

Intestinal mucosal barrier function in health and disease

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Abstract | Mucosal surfaces are lined by epithelial cells. These cells establish a barrier between sometimes hostile external environments and the internal milieu. However, mucosae are also responsible for nutrient absorption and waste secretion, which require a selectively permeable barrier. These functions place the mucosal epithelium at the centre of interactions between the mucosal immune system and luminal contents, including dietary antigens and microbial products. Recent advances have uncovered mechanisms by which the intestinal mucosal barrier is regulated in response to physiological and immunological stimuli. Here I discuss these discoveries along with evidence that this regulation shapes mucosal immune responses in the gut and, when dysfunctional, may contribute to disease.

Mucosa-associated lymphoid tissue

(MALT). The collections of B cells, T cells, plasma cells, macrophages and other antigen-presenting cells found in the mucosal linings of organs including the gastrointestinal tract, lungs, salivary glands and conjunctiva.

Tight junction

Also known as the zonula occludens, this is a site of close apposition of adjacent epithelial cell membranes — kiss points — that create a barrier against the free diffusion of water and solutes.

Complex multicellular organisms interface with their external environments at multiple sites, including mucosae of the airways, oral cavity, digestive tract and genitourinary tract, and the skin. Although the skin is the most visible site of interface, the combined area of the mucosal surfaces is much greater than that of the skin. Mucosae are also the primary sites at which the mucosa-associated lymphoid tissue (MALT) is exposed to and interacts with the external environment. The magnitude of these interactions is greatest in the gastrointestinal tract, which is the largest mucosal surface and is also in continuous contact with dietary antigens and diverse microorganisms. Thus, mucosal surfaces, particularly in the intestines, are crucial sites of innate and adaptive immune regulation.

A central mediator of interactions between MALT and the external environment, including the intestinal lumen, is the epithelium that covers the mucosa. Epithelial cells establish and maintain the barrier, which in the skin is a tight, although not impermeant, seal. By contrast, most mucosal epithelial cells form leaky barriers. This is necessary to support universal functions that include fluid exchange, which occurs at nearly all mucosal surfaces, as well as essential tissue-specific functions. Thus, the precise permeability characteristics of each barrier type differ based on the functions supported. For example, the ion-selective properties of the barrier vary considerably along the length of a nephron to promote or restrict transport of specific ions in each segment¹. Similarly, the ability of tight junctions to discriminate between and restrict passage of solutes based on size (a characteristic known

as size selectivity) varies with location in the intestine, as permeability to larger solutes decreases from the crypt to the villus². In addition to these fixed differences, mucosal permeability of many tissues is adaptable and may be regulated in response to extracellular stimuli, such as nutrients, cytokines and bacteria.

Recent advances have uncovered some of the mechanisms by which physiological and immunological stimuli affect cellular and extracellular components of the intestinal barrier. In this article I review our current understanding of the mechanisms that regulate intestinal barrier integrity, and discuss the hypothesis that the mucosal barrier can shape pro-inflammatory and immunoregulatory responses in the context of homeostasis and disease.

Although they may affect barrier function, dendritic cells (DCs), which extend dendritic processes across the tight junctions in the distal small intestine^{3,4}, and intraepithelial lymphocytes are not discussed here. In addition, the functions of M cells, which are specialized epithelial cells that deliver antigens directly to intraepithelial lymphocytes and to subepithelial lymphoid tissues by transepithelial vesicular transport from the gut lumen, are reviewed elsewhere⁵. Finally, detailed analyses of the interactions between epithelial cells and luminal microorganisms, as well as the intricacies of mucosal immune regulation, have also been recently reviewed elsewhere^{6,7}. This Review therefore focuses on the roles of the epithelial cell barrier in health and disease, with particular emphasis on the junctional complexes between intestinal epithelial cells, which have a crucial role in barrier regulation.

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Mucins

A family of heavily glycosylated proteins that are secreted as large aggregates by mucous epithelial cells.

Unstirred layer

A thin layer of fluid at epithelial cell surfaces that is separated from the mixing forces created by luminal flow and, in the intestine, peristalsis.

Coeliac disease

A chronic inflammatory condition of the upper small intestine in humans that is caused by immunological hypersensitivity to the α -gliadin component of wheat gluten. It can cause severe villous atrophy, which can lead to malabsorption and malnutrition if gluten-containing foods are not removed from the diet.

Anatomy of mucosal barriers

Extracellular components of the barrier. Most mucosal surfaces are covered by a hydrated gel formed by mucins (FIG. 1a). Mucins are secreted by specialized epithelial cells, such as gastric foveolar mucous cells and intestinal goblet cells, and create a barrier that prevents large particles, including most bacteria, from directly contacting the epithelial cell layer⁸. The importance of mucus gel hydration is shown by cystic fibrosis, in which the production of hyperviscous mucus contributes to pulmonary, pancreatic and intestinal disease⁹. Defective mucus production has also been reported in various immunemediated diseases, and spontaneous colitis develops in mice that lack specific mucin genes¹⁰.

Although small molecules pass through the heavily glycosylated mucus layer with relative ease, bulk fluid flow is limited and thereby contributes to the development of an unstirred layer of fluid at the epithelial cell surface. As the unstirred layer is protected from convective mixing forces, the diffusion of ions and small solutes is slowed. In the stomach, this property of the unstirred layer works with epithelial cell bicarbonate secretion to maintain a zone of relative alkalinity at the mucosal surface¹¹. The unstirred layer of the small intestine slows nutrient

absorption by reducing the rate at which nutrients reach the transporting protein-rich microvillus brush border, but may also contribute to absorption by limiting the extent to which small nutrients released by the activities of brush border digestive enzymes are lost by diffusion into the lumen. Unstirred layer defects have not been linked to specific diseases. However, it is interesting to note that increased unstirred layer thickness has been reported in coeliac disease¹², in which it may contribute to nutrient malabsorption.

Cellular components of the mucosal barrier. The primary responsibility for mucosal barrier function resides with the epithelial cell plasma membrane, which is impermeable to most hydrophilic solutes in the absence of specific transporters. Accordingly, direct epithelial cell damage, such as that induced by mucosal irritants or cytotoxic agents, including some drugs used for cancer chemotherapy, results in a marked loss of barrier function. However, in the presence of an intact epithelial cell layer, the paracellular pathway between cells must be sealed. This function is mediated by the apical junctional complex, which is composed of the tight junction and subjacent adherens junction (FIG. 1b). Both tight and

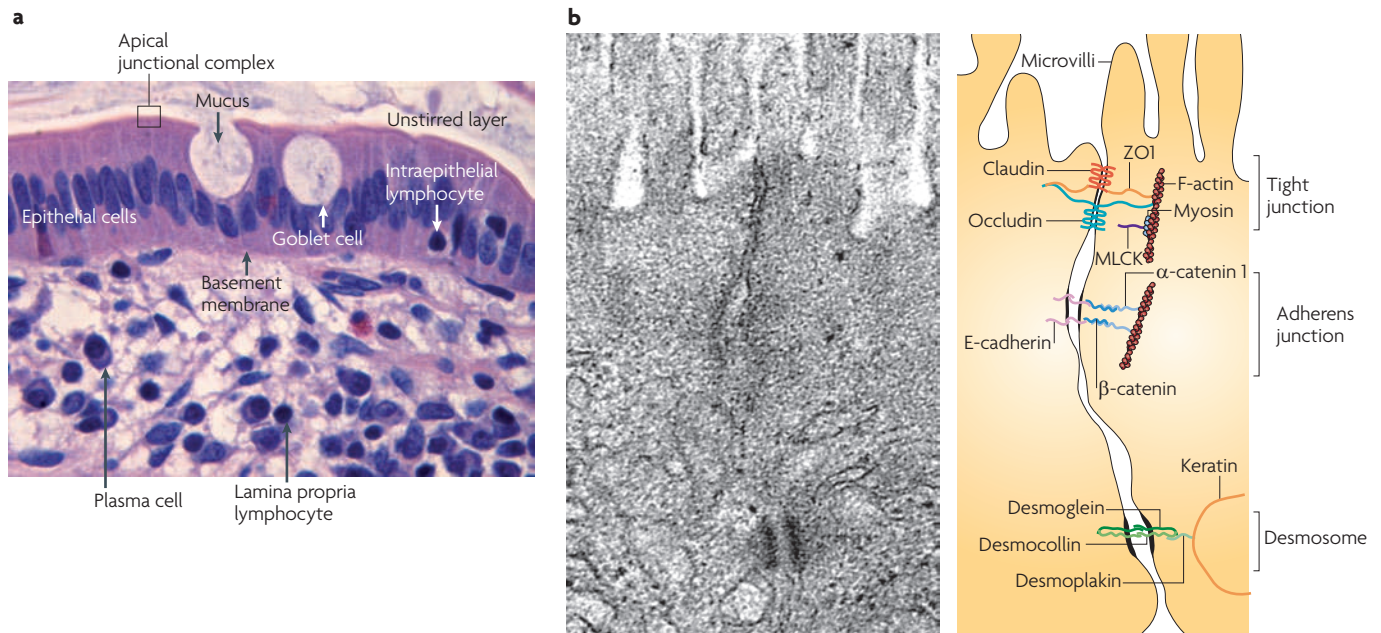


Figure 1 | Anatomy of the mucosal barrier. a | The human intestinal mucosa is composed of a simple layer of columnar epithelial cells, as well as the underlying lamina propria and muscular mucosa. Goblet cells, which synthesize and release mucin, as well as other differentiated epithelial cell types, are present. The unstirred layer, which cannot be seen histologically, is located immediately above the epithelial cells. The tight junction, a component of the apical junctional complex, seals the paracellular space between epithelial cells. Intraepithelial lymphocytes are located above the basement membrane, but are subjacent to the tight junction. The lamina propria is located beneath the basement membrane and contains immune cells, including macrophages, dendritic cells, plasma cells, lamina propria lymphocytes and, in some cases, neutrophils. **b** | An electron micrograph and corresponding line drawing of the junctional complex of an intestinal epithelial cell. Just below the base of the microvilli, the plasma membranes of adjacent cells seem to fuse at the tight junction, where claudins, zonula occludens 1 (ZO1), occludin and F-actin interact. E-cadherin, α -catenin 1, β -catenin, catenin δ 1 (also known as p120 catenin; not shown) and F-actin interact to form the adherens junction. Myosin light chain kinase (MLCK) is associated with the perijunctional actomyosin ring. Desmosomes, which are located beneath the apical junctional complex, are formed by interactions between desmoglein, desmocollin, desmoplakin and keratin filaments.

adherens junctions are supported by a dense perijunctional ring of actin and myosin that, as discussed below, can regulate barrier function.

As implied by the name, the adherens junctions, along with desmosomes, provide the strong adhesive bonds that maintain cellular proximity and are also a site of intercellular communication. Loss of adherens junctions results in disruption of cell–cell and cell–matrix contacts, ineffective epithelial cell polarization and differentiation, and premature apoptosis¹³. Adherens junctions are composed of cadherins, a family of transmembrane proteins that form strong, homotypic interactions with molecules on adjacent cells. The cytoplasmic tail of the epithelial cadherin, *E-cadherin* (also known as cadherin-1), interacts directly with *catenin δ 1* (also known as p120 catenin) and *β -catenin*. In turn, *β -catenin* binds to *α -catenin 1*, which regulates local actin assembly and contributes to development of the perijunctional actomyosin ring.

Adherens junctions are required for assembly of the tight junction, which seals the paracellular space. Tight junctions are multi-protein complexes composed of transmembrane proteins, peripheral membrane (scaffolding) proteins and regulatory molecules that include kinases. The most important of the transmembrane proteins are members of the claudin family, which define several aspects of tight junction permeability, as discussed below. Claudins are expressed in a tissue-specific manner, and mutation or deletion of individual family members can have profound effects on organ function. The role of occludin, a transmembrane tight junction protein that interacts directly with claudins and actin, is less well understood. Peripheral membrane proteins, such as zonula occludens 1 (*ZO1*) and *ZO2*, are crucial to tight junction assembly and maintenance, partly owing to the fact that these proteins include multiple domains for interaction with other proteins, including claudins, occludin and actin.

The tight junction limits solute flux along the paracellular pathway, which is typically more permeable than the transcellular pathway. The tight junction is, therefore, the rate-limiting step in transepithelial transport and the principal determinant of mucosal permeability. Thus, it is important to understand the specific barrier properties of the tight junction, which can be defined in terms of size selectivity and charge selectivity.

At least two routes allow transport across the tight junction, and emerging data suggest that the relative contributions of these types of paracellular transport may be regulated independently^{2,14,15}. One route, the leak pathway, allows paracellular transport of large solutes, including limited flux of proteins and bacterial lipopolysaccharides^{14,15}. Although the size at which particles are excluded from the leak pathway has not been precisely defined, it is clear that materials as large as whole bacteria cannot pass. As might be expected for a route that allows large solutes to cross, the leak pathway does not show charge selectivity. Flux across the leak pathway may be increased by cytokines, including interferon- γ (*IFN γ*) *in vitro* and tumour necrosis factor (*TNF*) *in vitro* and *in vivo*^{15–17}.

A second pathway is characterized by small pores that are thought to be defined by tight junction-associated claudin proteins, which are also primary determinants of charge selectivity^{18–20}. These pores have a radius that excludes molecules larger than 4 Å^{14,15}. Expression of specific claudins varies between organs and even within different regions of a single organ and, as detailed below, can be modified by external stimuli, such as cytokines. Thus, tight junctions show both size selectivity and charge selectivity, and these properties may be regulated individually or jointly by physiological or pathophysiological stimuli.

Interdependence of transport routes

Transepithelial transport requires a selectively permeable barrier. The vectorial nature of transcellular transport, which is required for effective absorption and secretion, generates a transepithelial concentration gradient. Transepithelial transport would, therefore, be ineffective without a tight junction barrier, as diffusion would allow equalization of concentrations on both sides of the epithelium. Thus, active transcellular transport depends on the presence of an intact tight junction barrier. The selective permeability of the tight junction barrier also allows transepithelial gradients to drive passive paracellular transport of ions and water^{17,20}. For example, *claudin-16*, which is necessary for tight junction cation selectivity, is mutated in the human disease familial hypomagnesaemia with hypercalciuria and nephrocalcinosis²⁰. Paracellular absorption of Mg²⁺ and Ca²⁺ in the thick ascending limb of Henle requires a cation-selective tight junction barrier, the absence of which results in urinary loss of Mg²⁺ and Ca²⁺ (REF. 20). Similarly, apical Na⁺–H⁺-exchange protein 3 (*NHE3*) is required for transcellular Na⁺ absorption in the renal tubule and intestine, and also contributes to the transepithelial Na⁺ gradient that drives paracellular water absorption (BOX 1). The tight junction barrier to paracellular Na⁺ efflux prevents dissipation of the gradient established by *NHE3*-mediated transcellular transport.

Tight junction barrier regulation modifies absorption. In addition to providing the driving force for paracellular transport, transcellular transport can activate intracellular signalling events that regulate the tight junction barrier. The best studied physiological example of this is the increased intestinal paracellular permeability that is induced by apical Na⁺–nutrient co-transport²¹. This allows passive paracellular absorption of nutrients and water to amplify transcellular nutrient absorption, particularly when high luminal nutrient concentrations exceed the capacity of apical Na⁺–nutrient co-transporters²². Ultrastructural analyses of intestinal epithelial cells during Na⁺–nutrient co-transport revealed condensation of the perijunctional actomyosin ring, suggesting that cytoskeletal contraction could be involved in this physiological process²¹. Subsequent studies confirmed this hypothesis and showed that the Ca²⁺–calmodulin-dependent serine-threonine protein kinase myosin light chain kinase (*MLCK*) is essential for Na⁺–nutrient co-transport-induced tight junction regulation^{23,24}. Moreover, *MLCK* activation alone is sufficient to increase tight junction permeability, both *in vitro* and *in vivo*^{25,26}. The mechanisms that mediate this

Paracellular pathway

The route of transepithelial transport that involves passive movement through the space between adjacent cells.

Adherens junction

Also known as the zonula adherens, this junction is immediately subjacent to the tight junction and requires the activity of lineage-specific Ca²⁺-dependent adhesion proteins, termed cadherins.

Desmosome

An adhesive junction that connects adjacent epithelial cells. These junctions are composed of multiple protein subunits and are the points where keratin filaments attach to the plasma membrane.

Claudin

From the Latin *claudere*, meaning 'to close', members of this family of transmembrane proteins are variably expressed by specific epithelial cell types and thereby contribute to the unique barrier properties of different tissues.

Occludin

The first transmembrane tight junction protein identified. The function of occludin remains controversial but it is likely to have roles in barrier regulation and tumour suppression. It also serves as a cofactor in hepatitis C virus entry.

Zonula occludens 1

A peripheral membrane, or plaque, protein containing multiple protein interaction domains that, along with the related protein zonula occludens 2, is required for tight junction assembly.

Transcellular pathway

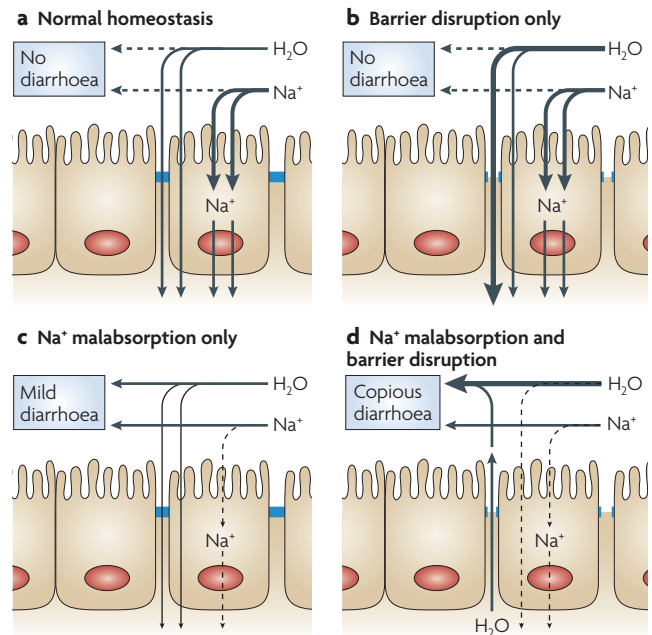
The route of transepithelial transport that involves active or passive movement across cell membranes, usually as a result of the action of specific transport channels.

Transepithelial transport

The sum of transport through the transcellular and paracellular pathways.

Box 1 | **Coordination of transcellular and paracellular transport**

The signal transduction pathway that enhances paracellular permeability following initiation of Na⁺-glucose co-transport has been characterized and includes activation of mitogen-activated protein kinase cascades, trafficking of Na⁺-H⁺-exchange protein 3 (NHE3) to the apical membrane and myosin light chain kinase (MLCK) activation^{23–24,107}. Increased NHE3 activity at the apical membrane enhances Na⁺ absorption, which (along with the Na⁺ co-transport) increases the transcellular Na⁺ gradient and promotes paracellular water absorption (see the figure, part a). The MLCK-dependent increase in tight junction permeability enhances paracellular absorption of water and small solutes, such as glucose, that are concentrated in the unstirred layer. This might contribute to the observation that rehydration after infectious diarrhoea, or even exercise, is more effective when oral rehydration solutions contain Na⁺ and carbohydrates¹⁰⁸ (see the figure, part b). By contrast, tumour necrosis factor activates both protein kinase Cα (PKCα) and MLCK to cause diarrhoea. NHE3 inhibition, mediated by PKCα, reduces the transcellular Na⁺ gradient that normally drives water absorption (see the figure, part c). This synergizes with MLCK-dependent increases in tight junction permeability to allow water to flow into the lumen, thereby causing large-volume diarrhoea¹⁷ (see the figure, part d).



MLCK-dependent tight junction regulation have been studied in detail owing to their contributions to nutrient and water absorption under normal physiological conditions and to the diarrhoea associated with acute barrier loss (BOX 1).

Barrier regulation by immune stimuli

The ability of cytokines, such as TNF and IFN γ , to regulate the function of the tight junction barrier was first described 20 years ago²⁷. Since then, increased tight junction protein transcription, vesicular removal of proteins from the tight junction, tight junction protein degradation, kinase activation and cytoskeletal modulation have all been proposed to mediate cytokine-induced loss of tight junction barrier function. Although extensive apoptosis of epithelial cells may also cause barrier loss, the relevance of single-cell apoptosis to barrier dysfunction remains controversial owing to differing results in diverse experimental systems.

Tight junction regulation through the cytoskeleton.

TNF and IFN γ modify tight junction barrier function in intestinal^{27,28}, renal²⁹, pulmonary³⁰ and salivary gland³¹ epithelia as well as between endothelial cells³². The effects of TNF on barrier integrity have been best studied in the gut, where this cytokine has a central role in many diseases associated with intestinal epithelial barrier dysfunction, including inflammatory bowel disease³³, intestinal ischaemia^{28,34} and graft-versus-host disease³⁵. For example, although the effect of therapy

with TNF-specific antibodies may be largely due to the overall reduction in inflammation, it is notable that this treatment corrects barrier dysfunction in patients with Crohn's disease³⁶. Increased mucosal TNF production may also contribute to increased intestinal permeability and susceptibility to colitis in mice with defective mucin biosynthesis^{10,37}.

MLCK has been shown to have a central role in TNF-induced epithelial and endothelial barrier dysregulation, both *in vitro* and *in vivo*^{16,38–41}. Similar to Na⁺-nutrient co-transport, TNF-induced MLCK activation seems to increase paracellular flux through the leak pathway^{16–17,23}. This MLCK activation occurs as a result of increased enzymatic activity and increased MLCK transcription and translation, both *in vitro* and *in vivo*^{16,40,42}. Similarly, MLCK expression and activity are increased in intestinal epithelial cells of patients with inflammatory bowel disease⁴³. The degree to which MLCK expression and activity are increased correlates with local disease activity, suggesting that these processes may be regulated by local cytokine signalling in these patients⁴³. MLCK is also a fundamental intermediate in barrier dysfunction induced by the TNF family member LIGHT (also known as TNFSF14)^{17,44}, interleukin-1 β (IL-1 β)⁴⁵, enteropathogenic *Escherichia coli* infection³⁹, *Helicobacter pylori* infection⁴⁶, giardiasis⁴⁷, lipopolysaccharide^{48,49} and the ethanol metabolite acetaldehyde⁵⁰. Thus, MLCK activation can be viewed as a common final pathway of acute tight junction regulation in response to a broad range of immune and infectious stimuli.

Thick ascending limb of Henle

The portion of the nephron just proximal to the distal tubule. This is a site of active Na⁺, K⁺ and Cl⁻ reabsorption, which generates an electrochemical gradient that drives paracellular reabsorption of Mg²⁺ and Ca²⁺.

Myosin light chain kinase

The Ca²⁺-calmodulin-dependent kinase that phosphorylates myosin II regulatory light chain at serine 19 and threonine 18 to activate myosin ATPase.

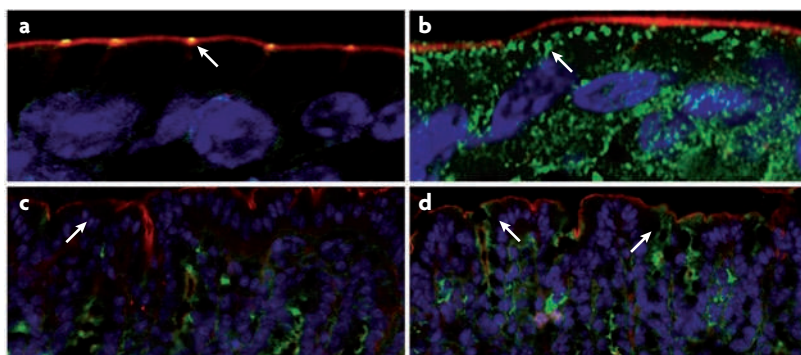


Figure 2 | Differential effects of cytokines on tight junction structure and function. **a** | Occludin is normally concentrated at the tight junction (arrow) in jejunal villus epithelium. The perijunctional actomyosin ring (red) and nuclei (blue) are shown for reference. **b** | Exogenous tumour necrosis factor increases myosin light chain kinase activity, which causes perijunctional myosin II regulatory light chain phosphorylation and triggers occludin (green) endocytosis (arrow). This increases flux across the tight junction leak pathway and enhances paracellular permeability to large solutes. **c** | Claudin-2 expression (green) is limited to crypt epithelial cells; it is not expressed by epithelial cells at the mucosal surface in the normal colon. F-actin (red) and nuclei (blue) are shown for reference. **d** | Interleukin-13 can stimulate claudin-2 expression (green) in surface epithelial cells (arrows). This increases flux across small tight junction pores, thereby enhancing paracellular cation permeability.

Although it is clear that MLCK phosphorylates myosin II regulatory light chain (MLC) within the perijunctional actomyosin ring to activate myosin ATPase activity, the subsequent molecular events that cause increased permeability are poorly defined. However, recent work suggests that ZO1, which interacts directly with actin, occludin, claudins and other proteins, may be an essential effector of perijunctional actomyosin ring-mediated tight junction regulation^{25,51,52}. Endocytic removal of the transmembrane protein occludin from the tight junction is also common in actomyosin-dependent, cytokine-mediated tight junction regulation^{38,53} (FIG. 2). *In vitro* studies of tight junction regulation induced by LIGHT suggest that occludin endocytosis occurs via caveolae and that inhibition of this process can prevent loss of barrier integrity despite MLCK activation⁴⁴. Caveolar endocytosis of occludin has also been associated with loss of epithelial and endothelial tight junction barrier function in response to actin disruption⁵⁴ and chemokine signalling⁵⁵, respectively, *in vitro*. However, other mechanisms of occludin removal may also be involved as *in vitro* studies have shown that IFN γ -induced occludin internalization is mediated by myosin ATPase-dependent macropinocytosis^{53,56}, and occludin cleavage may even modify the barrier to enhance transepithelial migration of inflammatory cells in the lung⁵⁷. Nevertheless, further work is necessary to define the contributions of occludin and endocytosis to *in vivo* tight junction regulation. This is particularly important as, despite numerous *in vitro* studies demonstrating a role for occludin in tight junction function, intestinal barrier function is intact in occludin-deficient mice⁵⁸. Future analyses of the response of occludin-deficient mice to stress, with particular reference to intestinal tight junction

Caveolae

Specialized flask-shaped invaginations of the plasma membrane that contain the protein caveolin-1 and cholesterol. These proteins mediate uptake of some extracellular materials and are involved in cell signalling.

function, as well as studies of potential compensatory changes in other, perhaps not yet discovered, proteins that allow these mice to avoid intestinal disease will be of great interest.

Although MLCK activation is clearly important, it is not the only means of cytoskeletal tight junction regulation. Other mediators include myosin ATPase⁵¹, the activity of which is regulated by MLC phosphorylation^{59–60}; members of the Rho kinase family^{41,51,53,61}, which can both phosphorylate MLC directly⁵⁹ and inhibit MLC phosphatase⁶²; and AMP-activated protein kinase⁵², which is activated during stress and can also directly phosphorylate MLC⁶³. Moreover, Rho kinases and AMP-activated protein kinase each have diverse effects that are separate from myosin function, and it is likely that at least some of these contribute to tight junction regulation.

Tight junction permeability and claudin expression.

As discussed above, regulation of perijunctional actomyosin provides a means of rapidly and reversibly regulating the paracellular leak pathway. By contrast, synthesis and trafficking of claudin proteins provides a means of regulating tight junction pores over longer periods. The perijunctional actomyosin ring does not seem to be directly involved in this mechanism of barrier regulation, which is consistent with the fact that claudins do not interact with actin directly. Expression of specific claudin proteins changes during development, differentiation and disease and in response to stressors — including cytokines — in intestinal⁶⁴, renal⁶⁵ and alveolar⁶⁶ epithelial cells. In addition to affecting barrier function, altered patterns of claudin expression may have other consequences. For example, claudin proteins have been associated with control of cell and organ growth. It may be, therefore, that some changes in claudin protein expression enhance cell proliferation and regeneration, as might be necessary to compensate for cell loss in colitis. This may explain the increased *claudin-1* expression by intestinal epithelial cells of patients with inflammatory bowel disease⁶⁷. However, claudin-1 has also been shown to enhance neoplastic transformation, tumour growth and metastasis in experimental models⁶⁸. Thus, changes in claudin expression may have undesired consequences.

One common change in claudin expression that directly affects barrier function is the increased *claudin-2* expression by intestinal epithelial cells in animal models of colitis (FIG. 2) and patients with inflammatory bowel disease⁶⁴. Consistent with this, IL-13 and IL-17, which are increased in the mucosa of patients with colitis^{64,69}, reduce barrier function and increase claudin-2 expression in cultured intestinal epithelium monolayers^{64,70}. *In vitro* studies have shown that claudin-2 expression increases the number of pores that allow paracellular flux of cations, predominantly Na⁺, and small molecules with radii less than 4 Å^{14,71}, and recent analyses have provided new insight into the structure of these pores⁷². However, it is not clear whether increased claudin-2 expression contributes to disease progression or, as proposed above for claudin-1, is an adaptive

Table 1 | Barrier defects associated with intestinal disease

Disease or model	Cause of barrier defect	Timing of barrier defect	Effects on tight junction proteins and cytoskeleton	Role of commensal microorganisms	Refs
Crohn's disease	Unknown, associated with a frameshift insertion at nucleotide 3020 of <i>NOD2</i>	Before clinical onset and before clinical relapse	Claudin-2 upregulation, MLCK activation and occludin downregulation	Antibiotics can be helpful in maintaining remission	43,64,109
Ulcerative colitis	Unknown	Not well studied	Claudin-2 upregulation, MLCK activation and occludin downregulation	Not defined	43,64,109
IL-10-deficient mice	Immune signalling (IL-10 deficiency)	Before clinical onset	Not defined	Eradication of microorganisms prevents disease	76,97
Coeliac disease	Not well studied	Not well studied	Not defined	No demonstrated role	110,111
Systemic T cell activation	MLCK activation	Associated with acute diarrhoea	MLCK activation and occludin endocytosis	Not defined	38
CD4 ⁺ CD45RB ^{hi} T cell adoptive transfer model of colitis	Cytokine release and epithelial cell damage	With clinical onset	MLCK activation, claudin-2 upregulation and occludin endocytosis	Eradication of microorganisms reduces severity	112–114
SAMP1/yit mice	Unknown	Before clinical onset	Claudin-2 upregulation and occludin downregulation	Not defined	77
DSS-induced colitis	Epithelial cell damage	After DSS treatment but before clinical onset	Not defined	Eradication of microorganisms exacerbates disease	80
TNBS-induced colitis	Immune signalling	Not well studied	Claudin-18 upregulation	Probiotics can reduce disease severity	92
Mucin-2-deficient mice	Not well studied	Not well studied	Not defined	Not defined	10,37
MDR1A-deficient mice	Unknown, follows increased CCL2 production	After immune cell activation	Reduced occludin phosphorylation	Increased epithelial cell response to LPS precedes disease	115,116
JAM-A-deficient mice	JAM-A deficiency	Not well studied	JAM-A deficiency	Not defined	89,90
<i>Clostridium difficile</i> -induced colitis	Actomyosin disruption and glucosylation of Rho proteins	With release of toxin and disease onset	Loss of ZO1 and ZO2	Antibiotics predispose to disease	117
EPEC infection	Type III secretion (of bacterial proteins)	After infection	MLCK activation and occludin endocytosis	Not defined	118
Graft-versus-host disease	Associated with elevated TNF production	After clinical onset	Not defined	Eradication of microorganisms limits disease	35

CCL2, CC-chemokine ligand 2; DSS, dextran-sulphate sodium; EPEC, enteropathogenic *Escherichia coli*; IL-10, interleukin-10; JAM-A, junctional adhesion molecule-A; LPS, lipopolysaccharide; MDR1A, multidrug resistance protein 1a; MLCK, myosin light chain kinase; *NOD*, nucleotide-binding oligomerization domain; TNF, tumour necrosis factor; TNBS, trinitrobenzene sulphonic acid; ZO, zonula occludens.

response that promotes homeostasis. Nevertheless, the consistency of intestinal epithelial claudin-2 upregulation in disease suggests that this should be a subject of further study.

Barrier function and immunity

Barrier loss is associated with disease risk. A large body of circumstantial evidence suggests that intestinal barrier dysfunction is associated with the pathogenesis of Crohn's disease. This includes the observation that a subset of first-degree relatives of patients with Crohn's disease has increased intestinal permeability despite being completely healthy⁷³. Interestingly, genetic analyses have linked increased intestinal permeability in these healthy relatives to the Crohn's disease-associated frameshift

insertion at nucleotide 3020 of the gene encoding the cytoplasmic sensor nucleotide-binding oligomerization domain-containing 2 (*NOD2*)⁷⁴ (TABLE 1), and the same *NOD2* mutation has been associated with decreased *IL-10* production by peripheral blood mononuclear cells⁷⁵. Thus, one explanation for the increased intestinal permeability observed in some of these healthy relatives is that they have subclinical mucosal immune activation, perhaps with increased TNF production, that leads to barrier dysfunction without overt disease. This hypothesis would also explain why IL-10-deficient and SAMP1/yit mice, which develop spontaneous colitis and enteritis, respectively, show increased intestinal permeability before disease onset^{76,77}. The suggestion that permeability is merely a sensitive indicator of mucosal

SAMP1/yit mice

An outbred mouse strain that spontaneously develops a chronic intestinal inflammation similar to human Crohn's disease.

immune activation would also explain the observation that, during clinical remission, increased intestinal permeability is a predictor of relapse in patients with Crohn's disease⁷⁸. However, barrier dysfunction must also be regarded as a potential contributor to disease progression.

The classic experiment showing a role for epithelial cell function in maintaining mucosal immune homeostasis used chimeric mice in which dominant-negative cadherin was expressed in some intestinal epithelial stem cells, resulting in disruption of the adherens junctions¹³. This led to profound epithelial cell defects that included incomplete polarization, brush border and actin cytoskeletal disruption, accelerated crypt-villus migration and premature apoptosis¹³. Moreover, the mucus layer overlying epithelial cells expressing dominant-negative cadherin was disrupted and numerous adherent bacteria were present at these sites¹³. Patchy, transmural enteritis developed in these mice and was limited to regions where epithelial cells expressed dominant-negative cadherin⁷⁹. In addition, epithelial cell dysplasia was occasionally present in these areas⁷⁹. This study has often been cited as evidence that tight junction barrier loss is sufficient to cause inflammatory bowel disease and, although tight junctions were not specifically examined, they were likely to be defective. However, although this important study shows that apical junctional complex disruption causes enteritis, the broad range of epithelial cell defects induced makes it impossible to draw conclusions regarding the specific contributions of the tight junction to homeostasis or disease. Similarly, although dextran-sulphate sodium (DSS)-induced colitis is associated with increased intestinal permeability, this is the result of widespread epithelial cell damage⁸⁰ rather than targeted dysfunction of the tight junction barrier. Altered sensitivity of genetically modified mice to DSS must therefore be viewed in the context of epithelial cell injury and repair^{81–83} and cannot be interpreted as a function of disrupted tight junction permeability alone.

Isolated barrier loss is insufficient to cause disease.

One study has reported an *in vivo* model of barrier dysfunction induced by direct activation of endogenous tight junction regulatory mechanisms²⁶. Although no overt developmental defects or disease developed in mice in which intestinal epithelial cells transgenically expressed constitutively active MLCK, increased intestinal tight junction permeability was observed²⁶. This increase in paracellular permeability was quantitatively similar to that induced by activation of Na⁺-glucose co-transport and was not an indiscriminate loss of barrier function²⁶, as occurs with exposure to DSS or dominant-negative cadherin expression. In addition, MLCK inhibition normalized MLC phosphorylation in epithelial cells and paracellular permeability²⁶, suggesting that compensatory changes in pathways that regulate MLC phosphorylation or barrier function were not induced. Moreover, growth of these mice was normal, and intestinal morphology, enterocyte structure, tight junction and adherens junction organization, actomyosin and brush border architecture, and

epithelial proliferation, migration and apoptosis were all similar to that observed in wild-type mice²⁶. This suggests that the effects of constitutively active MLCK expression in these mice were specific to its effect on the tight junction. Thus, consistent with the presence of barrier dysfunction in healthy relatives of patients with Crohn's disease, these mice provide evidence to suggest that increased tight junction permeability in the absence of more extensive epithelial cell dysfunction is insufficient to cause intestinal disease. Further analysis of mice expressing constitutively active MLCK did, however, provide evidence of mucosal immune cell activation, including increased numbers of lamina propria T cells, enhanced mucosal IFN γ , TNF and IL-10 transcription and repositioning of CD11c⁺ DCs to the superficial lamina propria²⁶. Thus, despite being insufficient to cause disease, chronic increases in intestinal permeability as a result of continuous activation of a physiological pathway of tight junction regulation does lead to mucosal immune cell activation.

Because a subset of healthy first-degree relatives of patients with Crohn's disease will ultimately develop the disease, and because a similar fraction of these healthy relatives have increased intestinal permeability, it has been suggested that tight junction barrier dysfunction is one factor that contributes to the development of inflammatory bowel disease⁸⁴. This hypothesis is consistent with a case report documenting increased intestinal permeability 8 years prior to disease onset in a healthy relative of a patient with Crohn's disease⁸⁵. However, given that the subject of the case report had two first-degree relatives with Crohn's disease and was, therefore, at increased risk of developing the disease regardless of intestinal permeability measures, this report does not provide evidence that barrier dysfunction itself is related to disease onset.

The relationship between barrier dysfunction and disease has been assessed using mice with a constitutively active MLCK transgene, which have increases in permeability that are quantitatively and qualitatively similar to those in healthy relatives of patients with Crohn's disease. Recombination-activating gene 1-knockout mice (which lack mature B and T cells) expressing constitutively active MLCK, as well as littermates that did not express constitutively active MLCK, received CD4⁺CD45RB^{hi} naive T cells from wild-type mice (the CD4⁺CD45RB^{hi} T cell adoptive transfer colitis model). Both groups of mice developed colitis. However, the disease developed more rapidly and was more severe, in clinical, biochemical and histological terms, in the mice that expressed constitutively active MLCK²⁶. Although one could argue that this accelerated disease progression was due to effects of constitutively active MLCK expression beyond tight junction regulation, other epithelial cell defects were not apparent in these mice (see above), perhaps because constitutively active MLCK targets an endogenous signalling pathway. Thus, targeted increases in intestinal epithelial tight junction permeability through constitutive activation of the pathophysiologically relevant MLCK pathway are sufficient to accelerate the onset and enhance the severity of immune-mediated colitis.

CD4⁺CD45RB^{hi} T cell adoptive transfer colitis model

A well-characterized model of chronic colitis induced by transfer of CD4⁺CD45RB^{hi} (naive) T cells from healthy wild-type mice into immunodeficient syngeneic recipients.

Conversely, the data above also suggest that inhibition of MLCK, which is sufficient to reverse acute TNF-induced barrier loss³⁸, may have therapeutic benefit in immune-mediated colitis. This notion is supported by a preliminary *in vivo* report using mice with a knockout of non-muscle MLCK⁸⁶. However, as non-muscle MLCK has many functions, including an essential role in neutrophil transendothelial migration⁸⁷, the results of this report may reflect changes beyond tight junction barrier preservation. Another recent study showed that a peptide capable of enhancing small-intestinal barrier function reduced the extent of mucosal inflammation in IL-10-deficient mice⁸⁸. However, this result must be interpreted with caution, as the peptide used (which is thought to antagonize the putative extracellular pathway signalling molecule zonulin) activates an undefined regulatory pathway and the overall physiology of peptide-treated mice has not been examined in detail. Thus, although the finding is intriguing, further investigation is necessary to determine whether barrier preservation, by MLCK inhibition or other means, can prevent or reverse intestinal disease.

Two studies have recently reported that junctional adhesion molecule-A (JAM-A)-deficient mice have reduced intestinal barrier function and increased rates of intestinal epithelial cell proliferation and apoptosis^{89,90}. These mice also have intestinal mucosal immune cell activation as defined by increased numbers of neutrophils in the intestinal mucosa^{89,90}. In addition, one study reported an increase in the number of distal-colonic lymphoid aggregates in JAM-A-deficient mice⁹⁰, although neither lymphoid aggregates nor enhanced mucosal cytokine expression was detected in a subsequent analysis⁸⁹. However, as JAM-A is widely expressed by epithelial cells, endothelial cells, platelets, antigen-presenting cells, circulating neutrophils, monocytes, lymphocytes and platelets, and as the mice studied were not epithelial cell-specific knockouts, it is not clear whether the observed effects were due to altered tight junction integrity, changes in epithelial cell shape⁹¹, an increased rate of epithelial cell apoptosis⁹⁰ or trapping of neutrophils within mucosal vessels owing to the loss of endothelial cell-expressed JAM-A. Nevertheless, it is intriguing that JAM-A-deficient mice showed increased sensitivity to DSS-induced colitis^{89,90}. By contrast, the sensitivity of mice with an endothelium-specific JAM-A deficiency to DSS was similar to that of wild-type control mice, indicating that the response of mice with universal JAM-A deficiency was not solely due to loss of endothelial JAM-A⁸⁹. Unfortunately, the use of mice with a universal JAM-A deficiency and the presence of other epithelial cell abnormalities, particularly increased epithelial cell turnover, in the absence of exogenous stimuli limits interpretation of these studies. Even so, data from JAM-A-deficient mice do support the hypothesis that mucosal barrier loss can enhance the severity of colitis.

Overall, data from both human subjects and mouse experimental models show that defects in tight junction barrier function are insufficient to cause disease. However, several lines of evidence suggest that increased paracellular permeability can increase mucosal immune

activity, enhance disease progression and severity and, possibly, be a risk factor for development of disease. Finally, although much more investigation is needed, early reports indicate that restoration of tight junction barrier function may be effective, either alone or in combination with other agents, in preventing disease in at-risk individuals or maintaining remission in patients with inflammatory bowel disease.

Barrier loss activates immunoregulatory processes. Why is tight junction barrier dysfunction alone insufficient to cause disease? Endoscopic mucosal resection, which removes the epithelium and mucosa completely and therefore causes barrier loss far greater than that caused by targeted tight junction dysfunction, is insufficient to cause chronic disease. Therefore, immunoregulatory mechanisms must be induced by the host following barrier loss to prevent inappropriate inflammatory responses, as mucosal damage is a daily occurrence in the gastrointestinal tract. Moreover, these immunoregulatory mechanisms must be robust because, even in patients with inflammatory bowel disease, endoscopic mucosal resection is insufficient to initiate a relapse to active disease.

The increased transcription of IL-10 in the intestinal mucosa of mice with a constitutively active MLCK transgene may provide a clue to which immunoregulatory processes are triggered by barrier dysfunction. In addition, responses to transient barrier loss have been used to explore mucosal immunoregulation⁹². In this model, intrarectal administration of ethanol caused transient epithelial cell damage, mucosal erosion and barrier loss. The induction of barrier loss was followed by an increase in the numbers of IFN γ - and IL-10-producing lamina propria mononuclear cells and lamina propria CD4⁺CD25⁺ T cells that express latency-associated peptide (LAP) on their surface⁹². Remarkably, such ethanol administration conferred protection from subsequent trinitrobenzene sulphonic acid (TNBS)-induced colitis, and this protection required the presence of LAP⁺ T cells⁹². The induction of these LAP⁺ T cells was shown to depend on CD11c⁺ DCs, Toll-like receptor 2 signalling and a normal luminal microorganism population⁹².

Although further study is needed to understand the mechanisms of LAP⁺ T cell induction by transient mucosal damage, it is interesting that in different experimental systems increased intestinal epithelial cell tight junction permeability increased the number of CD11c⁺ DCs in the superficial lamina propria⁸⁶, and that interactions with intestinal epithelial cells enhance the ability of bone marrow-derived CD11c⁺ DCs to induce the differentiation of regulatory T cells⁹³. Together, these observations support the hypothesis that the interactions between CD11c⁺ DCs and luminal materials are regulated by tight junction permeability and are also central to mucosal immune homeostasis.

Other data also support important roles for luminal material, particularly microorganisms and their products, in mucosal immune regulation⁹⁴. For example, antibiotics can be helpful in the management of Crohn's disease⁹⁵. Although the mechanisms by which antibiotics

Latency-associated peptide
A small peptide derived from the N-terminal region of the TGF β precursor protein; it can modulate TGF β signalling.

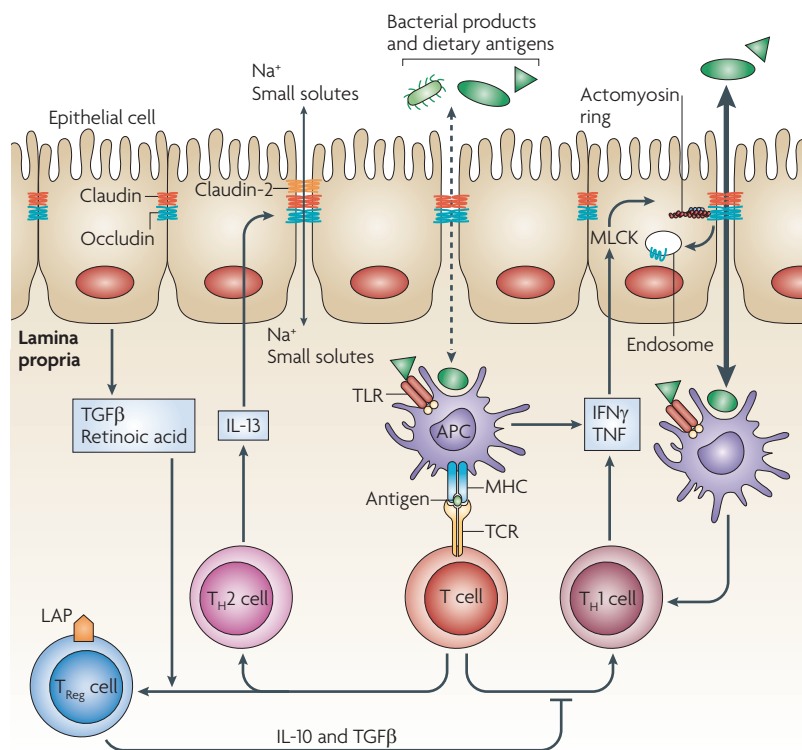


Figure 3 | The epithelium and tight junction as integrators of mucosal homeostasis.

Minor barrier defects allow bacterial products and dietary antigens to cross the epithelium and enter the lamina propria. This can lead to disease or homeostasis. If the foreign materials are taken up by antigen-presenting cells (APCs), such as dendritic cells, that direct the differentiation of T helper 1 (T_H1) or T_H2 cells, disease can develop. In this process, APCs and T_H1 cells can release tumour necrosis factor (TNF) and interferon- γ ($IFN\gamma$), which signal to epithelial cells to increase flux across the tight junction leak pathway, thereby allowing further leakage of bacterial products and dietary antigens from the lumen into the lamina propria and amplifying the cycle of inflammation. This may, ultimately, culminate in established disease. Alternatively, interleukin-13 (IL-13) released by T_H2 cells increases flux across small cation-selective pores, potentially contributing to ongoing disease. Conversely, homeostasis may dominate if APCs promote regulatory T (T_{Reg}) cell differentiation, which can be enhanced by epithelial cell-derived transforming growth factor- β (TGF β) and retinoic acid. The T_{Reg} cells display latency-associated peptide (LAP) on their surfaces and may secrete IL-10 and TGF β to prevent disease. MLCK, myosin light chain kinase; TLR, Toll-like receptor; TCR, T cell receptor.

promote maintenance of remission in patients with Crohn's disease are not defined, they may be related to the observation that luminal microorganisms are required in experimental models of inflammatory bowel disease that include IL-10-deficient mice^{96,97} and adoptive transfer colitis. Microorganisms are also necessary for the development of increased intestinal permeability before the onset of overt disease in IL-10-deficient mice⁷⁶. Although this observation is not entirely understood, one interpretation is that the limited flux of microbial products that normally occurs across intact intestinal epithelial tight junctions is sufficient to trigger mucosal immune activation in IL-10-deficient mice. This mucosal immune activation could, in turn, result in the release of cytokines that cause increased intestinal permeability and accelerate disease progression (FIG. 3). However, in addition to immune cells, epithelial cells can respond to foreign materials. Lipopolysaccharide, for

example, enhances paracellular permeability in pulmonary and intestinal epithelium^{48,49}. Conversely, epithelial Toll-like receptor 2 activation may restore barrier function⁹⁸. It will therefore be of interest to define the microbial products, dietary components and other factors that influence mucosal immune status following alterations in intestinal epithelial tight junction permeability.

Tight junctions integrate mucosal homeostasis

One interpretation of the available data is that the tight junction barrier integrates the relationship between luminal material and mucosal immune function (FIG. 3). In most individuals, this is a healthy relationship in which regulated increases in tight junction permeability or transient epithelial cell damage trigger the release of pro-inflammatory cytokines, such as TNF and $IFN\gamma$, as well as immunoregulatory responses. Immunoregulatory responses may include DC conditioning by epithelial cell-derived transforming growth factor- β (TGF β) and retinoic acid, both of which can enhance regulatory T cell differentiation^{99,100}. This precarious balance between pro-inflammatory and immunoregulatory responses can fail if there are exaggerated responses to pro-inflammatory cytokines, as may be associated with mutations in the endoplasmic reticulum stress response transcription factor X-box-binding protein 1 (XBP1) (REF. 101), insufficient IL-10 production (as in IL-10-deficient mice and, possibly, in patients with *IL10* promoter polymorphisms¹⁰²) or inadequate immune tolerance to luminal antigens and microbial products⁹⁴ (potentially because of *NOD2* mutations in patients with inflammatory bowel disease^{103–106}). As a result, mucosal immune cell activation may proceed unchecked and the release of cytokines, including TNF and IL-13, may enhance barrier loss that, in turn, allows further leakage of luminal material and perpetuates the pro-inflammatory cycle. This model highlights the roles of a susceptible host and defective epithelial cell barrier function as key components of the pathogenesis of intestinal inflammatory disease and explains the crucial role of the epithelial barrier in moulding mucosal immune responses.

Conclusions

Significant progress has been made in understanding the processes by which physiological and pathophysiological stimuli, including cytokines, regulate the tight junction. Early reports suggest that restoration of tight junction barrier function may have benefit^{38,86}. However, the mechanisms of tight junction regulation will have to be defined in greater detail if they are to be viable pharmacological targets. Conversely, recent data have emphasized the presence of immune mechanisms that maintain mucosal homeostasis despite barrier dysfunction, and some data suggest that the epithelium orchestrates these immunoregulatory events through direct interactions with innate immune cells. Future elucidation of the processes that integrate mucosal barrier function, or dysfunction, and immune regulation to prevent or perpetuate disease may lead to novel therapeutic approaches for diseases associated with increased mucosal permeability.

1. Kiuchi-Saishin, Y. *et al.* Differential expression patterns of claudins, tight junction membrane proteins, in mouse nephron segments. *J. Am. Soc. Nephrol.* **13**, 875–886 (2002).
2. Fihn, B. M., Sjoqvist, A. & Jodal, M. Permeability of the rat small intestinal epithelium along the villus–crypt axis: effects of glucose transport. *Gastroenterology* **119**, 1029–1036 (2000).
3. Chiappa, M., Rescigno, M., Huang, A. Y. & Germain, R. N. Dynamic imaging of dendritic cell extension into the small bowel lumen in response to epithelial cell TLR engagement. *J. Exp. Med.* **203**, 2841–2852 (2006).
4. Niess, J. H. *et al.* CX₃CR1-mediated dendritic cell access to the intestinal lumen and bacterial clearance. *Science* **307**, 254–258 (2005).
5. Neutra, M. R. & Kozlowski, P. A. Mucosal vaccines: the promise and the challenge. *Nature Rev. Immunol.* **6**, 148–158 (2006).
6. Artis, D. Epithelial-cell recognition of commensal bacteria and maintenance of immune homeostasis in the gut. *Nature Rev. Immunol.* **8**, 411–420 (2008).
7. Coombes, J. L. & Powrie, F. Dendritic cells in intestinal immune regulation. *Nature Rev. Immunol.* **8**, 435–446 (2008).
8. Johansson, M. E. *et al.* The inner of the two Muc2 mucin-dependent mucus layers in colon is devoid of bacteria. *Proc. Natl Acad. Sci. USA* **105**, 15064–15069 (2008).
9. Pilewski, J. M. & Frizzell, R. A. Role of CFTR in airway disease. *Physiol. Rev.* **79**, S215–S255 (1999).
10. Heazlewood, C. K. *et al.* Aberrant mucin assembly in mice causes endoplasmic reticulum stress and spontaneous inflammation resembling ulcerative colitis. *PLoS Med.* **5**, e54 (2008).
11. Baumgartner, H. K. & Montrose, M. H. Regulated alkali secretion acts in tandem with unstirred layers to regulate mouse gastric surface pH. *Gastroenterology* **126**, 774–783 (2004).
12. Stocchi, A. *et al.* Measurements of the jejunal unstirred layer in normal subjects and patients with celiac disease. *Am. J. Physiol.* **270**, G487–G491 (1996).
13. Hermiston, M. L. & Gordon, J. I. *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 (1995).
14. Van Itallie, C. M. *et al.* The density of small tight junction pores varies among cell types and is increased by expression of claudin-2. *J. Cell Sci.* **121**, 298–305 (2008).
This detailed analysis of tight junction size selectivity is the first study to show a role for claudins in the paracellular flux of uncharged solutes.
15. Watson, C. J., Hoare, C. J., Garrud, D. R., Carlson, G. L. & Warhurst, G. Interferon- γ selectively increases epithelial permeability to large molecules by activating different populations of paracellular pores. *J. Cell Sci.* **118**, 5221–5230 (2005).
16. Wang, F. *et al.* Interferon- γ and tumour necrosis factor- α synergize to induce intestinal epithelial barrier dysfunction by upregulating myosin light chain kinase expression. *Am. J. Pathol.* **166**, 409–419 (2005).
17. Clayburgh, D. R., Musch, M. W., Leitges, M., Fu, Y. X. & Turner, J. R. Coordinated epithelial NHE3 inhibition and barrier dysfunction are required for TNF-mediated diarrhea *in vivo*. *J. Clin. Invest.* **116**, 2682–2694 (2006).
18. Amasheh, S. *et al.* Claudin-2 expression induces cation-selective channels in tight junctions of epithelial cells. *J. Cell Sci.* **115**, 4969–4976 (2002).
19. Colegio, O. R., Van Itallie, C., Rahner, C. & Anderson, J. M. Claudin extracellular domains determine paracellular charge selectivity and resistance but not tight junction fibril architecture. *Am. J. Physiol. Cell Physiol.* **284**, C1346–C1354 (2003).
20. Simon, D. B. *et al.* Paracellin-1, a renal tight junction protein required for paracellular Mg²⁺ resorption. *Science* **285**, 103–106 (1999).
This is the first description of a human disease caused by tight junction defects.
21. Madara, J. L. & Pappenheimer, J. R. Structural basis for physiological regulation of paracellular pathways in intestinal epithelia. *J. Membr. Biol.* **100**, 149–164 (1987).
22. Meddings, J. B. & Westergaard, H. Intestinal glucose transport using perfused rat jejunum *in vivo*: model analysis and derivation of corrected kinetic constants. *Clin. Sci. (Lond.)* **76**, 403–413 (1989).
23. Turner, J. R. *et al.* Physiological regulation of epithelial tight junctions is associated with myosin light-chain phosphorylation. *Am. J. Physiol.* **273**, C1378–C1385 (1997).
This is the first report of MLCK-dependent physiological regulation of tight junction permeability.
24. Berglund, J. J., Riegler, M., Zolotarevsky, Y., Wenzl, E. & Turner, J. R. Regulation of human jejunal transmucosal resistance and MLC phosphorylation by Na⁺–glucose cotransport. *Am. J. Physiol. Gastrointest. Liver Physiol.* **281**, G1487–G1493 (2001).
25. Shen, L. *et al.* Myosin light chain phosphorylation regulates barrier function by remodeling tight junction structure. *J. Cell Sci.* **119**, 2095–2106 (2006).
26. Su, L. *et al.* Targeted epithelial tight junction dysfunction causes immune activation and contributes to development of experimental colitis. *Gastroenterology* **136**, 551–563 (2009).
This is the first *in vivo* analysis of physiologically relevant barrier dysfunction and consequent immunoregulation.
27. Madara, J. L. & Stafford, J. Interferon-gamma directly affects barrier function of cultured intestinal epithelial monolayers. *J. Clin. Invest.* **83**, 724–727 (1989).
28. Taylor, C. T., Dzus, A. L. & Colgan, S. P. Autocrine regulation of epithelial permeability by hypoxia: role for polarized release of tumour necrosis factor- α . *Gastroenterology* **114**, 657–668 (1998).
29. Mullin, J. M., Laughlin, K. V., Marano, C. W., Russo, L. M. & Soler, A. P. Modulation of tumour necrosis factor-induced increase in renal (LLC-PK1) transepithelial permeability. *Am. J. Physiol.* **263**, F915–F924 (1992).
30. Mazzon, E. & Cuzzocrea, S. Role of TNF- α in lung tight junction alteration in mouse model of acute lung inflammation. *Respir. Res.* **8**, 75 (2007).
31. Baker, O. J. *et al.* Proinflammatory cytokines tumour necrosis factor- α and interferon- γ alter tight junction structure and function in the rat parotid gland Par-C10 cell line. *Am. J. Physiol. Cell Physiol.* **295**, C1191–C1201 (2008).
32. Tiruppathi, C., Naqvi, T., Sandoval, R., Mehta, D. & Malik, A. B. Synergistic effects of tumour necrosis factor- α and thrombin in increasing endothelial permeability. *Am. J. Physiol. Lung Cell. Mol. Physiol.* **281**, L958–L968 (2001).
33. Baert, F. J. *et al.* Tumour necrosis factor α antibody (infliximab) therapy profoundly downregulates the inflammation in Crohn's ileocolitis. *Gastroenterology* **116**, 22–28 (1999).
34. Tamion, F. *et al.* Gut ischemia and mesenteric synthesis of inflammatory cytokines after hemorrhagic or endotoxic shock. *Am. J. Physiol.* **273**, G314–G321 (1997).
35. Brown, G. R. *et al.* Tumour necrosis factor inhibitor ameliorates murine intestinal graft-versus-host disease. *Gastroenterology* **116**, 593–601 (1999).
36. Suenaeert, P. *et al.* Anti-tumour necrosis factor treatment restores the gut barrier in Crohn's disease. *Am. J. Gastroenterol.* **97**, 2000–2004 (2002).
37. Van der Sluis, M. *et al.* Muc2-deficient mice spontaneously develop colitis, indicating that MUC2 is critical for colonic protection. *Gastroenterology* **131**, 117–129 (2006).
38. Clayburgh, D. R. *et al.* Epithelial myosin light chain kinase-dependent barrier dysfunction mediates T cell activation-induced diarrhea *in vivo*. *J. Clin. Invest.* **115**, 2702–2715 (2005).
This is the first *in vivo* demonstration of the role of MLCK in TNF-induced tight junction regulation.
39. Zolotarevsky, Y. *et al.* A membrane-permeant peptide that inhibits MLC kinase restores barrier function in *in vitro* models of intestinal disease. *Gastroenterology* **123**, 163–172 (2002).
This is the first report of the role of MLCK in TNF-induced tight junction regulation.
40. Ma, T. Y., Boivin, M. A., Ye, D., Pedram, A. & Said, H. M. Mechanism of TNF- α modulation of Caco-2 intestinal epithelial tight junction barrier: role of myosin light-chain kinase protein expression. *Am. J. Physiol. Gastrointest. Liver Physiol.* **288**, G422–G430 (2005).
41. McKenzie, J. A. & Ridley, A. J. Roles of Rho/ROCK and MLCK in TNF- α -induced changes in endothelial morphology and permeability. *J. Cell. Physiol.* **213**, 221–228 (2007).
42. Graham, W. V. *et al.* Tumour necrosis factor-induced long myosin light chain kinase transcription is regulated by differentiation-dependent signalling events. Characterization of the human long myosin light chain kinase promoter. *J. Biol. Chem.* **281**, 26205–26215 (2006).
43. Blair, S. A., Kane, S. V., Clayburgh, D. R. & Turner, J. R. Epithelial myosin light chain kinase expression and activity are upregulated in inflammatory bowel disease. *Lab. Invest.* **86**, 191–201 (2006).
44. Schwarz, B. T. *et al.* LIGHT signals directly to intestinal epithelia to cause barrier dysfunction via cytoskeletal and endocytic mechanisms. *Gastroenterology* **132**, 2383–2394 (2007).
45. Al-Sadi, R., Ye, D., Dokladny, K. & Ma, T. Y. Mechanism of IL-1 β -induced increase in intestinal epithelial tight junction permeability. *J. Immunol.* **180**, 5653–5661 (2008).
46. Wroblewski, L. E. *et al.* Helicobacter pylori dysregulation of gastric epithelial tight junctions by urease-mediated myosin II activation. *Gastroenterology* **136**, 236–246 (2009).
47. Scott, K. G., Meddings, J. B., Kirk, D. R., Lees-Miller, S. P. & Buret, A. G. Intestinal infection with Giardia spp. reduces epithelial barrier function in a myosin light chain kinase-dependent fashion. *Gastroenterology* **123**, 1179–1190 (2002).
48. Moriez, R. *et al.* Myosin light chain kinase is involved in lipopolysaccharide-induced disruption of colonic epithelial barrier and bacterial translocation in rats. *Am. J. Pathol.* **167**, 1071–1079 (2005).
49. Eutamene, H. *et al.* LPS-induced lung inflammation is linked to increased epithelial permeability: role of MLCK. *Eur. Respir. J.* **25**, 789–796 (2005).
50. Ferrier, L. *et al.* Impairment of the intestinal barrier by ethanol involves enteric microflora and mast cell activation in rodents. *Am. J. Pathol.* **168**, 1148–1154 (2006).
51. Van Itallie, C. M., Fanning, A. S., Bridges, A. & Anderson, J. M. ZO-1 stabilizes the tight junction solute barrier through coupling to the perijunctional cytoskeleton. *Mol. Biol. Cell* **20**, 3930–3940 (2009).
52. Scharl, M., Paul, G., Barrett, K. E. & McCoile, D. F. Adenosine monophosphate activated protein kinase mediates the interferon- γ -induced decrease in intestinal epithelial barrier function. *J. Biol. Chem.* **284**, 27952–27953 (2009).
53. Utech, M. *et al.* Mechanism of IFN- γ -induced endocytosis of tight junction proteins: Myosin II-dependent vacuolarization of the apical plasma membrane. *Mol. Biol. Cell* **16**, 5040–5052 (2005).
54. Shen, L. & Turner, J. R. Actin depolymerization disrupts tight junctions via caveolae-mediated endocytosis. *Mol. Biol. Cell* **16**, 3919–3936 (2005).
55. Stamatovic, S. M., Keep, R. F., Wang, M. M., Jankovic, I. & Andjelkovic, A. V. Caveolae-mediated internalization of occludin and claudin-5 during CCL2-induced tight junction remodeling in brain endothelial cells. *J. Biol. Chem.* **284**, 19053–19066 (2009).
56. Bruewer, M. *et al.* Interferon- γ induces internalization of epithelial tight junction proteins via a macropinosytosis-like process. *FASEB J.* **19**, 923–933 (2005).
57. Chun, J. & Prince, A. TLR2-induced calpain cleavage of epithelial junctional proteins facilitates leukocyte transmigration. *Cell Host Microbe* **5**, 47–58 (2009).
58. Saitou, M. *et al.* Complex phenotype of mice lacking occludin, a component of tight junction strands. *Mol. Biol. Cell* **11**, 4131–4142 (2000).
59. Amano, M. *et al.* Phosphorylation and activation of myosin by Rho-associated kinase (Rho-kinase). *J. Biol. Chem.* **271**, 20246–20249 (1996).
60. Higashihara, M., Takahata, K. & Kurokawa, K. Effect of phosphorylation of myosin light chain by myosin light chain kinase and protein kinase C on conformational change and ATPase activities of human platelet myosin. *Blood* **78**, 3224–3231 (1991).
61. Jou, T. S., Schneeberger, E. E. & James Nelson, W. Structural and functional regulation of tight junctions by rhoA and rac1 small GTPases. *J. Cell Biol.* **142**, 101–115 (1998).
62. Kimura, K. *et al.* Regulation of myosin phosphatase by Rho and Rho-associated kinase (Rho-kinase). *Science* **273**, 245–248 (1996).
63. Lee, J. H. *et al.* Energy-dependent regulation of cell structure by AMP-activated protein kinase. *Nature* **447**, 1017–1020 (2007).
64. Heller, F. *et al.* Interleukin-13 is the key effector Th2 cytokine in ulcerative colitis that affects epithelial tight junctions, apoptosis, and cell restitution. *Gastroenterology* **129**, 550–564 (2005).
Here, claudin-2 upregulation in inflammatory bowel disease is shown for the first time.
65. Balkovetz, D. F., Chumley, P. & Amlal, H. Downregulation of claudin-2 expression in renal epithelial cells by metabolic acidosis. *Am. J. Physiol. Renal Physiol.* **297**, F604–F611 (2009).

66. Wray, C. *et al.* Claudin-4 augments alveolar epithelial barrier function and is induced in acute lung injury. *Am. J. Physiol. Lung Cell. Mol. Physiol.* **297**, L219–L227 (2009).
67. Weber, C. R., Nalle, S. C., Tretiakova, M., Rubin, D. T. & Turner, J. R. Claudin-1 and claudin-2 expression is elevated in inflammatory bowel disease and may contribute to early neoplastic transformation. *Lab. Invest.* **88**, 1110–1120 (2008).
68. Dhawan, P. *et al.* Claudin-1 regulates cellular transformation and metastatic behaviour in colon cancer. *J. Clin. Invest.* **115**, 1765–1776 (2005).
69. Fujino, S. *et al.* Increased expression of interleukin 17 in inflammatory bowel disease. *Gut* **52**, 65–70 (2003).
70. Kinugasa, T., Sakaguchi, T., Gu, X. & Reinecker, H. C. Claudins regulate the intestinal barrier in response to immune mediators. *Gastroenterology* **118**, 1001–1011 (2000).
71. Yu, A. S. *et al.* Molecular basis for cation selectivity in claudin-2-based paracellular pores: identification of an electrostatic interaction site. *J. Gen. Physiol.* **133**, 111–127 (2009).
72. Angelow, S. & Yu, A. S. Structure-function studies of claudin extracellular domains by cysteine-scanning mutagenesis. *J. Biol. Chem.* **284**, 29205–29217 (2009).
73. Hollander, D. *et al.* Increased intestinal permeability in patients with Crohn's disease and their relatives. A possible etiologic factor. *Ann. Intern. Med.* **105**, 883–885 (1986).
This is the first demonstration of increased intestinal permeability in healthy relatives of patients with Crohn's disease.
74. Buhner, S. *et al.* Genetic basis for increased intestinal permeability in families with Crohn's disease: role of CARD15 3020insC mutation? *Gut* **55**, 342–347 (2006).
75. Noguchi, E., Homma, Y., Kang, X., Netea, M. G. & Ma, X. A Crohn's disease-associated NOD2 mutation suppresses transcription of human IL10 by inhibiting activity of the nuclear ribonucleoprotein hnRNP-A1. *Nature Immunol.* **10**, 471–479 (2009).
76. Madsen, K. L. *et al.* Interleukin-10 gene-deficient mice develop a primary intestinal permeability defect in response to enteric microflora. *Inflamm. Bowel Dis.* **5**, 262–270 (1999).
77. Olson, T. S. *et al.* The primary defect in experimental ileitis originates from a nonhematopoietic source. *J. Exp. Med.* **203**, 541–552 (2006).
78. Wyatt, J., Vogelsang, H., Hubl, W., Waldhoer, T. & Lochs, H. Intestinal permeability and the prediction of relapse in Crohn's disease. *Lancet* **341**, 1437–1439 (1993).
79. Hermiston, M. L. & Gordon, J. I. Inflammatory bowel disease and adenomas in mice expressing a dominant negative N-cadherin. *Science* **270**, 1203–1207 (1995).
The first demonstration that genetic insults in epithelial cells can cause experimental inflammatory bowel disease.
80. Yan, Y. *et al.* Temporal and spatial analysis of clinical and molecular parameters in dextran sodium sulfate induced colitis. *PLoS One* **4**, e6073 (2009).
81. Williams, K. L. *et al.* Enhanced survival and mucosal repair after dextran sodium sulfate-induced colitis in transgenic mice that overexpress growth hormone. *Gastroenterology* **120**, 925–937 (2001).
82. Brown, S. L. *et al.* Myd88-dependent positioning of Ptg2-expressing stromal cells maintains colonic epithelial proliferation during injury. *J. Clin. Invest.* **117**, 258–269 (2007).
83. Fukata, M. *et al.* Cox-2 is regulated by Toll-like receptor-4 (TLR4) signalling: Role in proliferation and apoptosis in the intestine. *Gastroenterology* **131**, 862–877 (2006).
84. Hollander, D. Crohn's disease — a permeability disorder of the tight junction? *Gut* **29**, 1621–1624 (1988).
85. Irvine, E. J. & Marshall, J. K. Increased intestinal permeability precedes the onset of Crohn's disease in a subject with familial risk. *Gastroenterology* **119**, 1740–1744 (2000).
86. Su, L., Nalle, S. C., Sullivan, E. A., Fu, Y.-X. & Turner, J. R. Genetic ablation of myosin light chain kinase limits epithelial barrier dysfunction and attenuates experimental inflammatory bowel disease. *Gastroenterology Abstr.* **136**, A81 (2009).
87. Xu, J. *et al.* Nonmuscle myosin light-chain kinase mediates neutrophil transmigration in sepsis-induced lung inflammation by activating $\beta 2$ integrins. *Nature Immunol.* **9**, 880–886 (2008).
88. Arrieta, M. C., Madsen, K., Doyle, J. & Meddings, J. Reducing small intestinal permeability attenuates colitis in the IL10 gene-deficient mouse. *Gut* **58**, 41–48 (2009).
89. Vetrano, S. *et al.* Unique role of junctional adhesion molecule-a in maintaining mucosal homeostasis in inflammatory bowel disease. *Gastroenterology* **135**, 173–184 (2008).
90. Laukoetter, M. G. *et al.* JAM-A regulates permeability and inflammation in the intestine *in vivo*. *J. Exp. Med.* **204**, 3067–3076 (2007).
91. Kang, L. I. *et al.* Deletion of JAM-A causes morphological defects in the corneal epithelium. *Int. J. Biochem. Cell Biol.* **39**, 576–585 (2007).
92. Boirivant, M. *et al.* A transient breach in the epithelial barrier leads to regulatory T-cell generation and resistance to experimental colitis. *Gastroenterology* **135**, 1612–1623 (2008).
This is the first detailed analysis of the effects of transient epithelial cell damage on immunoregulation in the gut.
93. Iliev, I. D., Milet, E., Matteoli, G., Chieppa, M. & Rescigno, M. Intestinal epithelial cells promote colitis-protective regulatory T-cell differentiation through dendritic cell conditioning. *Mucosal Immunol.* (2009).
This study shows that epithelial cells can direct DCs to facilitate regulatory T cell differentiation.
94. Sartor, R. B. Microbial influences in inflammatory bowel diseases. *Gastroenterology* **134**, 577–594 (2008).
95. Ewaschuk, J. B., Tejpar, O. Z., Soo, I., Madsen, K. & Fedorak, R. N. The role of antibiotic and probiotic therapies in current and future management of inflammatory bowel disease. *Curr. Gastroenterol. Rep.* **8**, 486–498 (2006).
96. Aranda, R. *et al.* Analysis of intestinal lymphocytes in mouse colitis mediated by transfer of CD4⁺, CD45RB^{high} T cells to SCID recipients. *J. Immunol.* **158**, 3464–3473 (1997).
97. Sellon, R. K. *et al.* Resident enteric bacteria are necessary for development of spontaneous colitis and immune system activation in interleukin-10-deficient mice. *Infect. Immun.* **66**, 5224–5231 (1998).
98. Cario, E. Barrier-protective function of intestinal epithelial Toll-like receptor 2. *Mucosal Immunol.* **1**, S62–S66 (2008).
99. Mucida, D. *et al.* Reciprocal T_H17 and regulatory T cell differentiation mediated by retinoic acid. *Science* **317**, 256–260 (2007).
100. Coombes, J. L. *et al.* A functionally specialized population of mucosal CD103⁺ DCs induces Foxp3⁺ regulatory T cells via a TGF- β and retinoic acid-dependent mechanism. *J. Exp. Med.* **204**, 1757–1764 (2007).
101. Kaser, A. *et al.* XBP1 links ER stress to intestinal inflammation and confers genetic risk for human inflammatory bowel disease. *Cell* **134**, 743–756 (2008).
102. Fisher, S. A. *et al.* Genetic determinants of ulcerative colitis include the ECM1 locus and five loci implicated in Crohn's disease. *Nature Genet.* **40**, 710–712 (2008).
103. Watanabe, T. *et al.* Muramyl dipeptide activation of nucleotide-binding oligomerization domain 2 protects mice from experimental colitis. *J. Clin. Invest.* **118**, 545–559 (2008).
104. Yang, Z. *et al.* NOD2 transgenic mice exhibit enhanced MDP-mediated downregulation of TLR2 responses and resistance to colitis induction. *Gastroenterology* **133**, 1510–1521 (2007).
105. Hedl, M., Li, J., Cho, J. H. & Abraham, C. Chronic stimulation of Nod2 mediates tolerance to bacterial products. *Proc. Natl Acad. Sci. USA* **104**, 19440–19445 (2007).
106. Kraus, T. A., Cheifetz, A., Toy, L., Meddings, J. B. & Mayer, L. Evidence for a genetic defect in oral tolerance induction in inflammatory bowel disease. *Inflamm. Bowel Dis.* **12**, 82–88 (2006).
107. Shiu, H., Musch, M. W., Wang, Y., Chang, E. B. & Turner, J. R. Akt2 phosphorylates ezrin to trigger NHE3 translocation and activation. *J. Biol. Chem.* **280**, 1688–1695 (2005).
108. Hirschhorn, N. *et al.* Decrease in net stool output in cholera during intestinal perfusion with glucose-containing solutions. *N. Engl. J. Med.* **279**, 176–181 (1968).
109. Zeissig, S. *et al.* 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 (2007).
110. Duerksen, D. R., Wilhelm-Boyles, C. & Parry, D. M. Intestinal permeability in long-term follow-up of patients with celiac disease on a gluten-free diet. *Dig. Dis. Sci.* **50**, 785–790 (2005).
111. Pearson, A. D., Eastham, E. J., Laker, M. F., Craft, A. W. & Nelson, R. Intestinal permeability in children with Crohn's disease and coeliac disease. *Br. Med. J. (Clin. Res. Ed)* **285**, 20–21 (1982).
112. Wang, F. *et al.* IFN- γ -induced TNFR2 expression is required for TNF-dependent intestinal epithelial barrier dysfunction. *Gastroenterology* **131**, 1153–1163 (2006).
113. Powrie, F. *et al.* Inhibition of Th1 responses prevents inflammatory bowel disease in scid mice reconstituted with CD45RB^{high} CD4⁺ T cells. *Immunity* **1**, 553–562 (1994).
114. Stepankova, R. *et al.* Segmented filamentous bacteria in a defined bacterial cocktail induce intestinal inflammation in SCID mice reconstituted with CD45RB^{high} CD4⁺ T cells. *Inflamm. Bowel Dis.* **13**, 1202–1211 (2007).
115. Collett, A. *et al.* Early molecular and functional changes in colonic epithelium that precede increased gut permeability during colitis development in mdr1a^{-/-} mice. *Inflamm. Bowel Dis.* **14**, 620–631 (2008).
116. Resta-Lenert, S., Smitham, J. & Barrett, K. E. Epithelial dysfunction associated with the development of colitis in conventionally housed mdr1a^{-/-} mice. *Am. J. Physiol. Gastrointest. Liver Physiol.* **289**, G153–G162 (2005).
117. Nusrat, A. *et al.* Clostridium difficile toxins disrupt epithelial barrier function by altering membrane microdomain localization of tight junction proteins. *Infect. Immun.* **69**, 1329–1336 (2001).
118. McNamara, B. P. *et al.* Translocated EspF protein from enteropathogenic Escherichia coli disrupts host intestinal barrier function. *J. Clin. Invest.* **107**, 621–629 (2001).

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