

Mast cell mediation of visceral sensation and permeability in irritable bowel syndrome

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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.

KEYWORDS

barrier function, IBS, mast cell, microbiome, visceral hypersensitivity

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; FGF, fibroblast growth factor; GI, gastrointestinal; IBS, irritable bowel syndrome; IgE, immunoglobulin E; IL-1 β , interleukin-1 β ; MAPK, extracellular signal-regulated kinase; NGF, nerve growth factor; NK, neurokinin; NO, nitric oxide; PAR, protease-activated receptor; PGE₂, prostaglandin E₂; PGH₂, prostaglandin H₂; PLA2, phospholipase A2; SCF, stem cell factor; TGF- β , transforming growth factor β ; TLR (1-9), toll-like receptor; TNFR, tumor necrosis factor receptor; TNF- α , tumor necrosis factor- α ; TRPA1, transient receptor potential ankyrin 1; TRPV1, transient receptor potential vanilloid 1; VEGF, vascular endothelial growth factor; VIP, vasoactive intestinal polypeptide; VPF, vascular permeability factor.

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1 | 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.

2 | MAST CELLS IN THE GUT

2.1 | 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, and 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

Key Points

- Abnormalities of epithelial barrier integrity resulting from activation by mast cell mediators may lead to increased mucosal permeability and development of visceral hypersensitivity.
- Recent studies have focused on interaction between gastrointestinal mast cells and the enteric microbiome which can modulate gut inflammatory process 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.

involved in gut sensation including calcitonin gene-related peptide (CGRP) and substance P.¹³

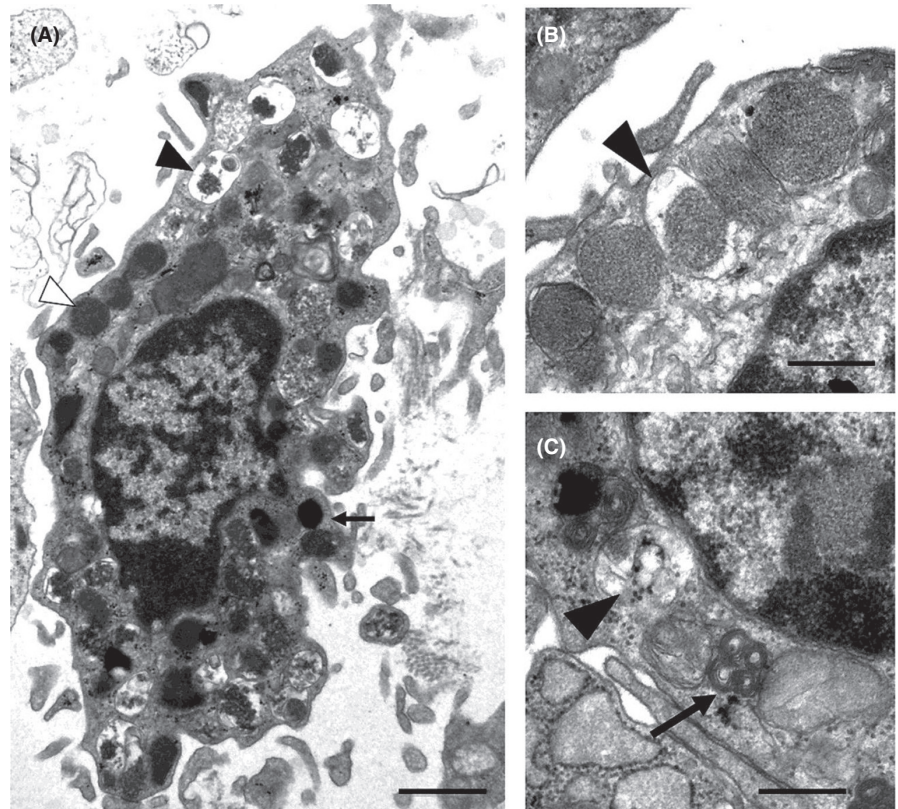
2.2 | Mast cell mediators

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

2.2.1 | 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 D₂ (PGD₂).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.¹⁸⁻²⁰ PARs are expressed by neurons in dorsal root ganglia (DRG) and the myenteric plexus. Tryptase specifically activates PAR₂.²¹ Upon activation of PAR₁ and PAR₂ receptors, sensory neurons release CGRP and substance P which then elicit neurogenic pain.

FIGURE 1 Ultrastructure of a mucosal mast cell is shown. Activated mast cells exhibit irregular plasma membranes and lipid bodies (arrow) and cytoplasmic granules (A). Intact (white arrowhead) and degranulated (black arrowhead) granules are seen. On high magnification, mucosal mast cell cytoplasmic granules can show either crystalloid structure (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 Wouters et al.⁷



2.2.2 | 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 proinflammatory (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-1 α (CCL3), MIP-1 β (CCL4), and CCL5.²³ In addition to participating in inflammation, cytokines (IL-3, IL-4, IL-6, IL-9, and 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- β 1, 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.

2.2.3 | 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.

2.3 | 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 PGD₂ synthesis. Non-IgE-mediated mast cell activation occurs after exposure to neuropeptides, complement, physical stimuli, and infection.

2.3.1 | 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 pronociceptive mediators. In a study of pleurisy in rats, injection of isologous serum promoted neutrophil infiltration which peaked at 4 h and was followed by eosinophilic influx lasting 24–48 h.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

2.3.2 | 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

2.3.3 | 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

3 | ROLE OF MAST CELLS IN IBS PATHOGENESIS

3.1 | 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

TABLE 1 Mast cell mediators

Preformed mediators	Neo-synthesized mediators	Neo-formed lipid mediators
Histamine	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)	PGD ₂
Trypsases (α , β , γ)		PGE ₂
Chymase		PGF ₂
Carboxypeptidase-A	Growth factors (basic FGF, FGF2, TGF- β 1, SCF, GM-CSF, M-CSF, VEGF, VPF, NGF, NT-3, LIF, LT- β , MIF, EGF, PDGF-AA, PDGF-BB)	PGI ₂
Heparin		TX
Chondroitin sulfates		LTB ₄
Cathepsin	Chemokines (CCL1, CCL2, CCL3, CCL3L1, CCL4, CCL5, CCL7, CCL8, CCL11, CCL13, CCL16, CCL17, CCL20, CCL22, CXCL1, CXCL2, CXCL3, CXCL8, CXCL10, XCL1)	LTC ₄
Major basic protein		LTD ₄
	Other neo-synthesized mediators (NO, superoxide, CRH, urocortin)	PAF

Note: Adapted from Buhner and Schemann.³⁰

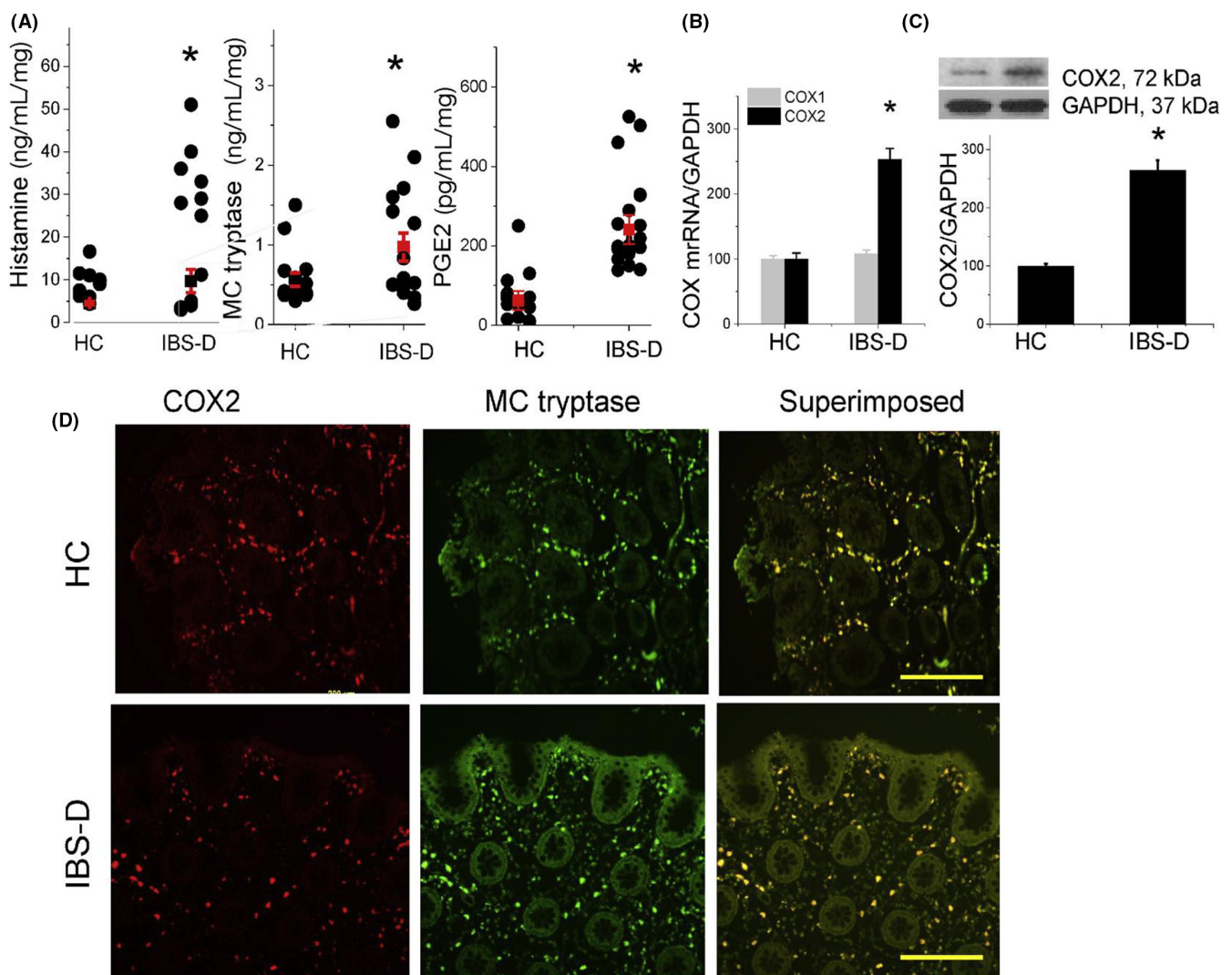


FIGURE 2 Proinflammatory mediators released by colonic mucosa of IBS-D patients and healthy controls (HC) are shown. Some IBS-D patients exhibit increased release of histamine, mast cell (MC) tryptase, and PGE₂ (A). IBS-D patients but not healthy controls (HCs) show increased COX2 mRNA (B) and COX2/GAPDH 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 Grabauskas et al.²⁴

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.

3.1.1 | 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 PGE₂ with associated increases in COX2 mRNA and protein expression (Figure 2).^{24,36} Histamine, protease, and PGE₂ 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 PAR₂ 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, IL-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-1 β 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

3.1.2 | 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 PGE₂ 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 CRH₁ and CRH₂ 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 CRH₁ and CRH₁/CRH₂ 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

3.1.3 | 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 H₁ antagonist properties, decreased IBS symptoms and improved quality of life but did not reduce mast cells.^{85,86}

Other studies suggest benefits of H₁ antagonists in IBS. In a placebo-controlled trial in 28 IBS patients, the H₁ 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

4 | PROPOSED MECHANISMS OF MAST CELL-MEDIATED IBS SYMPTOM PATHOGENESIS

4.1 | 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 these data originate from animal and in vitro investigations which provide plausibility for mast cell-induced visceral hypersensitivity in IBS.

4.1.1 | 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}

4.1.2 | 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 H₁ receptor activation from findings of a study employing IBS rectal supernatants.⁸⁷ Intracolonic PAR₂ agonist infusion promotes hypersensitivity to distention in rats and PAR₂-dependent mechanisms underlie hyperalgesia and increased sensory neuron calcium signaling in mice after exposure to IBS colon supernatants.^{18,19} PAR₂-deficient mice do not develop hypersensitivity to supernatant exposure. Histamine, PAR₂ agonists, and IBS colon supernatants fail to induce hypersensitivity in mast cell-deficient mice (Figure 3).²⁴

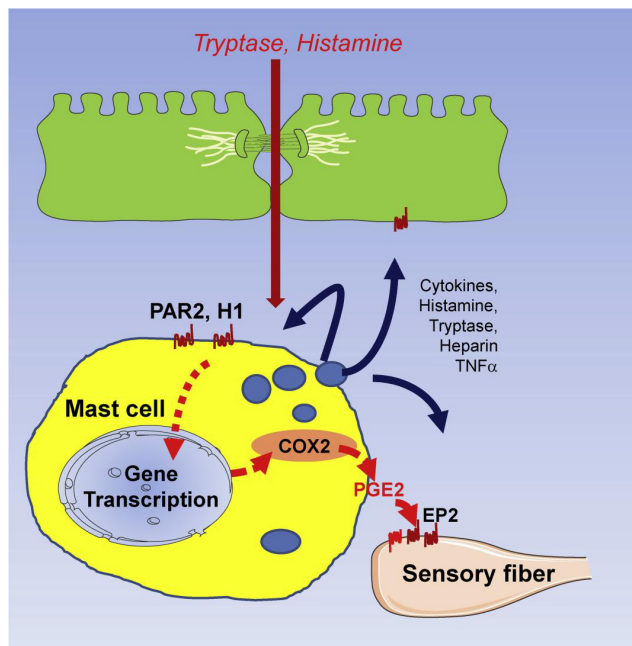


FIGURE 3 Role of PGE₂ produced by mucosal mast cells in generating visceral hypersensitivity in IBS is shown. Proinflammatory mediators such as histamine, tryptase, and LPS are increased in IBS. These activate mast cell GPCRs (H₁, PAR₂, TLR4, etc.) which lead to degranulation of vesicular mediators (histamine, tryptase, PGE₂, etc.) and induce transcription activation of COX2 which increases synthesis of prostaglandins. Mast cells in close proximity to submucosal sensory nerve fibers release PGE₂ 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 PGE₂ 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 Grabauskas et al.²⁴

Neo-synthesized mediators and associated pathways also participate in hypersensitivity. IL-1 β and TNF- α sensitize nociceptive neurons via p38 MAPK phosphorylation of Nav1.8, TRPV1, and transient receptor potential ankyrin-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. PGE₂ 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 κ B

(NF- κ B) in DRG neurons. PGE₂ 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 PGE₂ sensitizes gut afferent fibers to other mediators and participates in hypersensitivity in inflammation. PGE₂ 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} PGE₂ 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 PGE₂ increases which coincide with hypersensitivity development. Mast cell-deficient and Ptgs2^{Y385F} mutant mice develop hypersensitivity after PGE₂ 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-1 β -induced nociceptor sensitization.¹⁰⁴

4.1.3 | 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 PAR₂ agonism.²⁴ Hypersensitivity in stressed rats is prevented by the H₁ antagonists, fexofenadine, and ebastine.¹⁰⁷ Likewise, the H₁ 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 PGE₂ in another report.¹⁰⁸ Hypersensitivity elicited by colonic PAR₂ 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

4.2 | Mast cells and gut epithelial function

Increased gut permeability is observed in some IBS subgroups (IBS-D, postinfectious 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.

4.2.1 | Characterization of mast cell involvement in epithelial barrier dysfunction

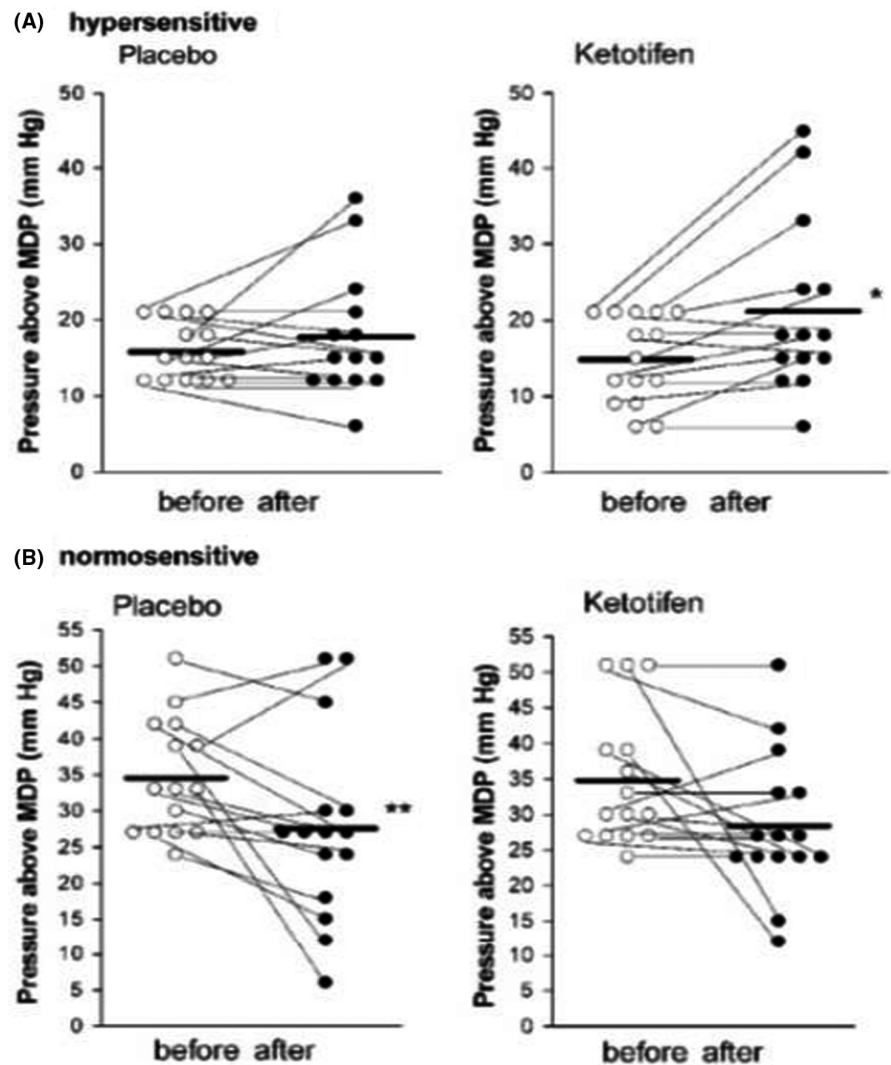
Mast cell influences on the epithelial barrier have been demonstrated in rodent models. Increased mast cell mediators and gut permeability are noted after parasitic infection in rats.¹¹⁰ Models of stress including water avoidance provide evidence for mast cell participation in epithelial barrier function.^{80,111-113} 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 (Mcp4). 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}

4.2.2 | Mast cell mediators as potential triggers of epithelial barrier dysfunction

Mast cell histamine, chymase, and PGD₂ 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 PAR₂ receptors. PAR₂ 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,

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 Klooker et al.⁸⁵



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 cytoplasm, 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.^{116,125} Of 54 IBS-D patients, 39% were found to have increased membrane permeability as measured by the lactulose/mannitol ratio.¹²⁶ 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 propria which in turn stimulate sensory neurons to induce visceral hypersensitivity.

4.2.3 | 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.^{112,127} In a rat model of postinfectious IBS, *Trichinella spiralis* increased mast cells, altered cytokine production, enhanced permeability, and elicited hypersensitivity.¹²⁸ Barrier and perceptual effects of *Trichinella spiralis* were normalized by a PAR₂ antagonist. In maternally separated rats, the sulfonylurea antidiabetic agent metformin inhibited loss of tight junction proteins and improved permeability and hypersensitivity.¹²⁹ Cromoglycate blocked increases in small intestinal permeability evoked by stress and CRH in healthy humans.⁸⁰

4.3 | 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.¹³⁰ Organisms like *Enterococcus faecalis* reduce in vitro mast cell degranulation.¹³¹

4.3.1 | 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.¹³² 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.¹³³ 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.¹³⁴

4.3.2 | 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.¹³⁵ Responses differ depending on the bacterial constituent. Microbial peptidoglycan triggers mast cell degranulation and cytokine release while LPS elicits cytokine release without degranulation.¹³⁶ *Clostridium difficile* toxin A binds to mast cell neurokinin-1 receptors to cause gut secretion.

4.3.3 | 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.^{60,137,138} Miltefosine, a treatment of leishmaniasis, reverses hypersensitivity in maternally separated rats in association with microbiome alterations and reduced mast cell degranulation.¹³⁹ Also in this model, fungicides including fluconazole and nystatin reversed gut sensitivity.¹⁴⁰ 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, and 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.¹⁴¹ 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.¹⁴²

5 | 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 E₂ 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.¹⁴²

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.¹⁴³ 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.^{126,144,145} Omalizumab, a medication that blocks IgE, elicited responses in a small study in IBS-D.¹⁴⁶ 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.¹⁴⁷ Randomized trials of these and other therapies will define roles of mast cell dysfunction in well-defined IBS subsets.

DISCLOSURE

No competing interests declared.

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