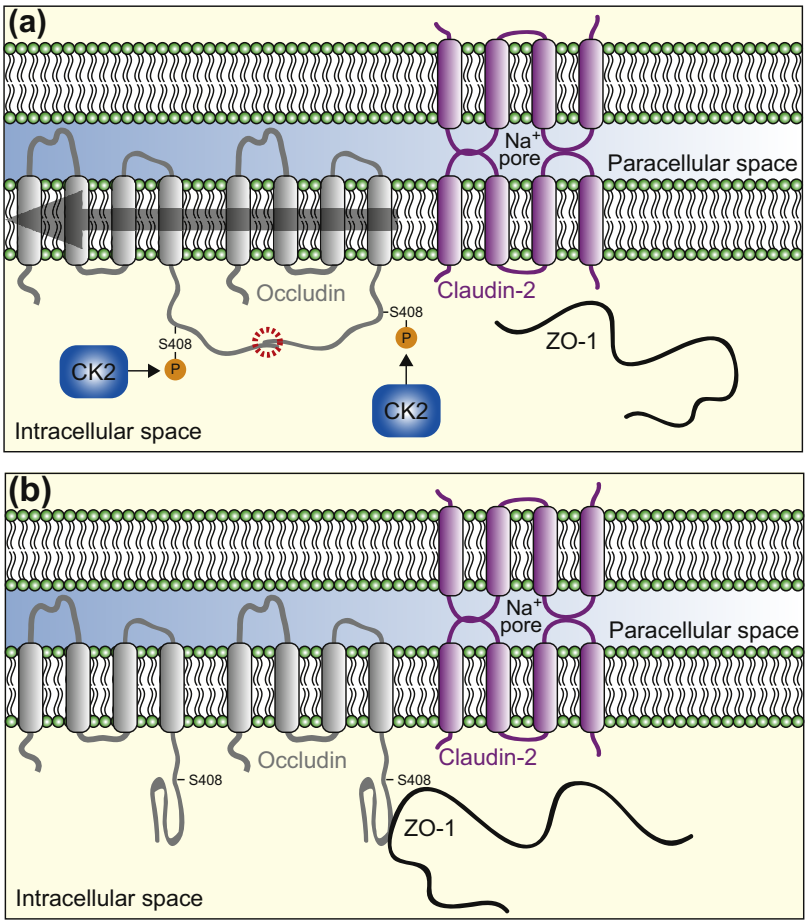

FIGURE 8 Occludin serine 408 phosphorylation in tight junction regulation. (a) The kinase CK2 phosphorylates occluding at serine 408 to induce formation of occluding homodimers. (b) CK2 inhibition and serine 408 dephosphorylation results in homodimer dissociation and promotes occluding binding to ZO-1. From Raleigh et al. (2011).

expressed on endothelia, epithelia, and immune cells (Martin-Padura et al., 1998), and they can mediate direct binding interactions between immune cells and either endothelial or epithelial cells (Ostermann et al., 2002). Although JAM-A has been implicated in the regulation of epithelial barrier function, it also contributes to cell polarity, migration, adhesion, and leukocyte migration (Ebnet et al., 2003; Martin-Padura et al., 1998; Ozaki et al., 1999). Further, JAM-A knockout mice exhibit reduced barrier function and increased enterocyte proliferation and apoptosis (Table 1; Vetrano et al., 2008; Laukoetter et al., 2007), indicating that JAM-A likely affects downstream signaling pathways in addition to its role at the tight junction. Therefore, it is difficult to determine whether JAM-A deletion results in altered tight junction integrity or if loss of barrier integrity is secondary to disruption of other JAM-A-mediated cellular processes.

Coxsackie adenovirus receptor (CAR), which mediates viral attachment and infection, is an integral membrane protein belonging to the Ig superfamily (Bergelson et al., 1997). CAR localizes to the epithelial tight junction (Cohen et al., 2001), likely by binding to the PDZ domain, which is found in the proteins PSD-95, Discs-large, and ZO-1. These domains that are specialized for protein–protein interactions are discussed in Kennedy (1995), and they are domains within tight junction proteins (Coyne et al., 2004). CAR also mediates migration of immune cells across endothelial or epithelial barriers (Witherden et al., 2010; Luissint et al., 2008), in part because it can serve as an epithelial receptor for JAM-like protein, which is expressed by neutrophils and, to a lesser extent, by monocytes and T cells (Luissint et al., 2008).

MEASURING BARRIER FUNCTION

In Vivo Measures of Barrier Function

Intestinal permeability is most commonly measured as the fractional urinary excretion of orally ingested probes. The probes selected must not be able to cross the epithelium by a transcellular route. Thus, probe entry into the bloodstream represents paracellular flux. Because the probes selected are not significantly metabolized in the blood stream, they persist and are freely filtered at the glomerulus. They then enter the urinary space and, via the renal tubules, arrive in the bladder. However, there are several important caveats to this approach. First, the probes must be selected carefully because some can be broken down by digestive enzymes or colonic bacteria (Meddings and Gibbons, 1998). Intestinal motility must also be considered because transit time affects the duration over which the probe is in contact with and can be transported across the epithelium (Camilleri et al., 2012; Rao et al., 2011). It is also important to consider that all of these probes can easily cross areas of damage, where epithelial cells, along with their intercellular junctions, are lost. In these cases, increases in absorption of the probe may not indicate changes in tight junction permeability.

Once probes enter the blood stream, they must be efficiently filtered into the urinary space without undergoing significant metabolism. The probes used to measure paracellular flux are generally unable to undergo metabolism, but this can change in certain clinical states, such as sepsis. In addition, fractional urinary recovery cannot be assessed reliably in subjects with altered glomerular filtration rates; therefore, these assays may not be useful in patients with renal disease.

Various probes have been used in an effort to overcome the limitations noted above. For measurements of small intestinal permeability, the lactulose:mannitol ratio has been most popular. Lactulose is large enough that it can only cross via the paracellular leak pathway or at sites of epithelial damage, and it can be thought of as measure of barrier integrity. Mannitol, which is 3 times smaller, with a radius of only approximately ~3.5 Å, crosses the pore pathway and can be thought of as an indicator that integrates surface area and exposure time. Thus, the lactulose:mannitol ratio can be interpreted as a unitless measure of the sum of leak pathway permeability and epithelial damage normalized to surface area. Because both probes are affected identically by changes in intestinal motility, this is not usually a complicating factor in lactulose:mannitol ratio measurements. However, lactulose and mannitol are degraded by colonic bacteria (Meddings and Gibbons, 1998), which makes them unsuitable for measures of colonic permeability. Sucralose and polyethlylene glycols have been used as probes that overcome the issue of bacterial degradation and can be used to measure colonic permeability (Meddings and Gibbons, 1998; Bjarnason et al., 1995). However, both can also be absorbed in the small intestine; therefore, any data obtained reflect a combination of small intestinal and colonic permeability. Polyethylene glycol also interacts with cell surfaces (Wu et al., 2004; Ramanathan et al., 2001) and does not always correlate with measures made using other probes (Chadwick et al., 1977; Olaison et al., 1988; Johansen et al., 1989). The use of other probes, including Cr^{51}-EDTA (Bjarnason et al., 1983, 1995) and creatinine (Turner et al., 2000), may simplify the sometimes complex task of measuring urinary probe concentration. However, Cr^{51}-EDTA has only been used by a few groups and, because it relies on radioisotopes, it is not suitable for many human studies. In one study using mice, fractional urinary recovery of creatinine was found to correlate closely with that of sucralose (Su et al., 2009). This suggests that in subjects without disease-related increases in serum creatinine, oral creatinine might be an easily measured probe of colonic permeability. However, further study is needed before it can be considered a standard assay.

Although collecting urine over time allows integration of recovery and is convenient in human subjects, the use of metabolic cages to collect urine in mice is labor-intensive

and time-consuming. Thus, many studies have measured serum recovery of the probes above as well as fluorescent-conjugated dextrans. This approach avoids potential artifacts of renal disease, but it has serious limitations to consider, including the measurement of a snapshot in time rather than integrated total absorption. In addition, most dextran probes are large enough that they generally reflect barrier defects due to intestinal damage (i.e., erosions) and are less informative as measures of tight junction permeability. Dextran uptake by the reticuloendothelial system is another potential source of artifact.

Lastly, the route of dextran administration is also an important consideration when selecting a measurement of epithelial permeability. Administration of dextran probes by oral gavage reduces potential artifacts that could be generated by using dextran enemas. For example, rectal instillation may cause unintentional damage to the colonic epithelium, resulting in a false-positive result. It is also difficult to ensure rectal instillation of the same volume of dextran between individual mice.

Thus, no perfect approach to in vivo analysis of intestinal permeability is available. At best, the standard approaches provide little indication of charge or size selectivity and cannot distinguish between increased tight junction permeability and epithelial cell damage. These shortcomings emphasize the need for development of improved assays of barrier function.

EX VIVO AND IN VITRO MEASURES OF BARRIER FUNCTION

Often, measurements of barrier function are reported using cultured epithelial monolayers or in tissue ex vivo. Intestinal epithelial permeability through either the pore or leak pathway can be assessed using several complementary methods (Figure 9). Currently, the methodology does not exist to measure local tight junction permeability; therefore, all measurements are averages of a global population of pore opening and closing events. Despite this, global measurements are useful for understanding the permeability properties of the tight junction.

Perhaps the most commonly used electrophysiological approach is measurement of TER, which measures the flux of all ions across the epithelium. Simply, the electrical potential across the epithelium is measured in the presence or absence of an applied current. These data are then used to calculate resistance using Ohm's law. The current is carried by the most common ions in physiological solutions, Na^+ and Cl^-. These ions are small and can cross the paracellular

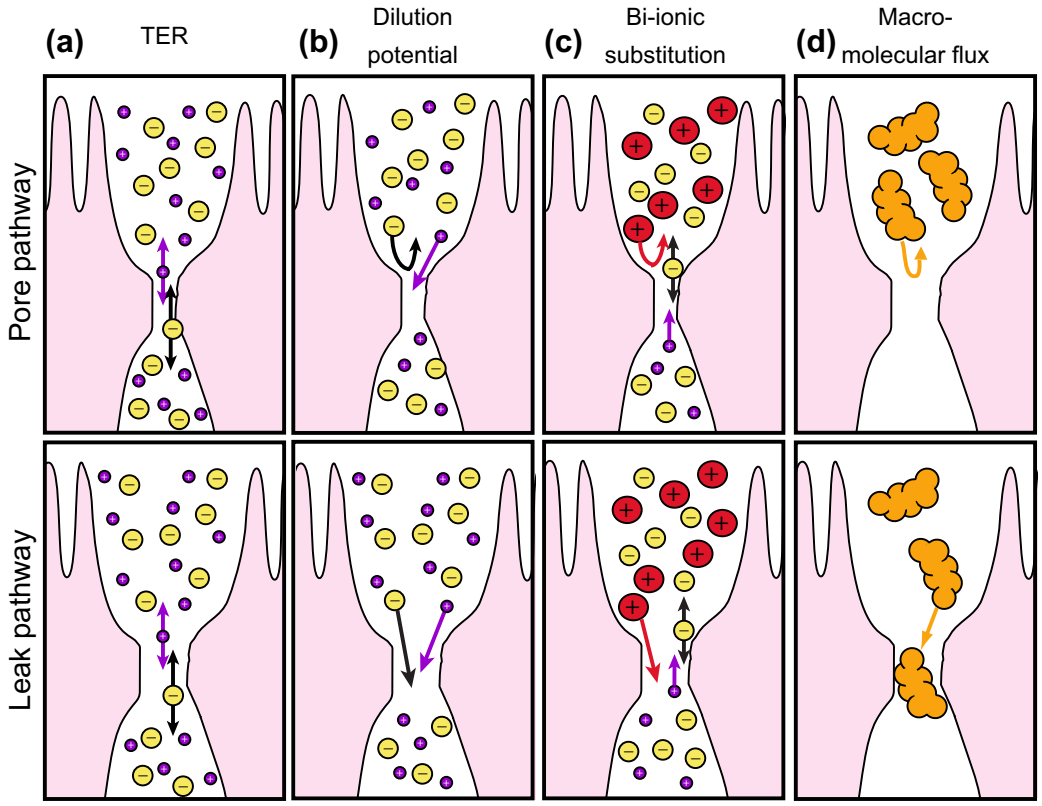

FIGURE 9 Measurements of epithelial permeability. Global measurements of pore opening and closing events provide information regarding the permeability properties of the tight junction. (a) TER measures the flux of all ions across the epithelium. (b) Measurement of the dilution potential shows changes in charge selectivity of the tight junction. (c) Bi-ionic substitution assesses size selectivity of the pore pathway. (d) Macromolecular flux of tracers across the epithelium assesses permeability through the leak pathway. *Adapted from Shen et al. (2011).*

space by either the pore or leak pathway; therefore, increased permeability through either pathway reduces TER.

There are several simple devices that can be used to measure the TER of cultured cells grown on semipermeable inserts with varying degrees of accuracy and reproducibility. Tissues are generally analyzed ex vivo using Ussing chambers or similar apparati. The use of Ussing chambers is technically challenging in that both the underlying muscle layer must be stripped from the epithelium to obtain meaningful data. Furthermore, the tissue becomes anoxic immediately after removal from the animal, thus making it difficult to assess barrier function over time because of the continuous deterioration of the tissue. Anoxia also occurs when assessing permeability in intestinal loops or by other methods in which portions of intestine are tied off at the ends, thus allowing luminal fluid accumulation to compress veins, thus blocking venous drainage preventing arterial blood flow and triggering ischemic tissue damage.

To determine changes in charge selectivity of the tight junction, the dilution potential is measured by inducing a transepithelial electrochemical gradient. By iso-osmotically altering the apical or basolateral NaCl concentration, a new equilibrium potential can be established based on the relative permeabilities of Na^+ and Cl^- (Figure 9). Alternatively, bi-ionic substitution assesses size selectivity of the pore pathway by replacing Na^+ on one side of the monolayer with various-sized organic cations, thus allowing measurement of the permeability of the different cations. Permeability through the leak pathway can be determined by the macromolecular flux of tracers across the epithelium, such as those described above for in vivo studies. The large size of these tracers allows specificity for the leak pathway, although some polyethylene glycols are small enough to traverse the pore pathway. Although these methods have been widely used on cultured epithelial monolayers and tissue, bi-ionic substitution has not yet been reported in tissue. Lastly, it is important to note that although the number of tight junction strands correlates with the permeability of individual tissues (Marcial et al., 1984), analysis of morphometric changes in the tight junction by transmission electron microscopy have not been validated as measures of permeability.

BARRIER FUNCTION AND DISEASE PATHOGENESIS

Intestinal epithelial barrier loss has been implicated in several immune-mediated diseases, including IBD, celiac disease, type I diabetes mellitus, multiple sclerosis, rheumatoid arthritis, and graft-versus-host disease. Other disease associations include irritable bowel syndrome, alcoholic cirrhosis, and acquired immunodeficiency syndrome. In general, the available data on barrier function are correlative and do not provide a detailed mechanistic understanding of the role of barrier loss in these diseases. Thus, questions of cause versus effect remain. Nevertheless, the strongest case for a pathogenic role of intestinal barrier loss in humans has been made for Crohn's disease, in which some healthy first-degree relatives of Crohn's disease patients exhibit increased intestinal permeability compared with unrelated control subjects (Hollander et al., 1986; May et al., 1992). Remarkably, subsequent studies showed that a subset of the healthy relatives with increased permeability tended to carry the 3020insC mutant of NOD2, and many also had the R702W mutation (Buhner et al., 2006). This raises the possibility that the increased permeability occurring in these healthy subjects reflects mild, subclinical immune activation and is consistent with the data above showing that the intestinal epithelial tight junction is exquisitely sensitive to regulation by several cytokines. Unfortunately, it remains unknown whether the healthy relatives with increased permeability are at greater risk of developing disease than the healthy relatives with normal intestinal permeability. This issue was addressed tangentially in a case report documenting the development of disease in a previously healthy relative with increased permeability (Irvine and Marshall, 2000). However, the patient in question had a mother and brother with Crohn's disease, thus elevating her risk and making it impossible to determine if disease development was related to the observed increase in intestinal permeability.

Maintenance or restoration of intestinal barrier function may also be important in Crohn's disease patients during remission. Two studies have shown that increased intestinal permeability is a strong risk factor for relapse into active disease (Wyatt et al., 1993; D'Inca et al., 1999). It is interesting to note that both of these studies found that permeability was increased at least 6 months before relapse in many patients. These data can be interpreted as evidence of a low-grade inflammatory state that reduces barrier function; alternatively, they could indicate that barrier loss promotes disease reactivation over extended intervals. Identification of the triggers for this increased permeability could help to resolve this issue. Nevertheless, it is notable that stress has been associated with relapse in Crohn's disease (Bitton et al., 2008) and can also induce intestinal barrier loss in rodents (Meddings and Swain, 2000). Finally, it is important to recognize that the presence of reduced barrier function in some healthy relatives of Crohn's disease patients indicates that tight junction barrier loss alone is insufficient to cause Crohn's or any other disease.

In addition to measuring changes in permeability, ultrastructural alterations of the tight junction have been observed in Crohn's disease and ulcerative colitis. Scanning, freeze fracture, and transmission electron microscopy revealed a reduced number of tight junction contacts and

disruption of the normal tight junction anastomosing strand pattern in diseased intestine (Marin et al., 1983a,b; Schmitz et al., 1999). Together with the functional correlations above, these data strongly suggest a link between loss of an intact barrier and IBD; however, whether an innate barrier defect promotes disease development or simply results from continuous activation of mucosal immunity remains unknown.

MODELS LINKING INTESTINAL BARRIER LOSS TO EXPERIMENTAL IBD

Several studies have addressed the contribution of intestinal epithelial barrier dysfunction to disease. The first among these used chimeric mice in which some intestinal epithelial cells expressed a dominant-negative N-cadherin transgene to inhibit E-cadherin function (Hermiston and Gordon, 1995a). Because E-cadherin is necessary for the assembly of adherens junctions, and in turn, adherens junctions are required for tight junction assembly and maintenance, these mice exhibited profound epithelial abnormalities (Hermiston and Gordon, 1995a). Mice expressing dominant-negative N-cadherin also developed spontaneous small intestinal inflammation and dysplasia (Hermiston and Gordon, 1995b). The latter is a marker of neoplastic transformation and is a known complication of human IBD (Leedham et al., 2009). However, the dysplasia likely reflects constitutive activation of the α-catenin/antigen-presenting cell signaling pathway in these mice. Dominant-negative N-cadherin expression also disrupted cell–cell contacts and interactions with the basement membrane. Furthermore, expression of the transgene disrupted epithelial polarization, increased migration along the crypt–villus axis, and triggered premature epithelial apoptosis (Hermiston and Gordon, 1995a). Taken together, dominant-negative N-cadherin expression created global defects that extended well beyond tight junction barrier loss. It is notable that E-cadherin, encoded by *cdh1*, has been linked to ulcerative colitis in genome-wide association studies (Barrett et al., 2009).

In contrast to the dominant-negative N-cadherin transgenic mice, most studies have found that knockout of genes encoding junctional proteins results in findings at two phenotypic extremes. Either the defects are so severe that genetic deletion causes embryonic lethality or loss of a specific junction protein does not induce obvious changes in unchallenged mice (Table 1; Saitou et al., 2000; Vetrano et al., 2008; Adachi et al., 2006; Katsuno et al., 2008; Xu et al., 2008). Nevertheless, some genetic knockouts have been useful tools in exploring the relationship between intestinal barrier function and colitis pathogenesis. These include studies of knockout mice lacking tight junction-associated proteins (Vetrano et al., 2008; Laukoetter et al., 2007), transgenic mice in which known regulatory pathways are activated inappropriately (Su et al., 2009), knockout mice that are unable to activate regulatory pathways (Su et al., 2013), and treatment of mice with novel therapeutic agents (Arrieta et al., 2009). Among these, several different experimental models have been used; therefore, it is important to distinguish those that model IBD and chronic disease from those that rely on acute epithelial injury.

Two separate studies have shown that intestinal permeability is markedly increased in JAM-A-deficient mice (Vetrano et al., 2008; Laukoetter et al., 2007). These JAM-A knockout mice demonstrate mucosal immune activation; inflammatory cell recruitment; and, as demonstrated by increased epithelial turnover, chronic mucosal damage (Vetrano et al., 2008; Laukoetter et al., 2007). However, these mice are clinically well in the absence of exogenous stressors. In contrast, dextran sodium sulfate (DSS)-induced colitis was far more severe in JAM-A knockout mice (Vetrano et al., 2008; Laukoetter et al., 2007). This striking result generates many questions. First, given that the mice used were universal JAM-A knockouts, the mechanism by which JAM-A contributed to these effects was unclear. For example, JAM-A is widely expressed in various tissues and is critical to inflammatory cell migration. Although one study showed that endothelial cell-specific JAM-A knockout did not affect DSS colitis severity (Vetrano et al., 2008), these studies have yet to be performed in intestinal epithelial cell-specific JAM-A knockout mice.

In addition, the cause of increased intestinal permeability in JAM-A knockout mice has not been clearly defined. Apoptosis is increased in intestinal epithelia of JAM-A knockout mice and after JAM-A knockdown in intestinal epithelial cell lines (Vetrano et al., 2008; Laukoetter et al., 2007). Furthermore, upregulation of claudin-10 and claudin-15 is observed after either JAM-A knockout in vivo or JAM-A knockdown in vitro (Laukoetter et al., 2007). However, the function of claudins-10 and -15 as paracellular ion channels (Van Itallie et al., 2006; Wada et al., 2013; Tamura et al., 2011; Colegio et al., 2002) is not consistent with the increased paracellular flux of large molecules after JAM-A loss. This implicates epithelial apoptosis as a major mechanism of barrier loss in JAM-A knockout mice. Given that direct epithelial damage and apoptosis are the primary mechanisms by which DSS induces colitis, the observed phenotype may simply indicate that JAM-A knockout mice have a reduced capacity for epithelial renewal after DSS-induced damage.

To address the specific effect of intestinal tight junction barrier dysfunction on intestinal physiology, transgenic mice were generated expressing an intestinal epithelial-specific constitutively active myosin light chain kinase (CA-MLCK; Su et al., 2009). This augmented intestinal epithelial myosin II regulatory light chain phosphorylation and also caused modest increases in intestinal epithelial tight junction permeability

(Su et al., 2009; Shen et al., 2006). Notably, the permeability increases observed were far less than those observed in JAM-A knockout mice, but they were quantitatively similar to the defects seen in healthy first-degree relatives of Crohn's disease patients (May et al., 1993; Hollander et al., 1986). On the basis of these findings, it is not surprising that CA-MLCK transgenic mice failed to develop spontaneous disease. However, mild mucosal immune activation and T helper 1 cell polarization was observed (Su et al., 2009). Further, immunodeficient (Rag1 knockout) CA-MLCK transgenic mice developed a more severe colitis with reduced survival after adoptive transfer of $CD4^+CD45RB^{hi}$ T cells relative to Rag1 knockout littermates (Su et al., 2009). More strikingly, onset of disease after $CD4^+CD45RB^{hi}$ T cell adoptive transfer was markedly accelerated in CA-MLCK mice. This suggests that intestinal epithelial tight junction barrier loss can enhance the rate of disease pathogenesis in a susceptible individual (e.g., immunodeficient mice that have received naïve T effector cells), but it is insufficient to cause disease in "normal" subjects. Thus, under homeostatic conditions, these mice may model the healthy first-degree relatives of Crohn's disease patients (May et al., 1992; Hollander et al., 1986), whereas stressed CA-MLCK mice may be similar to Crohn's disease patients with increased intestinal permeability during remission (D'Inca et al., 1999; Wyatt et al., 1993).

In contrast to the induction of a constitutive permeability defect, prevention of disease-associated increases in intestinal permeability has been recently explored using long MLCK knockout mice. As noted earlier, these mice are protected from acute TNF-driven, MLCK-dependent leak pathway barrier loss. Remarkably, they are also protected from leak pathway barrier loss and immune-mediated colitis after $CD4^+CD45RB^{hi}$ T cell adoptive transfer into immunodeficient recipients (Su et al., 2013). Tissue-specific restoration of MLCK activity in enterocytes eliminates the protective effect of long MLCK knockout, demonstrating that the phenotype results from loss of intestinal epithelial MLCK (Figure 10). Taken together, these data implicate the leak pathway during the early stages of disease pathogenesis. However, the pore pathway may also be involved because intestinal epithelial claudin-2 upregulation, which occurs in human IBD as well as adoptive transfer colitis (Figure 10(b); Su et al., 2013; Heller et al., 2005), is markedly attenuated in long MLCK knockout mice. The protection afforded by long MLCK knockout is not long-lasting; these mice eventually develop disease that is similar to that of their long MLCK sufficient cage-mates (Su et al., 2013). The onset of disease in long MLCK knockout mice is associated with increases in epithelial apoptosis and barrier loss (Figure 10(c)). Thus, although T cell-mediated disease in the adoptive transfer model is delayed by preventing MLCK-dependent leak pathway barrier loss, T cells are eventually activated to an extent necessary to induce epithelial apoptosis. The resulting tight junction-independent barrier loss then allows disease progression in the absence of increased pore or leak pathway permeability (Su et al., 2013). It is important to note that loss of MLCK expression was unable to prevent the development or progression of colitis. Therefore, these data show that long MLCK inhibition may be effective in limiting early disease but is unlikely to be helpful in advanced disease that includes a prominent component of direct epithelial damage. Finally, the divergent effects of long MLCK knockout on immune-mediated and epithelial damage-dependent (i.e., DSS-induced) disease emphasize the importance of using colitis models appropriate to the question being asked.

From a biological perspective, the IL-10 knockout mouse is one of the best models of IBD (Kuhn et al., 1993) because IL-10 promoter and receptor polymorphisms have been linked to severe, pediatric-onset ulcerative colitis (Tedde et al., 2008; Franke et al., 2008; Christodoulou et al., 2013; Glocker et al., 2009). IL-10 knockout mice generally develop spontaneous colitis during the first 3–6 months of life. In a manner that also recapitulates human disease, the presentation of colitis in these mice is variable in terms of severity and age at onset. This variability is present in

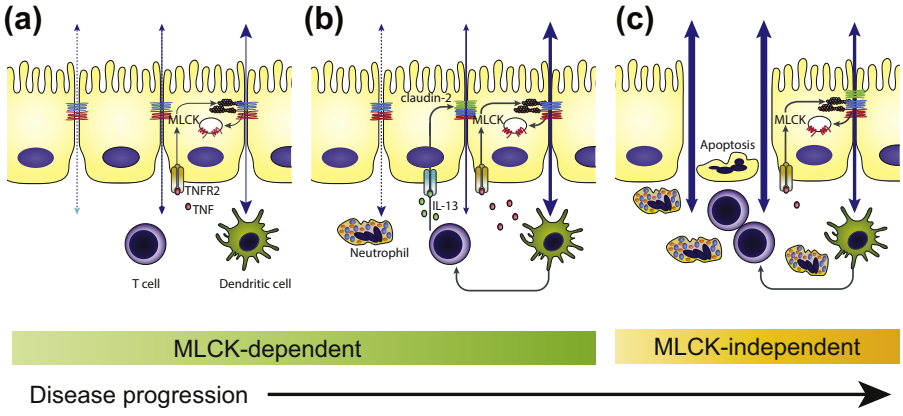

FIGURE 10 MLCK inhibition limits early immune-mediated intestinal disease. Initiating events in experimental colitis include (a) TNF-mediated tight junction barrier loss and (b) subsequent immune-mediated upregulation of claudin-2, both of which are MLCK-dependent processes. (c) However, MLCK inhibition is unable to prevent full-blown active disease characterized by significant epithelial damage resulting in massive barrier loss. *Adapted from Su et al. (2013).*

genetically identical littermates, but can be modified by genetic background (Farmer et al., 2001) and the environment. For example, disease penetrance is reduced in specific pathogen-free mouse facilities and absent when IL-10 knockout mice are housed under germ-free conditions (Sellon et al., 1998). Conversely, disease penetrance can be enhanced by *Helicobacter hepaticus* infection (Matharu et al., 2009; Kullberg et al., 1998) or by treatment with nonsteroidal anti-inflammatory drugs (Berg et al., 2002; Blum et al., 2004). IL-10 knockout mice may also be a powerful tool in studies of IBD-associated barrier loss because they display increased intestinal permeability long before disease presents (Madsen et al., 1999).

In contrast to IL-10 knockout mice, which do not develop disease under germ-free conditions (Madsen et al., 1999), increased permeability that develops before clinical disease in the outbred SAMP1/YitFc model of spontaneous ileocolitis is independent of the presence of microbiota (Corridoni et al., 2012; Olson et al., 2006). In an effort to take advantage of the increased permeability that precedes onset in IL-10 knockout mice, one study treated these mice with a peptide that enhances small intestinal barrier function and found reduced mucosal TNF expression and neutrophil infiltration (Arrieta et al., 2009). Unfortunately, the heterogeneity of disease presentation in IL-10 knockout mice made it difficult to assess the development of clinically evident disease in these mice. Together with the long MLCK knockout mouse study discussed above (Su et al., 2013), these data support further exploration of barrier restoration as a means to treat or potentially prevent disease.

INTESTINAL BARRIER LOSS PROMOTES COMPENSATORY IMMUNOREGULATORY PROCESSES

Although the data obtained using the models described above indicate that intestinal barrier restoration may be effective therapeutically, they do not explain the failure of barrier loss, whether induced by tight junction dysregulation or enhanced cell turnover, to cause disease. As noted earlier, this finding correlates with the clinical observation that some first-degree relatives of Crohn's disease patients are healthy despite having increased intestinal permeability (Hollander et al., 1986; May et al., 1992; Buhner et al., 2006). This had led many to investigate why intestinal barrier loss is insufficient to cause disease (Boirivant et al., 2008; Su et al., 2009; Khounlotham et al., 2012; Turner, 2009). This question is addressed most directly in a study that assessed disease susceptibility after intrarectal ethanol administration, which causes transient epithelial cell damage, mucosal erosion, and barrier loss (Boirivant et al., 2008). After this transient insult, the number of IFNγ- and IL-10-producing mononuclear cells increased in the lamina propria (Figure 11; Boirivant et al., 2008). Mucosal $CD4^+CD25^+$ regulatory T cells were also increased, and more of these cells expressed surface latency-associated peptide (LAP), a transforming growth factor-β (TGFβ) precursor, than in untreated mice. After a period of recovery, ethanol-treated mice were resistant to trinitrobenzene sulphonic acid (TNBS)-induced colitis. This protection required the presence of LAP^+ T cells, the induction of which depended on $CD11c^+$ DC function, Toll-like receptor-2 signaling, and the microbiota (Boirivant et al., 2008). It is interesting to note that lamina propria $CD11c^+$ cells have been reported to move toward the luminal surface of CA-MLCK transgenic mice (Su et al., 2009), which also have an increased complement of IgA-producing plasma cells within the lamina propria, possibly reflecting an increase in TGFβ production similar to that observed after transient epithelial damage. Consistent with this, similar data have recently been reported in JAM-A knockout mice, in which increased IgA production and TGFβ-producing $CD4^+$ T cells reduce DSS colitis severity (Khounlotham et al., 2012).

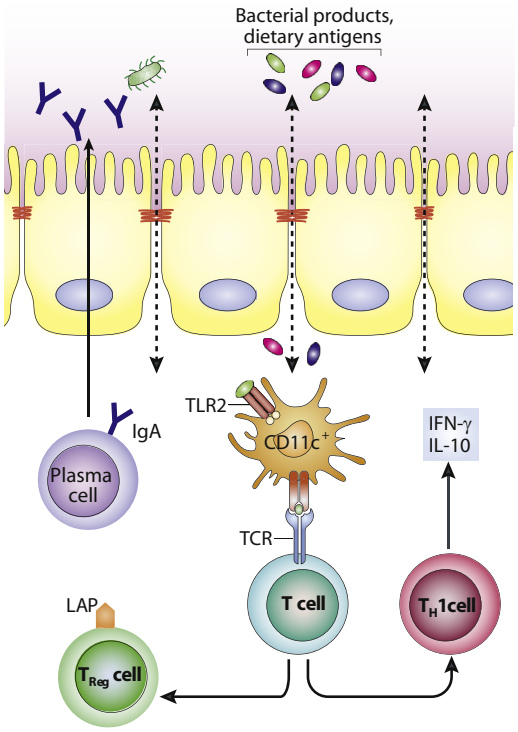

FIGURE 11 Transient paracellular permeability promotes regulatory immune responses. Transient loss of barrier function allows an influx of luminal antigen resulting in a regulatory immune response characterized by IFNγ and IL-10 production by lamina propria mononuclear cells and an increased number of $CD4^+CD25^+$ regulatory T cells expressing surface LAP, a TGFβ precursor. This effect appears to be dependent on antigen presentation by $CD11c^+$ dendritic cells, Toll-like receptor-2 signaling, and the microbiota. TGFβ may also contribute to observed increases in IgA-producing plasma cells.

CONCLUSION

Significant progress has been made toward understanding the relationship between epithelial barrier loss and inflammatory disease; however, it is evident that many questions remain. Although inflammation can lead to barrier dysfunction, barrier dysfunction may also precede disease development. Further, increased intestinal permeability activates regulatory immune processes that may be tolerogenic and limit responses to transient luminal antigen exposure. This improved understanding of the molecular regulation of barrier function and the direct interactions between the epithelium and mucosal immunity is needed if we are to develop targeted therapeutic approaches for diseases associated with increased mucosal permeability.

REFERENCES

Adachi, M., Inoko, A., Hata, M., Furuse, K., Umeda, K., Itoh, M., Tsukita, S., 2006. Normal establishment of epithelial tight junctions in mice and cultured cells lacking expression of ZO-3, a tight-junction MAGUK protein. Mol. Cell. Biol. 26, 9003–9015.

Al-Sadi, R., Khatib, K., Guo, S., Ye, D., Youssef, M., Ma, T., 2011. Occludin regulates macromolecule flux across the intestinal epithelial tight junction barrier. Am. J. Physiol. Gastrointest. Liver Physiol. 300, G1054–G1064.

Al-Sadi, R., Ye, D., Said, H.M., Ma, T.Y., 2010. IL-1beta-induced increase in intestinal epithelial tight junction permeability is mediated by MEKK-1 activation of canonical NF-kappaB pathway. Am. J. Pathol. 177, 2310–2322.

Amasheh, S., Meiri, N., Gitter, A.H., Schoneberg, T., Mankertz, J., Schulzke, J.D., Fromm, M., 2002. Claudin-2 expression induces cation-selective channels in tight junctions of epithelial cells. J. Cell. Sci. 115, 4969–4976.

Anderson, J.M., Van Itallie, C.M., 2009. Physiology and function of the tight junction. Cold Spring Harb. Perspect. Biol. 1, a002584.

Andersoj, J.M., Van Itallie, C.M., Fanning, A.S., 2004. Setting up a selective barrier at the apical junction complex. Curr. Opin. Cell. Biol. 16, 140–145.

Ando-Akatsuka, Y., Yonemura, S., Itoh, M., Furuse, M., Tsukita, S., 1999. Differential behavior of E-cadherin and occludin in their colocalization with ZO-1 during the establishment of epithelial cell polarity. J. Cell. Physiol. 179, 115–125.

Andreeva, A.Y., Krause, E., Muller, E.C., Blasig, I.E., Utepbergenov, D., 2001. Protein kinase C regulates the phosphorylation and cellular localization of occludin. J. Biol. Chem. 276, 38480–38486.

Angelow, S., Yu, A.S., 2009. Structure-function studies of claudin extracellular domains by cysteine-scanning mutagenesis. J. Biol. Chem. 284, 29205–29217.

Arrieta, M.C., Madsen, K., Doyle, J., Meddings, J., 2009. Reducing small intestinal permeability attenuates colitis in the IL10 gene-deficient mouse. Gut 58, 41–48.

Atisook, K., Carlson, S., Madara, J.L., 1990. Effects of phlorizin and sodium on glucose-elicited alterations of cell junctions in intestinal epithelia. Am. J. Physiol. 258, C77–C85.

Baas, A.F., Kuipers, J., Van Der Wel, N.N., Batlle, E., Koerten, H.K., Peters, P.J., Clevers, H.C., 2004. Complete polarization of single intestinal epithelial cells upon activation of LKB1 by STRAD. Cell 116, 457–466.

Balda, M.S., Flores-Maldonado, C., Cereijido, M., Matter, K., 2000. Multiple domains of occludin are involved in the regulation of paracellular permeability. J. Cell. Biochem. 78, 85–96.

Barker, N., Van Es, J.H., Kuipers, J., Kujala, P., Van Den Born, M., Cozijnsen, M., Haegebarth, A., Korving, J., Begthel, H., Peters, P.J., Clevers, H., 2007. Identification of stem cells in small intestine and colon by marker gene Lgr5. Nature 449, 1003–1007.

Barrett, J.C., Lee, J.C., Lees, C.W., Prescott, N.J., Anderson, C.A., Phillips, A., Wesley, E., Parnell, K., Zhang, H., Drummond, H., Nimmo, E.R., Massey, D., Blaszczyk, K., Elliott, T., Cotterill, L., Dallal, H., Lobo, A.J., Mowat, C., Sanderson, J.D., Jewell, D.P., Newman, W.G., Edwards, C., Ahmad, T., Mansfield, J.C., Satsangi, J., Parkes, M., Mathew, C.G., Donnelly, P.C., Peltonen, L.D.C., Blackwell, J.M., Bramon, E., Brown, M.A., Casas, J.P., Corvin, A., Craddock, N., Deloukas, P., Dunvanson, A., Jankowski, J., Markus, H.S., McCarthy, M.I., Palmer, C.N., Plomin, R., Rautanen, A., Sawcer, S.J., Samani, N., Trembath, R.C., Viswanathan, A.C., Wood, N., Spencer, C.C., Bellenguez, C., Davison, D., Freeman, C., Strange, A., Donnelly, P., Langford, C., Hunt, S.E., Edkins, S., Gwilliam, R., Blackburn, H., Bumpstead, S.J., Dronov, S., Gillman, M., Gray, E., Hammond, N., Jayakumar, A., McCann, O.T., Liddle, J., Perez, M.L., Potter, S.C., Ravindrarajah, R., Ricketts, M., Waller, M., Weston, P., Attwood, A.P., Stephens, J., Sambrook, J., Ouwehansd, W.H., Mcardle, W.L., Ring, S.M., Strachan, D.P., 2009. Genome-wide association study of ulcerative colitis identifies three new susceptibility loci, including the HNF4A region. Nat. Genet. 41, 1330–1334.

Ben-Yosef, T., Belyantseva, I.A., Saunders, T.L., Hughes, E.D., Kawamoto, K., Van Itallie, C.M., Beyer, L.A., Halsey, K., Gardner, D.J., Wilcox, E.R., Rasmussen, J., Anderson, J.M., Dolan, D.F., Forge, A., Raphae, Y., Camper, S.A., Friedman, T.B., 2003. Claudin 14 knockout mice, a model for autosomal recessive deafness DFNB29, are deaf due to cochlear hair cell degeneration. Hum. Mol. Genet. 12, 2049–2061.

Berg, D.J., Zhang, J., Weinstock, J.V., Ismail, H.F., Earle, K.A., Alila, H., Pamukcu, R., Moore, S., Lynch, R.G., 2002. Rapid development of colitis in NSAID-treated IL-10-deficient mice. Gastroenterology 123, 1527–1542.

Bergelson, J.M., Cunningham, J.A., Droguteet, G., Kurt-Jones, E.A., Krithivas, A., Hong, J.S., Horwitz, M.S., Crowell, R.L., Finberg, R.W., 1997. Isolation of a common receptor for coxsackie B viruses and adenoviruses 2 and 5. see reference - Cohen et al. Science 275, 1320–1323.

Bitton, A., Dobkin, P., Edwardes, M.D., Sweeitch, M., Meddings, J., Rawal, S., Cohen, A., Vermeire, S., Dufresne, L., Franchimont, D., Wild, G.E., 2008. Predicting relapse in Crohn's disease: a biopsychosocial model. Gut 57, 1386–1392.

Bjarnsason, I., MacPherson, A., Hollander, D., 1995. Intestinal permeability: an overview. Gastroenterology 108, 1566–1581.

Bjarnason, I., O'Morain, C., Levi, A.J., Peters, T.J., 1983. Absorption of 51chromium-labeled ethylenediaminetetraacetate in inflammatory bowel disease. Gastroenterology 85, 318–322.

Blasig, I.E., Winkler, L., Lassowski, B., Mueller, S.L., Zuleger, N., Krause, E., Krause, G., Gast, K., Kolbe, M., Piontek, J., 2006. On the self-association potential of transmembrane tight junction proteins. Cell. Mol. Life Sci. 63, 505–514.

Blum, A.M., Metwali, A., Elliott, D.E., Berg, D.J., Weinstock, J.V., 2004. $CD4^+$ T cells from IL-10-deficient mice transfer susceptibility to NSAID-induced Rag colitis. Am. J. Physiol. Gastrointest. Liver Physiol. 287, G320–G325.

Boirivant, M., Amendola, A., Butera, A., Sanchez, M., Xu, L., Marinaro, M., Kitani, A., Di Giacinto, C., Strober, W., Fuss, I.J., 2008. A transient breach in the epithelial barrier leads to regulatory T-cell generation and resistance to experimental colitis. Gastroenterology 135, 1612–1623.

Buhner, S., Buning, C., Genschel, J., Kling, K., Herrmann, D., Dignass, A., Kuechler, I., Krueger, S., Schmidt, H.H., Lochs, H., 2006. Genetic basis for increased intestinal permeability in families with Crohn's disease: role of CARD15 3020insC mutation? Gut 55, 342–347.

Burton, P.R., Clayton, D.G., Cardon, L.R., Craddock, N., Deloukas, P., Duncanson, A., Kwiatkowski, D.P., McCarthy, M.I., Ouwehand, W.H., Samani, N.J., Todd, J.A., Donnelly, P., Barrett, J.C., Burton, P.R., Davison, D., Donnelly, P., Easton, D., Evans, D., Leung, H.T., Marchini, J.L., Morris, A.P., Spencer, C.C., Tobin, M.D., Cardon, L.R., Clayton, D.G., Attwood, A.P., Boorman, J.P., Cant, B., Everson, U., Hussey, J.M., Jolley, J.D., Knight, A.S., Koch, K., Meech, E., Nutland, S., Prowse, C.V., Stevens, H.E., Taylor, N.C., Walters, G.R., Walker, N.M., Watkins, N.A., Winzer, T., Todd, J.A., Ouwehand, W.H., Jones, R.W., McArdle, W.L., Ring, S.M., Strachan, D.P., Pembrey, M., Breen, G., St Clair, D., Caesar, S., Gordon-Smith, K., Jones, L., Fraser, C., Green, E.K., Grozeva, D., Hamshere, M.L., Holmans, P.A., Jones, I.R., Kirov, G., Moskvina, V., Nikolov, I., O'Donovan, M.C., Owen, M.J., Craddock, N., Collier, D.A., Elkin, A., Farmer, A., Williamson, R., McGuffin, P., Young, A.H., Ferrier, I.N., Ball, S.G., Balmforth, A.J., Barrett, J.H., Bishop, D.T., Iles, M.M., Maqbool, A., Yuldasheva, N., Hall, A.S., Braund, P.S., Burton, P.R., Dixon, R.J., Mangino, M., Suzanne, S., Tobin, M.D., Thompson, J.R., Samani, N.J., Bredin, F., Tremelling, M., Parkes, M., Drummond, H., Lees, C.W., Nimmo, E.R., Satsangi, J., Fisher, S.A., Forbes, A., Lewis, C.M., Onnie, C.M., Prescott, N.J., Sanderson, J., Mathew, C.G., Barbour, J., Mohiuddin, M.K., Todhunter, C.E., Mansfield, J.C., Ahmad, T., Cummings, F.R., Jewell, D.P., Webster, J., Brown, M.J., Clayton, D.G., Lathrop, G.M., Connell, J., Dominczak, A., Samani, N.J., Marcano, C.A., Burke, B., Dobson, R., Gungadoo, J., Lee, K.L., Munroe, P.B., Newhouse, S.J., Onipinla, A., Wallace, C., Xue, M., Caulfield, M., Farrall, M., Barton, A., Bruce, I.N., Donovan, H., Eyre, S., Gilbert, P.D., Hider, S.L., Hinks, A.M., John, S.L., Potter, C., Silman, A.J., Symmmons, D.P., Thomson, W., Worthington, J., Clayton, D.G., Dunger, D.B., Nutland, S., Stevens, H.E., Walker, N.M., Widmer, B., Todd, J.A., Frayling, T.A., Freathy, R.M., Lango, H., Perry, J.R., Shields, B.M., Weedon, M.N., Hattersley, A.T., Hitman, G.A., Walker, M., Elliott, K.S., Groves, C.J., Lindgren, C.M., Rayner, N.W., Timpson, N.J., Zeggini, E., McCarthy, M.I., Newport, M., Sirugo, G., Lyons, E., Vannberg, F., Hill, A.V., Bradbury, L.A., Farrar, C., Pointon, J.J., Wordsworth, P., Brown, M.A., Franklyn, J.A., Heward, J.M., Simmonds, M.J., Gough, S.C., Seal, S., Stratton, M.R., Rahman, N., Ban, M., Goris, A., Sawcer, S.J., Compston, A., Conway, D., Jallow, M., Newport, M., Sirugo, G., Rockett, K.A., Kwiatowski, D.P., Bumpstead, S.J., Chaney, A., Downes, K., Ghori, M.J., Gwilliam, R., Hunt, S.E., Inouye, M., Keniry, A., King, E., McGinnis, R., Potter, S., Ravindrarajah, R., Whittaker, P., Widden, C., Withers, D., Deloukas, P., Leung, H.T., Nutland, S., Stevens, H.E., Walker, N.M., Todd, J.A., Easton, D., Clayton, D.G., Burton, P.R., Tobin, M.D., Barrett, J.C., Evans, D., Morris, A.P., Cardon, L.R., Cardin, N.J., Davison, D., Ferreira, T., Pereira-Gale, J., Hallgrimsdottir, I.B., Howie, B.N., Marchini, J.L., Spencer, C.C., Su, Z., Teo, Y.Y., Vukcevic, D., Donnelly, P., Bentley, D., Brown, M.A., Gordon, L.R., Caulfield, M., Clayton, D.G., Compston, A., Craddock, N., Deloukas, P., Donnelly, P., Farrall, M., Gough, S.C., Hall, A.S., Hattersley, A.T., Hill, A.V., Kwiatkowski, D.P., Mathew, C., McCarthy, M.I., Ouwehand, W.H., Parkes, M., Pembrey, M., Rahman, N., Samani, N.J., Stratton, M.,R., Todd, J.A., Worthington, J., 2007. Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls. Nature 447, 661–678.

Camilleri, M., Lasch, K., Zhou, W., 2012. Irritable bowel syndrome: methods, mechanisms, and pathophysiology. The confluence of increased permeability, inflammation, and pain in irritable bowel syndrome. Am. J. Physiol. Gastrointest. Liver Physiol. 303, G775–G785.

Chadwick, V.S., Phillips, S.F., Hofmann, A.F., 1977. Measurements of intestinal permeability using low molecular weight polyethylene glycols (PEG 400). II. Application to normal and abnormal permeability states in man and animals. Gastroenterology 73, 247–251.

Cheng, H., Merzel, J., Leblond, C.P., 1969. Renewal of Paneth cells in the small intestine of the mouse. Am. J. Anat. 126, 507–525.

Chisti, M.S., Bhatti, A., Tamim, S., Lee, K., McDonald, M.L., Leal, S.M., Ahmad, W., 2008. Splice-site mutations in the TRIC gene underlie autosomal recessive nonsyndromic hearing impairment in Pakistani families. J. Hum. Genet. 53, 101–105.

Christodoulou, K., Wiskin, A.E., Gibson, J., Tapper, W., Willis, C., Afzal, N.A., Upstill-Goddard, R., Hollowy, J.W., Simpson, M.A., Beattie, R.M., Collins, A., Ennis, S., 2013. Next generation exome sequencing of paediatric inflammatory bowel disease patients identifies rare and novel variants in candidate genes. Gut 62, 977–984.

Claude, P., 1978. Morphological factors influencing transepithelial permeability: a model for the resistance of the zonula occludens. J. Membr. Biol. 39, 219–232.

Clayburgh, D.R., Barrett, T.A., Tang, Y., Meddings, J.B., Van Eldik, L.J., Watterson, D.M., Clarke, L.L., Mrsny, R.J., Turner, J.R., 2005. Epithelial myosin light chain kinase-dependent barrier dysfunction mediates T cell activation-induced diarrhea in vivo. J. Clin. Invest. 115, 2702–2715.

Clayburgh, D.R., Musch, M.W., Leitges, M., Fu, Y.X., Turner, J.R., 2006. Coordinated epithelial NHE3 inhibition and barrier dysfunction are required for TNF-mediated diarrhea in vivo. J. Clin. Invest. 116, 2682–2694.

Cohen, C.J., Shieh, J.T., Pickles, R.J., Okegawa, T., Hsieh, J.T., Bergelson, J.M., 2001. The coxsackievirus and adenovirus receptor is a transmembrane component of the tight junction. Proc. Natl. Acad. Sci. U.S.A. 98, 15191–15196.

Colegio, O.R., Van Itallie, C.M., Mccrea, H.J., Rahner, C., Anderson, J.M., 2003. Claudin extracellular domains determine paracellular charge selectivity and resistance but not tight junction fibril architecture. Am. J. Physiol. Cell. Physiol. 284, C1346–C1354.

Colegio, O.R., Van Itallie, C.M., Mccrea, H.J., Rahner, C., Anderson, J.M., 2002. Claudins create charge-selective channels in the paracellular pathway between epithelial cells. Am. J. Physiol. Cell. Physiol. 283, C142–C147.

Corridoni, D., Pastorelli, L., Mattioli, B., Locovei, S., Ishikawa, D., Arseneau, K.O., Chieppa, M., Cominelli, F., Pizarro, T.T., 2012. Probiotic bacteria regulate intestinal epithelial permeability in experimental ileitis by a TNF-dependent mechanism. PLoS. ONE 7, e42067.

Coyne, C.B., Voelker, T., Pichla, S.L., Bergelson, J.M., 2004. The coxsackievirus and adenovirus receptor interacts with the multi-PDZ domain protein-1 (MUPP-1) within the tight junction. J. Biol. Chem. 279, 48079–48084.

Cummins, P.M., 2012. Occludin: one protein, many forms. Mol. Cell. Biol. 32, 242–250.

D'Inca, R., Di leo, V., Corrao, G., Martines, D., D'Odorico, A., Mestriner, C., Venturi, C., Longo, G., Sturniolo, G.C., 1999. Intestinal permeability test as a predictor of clinical course in Crohn's disease. Am. J. Gastroenterol. 94, 2956–2960.

Ebnet, K., Aurrand-Lions, M., Kuhn, A., Kiefer, F., Butz, S., Zander, K., Meyer zu Brickwedde, M.K., Suzuki, A., Imhof, B.A., Vesteweber, D., 2003. The junctional adhesion molecule (JAM) family members JAM-2 and JAM-3 associate with the cell polarity protein PAR-3: a possible role for JAMs in endothelial cell polarity. J. Cell. Sci. 116, 3879–3891.

Elias, B.C., Suzuki, T., Seth, A., Giorgianni, F., Kale, G., Shen, L., Turner, J.R., Naren, A., Desiderio, D.M., Rao, R., 2009. Phosphorylation of Tyr-398 and Tyr-402 in occludin prevents its interaction with ZO-1 and destabilizes its assembly at the tight junctions. J. Biol. Chem. 284, 1559–1569.

Fanning, A.S., Anderson, J.M., 2009. Zonula occludens-1 and -2 are cytosolic scaffolds that regulate the assembly of cellular junctions. Ann. N.Y. Acad. Sci. 1165, 113–120.

Fanning, A.S., Jameson, B.J., Jesaitis, L.A., Anderson, J.M., 1998. The tight junction protein ZO-1 establishes a link between the transmembrane protein occludin and the actin cytoskeleton. J. Biol. Chem. 273, 29745–29753.

Fanning, A.S., Jameson, B.J., Rahner, C., Utepbergenov, D., Walther, Z., Anderson, J.M., 2007a. The unique-5 and -6 motifs of ZO-1 regulate tight junction strand localization and scaffolding properties. Mol. Biol. Cell. 18, 721–731.

Fanning, A.S., Lye, M.F., Anderson, J.M., Lavie, A., 2007b. Domain swapping within PDZ2 is responsible for dimerization of ZO proteins. J. Biol. Chem. 282, 37710–37716.

Fanning, A.S., Lye, M.F., Anderson, J.M., 2002. Isolation and functional characterization of the actin binding region in the tight junction protein ZO-1. FASEB J. 16, 1835–1837.

Fanning, A.S., Van Itallie, C.,M., Anderson, J.M., 2012. Zonula occludens-1 and -2 regulate apical cell structure and the zonula adherens cytoskeleton in polarized epithelia. Mol. Biol. Cell. 23, 577–590.

Farmer, M.A., Sundberg, J.P., Bristol, I.J., Churchill, G.A., Li, R., Elson, C.O., Leiter, E.H., 2001. A major quantitative trait locus on chromosome 3 controls colitis severity in IL-10-deficient mice. Proc. Natl. Acad. Sci. U.S.A. 98, 13820–13825.

Fihn, B.M., Sjoqvist, A., Jodal, M., 2000. Permeability of the rat small intestinal epithelium along the villus- crypt axis: effects of glucose transport. Gastroenterology 119, 1029–1036.

Franke, A., Balschun, T., Karlsen, T.H., Sventoraityte, J., Nikolaus, S., Mayr, G., Domingues, F.S., Albrecht, M., Nothnagel, M., Ellinghaus, D., Sina, C., Onnie, C.M., Weersma, R.K., Stokkers, P.C., Wijmenga, C., Gazouli, M., Strachan, D., Mcardle, W.L., Vermeire, S., Rutgeerts, P., Rosenstiel, P., Krawczak, M., Vatn, M.H., Mathew, C.G., Schreiber, S., 2008. Sequence variants in IL-10, ARPC2 and multiple other loci contribute to ulcerative colitis susceptibility. Nat. Genet. 40, 1319–1323.

Furuse, M., Fujita, K., Hiiragi, T., Fujimoto, K., Tsukita, S., 1998a. Claudin-1 and -2: novel integral membrane proteins localizing at tight junctions with no sequence similarity to occludin. J. Cell Biol. 141, 1539–1550.

Furuse, M., Hata, M., Furuse, K., Yoshida, Y., Haratake, A., Sugitani, Y., Noda, T., Kubo, A., Tsukita, S., 2002. Claudin-based tight junctions are crucial for the mammalian epidermal barrier: a lesson from claudin-1-deficient mice. J. Cell Biol. 156, 1099–1111.

Furuse, M., Hirase, T., Itoh, N., Nagafuchi, A., Yonemura, S., Tsukita, S., Tsukita, S., 1993. Occludin: a novel integral membrane protein localizing at tight junctions. J. Cell Biol. 123, 1777–1788.

Furuse, M., Sasaki, H., Fujimoto, K., Tsukita, S., 1998b. A single gene product, claudin-1 or -2, reconstitutes tight junction strands and recruits occludin in fibroblasts. J. Cell Biol. 143, 391–401.

Glocker, E.O., Kotlarz, D., Boztug, K., Gertz, E.M., Schäffer, A.A., Noyan, F., Perro, M., Diestelhorst, J., Allroth, A., Murugan, D., Hätscher, N., Pfeifer, D., Sykora, K.W., Sauer, M., Kreipe, H., Lacher, M., Nustede, R., Woellner, C., Baumann, U., Salzer, U., Koletzko, S., Shah, N., Segal, A.W., Sauerbrey, A., Buderus, S., Snapper, S.B., Grimbacher, B., Klein, C., 2009. Inflammatory bowel disease and mutations affecting the interleukin-10 receptor. N. Engl. J. Med. 361, 2033–2045.

Gow, A., Southwood, C.M., Li, J.S., Pariali, M., Riordan, G.P., Brodie, S.E., Danias, J., Bronstein, J.M., Kachar, B., Lazzarini, R.A., 1999. CNS myelin and Sertoli cell tight junction strands are absent in Osp/claudin-11 null mice. Cell 99, 649–659.

Heller, F., Florain, P., Bojarski, C., Richter, J., Christ, M., Hillenbrand, B., Mankertz, J., Gitter, A.H., Burgel, N., Fromm, M., Zeitz, M., Fuss, I., Strober, W., Schulzke, J.D., 2005. Interleukin-13 is the key effector Th2 cytokine in ulcerative colitis that affects epithelial tight junctions, apoptosis, and cell restitution. Gastroenterology 129, 550–564.

Hermiston, M.L., Gordon, J.I., 1995a. In vivo analysis of cadherin function in the mouse intestinal epithelium: essential roles in adhesion, maintenance of differentiation, and regulation of programmed cell death. J. Cell Biol. 129, 489–506.

Hermiston, M.L., Gordon, J.I., 1995b. Inflammatory bowel disease and adenomas in mice expressing a dominant negative N-cadherin. Science 270, 1203–1207.

Hollander, D., Vadheim, C.M., Brettholz, E., Petersen, G.M., Delahunty, T., Rotter, J.I., 1986. Increased intestinal permeability in patients with Crohn's disease and their relatives. A possible etiologic factor. Ann. Intern. Med. 105, 883–885.

Holmes, J.L., Van Itallie, C.M., Rasmussen, J.E., Anderson, J.M., 2006. Claudin profiling in the mouse during postnatal intestinal development and along the gastrointestinal tract reveals complex expression patterns. Gene Expr. Patterns 6, 581–588.

Huber, D., Balda, M.S., Matter, K., 2000. Occludin modulates transepithelial migration of neutrophils. J. Biol. Chem. 275, 5773–5778.

Huppert, J., Closhen, D., Croxford, A., White, R., Kulig, P., Pietrowski, E., Bechmann, I., Becher, B., Luhmann, H.J., Waisman, A., Kuhlmann, C.R., 2010. Cellular mechanisms of IL-17-induced blood-brain barrier disruption. FASEB J. 24, 1023–1034.

Ikenouchi, J., Furuse, M., Furuse, K., Sasaki, H., Tsukita, S., 2005. Tricellulin constitutes a novel barrier at tricellular contacts of epithelial cells. J. Cell Biol. 171, 939–945.

Ikenouchi, J., Sasaki, H., Tsukita, S., Furuse, M., 2008. Loss of occludin affects tricellular localization of tricellulin. Mol. Biol. Cell. 19, 4687–4693.

Irvine, E.J., Marshall, J.K., 2000. Increased intestinal permeability precedes the onset of Crohn's disease in a subject with familial risk. Gastroenterology 119, 1740–1744.

Itoh, M., Furuse, M., Morita, K., Kubota, K., Saitou, M., Tsukita, S., 1999. Direct binding of three tight junction-associated MAGUKs, ZO-1, ZO-2, and ZO-3, with the COOH termini of claudins. J. Cell Biol. 147, 1351–1363.

Johansen, K., Stintzing, G., Magnusson, K.E., Sundqvist, T., Jalil, F., Murtaza,A.,Khan,S.R.,Lindblad,B.S.,Mollby,R.,Orusild,E.,Svensson, L., 1989. Intestinal permeability assessed with polyethylene glycols in children with diarrhea due to rotavirus and common bacterial pathogens in a developing community. J. Pediatr. Gastroenterol. Nutr. 9, 307–313.

Kaetzel, C.S., 2014. Cooperativity among secretory IgA, the polymeric immunoglobin receptor, and the gut microbiota promotes host–microbial mutualism. Immunol. Lett. In press.

Katsuno, T., Umeda, K., Matsui, T., Hata, M., Tamura, A., Itoh, M., Takeuchi, K., Fujimori, T., Nabeshima, Y., Noda, T., Tsukita, S., 2008. Deficiency of zonula occludens-1 causes embryonic lethal phenotype associated with defected yolk sac angiogenesis and apoptosis of embryonic cells. Mol. Biol. Cell. 19, 2465–2475.

Kennedy, M.B., 1995. Origin of PDZ (DHR, GLGF) domains. Trends Biochem. Sci. 20 (350).

Khounlotham, M., Kim, W., Peatman, E., Nava, P., Medina-Contreras, O., Addis, C., Koch, S., Fournier, B., Nusrat, A., Denning, T.L., Parkos, C.A., 2012. Compromised intestinal epithelial barrier induces adaptive immune compensation that protects from colitis. Immunity 37, 563–573.

Kinugasa, T., Sakaguchi, T., Gu, X., Reinecker, H.C., 2000. Claudins regulate the intestinal barrier in response to immune mediators. Gastroenterology 118, 1001–1011.

Kitajiri, S., Miyamoto, T., Mineharu, A., Sonoda, N., Furuse, K., Hata, M., Sasaki, H., Mori, Y., Kubota, T., Ito, J., Furuse, M., Tsukita, S., 2004a. Compartmentalization established by claudin-11-based tight junctions in stria vascularis is required for hearing through generation of endocochlear potential. J. Cell. Sci. 117, 5087–5096.

Kitajiri, S.I., Furuse, M., Morita, K., Saishin-Kiuchi, Y., Kido, H., Ito, J., Tsukita, S., 2004b. Expression patterns of claudins, tight junction adhesion molecules, in the inner ear. Hear. Res. 187, 25–34.

Krause, G., Winkler, L., Mueller, S.L., Haseloff, R.F., Piontek, J., Blasig, I.E., 2008. Structure and function of claudins. Biochim. Biophys. Acta 1778, 631–645.

Krause, G., Winkler, Piehl, C., Blasig, I., Piontek, J., Muller, S.L., 2009. Structure and function of extracellular claudin domains. Ann. N.Y. Acad. Sci. 1165, 34–43.

Kuhn, R., Lohler, J., Rennick, D., Rajewsky, K., Muller, W., 1993. Interleukin-10-deficient mice develop chronic enterocolitis. Cell 75, 263–274.

Kullberg, M.C., Ward, J.M., Gorelick, P.L., Caspar, P., Hieny, S., Cheever, A., Jankovic, D., Sher, A., 1998. *Helicobacter hepaticus* triggers colitis in specific-pathogen-free interleukin-10 (IL-10)-deficient mice through an IL-12- and gamma interferon-dependent mechanism. Infect. Immun. 66, 5157–5166.

Kuwabara, H., Kokai, Y., Kojima, T., Takakuwa, R., Mori, M., Sawada, N., 2001. Occludin regulates actin cytoskeleton in endothelial cells. Cell. Struct. Funct. 26, 109–116.

Laukoetter, M.G., Nava, P., Lee, W.Y., Severson, E.A., Capaldo, C.T., Babbin, B.A., Williams, I.R., Koval, M., Peatman, E., Campbell, J.A., Dermody, T.S., Nusrat, A., Parkos, C.A., 2007. JAM-A regulates permeability and inflammation in the intestine in vivo. J. Exp. Med. 204, 3067–3076.

Leedham, S.J., Graham, T.A., Oukrif, D., McDonald, S.A., Rodriguez-Justo, M., Harrison, R.F., Shepherd, N.A., Novelli, M.R., Jankowski, J.A., Wright, N.A., 2009. Clonality, founder mutations, and field cancerization in human ulcerative colitis-associated neoplasia. Gastroenterology 136, 542–550. e6.

Li, Y., Fanning, A.S., Anderson, J.M., Lavie, A., 2005. Structure of the conserved cytoplasmic C-terminal domain of occludin: identification of the ZO-1 binding surface. J. Mol. Biol. 352, 151–164.

Lin, R., Murtazina, R., Cha, B., Chakraborty, M., Sarker, R., Chen, T.E., Lin, Z., Hogema, B.M., De Jonge, H.R., Seidler, U., Turner, J.R., Li, X., Kovbasnjuk, O., Donowitz, M., 2010. D-glucose acts via SGLT1 to increase NHE3 in mouse jejunal brush border by a NHERF2-dependent process. Gastroenterology 140, 560–571.

Luissint, A.C., Lutz, P.G., Calderwood, D.A., Couraud, P.O., Bourdoulous, S., 2008. JAM-L-mediated leukocyte adhesion to endothelial cells is regulated in cis by alpha4beta1 integrin activation. J. Cell Biol. 183, 1159–1173.

Madara, J.L., Pappenheimer, J.R., 1987. Structural basis for physiological regulation of paracellular pathways in intestinal epithelia. J. Membr. Biol. 100, 149–164.

Madsen, K.L., Malfair, D., Gray, D., Doyle, J.S., Jewell, L.D., Fedorak, R.N., 1999. Interleukin-10 gene-deficient mice develop a primary intestinal permeability defect in response to enteric microflora. Inflamm. Bowel Dis. 5, 262–270.

Mandel, L.J., Bacallao, R., Zamphighi, G., 1993. Uncoupling of the molecular 'fence' and paracellular 'gate' functions in epithelial tight junctions. Nature 361, 552–555.

Marano, C.W., Laughlin, K.V., Russo, L.M., Peralta Soler, A., Mullin, J.M., 1993. Long-term effects of tumor necrosis factor on LLC-PK1 transepithelial resistance. J. Cell. Physiol. 157, 519–527.

Marchiando, A.M., Shen, L., Graham, W.V., Weber, C.R., Schwarz, B.T., Austin, J.R., Raleigh, R.D., Guan, Y., Watson, A.J., Montrose, M.H., Turner, J.R., 2010. Caveolin-1-dependent occludin endocytosis is required for TNF-induced tight junction regulation in vivo. J. Cell Biol. 189, 111–116.

Marcial, M.A., Carlson, S.L., Madara, J.L., 1984. Partitioning of paracellular conductance along the ileal crypt-villus axis: a hypothesis based on structural analysis with detailed consideration of tight junction structure-function relationships. J. Membr. Biol. 80, 59–70.

Marin, M.L., Geller, S.A., Greenstein, A.J., Marin, R.H., Gordon, R.E., Aufses Jr., A.H., 1983a. Ultrastructural pathology of Crohn's disease: correlated transmission electron microscopy, scanning electron microscopy, and freeze fracture studies. Am. J. Gastroenterol. 78, 355–364.

Marin, M.L., Greenstein, A.J., Geller, S.A., Gordon, R.E., Aufses Jr., A.H., 1983b. A freeze fracture study of Crohn's disease of the terminal ileum: changes in epithelial tight junction organization. Am. J. Gastroenterol. 78, 537–547.

Martin-Padura, I., Lostaglio, S., Schneemann, M., Williams, L., Romano, M., Fruscella, P., Panzeri, C., Stoppacciaro, A., Ruco, L., Villa, A., Simmons, D., Dejana, E., 1998. Junctional adhesion molecule, a novel member of the immunoglobulin superfamily that distributes at intercellular junctions and modulates monocyte transmigration. J. Cell Biol. 142, 117–127.

Matharu, K.S., Mizoguchi, E., Cotoner, C.a., Nguyen, D.D., Mingle, B., Iweala, O.I., McBee, M.E., Stefka, A.T., Prioult, G., Haigis, K.M., Bhan, A.K., Snapper, S.B., Murakami, H., Schauer, D.B., Reinecker, H.C., Mizoguchi, A., Nagler, C.R., 2009. Toll-like receptor 4-mediated regulation of spontaneous Helicobacter-dependent colitis in IL-10-deficient mice. Gastroenterology 137, 1380–1390. e1–3.

May, G.R., Sutherland, L.M., Meddings, J.B., 1992. Lactulose/mannitol permeability is increased in relatives of patients with Crohn's disease. Gastroenterology 102, A934.

May, G.R., Sutherland, L.R., Meddings, J.B., 1993. Is small intestinal permeability really increased in relatives of patients with Crohn's disease? Gastroenterology 104, 1627–1632.

Mayer, L., Eisenhardt, D., Salomon, P., Bauer, W., Plous, R., Piccinini, L., 1991. Expression of class II molecules on intestinal epithelial cells in humans: differences between normal and inflammatory bowel disease. Gastroenterology 100, 3–12.

Meddings, J.B., Gibbons, I., 1998. Discrimination of site-specific alterations in gastrointestinal permeability in the rat. Gastroenterology 114, 83–92.

Meddings, J.B., Swain, M.G., 2000. Environmental stress-induced gastrointestinal permeability is mediated by endogenous glucocorticoids in the rat. Gastroenterology 119, 1019–1028.

Meddings, J.B., Westergaard, H., 1989. Intestinal glucose transport using perfused rat jejunum in vivo: model analysis and derivation of corrected kinetic constants. Clin. Sci. (Lond.) 76, 403–413.

Merzel, J., Leblond, C.P., 1969. Origin and renewal of goblet cells in the epithelium of the mouse small intestine. Am. J. Anat. 124, 281–305.

Mineta, K., Yamamoto, Y., Yamazaki, Y., Tanaka, H., Tada, Y., Saito, K., Tamura, A., Igarashi, M., Endo, T., Takeuchi, K., Tsukita, S., 2011. Predicted expansion of the claudin multigene family. FEBS Lett. 585, 606–612.

Miyamoto, T., Morita, K., Takemoto, d., Takeuchi, K., Kitano, Y., Miyakawa, T., Nakayama, K., Okamura, Y., Sasaki, H., Miyachi, Y., Furuse, M., Tsukita, S., 2005. Tight junctions in Schwann cells of peripheral myelinated axons: a lesson from claudin-19-deficient mice. J. Cell Biol. 169, 527–538.

Murakami, T., Felinski, E.A., Antonetti, D.A., 2009. Occludin phosphorylation and ubiquitination regulate tight junction trafficking and vascular endothelial growth factor-induced permeability. J. Biol. Chem. 284, 21036–21046.

Muto, S., Hata, M., Taniguchi, J., Tsuruoka, S., Moriwaki, K., Saitou, M., Furuse, K., Sasaki, H., Fujimura, A., Imai, M., Kusano, E., Tsukita, S., Furuse, M., 2010. Claudin-2-deficient mice are defective in the leaky and cation-selective paracellular permeability properties of renal proximal tubules. Proc. Natl. Acad. Sci. U.S.A. 107, 8011–8016.

Nitta, T., Hata, M., Gotoh, S., Seo, Y., Sasaki, H., Hashimoto, N., Furuse, M., Tsukita, S., 2003. Size-selective loosening of the blood-brain barrier in claudin-5-deficient mice. J. Cell Biol. 161, 653–660.

Nomme, J., Fanning, A.S., Caffrey, M., Lye, M.F., Anderson, J.M., Lavie, A., 2011. The Src homology 3 domain is required for junctional adhesion molecule binding to the third PDZ domain of the scaffolding protein ZO-1. J. Biol. Chem. 286, 43352–43360.

Olaison, G., Leandersson, P., Sjodahl, R., Tagesson, C., 1988. Intestinal permeability to polyethyleneglycol 600 in Crohn's disease. Peroperative determination in a defined segment of the small intestine. Gut 29, 196–199.

Olson, T.S., Reuter, B.K., Scott, K.G., Morris, M.A., Wang, X.M., Hancock, L.N., Burcin, T.L., Cohn, S.M., Ernst, P.B., Cominelli, F., Meddings, J.B., Ley, K., Pizarro, T.T., 2006. The primary defect in experimental ileitis originates from a nonhematopoietic source. J. Exp. Med. 203, 541–552.

Ostermann, G., Weber, K.S., Zernecke, A., Schroder, A., Weber, C., 2002. JAM-1 is a ligand of the beta(2) integrin LFA-1 involved in transendothelial migration of leukocytes. Nat. Immunol. 3, 151–158.

Ozaki, H., Ishii, K., Horiuchi, H., Arai, H., Kawamoto, T., Okawa, K., Iwamatsu, A., Kita, T., 1999. Cutting edge: combined treatment of TNF-alpha and IFN-gamma causes redistribution of junctional adhesion molecule in human endothelial cells. J. Immunol. 163, 553–557.

Piehl, C., Piontek, J., Cording, J., Wolburg, H., Blasig, I.E., 2010. Participation of the second extracellular loop of claudin-5 in paracellular tightening against ions, small and large molecules. Cell. Mol. Life Sci. 67, 2131–2140.

Piontek, J., Fritzsche, S., Cording, J., Richter, S., Hartwig, J., Walter, M., Yu, D., Turner, J.R., Gehring, C., Rahn, H.P., Wolburg, H., Blasig, I.E., 2011. Elucidating the principles of the molecular organization of heteropolymeric tight junction strands. Cell. Mol. Life Sci. 68, 3903–3918.

Piontek, J., Winkler, L., Wolburg, H., Muller, S.L., Zuleger, N., Piehl, C., Wiesner, B., Krause, G., Blasig, I.E., 2008. Formation of tight junction: determinants of homophilic interaction between classic claudins. FASEB J. 22, 146–158.

Potten, C.S., Loeffler, M., 1990. Stem cells: attributes, cycles, spirals, pitfalls and uncertainties. Lessons for and from the crypt. Development 110, 1001–1020.

Rajasekaran, A.K., Hojo, M., Huima, T., Rodriguez-Boulan, E., 1996. Catenins and zonula occludens-1 form a complex during early stages in the assembly of tight junctions. J. Cell Biol. 132, 451–463.

Raleigh, D.R., Boe, D.M., Yu, D., Weber, C.R., Marchiando, A.M., Bradford, E.M., Wang, Y., Wu, L., Schneeberger, E.E., Shen, L., Turner, J.R., 2011. Occludin S408 phosphorylation regulates tight junction protein interactions and barrier function. J. Cell Biol. 193, 565–582.

Raleigh, D.R., Marchiando, A.M., Zhang, Y., Shen, L., Sasaki, H., Wang, Y., Long, M., Turner, J.R., 2010. Tight junction-associated MARVEL proteins marvelD3, tricellulin, and occludin have distinct but overlapping functions. Mol. Biol. Cell. 21, 1200–1213.

Ramkrishna, B.S., Venkataraman, S., Srinivasan, P., Dash, P., Young, G.P., Binder, H.J., 2000. Amylase-resistant starch plus oral rehydration solution for cholera. N. Engl. J. Med. 342, 308–313.

Ramanathan, S., Qiu, B., Pooyan, S., Zhang, G., Stein, S., Leibowitz, M.J., Sinko, P.J., 2001. Targeted PEG-based bioconjugates enhance the cellular uptake and transport of a HIV-1 TAT nonapeptide. J. Control Release 77, 199–212.

Rao, A.S., Camilleri, M., Eckert, D.J., Busciglio, I., Burton, D.D., Ryks, M., Wong, B.S., Lamsam, J., Singh, R., Zinsmeister, A.R., 2011. Urine sugars for in vivo gut permeability: validation and comparisons in irritable bowel syndrome-diarrhea and controls. Am. J. Physiol. Gastrointest. Liver Physiol. 301, G919–G928.

Riazuddin, S., Ahmed, Z.M., Fanning, A.S., Lagziel, A., Kitajiri, S., Ramzan, K., Khan, S.N., Chattaraj, P., Friedman, P.L., Anderson, J.M., Belyantseva, I.A., Forge, A., Friedman, T.B., 2006. Tricellulin is a tight-junction protein necessary for hearing. Am. J. Hum. Genet. 79, 1040–1051.

Robertson, S.L., Smedley 3rd, J.G., McClane, B.A., 2010. Identification of a claudin-4 residue important for mediating the host cell binding and action of *Clostridium perfringens* enterotoxin. Infect. Immun. 78, 505–517.

Rosenthal, R., Milatz, S., Krug, S.M., Oelrich, B., Schulzke, J.D., Amasheh, S., Gunzel, D., Fromm, M., 2010. Claudin-2, a component of the tight junction, forms a paracellular water channel. J. Cell. Sci. 123, 1913–1921.

Russell, M.W., Brown, T.A., Mestecky, J., 1981. Role of serum IgA. Hepatobiliary transport of circulating antigen. J. Exp. Med. 153, 968–976.

Saitou, M., Furuse, M., Sasaki, H., Schulzke, J.D., Fromm, M., Takano, H., Noda, T., Tsukita, S., 2000. Complex phenotype of mice lacking occludin, a component of tight junction strands. Mol. Biol. Cell. 11, 4131–4142.

Sanchez-Pulido, L., Martin-Belmonte, F., Valencia, A., Alonso, M.A., 2002. MARVEL: a conserved domain involved in membrane apposition events. Trends Biochem. Sci. 27, 599–601.

Sasaki, H., Matsui, C., Furuse, K., Mimori-Kiyosue, Y., Furuse, M., Tsukita, S., 2003. Dynamic behavior of paired claudin strands within apposing plasma membranes. Proc. Natl. Acad. Sci. U.S.A. 100, 3971–3976.

Schluter, M.A., Margolis, B., 2009. Apical lumen formation in renal epithelia. J. Am. Soc. Nephrol. 20, 1444–1452.

Schmitz, H., Barmeyer, C., Fromm, M., Runkel, N., Foss, H.D., Bentzel, C.J., Riecken, E.O., Schulzke, J.D., 1999. Altered tight junction structure contributes to the impaired epithelial barrier function in ulcerative colitis. Gastroenterology 116, 301–309.

Schulzke, J.D., Gitter, A.H., Makertz, J., Spiegel, S., Seidler, U., Amasheh, S., Saitou, M., Tsukita, S., Fromm, M., 2005. Epithelial transport and barrier function in occludin-deficient mice. Biochim. Biophys. Acta 1669, 34–42.

Schwarz, B.T., Wang, F., Shen, L., Clayburgh, D.R., Su, L., Wang, Y., Fu, Y.X., Turner, J.R., 2007. LIGHT signals directly to intestinal epithelia to cause barrier dysfunction via cytoskeletal and endocytic mechanisms. Gastroenterology 132, 2383–2394.

Sellon, R.K., Tonkonogy, S., Schultz, M., Dielem, L.A., Grenther, W., Balish, E., Rennick, D.M., Sartor, R.B., 1998. Resident enteric bacteria are necessary for development of spontaneous colitis and immune system activation in interleukin-10-deficient mice. Infect. Immun. 66, 5224–5231.

Seth, A., Sheth, P., Elias, B.C., Rao, R., 2007. Protein phosphatases 2A and 1 interact with occludin and negatively regulate the assembly of tight junctions in the CACO-2 cell monolayer. J. Biol. Chem. 282, 11487–11498.

Shen, l., Black, E.D., Witkowski, E.D., Lencer, W.I., Guerriero, V., Schneeberger, E.E., Turner, J.R., 2006. Myosin light chain phosphorylation regulates barrier function by remodeling tight junction structure. J. Cell. Sci. 119, 2095–2106.

Shen, L., Turner, J.R., 2005. Actin depolymerization disrupts tight junctions via caveolae-mediated endocytosis. Mol. Biol. Cell. 16, 3919–3936.

Shen, L., Weber, C.R., Raleigh, D.R., Yu, D., Turner, J.R., 2011. Tight junction pore and leak pathways: a dynamic duo. Annu. Rev. Physiol. 73, 283–309.

Shen, L., Weber, C.R., Turner, J.R., 2008. The tight junction protein complex undergoes rapid and continuous molecular remodeling at steady state. J. Cell Biol. 181, 683–695.

Shin, K., Fogg, V.C., Margolis, B., 2006. Tight junctions and cell polarity. Annu. Rev. Cell. Dev. Biol. 22, 207–235.

Shiue, H., Musch, M.W., Wang, Y., Chang, E.B., Turner, J.R., 2005. Akt2 phosphorylates ezrin to trigger NHE3 translocation and activation. J. Biol. Chem. 280, 1688–1695.

Smales, C., Ellis, M., Baumber, R., Hussain, N., Desmond, H., Staddon, J.M., 2003. Occludin phosphorylation: identification of an occludin kinase in brain and cell extracts as CK2. FEBS Lett. 545, 161–166.

Steed, E., Rodrigues, N.T., Balda, M.S., Matter, K., 2009. Identification of MarvelD3 as a tight junction-associated transmembrane protein of the occludin family. BMC. Cell. Biol. 10. Abst.95.

Stevenson, B.R., Siliciano, J.D., Mooseker, M.S., Goodenough, D.A., 1986. Identification of ZO-1: a high molecular weight polypeptide associated with the tight junction (Zonula Occludens) in a variety of epithelia. J. Cell Biol. 103, 755–766.

Su, L., Nalle, S.C., Shen, L., Turner, E.S., Singh, G., Breskin, L.A., Khramtsova, E.A., Khramtsova, G., Tsai, P.Y., Fu, Y.X., Abraham, C., Turner, J.R., 2013. TNFR2 activates MLCK-dependent tight junction dysregulation to cause apoptosis-mediated barrier loss and experimental colitis. Gastroenterology 145, 407–415.

Su, L., Shen, L., Clayburgh, D.R., Nalle, S.C., Sullivan, E.A., Meddings, J.B., Abraham, C., Turner, J.R., 2009. Targeted epithelial tight junction dysfunction causes immune activation and contributes to development of experimental colitis. Gastroenterology 136, 551–563.

Suzuki, T., Elias, B.C., Seth, A., Shen, L., Turner, J.R., 2009. PKC eta regulates occludin phosphorylation and epithelial tight junction integrity. Proc. Natl. Acad. Sci. U.S.A. 106, 61–66.

Suzuki, T., Yoshinaga, N., Tanabe, S., 2011. Interleukin-6 (IL-6) regulates claudin-2 expression and tight junction permeability in intestinal epithelium. J. Biol. Chem. 286, 31263–31271.

Tamagawa, H., Takahashi, I., Furuse, M., Yoshitake-Kitano, Y., Tsukita, S., Ito, T., Matsuda, H., Kiyono, H., 2003. Characteristics of claudin expression in follicle-associated epithelium of Peyer's patches: preferential localization of claudin-4 at the apex of the dome region. Lab. Invest. 83, 1045–1053.

Tamura, A., Hayashi, H., Imasato, M., Yamazaki, Y., Hagiwara, A., Wada, M., Noda, T., Watanabe, M., Suzuki, Y., Tsukita, S., 2011. Loss of claudin-15, but not claudin-2, causes Na^+ deficiency and glucose malabsorption in mouse small intestine. Gastroenterology 140, 913–923.

Tamura, A., Kitano, Y., Hata, M., Katsuno, T., Moriwaki, K., Sasaki, H., Hayashi, H., Suzuki, Y., Noda, T., Furuse, M., Tsukita, S., 2008. Megaintestine in claudin-15-deficient mice. Gastroenterology 134, 523–534.

Tedde, A., Laura Putignano, A., Bagnoli, S., Congregati, C., Milla, M., Sorbi, S., Genuardi, M., Papi, L., 2008. Interleukin-10 promoter polymorphisms influence susceptibility to ulcerative colitis in a gender-specific manner. Scand. J. Gastroenterol. 43, 712–718.

Turksen, K., Troy, T.C., 2002. Permeability barrier dysfunction in transgenic mice overexpressing claudin 6. Development 129, 1775–1784.

Turner, J.R., 2009. Intestinal mucosal barrier function in health and disease. Nat. Rev. Immunol. 9, 799–809.

Turner, J.R., Cohen, D.E., Mrsny, R.J., Madara, J.L., 2000. Noninvasive in vivo analysis of human small intestinal paracellular absorption: regulation by Na^+-glucose cotransport. Dig. Dis. Sci. 45, 2122–2126.

Turner, J.R., Rill, B.K., Carlson, S.L., Carnes, D., Kerner, R., Mrsny, R.J., Madara, J.L., 1997. Physiological regulation of epithelial tight junctions is associated with myosin light-chain phosphorylation. Am. J. Physiol. 273, C1378–C1385.

Umeda, K., Ikenouchi, J., Katahira-Tayama, S., Furuse, K., Sasaki, H., Nakayama, M., Matsui, T., Tsukita, S., Furuse, M., 2006. ZO-1 and ZO-2 independently determine where claudins are polymerized in tight-junction strand formation. Cell 126, 741–754.

Umeda, K., Ikenouchi, J., Katahira-Tayama, S., Furuse, K., Sasaki, H., Nakayama, M., Matsui, T., Tsukita, S., Furuse, M., 2004. Establishment and characterization of cultured epithelial cells lacking expression of ZO-1. J. Biol. Chem. 279, 44785–44794.

Vaishnava, S., Yamamoto, M., Severson, K.M., Ruhn, K.A., Yu, S., Koren, O., Ley, R., Wakeland, E.K., Hooper, L.V., 2011. The antibacterial lectin RegIIIgamma promotes the spatial segregation of microbiota and host in the intestine. Science 334, 255–258.

Van Der Flier, L.G., Clevers, H., 2009. Stem cells, self-renewal, and differentiation in the intestinal epithelium. Annu. Rev. Physiol. 71, 241–260.

Van Itallie, C.M., Anderson, J.M., 2006. Claudins and epithelial paracellular transport. Annu. Rev. Physiol. 68, 403–429.

Van Itallie, C.M., Fanning, A.S., Bridges, A., Anderson, J.M., 2009. ZO-1 stabilizes the tight junction solute barrier through coupling to the perijunctional cytoskeleton. Mol. Biol. Cell. 20, 3930–3940.

Van Itallie, C.M., Fanning, A.S., Holmes, J., Anderson, J.M., 2010. Occludin is required for cytokine-induced regulation of tight junction barriers. J. Cell. Sci. 2844–2852.

Van Itallie, C.M., Rogan, S., Yu, A., Vidal, L.S., Holmes, J., Anderson, J.M., 2006. Two splice variants of claudin-10 in the kidney create paracellular pores with different ion selectivities. Am. J. Physiol. Renal. Physiol. 291, F1288–F1299.

Van Itallie, C.M., Tietgens, A.J., Logrande, K., Aponte, A., Gucek, M., Anderson, J.M., 2012. Phosphorylation of claudin-2 on serine 208 promotes membrane retention and reduces trafficking to lysosomes. J. Cell. Sci. 125, 4902–4912.

Vetrano, S., Rescigno, M., Rosaria Cera, M., Correale, C., Rumio, C., Doni, A., Fantini, M., Sturm, A., Borroni, E., Repici, A., Locati, M., Malesci, A., Dejana, E., Danese, S., 2008. Unique role of junctional adhesion molecule-a in maintaining mucosal homeostasis in inflammatory bowel disease. Gastroenterology 135, 173–184.

Wada, M., Tamura, A., Takahashi, N., Tsukita, S., 2013. Loss of claudins 2 and 15 from mice causes defects in paracellular Na(+) flow and nutrient transport in gut and leads to death from malnutrition. Gastroenterology 144, 369–380.

Watson, A.J., Chu, S., Sieck, L., Gerasimenko, O., Bullen, T., Campbell, F., McKenna, M., Rose, T., Montrose, M.H., 2005a. Epithelial barrier function in vivo is sustained despite gaps in epithelial layers. Gastroenterology 129, 902–912.

Watson, C.J., Hoare, C.J., Garrod, D.R., Carlson, G.L., Warhurst, G., 2005b. Interferon-gamma selectively increases epithelial permeability to large molecules by activating different populations of paracellular pores. J. Cell. Sci. 118, 5221–5230.

Weber, C.R., Nalle, S.C., Tretiakova, M., Rubin, D.T., Turner, J.R., 2008. Claudin-1 and claudin-2 expression is elevated in inflammatory bowel disease and may contribute to early neoplastic transformation. Lab. Invest. 88, 1110–1120.

Weber, C.R., Raleigh, D.R., Su, L., Shen, L., Sullivan, E.A., Wang, Y., Turner, J.R., 2010. Epithelial myosin light chain kinase activation induces mucosal interleukin-13 expression to alter tight junction ion selectivity. J. Biol. Chem. 285, 12037–12046.

Westphal, J.K., Dorfel, M.J., Krug, S.M., Cording, J.D., Piontek, J., Blasig, I.E., Tauber, R., Fromm, M., Huber, O., 2010. Tricellulin forms homomeric and heteromeric tight junctional complexes. Cell. Mol. Life Sci. 67, 2057–2068.

Witherden, D.A., Verdino, P., Rieder, S.E., Garijo, O., Mills, R.E., Teyton, L., Fischer, W.H., Wilson, I.A., Havran, W.L., 2010. The junctional adhesion molecule JAML is a costimulatory receptor for epithelial gammadelta T cell activation. Science 329, 1205–1210.

Wong, V., 1997. Phosphorylation of occludin correlates with occludin localization and function at the tight junction. Am. J. Physiol. 273, C1859–C1867.

Wright, E.M., 1993. The intestinal Na^+/glucose cotransporter. Annu. Rev. Physiol. 55, 575–589.

Wu, L., Zaborina, O., Zaborin, A., Chang, E.B., Musch, M., Holbrook, C., Shapiro, J., Turner, J.R., Wu, G., Lee, K.Y., Alverdy, J.C., 2004. High-molecular-weight polyethylene glycol prevents lethal sepsis due to intestinal *Pseudomonas aeruginosa*. Gastroenterology 126, 488–498.

Wu, S., Lim, K.C., Huang, J., Saidi, R.F., Sears, C.L., 1998. Bacteroides fragilis enterotoxin cleaves the zonula adherens protein, E-cadherin. Proc. Natl. Acad. Sci. U.S.A. 95, 14979–14984.

Wyatt, J., Vogelsang, H., Hubl, W., Waldhoer, T., Lochs, H., 1993. Intestinal permeability and the prediction of relapse in Crohn's disease. Lancet 341, 1437–1439.

Xu, J., Kausalya, P.J., Phua, D.C., Ali, S.M., Hossain, Z., Hunziker, W., 2008. Early embryonic lethality of mice lacking ZO-2, but not ZO-3, reveals critical and nonredundant roles for individual zonula occludens proteins in mammalian development. Mol. Cell. Biol. 28, 1669–1678.

Yamazaki, Y., Umeda, K., Wada, M., Nada, S., Okada, M., Tsukita, S., 2008. ZO-1- and ZO-2-dependent integration of myosin-2 to epithelial zonula adherens. Mol. Biol. Cell. 19, 3801–3811.

Yoshimoto, S., Loo, T.M., Atarashi, K., Kanda, H., Sato, S., Oyadomari, S., Iwakura, Y., Oshima, K., Morita, H., Hattori, M., Honda, K., Ishikawa, Y., Hara, E., Ohtani, N., 2013. Obesity-induced gut microbial metabolite promotes liver cancer through senescence secretome. Nature 499, 97–101.

Yu, A.S., Cheng, M.H., Angelow, S., Gunzel, D., Kanzawa, S.A., Schneeberger, E.E., Rromm, M., Coalson, R.D., 2009. Molecular basis for cation selectivity in claudin-2-based paracellular pores: identification of an electrostatic interaction site. J. Gen. Physiol. 133, 111–127.

Yu, A.S., McCarthy, K.M., Francis, S.A., McCormack, J.M., Lai, J., Rogers, R.A., Lynch, R.D., Schneeberger, E.E., 2005. Knockdown of occludin expression leads to diverse phenotypic alterations in epithelial cells. Am. J. Physiol. Cell. Physiol. 288, C1231–C1241.

Yu, D., Marchiando, A.M., Weber, C.R., Raleigh, D.R., Wang, Y., Shen, L., Turner, J.R., 2010. MLCK-dependent exchange and actin binding region-dependent anchoring of ZO-1 regulate tight junction barrier function. Proc. Natl. Acad. Sci. U.S.A. 107, 8237–8241.

Zeissig, S., Burgel, N., Gunzel, D., Richter, J., Mankertz, J., Wahnschaffe, U., Kroesen, A.J., Zeitz, M., Fromm, M., Schulzke, J.D., 2007. Changes in expression and distribution of claudin 2, 5 and 8 lead to discontinuous tight junctions and barrier dysfunction in active Crohn's disease. Gut 56, 61–72.

Zhang, J.R., Mostov, K.E., Lamm, M.E., Nanno, M., Shimida, S., Ohwaki, M., Tuomanen, E., 2000. The polymeric immunoglobulin receptor translocates pneumococci across human nasopharyngeal epithelial cells. Cell 102, 827–837.

Zhang, S., Castro, G.A., 1992. Boosted mucosal immune responsiveness in the rat intestine by actively transported hexose. Gastroenterology 103, 1162–1166.

Zhao, H., Shiue, H., Palkon, S., Wang, Y., Cullinan, P., Burkhardt, J.K., Musch, M.W., Chang, E.B., Turner, J.R., 2004. Ezrin regulates NHE3 translocation and activation after Na^+-glucose cotransport. Proc. Natl. Acad. Sci. U.S.A. 101, 9485–9490.

Zolotarevsky, Y., Hecht, G., Koutsouris, A., Gonzalez, D.E., Quan, C., Tom, J., Mrsny, R.J., Turner, J.R., 2002. A membrane-permeant peptide that inhibits MLC kinase restores barrier function in in vitro models of intestinal disease. Gastroenterology 123, 163–172.