DR W.L. HASLER (Orcid ID : 0000-0002-6158-2871)

DR CHUNG OWYANG (Orcid ID : 0000-0002-1746-2370)



Article type : Review

# MAST CELL MEDIATION OF VISCERAL SENSATION AND PERMEABILITY IN IRRITABLE BOWEL SYNDROME

**William L. Hasler,** Gintautas Grabauskas, Prashant Singh, Chung Owyang

Division of Gastroenterology and Hepatology, University of Michigan Health System, Ann Arbor MI, USA

**SHORT TITLE:** MAST CELL INVOLVEMENT IN IBS PATHOGENESIS

**ADDRESS CORRESPONDENCE TO:** Chung Owyang, MD

Chief, Division of Gastroenterology and Hepatology

3912 Taubman Center, SPC 5362

Ann Arbor, MI 48109

Telephone: (734) 936-4785

FAX: 734-936-7392

Email: cowyang@med.umich.edu



**ABSTRACT**

Abnormalities of mast cell structure or function may play prominent roles in irritable bowel syndrome (IBS) symptom genesis. Mast cells show close apposition to sensory nerves and release bioactive substances in response to varied stimuli including infection, stress, and other neuroendocrine factors. Most studies focus on patients who develop IBS after enteric infection or who report diarrhea-predominant symptoms. Three topics underlying IBS pathogenesis have been emphasized in recent investigations. Visceral hypersensitivity to luminal stimulation is found in most IBS patients and may contribute to abdominal pain. Mast cell dysfunction also may disrupt epithelial barrier function which alters mucosal permeability potentially leading to altered bowel function and pain. Mast cell products including histamine, proteases, prostaglandins, and cytokines may participate in hypersensitivity and permeability defects, especially with diarrhea-predominant IBS. Recent experimental evidence indicates that the pronociceptive effects of histamine and proteases are mediated by the generation of prostaglandins in the mast cell. Enteric microbiome interactions including increased mucosal bacterial translocation may activate mast cells to elicit inflammatory responses underlying some of these pathogenic effects. Therapies to alter mast cell activity (mast cell stabilizers) or function (histamine antagonists) have shown modest benefits in IBS. Future investigations will seek to define patient subsets with greater potential to respond to therapies that address visceral hypersensitivity, epithelial permeability defects, and microbiome alterations secondary to mast cell dysfunction in IBS.

**KEY POINTS**

- Mast cells are increased in many irritable bowel syndrome (IBS) patients, show proximity to sensory nerves, and release bioactive substances that may underlie pain and altered bowel function.

- Therapies to alter mast cell activity (mast cell stabilizers) or function (histamine antagonists) show modest benefits reducing symptoms in some IBS subsets, especially after enteric infection or with diarrhea-predominant symptoms.
- Visceral hypersensitivity to luminal distention may be elicited by mast cell activation and release of mediators and may contribute to reports of pain in this condition.
- Pronociceptive effects of histamine and proteases are prevented by COX2 inhibitors and cromolyn sodium suggesting mediation by mast cell-deficient prostaglandin synthesis.
- Abnormalities of epithelial barrier integrity resulting from mast cell mediators may lead to increased mucosal permeability and development of bowel habit alterations and pain in IBS.
- Recent studies have focused on interactions between gastrointestinal mast cells and the enteric microbiome which can modulate gut inflammatory processes underlying IBS symptom exacerbations.
- Future studies addressing mast cell participation in IBS symptom genesis will define patient subsets who respond to treatments that reverse visceral hypersensitivity, permeability defects, and microbiome alterations in this condition.
-

## INTRODUCTION

Irritable bowel syndrome (IBS) is the most prevalent gastrointestinal disorder and presents with abdominal pain and altered bowel habits[1]. IBS pathophysiology is heterogeneous and variable from patient to patient. Visceral hypersensitivity may underlie symptoms in large IBS subsets[2, 3] Dysfunction of epithelial barrier function with increases in permeability may contribute to altered defecation and pain in IBS[4]. Alterations in gut bacterial populations are common and may participate in IBS pathophysiology[5]. Each of these factors interact with each other and other factors including bile acids, enteric and central nervous activity, and the immune system to produce IBS symptoms[4]. Better understanding of mechanisms underlying development of hypersensitivity, epithelial dysfunction, and gut dysbiosis in IBS will provide insight into symptom pathogenesis and facilitate drug discovery for improved treatment of this condition.

Mucosal mast cells are increased and show heightened activation in some IBS subsets. Mast cells can elicit visceral hypersensitivity, influence epithelial function, and interact with gut

microbes providing a possible link between the neuroimmune system and other contributors to IBS pathogenesis[6]. The aims of this review are to describe gut mast cell biology, characterize mast cell abnormalities in IBS, detail roles of mast cell activity in visceral hypersensitivity, epithelial barrier function, and enteric microbial activity, and to speculate on the potential for future therapies targeting mast cell functions in IBS.

## MAST CELLS IN THE GUT

### Structural Considerations:

Mast cells represent up to 5% of gut mononuclear cells and are present in the mucosa, lamina propria, submucosa, smooth muscle, and serosa. On ultrastructural analyses, activated mast cells contain cytoplasmic granules with bioactive mediators (Figure 1)[7]. Mast cells are derived from pluripotent bone marrow progenitors including CD34+/CD117+ cells. These differentiate into tissue mast cells after exposure to growth factors and other agents promoting maturation including interleukins (IL-3, IL-4, IL-9, IL-10, IL-33), transforming growth factor-β (TGF-β), nerve growth factor (NGF), stem cell factor (SCF), and the chemokine CXCL12[8]. Two subtypes of gut mast cells have been identified, mucosal mast cells ($MC_T$) and connective tissue mast cells ($MC_{TC}$)[7, 9, 10]. Small intestinal mucosal mast cell density increases from the jejunum to the distal ileum; colon mast cells decrease from the cecum to the rectum[11]. Mast cell numbers increase from the mucosa villous tips to the bases of the crypts. Forty-seven to 77% of mucosal mast cells are closely apposed to sensory nerve fibers in different gut regions[12]. Most nerve fibers adjacent to mast cells are unmyelinated and stain positive for neurotransmitters involved in gut sensation including calcitonin gene-related peptide (CGRP) and substance P[13].

### Mast Cell Mediators

Gut mast cells release biologically active substances which can be stratified into preformed, neo-synthesized, and neo-formed lipid mediators (Table 1)[9].

#### *Preformed Mediators*

Preformed mediators are stored in cytoplasmic granules and include histamine, proteases, and heparin which can be rapidly replenished after mast cell activation[9]. Contents of restored mast cell granules may be markedly different from the original mediator profile prior to degranulation[14]. Histamine is synthesized by histidine decarboxylase and influences gut motor function, fluid transport, and inflammation by action on submucosal and primary afferent neurons[15, 16]. Activated mucosal $MC_T$ mast cells release relatively less histamine than cysteinyl leukotrienes, while connective tissue $MC_{TC}$ mast cells release higher levels of histamine and prostaglandin $D_2$ ($PGD_2$)[17]. Proteases produced by $MC_{TC}$ cells include tryptase, chymase and carboxypeptidase; the main protease produced by $MC_T$ cells is tryptase[7, 9, 10]. In addition to proteolytic activity; tryptase and other proteases cleave protease-activated receptors (PARs), which regulate motility, pain perception, epithelial permeability and secretion, and inflammation[18, 19, 20]. PARs are expressed by neurons in dorsal root ganglia (DRG) and the myenteric plexus. Tryptase specifically activates $PAR_2$[21]. Upon activation of $PAR_1$ and $PAR_2$ receptors, sensory neurons release CGRP and substance P which then elicit neurogenic pain.

***Neo-Synthesized Mediators***

Neo-synthesized mediators, including cytokines, chemokines, and growth factors, are produced by transcriptional activation after exposure to a mast cell stimulus. Cytokines synthesized by gut mast cells include those which are pro-inflammatory (IL-1, IL-3, IL-4, IL-5, IL-6, IL-12, IL-13, IL-16, IL-18, tumor necrosis factor-α [TNF-α], interferon-γ [IFN-γ]) and anti-inflammatory (IL-10) and are produced within hours of activation[22]. Gut mast cell chemokines include CXCL8, MCP-1 (CCL2), MIP-1α (CCL3), MIP-1β (CCL4), and CCL5[23]. In addition to participating in inflammation, cytokines (IL-3, IL-4, IL-6, IL-9, IL-10) and NGF participate in mast cell differentiation in rodents[9]. Growth factors secreted by gastrointestinal mast cells include fibroblast growth factor-2 (FGF2), basic FGF, TGF-β1, SCF, granulocyte-macrophage colony-stimulating factor (GM-CSF), vascular endothelial growth factor (VEGF), vascular permeability factor (VPF), and NGF[23]. NGF regulates maturation, growth, and maintenance of central and peripheral neurons.

***Neo-Formed Lipid Mediators***

Neo-formed lipid mediators synthesized after mast cell activation include eicosanoid compounds. Prostaglandin $G_2$ is an arachidonic acid product converted by cyclooxygenases (COX) into an intermediary molecule, prostaglandin $H_2$ ($PGH_2$). There are three COX isoforms. COX1 is a constitutive form expressed in mast cells and is responsible for basal prostanoid synthesis. COX3 is a splice variant of COX1 mostly expressed in the brain and heart. COX2 is induced in several cell types by cytokines, hormones, and mitogens and elicits prostaglandin production in inflammation. Rapid increases in COX2 gene expression in inflamed tissues are followed by $PGD_2$, $PGE_2$, $PGF_2$, $PGI_2$ and thromboxane (TX) biosynthesis. In a rigorous study, immunoreactivities for mast cell COX2 and tryptase extensively overlapped in human and animal colonic tissues confirming a mast cell origin for mucosal prostaglandins[24]. Prostaglandin D synthase is responsible for $PGD_2$ generation, which is abundantly released by mast cells and fibroblasts and regulates central and peripheral nerve function[25]. Many symptoms of IBS can be mimicked by exogenous prostaglandin administration which are ameliorated by prostaglandin synthase inhibition[26].

Prostaglandin pathways overlap with nitric oxide (NO) processes. Activated mast cells express inducible nitric oxide synthase (iNOS); mast cell iNOS expression is increased after cytokine exposure[27]. In iNOS knockout mice, $PGE_2$ formation after proinflammatory stimulation decreased by ~80% although COX2 protein expression was not impaired, confirming the importance of NO generation for prostaglandin synthesis[28]. NO increases COX2 activity by reacting with the heme-component of the enzyme to increase prostaglandin synthesis and acts at transcriptional and translational levels to augment COX2 expression.

**Mast Cell Activation**

Stimuli including allergens, infections, stress, and neurotransmitters promote mast cell activation. For example, substance P increases mast cell histamine content and causes degranulation[29]. Alternatively, transmitters like somatostatin blunt mast cell function[30]. Mast cells are activated when antigens crosslink immunoglobulin E (IgE) to high affinity Fc epsilon receptors with subsequent degranulation and release of stored mediators (histamine, tryptase, proteoglycans) and subsequent leukotriene and $PGD_2$ synthesis. Non-IgE-mediated mast cell activation occurs after exposure to neuropeptides, complement, physical stimuli, and infection.

### *Activated Mast Cell Involvement in Inflammation*

Mast cells participate in inflammation by virtue of their proximity to nerve fibers, epithelial cells, and blood vessels. Sensorimotor dysfunction induced by inflammation may be mediated by proinflammatory cytokines and persists after resolution of the acute inflammatory response[31]. Mast cell mediators also contribute to recruiting neutrophils, macrophages, and T-lymphocytes which then release additional pro-nociceptive mediators. In a study of pleurisy in rats, injection of isologous serum promoted neutrophil infiltration which peaked at 4 hours and was followed by eosinophilic influx lasting 24-48 hours[32]. Mast cell deficient (*Ws/Ws*) rats exhibited reduced neutrophil recruitment and myeloperoxidase activity in pleural lavage extracts which increased after local reconstitution with mast cells from wild type rat peritoneum. Mast cells support polarization of T-lymphocyte responses through secretion of IL-12, IFN-γ, Th-1, IL-4, and IL-6 and internalize and process antigens presented to T-lymphocytes by MHC class II pathways[33]. Activated mast cells release TNF-α, which binds T-cell TNF receptor I (TNFRI) and TNFRII to regulate T-lymphocyte activation[34]. Mast cells also contribute to B-lymphocyte proliferation and differentiation into IgA-secreting plasma cells by direct interaction with costimulatory proteins (CD40, CD40L) and secretion of IL-5, IL-6, and TGF-β[35].

### *Activated Mast Cell Involvement in Gut Neural Function*

Activated mast cells elicit nerve-mediated sensorimotor responses that increase perception. Mast cell histamine and proteases extracted from supernatants of colon biopsy specimens from IBS patients activate enteric and primary afferent neurons in experimental models[36]. Using calcium imaging, mast cell degranulation activates DRG neurons in co-culture[37]. Cell adhesion molecule 1 (CADM1) couples mast cells to sensory neurons. CADM1 blocking peptide or knockdown prevents mast cell degranulation and IL-6 secretion[38].

### *Self-Amplification of Mast Cell Activation and Response*

Mast cell activation can be stimulated by other mediators, reflecting self-amplification of mast cell-regulated processes that sustain inflammatory responses[7, 39]. Chymase, tryptase, histamine, and IL-29 promote inflammatory cell accumulation[40]. Chymase also is a potent chemoattractant for eosinophils, monocytes, and neutrophils by extracellular signal-regulated

kinase (ERK) and p38 mitogen-activated protein kinase (MAPK) pathways[41]. These reciprocal interactions activate nerve-mediated responses that modulate subsequent mast cell functions[42].

## ROLE OF MAST CELLS IN IBS PATHOGENESIS

### Mast Cell Abnormalities in IBS

Mast cell alterations are prominent in IBS, including changes in mast cell number, mediator release during stimulation, and proximity to nerve tissue. Studies show benefits of treating mast cell dysfunction in IBS subsets. Mutations in the tyrosine kinase Kit gene are described in IBS, suggesting a possible genetic basis for mast cell dysregulation. In one study, 13 of 19 IBS patients showed one or multiple Kit mutations including D 419 H and D 816V[43].

Some studies report mast cell increases in IBS, but cell counts overlap with healthy values[44]. Meta-analyses report increased mast cell counts in the small intestine and colon of IBS patients with greater overall lamina propria area occupied by mast cells[36, 44- 46]. Mast cell numbers were similar in IBS and ulcerative colitis in remission in one study[47]. Regional colon mast cell differences are found, being higher in the cecum in one report[48]. Small intestinal mast cells were higher in 10 of 11 studies from one meta-analysis and two systematic reviews[44, 45]. There also are regional differences in small bowel distributions in IBS, being higher in the ileum than the duodenum and jejunum in one meta-analysis[45]. Mast cell numbers correlated with bloating and dysmotility-like dyspepsia in another study[49].

Mast cell increases have been related to specific IBS subsets. Female patients had higher mast cell numbers versus males in one report[49]. Increased lamina propria mast cells are described in those with chronic symptoms after Campylobacter-induced gastroenteritis[50]. Some researchers propose that patients with postinfectious IBS selectively develop low grade mast cell responses, while others observe no mast cell elevations in IBS patients without prior infection[51]. In a recent review, no overall differences in mast cells were seen in postinfectious- versus non-postinfectious-IBS[52]. Some groups report higher cell counts in diarrhea-predominant IBS (IBS-D), while others also note prominent mast cells in constipation-predominant IBS (IBS-C)[52-55]. In a meta-analysis of 22 studies, mast cells were increased to similar degrees in both IBS-C and IBS-D patients in the descending (standardized mean difference 1.69, 95% CI 0.65-2.73, P=0.001) and rectosigmoid (SMD 0.38, 95% CI 0.06-0.71, P=0.02) colon[44]. A study of IBS-D

patients noted higher mast cell counts in those with lactose intolerance and symptoms versus asymptomatic patients who were lactose intolerant[56].

Mast cells show important morphologic differences in IBS. The proximity of mast cells to nerve endings is closer (within 5-10 microns) in IBS patients versus healthy controls, which correlates with abdominal pain severity[36, 51, 55, 57, 58]. Substance P containing nerve fibers and nerve endings that express TRPV1 show close proximity to ileal and colonic mast cells in postinfectious IBS[6, 51, 59]. These findings correlate with abdominal pain severity and frequency.

### *Alterations in Mast Cell Mediator Release and Responses in IBS*

Electron microscopic evidence of colonic mast cell activation is often observed in IBS, including increases in degranulation with labyrinthic arrays and clearing of individual granules[36, 55]. In one study, 77% of IBS patients showed higher mast cell density including 150% increases in degranulating mast cells[55].

Mast cell mediators are increased in IBS. Mucosal biopsies in IBS exhibit higher stored mediators like histamine and tryptase and neo-formed lipid mediators like $PGE_2$ with associated increases in COX2 mRNA and protein expression (Figure 2)[24, 36]. Histamine, protease, and $PGE_2$ release is increased in colon biopsy and fecal supernatants from IBS patients[16, 19, 36, 55, 60-62]. These findings have been related to increased tryptase and $PAR_2$ mRNA and tryptase protein[63]. Tryptase expression is increased in IBS-D versus IBS-C and levels correlate with stool frequency and consistency in IBS-D[64-66]. Serum cytokines including IL-6, -8 and TNF-α are increased in some studies in IBS[67]. In one report, elevated mast cell NGF correlated with higher mast cell numbers[68]. In another IBS study, mucosal NGF, neurotrophic receptor tyrosine kinase 1 (NTRK1), and tropomyosin receptor kinase A (TrkA) expression were increased[69]. Mast cell NGF release can increase neuronal sprouting and neuroplastic changes in colon mucosa in IBS[69]. In colon mucosa from IBS-D patients, mast cell numbers increase in association with upregulated tryptase, iNOS, and IL-1β expression showing involvement of NO pathways in mast cell function[27]. Mast cell counts correlated with mucosal substance P and vasoactive intestinal polypeptide (VIP) content in female IBS patients in one report[54]. In another study, mucosal serotonin release and abdominal pain intensity correlated with higher mast cell numbers[70].

### *Activation of Mast Cells in IBS*

Triggers for mast cell activation in animal models include gastrointestinal infection, food intolerance, and stress[7]. After remission of experimental colitis in C57BL/6 mice, increases in tryptase-positive mast cells were associated with prolonged gastrointestinal transit[71]. In another report in guinea pigs, ileal and colon mast cells remained increased after *Trichinella spiralis* infection[72]. Supernatants from these regions increased mesenteric afferent nerve firing, an effect blunted by cromolyn disodium. Dietary fructooligosaccharide increases ileal mast cells and stimulates interleukin production in water avoidance-stressed mice[73]. Modulators of stress effects on gut sensorimotor and immune function include glucocorticoids, VIP, substance P, corticotropin releasing hormone (CRH), neurotensin, adrenomedullin, and catecholamines[74]. Stress alters mast cell degranulation, increases $PGE_2$ production, activates histamine and tryptase release, stimulates COX2 mRNA expressions and impairs epithelial barrier function; together, these actions accelerate colon transit and increase fecal expulsion[75].

Stress pathways play prominent roles in mast cell activation in humans. Cold pain stress induces jejunal release of mast cell mediators in patients with food allergy[76]. CRH mediates stress-induced disruption of human gut motor, epithelial barrier, and perceptual activity[77, 78]. High numbers of $CRH_1$ and $CRH_2$ receptors are expressed by human colon mast cells[77, 79]. CRH receptor stimulation elicits mast cell degranulation and releases cytokines and growth factors[79]. Stress- and CRH-induced changes in intestinal motor and epithelial function are absent in mast cell deficient-rodents and are abolished by mast cell stabilizers[80]. A recent review emphasized the ability of $CRH_1$ and $CRH_1$/$CRH_2$ receptor antagonists to reduce stress-induced mast cell activation in experimental models, but clinical studies of such therapies have been limited by unfavorable pharmacokinetics and formation of reactive metabolites[75].

***Treatments That Target Mast Cell Pathways in IBS***

Treatments to control mast cell activation or reduce actions of mast cell mediators have been studied in IBS. Disodium cromoglycate, a mast cell stabilizer that inhibits histamine and leukotriene release, decreased tryptase release and TLR2 and TLR4 expression in preliminary studies in IBS-D[81]. In double-blind trials, cromoglycate was superior to placebo in reducing symptoms in IBS patients with food intolerance[82-84]. A placebo-controlled trial found that ketotifen, a mast cell stabilizer with histamine $H_1$ antagonist properties, decreased IBS symptoms and improved quality of life but did not reduce mast cells[85, 86].

Other studies suggest benefits of $H_1$ antagonists in IBS. In a placebo-controlled trial in 28 IBS patients, the $H_1$ antagonist ebastine reduced abdominal pain and overall symptoms and improved quality of life[87]. A retrospective analysis from 307 children with functional gastrointestinal disorders reported symptom improvement with cyproheptadine—an antihistamine with anticholinergic and antiserotonergic properties[88]. Some propose that benefits of tricyclic antidepressants in IBS may result from histamine antagonism[89].

Other drugs which influence mast cell function have been proposed as IBS treatment. Mesalamine, an anti-inflammatory agent which acts by COX and prostaglandin inhibition, reduced symptoms in some early studies in IBS-D[90, 91]. However, two more recent controlled trials in IBS failed to show benefit of mesalamine over placebo[92, 93]. Corticosteroids were ineffective in one report, possibly due to an inability to affect mast cell appearance and degranulation[94].

## PROPOSED MECHANISMS OF MAST CELL-MEDIATED IBS SYMPTOM PATHOGENESIS

### Mast Cells and Gut Hypersensitivity

Depending on geography and symptom characteristics, heightened gut perception is reported by 20-94% of IBS patients in different investigations[2, 3]. Hypersensitivity likely is influenced by mast cell activation and mediators as detailed in the following sections. Much of this data originates from animal and in vitro investigations which provide plausibility for mast cell-induced visceral hypersensitivity in IBS.

#### *Characterization of Mast Cell Involvement in Hypersensitivity Development*

Support for mast cell mediation of visceral hypersensitivity is offered by rodent models. Mast cell hyperplasia and increased granulation are found in hypersensitive rodents[95]. Mast cell-deficient mice do not exhibit hypersensitivity to 2,4,6-trinitrobenzene sulfonic acid (TNBS). Mast cell deficiency does not affect normal nociception to colon distention, but abolishes hypersensitivity evoked by IBS-D colon biopsy supernatants[24, 96]. Reconstitution of mast cell-deficient mice with bone marrow-derived mast cells from wild type mice restores the ability of

IBS colon supernatants to elicit hypersensitivity, verifying mast cell participation for this potential mechanism for IBS symptoms[24].

Based on these animal studies, mast cell pathways have been proposed to modulate visceral hypersensitivity in IBS[24, 55, 57]. One study noted lower ileal and colonic mast cells in IBS patients with rectal hypersensitivity, while another noted no difference in cell counts in relation to sensation[57, 97].

***Mast Cell Mediators as Potential Triggers of Hypersensitivity***

Preformed mast cell mediators contribute to hypersensitivity in animal models. Histamine activation of afferent neurons adjacent to mast cells promotes sensitization to painful stimuli. Abdominal pain in IBS is proposed to result from TRPV1 sensitization after $H_1$ receptor activation from findings of a study employing IBS rectal supernatants[87]. Intracolonic $PAR_2$ agonist infusion promotes hypersensitivity to distention in rats and $PAR_2$-dependent mechanisms underlie hyperalgesia and increased sensory neuron calcium signaling in mice after exposure to IBS colon supernatants[18, 19]. $PAR_2$-deficient mice do not develop hypersensitivity to supernatant exposure. Histamine, $PAR_2$ agonists, and IBS colon supernatants fail to induce hypersensitivity in mast cell-deficient mice (Figure 3)[24].

Neo-synthesized mediators and associated pathways also participate in hypersensitivity. IL-1β and TNF-α sensitize nociceptive neurons via p38 MAPK phosphorylation of Nav1.8, TRPV1, and transient receptor potential ankyrine-1 (TRPA1) channels which then induces hyperalgesia to mechanical and thermal stimuli[98, 99]. Estrogen and an agonist of G-protein coupled estrogen receptor (GPER) increase mast cell degranulation, tryptase and histamine release, and hypersensitivity in a rat stress model while ovariectomy decreases these activities[100].

Prostaglandin involvement in gut hypersensitivity is incompletely understood. $PGE_2$ signaling directly sensitizes peripheral nociceptors in inflamed tissues by activating TRPV1, hyperpolarization-activated cyclic nucleotide-2 (HCN2), and tetrodotoxin-resistant sodium channels on sensory neurons and induces hyperalgesia via protein kinase A- and C-mediated activation of nuclear factor κB (NF-kB) in DRG neurons. $PGE_2$ production and COX2 expression are upregulated in IBS-D colon supernatants[24, 55, 61]. This response is absent in mast cell-deficient rats but is restored after reconstitution with bone marrow-derived mast cells from wild type mice but not bone marrow mast cells from COX2-(Ptgs2Y385F) mutant mice[24]. Mast

cell $PGE_2$ sensitizes gut afferent fibers to other mediators and participates in hypersensitivity in inflammation. $PGE_2$ facilitates substance P and CGRP release, promotes IL-6 and brain-derived neurotrophic factor (BDNF) synthesis by DRG neurons, and enhances neuronal sensitivity to serotonin, bradykinin, and cytokines[101, 102]. $PGE_2$ alone enhances serotonin-evoked currents in stomach- and ileum-innervating afferent neurons[103].

Support for prostaglandins as final mediators of nociception come from studies showing that intracolonic histamine or tryptase causes delayed colon $PGE_2$ increases which coincide with hypersensitivity development. Mast cell-deficient and $PtgS2^{Y38SF}$ mutant mice develop hypersensitivity after $PGE_2$ but not histamine or tryptase administration, confirming intermediary roles of histamine and proteases and final mediation by prostaglandins in rat models (Figure 3)[24]. Similarly, prostaglandins may mediate hypersensitivity induced by cytokines as shown by the ability of COX2 inhibitors to block TNF-α and IL-1B induced nociceptor sensitization[104].

***Reversal of Mast Cell-Mediated Visceral Hypersensitivity***

Mast cell stabilizers and agents which target preformed mast cell mediators blunt hypersensitivity in animal studies. Stress-induced hypersensitivity is prevented by the mast cell stabilizer doxantrazole in rats[105]. Mast cell stabilizer reductions in hypersensitivity are associated with lower mast cell degranulation and TLR4 mRNA and protein expression[106]. In rats, cromolyn sodium prevents hypersensitivity induced by supernatants from IBS-D colon biopsies, histamine, and $PAR_2$ agonism[24]. Hypersensitivity in stressed rats is prevented by the $H_1$ antagonists, fexofenadine and ebastine[107]. Likewise, the $H_1$ antagonist olopatadine blunts hypersensitivity elicited by IBS-D colon supernatants[24].

COX2 inhibition and other agents have impact on mast cell-mediated hypersensitivity. In rats, the COX2 inhibitor celecoxib prevented hypersensitivity induced by IBS colon supernatants[24]. Bradykinin actions on serosal afferent nerves were blunted by the COX inhibitor naproxen but were restored by adding $PGE_2$ in another report[108]. Hypersensitivity elicited by colonic $PAR_2$ agonist infusion was prevented by a neurokinin-1 antagonist in a different report[18]. Electroacupuncture reduced hypersensitivity in rats which was associated with decreased TLR4 mRNA and protein and mast cell degranulation[106].

These animal models offer plausible support for clinical observations in IBS. Ketotifen was shown in one investigation to reduce perception of distention in IBS patients with defined

hypersensitivity (Figure 4)[85]. In another controlled trial in IBS-D, ketotifen reduced hypersensitivity to noxious distention versus placebo[86].

**Mast Cells and Gut Epithelial Function**

Increased gut permeability is observed in some IBS subgroups (IBS-D, post-infectious IBS), and is associated with altered bowel habits and increased abdominal pain[4]. Positive correlations of mast cell numbers with intestinal permeability defects have been reported mostly in animal studies as detailed in the following sections[109]. These findings support roles for mast cells in modulating epithelial dysfunction clinically observed in IBS.

***Characterization of Mast Cell Involvement in Epithelial Barrier Dysfunction***

Mast cell influences on the epithelial barrier have been demonstrated in rodent models. Increased mast cell mediators and gut permeability are noted after parasitic infection in rats[110]. Models of stress including water avoidance provide evidence for mast cell participation in epithelial barrier function[80, 111, 112, 113]. Mast cell-deficient mice models verify dependence of nerve-mediated chloride secretion on mucosal mast cells[114]. Intestinal barrier alterations in mast cell-deficient (*Wsh*) mice lead to reduced epithelial migration and permeability[115]. Claudin-3 expression is linked to regulation of barrier function by mast cell protease-4 (Mcpt-4). Mcpt-4 deficient mice exhibit similar permeability alterations as *Wsh* mice, but reconstitution of *Wsh* mice with bone marrow mast cells from wild type mice but not Mcpt-4 deficient mice restores epithelial architecture and permeability. Water avoidance stress effects on epithelial function are seen in wild type but not mast cell-deficient mice.

Epithelial barrier alterations with increased transcellular and paracellular mucosal permeability may underlie symptoms in some IBS subsets, especially relating to bowel habits[60, 111, 116, 117]. Transcellular permeability across the rectal mucosa of IBS-D patients measured with horseradish peroxidase correlates with mast cell numbers and increased tryptase activity, offering a clinical correlate to observations from animal studies[111, 116, 117].

***Mast Cell Mediators as Potential Triggers of Epithelial Barrier Dysfunction***

Mast cell histamine, chymase, and $PGD_2$ increase epithelial secretion and other mast cell products also impair epithelial function[115]. Proteases disrupt paracellular permeability by direct proteolysis and action on epithelial PAR receptors. Tryptase and chymase also cleave tight junction proteins including claudin-1, claudin-3, claudin-5, and junctional adhesion molecule-A (JAM-A)[115, 117, 118]. Elevated colon paracellular permeability in IBS-D results from tryptase action on $PAR_2$ receptors. $PAR_2$ receptor-mediated effects may involve calmodulin-dependent activation of myosin light chain kinase (MLCK) or β-arrestin-dependent activation of cofilin, a regulatory protein that severs actin[119, 120]. In knock out mice, microRNAs (MIR29) may regulate expression of tight junction proteins (cingulin, claudin-1) and NFRF to increase intestinal permeability[121]. Mast cell-dependent pathways involving substance P contribute to *Clostridium difficile* induced secretion in mice[122].

Human studies provide support for mast cell mediation of gut barrier function. In IBS-D, tryptase levels correlate with epithelial tight junction ultrastructural changes including increases in dilated junctions and intercellular distance plus enhanced myosin phosphorylation, redistribution of tight junction zonula occludens-1 (ZO-1) and occludin from the membrane to the cytoplasmin, decreased ZO-1 protein expression, and increased claudin-2 expression [66, 123]. Reductions in JAM-A in IBS are associated with worse abdominal pain and longer symptom durations[117]. Tight junctions are disrupted by cytokines like TNF-α that act by MLCK-mediated myosin light chain phosphorylation and ZO-1 and occludin reorganization[124]. A recent study demonstrated that intestinal tissues from patients with IBS-D had increased levels of MIR29A[121]. Clinical studies in IBS indicate that the magnitude of barrier loss and mast cell activation correlate with pain severity[125, 126]. Of 54 IBS-D patients, 39% were found to have increased membrane permeability as measured by the lactulose/mannitol ratio[127]. Interestingly the same group of patients also demonstrated increased visceral and thermal sensitivity. It is conceivable that increased permeability might allow access of luminal bacterial products into lamina proper which in turn stimulate sensory neurons to induce visceral hypersensitivity.

***Reversal of Mast Cell-Mediated Epithelial Barrier Dysfunction***

Treatments targeting mast cell function can reverse epithelial abnormalities in animal and human models. The mast cell stabilizer doxantrazole reversed increased secretion and permeability in stressed rodents and reduced secretion elicited by substance P[112, 128]. In a rat

model of postinfectious IBS, *Trichinella spiralis* increased mast cells, altered cytokine production, enhanced permeability, and elicited hypersensitivity[129]. Barrier and perceptual effects of *Trichinella spiralis* were normalized by a $PAR_2$ antagonist. In maternally separated rats, the sulfonylurea antidiabetic agent metformin inhibited loss of tight junction proteins and improved permeability and hypersensitivity[130]. Cromoglycate blocked increases in small intestinal permeability evoked by stress and CRH in healthy humans[80].

**Mast Cell Interactions with Microbiome**

IBS patients exhibit gut microbiota alterations including increases in *Firmicutes* and reductions in *Bacteroides* species, but findings are inconsistent between studies and geographic regions[5]. Changes in bacterial populations may cause symptoms by altering cytokine levels[131]. Organisms like *Enterococcus faecalis* reduce in vitro mast cell degranulation[132].

***Characterization of Mast Cell Interactions with Enteric Flora***

Interactions between mast cells and gut microbes may underlie some manifestations of IBS. Enteric bacteria promote mast cell histamine and protease release and activate inflammation through production of bile acids, organic acids, amino acids, phenols, polyunsaturated fatty acids, and short chain fatty acids[133]. Increased cellular translocation of bacteria promotes up-regulation of mast cell signaling in IBS-D[60]. Physical contact is not required for bacteria to activate mast cells; rather, bacterial toxins, metabolites such as histamine, and cell wall constituents accomplish this function after breaching the epithelial barrier[134]. Enteric flora modulate gut functions other than inflammation. Hypersensitivity to colonic distention of IBS patients can be transferred to rats through their fecal bacteria, demonstrating contributions of gut microbiota to sensorimotor dysfunction[135].

***Mast Cell Mediators Involved in Interactions with Enteric Bacteria***

Mast cell recognition of bacterial products involves activation of: (i) TLR4 receptors by lipopolysaccharide (LPS), (ii) TLR5 receptors by flagellin, and (iii) TLR2 receptors by the gram-positive bacterial component peptidoglycan[60]. Receptors for *Clostridium difficile*, *Bordetella pertussis*, and *Vibrio cholerae* toxins are expressed by mast cells[136]. Responses differ depending

on the bacterial constituent. Microbial peptidoglycan triggers mast cell degranulation and cytokine release while LPS elicits cytokine release without degranulation[137]. *Clostridium difficile* toxin A binds to mast cell neurokinin-1 receptors to cause gut secretion.

***Reversal of Mast Cell-Associated Gut Microbiota Interactions***

Animal and in vitro studies of therapies with dual action on mast cells and enteric bacteria illustrate possible roles of mast cell-microbiome interactions in gut illness. Ketotifen reduces enteritis in rodents exposed to *Clostridium difficile* toxin, blunts effects of *Vibrio cholerae* toxin in rat ileum, decreases epithelial passage of *Escherichia coli and Salmonella typhimurium*, and reverses effects of *Salmonella* to decrease occludin levels[60, 138, 139]. Miltefosine, a treatment of leishmaniasis, reverses hypersensitivity in maternally-separated rats in association with microbiome alterations and reduced mast cell degranulation[140]. Also in this model, fungicides including fluconazole and nystatin reversed gut sensitivity[141]. The human mast cell line HMC-1 released histamine in response to fungal antigens in this study.

Roles of mast cells in responding to therapies that modulate microbiome populations in IBS (probiotics, antibiotics, prebiotics, fecal transplant) are poorly understood. However, foods which are high in fermentable oligosaccharides, disaccharides, monosachrides and polyols (FODMAPs) increase visceral nociception by inducing gut dysbiosis and elevated fecal LPS level which mediate intestinal inflammation and barrier dysfunction, providing a potential mechanism for clinically observed IBS symptom exacerbation with FODMAP intake[142]. These abnormalities were reversed by low FODMAP diet. Subsequent studies in IBS-D patients showed that low FODMAP diets normalize fecal LPS levels and improve IBS symptoms, accompanied by improved colon barrier functions and reduced mast cell activation[143].

## CONCLUSIONS AND CLINICAL IMPLICATIONS

Prominent abnormalities in mast cell numbers, connectivity, and mediator release have been identified in IBS. Small intestine and colon mast cells show close apposition to sensory nerves which modulate sensorimotor and secretory activities. Mast cells release preformed, neo-synthesized, and neo-formed lipid bioactive substances in response to stimuli including infection, stress, allergens, and neuroendocrine factors. Studies of mast cells in different IBS subsets have

yielded inconsistent results, but research suggests that IBS that develops after an enteric infection or is diarrhea-predominant most often has mast cell dysfunction.

Research has focused on three factors as contributors to IBS symptom development. Visceral hypersensitivity is detectable in many patients and may influence abdominal pain pathogenesis. Important recent investigations have defined prominent abnormalities of mast cell prostaglandin $E_2$ synthesis which show interactions with histamine and tryptase release and which induce hypersensitivity in IBS-D. Mast cell dysfunction with abnormal protease and cytokine release also produces epithelial barrier dysfunction in IBS, which alters mucosal permeability and may disrupt defecation patterns. Epithelial abnormalities frequently coexist with hypersensitivity in IBS, worsening abdominal pain in this disorder. Lastly, enteric microbe interactions with mast cells may affect symptom reports in some patients. This is evidenced by the observation that low FODMAP diet corrects gut dysbiosis and improves IBS symptoms. This is accompanied by reduction of mast cell activation and normalization of colonic barrier function[143].

Validating the importance of any purported pathogenic factor in IBS should include characterizing effective treatments which target underlying mechanistic defects. To date, treatments that alter mast cell activity (mast cell stabilizers) or function (histamine antagonists) have shown only modest benefits in IBS and are not widely adopted in clinical practice. Limitations of published studies include recruitment of small samples and poor experimental designs. Currently, no biomarkers are available to define mast cell causation of symptoms in specific IBS subsets. A blood panel that measures interleukins released by mast cells has shown 88% sensitivity and 86% specificity in distinguishing IBS patients from healthy controls, but these findings have not been specifically ascribed to mast cell abnormalities[144]. Studies in IBS and animal models suggest potential treatments to reverse visceral hypersensitivity, epithelial dysfunction, or microbial abnormalities. Novel pharmaceuticals have been proposed which reduce IBS symptoms by modifying mast cell activity include next generation histamine antagonists, anti-Th2 cytokine antibodies, PAR antagonists, anti-IgE antibodies, tyrosine kinase inhibitors, miRNA inhibitors or precursors, and dietary therapies[127, 145, 146]. Omalizumab, a medication that blocks IgE, elicited responses in a small study in IBS-D[147]. A recent 12-week controlled trial of palmitoylethanolamide and polydatin, two dietary compounds which synergistically reduce mast cell activation, reported reductions in abdominal pain in IBS patients

without decreasing mast cell numbers[148]. Randomized trials of these and other therapies will define roles of mast cell dysfunction in well-defined IBS subsets.

**ABBREVIATIONS**

CCL, cytokine

CGRP, calcitonin gene-related peptide

COX1/2, cyclooxygenase1/2

CRH, corticotropin-releasing hormone

CXCL, cytokine of CXC chemokine family

DRG, dorsal root ganglia

MAPK, extracellular signal-regulated kinase

GI, gastrointestinal

FGF, fibroblast growth factor

IBS, irritable bowel syndrome

IgE, immunoglobulin E

IL-1β, interleukin-1β

NGF, nerve growth factor

NO, nitric oxide

NK, neurokinin

PAR, protease-activated receptor

$PGE_2$, prostaglandin $E_2$

$PGH_2$, prostaglandin $H_2$

PLA2, phospholipase A2

SCF, stem cell factor

TGF-β, transforming growth factor β

TLR (1-9), tool-like receptor

TNF-α, tumor necrosis factor-α

TNFR, tumor necrosis factor receptor

TRPA1, transient receptor potential ankyrine 1

TRPV1, transient receptor potential vanilloid 1

VEGF, vascular endothelial growth factor

VIP, vasoactive intestinal polypeptide

VPF, vascular permeability factor

**COMPETING INTERESTS**

**GRANT SUPPORT:** The studies were supported by the National Institutes of Health grants R01DK110436 and P30DK34933.

**REFERENCES**

1. Mearin, F., et al. Bowel disorders. *Gastroenterology* doi: 10.1053/j.gastro.2016.02.031 (2016).
2. Mertz, H., Naliboff, B, Munakara, J., Niazi N., & Mayer, E.A. Altered rectal perception is a biological marker of patients with irritable bowel syndrome. *Gastroenterology* **109,** 40-52 (1995).
3. Camilleri, M., et al., Prospective study of motor, sensory, psychologic, and autonomic functions in patients with irritable bowel syndrome. *Clin. Gastroenterol. Hepatol.* **6,** 772-781 (2008).
4. Camilleri, M., Lasch, K., & Zhou, K. Irritable bowel syndrome: methods, mechanisms, and pathophysiology. The confluence of increased permeability, inflammation, and pain in irritable bowel syndrome. *Am. J. Physiol. Gastrointest. Liver Physiol.* **303,** G775-G785 (2012).
5. Pittayanon, R., et al. Gut microbiota in patients with irritable bowel syndrome—a systematic review. *Gastroenterology* **157,** 97-108 (2019).
6. Hughes, P.A., et al. Immune activation in irritable bowel syndrome: can neuroimmune interactions explain symptoms? *Am. J. Gastroenterol.* **108,** 1066-1074 (2013).
7. Wouters, M.M., Vicario, M., & Santos, J. The role of mast cells in functional GI disorders. *Gut* **65,** 155-168 (2016).
8. Gilfillan, A.M., Austin, S.J., & Metcalfe, D.D. Mast cell biology: introduction and overview. *Adv. Exp. Med. Biol.* **716,** 2-12 (2011).
9. Metcalfe, D.D., Baram, D., & Mekori, M.A. Mast cells. *Physiol. Rev.* **77,** 1033-1079 (1997).

10. Irani, A.A., Schechter, N.M., Craig, S.S., DeBlois, G., & Schwartz, L.B. Two types of human mast cells that have distinct neural protease compositions. *Proc. Natl. Acad. Sci. USA* **83,** 4464-4468 (1986).
11. German, A.J., Hall, E.J., & Day, M.J. Analysis of leucocyte subsets in the canine intestine. *J. Comp. Pathol.* **120,** 129-145 (1999).
12. Stead, R.H., Dixon, M.F., Bramwell, N.H., Riddell, R.H., & Bienenstock, J. Mast cells are closely apposed to nerves in the human gastrointestinal mucosa. *Gastroenterology* **97,** 575-585 (1989).
13. Stead, R.H., et al. Intestinal mucosal mast cells in normal and nematode-infected rat intestines are in intimate contact with peptidergic nerves. *Proc. Natl. Acad. Sci. USA* **84,** 2975-2979 (1987).
14. Theoharides, T.C., Kempuraj, D., Tagen, M., Conti, P., & Kalogeromitros, D. Differential release of mast cell mediators and the pathogenesis of inflammation. *Immunol. Rev.* **217,** 65-78 (2007).
15. Wood, J.D. Histamine, mast cells, and the enteric nervous system in the irritable bowel syndrome, enteritis, and food allergies. *Gut* **55,** 445-447 (2006).
16. Buhner, S., et al. Activation of human enteric neurons by supernatants of colonic biopsy specimens from patients with irritable bowel syndrome. *Gastroenterology* **137,** 1425-1434 (2009).
17. Heavey, D.J., et al. Generation of leukotriene C4, leukotriene B4, and prostaglandin D2 by immunologically activated rat mucosa mast cells. *J. Immunol.* **140,** 1953-1957 (1988).
18. Coelho, A.M., Vergnolle, N., Guiard, B., Fioramonti, J., & Bueno, L. Proteinases and proteinase-activated receptor 2: a possible role to promote visceral hyperalgesia in rats. *Gastroenterology* **122,** 1035-1047 (2002).
19. Cenac, N, et al. Role for protease activity in visceral pain in irritable bowel syndrome. *J. Clin. Invest.* **117,** 636-647 (2007).
20. Valdez-Morales, E.E., at al. Sensitization of peripheral sensory nerves by mediators from colonic biopsies of diarrhea-predominant irritable bowel syndrome patients: a role for PAR2. *Am. J. Gastroenterol.* **108,** 1634-1643 (2013).

21. Molino, M., et al. Interactions of mast cell tryptase with thrombin receptors and PAR-2. *J. Biol. Chem.* **272,** 4043-4049 (1997).
22. Wedemeyer, J., Tsai, M., & Galli, S.J. Roles of mast cells and basophils in innate and acquired immunity. *Curr. Opin. Immunol.* **12,** 624-631 (2000).
23. Mukai, K., Tsai, M, Saito, H, & Galli, S.J. Mast cells as sources of cytokines, chemokines, and growth factors. *Immunol. Rev.* **282,** 121-150 (2018).
24. Grabauskas, G., et al. Prostaglandin $E_2$, produced by mast cells in colon tissues from patients with irritable bowel syndrome, contributes to visceral hypersensitivity in mice. *Gastroenterology* **158,** 2195-2207 (2020).
25. Zhang, S., et al. Role of prostaglandin $D_2$ in mast cell activation-induced sensitization of esophageal vagal afferents. *Am. J. Physiol. Gastrointest. Liver Physiol.* **304,** G908-G916 (2013).
26. Ferreira, S.H. Prostaglandins, aspirin-like drugs and analgesia. *Nat. New Biol.* **240,** 200-203 (1972).
27. An, S., et al. Expression of inducible nitric oxide synthase in mast cells contributes to the regulation of inflammatory cytokines in irritable bowel syndrome with diarrhea. *Neurogastroenterol. Motil.* **28,** 1083-1093 (2016).
28. Marnett, L.J., Wright, T.L., Crews, B.C., Tannenbaum, S.R., & Morrow J.D. Regulation of prostaglandin biosynthesis by nitric oxide is revealed by targeted deletion of inducible nitric-oxide synthase. *J. Biol. Chem.* **275,** 13427-13430 (2000).
29. Shanahan, F., Denburg, J.A., Fox, J., Bienenstock, J., & Befus, D. Mast cell heterogeneity: effects of neuroenteric peptides on histamine release. *J. Immunol.* **135,** 1331-1337 (1985).
30. Buhner, S. & Schemann, M. Mast cell-nerve axis with a focus on the human gut. *Biochim. Biophys. Acta* **1822,** 85-92 (2012).
31. Collins, S.M., et al. Previous inflammation alters the response of the rat colon to stress. *Gastroenterology* **111,** 1509-1515 (1996).
32. Nishida, M., et al. Migration of neutrophils is dependent on mast cells in nonspecific pleurisy in rats. *APMIS.* **107,** 929-936 (1999).

33. Theiner, G., Gessner, A., & Lutz, M.B. The mast cell mediator $PGD_2$ suppresses IL-12 release by dendritic cells leading to Th2 polarized immune responses in vivo. *Immunobiology* **211,** 463-472 (2006).
34. Nakae, S., et al. Mast cells enhance T cell activation: importance of mast cell-derived TNF. *Proc. Natl. Acad. Sci. USA* **1021,** 6467-6472 (2005).
35. Merluzzi, S., et al. Mast cells enhance proliferation of B lymphocytes and drive their differentiation toward IgA-secreting plasma cells. *Blood* **115,** 2810-2817 (2010).
36. Barbara, G., et al. Mast cell-dependent excitation of visceral-nociceptive sensory neurons in irritable bowel syndrome. *Gastroenterology* **132,** 26-37 (2007).
37. De Jonge, F., et al. In vitro activation of murine DRG neurons by CGRP-mediated mucosal mast cell degranulation. *Am. J. Physiol. Gastrointest. Liver Physiol.* **287,** G178-G191 (2004).
38. Magadmi, R., Meszaros, J., Damanhouri, Z.A., & Seward, E.P. Secretion of mast cell inflammatory mediators is enhanced by CADM1-dependent adhesion to sensory neurons. *Front. Cell. Neurosci.* **13,** 262 (2019).
39. He, S., Zhang, H., Zeng, X., & Yang, P. Self-amplification mechansism of mast cell activation: a new look in allergy. *Curr. Mol. Med.* **12,** 1329-1339 (2012).
40. He, S., et al. Expression and release of IL-29 by mast cells and modulation of mast cell behavior by IL-29. *Allergy* **65,** 1234-1241 (2010).
41. Terakawa, M., et al. Eosinophil migration induced by mast cell chymase is mediated by extracellular signal-regulated kinase pathway. *Biochim. Biophys. Res. Commun.* **332,** 969-975 (2005).
42. Wood, J.D. Enteric neuroimmunophysiology and pathophysiology. *Gastroenterology* **127,** 635-657 (2004).
43. Frieling, T., et al. Evidence for mast cell activation in patients with therapy-resistant irritable bowel syndrome. *Z. Gastroenterol.* **49,** 191-194 (2011).
44. Bashashati, M., et al. Colonic immune cells in irritable bowel syndrome: a systematic review and meta-analysis. *Neurogastroenterol. Motil.* doi: 10.1111/nmo.13192 (2018).
45. Robles, A., et al. Mast cells are increased in the small intestinal mucosa of patients with irritable bowel syndrome : a systematic review and meta-analysis. *Neurogastroenterol. Motil.* **31,** e13718 (2019).

46. Burns, G., et al. Evidence for local and systemic immune activation in functional dyspepsia and irritable bowel syndrome: a systematic review. *Am. J. Gastroenterol.* **114,** 429-436 (2019).
47. Ahn, J.Y. et al. Colonic mucosal immune activity in irritable bowel syndrome: comparison with healthy controls and patients with ulcerative colitis. *Dig. Dis. Sci.* **59,** 1001-1011 (2014).
48. O'Sullivan, M., et al. Increased mast cells in the irritable bowel syndrome. *Neurogastroenterol. Motil.* **12,** 449-457 (2000).
49. Cremon, C., et al. Mucosal immune activation in irritable bowel syndrome: gender-dependence and association with digestive symptoms. *Am. J. Gastroenterol.* **104,** 392-400 (2009).
50. El-Salhy, M., Mazzawi, T., Gundersen, D., Hatlebakk, J.G., & Hausken, T. Changes in the symptom pattern and the densities of large-intestinal endocrine cells following Campylobacter infection in irritable bowel syndrome: a case report. *BMC Res. Notes* **6,** 391 (2013).
51. Wang, L.H., Fang, X.C., & Pan, G.Z. Bacillary dysentery as a causative factor of irritable bowel syndrome and its pathogenesis. *Gut* **53,** 1096-1101 (2004).
52. Schmulson, M., et al. Microbiota, gastrointestinal infections, low-grade inflammation, and antibiotic therapy in irritable bowel syndrome: an evidence-based review. *Rev. Gastroenterol. Mex.* **79,** 96-134 (2014).
53. Chadwick, V.S., et al. Activation of the mucosal immune system in irritable bowel syndrome. *Gastroenterology* **122,** 1778-1783 (2002).
54. Sohn, W., et al. Mast cell number, substance P and vasoactive intestinal polypeptide in irritable bowel syndrome with diarrhea. *Scand. J. Gastroenterol.* **49,** 43-51 (2014).
55. Barbara, G., et al. Activated mast cells in proximity to colonic nerves correlate with abdominal pain in irritable bowel syndrome. *Gastroenterology* **126,** 693-702 (2004).
56. Yang, J., et al. Lactose intolerance in irritable bowel syndrome patients with diarrhoea: the roles of anxiety, activation of the innate mucosal immune system and visceral sensitivity. *Aliment. Pharmacol. Ther.* **39,** 302-311 (2014).

57. Park, J.H., et al. Mucosal mast cell counts correlate with visceral hypersensitivity in patients with diarrhea predominant irritable bowel syndrome. *J. Gastroenterol. Hepatol.* **21,** 71-78 (2006).
58. Di Nardo, G., et al. Neuroimmune interactions at different intestinal sites are related to abdominal pain symptoms in children with IBS. *Neurogastroenterol. Motil.* **26,** 196-204 (2014).
59. Schemann, M. & Camilleri, M. Functions and imaging of mast cell and neural axis of the gut. *Gastroenterology* **144,** 698-704 (2013).
60. Bednarska, O, et al. Vasoactive intestinal polypeptide and mast cells regulate increased passage of colonic bacteria in patients with irritable bowel syndrome. *Gastroenterology* **153,** 948-960 (2017).
61. Cenac, N., et al. Quantification and potential functions of endogenous agonists of transient receptor potential channels in patients with irritable bowel syndrome. *Gastroenterology* **149,** 433-444 (2015).
62. Barbara, G., De Giorgio, R., Stanghellini, V., Cremon, C., & Corinaldesi, R. A role for inflammation in irritable bowel syndrome? *Gut* **51 (Suppl 1),** 41-4 (2002).
63. Liang, W.J., et al. Tryptase and protease-activated receptor 2 expression levels in irritable bowel syndrome. *Gut Liver* **10,** 382-390 (2016).
64. Gecse, K., et al. Increased faecal serine protease activity in diarrhoeic IBS patients: a colonic lumenal factor impairing colonic permeability and sensitivity. *Gut* **57,** 591-599 (2008).
65. Tooth, D., et al. Characterisation of faecal protease activity in irritable bowel syndrome with diarrhoea: origin and effect of gut transit. *Gut* **63,** 753-760 (2014).
66. Martinez, C., et al. Diarrhoea-predominant irritable bowel syndrome: an organic disorder with structural abnormalities in the jejunal epithelial barrier. *Gut* **62,** 1160-1168 (2013).
67. Scully, P., et al. Plasma cytokine profiles in females with irritable bowel syndrome and extra-intestinal co-morbidity. *Am. J. Gastroenterol.* **105,** 2235-2243 (2010).
68. Willot, S., Gauthier, C., Patey, N., & Faure, C. Nerve growth factor content is increased in the rectal mucosa of children with diarrhea-predominant irritable bowel syndrome. *Neurogastroenterol. Motil.* **24,** 734-739 (2012).

69. Dothel, G., et al. Nerve fiber outgrowth is increased in the intestinal mucosa of patients with irritable bowel syndrome. *Gastroenterology* **148,** 1002-1011 (2015).
70. Cremon, C., et al. Intestinal serotonin release, sensory neuron activation, and abdominal pain in irritable bowel syndrome. *Am. J. Gastroenterol.* **106,** 1290-1298 (2011).
71. Kodani, M., et al. Association between gastrointestinal motility and macrophage/mast cell distribution in mice during the healing phase after DSS-induced colitis. *Mol. Med. Rep.* **17,** 8167-8172 (2018).
72. Song, J., et al. Mast cell-dependent mesenteric afferent activation by mucosal supernatant from different bowel segments of guinea pigs with post-infectious irritable bowel syndrome. *J. Neurogastroenterol. Motil.* **21,** 236-246 (2015).
73. Chen, B.R., et al. Fructo-oligosaccharide intensifies visceral hypersensitivity and intestinal inflammation in a stress-induced irritable bowel syndrome mouse model. *World J. Gastroenterol.* **23,** 8321-8333 (2017).
74. Ibeakanma, C., et al. Brain-gut interactions increase peripheral nociceptive signaling in mice with postinfectious irritable bowel syndrome. *Gastroenterology* **141,** 2098-2108 (2011).
75. Tache, Y. & Million, M. Role of corticotropin-releasing factor signaling in stress-related alterations of colonic motility and hyperalgesia. *J. Neurogastroenterol. Motil.* **21,** 8-24 (2015).
76. Santos, J., et al. Release of mast cell mediators into the jejunum by cold pain stress in humans. *Gastroenterology* **114,** 640-648 (1998).
77. Sagami, Y., et al. Effect of a corticotropin releasing hormone receptor antagonist on colonic sensory and motor function in patients with irritable bowel syndrome. *Gut* **53,** 958-964 (2004).
78. Theoharides, T.C., et al. Mast cells as targets of corticotropin-releasing factor and related peptides. *Trends Pharmacol. Sci.* **25,** 563-568 (2004).
79. Cao, J., et al. Human mast cells express corticotropin-releasing hormone (CRH) receptors and CRH leads to selective secretion of vascular endothelial growth factor. *J. Immunol.* **174,** 7665-7675 (2005).

80. Vanuytsel, T., et al. Psychological stress and corticotropin-releasing hormone increase intestinal permeability in humans by a mast cell-dependent mechanism. *Gut* **63,** 1293-1299 (2014).
81. Lobo, B., et al. Clinical improvement in IBS after disodium cromoglycate involves mast cell-mediated toll-like receptor signaling downregulation (abstract). *Gastroenterology* **140 (Suppl 1),** 499-500 (2011).
82. Lunardi, C., et al. Double-blind cross-over trial of oral sodium cromoglycate in patients with irritable bowel syndrome due to food intolerance. *Clin. Exp. Allergy* **21,** 569-572 (1991).
83. Stefanini, G.F., et al. Oral cromolyn sodium in comparison with elimination diet in the irritable bowel syndrome, diarrheic type. Multicenter study of 428 patients. *Scand. J. Gastroenterol.* **30,** 535-541 (1995).
84. Leri, O., et al. Management of diarrhoeic type of irritable bowel syndrome with exclusion diet and disodium cromoglycate. *Inflammopharmacology* **5,** 153-158 (1997).
85. Klooker, T.K. et al. The mast cell stabilizer ketotifen decreases visceral hypersensitivity and improves intestinal symptoms in patients with irritable bowel syndrome. *Gut* **59,** 1213-1221 (2010).
86. Wang, J., et al. Clinical efficacy and safety of ketotifen in treating irritable bowel syndrome with diarrhea. *Eur. J. Gastroenterol. Hepatol.* **32,** 706-712 (2020).
87. Wouters, M.M., et al. Histamine receptor H1-mediated sensitization of TRPV1 mediates visceral hypersensitivity and symptoms in patients with irritable bowel syndrome. *Gastroenterology* **150,** 875-887 (2016).
88. Modani, S., Cortes, O., & Thomas, R. Cyproheptadine use in children with functional gastrointestinal disorders. *J. Pediatr. Gastroenterol. Nutr.* **62,** 409-413 (2016).
89. Gillman, P.K. Tricyclic antidepressant pharmacology and therapeutic drug interactions updated. *Br. J. Pharmacol.* **151,** 737-748 (2007).
90. Dorofeyev, A.E., Kiriyan, E.A., Vasilenko, I.V., Rassokhina, O.A., & Elin, A.F. Clinical, endoscopical and morphological efficacy of mesalazine in patients with irritable bowel syndrome. *Clin. Exp. Gastroenterol.* **4,** 141-153 (2011).

91. Tuteja, A.K., Fang, J.C., Al-Suqi, M., Stoddard, G.J., & Hale, D.C. Double-blind placebo-controlled study of mesalamine in post-infective irritable bowel syndrome—a pilot study. *Scand. J. Gastroenterol.* **47,** 1159-1164 (2012).
92. Barbara, G., et al. Randomised controlled trial of mesalazine in IBS. *Gut* **65,** 82-90 (2016).
93. Lam, C., et al. A mechanistic multicentre, parallel group, randomised placebo-controlled trial of mesalazine for treatment of IBS with diarrhoea (IBS-D). *Gut* **65,** 91-99 (2016).
94. Dunlop, S.P., et al. Randomized, double-blind, placebo-controlled trial of prednisolone in post-infectious irritable bowel syndrome. *Aliment. Pharmacol. Ther.* **18,** 77-84 (2003).
95. La, J.H., et al. Role of mucosal mast cells in visceral hypersensitivity in a rat model of irritable bowel syndrome. *J. Vet. Sci.* **5,** 319-324 (2004).
96. Ohashi, K., Sato, Y., Kawai, M., & Kurebayashi, Y. Abolishment of TNBS-induced visceral hypersensitivity in mast cell deficient rats. *Life Sci.* **82,** 419-423 (2008).
97. Braak, B., et al. Mucosal immune cell numbers and visceral sensitivity in patients with irritable bowel syndrome: is there a relationship? *Am. J. Gastroenterol.* 107, 715-726 (2012).
98. Jin, X. & Gereau, R.W. Acute p38-mediated modulation of tetrodotoxin-resistant sodium channels in mouse sensory neurons by tumor necrosis factor-alpha. *J. Neurosci.* **26,** 246-255 (2006).
99. Binshtok, A.M. et al. Nociceptors are interleukin-1 beta sensors. *J. Neurosci.* **28,** 14062-14073 (2008).
100. Xu, S., et al. GPER-mediated, oestrogen-dependent visceral hypersensitivity in stressed rats is associated with mast cell tryptase and histamine expression. *Fundam. Clin. Pharmacol.* **34,** 433-443 (2020).
101. St-Jacques, B. & Ma, W. Role of prostaglandin E2 in the synthesis of the pro-inflammatory cytokine interleukin-6 in primary sensory neurons: an in vivo and in vitro study. *J. Neurochem.* **118,** 841-854 (2011).
102. Cruz Duarte, P., St-Jacques, B, & Ma, W. Prostaglandin E2 contributes to the synthesis of brain-derived neurotrophic factor in primary sensory neuron in ganglion explant cultures and in a neuropathic pain model. *Exp. Neurol.* **234,** 466-481 (2012).

103. Kim, S., et al. Prostaglandin potentiates 5-HT responses in stomach and ileum innervating visceral afferent sensory neurons. *Biochem. Biophys. Res. Commun.* **456,** 167-172 (2015).

104. Cunha, T.M., et al. A cascade of cytokines mediates mechanical inflammatory hypernociception in mice. *Proc. Natl. Acad. Sci. USA* **102,** 1755-1760 (2005).

105. van den Wijngaard, R.M., et al. Susceptibility to stress induced visceral hypersensitivity in maternally separated rats in transferred across generations. *Neurogastroenterol. Motil.* **25,** e780-e790 (2013).

106. Yang., J. et al. The role of toll-like receptor 4 and mast cell in the ameliorating effect of electroacupuncture on visceral hypersensitivity in rats. *Neurogastroenterol. Motil.* **31,** e13583 (2019).

107. Stanisor, O.I., et al. Stress induced visceral hypersensitivity in maternally separated rats can be reversed by peripherally restricted histamine-1-receptor antagonists. *PLoS One* **8,** e66884 (2013).

108. Maubach, K.A. & Grundy, D. The role of prostaglandins in the bradykinin-induced activation of serosal afferents of the rat jejunum in vitro. *J. Physiol.* **515,** 277-285 (1999).

109. Kamphuis, J.B., et al. Lactose and fructo-oligosaccharides increase visceral sensitivity in mice via glycation processes, increasing mast cell density in colonic mucosa. *Gastroenterology* **158,** 652-663 (2020).

110. Ramage, J.K., Hunt, R.H., & Perdue, M.H. Changes in intestinal permeability and epithelial differentiation during inflammation in the rat. *Gut* **29,** 57-61 (1988).

111. Lee, H., et al. Mucosal mast cell count is associated with intestinal permeability in patients with diarrhea predominant irritable bowel syndrome. *J. Neurogastroenterol. Motil.* **19,** 244-250 (2013).

112. Demaude, J., Salvador-Cartier, C., Fioramonti, J., Ferrier, L., & Bueno, L. Phenotypic changes in colonocytes following acute stress or activation of mast cells in mice: implications for delayed epithelial barrier dysfunction. *Gut* **55,** 655-661 (2006).

113. Santos, J., Benjamin, M., Yang, P.C., Prior, T, & Perdue, M.H. Chronic stress impairs rat growth and jejunal epithelial barrier function: role of mast cells. *Am. J. Physiol. Gastrointest. Liver. Physiol.* **278,** G847-G854 (2000).

114. Perdue, M.H., Masson, S., Wershil, B.K., & Galli S.J. Role of mast cells in ion transport abnormalities associated with intestinal anaphylaxis. Correction of the diminished secretory response in genetically mast cell-deficient W/Wv mice by bone marrow transplantation. *J. Clin. Invest.* **87,** 687-693 (1991).

115. Groschwitz, K.R., et al. Mast cells regulate homeostatic intestinal epithelial migration and barrier function by a chymase/Mcpt4-dependent mechanism. *Proc. Natl. Acad. Sci. USA* **106,** 22381-22386 (2009).

116. Piche, T., et al. Impaired intestinal barrier integrity in the colon of patients with irritable bowel syndrome: involvement of soluble mediators. *Gut* **58,** 196-201 (2009).

117. Wilcz-Villega, E.M., McClean, S., & O'Sullivan, M.A. Mast cell tryptase reduces junctional adhesion molecule-A (JAM-A) expression in intestinal epithelial cells: implications for the mechanisms of barrier dysfunction in irritable bowel syndrome. *Am. J. Gastroenterol.* **108,** 1140-1151 (2013).

118. Groschwitz, K.R., Wu, D., Osterfeld, H., Ahrens, R., & Hogan, S.P. Chymase-mediated intestinal epithelial permeability is regulated by a protease-activating receptor/matrix metalloproteinase-2-dependent mechanism. *Am. J. Physiol. Gastrointest. Liver. Physiol.* **304,** G479-G489 (2013).

119. Cenac, N., et al. PAR2 activation alters colonic paracellular permeability in mice via IFN-gamma-dependent and -independent pathways. *J. Physiol.* **558,** 913-925 (2004).

120. Jacob, C., et al. Mast cell tryptase controls paracellular permeability of the intestine. Role of protease-activated receptor 2 and beta-arrestins. *J. Biol. Chem.* **280,** 31936-31948 (2005).

121. Zhou Q, et al. MicroRNA 29 targets nuclear factor-κB-repressing factor and Claudin 1 to increase intestinal permeability. *Gastroenterology* **148,** 158-169 (2015).

122. Wershil, B.K., Castagliuolo, I., & Pothoulakis, C. Direct evidence of mast cell involvement in Clostridium difficile toxin A-induced enteritis in mice. *Gastroenterology* **114,** 956-964 (1998).

123. Martinez, C., et al. The jejunum of diarrhea-predominant irritable bowel syndrome shows molecular alterations in the tight junction signaling pathway that are associated with mucosal pathobiology and clinical manifestations. *Am. J. Gastroenterol.* **107,** 736-746 (2012).

124. Shen, Q., et al. Improving RhoA-mediated intestinal epithelial permeability by continuous blood purification in patients with severe acute pancreatitis. *Int. J. Artif. Organs* **36,** 812-820 (2013).
125. Piche T, et al. Impaired intestinal barrier integrity in the colon of patients with irritable bowel syndrome: involvement of soluble mediators. *Gut* **58**, 196-201 (2009)
126. Bertiaux-Vandaële N, et al. The expression and the cellular distribution of the tight junction proteins are altered in irritable bowel syndrome patients with differences according to the disease bubtype, *Am. J. Gastroenterol.* **106**, 2165-2173 (2011).
127. Zhou Q, et al. Intestinal membrane permeability and hypersensitivity in the irritable bowel syndrome. *Pain* **146**, 41-46 (2009).
128. Yang, X., Sheng, L., Guan, Y., Qian, W., & Hou, X. Synaptic plasticity: the new explanation of visceral hypersensitivity in rats with Trichinella spiralis infection. *Dig. Dis. Sci.* **54,** 937-946 (2009).
129. Du, L., et al. Protease activated receptor-2 induces immune activation and visceral hypersensitivity in post-infectious irritable bowel syndrome mice. *Dig. Dis. Sci.* **64,** 729-739 (2019).
130. Li, Y., et al. Metformin prevents colonic barrier dysfunction by inhibiting mast cell activation in maternal separation-induced IBS-like rats. *Neurogastroenterol. Motil.* **31,** e13556 (2019).
131. Camilleri, M. Halawi, H., & Oduyebo, I. Biomarkers as a diagnostic tool for irritable bowel syndrome: where are we? *Expert Rev. Gastroenterol. Hepatol.* **11,** 303-316 (2017).
132. Kasakura, K., et al. Commensal bacteria directly suppress in vitro degranulation of mast cells in a MyD88-independent manner. *Biosci. Biotchnol. Biochem.* **78,** 1669-1676 (2014).
133. Tana, C., et al. Altered profiles of intestinal microbiota and organic acids may be the origin of symptoms in irritable bowel syndrome. *Neurogastroenterol. Motil.* **22,** 512-519 (2010).
134. Galli, S.J., Nakae, S., & Tsai, M. Mast cells in the development of adaptive immune responses. *Nat. Immunol.* **6,** 135-142 (2005).

135. Crouzet, L., et al. The hypersensitivity to colonic distension of IBS patients can be transferred to rats through their fecal microbiota. *Neurogastroenterol. Motil.* **25,** e272-e282 (2013).

136. Moreno, L. & Gatheral, T. Therapeutic targeting of NOD1 receptors. *Br. J. Pharmacol.* **170,** 475-485 (2013).

137. Supajatura, V., et al. Differential responses of mast cell Toll-like receptors 2 and 4 in allergy and innate immunity. *J. Clin. Invest.* **109,** 1351-1359 (2002).

138. Pothoulakis, C., et al. Ketotifen inhibits Clostridium difficile toxin A-induced enteritis in rat ileum. *Gastroenterology* **105,** 701-707 (1993).

139. Rocha, M.F., et al. Role of mast cells and pro-inflammatory mediators on the intestinal secretion induced by cholera toxin. *Toxicon* **42,** 183-189 (2003).

140. Botschuijver, S., et al. Miltefosine treatment reduces visceral hypersensitivity in a rat model for irritable bowel syndrome via multiple mechanisms. *Sci. Rep.* **9,** 12530 (2019).

141. Botschuijver, S., et al. Intestinal fungal dysbiosis is associated with visceral hypersensitivity in patients with irritable bowel syndrome and rats. *Gastroenterology* **153,** 1026-1039 (2017).

142. Zhou, S.Y., et al. FODMAP diet modulates visceral nociception by lipopolysaccharide-mediated intestinal inflammation and barrier dysfunction. *J. Clin. Invest*. **128**, 267-280, (2018).

143. Singh, P., et al. High FODMAP diet causes barrier loss via liposaccharide mediated mast cell activation. *JCI Insight* (epub ahead of print) (2021).

144. Mujagic, Z., et al. A novel biomarker panel for irritable bowel syndrome and the application in the general population. *Sci. Rep.* **6,** 26420 (2016).

145. Eswaran S, et al. A randomized controlled trial comparing the low FODMAP diet vs. modified NICE guidelines in US adults with IBS-D. *Am. J. Gastroenterol.* **111**, 1824-1832 (2016).

146. Dionne, J., et al. A systematic review and meta-analysis evaluating the efficacy of a gluten-free diet and a low FODMAP diet in treating symptoms of irritable bowel syndrome. *Am. J. Gastroenterol.* **113,** 1290-1300, (2018).

147. Barbara, G., Stanghellini, V., De Giorgio, R., & Corinaldesi. R. Functional gastrointestinal disorders and mast cells: implications for therapy. *Neurogastroenterol. Motil.* **18,** 6-17, (2006).

148. Cremon, C., et al. Randomised clinical trial: the analgesic properties of dietary supplementation with palmitoylethanolamide and polydatin in irritable bowel syndrome. *Aliment. Pharmacol. Ther.* **45,** 909-922 (2017).

**TABLES**

**Table 1: MAST CELL MEDIATORS**

| Preformed Mediators | Neo-Synthesized Mediators | Neo-Formed Lipid Mediators |
|---|---|---|
| Histamine<br>Tryptases (α, β, γ)<br>Chymase<br>Carboxypeptidase-A<br>Heparin<br>Chondroitin sulfates<br>Cathepsin<br>Major basic protein | Cytokines (IL-1, IL-1R antagonist, IL-3, IL-4, IL-5, IL-6, IL-8, IL-9, IL-10, IL-11, IL-12, IL-13, IL-14, IL-15, IL-16, IL-18, TNF-α, TNF-β, INF-γ)<br>Growth factors (basic FGF, FGF2, TGF-β1, SCF, GM-CSF, M-CSF, VGEF, VPF, NGF, NT-3, LIF, LT-β, MIF, EGF, PDGF-AA, PDGF-BB)<br>Chemokines (CCL1, CCl2, CCL3, CCL3L1, CCL4, CCL5, CCL7, CCL8, CCL11, CCL13, CCL16, CCL17, CCL20, CCL22, CXCL1, CXCL2, CXCL3, CXCL8, CXCL10, XCL1)<br>Other neo-synthesized mediators (NO, superoxide, CRH, urocortin) | $PGD_2$<br>$PGE_2$<br>$PGF_2$<br>$PGI_2$<br>TX<br>LTB4<br>LTC4<br>LTD4<br>PAF |

Adapted from reference 30

**FIGURE LEGENDS:**

**Figure 1:** The ultrastructure of a mucosal mast cell is shown. Activated mast cells exhibit irregular plasma membranes and lipid bodies (arrow) and cytoplastic granules (A). Intact (white arrowhead) and degranulated (black arrowhead) granules are seen. On high magnification, mucosal mast cell cytoplasmic granules can show either crystalloid stricture (B) or scroll patterns (black arrow)(C). Enlarged empty and partly empty granules (black arrowhead) reflect piecemeal degranulation. Bars: 1 μm (A) and 0.5 μm (B, C). From reference 7.

**Figure 2:** Proinflammatory mediators released by colonic mucosa of IBS-D patients and healthy controls (HC) are shown. Some IBS-D patients exhibit increased release of histamine, mast cell (MC) tryptase, and $PGE_2$ (A). IBS-D patients but not healthy controls (HCs) show increased COX2 mRNA (B) and COX2//GAPDH protein (C) expression. Immunofluorescence staining for COX2 (red) and MC tryptase (green) is shown for HCs and IBS-D patients (D). Superimposed staining shows significant overlap of COX2 and MC tryptase immunoreactivity (yellow). Scale bar: 200 mm. From reference 24.

**Figure 3:** The role of $PGE_2$ produced by mucosal mast cells in generating visceral hypersensitivity in IBS is shown. Proinflammatory mediators such as histamine, tryptase, and LPS are increased in IBS. These activate mast cell GPCRs ($H_1$, PAR2, TLR4, etc.) which lead to degranulation of vesicular mediators (histamine, tryptas3, $PGE_2$, etc.) and induce transcription activation of COX2 which increases synthesis of prostaglandins. Mast cells in close proximity to submucosal sensory nerve fibers release $PGE_2$ which acts on sensory fiber EP2 receptors and potentiates action of pronociceptive mediators released by mechanical or chemical stimulation, leading to hypersensitivity. Histamine and tryptase are critical mediators released by mast cells to activate COX2 synthesis as blockade of either molecule prevents hypersensitivity development. However, histamine and tryptase are not the final mediators; rather their actions are dependent on $PGE_2$ synthesized and released by mast cells. Histamine, tryptase, TNF-α, and other mediators also may activate receptors on epithelial cells and enteric neurons causing dysmotility and epithelial barrier dysfunction via modulation of tight junction proteins. From reference 24.

**Figure 4:** Thresholds for discomfort/pain during rectal distention before and after 8 weeks of treatment with placebo or ketotifen are shown for individual subjects with IBS with hypersensitivity (A) and without hypersensitivity (B). The horizontal lines represent mean thresholds for discomfort. * P=0.015, **P=0.024 versus before treatment. From reference 85.