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Clinical Reviews

The Small Intestine and Nutrition

Intestinal Permeation and Gastrointestinal Disease

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Abstract

The gastrointestinal tract constitutes one of the largest sites of exposure to the outside environment. The function of the gastrointestinal tract in monitoring and sealing the host interior from intruders is called the gut barrier. A variety of specific and nonspecific mechanisms are in operation to establish the host barrier; these include luminal mechanisms and digestive enzymes, the epithelial cells together with tight junctions in between them, and the gut immune system. Disruptions in the gut barrier follow injury from various causes including nonsteroidal anti-inflammatory drugs and oxidant stress, and involve mechanisms such as adenosine triphosphate depletion and damage to epithelial cell cytoskeletons that regulate tight junctions. Ample evidence links gut barrier dysfunction to multiorgan system failure in sepsis and immune dysregulation. Additionally, contribution of gut barrier dysfunction to gastrointestinal disease is an evolving concept and is the focus of this review. An overview of the evidence for the role of gut barrier dysfunction in disorders such as Crohn's disease, celiac disease, food allergy, acute pancreatitis, non-alcoholic fatty liver disease, and alcoholic liver disease is provided, together with critical insight into the implications of this evidence as a primary disease mechanism.

Key Words: Intestinal permeation—Gastrointestinal disease—Gastrointestinal tract.

The gastrointestinal tract is not only the primary organ of digestion and absorption, but also constitutes the largest site of exposure to the outside environment in the human body, and it is the site for a symbiotic relationship between the microbial cosmos of the intestinal lumen and the host. Maintenance of host integrity requires effective monitoring of this site and sealing of the host interior against potential intruders such as bacteria, toxins, or other antigenic molecules. It is this function of the gastrointestinal tract that has been called the gut barrier. This review introduces some of the basic mechanisms of gut barrier function and the tools used in its assessment. It examines the role of barrier dysfunction in gastrointestinal disease, an evolving and growing concept.

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THE ROLE OF THE GASTROINTESTINAL TRACT AS A BARRIER

The gastrointestinal system has several layers of defense against toxins, antigens, and microbes. These include nonspecific protective mechanisms in the intestinal lumen, the intestinal epithelial cells, and immunologic responses to intruders.

Intestinal luminal mechanisms begin with gastric acid and digestive enzymes. The low pH of the gastric juice is bactericidal against infectious organisms, and pancreatic enzymes are able to damage the cell walls of bacteria.[1] Neutralization of gastric acidity can increase susceptibility to pathogenic bacteria such as Cholera and Shigella. Decreased secretion of pancreatic enzymes such as in chronic pancreatitis has been associated with an increased incidence of bacterial overgrowth.[1]

Additionally, epithelial crypt cells secrete fluid into the lumen, which, combined with gastrointestinal motility, tends to "wash away" bacteria and toxins. Large amounts of mucous secreted by epithelial villous cells decreases the ability of luminal microbes to adhere to surface receptors on epithelial cells, thereby diminishing access.

Other nonspecific barrier mechanisms involve the release of self-protective molecules such as immunoglobulin A (IgA) and a class of anti-microbial peptides, collectively known as defensins. Immunoglobulin A is the primary immunoglobulin secreted into the gut, and, unlike other immunoglobulins, it does not participate in proinflammatory or cytotoxic reactions. Its primary contribution to host defense is binding of foreign antigens.[2] This binding usually occurs after its secretion into the gut lumen, which consequently limits access to the epithelium. However, IgA also may bind antigens that have penetrated epithelial cell walls. Once bound, the immunoglobulin–antigen complex is transported through the cytoplasm of the cell and is excreted into the gut lumen and eliminated.[2] Conversely, defensins, produced by cells of myeloid lineage and by gut Paneth cells, are directly toxic by disrupting cell membranes of bacteria. They also can stimulate chloride secretion in the crypt, serving to "flush the crypt of microbial invaders," display che-

motactic activity, and act as links between innate and adaptive immunity.[3,4]

The next line of defense is the epithelial cell, providing the main physical barrier between the lumen and the host. Rapid epithelial cell turnover, combined with peristalsis, contributes to barrier function by clearing adherent or entrapped microbes into the gut lumen.[1] The epithelial cell is supported by a cytoskeleton, and the extension of this framework to the lateral apical portion of the cell forms the intercellular tight junctions (zonula occludens). The cellular cytoskeleton and the intercellular tight junction are dynamic barriers that allow for the bidirectional passage of various substances, possibly including inflammatory cells. This aspect provides the basis of permeation tests that have been used to assess the barrier function of the gastrointestinal tract. These cells also play an important role in antigen sampling and presentation to immune naïve cells to develop a "primed" mucosal defense system.

The maintenance of the integrity and dynamic nature of the epithelial barrier is an energy-requiring process. Thus, in addition to physical damage to the epithelial cells, which can disrupt this barrier, other processes that lead to a decrease in cellular adenosine triphosphate (ATP) also compromise intestinal barrier function, at least as measured by permeation tests. Depletion of cellular ATP has been proposed as the mechanism for damage by nonsteroidal antiinflammatory drugs (NSAIDs) and recently has been shown to also occur with the use of tacrolimus (FK506).[5–7] Although cellular energy depravation has not been evaluated specifically in other disease states that are associated with bowel injury or increased permeation such as bowel ischemia, these processes may play a role in permeation abnormalities.

Clearly, however, other inflammatory conditions may not directly involve the bowel or injure the gut epithelial cell yet may alter barrier integrity, as measured by increased gut permeation. Thus, another mechanism leading to increased permeation has to be invoked. An alternative mechanism may be the proinflammatory cytokines, which can be produced locally by epithelial cells or may reach the intestinal mucosa from an inflammatory focus distant from the bowel.[8] Interestingly, in vitro studies in cell monolayers suggest that cytokines may mediate these permeation effects by changes in the production of nitric oxide.[9] The mechanism for this effect is not known but may involve relaxation of the cytoskeleton[9] or oxidation/nitration of cytoskeletal proteins.[10] Changes in the cytoskeleton resulting in increased permeation appears to be an advantageous response because it may result in increased antigen sampling and also may have an effect on ion transport (net luminal secretion).[9] However, under local inflammatory or other adverse conditions, nitric oxide may adversely contribute to cellular permeability by the formation an oxidant, peroxynitrate, which subsequently can cause DNA damage. The cellular response to this injury is the activation of a nuclear enzyme poly (ADP-ribose) synthetase, which, during the repair process, uses ATP. Thus, in this setting, both cellular damage and cellular ATP depletion can result in increased permeation.[11,12]

The last line of defense is the immunologic response (Fig. 1). The immune system monitors the gut lumen by sampling of luminal antigens, which occurs in an area of specialized epithelium. This area overlies lymphoid follicles, and hence is termed "follicle associated," and is composed of cells that are characterized by paucity of microvilli, a poorly developed glycocalyx, and absence of lysosomal organelles under the electron microscope. The latter cells, called M cells, are the main portal of antigen entry. They internalize microorganisms and macromolecules, which then undergo intracellular processing and are transported to the cell surface. Here, they bind to HLA class I or II receptors on undifferentiated immunocompetent lymphocytes,[2,13] which lie beneath antigen processing cells in gut lymphoid tissue. Once stimulated, the lymphocytes mature to immunocompetent cells with specific memory to the presented antigen. The lymphocytes then can migrate to the lamina propria and intraepithelial regions or can reside in mesenteric lymph nodes, superior mesenteric duct, and along the thoracic duct. Some of these cells then enter the systemic circulation and migrate to other mucosal surfaces, such as the bronchus and breast, and others hone back to the gastrointestinal mucosa. In so doing, they provide a line of defense to invading organisms that breech the epithelium. Malnutrition and other factors that negatively impact the mucosal associated immune system can compromise the integrity of the gastrointestinal barrier.[14]

PERMEATION TESTING

Currently, no tests comprehensively examine the barrier function of the gut in entirety, and most methods test mainly the epithelial barrier. A noninvasive method that has been used in assessing the integrity of the epithelial barrier is the oral administration of test substances and the subsequent measurement of these substances in the urine. Probes used differ in molecular size and sometimes shape but have in common a relatively poor uptake in normal bowel and are poorly metabolized. Thus, significant uptake of a probe occurs only when there is an actual or relative breech in the mucosal barrier. Probes, taken up by the bowel, ultimately are filtered and recovered in the urine. The urine usually is collected for 5 hours, and these probes are measured in the collected specimen. An increased amount of these probes in the urine implies that the integrity of the gut as a barrier to these probes has been compromised.

Initially, a single test substance was used for permeation testing; however, this approach was not ideal because premucosal (i.e., gastric emptying, bacterial degradation) and

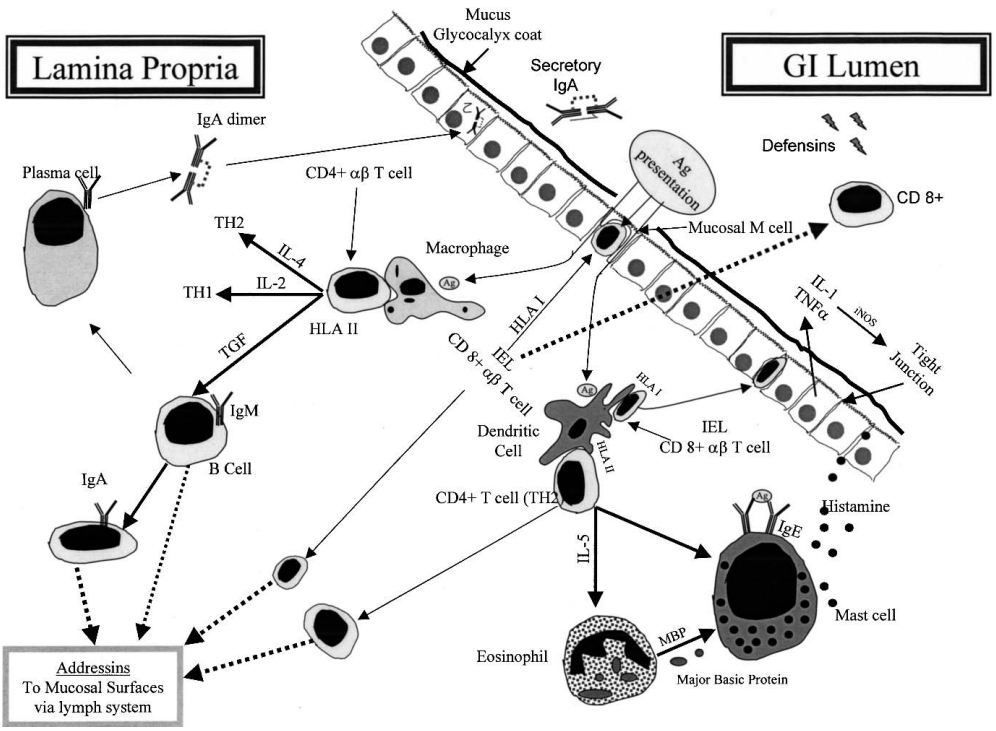

FIG. 1. Gut barrier. The innate protection of the gut barrier is facilitated by the glycocalyx, tight junctions, secretory IgA, antimicrobial defensins (from Paneth cells and neutrophils), gastric acid, and digestive enzymes. These luminal forces allow "washing away" of potential pathogens. The epithelial cell through it cytoskeletal structure maintains intracellular junctions, which can be altered by proinflammatory cytokines produced locally by epithelial cells (interleukin [IL]-1, tumor necrosis factor [TNF]-alfa) themselves or from a distant focus. The cytokines may mediate changes in permeation via nitric oxide. If antigen penetrates the epithelium into the lamina propria, mast cells can recognize and respond via mediator and cytokine release. The antigen processing of the "M" cell facilitates the adaptive immunologic response. The M cell itself, dendritic cell, or macrophage present peptides to naïve T cells, which evolve to aB CD4+ T cells, or CD8+ T cells. The CD4+ cells via cytokines (IL-2,IL-4) influence the expression of Th1 or Th2 subpopulations, which can be altered in disease states. CD4+ cells also assist in the maturation of B cells to produce antibody. CD8+ T cells play an important regulatory role as intraepithelial lymphocytes (IEL). These lymphocytes are then directed via addressins to other mucosal sites or return to the lamina propria.

postmucosal (i.e., renal disease, volume of distribution) factors, as well as intestinal permeability, influenced urinary recovery of the test substance. To address this shortcoming, the urinary ratio of two probes was used (usually, a disaccharide and a monosaccharide) as an index of intestinal permeability. The ratio of administered test probes was a more accurate indicator of permeation because the premucosal and postmucosal factors should influence the probes equally, and, therefore, the urinary excretion ratio should not be affected.[15,16] The four major classes of probes include the ethylene glycol polymers (of varying molecular weights but most commonly PEG 400), oligosaccharides (most commonly lactulose), monosaccharides (either L-rhamnose or mannitol), and radiolabeled chelates (chromium–ethylenediaminetetraacetic acid [Cr-EDTA]).

Which transepithelial pathway is taken by these probes to access the bloodstream remains controversial. The probes appear to traverse the epithelium in one of three ways: paracellular, transcellular aqueous, and transcellular lipid. Thus, the route that a particular substance would take could depend on the properties of the probe. An alternate model proposed by Hollander suggests that all probes cross through the paracellular pathway. The model predicts that there is increasing paracellular junctional tightness from the crypt junctions to the villous junctions. The villous tight junctions are more accessible to luminal compounds but at the same time are "tighter" and more selective for smaller compounds than the crypt tight junctions.[17] Disease states would be predicted to change permeation either by influencing the tightness of the paracellular junctions or through villous injury. In the latter setting, razing the villous structure would physically decrease the tighter junctions in the villous tip and increase exposure of the probes to the "looser" junctions in the crypts.[15,17] This theory by Hollander is supported in a recent study by Fihn et al. using a rat model to assess differential permeability along the villous–crypt axis.[18]

Although it is not clear that increases in intestinal permeability, as measured by these different probes, necessarily implies dysfunction in the mucosal barrier, it may be an early event in the potential disruption of this defense. If, however, increased permeation implies a breakdown of barrier function, it seems likely that additional factors need to be present. The progression from permeation changes to disease likely implies either immune dysregulation or relative immunodeficiency.

THE UNDENIABLE ASSOCIATION

The concept of a breach in the integrity of the gut as a barrier and its consequences in the clinical setting was first recognized in critically ill or injured patients. A portion of these patients would inexplicably develop an inflammatory response or an infectious-like event after a period of stabilization after the initial insult.[19] If the inflammatory response were severe enough, the patient's hospital course could be complicated by multiorgan failure and death. These patients often were malnourished and immunocompromised, and may have undergone surgery during that admission. Although an infectious source responsible for this systemic inflammatory response frequently was not found, when isolated, the culpable microbe often was considered to be of gut origin. In the absence of frank perforation of hollow viscera, some breakdown in the integrity of the intestine's barrier function was implicated.

However, it was realized later that the actual translocation of viable bacteria across the border of the intestines was not a necessary event for subsequent morbidity. It was hypothesized that an inflammatory response with similar morbidity and mortality could be elicited by the sampling of endotoxin or other gut antigens by mononuclear cells, mainly Kupffer cells in the liver. These immunocompetent cells are continually exposed to portal blood and, when stimulated, can release proinflammatory prostaglandins and cytokines.[20] Similar to the "permeation response" of the epithelial cell, this reaction is likely an adaptive reaction to prime the host's immune defenses to a potential gut challenge. However, excessive release of immunomodulatory cytokines can have a detrimental effect on an already compromised host. The cytokine release by Kupffer cells can contribute to the escalating inflammatory response and ultimately result in conditions such as acute respiratory distress syndrome or acute tubular necrosis.[21–23]

These clinical observations have lead to an enormous amount of elegant and interesting research in laboratory animals. Studies were designed to ascertain which situations lead to a compromise in the barrier function of the intestine, usually determined by translocation of intestinal bacteria to mesenteric lymph nodes and sites beyond. Studies determined which interventions could be undertaken to retard this process, and, in a few studies, whether the "translocation event" and the magnitude of the barrier disruption were associated with a poorer outcome. In summary, animals that were subjected to severe injury, exposed to endotoxin, were malnourished, or had decreased or altered enteral stimulation of their intestinal tract were predisposed to bacterial translocation.[24,25]

Supported by these animal data, the extrapolation to the hospitalized patient population was both logical and enticing. Surgical studies already were in place demonstrating a reduction in postoperative septic complications in trauma patients who had early gut stimulation with enteral feeding as opposed to parenteral nutrition.[23] Studies also were undertaken with selective gut decontamination to address and perhaps attenuate this problem.

However, as information began to accrue in human subjects, the clinical relevance of bacterial translocation began to be questioned.[26,27] Overall, although not nearly as dramatic as in the animal studies, there appears to be a clear association between septic complications and bacterial translocation. In two recent surgical reports from the same group, when bacterial translocation was documented, there was a significantly increased incidence of postoperative sepsis.[27,28] Additionally, a recent study in critically ill patients in intensive care units demonstrated a significant correlation between increased intestinal permeation on admission to the intensive care unit and multiorgan dysfunction (MODS). In that study, the severity of the permeation defect also correlated with the severity of the MODS. This observational study lends credence to the theory that gut barrier dysfunction can contribute to MODS.[29] Perhaps one reason for less-than-ideal results in human studies is the inaccessibility of the gut to examination, particularly in critically ill patients, and the heterogeneity of the patients under study. Recent availability of noninvasive ways to test at least epithelial permeability may provide an opportunity to test the possibility of gut barrier dysfunction in critically ill patients.

GASTROINTESTINAL DISEASES AND GUT BARRIER DYSFUNCTION

Breeches in the integrity of the gut barrier have been associated with many inflammatory gastrointestinal diseases, leading to the "leaky gut" hypothesis. It has been postulated that a leaky gut occurs as a result of a primary genetic weakness in barrier function in conjunction with factors that "stress" it, such as NSAIDs or alcohol. Subsequently, excess amount of antigens or bacteria enter the body from the gut lumen, initiating a detrimental immune response initiating disease or worsening disease outcomes. This section reviews the evidence for and against the leaky gut hypothesis in a selected set of gastrointestinal diseases.

Crohn's Disease

The pathogenesis of Crohn's disease (CD) is unknown, but the currently accepted theory postulates several mecha-

nisms. These include environmental factors such as antigens in the diet or the bacterial flora; increased gut permeability to environmental antigens; and increased immune responsiveness to these environmental antigens in genetically susceptible individuals. Of these mechanisms, the most important one is the presence of environmental factors because the disease fails to develop under germ-free conditions in susceptible animal models such as interleukin10–deficient mice.[30] Although the search for a single pathogenetic environmental antigen or an autoantigen has been unfruitful, patients with CD are noted to have an immune reactivity to foods (e.g., milk antibodies), autoantigens (e.g., antinuclear antibodies to histones), and microbes that are part of the normal gut flora (e.g., antibodies against endotoxin core/lipid A [a cell wall component of gram-negative bacteria], and antibodies against a yeast commonly found in baked goods called *Saccharomyces cerevisiae*).

Looking for an explanation for such reactivity, research has focused on intestinal permeability in CD. Many of the animal models for the disease suggest that breech of the intestinal barrier occurs early in the course of disease development. Additionally, knockout mice that are deficient in a major adhesion molecule of the epithelium, *N*-cadherin, develop intestinal inflammatory lesions similar to those seen in CD.[31]

In CD patients, much of the earlier work examined passive permeation to polyethylene glycol, yielding conflicting results. Currently, the technical limitations of measuring polyethylene glycol and a lack of understanding of the mechanisms governing polyethylene glycol permeability prohibit an accurate interpretation of these results. Measurements of gut permeability with Cr-EDTA or differential urinary excretion of monosaccharides and disaccharides (such as the lactulose-to-mannitol excretion ratio) have shown increased intestinal permeability in many studies.[32–36] Also, other studies demonstrate increased pulmonary permeability in patients with CD, suggesting a systemic epithelial barrier dysfunction.[37]

It is unclear whether the observed changes in epithelial permeability in CD cause the disease process or are a consequence of it. To establish a role for permeability in causality, the changes have to precede disease onset. Currently, only one case report exists in the literature that shows changes in permeation before disease onset.[38] In this case, changes occurred 8 years before manifestation of any symptoms, and a thorough examination had excluded subclinical CD at the time of permeation changes.

It has been difficult to study permeation before CD onset; as a result, several alternative approaches have been taken. Initially, focus was directed at relatives of patients with CD, and measurements of epithelial permeability in first-degree relatives have shown increases in some studies but no statistically significant changes in most.[35,39–43] Considering that there is a small absolute risk of disease development in relatives, most negative studies lack adequate power to detect such small differences. In fact, under close scrutiny, even in negative studies, a proportion of relatives (about 10– 25%) have increased epithelial permeation, and clustering in certain families has been observed. In at least one large family study from Europe, however, absence of a typical family pattern and the relatively high prevalence of increased permeability in spouses suggests a role for common environmental factors rather than a genetic disturbance (especially a single gene malfunction) adversely affecting intestinal permeation.[44] In another study, all spouses with increased permeability above the 95th percentile of controls had lived with their partners with CD for more than 10 years.[45] Such a link between permeability and length of contact with CD patients was not seen for relatives of patients.[45]

Although intestinal permeability is not increased in all healthy relatives at baseline, in a significant portion, there is an exaggerated increase in permeability in response to insults causing barrier dysfunction. Hilsden et al.[45A] have shown that small doses of acetylsalicylic acid given to healthy relatives can raise urinary excretion of permeability probes much higher than those observed in controls.[45] Others have confirmed this finding, and one study shows that this provoked elevation is restricted to the relatives rather than spouses of patients.[45] This is especially intriguing when occurrence of flare ups of CD after NSAID consumption are considered and suggests that other stressors may have similar effects.

Currently, no studies directly demonstrate that baseline or post-NSAID (or other stressor) increases in permeability in spouses or relatives can trigger the inflammatory response in CD. In a study by Yacyshyn et al.,[46] when intestinal permeation defects were present in healthy relatives, all of these relatives showed immune activation of B cells indicated by a change in isoform expression of the leukocyte marker CD45RA to CD45RO. Although this association does not confer causality, it suggests that permeation and immunologic changes occur early in the course of disease development and intestinal barrier dysfunction.

A second approach to examine intestinal permeability as a primary or causative factor in CD development was to look at permeability of the normal-appearing intestine, both in vitro and in vivo. However, considering that the immune reaction in CD and the cytokines and prostaglandins involved in this reaction easily may exert systemic or spillover effects in normal tissue, this line of evidence supporting a primary permeability defect in CD can be refuted easily. Nevertheless, in vitro biopsy specimens of normal tissue have been found to have increased permeability,[47] and examination of tight junctions in normal small bowel from CD patients show that these junctions are composed of fragmented and irregularly distributed strands.[48] In vivo, perfusion of macroscopically normal je-

junum (as assessed by small bowel follow-through or enteroclysis) in patients with CD, using an orally inserted catheter that delivers the permeability probes only to the first 100 cm of intestinal mucosa, showed increased permeability in 13 of 19 patients.[49] Conversely, gastroduodenal permeability studies demonstrate no increases in sucrose permeation in endoscopically and histologically normal stomach and duodenum in CD patients without upper gastrointestinal symptoms.[50,51]

A third approach was to examine the ability of permeation testing to identify patients who will have relapse of disease. Several studies show that permeability increases in CD patients preceding clinical flare-ups, suggesting a role for gut barrier dysfunction in perpetuation of disease.[36,52,53] Although the possibility of subclinical disease in these studies cannot be excluded because of the poor correlation between the CD activity index and permeability findings, the magnitude of permeability changes are well correlated with endoscopic extent of disease,[54] making it possible to contemplate a role for permeability testing as an objective, noninvasive way to evaluate patients at follow-up. Further studies with more patients and simplification of the measurement technique may enable this in the future.

In short, the role of increased permeability in initiation of CD is uncertain; however, there is ample evidence suggesting that it is an important factor in disease perpetuation and reflects an early change in the course of disease.

Celiac Disease

Epithelial permeation tests in patients with celiac disease (CS) demonstrate a reduced absorption of small markers of permeation, such as mannitol, and an increased absorption of relatively larger markers, such as lactulose. This finding fits well with the permeation hypothesis as proposed by Hollander.[17] The increased absorption of large molecules may result from villous epithelial injury with greater exposure of these probes to the crypt tight junctions, increased cell shedding with less "mature" tight junctions, or changes in the tight junctions secondary to inflammatory cells and their mediators. The decreased absorption of small molecules may result from a reduction in the total number of paracellular junctions because of a reduced surface area.[55,56] Sucrose also has been introduced as a probe to assess increased permeation in CS. Its applicability to the diagnosis of this disease arises from the usual digestive pathway of this disaccharide. Sucrose, which normally is broken down by brush border disaccharidases, is spared that metabolic fate in the proximal gut because of the effects of CS on abolishing sucrase activity; hence, the ingested sucrose is absorbed intact. As the disaccharide progresses through the gastrointestinal tract, it is rapidly degraded, unlike other relatively large molecular probes. Thus, sucrose appearing in the urine collection after an oral challenge should reflect increased permeation through the proximal diseased small intestine in this disease. An important requirement for the specificity of the test is the absence of gastric disease because changes in this probe also can reflect NSAID-induced gastric disease.[57,58] Smecuol et al.[55] verified increased sucrose absorption in patients with known CS. Most patients in this study had advanced histologic disease, and in this subgroup of patients, the sensitivity of this probe was comparable with other noninvasive diagnostic measures. Additionally, patients on a gluten-free diet for 2 months had improvement in their urinary sucrose excretion, implying that this probe may be valuable in monitoring response to therapy.[55]

Although patients with symptomatic CS clearly have a permeation defect, it is unclear if the permeation defect is a primary factor for initiation of the disease process, contributes to some of the associated manifestations of the disease, or is solely the result of the disease process and tissue injury as an epiphenomenon. Regarding the first possibility, an underlying permeability defect may trigger the expression of CS in susceptible individuals. The increase in intestinal permeability may allow ingested gluten or gluten-derived peptides to cross the epithelial barrier and incite an immune reaction. In a study by Hall and Batt,[59] Irish setter dogs, bred from parent dogs with a gluten enteropathy, were fed either a regular or gluten-free diet from birth. Intestinal permeation was measured at 4, 6, 8, and 12 months of age and correlated with small bowel biopsy specimens. Intestinal permeation was greater in the dogs fed a regular diet than those fed a gluten-free diet and progressively increased over the subsequent determinations, reaching statistical significance between groups at 8 months. The permeation difference in both groups of affected dogs was statistically significant from control dogs on the first determination at 4 months. The permeation changes in the affected dogs fed a regular diet occurred before histologic changes were evident. These findings suggest that a baseline permeation defect exacerbated by continued exposure to gluten finally resulted in morphologic damage.[59] However, human studies yield conflicting results. In both untreated patients and in most patients successfully treated with gluten withdrawal, a residual permeation defect exists despite apparent histologic normalization. The possibility of ingestion of a small amount of gluten despite being "gluten free," as well as the overlap in permeation values with normal control patients, casts doubt as to whether the defect in the intestinal barrier is a primary problem or is a result of intestinal damage.[59] Because there is a 10% prevalence of CS among first-degree relatives, permeation studies have been conducted in relatives of affected individuals before disease manifestation to determine if abnormalities in the intestinal barrier precede expression of the disease. Increased intestinal permeation has been inconsistently found in first-degree relatives of patients.[60,61] Using a combination of jejunal biopsies and standard permeation probes, Marsh et al.[62] found that 38%

of "largely asymptomatic" relatives demonstrated increased intraepithelial lymphocytes, an early histopathologic finding in CS, yet epithelial permeability was normal. However, because there were no mucosal morphologic abnormalities or increased permeation associated with this finding, the authors could only speculate as to their significance as a harbinger of clinical disease.[62] In contrast, in a study by Vogelsang et al.,[60] increased epithelial permeation was found in 31% of 111 relatives of patients with CS. Of these relatives, 8% ultimately were diagnosed with the disease. In a subset of these relatives who sought medical attention because of mild gastrointestinal symptoms and had a family member with CS, 42% ultimately were diagnosed with CS, and all of these patients had increased intestinal permeation. An additional 4% of asymptomatic individuals also were diagnosed with CS and also had increased permeation. In the remaining 22% of relatives, greater than 70% either had no repeat permeation test or the permeation had decreased, but not necessarily normalized, on a subsequent examination. Finally, the prevalence of abnormal intestinal permeability in relatives of patients with CS was found to be similar to the 40% prevalence encountered in the control subjects with nonspecific gastrointestinal symptoms.[60] Therefore, an inherent intestinal permeation defect does not seem to play a central role in disease development. Further longitudinal studies with serial permeability testing and intestinal biopsies in both asymptomatic controls and relatives of patients with CS are needed to confirm this observation and to determine whether there is a subgroup of celiac patients with inherent permeation defects[41] or increased susceptibility to acquisition of such defects.

Regarding the second concept of increased morbidity associated with permeation changes in CS, there also are interesting, although not definitive, studies. Permeation to ^{51}Cr-EDTA in experimental models of intestinal inflammation has been shown to correlate with permeation to oligopeptides and macromolecules and with the formation of circulating immune complexes.[59,63–65] The increased exposure of the gut immune system or the systemic immune system to these macromolecules may lead to autoimmune disease in susceptible individuals. It also has been suggested that IgA deficiency leads to enhanced macromolecular absorption, a mechanism that may explain its association with autoimmune disease.[65] A report by Ventura et al. corroborates the idea of increased intestinal permeation and exposure to macromolecules leading to autoimmune disorders. These authors found that the prevalence of autoimmune disorders in CS was related to the duration of exposure to gluten.[66]

Clearly, many individuals who demonstrate the classic manifestations of CS do so during infancy with the transition from milk to solids. Breast-feeding also has been reported to delay the onset or change the clinical manifestation of CS. This may be as much a role of IgA-mediated binding of potentially toxic antigen during a time when the intestinal barrier is not fully mature because it is a delay in the exposure to wheat. Additionally, some individuals do not manifest symptoms until later in life, and many of those individuals may not present with classic clinical symptoms. In some individuals, an antecedent insult, such as surgery or a viral illness, may be the harbinger of the disease. Various exposures to the immature gut barrier or insults to the more mature gut barrier may result in increased antigen exposure. This exposure to the gut immune system may, in turn, be the mechanism that ultimately causes the genetically susceptible individual to express clinical manifestation of the disease. Finally, the association of CS with IgA deficiency is curious and may represent a decrease in mucosal defense and a subsequent increase in exposure risk in the susceptible individual.

Food Allergy

Animal studies show that intestinal uptake of macromolecules is at a maximum during the neonatal period and decreases overtime with maturation of the intestinal barrier. In humans, maturation of the intestinal barrier occurs at around 38 weeks of gestation, continues in the neonatal period and infancy and is influenced by a variety of factors.[67] One such important factor is breast-feeding, which has been shown to increase production of neonatal secretory IgA.[68] Another is indigenous flora, which may modulate the immune response to luminal antigens by acting as adjuvants.[67]

It is plausible that any alterations in maturation of the gut barrier can lead to increased exposure to orally ingested antigens and cause a primed immune response. Evidence supporting this hypothesis comes from the presence of circulating antibodies to common food antigens during the first 3 months of life.[67,69] The common nature of food allergies in the pediatric population also attests to this possibility.

Most of the existing data in permeability and food allergy concern children. Children with cow's milk allergy have increased ^{51}Cr-EDTA and lactulose–mannitol permeation.[70–73] Additionally, asymptomatic children who have been fed cow's milk formula versus those who were breast-fed also have increased intestinal permeation.[74] However, these results are contradicted by Falth-Magnusson et al., who found normal permeability even after challenge with cow's milk.[75] Another study by Jalonen shed light on these findings.[76] Lactulose–mannitol ratios that were measured before cow milk's challenge were normal in control subjects and allergic children, whereas after 3 days of continuous challenge, there was a significant increase in permeability. These findings suggest that intestinal permeability is altered as a result of the initial reaction to the allergen, possibly through the release of inflammatory cytokines. The latter hypothesis also is supported by findings of increased tumor necrosis factor and interferon gamma release after cow

milk's challenges,[77,78] up-regulation of transport and processing of food antigens,[78] and increase in paracellular transport with in vitro experiments as a consequence of the release of these cytokines.[78]

Aside from cow milk's allergy, Dumont et al. demonstrated normal intestinal permeability in food-allergic eczematous children.[79] However, Barau and Dupont showed increased permeability in children with inflammatory bowel syndrome after challenge with symptom-causing foods.[80] Similarly, Paganelli et al. found normal baseline permeability using beta-lactoglobulin but decreases in permeation after a hypoallergenic diet or sodium chromoglycate in patients with inflammatory bowel syndrome and food sensitivities.[81] Several other studies have shown a utility for lactulose–mannitol test in food allergy in atopic children,[82,83] and reversibility of permeation changes with treatment of mast cell stabilizers such as chromoglycate.[82] In contrast, other studies in adults have demonstrated normal or close-to-normal intestinal permeability in vivo and in vitro in food-intolerant subjects.[84–87]

In conclusion, technical difficulties of confirming or establishing a diagnosis of food allergy, lack of uniformly applied criteria, small sample sizes, and use of variety of techniques to assess permeability have resulted in a complex body of literature. Current evidence supports alteration of permeability through cytokine release in response to food allergens, but further studies are needed to define the contribution of altered permeation to food allergies and sensitivities.

Acute Pancreatitis

Although increased intestinal permeation does not appear to play a role in the initiation of acute pancreatitis, it may contribute to an increase in the morbidity and mortality of this disease. Severe attacks of pancreatitis often are complicated by cardiopulmonary and renal abnormalities related to the systemic inflammatory response syndrome. Pancreatitis-related infections occur in 8% to 10% of these patients and account for more than 80% of the mortality in these patients.[88] The infecting organism usually is a gram-negative aerobic bacterium, suggesting an enteric origin.

The relationship between acute pancreatitis and changes in gut permeation has been studied in animal models. Using a model of cerulein-induced pancreatitis, Ryan et al. demonstrated that acute pancreatitis resulted in increased intestinal permeation to the macromolecular probe polyethylene glycol 3350. Additionally, the magnitude of this permeation defect correlated with the severity of the pancreatitis.[89] This process was further studied by Gianotti et al. in two different models of pancreatitis, demonstrating significant translocation of gut bacteria into peritoneal fluid, mesenteric lymph nodes, liver, lungs, and pancreas.[24,90] Translocation was determined by homogenizing the solid tissue, or isolating peritoneal fluid–blood, and plating the mixture. After incubation, colony-forming units (CFUs) were counted. Interestingly, the study used gavaged radiolabeled *Escherichia coli* in some animals. With this technique, the authors were able to assess the total amount of translocated bacteria (nuclide ratio) and compare this with the translocation of viable bacteria (CFU ratio). The high nuclide-to-CFU ratios suggest that the tissue to which the bacteria translocated was effective in killing or controlling the spread of the organism. As might be expected, lymph nodes and liver had high nuclide-to-CFU ratios, whereas the pancreas, lung, and peritoneal fluid were less effective in controlling the spread of these bacteria. These findings parallel the sites of septic complications seen clinically in this disease.[24]

Wang et al. have further added to the understanding of the pathogenesis of bacterial translocation and spread during acute pancreatitis.[91] In a model of experimental pancreatitis, they confirmed the presence of translocation but also found impairments in Kupffer cell phagocytic function, hypothesized to be partly related to the release of pancreatic proteases into the portal circulation. Interestingly, there also was a decrease in gut oxygen extraction,[91] which could result in epithelial dysfunction, and bacterial overgrowth was present. Bacterial overgrowth has been associated with an increased propensity for translocation in other studies and has been attributed to bowel dysmotility during the acute pancreatic insult. Finally, in contrast to defective phagocytic function of Kupffer cells, intestinal macrophages were found to be overactivated. Data from the same group of researchers also demonstrated that blocking these intestinal macrophages could decrease the incidence of translocation. One hypothesis of Wang et al. is that the facilitating effect of activated intestinal macrophages on translocation may be caused by release of tissue-destructive agents, inflammatory mediators, or physical shunting of the bacteria through the gut wall.[91]

Studies to improve the integrity of the intestinal barrier in the setting of experimentally induced pancreatitis also have been undertaken. Epidermal growth factor, a peptide substantially involved in maintaining the integrity of the intestinal barrier by promoting growth and maturation of epithelial cells as well as migration of epithelial cells, was shown to be effective in decreasing bacterial translocation to mesenteric lymph nodes.[92]

The protective role of enteral nutrition and stimulation in maintaining the integrity of the gut barrier has been demonstrated in other animal models of sepsis and stress. Therefore, Kotani et al., in a rat model of acute pancreatitis, evaluated the role of enteral versus parenteral feeding. The enteral nutrition group was found to have significantly less bacterial translocation and a lower blood endotoxin level than the parenterally treated group. Unfortunately, the authors were not able to demonstrate a difference in survival.[93]

Similar increases in intestinal permeability have been found in human studies of acute pancreatitis, with the severity of the insult correlating with the degree of increased permeation.[94] Attempts to address breakdown of the intestinal barrier, with resultant potential increases in the morbidity and mortality of acute pancreatitis in humans, largely have consisted of selective gut decontamination and enteral stimulation.

In a study by Luiten et al.,[95] 102 patients with severe acute pancreatitis were randomly assigned to receive either standard therapy or selective gut decontamination. Gut decontamination was performed with an oral solution consisting of colistin, amphotericin, and norfloxacin. The authors found a significantly reduced rate of pancreatic infection in the treated group (38% vs. 18%). This decrease in the incidence of infected pancreatic necrosis was associated with a reduction in the number of laparotomies and deaths. Perhaps more significantly, the treated group had fewer pancreatic infections with gram-negative organisms (33% vs. 8%). The authors determined that gram-negative pancreatic infection increased mortality, no matter which group the patient was assigned. Thus, selective gut decontamination appears to improve outcome by decreasing gram-negative infection of pancreatic necrosis.[95,96]

Despite potential benefits of enteral stimulation on the maintenance of gut barrier integrity, the use of enteral nutrition in acute pancreatitis largely has been avoided until recently because of the perceived untoward effects of enteral stimulation on the inflamed pancreas. A pioneer study by McClave et al. compares enteral jejunal feedings to parenteral nutrition in patients with mild pancreatitis, generally alcoholic in etiology.[97] The authors found that the enteral route was safe, and there were no differences in clinical or biochemical resolution of pancreatitis in their study patients.[97] Kalfarentzos et al.[98] then performed a randomized prospective trial of enteral versus parenteral feeding in severe acute pancreatitis (Apache II score, 8–15). These authors not only showed that enteral feeding was safe, but patients in that group also had a significantly lower rate of septic complication than the parenteral nutrition group. Other morbidities, including the incidence of infected pancreatic or peripancreatic necrosis, also were more favorable in the enteral nutrition group but did not reach statistical significance, likely secondary to the small numbers of patients in the groups.[98]

Although these studies establish safety and suggest better outcomes and decreased infectious complications, a clear relationship between maintenance of gut barrier function and decreased septic complications has not been proven. A study by Windsor et al.[99] examines endpoints that were not only related to nutrition and outcome, but also the immunoinflammatory response and its possible modulation with enteral feeding. In addition to finding that enteral nutrition was safe and associated with downward trends in sepsis and multiorgan failure, the authors report significant reductions in the APACHE II scores of the enteral feeding group versus the parenteral, and significant decreases in C-reactive protein. Interestingly, there was significant escalation in the IgM anti-endotoxin response in parenterally fed patients whereas levels of these antibodies remained unchanged in the enterally fed ones. Windsor et al.[99] state that enteral feeding seemed to improve disease severity and clinical outcome by modifying the acute-phase response and by maintaining the integrity of the gut as a barrier against translocation, a major contributor to the inflammatory response in pancreatitis. Although animal and human studies suggest that a breakdown of the gut barrier is instrumental in the local and systemic infectious complications associated with severe acute pancreatitis, larger multicenter studies are underway to firmly establish this link.

Liver Diseases

Increased intestinal permeability has been implicated in at least two common liver diseases: alcoholic liver disease and fatty liver disease. Fatty liver disease is the most common cause for chronic abnormal results of liver function tests, and 25% of Americans have fatty liver. However, few patients with fatty liver have advanced liver disease such as steatohepatitis, fibrosis, and cirrhosis. Yet, advanced liver disease related to fatty liver appears to constitute a major cause of liver failure and is the most common underlying cause of cryptogenic cirrhosis. Therefore, a better understanding of the cofactor responsible for progression of fatty liver to advanced liver disease will have an important therapeutic impact. Several recent studies strongly suggest that endotoxin could be such a cofactor. Increased intestinal permeability, therefore, has been implicated in pathogenesis of steatohepatitis and severe fatty liver.[100,101] However, there has been no direct measurement of intestinal permeability in patients with fatty liver.

Another liver disease that has been associated with increased intestinal permeability is alcoholic liver disease. It is well known that only 30% of alcoholics develop chronic liver disease. This observation indicates that there is a cofactor for the development of liver disease in alcoholics. Several experimental studies strongly suggest that endotoxin is essential in causing alcohol-related liver injury. For example, reducing endotoxemia in alcohol-fed animals has successfully prevented alcohol-induced liver injury.[102] Furthermore, increased serum endotoxin has been demonstrated in patients with alcoholic cirrhosis, strongly suggesting that endotoxin plays a pivotal factor in developing alcohol-induced liver disease.[103] Because the major source of endotoxin is the gut lumen, increased intestinal permeability has been suggested as a major factor in alcohol-induced endotoxemia. We recently demonstrated increased intestinal permeability and increased absorption of endotoxin from intestinal lumen in an animal model of al-

coholic liver disease.[104] Furthermore, we also demonstrated increased intestinal permeability in alcoholics with liver disease, but not in alcoholics without liver disease or in nonalcoholics with chronic liver disease.[105] Additionally, "gut leakiness" and endotoxemia have been implicated as cofactors for exacerbation of liver failure in patients with advanced liver disease, and they are proposed as important factors in the worsening of encephalopathy and spontaneous bacterial peritonitis.[106] These examples suggest that increased intestinal permeability and endotoxemia play a role in either initiation of liver injury or in the progression of liver disease. The mechanism through which abnormal intestinal permeability occurs in patients with liver disease is not known and remains to be investigated.

CONCLUSIONS

Human defense strategies, although far from perfect, have evolved since inception. As such, innate reactions that appear to contribute to disease initiation and propagation likely have an underlying initial advantageous response for the individual. An alteration in intestinal permeation in response to insult or antigenic challenge is no exception to this rationale. Normally, luminal antigens are sampled in a more controlled fashion by M cells and other antigen-presenting cells in the bowel. Antigens then are presented to undifferentiated immunocompetent cells that mature and hone back to mucosal surfaces, including the gut. This is an essential function because it provides a sensitized line of defense should an insult from a like or similar antigen be mounted.

Permeation can be increased through several mechanisms. One is an acquired or inherent defect in the gut barrier, which includes physical injury to the barrier or possibly a genetic predisposition to gut leakiness. Other acquired but nonphysically injurious mechanisms would cause ATP deficiency either through noncytotoxic insult to the cell or by interference with energy-generating pathways. Because ATP is essential for the maintenance of a normal functioning cytoskeleton and tight junctions, its depletion has negative effects on gut permeation. A second broad mechanism is through the action of inflammatory cytokines. This latter reaction likely is a mechanism to increase luminal antigen sampling and "prime" Kupffer cells for a potential impending insult. However, in the setting of too vigorous a cytokine response, an overwhelming bacterial or antigenic stimulus can result in further up-regulation of the inflammatory response, resulting in multiorgan dysfunction. Additionally, less overwhelming antigenic challenge in the setting of an intrinsic immune dysregulation, or relative immunodeficiency, also can lead to untoward detrimental effects. Thus, more studies are needed to further address the relationship between gut barrier dysfunction and immunity in light of the potential contributions to disease initiation and propagation.

If it leaks and we are able to spot the breech, we are likely to be stronger for the event, but if the craft is faulty or we take on too much water, we will likely go down.

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