Irritable Bowel Syndrome: Methods, Mechanisms, and Pathophysiology.

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Am J Physiol Gastrointest Liver Physiol 303: G775–G785, 2012. First published July 26, 2012; doi:10.1152/ajpgi.00155.2012.—Irritable bowel syndrome (IBS) is one of the most common gastrointestinal ailments among those seeking health care for gastrointestinal disorders. Despite its prevalence, IBS pathophysiology is still not completely understood. Continued elucidation of IBS etiological mechanisms will lead to a greater appreciation of possible therapeutic targets. In the past decade, there has been increasing focus on the possible connection between increased intestinal mucosal permeability, inflammation, and visceral hypersensitivity. Increased permeability in subsets of IBS patients has been observed and the possible mechanisms underlying this defect are just beginning to be understood. The objectives of this review are to summarize the role of the healthy intestinal epithelium as a barrier between the lumen and the rest of the body with a focus on tight junctions; to examine the lines of evidence that suggest that different triggers lead to increased intestinal mucosal permeability and disruption of tight junctions in IBS patients; and to explore how this increased permeability may elicit immune responses that then affect afferent nerves, resulting in pain associated with IBS.

Irritable bowel syndrome (IBS) is a functional gastrointestinal disorder characterized by recurrent abdominal pain associated with changes in stool frequency and form, with no recognized underlying pathological or organic etiology (35, 74). It affects up to 18% of adults in Western countries, predominantly women, especially among those seeking health care (26). IBS has a significant impact on quality of life and health care utilization (35, 77). IBS subtypes based on bowel dysfunction (diarrhea-predominant [IBS-D], constipation-predominant [IBS-C], alternating stool forms [IBS-A], and unsubtyped [IBS-U]) appear to be equally distributed in affected populations (26, 74).

Traditional management of IBS has been symptom based, but recent developments in the understanding of complex interactions between the gut, immune system, and nervous system have led to an expanded arsenal of therapeutic options for relief of both bowel movement-related symptoms and pain (18, 75). There is growing interest in the connection between increased intestinal permeability, immune responses, and visceral hypersensitivity, especially as a potential target for therapeutic intervention. In this paper, we will review the role of the healthy intestinal epithelium as a barrier between the lumen and the rest of the body, examine the lines of evidence that suggest that different triggers lead to increased intestinal mucosal permeability, and explore how this increased permeability may elicit immune responses that then affect afferent nerves, resulting in pain.

The Intestinal Barrier

In healthy individuals, the intestinal barrier provides a “gated wall” between the luminal contents of the gut (e.g., food antigens, microflora, ingested bacteria) and the rest of the body, selectively regulating what crosses the epithelium via transcellular transport mechanisms and regulated paracellular permeability. The intestinal barrier is comprised of several defensive layers: 1) the lumen, where gastric acids and pancreatic and biliary secretions degrade bacteria and antigens; 2) host luminal bacteria, which inhibit colonization by pathogens through the production of antimicrobial substances, through modification of the luminal pH and luminal content, and by competing for nutrients that are required for pathogen growth; 3) the microclimate, which includes the unstirred water layer, the glyocalyx, and the mucus layer with secreted IgA and prevents adhesion of pathogenic bacteria to the epithelium; 4) the epithelium, which consists of cells connected to each other via junctional complexes to create a physical barrier and reacts to noxious stimuli with chloride secretion and release of antimicrobial peptides; and 5) the lamina propria. The latter contains several components: cells that participate in innate and acquired immunity and secrete immunoglobulins and cytokines; the enteric nervous system and endocrine system; myofibroblasts; and other components (58).

Lipid-soluble and some small hydrophilic molecules can passively move through the epithelial cell via diffusion across the cell membrane, whereas energy-dependent, carrier-mediated mechanisms transport nutrients (58). Transcellular movement of large molecules and particles across the intestinal epithelium is facilitated by different forms of vesicular-mediated transport, including clathrin-mediated endocytosis, phagocytosis, caveolar membrane trafficking, and macropinocytosis (58). Paracellular movement of small, hydrophilic molecules is highly regulated by tight junctions (TJs). In healthy, intact mucosa, TJs seal the paracellular space and form an ion- and size-selective paracellular gateway (42). Degrees of permeability vary throughout the gastrointestinal tract; for example, the small intestine has higher permeability than the colon to the saccharide mannitol (83), whereas ion permeability differs between the ileum, jejunum, and colon (32). In addition, TJs create a “belt” around each epithelial cell that physically

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demarcates the apical and basolateral domains of the cell. Intracellular trafficking may involve key proteins, including caveolin-1 and Rab5; chloride channel protein 2 (CIC-2) was recently reported to play an important role in the modulation of TJ integrity by influencing caveolar trafficking of the TJ protein occludin (85).

A variety of proteins involved in TJ structure and function has been identified and characterized; full review of all of these proteins is beyond the scope of this paper. Among the transmembrane protein components of TJs, the claudin family of proteins comprises the backbone of TJ strands and is involved in the ion and size selectivity of the TJs (42, 58, 118). Occludin makes up part of the extracellular TJ “fencing,” but its function in regulating paracellular permeability is still being elucidated (42, 97). Tricellulin, which shares some structural similarities with occludin, is located primarily at the tricellular TJs and to a lesser extent at bicellular TJs (53, 63). Varying expression levels of tricellulin are thought to influence epithelial permeability to macromolecules at the tricellular junctions and to solutes of all sizes at bicellular junctions (63). Occludin, tricellulin, and a third protein, marvedel3, belong to a family of proteins called tight junction-associated MARVEL proteins (TAMP). They contain the tetra-spanning MARVEL [MAL (myelin and lymphocyte protein) and related proteins for vesicle trafficking and membrane link] domain that is present in proteins involved in membrane apposition and concentrated in cholesterol-rich microdomains (102). The TAMP group of proteins provides both overlapping and unique contributions to epithelial function and TJ regulation (98). Junction adhesion molecules (JAM) are another class of transmembrane TJ proteins. JAM-A appears to be involved in the regulation of permeability, TJ assembly, and inflammation (66, 73).

Zonula occludens (ZO) proteins ZO-1, ZO-2, and ZO-3, interact with claudins, occludin, JAM-A, and each other and connect the transmembrane TJ proteins with the actomyosin fibers of the cytoskeleton (42, 58, 118). Redistribution of ZO proteins to the cytosol has been observed in multiple scenarios of increased paracellular permeability (13, 25, 27, 45, 55, 79, 107). Phosphorylation of the myosin light chain by myosin light chain kinase (MLCK) and Rho GTPases has been shown to regulate the size selectivity of TJs and, thus, paracellular permeability via redistribution of ZO-1 and occludin (51, 107). Upregulation of MLCK expression and activity has been noted in patients with Crohn’s disease (15). Increased activity of intestinal epithelial MLCK is the primary mechanism of TNF-induced barrier dysfunction. However, in elegant studies in transgenic mice that express constitutively active MLCK, Su et al. (114) have demonstrated that pathophysiologically relevant intestinal epithelial barrier dysfunction is insufficient to cause experimental intestinal disease, but it can broadly activate mucosal immune responses and accelerate the onset and severity of immune-mediated colitis. Rho GTPase inhibition or activation both appear to induce TJ disassembly, more than regulating intact junctions (51). Zonulin has also been identified as a regulator of TJs. Elevated zonulin expression increases intestinal permeability, and it has been suggested that this may contribute to antigen exposure in autoimmune disorders such as celiac disease and Type 1 diabetes (38). Additional studies are needed to independently confirm the role of zonulin and occludin in intestinal permeability. Indeed, the role of ZO-1 in TJ regulation is somewhat controversial (54), and TJs have been observed in occludin-deficient cells (101).

Along with TJ proteins, ion channels may play a role in maintaining the integrity of the intestinal barrier. CIC-2 is localized near TJs in murine small intestine mucosal epithelium (50) and in the apical and supranuclear areas of human colonic enterocytes (72). CIC-2 may play a critical role in the modulation of TJ barrier by promoting the presence of occludin in the cell membrane (85). In CIC-2 knockout mice, recovery of intestinal mucosal barrier function after ischemia is impaired relative to wild-type mice, with an observed failure to properly distribute TJ proteins occludin and claudin-1 to the apical portion of epithelial cells (86). In Na+/H+ exchanger 2 knockout mice, recovery of ischemic-induced intestinal permeability was impaired, with a shift in the expression of occludin and claudin-1 and disruption of their localization patterns (81). Although the above-mentioned animal models are not completely representative of the histopathology associated with IBS in humans, the role of CIC-2 in barrier repair continues to be elucidated and warrants further investigation in an IBS model. A similar association has been suggested between altered ion transport due to mutations in the cystic fibrosis conductance regulator (CFTR) and disrupted TJ function in the compromised respiratory epithelial barrier function observed in cystic fibrosis (69, 128).

Increased intestinal permeability has been recognized as an underlying pathophysiology for other gastrointestinal diseases, including Crohn’s disease (47, 108), ulcerative and microscopic colitis (47, 71), and celiac disease (120). However, it is unclear whether there is a common molecular mechanism that causes the altered permeability in these disease states. On the other hand, some of the genetic predispositions to altered barrier function in Crohn’s disease (12) have also been associated with symptoms of IBS, particularly TNF ligand superfamily member 15 (135), and Toll-like receptor 9, interleukin-6 (IL-6), and cadherin-1 (CDH1) (122). Genetic variation in loci associated with susceptibility to immune activation is also univariately associated with increased colonic transit, a clinical marker in ~45% of patients with IBS-D (19).

Increased Permeability in IBS

Intestinal permeability can be assessed by multiple techniques. The four primary modes often used to explore permeability in IBS are 1) following the absorption and urinary secretion of molecules (oral probes) known to be unchanged in urine and whose site of intestinal absorption is known, such as polyethylene glycol, the saccharides mannitol, lactulose, and sucralose, or chromium-labeled EDTA (51Cr-EDTA); 2) examining the migration of a probe across mucosal biopsies in vitro; 3) studying the effects of biopsy extracts or colonic supernatants from IBS patients (compared with healthy controls) when applied to intestinal epithelial monolayers, such as Caco-2 monolayers, or murine intestinal tissue (in vivo or in vitro); and 4) analyzing expression levels of TJ proteins by immunohistochemistry, immunofluorescence, or mRNA quantitation. Each technique has its own strengths and weaknesses (Table 1).

With use of oral probe excretion assays, increased small bowel and colonic permeability has been noted in both adult and pediatric patients, primarily with postinfectious IBS (PI-
IBS) and IBS-D (37, 46, 76, 100, 110, 113, 134) (Table 2). However, analyses of colonic and jejunal biopsies have revealed evidence of significantly increased permeability compared with healthy controls regardless of IBS subtype and disruption in the expression and distribution of TJ proteins (13, 29, 60, 78, 79, 92, 123). In colonic biopsies of both IBS-D and IBS-C patients, there is increased ubiquitin proteasome activity compared with that of healthy controls, with no difference observed between the two IBS groups. Ubiquitin proteasomes degrade ubiquitin-tagged proteins. This proteasome activity may be responsible for the observed degradation of occludin, since occludin protein levels were lower in biopsy specimens of IBS patients compared with controls, whereas occludin mRNA levels were not significantly different in IBS and controls (29). A subsequent study by the same group of investigators found that expression of ZO-1 and occludin proteins was significantly lower in the colonic mucosa of IBS patients compared with controls (13). There was no difference in ZO-1 protein expression between IBS subtypes, but occludin expression was reduced in IBS-D compared with controls and claudin-1 expression was significantly lower in IBS-D compared with both IBS-C and controls. Ocludin and claudin-1 expression were not different in IBS-C and IBS-A compared with controls; mRNA levels of ZO-1, occludin, and claudin-1 were not different in IBS patients, regardless of subtype, compared with controls (13). These observed changes in TJ protein expression resulted in the disrupted and irregular distribution of claudin-1, occludin, and ZO-1 staining patterns in both IBS-C and IBS-D patients. In colonic samples from controls, claudin-1, occludin, and ZO-1 staining was mainly at the apical end of epithelial cells. However, in IBS-C patients staining for occludin and claudin-1 was mainly cytosolic and completely abolished in IBS-D patients. ZO-1 staining was less intense in both IBS-C and IBS-A (13).

Piche et al. (92) demonstrated significantly increased paracellular permeability in colonic biopsy samples of IBS patients, regardless of subtype, and decreased ZO-1 mRNA expression compared with that observed in samples from healthy controls. Incubation of Caco-2 cell monolayers with the supernatants of IBS colonic biopsy samples led to significantly increased paracellular permeability compared with the supernatants of biopsies from healthy controls (92). ZO-1 mRNA expression was significantly lower in Caco-2 monolayers incubated with colonic supernatants of IBS patients compared with healthy controls, whereas expression of occludin mRNA was similar with no differences observed between the IBS subtypes (92). Application of biopsy supernatants from IBS-D patients to the mucosal side of mouse colon tissue samples led to significant increases in paracellular permeability, whereas supernatants from IBS-C patients and healthy controls had no effect (45). In addition, intracolonic infusion of fecal supernatants from IBS-D patients resulted in the phosphorylation of myosin light

Table 1. Strengths and weaknesses of different techniques for the measurement of intestinal epithelial permeability

<table>
<thead>
<tr>
<th>Method</th>
<th>Overall Barrier Function In Vivo</th>
<th>Barrier Function In Vitro</th>
<th>Neuroimmune Function</th>
<th>Tight Junction Morphology</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caco-2/H11002 cell monolayers</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Ussing chambers with human mucosa</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Urine excretion of oral probes</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Human fecal or biopsy supernatant applied to animal tissue</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>+/–</td>
</tr>
<tr>
<td>Zonula occludens-1 immunohistochemistry</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>mRNA expression of tight junction proteins</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

Table 2. Studies assessing intestinal permeability in IBS patients

<table>
<thead>
<tr>
<th>Reference</th>
<th>IBS Group(s)</th>
<th>Method of Assessment</th>
<th>Permeability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spiller et al., 2000 (113)</td>
<td>PI-IBS</td>
<td>Lactulose/mannitol excretion</td>
<td>Increased</td>
</tr>
<tr>
<td>Marshall et al., 2004 (76)</td>
<td>PI-IBS</td>
<td>Sucrose/lactulose/mannitol excretion</td>
<td>Increased in 36% of pts</td>
</tr>
<tr>
<td>Dunlop et al., 2006 (37)</td>
<td>IBS-D (PI and nonPI)</td>
<td>51Cr-EDTA excretion</td>
<td>Increased</td>
</tr>
<tr>
<td>Shulman et al., 2008 (110)</td>
<td>Children with IBS or FAP</td>
<td>Lactulose/mannitol and sucrose/lactulose excretion</td>
<td>Increased</td>
</tr>
<tr>
<td>Gesce et al., 2008 (45)</td>
<td>All IBS subtypes</td>
<td>Fecal supernatants applied to murine colonic strips mounted in Ussing chambers; FITC-dextran transfer</td>
<td>Increased with IBS-D supernatants, no difference with IBS-C</td>
</tr>
<tr>
<td>Park et al., 2009 (89)</td>
<td>All IBS subtypes</td>
<td>PEG excretion</td>
<td>Increased in all IBS subtypes</td>
</tr>
<tr>
<td>Piche et al., 2009 (92)</td>
<td>All IBS subtypes</td>
<td>Colonic biopsies mounted in Ussing chambers; fluorescein-5.6 sulfonic acid</td>
<td>Increased in all IBS subtypes</td>
</tr>
<tr>
<td>Zhou et al., 2009 (134)</td>
<td>IBS-D</td>
<td>Lactulose/mannitol excretion</td>
<td>Increased in 39% of patients</td>
</tr>
<tr>
<td>Kerckhoffs et al., 2010 (59)</td>
<td>All IBS subtypes</td>
<td>PEG excretion</td>
<td>No difference</td>
</tr>
<tr>
<td>Lee et al., 2010 (68)</td>
<td>IBS-D</td>
<td>Colonic biopsies mounted in Ussing chambers; horseradish peroxidase</td>
<td>Increased</td>
</tr>
<tr>
<td>Zhou et al., 2010 (133)</td>
<td>IBS-D</td>
<td>Lactulose/mannitol excretion</td>
<td>Increased in 42% of patients</td>
</tr>
<tr>
<td>Rao et al., 2011 (99)</td>
<td>IBS-D</td>
<td>Lactulose/mannitol excretion</td>
<td>Increased</td>
</tr>
<tr>
<td>Gesce et al., 2012 (46)</td>
<td>IBS-D and IBS-C</td>
<td>51Cr-EDTA excretion</td>
<td>Increased in 2% of patients</td>
</tr>
<tr>
<td>Vivinus-Nébot et al., 2012 (125)</td>
<td>All IBS subtypes</td>
<td>Colonic biopsies mounted in Ussing chambers; fluorescein-5.6 sulfonic acid</td>
<td>Increased in all IBS subtypes</td>
</tr>
</tbody>
</table>

Table from Rao et al. (100) updated. Permeability is compared with healthy controls. FAP, functional abdominal pain; FITC, fluorescein isothiocyanate-labeled; IBS, irritable bowel syndrome; D, diarrhea predominant; C, constipation predominant; PI, postinfectious; PEG, polyethylene glycol.
chains and redistribution of ZO-1 protein in mouse colonic epithelial cells, leading to the disruption of TJs (45). These data suggest that there is some degree of increased intestinal permeability and TJ disruption in response to fecal supernatants from a subset of IBS patients. The recognized heterogeneity of clinical symptoms associated with IBS may reflect differences in the extent of increased permeability in each patient. The chemical nature of the factor in the supernatants is unclear. Candidates include serine proteases (45) or bile acids (36, 131) and other organic acids including short-chain fatty acids (82), which are all increased in stool of patients with IBS-D.

**Triggers of Altered Permeability in IBS**

**Stress.** There is a well-recognized association between psychological factors, particularly anxiety and stress, and the development of IBS (16, 62). Increased colonic paracellular permeability has been extensively observed in both chronic and acute stress murine models (9, 10, 33, 39, 44, 104, 105, 111, 121). One of the key mediators linking stress to increased permeability appears to be corticotropin-releasing factor (CRF) (9, 33, 44, 103, 105, 111, 121). In adult rats previously subjected to neonatal maternal deprivation, CRF levels, circulating cortisone levels, and colonic permeability, assessed by $^{51}$Cr-EDTA urinary excretion were significantly higher than those in control rats (9). CRF appeared to promote permeability by stimulating the release of nerve growth factor (NGF) by mast cells, since administration of anti-NGF antibodies to control rats administered exogenous CRF (to promote permeability) led to a significant decrease in permeability compared with rats given only CRF (9). Similarly, in rats chronically stressed by being housed in crowded cages, increased corticosterone release, mild inflammation, mast cell degranulation, and barrier dysfunction in the bowel were observed (121). This was measured by increased flux of horseradish peroxidase (HRP) across jejunal tissues suspended in Ussing chambers; the increased HRP flux indicated barrier dysfunction and increased permeability, which correlated with mast cell degranulation (121). Santos and colleagues (103) also found increased HRP flux via endosomes in the colonocytes of rats, either stressed by being restrained or injected with CRF. In response to a single episode of acute stress or the induction of mast cell degranulation, there was increased colonic paracellular permeability, as assessed by in vivo $^{51}$Cr-EDTA lumen-to-blood ratio, in mice (33). This was attributable to decreased expression of ZO-2 and occludin, whereas the expression of ZO-1 remained unchanged. In addition, there was incomplete or delayed colonocyte differentiation, which led to increased intercellular space and colonic paracellular permeability (33). Increased colonic paracellular permeability in chronically stressed mice coincided with increased IFN-γ expression and myosin light chain phosphorylation (39). Elevated myosin light chain phosphorylation is associated with redistribution of ZO-1 and occludin (107).

In the first study of the functional effects of CRF in human intestinal mucosa, Wallon et al. (125) found that CRF activated subepithelial mast cells. Moreover, when CRF was applied to the serosal side of healthy colonic biopsy tissues mounted in Ussing chambers, there was increased transepithelial uptake of HRP, but not $^{51}$Cr-EDTA (125). CRF receptors R1 and R2 were expressed on mast cells, and CRF receptor antagonists inhibited the permeability increase evoked by CRF. This suggested that CRF, acting via mast cells, is involved in regulating permeability to macromolecules in the normal human colon (125). Taken together, these data suggest a role for stress and the stress-induced mediator CRF as a promoter of both transepithelial and paracellular intestinal permeability. However, the connection between stress and alterations in barrier function in human IBS is still evolving. Recent studies by Martinez and colleagues (78, 79), while demonstrating significant alterations in TJ structure and regulation, found no correlation between IBS-D patients’ basal stress level and the above-mentioned alterations, although stress levels were correlated with mast cell activation. Additional studies in greater numbers of IBS patients are needed to further explore the connection between stress and TJ dysfunction. Experimental animal (e.g., rat, mice) studies show that stress is associated with acceleration of colonic transit (65). It is unclear whether the increase in colonic permeability or the release of mediators from mast cells contributes to the observed colonic transit acceleration.

**Food.** Increased intestinal permeability is frequently observed in patients with food allergies or hypersensitivities. In celiac disease, gliadin, the toxic component of wheat gluten, increases intestinal permeability, leading to the associated immune responses. When Caco-2 cell monolayers are exposed to gliadin, the cells release zonulin, leading to rearrangement of the cell cytoskeleton, loss of occludin/ZO-1 protein interaction, and increased monolayer permeability. Similarly, when exposed to gliadin, intestinal biopsies from both celiac and nonceliac patients demonstrate zonulin release and resulting increases in permeability, although this response is higher in celiac biopsies (34). Chronic gliadin exposure causes down-regulation of both ZO-1 and occludin gene expression, promoting TJ disassembly (34, 64).

Although the exact proportion of patients with IBS symptoms who have proven celiac disease is not certain and thought to be ~1% (23, 41), it is likely that a proportion of IBS patients have gluten sensitivity without the presence of pathological disease (22, 120). Wahnshaffe et al. (124) found that IBS-D patients, who were positive for human leukocyte antigen (HLA) DQ2, a genetic biomarker for celiac disease, were approximately five times more likely to respond to a gluten-free diet than IBS patients who were HLA DQ2 negative. Although some IBS patients do experience symptom relief on a gluten-free diet, the link between gluten and increased permeability in IBS has not yet been proven. A recent, double-blind, placebo-controlled study enrolled 39 subjects with IBS (all subtypes) who reported control of symptoms on a gluten-free diet (14). Subjects were randomized 1:1 to a gluten-containing diet or a placebo diet. Throughout the duration of the 6-wk study, subjects on the gluten diet reported significantly higher severity scores of pain and tiredness (14). However, neither group had significant changes in gliadin IgA or IgG levels or in measures of intestinal permeability, and there were no differences in any of the end points with regards to the presence or absence of HLA DQ2 or DQ8 (another genetic marker for celiac disease). It may be that all of these individuals already had increased permeability and that induction of symptoms by gluten in this study was wheat specific, not gluten specific (14). Furthermore, the study was biased by the inclusion of subjects already known to be responders to a gluten-free diet. Recently, a blinded, randomized trial with
IBS-D patients not selected for prior response to a gluten-free diet or HLA DQ status demonstrated that a gluten-containing diet resulted in increased colonic mucosal permeability in patients who were HLA DQ2 or DQ8 positive, but not in patients who were negative for either genetic marker (119).

IBS symptoms triggered by food are not limited to gluten sensitivity, but have been shown to extend to other foods in both adults and children (3, 5). Liljestol et al. (70) found a high prevalence of IBS and atopic disease (rhinoconjunctivitis, dermatitis, eczema) among patients with self-reported food hypersensitivity. Atopic patients had increased intestinal permeability compared with nonatopic patients, but gastrointestinal symptoms did not differ between the two groups. Along these lines, IBS patients are significantly more likely to report self-perceived suspected food allergies ($P = 0.001$) or have a positive skin test against food allergens ($P = 0.07$) (123).

Bile. Up to one-third of patients with IBS-D or functional diarrhea may have adult-onset idiopathic bile acid malabsorption (127, 130). In vitro studies using Caco-2 monolayers have shown that certain bile acids, deoxycholic acid and chenodeoxycholic acid, can induce epidermal growth factor receptor phosphorylation, which induces occludin dephosphorylation and TJ cytoskeletal rearrangement, thereby increasing paracellular permeability (95). In addition, bile acids may increase colonic permeability via alteration of functions of enteric nicotinic and muscarinic submucosal neurons. In rats, permeability induced by deoxycholic acid was reduced by the intravenous administration of the nerve blockers hexamethonium and atropine and the topicalosal serumal application of lidocaine (115).

The membrane bound G protein-coupled bile acid receptor 1 (GpBAR1, also called TGR5) is highly expressed in the human and murine colon (80, 93). Specifically, in the mouse large intestine, it is expressed in the myenteric and submucosal ganglia; in ~50% of all myenteric neurons, and in >80% of inhibitory motor neurons and descending interneurons expressing nitric oxide synthetase (93). When applied to isolated colonic strips, deoxycholic acid, a GpBAR1 agonist, causes inhibition of spontaneous phasic activity by a neurogenic, cholinergic, and nitrergic mechanism, and it delays gastrointestinal transit (93). Its presence in submucosal neurons, many of which are cholinergic secretomotor neurons, suggests it plays a role in stimulating secretory reflexes as a defensive mechanism to eliminate bile acids and other noxious agents (93). GpBAR1 may play a role in regulating intestinal barrier function. GpBAR1 knockout mice develop abnormal colonic mucosal cell morphology, altered molecular architecture of epithelial TJs, and resulting increased intestinal permeability. This occurs owing to decreased occludin expression, whereas ZO-1 expression was increased and its subcellular distribution disrupted (27). Genetic variation in GpBAR1 has been associated with altered small bowel transit in IBS-D (21); the link with permeability is unknown.

Infection and dysbiosis. Gastroenteritis is a common trigger for IBS; mathematical modeling suggests that PI-IBS may account for most cases of IBS (106). The risk of developing IBS after acute gastroenteritis is sixfold greater than that for an individual with no prior gastrointestinal infection and occurs in ~10% of individuals postinfection (117). Infectious diarrhea can frequently lead to increased permeability (136) due to disruption of TJs (43, 94, 109, 132), and significant proportions of patients with PI-IBS have increased intestinal permeability (37, 76, 113). The underlying mechanisms that lead to the development of PI-IBS after infectious gastroenteritis are not yet known, although other risk factors associated with development of IBS in general, such as stress, young age, and female gender are thought to increase the likelihood of persistent symptoms after the acute infection (112). Recently, genetic analysis of PI-IBS patients have identified a polymorphism of CDH1, which encodes for the TJ protein E-cadherin, as significantly associated with the development of PI-IBS (122).

Acute gastroenteritis may increase the likelihood of increased epithelial permeability and PI-IBS by triggering inflammation and by disruption of normal intestinal flora (20, 112). The correlation between increased inflammation and barrier function is discussed below. Preliminary studies with commensal bacteria suggest their ability to reinforce barrier integrity. For example, Lactobacillus planarum and Escherichia coli Nissle 1917, known to have beneficial probiotic effects, may protect epithelial barrier function by enhancing the translocation of ZO-1 and ZO-2 to the TJ region (56, 137). Commensal E. coli produce indole, which has been shown to strengthen barrier function in vitro via increased TJ protein expression and decreased paracellular permeability (4). Both mucosal- and fecal-associated microbiota in IBS patients display significant alterations in numbers and composition compared with healthy controls, and noted alterations in gut microbial populations often correlate with symptoms (28, 57, 91, 96). Thus the dysbiosis induced by infection or by antibiotic use could alter the composition of gut microbiota and possibly reduce those populations of bacteria that normally enhance or help to maintain intestinal epithelial barrier function. A comprehensive discussion about the roles of acute gastroenteritis and gut dysbiosis in the pathophysiology of IBS is beyond the scope of the article; the reader is referred to a few of many excellent reviews on the matter, including references (48, 67, 112).

Increased Permeability, Immune Responses, and Pain in IBS

Various lines of evidence point toward an interplay between increased intestinal permeability, low levels of inflammation, and visceral hypersensitivity in IBS, in both human studies and animal models. Increased infiltration of mast cells in the gut mucosa of IBS patients has been reported in many studies (1, 6, 7, 17, 29–31, 49, 79, 87, 90, 123, 126) but not in others (24, 61) (Table 3). Higher levels of mast cell mediators, such as histamine, tryptase, and trypsin, have been documented in colonic and jejunal biopsy supernatants of both IBS-D and IBS-C patients, both in studies that report increase mast cell counts (6, 7, 17, 29, 30, 123) and in those that found no increase in mast cell numbers (24, 61). Along with mast cells, significantly higher concentrations of total immunocytes, CD3+, CD4+, and CD8+ T cells in IBS patients compared with healthy controls have been demonstrated in some studies (31, 49, 88). Increased levels of mast cell mediators may be involved in increased permeability. Application of tryptase to the basolateral side of normal rectal biopsy samples increased permeability in a dose-dependent manner (68). In vitro, increased paracellular permeability was noted in Caco-2 monolayers exposed to tryptase, and this was accompanied by reduced JAM-A expression and redistribution of JAM-A pro-
Two recent studies by Martinez and colleagues (78, 79) found significantly increased levels of mast cells in the jejunum of IBS-D patients compared with healthy controls. Microarray studies of these patients’ jejuna mucosa revealed a distinctive...

Teins away from TJ regions (129). In addition, intracolonic administration of IBS-D fecal (45) or colonic (24) supernatants led to increased paracellular permeability in mice (24, 45).

In rats, stress-induced colonic barrier dysfunction is paralleled by mucosal mast cell hyperplasia and activation (11, 104); elevated mRNA expression of the cytokines IFN-γ, IL-1β, IL-2, IL-4, and IL-10 (10); increased mucosal innervations and synaptogenesis (11); and greater concentration of mast cells in close proximity to nerve fibers (11). As previously discussed, in murine models of stress, CRF appears to be a key factor in mitigating changes in epithelial barrier function. Cortagine, a CRF1 agonist, increased the colonic permeability along with increased visceral hyperalgesia in rats through selective peripheral activation of CRF1 receptors (65). In stressed mice with prior infectious gastroenteritis, increased protease activity and stress mediators, which are released by mast cells, enterocytes, and activated immune cells, led to afferent neuronal excitability and increased visceral hypersensitivity and alldynia (52). The mast cell mediator NGF is likely to be one factor involved in the increased visceral hypersensitivity observed in murine models of stress, since administration of anti-NGF antibodies reduced visceral sensitivity (8, 11).

Mast cell counts and mediator levels are compared with healthy controls. IBS, irritable bowel syndrome; D, diarrhea predominant; C, constipation predominant. A, alternating bowel pattern.

<table>
<thead>
<tr>
<th>Study</th>
<th>IBS Groups</th>
<th>Mast Cells</th>
<th>Mast Cell Mediators</th>
</tr>
</thead>
<tbody>
<tr>
<td>O’Sullivan et al., 2000 (87)</td>
<td>All IBS subtypes</td>
<td>Significantly higher in the cecum ((P &lt; 0.05)), but not ascending or descending colon, or rectum of IBS pts</td>
<td>Not assessed</td>
</tr>
<tr>
<td>Barbara et al., 2004 (6)</td>
<td>IBS-D and IBS-C</td>
<td>34/44 IBS patients had increased mucosal cell area occupied by mast cells (descending colon: (P &lt; 0.001)); no difference observed between IBS-D and IBS-C pts.</td>
<td>Significantly higher proportions of mast cells were degranulating in IBS. Both levels of tryptase and histamine were significantly higher in IBS patients ((P = 0.015) for both comparisons).</td>
</tr>
<tr>
<td>Park et al., 2006 (90)</td>
<td>IBS-D</td>
<td>Significantly increased in terminal ileum, ascending colon, and rectum ((P &lt; 0.01)). No increase in duodenum or jejunum.</td>
<td>Not assessed</td>
</tr>
<tr>
<td>Wang et al., 2007 (126)</td>
<td>IBS-D and IBS-C</td>
<td>Increased in ileum of IBS-D and IBS-C ((P &lt; 0.05)). No increase in duodenum or jejunum.</td>
<td>Not assessed</td>
</tr>
<tr>
<td>Cenac et al., 2007 (24)</td>
<td>All IBS subtypes</td>
<td>No differences in the number of mast cells in ascending colon or rectum.</td>
<td>Significantly increased serine protease activity ((P &lt; 0.005)). Significant increases in tryptase and trypsin mRNA expression in tissue from IBS patients from ascending colon and rectum ((P &lt; 0.05)).</td>
</tr>
<tr>
<td>Barbara et al., 2007 (7)</td>
<td>IBS-D and IBS-C</td>
<td>Greater mean area of colonic mucosa occupied by mast cells in IBS patients ((P &lt; 0.001)).</td>
<td>IBS colonic mucosal biopsies released significantly higher levels of tryptase ((P &lt; 0.001)), histamine ((P &lt; 0.01)) and prostaglandin-E_{2}.</td>
</tr>
<tr>
<td>Guilarte et al., 2007 (49)</td>
<td>IBS-D</td>
<td>Significantly increased in the jejunum ((P &lt; 0.001)).</td>
<td>Significantly higher levels of tryptase in the jejunum ((P = 0.005)).</td>
</tr>
<tr>
<td>Akbar et al., 2008 (1)</td>
<td>All IBS subtypes</td>
<td>Significantly elevated in colonic mucosa ((P = 0.02)).</td>
<td>Not assessed</td>
</tr>
<tr>
<td>Cremon et al., 2009 (31)</td>
<td>IBS-D and IBS-C</td>
<td>Greater mean area of colonic mucosa occupied by mast cells in IBS patients ((P &lt; 0.001)).</td>
<td>Not assessed</td>
</tr>
<tr>
<td>Buhner et al., 2009 (17)</td>
<td>IBS-D and IBS-C</td>
<td>Significantly higher ((P = 0.002)).</td>
<td>Significantly higher tryptase ((P &lt; 0.001)) and histamine ((P = 0.008)) concentrations IBS supernatants.</td>
</tr>
<tr>
<td>Klooiker et al., 2010 (61)</td>
<td>All IBS subtypes</td>
<td>Significantly lower in rectum and descending colon in IBS patients with visceral normosensitivity ((P &lt; 0.05)), but not significantly differently in those with hypersensitivity.</td>
<td>Histamine release was higher in all IBS patients ((P = 0.04)), while spontaneous release of tryptase was lower ((P = 0.04)).</td>
</tr>
<tr>
<td>Coeffier et al., 2010 (29)</td>
<td>All IBS subtypes</td>
<td>Significantly higher in colonic (descending) mucosa of IBS-D ((P &lt; 0.05)), but not IBS-C patients.</td>
<td>Tryptsin-like proteasome activity was significantly increased in IBS patients, regardless of bowel pattern ((P &lt; 0.05)).</td>
</tr>
<tr>
<td>Cremon et al., 2011 (30)</td>
<td>IBS-D and IBS-C</td>
<td>Significantly greater mean area occupied by mast cells ((P &lt; 0.01)), regardless of subtype.</td>
<td>Significantly higher spontaneous release of tryptase ((P = 0.037)) and histamine ((P = 0.01)) in IBS patients, regardless of subtype.</td>
</tr>
<tr>
<td>Martinez et al., 2012 (78, 79)</td>
<td>IBS-D</td>
<td>Significantly higher in the jejunum ((P = 0.008)).</td>
<td>Up-regulation of tryptase mRNA expression and increase protein expression in jejunal biopsies of IBS patients ((P = 0.0007)).</td>
</tr>
<tr>
<td>Vivinus-Nébot et al., 2012 (123)</td>
<td>All IBS subtypes</td>
<td>Significantly higher in all IBS subtypes ((P = 0.003)).</td>
<td>Significantly higher levels of tryptase in IBS-D ((P = 0.0007)) and IBS-A ((P = 0.001)) compared with IBS-C.</td>
</tr>
</tbody>
</table>
transcription profile in intestinal permeability, mast cell function, and TJ signaling. Additional mRNA expression analyses found tryptase mRNA expression to be consistently upregulated in IBS-D patients. Furthermore, the authors found significant downregulation of ZO-1 and ZO-3 mRNA, along with intensive internalization of ZO-1 proteins to the cytoplasm of jejuna epithelial cell, as opposed to normal distribution on the cell surface. The expression of ZO mRNAs was inversely correlated to tryptase mRNA expression (79). Additional analyses of jejunal biopsy samples from patients with IBS-D compared with those of healthy controls found increased mast cell activation (tryptase release), altered expression levels of claudin proteins, decreased phosphorylation of occludin leading to its redistribution to the cytoplasm, and increased myosin light chain phosphorylation. Furthermore, ultrastructural alterations were noted at the apical junction complexes, including increased apical intercellular distance, a higher proportion of dilated junctions, and a higher percentage of junctions with perijunctional cytoskeletal condensation (78).

IBS-D patients with increased intestinal permeability have more severe symptoms and hypersensitivity to both somatic and visceral stimuli compared with IBS-D patients with normal permeability and to healthy controls (134). Inflammation detected in children with functional abdominal pain/IBS and increased colonic permeability has been correlated with pain interfering with activity (110). In both IBS-C and IBS-D patients, compared with healthy controls, there is a greater number of activated mast cells close to nerve endings (6, 7). This correlates with the severity and frequency of perceived painful abdominal sensations (6). The severity of abdominal pain experienced by IBS patients is positively correlated with the degree to which their colonic supernatants induce paracellular permeability and downregulate ZO-1 mRNA expression in Caco-2 monolayers (92). In contrast, however, Cremon et al. (31) found no significant correlation between the concentration of total or subtypes of immune cells and abdominal pain or discomfort in IBS patients. More recently, Vivinus-Nébot et al. (123) reported that IBS severity score was positively and significantly correlated with mast cell counts and the degree of paracellular permeability, as was the release of tryptase, in IBS patients. Conflicting data regarding the presence of increased permeability in IBS patients, elevated concentrations of mast cells and their mediators, and how these correlate with pain suggest there is heterogeneity in IBS and may reflect differences in study designs.

Akbar et al. (1) examined transient receptor potential potential vanilloid type-1 (TRPV1) immunoreactive sensory nerve fibers in colonic biopsies of IBS patients (8 with IBS-D, 8 with IBS-C, 7 with IBS-A). There was a threefold higher number of TRPV1 fibers in IBS patients compared with controls with no significant difference between subgroups. There was also a significantly greater percentage area of CD3+ cells and significantly higher levels of mast cells in IBS patients compared with controls. Stepwise multivariate linear regression analyses showed that numbers of TRPV1 fibers and mast cells were significant predictors of pain (1).

Both in vivo and in vitro exposure of murine tissues to colonic or fecal supernatants of IBS patients provides additional evidence to support the suggested confluence of increased intestinal permeability, inflammation, and pain. Increased levels of serine protease activity in the fecal (45) and colonic (24) supernatants of IBS-D patients leads to increased colonic paracellular permeability and visceral hypersensitivity in mice, suggesting that a soluble factor present in the colon of humans may conceivably mediate the biological effects on the barrier and activation of visceral afferents (24, 45). It was postulated that this effect is modulated by the protease-activated receptor 2 (PAR-2), as these responses are not observed in PAR-2 knockout mice; however, other potential mechanisms have not been explored. For example, there are data that suggest activation of tachykininergic mechanisms (18). Supernatants of colonic mucosal IBS biopsy samples, compared with those of healthy controls, induce marked increases in the firing rates of rat and mouse visceral sensory nerves and enhance Ca2+ mobilization in the vast majority of capsaicin-sensitive afferent neurons (7, 24). Similarly, supernatants from IBS colonic biopsy samples have an excitatory effect on human enteric afferent neurons; excitations were not related to IBS subtypes and were mediated by tryptase, histamine, and serotonin (17). Although serotonin, histamine, and trypotase concentrations in the supernatants were significantly correlated with the rate of neuronal firing, only trypotase concentrations correlated with the percentage of responding neurons, providing further support for the role of PAR-2 in visceral hypersensitivity (17). In vitro, tryptase induces the redistribution of ZO-1 and occludin to the cytoplasm of colonocytes via the activation of PAR-2, leading to increased paracellular permeability (55). PAR-2 activation has been shown to increase colonic paracellular permeability in mice via alterations in ZO-1 localization and calmodulin-mediated phosphorylation of myosin light chain cytoskeletal components, which are bound to the cytoplasmic plaques of TJ and are involved in regulating TJ permeability (25).

Endogenous organic acids, such as bile acids, are another class of potential soluble factors in feces that may increase permeability, activate inflammation, and stimulate afferents (95, 115). In contrast, short-chain fatty acids, which are products of digestion, enhance intestinal barrier function (116). Serotonin (5-HT) levels are known to be altered in IBS patients (2, 126), and serotonin is certainly released into the lumen in response to luminal activation. The pharmaceutical management of IBS with 5-HT3 receptor antagonists or 5-HT4 agonists provides symptomatic relief for some patients (40). In the gut, serotonin is released by enterochromaffin (EC) cells. Persistent elevated EC cell counts have been demonstrated in FI-IBS patients up to 4 years after initial infection (113). In a recent study by Cremon and colleagues (30), serotonin-positive EC cell counts were significantly higher in IBS patients compared with healthy controls. When analyzed by bowel patterns, EC cell counts were significantly greater in IBS-D vs. IBS-C patients. However, there was no significant difference in serotonin release between either IBS subgroup, and spontaneous release of serotonin was significantly higher in IBS patients than in controls (30). Spontaneous serotonin release correlated with mast cell counts, spontaneous release of tryptase and histamine, and severity of abdominal pain. Levels of released serotonin positively and significantly correlated with the severity, but not frequency of abdominal pain and discomfort in IBS patients, regardless of bowel patterns (30). Similar to other studies discussed above, exposure of mouse afferent nerves to IBS colonic supernatants led to increased neuronal firing, which was mitigated by the addition of a 5-HT3 receptor antagonist. Because there was no observed increase in EC cell counts, whereas mast cell counts and mediator levels correlated with serotonin levels, the authors suggested that mast
cell media may drive the increased release of serotonin from EC cells (30).

Activation of certain enteric nervous system pathways, perhaps by mast cell mediators and serotonin, may be involved in the control of intestinal epithelial permeability by regulating expression of TJ proteins (84). Bertaux-Vandaele et al. (13) found that occludin expression is lower in IBS patients with Visual Analog Scale (VAS) score >6 compared with patients with VAS score <6, regardless of IBS subtype, whereas claudin-1 expression is only correlated to abdominal pain in IBS-D patients (13). ZO-1 mRNA expression was negatively correlated with the severity of pain measured by means of a VAS. Furthermore, cellular distribution of claudin-1, occludin, and ZO-1 was disrupted and irregular in IBS patients in this study. Multivariate analysis indicated that only occludin expression was associated with visceral pain (13).

Conclusions
In subsets of IBS patients, increased intestinal permeability, brought on by a variety of different triggers, is likely connected to facilitating mucosal inflammation and activation of visceral pain. Continued elucidation of the underlying pathophysiology of IBS, along with other related bowel disorders, will provide additional data to allow for more targeted, effective therapies. It may be that the most effective management of IBS patients involves a multipronged approach that targets environmental and intraluminal triggers, immune responses, epithelial dysfunction, and the gut-brain axis.

ACKNOWLEDGMENTS
Writing support was provided by Meryl Gersh, PhD, of AlphaBioCom, LLC, King of Prussia, PA, and was funded by Takeda Pharmaceuticals International, Inc.

DISCLOSURES
K. Lasch and W. Zhou are employees of Takeda Pharmaceuticals, Inc.

AUTHOR CONTRIBUTIONS
Author contributions: M.C., K.L., and W.Z. conception and design of research; M.C., K.L., and W.Z. edited and revised manuscript; M.C., K.L., and W.Z. approved final version of manuscript.

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