

ADVANCES IN TRANSLATIONAL SCIENCE

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Intestinal Permeability Defects: Is It Time to Treat?

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An essential role of the intestinal epithelium is to separate luminal contents from the interstitium, a function primarily determined by the integrity of the epithelium and the tight junction that seals the paracellular space. Intestinal tight junctions are selectively permeable, and intestinal permeability can be increased physiologically in response to luminal nutrients or pathologically by mucosal immune cells and cytokines, the enteric nervous system, and pathogens. Compromised intestinal barrier function is associated with an array of clinical conditions, both intestinal and systemic. Although most available data are correlative, some studies support a model where cycles of increased intestinal permeability, intestinal immune activation, and subsequent immune mediated barrier loss contribute to disease progression. This model is applicable to intestinal and systemic diseases. However, it has not been proven, and both mechanistic and therapeutic studies are ongoing. Nevertheless, the correlation between increased intestinal permeability and disease has caught the attention of the public, leading to a rise in popularity of the diagnosis of “leaky gut syndrome,” which encompasses a range of systemic disorders. Proponents claim that barrier restoration will cure underlying disease, but this has not been demonstrated in clinical trials. Moreover, human and mouse studies show that intestinal barrier loss alone is insufficient to initiate disease. It is therefore uncertain whether increased permeability in these patients is a cause or effect of the underlying disorder. Although drug targets that may mediate barrier restoration have been proposed, none have been proven effective. As such, current treatments for barrier dysfunction should target the underlying disease.

Keywords: Tight Junction; Myosin Light Chain Kinase; Claudin; Inflammatory Bowel Disease; Graft vs Host Disease; Irritable Bowel Syndrome; Leaky Gut Syndrome.

A critical function of the intestinal mucosa, particularly the epithelium, is to form a barrier that prevents potentially noxious contents of the intestinal lumen, including the microbiota, from accessing internal sites and the systemic circulation.¹ Barrier defects have been associated with a variety of human diseases, including those primarily affecting the gut, eg, in inflammatory bowel disease (IBD), celiac disease, and irritable bowel syndrome (IBS), as well as systemic diseases or diseases involving other organ systems, eg, type I diabetes, graft versus host disease (GVHD), AIDS, multiple sclerosis, rheumatoid arthritis, and autism. The breadth of related diseases is expanded further by use of the term *leaky gut syndrome* within the lay and alternative medicine communities and even by some

physicians. Proponents of leaky gut syndrome claim that it is responsible for a dizzying array of disorders, including chronic fatigue syndrome, fibromyalgia, allergies, depression, and skin disorders (Table 1). In part, the speculation and uncertainty regarding both bona fide disease associations and those ascribed to leaky gut syndrome reflect the absence of conclusive human data. To date, all clinical studies have focused on correlation, precluding distinction between cause and effect. Furthermore, no therapies that have been shown to directly target and restore the intestinal barrier in human subjects are currently available as Food and Drug Administration approved or even investigational drugs. Here we review the determinants of intestinal barrier function, real and claimed associations with disease, experimental data that may shed light on the pathogenic contributions of intestinal permeability defects, and the potential of future advances to provide therapeutic tools for barrier restoration.

Technological Primer

Definitions

Intestinal permeability is the property that allows solute and fluid exchange between the lumen and tissues. Conversely, intestinal barrier function refers to the ability of the mucosa and extracellular barrier components, eg, mucus, to prevent this exchange. Neither permeability nor barrier function is absolute, and the relative magnitudes of these opposing characteristics vary inversely. However, the term *intestinal barrier* has become a catch phrase that is being increasingly applied to related, but distinct, properties, eg, immunoregulatory and antibacterial barriers. Although these may be barriers, they should not be characterized as permeability or barrier function. To avoid confusion caused by the pervasive indiscriminate use of these terms, it is critical to define them. In this article we will use barrier function as the converse of intestinal permeability, and both will refer exclusively to changes in flux of solutes and fluids across the epithelium.

The small intestinal and colonic mucus layers,² which are composed of goblet cell secretions, form the first barrier. The apical mucus also contributes to the development of the zone of limited luminal flow, ie, the unstirred layer,³ that directly

Abbreviations used in this paper: CA MLCK, constitutively active-myosin light chain kinase; CD, Crohn's disease; GVHD, graft versus host disease; IBD, inflammatory bowel disease; IBS, irritable bowel syndrome; IL, interleukin; MLCK, myosin light chain kinase; TNF, tumor necrosis factor.

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Table 1. Relationships Between Intestinal Barrier Function and Selected Diseases

Disease	Human data regarding intestinal permeability	Is association supported by animal model(s)?	Prognostic implications of increased permeability	Role of microbiota	Does disease treatment improve disease associated permeability defects?
Extensive supporting data					
IBD (CD and ulcerative colitis)	Positive correlation with disease activity (discussed in text); present in some healthy first degree relatives (CD)	Yes ^{16,47,51,54}	Increased risk of relapse in some studies of CD patients ^{39,40}	<i>Trichuris suis</i> (whipworm) infection and fecal microbial transplantation data are encouraging. ^{52,53} Germ free rodents do not develop disease in most experimental models. ⁵⁴	Yes (in patients and experimental models) ^{16,55,56}
GVHD	Positive correlation of preconditioning gastrointestinal toxicity (presumed to indicate degree of transient barrier loss) with disease activity ⁵⁷	Yes	Unknown	Antibiotics reduce disease incidence in patients and experimental animals. ^{59,60}	Yes (in experimental models) ⁵⁸
Type I diabetes	Increased in pre diabetic and diabetic patients ⁶¹	Yes ⁶²	Unknown	Changes in microbiome modify incidence of experimental disease. ^{63,64}	Unknown
HIV/AIDS	Increased in HIV enteropathy ⁶⁵ ; positive correlation with disease stage ⁶⁶	Yes ⁶⁷	Unknown	Serum LPS is elevated in patients. Bacterial translocation has been postulated to cause immune activation. ⁶⁷	Yes (in patients) ⁶⁸
Multiple organ dysfunction syndrome	Correlates with increased disease severity ⁶⁹	Yes ^{70,71}	Unknown	Controversial	Unknown
Some supporting data					
IBS	Increased in diarrhea predominant, postinfectious, and non postinfectious IBS ^{72,73}	No	Unknown	Unknown	Unknown
Celiac disease	Positive correlation with disease activity ⁷⁴ ; increased in patients and healthy relatives ⁷⁵	Yes ^{76,77}	Unknown	Unknown	Yes ⁷⁴
Limited or no supporting data					
Autism	Increased in patients and relatives; correlates with food intolerance ⁷⁸	No	Unknown	Unknown	Unknown
Eczema	Increased in subset of patients; possible correlation with disease activity ^{79,80}	No	Unknown	Unknown	Unknown
Psoriasis	Inconclusive	No	Unknown	Unknown	Unknown
Acute pancreatitis	Positive correlation with disease activity ⁸¹	Yes ^{82,83}	Unknown	Unknown	Yes (in experimental models) ^{82,84}

Table 1. Continued

Disease	Human data regarding intestinal permeability	Is association supported by animal model(s)?	Prognostic implications of increased permeability	Role of microbiota	Does disease treatment improve disease associated permeability defects?
Parkinson's disease	Increased in subset of patients ^{85,86}	No	Unknown	Unknown	Unknown
Fibromyalgia	Increased in subset of patients; correlation with disease activity not studied ⁸⁷	No	Unknown	Unknown (no experimental models, but extent of bacterial overgrowth correlates with self reported pain intensity) ⁸⁸	Unknown
Depression	Unknown	No	Unknown	Unknown	Unknown
Chronic fatigue syndrome	Unknown	No	Unknown	Unknown	Unknown
Asthma	Increased in asthmatic patients; no correlation with disease activity ⁸⁹	No	Unknown	Hypothesized to provide possible protective role ⁹⁰	No
Multiple sclerosis	Increased in subset of patients; normal in remission ⁹¹	No	Unknown	Reduced experimental disease in germ free mice ⁹²	Unknown
Rheumatic diseases (arthritis and ankylosing spondylitis)	Increased in patients ⁹³ ; NSAID treatment is confounding factor ⁹⁴	No	Unknown	Symptoms correlate with germ free status in rat model ⁹⁵ ; gut flora worsens disease in mouse models. ⁹⁶	Unknown
Nonalcoholic fatty liver disease	Positive correlation with disease activity ^{97,98}	No	Unknown	Correlation in humans. ⁹⁷ Strong association in mice. ^{99,100}	Unknown
Alcoholic cirrhosis	Increased in subset of patients; correlation with disease activity not studied ¹⁰¹	No	Unknown	Postulated to be the source of LPS that leads to liver damage and inflammation. ¹⁰²	Unknown
Environmental enteropathy	Correlation with growth failure ^{103 107}	No	Unknown	Likely ^{104 106}	Unknown
Kwashiorkor	Positive correlation with disease severity ¹⁰⁸	Yes ¹⁰⁹	Unknown	Association, in conjunction with diet ¹¹⁰	Slowly ¹⁰⁸

AIDS, acquired immune deficiency syndrome; HIV, human immunodeficiency virus; LPS, lipopolysaccharide; NSAID, nonsteroidal anti inflammatory drug.

overlies the epithelium. Although the mucus and unstirred layers prevent some organisms and large molecules, eg, food particles, from directly accessing the epithelium, they do little to prevent flux of small molecules, ions, and water. Nevertheless, studies with experimental animals have shown that disruption of mucus production can lead to intestinal damage and inflammatory disease.⁴

Beneath the mucus and unstirred layers lies a simple columnar epithelium. The membranes of these epithelial cells provide an effective barrier to most hydrophilic solutes but would not be an adequate barrier if the space between individual cells were not sealed by a series of intercellular junctions. Of

these, the tight junction is the primary determinant of paracellular flux in an intact epithelium.⁵ However, intestinal tight junctions do not form an absolute seal. Further, it should be apparent that disruptions of intestinal barrier function, ie, increased permeability, that are due to direct epithelial damage do not reflect tight junction barrier function.

Permeability Pathways

The tight junction is a highly dynamic protein complex that forms within specialized plasma membrane lipid domains.^{6,7} The specific proteins involved have been reviewed elsewhere,^{6,8 11} and except where linked to disease, individual

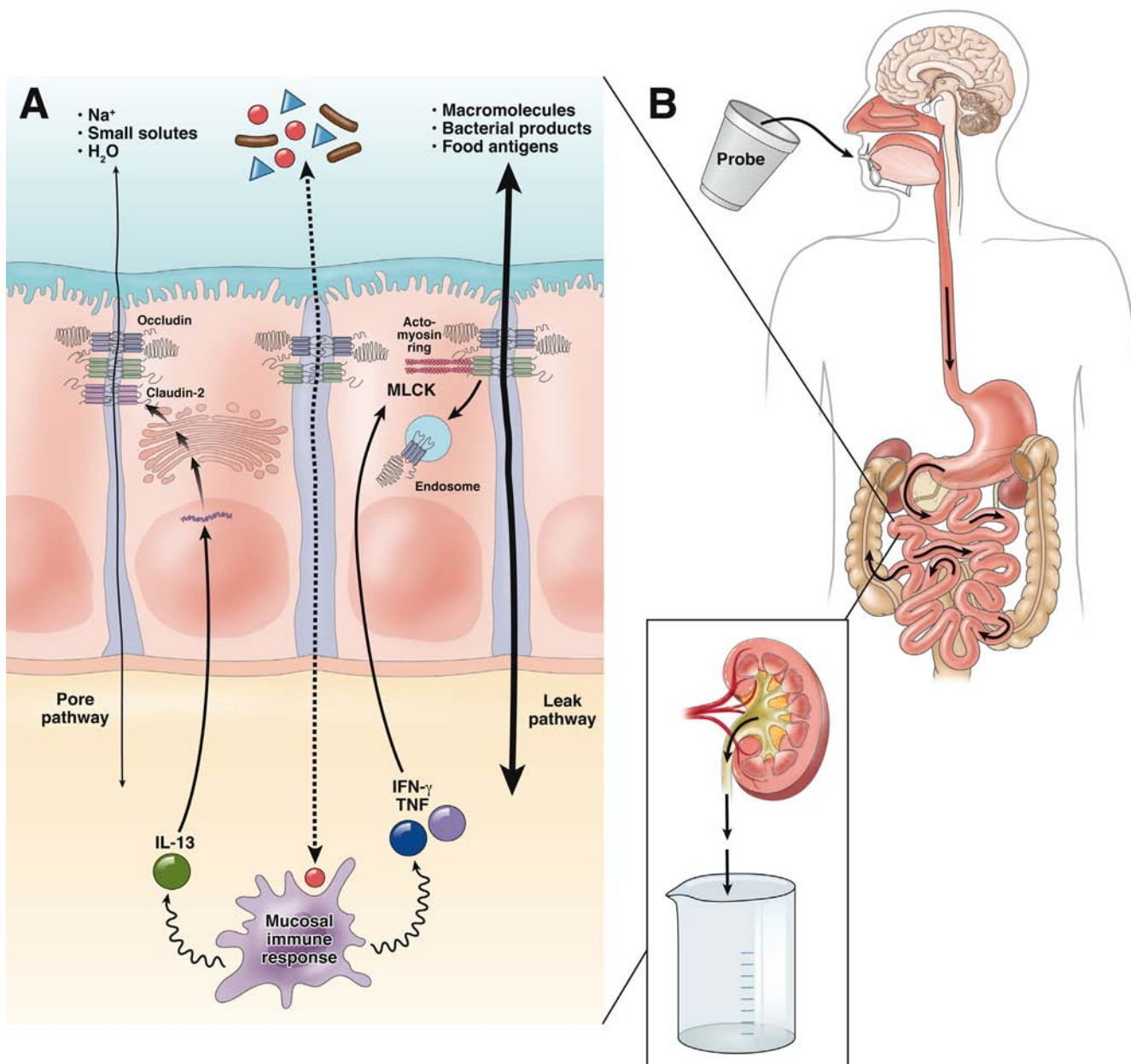


Figure 1. Intestinal permeability: pathophysiological mechanisms and methods of analysis. (A) The intestinal epithelium normally provides an effective barrier to macromolecules, bacterial products, and food antigens, but a small percentage can cross the tight junction (*dashed arrow*). In genetically susceptible individuals, translocation of luminal materials has been proposed to trigger exaggerated mucosal immune activation and cytokine release (eg, IL 13, TNF, interferon [IFN] γ), which subsequently activates 2 distinct pathways of trans tight junction flux. These are referred to as the pore (*thin arrow*) and leak (*bold arrow*) pathways and are selectively activated by IL 13 and TNF, respectively. Increased tight junction permeability may lead to further translocation of macromolecules from the lumen into the lamina propria and amplification of mucosal immune activation. In the absence of appropriate regulatory signals, this vicious cycle may progress to disease. (B) Intestinal permeability is most commonly measured in human subjects as fractional urinary excretion of orally ingested probes. These probes can cross the intestinal epithelium by the paracellular pathway and enter the bloodstream. They are then filtered by the glomerulus and excreted in the urine. Ideal probes are not metabolized in the intestinal lumen or blood, are readily filtered by the glomerulus, and are not actively absorbed or secreted in the kidney. Fractional urinary excretion can therefore be used as an indirect measure of intestinal permeability.

tight junction proteins will not be discussed here. It has been recognized for many years that tight junctions restrict molecular flux on the basis of both size and charge, ie, tight junctions display size selectivity and charge selectivity.¹²⁻¹⁵ Recent *in vitro* and *in vivo* studies with cultured human intestinal epithelia and mice, respectively, have shown that 2 disease related cytokines,

tumor necrosis factor (TNF) and interleukin 13 (IL 13), can differentially regulate tight junction size selectivity and charge selectivity (Figure 1A).^{16,17} Together with high resolution *in vitro* analyses of tight junction size selectivity,¹⁸⁻²¹ this has led to a model in which there are at least 2 distinct routes of paracellular flux across the tight junction.^{1,6,10} These have been

termed pore and leak pathways and refer to a high capacity, size and charge selective route and a low capacity, nonselective route, respectively.^{1,10} IL 13 specifically increases flux across the pore pathway by up regulating expression of the tight junction protein claudin 2, which forms a paracellular cation and water channel.^{17,22,24} In contrast, TNF increases leak pathway flux by a rapid mechanism that involves reorganization of the tight junction and perijunctional actomyosin ring by a process that requires myosin light chain kinase (MLCK).^{16,25} Notably, claudin 2 expression and MLCK activity are both increased in active IBD,^{22,26} suggesting that both pathways are involved in disease associated barrier loss.^{15,17}

Measures of Intestinal Permeability

Many tools exist to measure intestinal permeability *ex vivo* and *in vitro*.^{6,27} Although simple *in vivo* methods are also available and readily applicable to human subjects and experimental animals, they offer less resolution, and interpretation can be complex. These approaches most frequently assess fractional urinary excretion of orally ingested probes (Figure 1B).²⁸ The probes selected must not be able to cross the epithelium by a transcellular route, and any probe that enters the bloodstream is assumed to have crossed the tight junction or a site of epithelial damage. Probes must also be inert within the bloodstream and freely filtered at the glomerulus, which allows their collection in the urine. However, many factors, including intestinal transit time, intestinal surface area, probe degradation within the lumen or bloodstream, and renal function can affect fractional urinary excretion.^{29,30} It is therefore not surprising that a variety of probes have been used and that each has a unique constellation of limitations.

For measurements of small intestinal permeability, the lactulose:mannitol ratio (sometimes referred to as LAMA) has been popular. The use of 2 probes in this case partially corrects for absorptive differences resulting from changes in motility. However, lactulose and mannitol are degraded by colonic bacteria²⁸ and are not useful as measures of colonic permeability; probes including sucralose, polyethylene glycols, and ⁵¹Cr EDTA have been used instead. Importantly, none of these probes are able to assess charge selectivity.

Lactulose is large and can only cross via the leak pathway or at sites of epithelial damage. It can therefore be considered a marker of barrier integrity. Mannitol, which is one third as large, crosses the pore pathway and can be thought of as a measure of surface area. Thus, the lactulose:mannitol ratio can be interpreted as a measure of the sum of leak pathway permeability and epithelial damage normalized to surface area. For example, mannitol recovery is reduced in celiac disease, reflecting loss of villous surface area, whereas lactulose recovery is increased because of epithelial damage. This results in an increased lactulose:mannitol ratio that is only partly due to increases in intestinal permeability. Thus, it is critical to assess both lactulose and mannitol recoveries separately, as well as the lactulose:mannitol ratio, when interpreting test results.

Remarkably, several Internet vendors promoting leaky gut syndrome treatments also provide mail order lactulose:mannitol assays. Thus, patients may arrive with their own lactulose:mannitol data. However, it is not yet clear how these data can or should be used to guide diagnosis or treatment.

Findings

Physiological Regulation of Intestinal Barrier Function

The most studied instance of physiological intestinal barrier regulation is that triggered by Na⁺ glucose cotransport. This leads to activation of epithelial MLCK, which drives size selective, ie, pore pathway, increases in paracellular permeability.^{15,31,33} This enhances paracellular water flux as a result of the osmotic gradient created by transcellular Na⁺ and glucose transport. This combination of paracellular water flow and increased paracellular permeability also allows paracellular absorption of nutrient sized molecules, such as glucose, that can reach very high concentrations within the unstirred layer as a result of brush border digestive enzyme activity. Thus, nutrients are carried passively as solutes within water, ie, the solvent.³⁴ This process, termed solvent drag, likely explains the inability of excess luminal glucose to saturate intestinal absorption. This mechanism also contributes to the efficacy of Na⁺ based and carbohydrate based oral rehydration solutions.³⁵ In contrast, Na⁺ glucose cotransport dependent barrier regulation has not been described in the kidney, where tubular reabsorption is saturated at glucose concentrations exceeding ~300 mg/dL and leads to spilling in the urine, eg, in diabetes.

Disease Correlates in Human Subjects

Intestinal permeability has been most extensively studied in the context of IBD and IBS. The lactulose:mannitol ratio is most relevant for analysis of small intestinal permeability; thus, most IBD studies have focused on Crohn's disease (CD) patients with small intestinal disease. This limitation of the lactulose:mannitol ratio, as well as the impact of intestinal motility and transit time on probe absorption, complicates studies of IBS. Most importantly, although permeability by these measures is increased in CD, IBS, and other diseases, it is well recognized that disease processes, such as proinflammatory cytokine release, can impact intestinal permeability. This makes it impossible to separate cause from effect. The strongest case for a pathogenic role of barrier loss has been made in CD, where some healthy first degree relatives of CD patients exhibit increased intestinal permeability compared with unrelated control subjects.^{36,37} Subsequent studies showed that these healthy relatives with increased permeability tended to carry NOD2 mutations,³⁸ suggesting that the increased permeability observed may reflect subclinical immune activation. Unfortunately, we do not know whether these healthy relatives with increased permeability are at greater risk of developing disease than the healthy relatives with normal intestinal permeability.

The intestinal barrier may also be critical to maintenance of CD remission, as increased intestinal permeability can be a strong risk factor for CD reactivation.^{39,40} The factors that contribute to increased permeability during remission have not been defined but could include stress, which has been associated with CD relapse⁴¹ and has also been shown to induce intestinal barrier loss in rodents.⁴² However, the data can also be interpreted as evidence of a mild inflammatory state and smoldering disease that are poised to become clinically active (Figure 1A). Despite these uncertainties, it should be clear that barrier loss alone is insufficient to cause CD or any other disease. Indeed, data from some mouse studies indicate

that mild permeability increases may activate immunoregulatory pathways that can limit colitis on subsequent challenge.^{1,43}

Importance

Implications of Animal Model Data for Human Disease

Intestinal barrier loss, ie, permeability increases beyond the normal range, precedes disease onset in mouse models of type I diabetes, GVHD, necrotizing enterocolitis, and IBD. Unfortunately, like human data, most studies that use these models have failed to distinguish between cause and effect. However, there are several exceptions. For example, a zonulin antagonist (larazotide) has been shown to reduce diabetes and IBD in the nonobese diabetic and IL 10 knockout mouse models of these diseases, respectively.^{44,45} Regrettably, clinical results have shown that larazotide does not prevent gluten induced permeability increases in celiac disease patients.⁴⁶

A range of experimental models has been used to study disease associated barrier loss. In these models it is important to distinguish between tight junction dependent and tight junction independent barrier loss. For example, the commonly used dextran sulfate sodium model of colitis causes direct colonic epithelial cell injury and death. Although there is documented tight junction disruption in this disease, the bulk of observed barrier loss is due to epithelial damage, which is far greater in magnitude than that resulting from tight junction dysregulation. Thus, although dextran sulfate sodium is an excellent model of acute intestinal damage, it does not reflect IBD, IBS, or celiac disease in mechanistic terms, and therapies effective in dextran sulfate sodium colitis have failed in human IBD trials. The most relevant information may come from mouse models that use mechanisms similar to those activated in human disease. In IBD, these include the IL 10 knockout mouse and models that rely on adoptive transfer of effector T lymphocytes into immunodeficient mice that lack essential immunoregulatory processes. Regardless, it is critical to assess the model used when determining relevance of experimental data to human disease pathogenesis.

One disease model that may provide insight into the role of intestinal permeability in disease initiation and propagation is the constitutively active MLCK (CA MLCK) mouse. In this mouse, expression of CA MLCK within the epithelium induced intestinal epithelial tight junction barrier loss without associated epithelial damage.⁴⁷ This increased intestinal permeability was quantitatively similar to that seen in healthy first degree relatives of CD patients.^{36,48} Therefore, it is not surprising that CA MLCK transgenic mice failed to develop spontaneous disease. However, they did display subclinical immune activation. Furthermore, CA MLCK transgenic mice developed a more severe colitis with reduced survival in an adoptive transfer colitis model.⁴⁷ Perhaps more strikingly, onset of disease was markedly accelerated in CA MLCK transgenic mice. These data show that intestinal epithelial tight junction barrier loss can trigger mucosal immune activation, without causing disease, but it can also enhance the rate of disease progression in a susceptible individual.

The data showing that a tight junction barrier defect enhances disease progression in a susceptible host may also relate to human studies of postinfectious IBD and IBS,^{49,50} where the barrier loss induced by infection may be the trigger that drives pathogenesis. Support for this general idea comes

from a recent study of GVHD. When CA MLCK transgenic mice received cells from a minor antigen mismatched donor, they developed mild GVHD, whereas mice lacking the CA MLCK transgene remained healthy. Conversely, mice lacking the epithelial MLCK isoform (long MLCK) were protected from chronic GVHD, suggesting that targeted MLCK inhibition may be beneficial in GVHD.

Epithelial MLCK knockout mice have also been studied in the context of IBD.⁵¹ In this case, mice were significantly protected from adoptive transfer colitis and disease associated barrier loss. However, epithelial MLCK knockout mice ultimately developed disease and barrier loss. Disease progression was associated with epithelial cell death and tight junction independent barrier loss. This contrasts sharply with the durable protection from GVHD provided by epithelial MLCK knockout. The difference likely reflects an important distinction between advanced IBD and advanced GVHD. The former is characterized by epithelial damage and ulcers, whereas the latter typically has only rare apoptotic crypt epithelial cells. In addition to reinforcing the idea that barrier loss can be due to many processes other than tight junction dysregulation, these data suggest that MLCK inhibition may be helpful in mild IBD or possibly in maintenance of remission, but it is unlikely to be of benefit in advanced IBD.

Translation and Roadblocks

Implications for Clinical Diagnosis

The data above show that progress is being made in understanding barrier loss in disease, including the means by which such barrier loss contributes to disease. However, they also make it clear that intestinal barrier loss can occur by several mechanisms, only some of which reflect tight junction dysregulation, and that tight junction barrier loss can be divided into distinct pathways that are differentially modulated by disease effectors. Finally, increased intestinal permeability can be beneficial in some contexts, eg, in promoting nutrient and water absorption or activating protective immunoregulatory processes. Thus, even if clinical assays that could measure intestinal barrier function more precisely than those available today were used, it is not clear what could or should be done for a patient with increased intestinal permeability or even if the magnitude of permeability changes should be a factor in clinical decision making. Nonetheless, one case where an argument for therapeutic intervention might be made is that of a CD patient during clinical remission. Unfortunately, no barrier restorative therapies are available. In IBD and type I diabetes, data from mouse models suggest that restoring barrier function could be beneficial, but there is no support from human trials. Finally, both human and mouse studies have made it clear that intestinal barrier loss alone, whether from tight junction dysregulation or epithelial damage, is insufficient to cause disease in an otherwise healthy individual.

Therapeutic Approaches

For now, the best therapy for barrier loss should target the disease itself. For example, anti TNF agents have been shown to restore barrier function in CD patients. For those complaining of leaky gut syndrome, the increased intestinal permeability present is as likely to reflect the underlying disorder as to be a cause

of the pathogenic process. Thus, the leaky gut cures being sold at a variety of Internet sites and alternative medicine stores should be considered with caution. None have been tested in randomized clinical trials, and they may do more harm than good.

Conclusions

Advances have been made in understanding the cellular mechanisms of intestinal barrier loss in disease. Unfortunately, the only agent purported to restore the barrier failed to do so in clinical trials. More detailed data are available for inflammatory mediators such as TNF and IL 13, as well as some infectious agents. However, this information has not yet led to therapeutic agents suitable for clinical trials. MLCK could be a promising therapeutic target, but the inhibitors presently available target MLCK enzymatic activity. Because the catalytic domain of MLCK is identical in epithelial and smooth muscle MLCK, toxicities of such an approach are likely to be unacceptable. Alternative means of specifically targeting intestinal epithelial MLCK must be sought if progress is to be made in this area. Similarly, no inhibitors of claudin 2 pore function are presently undergoing clinical trials. Finally, although a great deal has been learned about mechanisms of cell death, agents that prevent this to maintain barrier function have not been studied in humans. Thus, although much has been accomplished, further insight into both mechanisms of disease and development of novel therapeutic agents is needed before direct therapy of intestinal barrier function can be considered seriously.

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Conflicts of interest

The authors disclose no conflicts.

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