"All Disease Begins in the Gut": Elucidating Disease Mechanism Related to Intestinal Barrier Dysfunction

Daniel Keszthelyi, MD
Maastricht University Medical Center
Department of Internal Medicine
Division of Gastroenterology-Hepatology
Maastricht, The Netherlands
Email: daniel.keszthelyi@maastrichtuniversity.nl

Abstract

Tight junctions between intestinal epithelial cells form a selective barrier, which regulate paracellular traffic of luminal substances into the lamina propria. As the gut is the primary site of exposure to antigens, this barrier function plays an important role in systemic immune function. Accumulating evidence suggests that the disturbance in intestinal barrier function has a causative role in the pathogenesis of several systemic diseases, including diabetes mellitus.

Glossary of Abbreviations
DH: Dermatitis Herpetiformis
GI: Gastrointestinal
IBD: Inflammatory Bowel Disease
IL-4: Interleukin-4
IFN-γ: Interferon-γ
IP: Intestinal Permeability
NF-κB: Nuclear Factor-κB
NSAIDs: Nonsteroidal Anti-Inflammatory Drugs
TNF-α: Tumor Necrosis Factor-α

Intestinal Barrier Function and the Role of Tight Junctions

Along the gastrointestinal (GI) tract, an adjacent layer of cells separates the internal body systems from the external environment. This separation ensures protection from a wide range of environmental pathogens entering the lumen, thereby preventing infection, inflammation and alteration of normal body functions. Besides the tight lining of epithelial cells, other products, such as mucus, immunoglobulins and other antimicrobial agents, are important in maintaining a proper barrier function. The absorptive functions of the small intestine are regulated through two mechanisms. The first is transcellular transportation across the enterocyte brush border, usually facilitated by transport carriers or by means of passive diffusion. The second path is movement through paracellular spaces, not mediated by carriers and thus based solely on passive diffusion of molecules.

Several recent reports have reviewed the structure and function of tight junctions, which appear to have a principal role in regulating paracellular transport across the intestinal epithelium.[1,2] In brief, the junctions between adjacent epithelial cells consist of the more luminally situated tight junctions. Tight junctions are composed of transmembrane proteins (occludins, claudins) and plaque proteins (ZO protein family, among others) and are associated with the intracellular actin-myosin cytoskeleton. Components of the diet, such as glucose and amino acids, are able to induce openings of the tight junctions and increase paracellular permeability. These openings are regulated through a series of signal transductory pathways, all resulting in the increased activity of myosin light chain kinase, which phosphorylates myosin and causes a contraction of cytoskeletal components and conformational changes in structures associated with it, such as the tight junctions. Hence, this dynamic process of the opening and closing of the tight junction complex regulates the paracellular transport of luminal substances into the lamina propria.

Measuring Intestinal Permeability

When evaluating intestinal permeability (IP), researchers are particularly interested in the regulatory mechanisms and properties concerning the intrinsic permeability of the gut barrier. To measure the barrier function, different sets of probes have been used, such as monosaccharides (mannitol, L-rhamnose), disaccharides (lactulose, sucralose), polyethylene glycol, and nondegraded radiolabeled chelates (^{51}Cr-EDTA). The probes share specific characteristics: They are small-sized, water-soluble, not degraded or metabolized in the gut lumen, nontoxic, totally excreted by the kidney, and therefore can easily be detected in urine samples. Measurements using a single molecule (such as ^{51}Cr-EDTA) may be influenced by inter-individual differences not related to permeability, such as intestinal transit or urinary excretion. Thus far, human intestinal permeability has been measured by urinary excretion of two probes of different sizes but similar transit and uptake processes, calculating the excretion ratio of a monosaccharide and a disaccharide, such as mannitol and lactulose, respectively.[3] These probes differ in manner of transport, i.e., paracellular or transcellular. In this way two routes of uptake are compared. The most widely accepted method of measuring IP in the small intestine in humans is the lactulose/mannitol or lactulose/rhamnose urine excretion test. In the healthy small bowel, the permeability for larger sugars, such as lactulose, is much lower than for smaller sugars, such as mannitol or rhamnose. Lactulose and other larger

From the Purina Companion Animal Nutrition Summit: *The Gastrointestinal Tract in Health and Disease*; Lisbon, Portugal; March 22-24, 2012.

molecules pass through the intercellular spaces, which are regulated by intercellular tight junctions. Under pathological conditions, such as mucosal inflammation, the permeability of the larger sugars increases, whereas the permeability of the smaller sugars remains stable or decreases. This results in an increased urinary excretion ratio of large to small sugars.[4]

The Role of Intestinal Barrier Function in Systemic Disease

An increased intestinal permeability, often referred to as a "leaky gut," has been proposed to be associated with several gastrointestinal disorders, including intestinal and liver diseases, such as inflammatory bowel disease (IBD)[5] and nonalcoholic steatohepatitis,[6] but also diseases that are not primarily related to GI malfunction, such as type 1 and type 2 diabetes.[7]

Although an altered intestinal barrier function can be a consequence of disease exacerbation, clinical evidence suggests that it may be a primary causative factor predisposing to disease development.[1] For example, healthy, first-degree relatives of patients with IBD and celiac disease have increased intestinal permeability.[8-10] Although the diseases associated with increased permeability differ in terms of pathogenesis and clinical presentation, there seems to be a common denominator: An altered barrier function is believed to facilitate increased exposure to antigens that can trigger immune reaction and autoimmune destruction and alter normal body function. Within this model, the specificity for disease location (target tissue) is provided by both the antigen and the genetic abnormality of the immune system. For instance, the target may be the beta cells of the pancreatic islets (diabetes), the epithelial cells of the gut (celiac disease), or the myelin sheaths surrounding nerves (multiple sclerosis).[11]

This model also does not place any requirements on how the increase in permeability arises. This increase can occur during an infectious process by activation of endogenous humoral pathways or by microbial manipulation of the host's epithelial cell pathways. It may also be a transient event, which may explain the lack of detectable permeability abnormalities in some patients.

Perhaps the most convincing evidence for such a disease model exists for type 1 diabetes mellitus. Moordian et al. were the first to demonstrate increased permeability in diabetic patients by measuring urinary secretion of lactulose and rhamnose.[12] Later, a significantly increased lactulose/mannitol ratio was observed in diabetic patients in comparison to controls, but no significant correlation was found with duration of disease or mean HbA1c values. These findings have been confirmed in other studies.[13,14] Prediabetic subjects had the greatest increase, suggesting that increased IP precedes the onset of clinical diabetes. Accordingly, Bosi et al.[15] observed no differences in enteropathy, measured by the lactulose/mannitol test, between preclinical and long-standing diabetes, suggesting that the duration of diabetes does not further influence IP and that an increased IP precedes, rather than is caused by, type 1 diabetes mellitus. Furthermore, studies in biobreed rats have indicated that the increased permeability detected in prediabetic rats is related to decreased expression of claudin-1 and occludin,[16,17] suggesting a role for tight junctions in altered barrier function in diabetes.

These findings demonstrated that increased IP is observed not only in patients who have developed type 1 diabetes but also in those who are already in preclinical condition. Subclinical inflammation, found in young diabetic patients and characterized by increased interleukin-4 (IL-4), tumor necrosis factor-α (TNF-α) and interferon-γ (IFN-γ), is possibly involved in compromising the integrity of epithelial barrier leading to increased IP of the gut.[18-20] Whether subclinical inflammation precedes or is caused by increased IP requires further investigation. Nevertheless, increased IP makes the host more susceptible and prone to immune reactions against antigens from dietary (cow milk substances like bovine insulin[21] or wheat gliadins), viral or bacterial origin. These agents can activate humoral responses and provided there is genetic susceptibility may trigger autoimmune reactions against insulin-producing beta cells. According to this proposed disease model, expression of diabetes requires genetic predisposition, a dietary provocative agent and abnormal permeability. Removal of either the luminal antigen or the permeability defect prevents disease despite retaining the genetic predisposition. This offers an unprecedented opportunity to prevent disease by counteracting dysbalances in intestinal barrier function.

In case of celiac disease patients, for instance, removal of the antigen (gluten) prompts complete remission of all attributes of the disease, including a return of abnormal intestinal permeability to almost the normal range in the majority of subjects.[22] Furthermore, an inbred Irish Setter line was shown to develop a gluten-sensitive enteropathy that mimics human celiac disease. In these animals, the disease can be completely prevented by weaning the animal onto a gluten-free diet. However, subsequent exposure to the antigen immediately prompts development of the disease. Importantly, animals that have never been exposed to dietary gluten have increased small intestinal permeability.[23] This strongly suggests that in this animal model, abnormal permeability precedes disease. Patients with dermatitis herpetiformis (DH) provide an interesting perspective in this regard. Subjects with this condition exhibit an enormous range of associated bowel pathology from frank celiac disease to a completely normal intestinal biopsy and no evidence of bowel disease. DH patients exhibit increased intestinal permeability, including those patients without evidence of intestinal disease.[24] As some patients may go on to develop celiac disease, it would appear that, in these cases, increased permeability precedes development of disease.

Rheumatological conditions have long been associated with abnormalities of intestinal function, and the concept of abnormal reactivity to a luminal antigen in these conditions is prevalent. Perhaps the best evidence for this comes from the literature on ankylosing spondylitis. Increased gastrointestinal permeability had

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been recognized in these patients for decades, but it was unclear whether this was due to the disease or treatment with nonsteroidal anti-inflammatory drugs (NSAIDs),[25] a drug group known to influence intestinal permeability. With more recent work, the effect of NSAIDs has been isolated, and it is apparent that these patients appear to have a primary defect in intestinal permeability that is shared by a subgroup of relatives.[26] Also, increased gut permeability was observed in patients with juvenile chronic arthritides[27] irrespective whether they were taking NSAIDs, indicating that the disrupted permeability is disease-related.

Accumulating evidence therefore suggests the involvement of barrier function in the pathogenesis of a wide variety of diseases. Another mechanism related to intestinal barrier dysfunction is bacterial translocation. An increase in intestinal barrier permeability can facilitate translocation of luminal bacteria. This can lead to macrophage activation and an increased systemic production of pro-inflammatory cytokines (interleukins, TNF-α) and C-reactive protein, resulting in a systemic inflammatory reaction. These cytokines can thereafter induce systemic changes, such as induction of peripheral insulin resistance by activating nuclear factor-κB (NF-κB), which results in serine phosphorylation of insulin receptor substrate-1 and insulin resistance.[28] Similarly, bacterial translocation has been implicated to play a role in other systemic diseases, as higher levels of antibodies to *Klebsiella pneumoniae* have been found in the serum of patients with ankylosing spondylitis, rheumatoid arthritis and IBD.[29] More recently, it has been proposed that translocation of endotoxin, a constituent of the wall of gram negative bacteria, through a "leaky gut" can exert cardiotoxic effects and contribute to the development of chronic heart failure.[30]

Novel Therapeutic Target: Reinforcement of the Intestinal Barrier Function

Although the diseases listed above clearly differ with respect to pathophysiological mechanisms and clinical presentation, they possibly share an important initiating organ in common: the gut. Reinforcement of the intestinal barrier may therefore become a major goal. There are several routes through which intervention on gut barrier can be established: (1) by altering exposure to nutrients (antigens, especially at young age); (2) by alterations in microbiota composition (pre-, pro- and antibiotics); (3) by modification of gut-barrier proteins and other regulatory proteins; and (4) by restraining the inflammation responsible for the autoimmune reaction. It has become apparent that when the finely tuned trafficking of macromolecules through the intestinal barrier is dysregulated, both intestinal and extraintestinal disorders can occur, particularly in genetically susceptible individuals. This new paradigm subverts traditional theories underlying the development of certain diseases, suggesting that the unfavorable immune activation can be counteracted if the interplay between genes and environmental triggers is prevented by re-establishing intestinal barrier function. Acknowledging the role of the intestinal barrier in the pathophysiology of systemic diseases, a limited number of studies, albeit with varying success, have attempted to reinforce the barrier function using nutritional interventions.[7] Further studies will be needed to verify the true therapeutic potential of enhancing intestinal barrier function.

Conclusion

The intestinal epithelial cells form a selective barrier and ensure the regulation of the trafficking of macromolecules between the environment and the host. Alteration in this barrier function can have profound effects on the interactions between the mucosal immune system and luminal contents, including dietary antigens and microbial products. Increased permeability can therefore contribute to systemic malfunctioning and disease development. Clinical and experimental evidence supports that diseases such as diabetes, celiac disease, IBD and rheumatoid disorders, among others, are associated with an increased intestinal permeability. Whether intestinal epithelial barrier function is a primary causative factor in the predisposition to disease development needs further elucidation. However, recent studies have identified a number of plausible mechanisms that could account for an increased exposure of luminal contents to immunoreactive host cells contributing to altered immune reactions. This increased exposure to luminal antigens can result in an autoimmune destruction of certain target cells leading to disease manifestation or can contribute to augmentation of a systemic immune reaction. Therefore, reinforcing intestinal barrier function may become an important objective to help prevent or counteract pathophysiological mechanisms. A more complete understanding of the molecular pathways involved in the regulation of intestinal barrier function will have important clinical implications by opening new horizons in the treatment and prevention of several systemic diseases, including diabetes mellitus.

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