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The role of mast cells in functional gastrointestinal disorders

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Abbreviations used in this paper: FGIDs, functional gastrointestinal disorders; FD, Functional dyspepsia; IBS, irritable bowel syndrome; MCs, mast cells; GI, gastrointestinal; IL, interleukin; IgLC, immunoglobulin free-light chains; SP, substance P; NGF, nerve growth factor; TRPV, transient receptor potential vanilloid; PI, post infection; IBS-D,

diarrhea predominant IBS; IBD, inflammatory bowel disease; CRF, corticotropin releasing hormone; DSCG, disodium cromoglycate; DRG, dorsal root ganglia; PAR, protease-activated receptor; TJ, tight junctions; DSCG, disodium cromoglycate; TLR, toll-like receptor.

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Introduction

Functional gastrointestinal disorders (FGIDs) are characterized by chronic complaints arising from disorganized brain-gut interactions leading to dysmotility and hypersensitivity. FGIDs diagnosis is made by symptom-based approach using the corresponding Rome criteria. Functional dyspepsia (FD) and irritable bowel syndrome (IBS) are the two most prevalent FGIDs, affecting up to 16-26% of worldwide population[1,2]. However, despite these figures, their etiopathogenic mechanisms remain unclear, accounting for the lack of diagnostic biomarkers and the paucity of therapeutic options providing satisfactory long-standing clinical remission[3].

FGIDs are associated with a high prevalence of psychiatric comorbidities, chronic fatigue and chronic somatic and visceral pain disorders, rendering substantial social, humanistic and direct and indirect health care costs[4]. Recent observations revealing the presence of low-grade mucosal inflammation and immune activation, in association with impaired epithelial barrier function[5,6] and aberrant neuronal sensitivity come to challenge the traditional view of FGIDs as pure functional disorders, and relate the origin to a tangible organic substrate that stimulates the search for innovative diagnostic and therapeutic approaches. Mast cells (MCs), eosinophils and intraepithelial lymphocytes dominate the inflammatory infiltrate in the intestine of FGIDs. MC activation can generate epithelial and neuro-muscular dysfunction and promote visceral hypersensitivity and altered motility patterns in FGIDs[7,8,9], postoperative ileus, food allergy and inflammatory bowel disease (IBD)[10]. This review will discuss the role of mucosal MCs in the gastrointestinal (GI) tract with a specific focus on recent advances in disease mechanisms and management in IBS and FD.

The origin, phenotypes, and function of gastrointestinal MCs

MCs are long-lived granulated cells derived from bone marrow myeloid-cell progenitors ($CD34^+$), under the influence of stem cell factor and interleukin (IL)-4, cytokines that also regulate the development of MCs subtypes[11]. MC progenitor cells ($CD34^+$, $CD13^+$, c-kit^+, $Fc\varepsilon RI^-$) circulate in low numbers in the blood and migrate to locate in close proximity to blood and lymphatic vessels, glands, smooth muscle, and nerves. In the tissue, they remain as a homeostatic pool or they complete their differentiation process into mature MCs, as a direct consequence of genetic background, and inflammatory or bacterial-derived molecules released in the local micro-environment including IL-3, IL-4, IL-9, IL-10, IL-33, CXCL12, transforming growth factor-β, nerve growth factor (NGF), and stem cell factor[12]. Intestinal homing of MCs progenitor cells depends mostly on the binding of α4β7 integrin with their corresponding adhesion molecules such as cell adhesion molecule-1 or vascular cell adhesion molecule-1 on the endothelium, although the CXC chemokine receptor 2, expressed on MC progenitors, has been also implicated[13]. Mature MCs are particularly abundant in body barriers, ready for optimal interaction with the local environment. In the GI tract, MCs comprise 1-5% of mononuclear cells in the lamina propria and the submucosa, and are also found intraepithelial and deep in the muscle and serosal layers. Based on the anatomical location, human MCs are classified into mucosal MCs and connective tissue MCs, while depending on protease content, MCs are divided in two large subsets: MC_T, containing tryptase but little or no chymase, and MC_{TC}, containing tryptase, chymase, and carboxypeptidase[12,13]. MC_C, which express chymase but little or no tryptase also have been described, but they appear to be infrequent[12,13]. MC_T prevail in the intestinal and pulmonary mucosa, near T cells, whereas MC_{TC} are found in the skin and lymph nodes, in addition to the lung and the gut submucosa[11]. In the human small intestine, MC_T

represent ~98% of all MCs in the mucosa and ~13% of MCs in the submucosa are MC_T[12]. Recently, a new phenotype of MCs expressing tryptase and carboxypeptidase A3, but not chymase, has been described in the airway epithelium in asthmatic subjects and in esophageal samples of patients with eosinophilic esophagitis[14]. Heterogeneity of MCs also includes differential content in heparin, cytokines, and the receptor for the complement C5a, and the trans-differentiation between subtypes[12,13]. Therefore, location and granule content will determine the nature of mediators released to the extracellular milieu, accounting for modulation of specific functions in the GI tract[11]

MCs have been viewed, for the most part, as effectors of allergy and anaphylaxis and are best known for their association with pathological conditions such as asthma. However, the advent of MC lines, mouse strains deficient in MCs, and the reconstitution of these strains with bone marrow-derived MCs, has greatly facilitated the characterization of various aspects of MC function in vivo and their involvement in several disease states by interacting with a variety of other cells implicated in physiological and immunological responses. In the GI tract, MCs regulate vascular and epithelial permeability, ion secretion, angiogenesis, peristalsis, fibrosis and tissue repair, innate and adaptive immunity, bacterial defence, chemotaxis, and nociception[11]. Hence, uncontrolled or dysregulated MC activation may interfere with gut homeostasis and generate tissue dysfunction and promote inflammation in diverse GI diseases such as food allergy, IBD, postoperative ileus,, autoimmune disorders, cancer, and FGIDs[11]. However, at the same time, MCs are indispensable for controlling a wide range of pathogenic infections, and for modulation innate and adaptive immune responses[15]. Indeed, MCs can be intentionally activated to enhance protective host responses, including the production of high-affinity antibodies and

immunological memory, raising the possibility of incorporating MC activators in vaccine formulations to harness the inherent adjuvant activity of MC activation[15].

Regulation and activation of MCs

The classical and most effective stimulus for MC activation is crosslinking of cell surface-bound IgE to its high-affinity receptor (FcεRI) by allergen in sensitized individuals[16]. This results in a sequence of phosphorylation cascades and activation motifs that leads to intracellular calcium flux, activation of certain transcription factors such as AP-1 (c-FOS, v-Jun), MITF and STAT-5, and MC degranulation and cytokine production[17]. MCs also express receptors for IgG (FcγRI), immunoglobulin free-light chains (IgLC), other Ig-associated receptors, complement fractions, and toll-like receptors (TLRs). Moreover, MCs can be activated by neurotransmitters, neuropeptides, growth factors, and hormones (**Table 1**), accounting for MC versatility. Upon activation, MCs release newly synthesized (lipid mediators and cytokines) and stored (histamine, heparin, proteases) bioactive substances contained in cytoplasmic lipid bodies and granules (**Figure 1**). Secretion is achieved by IgE-mediated rapid release of all granule contents by fusion of granules and extrusion (anaphylactic degranulation) or by partial or total granule emptying without inter-granule fusion (piecemeal degranulation)[18]. Neuropeptides, cytokines, and microbial products induce piecemeal degranulation as frequently seen in diverse diseases, including IBD, IBS and FD[19].

Factors and mechanisms underlying MC activation in the gut

Food antigens as trigger for MC activation

The majority of FGIDs patients consider their symptoms to be related to meals. For example, more than 60% of patients with IBS report the onset or worsening of symptoms after meals, within 15 min in 28% and within 3 h in 93% of these patients[20,21]. Classically,

in food allergy, MCs are activated by food antigen-dependent cross-linking of antigen-specific IgE to FcЄRI. Although some patients with IBS have a higher incidence of atopy[22], food allergy has not convincingly been associated to FGIDs pathogenesis. Of note, adverse reactions to food, including some types of food intolerance, may occur through IgG-mediated sensitization of MCs but the role for these IgG-mediated immune reactions remains to be established[20,22]. When candidate food antigens are directly applied to the duodenal mucosa of IBS patients with suspected food intolerance through an endoscope it caused immediate epithelial breaks, increased intervillous spaces, and increased IEL numbers in the intestinal mucosa[23], and an individualized exclusion diet improved symptoms in 74% of patients at 1 year follow-up. The underlying mechanism and the potential role for mast cells, requires further study. On the other hand, the response to food is also partly regulated by neuroendocrine factors including peripheral serotonergic responses[24]. Although MCs can secrete and synthesize serotonin from tryptophan and serotonin is a chemotactic molecule for MCs[25], and some adverse reactions to diet in FGIDs involve foods containing serotonin, including cheese, meat, soya beans, cereals, nuts and vegetables[26], the role of MCs in such responses, if any, is mostly ignored. Finally, spice intake correlates directly with the likelihood of developing IBS in females[27]. Spicy foods contain capsaicin, the natural ligand of transient receptor potential vanilloid 1 (TRPV1) receptors on nociceptive afferent C-fibers. The increased density of sensory fibers expressing TRPV1 receptors reported in patients with FGIDs and visceral hypersensitivity[28], the genetic polymorphism of TRPV1 gene in FD[29], the potential TRPV1 sensitization in IBS patients[30], the close proximity of MCs to TRPV1 expressing sensory nerve fibers, and the ability of capsaicin to modulate MCs[31] all suggest that transmission of pain signals, including those generated by spicy foods, may be enhanced in FGIDs. In

contrast, desensitization of afferent terminals by a high capsaicin diet seems also plausible, as one study reported beneficial effects on abdominal bloating and pain in response to the ingestion of encapsulated red pepper for 6 weeks in IBS[32].

The role of infections

Post-infectious (PI)-FGIDs represent common entities in daily clinical practice. Infectious gastroenteritis is associated with an increased risk for FD and IBS, however the mechanisms leading to chronicity remain unknown[33]. MCs are potential regulatory linkers between innate and adaptive immunity and have been demonstrated to play critical roles in host defense, participating in effective immune responses to a number of bacterial, parasitic, viral and fungal pathogen products[15]. Antibody titers against bacterial flagellin are increased in IBS patients and are even higher in PI-IBS[34]. Recently, increased mucosal Ig production and up-regulation of germline transcripts and Ig genes have been identified in diarrhea predominant IBS (IBS-D) together with increased proximity between MC and plasma cell, suggesting MC activation by Ig[35]. Whether FGIDs individuals may become sensitized to food and microbial antigens during an acute infection and subsequently develop antibodies that will activate MCs upon antigen exposure remains to be established.

The role of stress

Chronic stress may also lead to MC activation. In preclinical studies, several types of stresses and stress mediators such as corticotropin releasing hormone (CRF) and related peptides have been shown to modulate ion and water secretion as well as intestinal and colonic paracellular and transcellular permeability, primarily via nerve-MC interactions[36,37]. Similarly, stress-induced rectal hyperalgesia could be prevented and reversed by administration of a MC stabilizer[38]. Other studies have confirmed and extended this paradigm to the human intestine. Santos et al. showed that a cold stress increased jejunal

MC tryptase and histamine release along with intestinal water secretion[39], and intestinal permeability, with larger responses in women with moderate levels of background stress[40]. CRF has been shown to enhance transcellular uptake of macromolecules in human colonic mucosa via CRF-R1 and CRF-R2 receptors located on subepithelial MCs[41]. More recently, acute psychological stress (public speech) has been shown to increase small intestinal permeability in humans[42]. This effect could be reproduced by peripheral administration of CRF, and blocked by the MC stabilizer disodium cromoglycate (DSCG). Preclinical models showed that chronic stress can induce substance P (SP) release by efferent nerves in the periphery, leading to CRF expression and release by intestinal eosinophils. Eosinophil-derived CRF was then capable of activating MCs resulting in jejunal epithelial barrier dysfunction[43]. SP, NGF and sex steroids also induce the release of vasoactive mediators from MCs, contributing to chloride secretion, barrier dysfunction, hyperalgesia, diarrhea, inflammation and motility changes[44,45].

MC infiltration in the GI tract in FGIDs

Since the description by Weston et al in 1993 on the infiltration of the terminal ileum by MCs in IBS[46], numerous studies evaluated MC numbers in the gastrointestinal mucosa of FGIDs (**Table 2**). It is interesting to note here that the presence of low-grade intestinal inflammation in the gut of these patients also involves an increase in intraepithelial T lymphocytes, and less consistently, enterochromaffin cells, plasma cells, B lymphocytes, neutrophils, and other immunocytes[47,48].

MCs have been identified by metachromatic stains such as Giemsa or toluidine blue, but these methods have been replaced by immunohistochemistry (antibodies for *c-kit* (CD117) or tryptase)[49,50,51] because it is more sensitive and specific. MC counts are comparable with both stains, yet $CD117^+$ cells display a more stable membranous staining whereas $tryptase^+$

cells display cytoplasmic staining that could be influenced by cell degranulation[52]. FGIDs biopsies contain singly dispersed MCs with no aggregates[52]. When elevated MC counts are detected, it may be helpful to exclude systemic mastocytosis by staining for the low-affinity receptor for interleukin-2 (CD25)[52]. A reference range for significant increased MC counts is still lacking. This is partly due to the absence of agreement and standardization on the methodology used to count MCs, to differences in patient and control selection, inter-individual variation, location of the biopsy, the relatively small cohort numbers for the majority of individual studies, and to other uncontrolled potential confounding factors (**Table 3**) [see Nasser et al for a detailed review]. The great variation in reporting mean mucosal MC numbers in the GI tract makes the interpretation of discriminatory cutoff values very complicated and currently un-interpretable according to some pathologists[53]. MC counts have been found to be normal, increased or decreased in IBS (**Table 2**). However, although the numbers vary across studies and segments, the analysis of more than one thousand IBS biopsies detects a mean, modest 1.2-2.5 fold increase in MC numbers throughout the entire gastrointestinal tract[54,55]. This is also true for cases of chronic undefined diarrhea, mostly studied in the upper small bowel and left colon, to the point that some pathologists debate the convenience of coining the term mastocytic enterocolitis for this clinical-pathological association[56]. A significant finding is that mucosal MC "hyperplasia", when present, is not limited to the lower small intestine[57-58] and colon[59,60] but also involves the duodenum[61], the jejunum[62], and the rectum[63]. While there is discrepancy in IBS, available studies in FD reveal that MC numbers are significantly increased in the antrum and corpus of H. pylori negative FD[64,65], and in the duodenum of FD patients (**Table 2**)[9,61,66,67]. Moreover, increased MCs have been recently reported in the esophagus of patients suffering noncardiac chest pain[68]. Even so, it is hard to dismiss the

physiological relevance of such "modest" increases because, on one side, similar incremental changes in leukocyte counts in circulating blood occur in infectious and inflammatory conditions, and on the other side, the magnitude of cell change is enormous if we consider the total mucosal surface of the gastrointestinal tract.

When evaluating MCs in IBS subtypes, some studies show that MC hyperplasia is more common in IBS-D[69,70] and in non-PI IBS[71] than in other subtypes, though in many other studies this is not the case[72,73,74]. In contrast, MCs are increased similarly in gastric biopsies in PI-FD and nonspecific FD[75]. Moreover, others found MC numbers decreased in the descending colon of diarrhea and alternating predominant IBS, but not constipation predominant IBS compared to health[51]. There is also some indication that MC numbers remain increased compared to both non-PI IBS and controls, three years after Shigella infection[73]. Although not the scope of this review, an increased number of MCs has been reported in the colorectal mucosa, in the lamina propria and in the submucosa from patients with Crohn's disease and ulcerative colitis[10].

The role of gender differences in MC number is unclear. Several lines of evidence indicate that gonadal steroids are involved in gender-related differences in tissue MC infiltration in the colon. This difference in the number of MCs has been described in a variety of tissues from rodents, such as skin, myocardium and rat colon. When specifically analyzed, some authors found increased MC counts in the terminal ileum, ascending and descending colon, and rectum of female vs male controls[57,60,74] , with females showing 43% increase in the area occupied by MCs[7], similar to observations in patients with chronic undefined diarrhea[53], while others do not[51,60,63]. These data raise the hypothesis that gender-dependent differences in immune responses are involved in the observed higher prevalence of IBS in

females, in the described gender-related differences in IBS pathophysiology, and in the known effects of the menstrual cycle in the modulation of rectal sensitivity[76].

Differences in MC numbers in the jejunum, cecum, colon, or rectum of IBS are not attributable to age, stress and cortisol levels, anxiety or depression, or duration of the disease[51,60,62,69]. Although disputed, it seems that changes in MC counts cannot easily be explained by differences in bowel preparation[7,48]. The role of diet on MC counts remains to be established. Thus, the diagnostic utility of routine MC stains in gastrointestinal biopsies remains unclear and requires further investigation.

MC activation in the gastrointestinal tract in FGIDs

MC activation in the gastrointestinal tract may be evaluated by: 1-Morphological analysis, most commonly by checking ultrastructural characteristics of piecemeal or anaphylactic degranulation on transmission electron microscopy (TEM); 2-Measuring the spontaneous or stimulated release of mediators in tissue, intestinal fluid, and blood, most commonly tryptase and histamine, and less often hexosaminidase, carboxypeptidase A, heparin, chromogranin A, leukotriene E4, prostaglandin D2, and prostaglandin 9α,11βPGF2, and methylhistamine in urine, and; 3-The expression of related genes and proteins in the mucosa (**Figure 2**).

Based on TEM studies, it has been shown that MCs display higher activation rates in the cecum and rectum in IBS-D, and that activation rates increase even more when nerve-MC distance is less than 2 μm[59]. Moreover, MCs located within 5 μm of nerve fibers were 3.1-times more frequent in the descending colon of IBS than in controls, and there was a 150% increase in the number of degranulating MCs[7]. Furthermore, the ileal and colonic density of neuronal specific enolase, SP, and 5-hydroxytryptamine positively stained nerve fibres

increased and appeared in clusters, surrounding an increased number of MCs with no differences between PI and non-post-infection IBS patients[73,77].

Supernatants of mucosal biopsies of IBS patients contain increased concentrations of histamine, serotonin, trypsin, tryptase, prostaglandin E2, other proteases, and cytokines[7,78,79,80]. Moreover, jejunal luminal tryptase release was 5-times higher[62] and the expression of both tryptase mRNA and protein enhanced in jejunal tissue[8] in IBS-D, while serum tryptase remained unaltered. Tryptase protein expression was also higher in both postinfectious FD and nonspecific FD gastric biopsies[64].

It is interesting that λIgLC$^+$ MCs but not IgE or IgG$^+$ MCs are reduced in the colon of IBS[51]. This finding, together with the description of elevated serum concentrations of λ and κ IgFLC in IBS[81], suggests that Ig light chain-mediated MC activation may be associated with IBS.

Taken together, evidence indicates that the activity of MCs rather than an increased number is essential in the pathophysiology of FGIDs, a point that has been recently raised by several experts in the field.

Linking MC infiltration and activation in the gastrointestinal tract with clinical manifestations in FGIDs

Role of MCs in visceral hypersensitivity and motility changes: motor and neuronal activation and sensitization

In the human gut, MCs lie in close proximity to gastrointestinal mucosal sensory nerve fibers containing neuropeptides, including visceral afferents expressing TRPV1 receptors[82]. This close spatial association, when coupled with MC activation, has been suggested to be of functional relevance for neuromuscular function and altered pain perception in response to insults such as infections, stress, and emotions in FGIDs[47,83]. Indeed, afferent innervation

of enteric MCs can trigger the release of histamine and mast cell protease II, mediators that act in a paracrine manner to elevate the sensitivity of spinal afferent terminals[84]. The use of supernatants obtained from biopsies allows studying the effect of these mediators on neuronal activation and sensitization. Injection of IBS-derived supernatants into rat mesenteric arteries evoked a marked increase in afferent nerve discharge, whereas injection of control supernatants had no effect[79]. In addition, IBS-dependent excitation of dorsal root ganglia (DRG) was inhibited by histamine H1 receptor blockade and serine protease inactivation[78], underscoring the role of MC mediators in neuronal activation. These findings were confirmed by Buhner et al. who reported that IBS biopsy supernatants, but not those of healthy controls, significantly increased the spike discharge of human submucosal neurons. This effect was inhibited by histamine receptor (H1-H3) antagonists, 5-HT3 receptor antagonist and protease inhibition[80]. Moreover, supernatants from hypersensitive IBS patients caused stronger activation of guinea pig enteric and mouse DRG neurons compared to supernatants of normosensitive patients[85], indicating that neuronal activation responses *in vitro* correlate with the individual pain threshold pressure values. Others showed that intracolonic infusion of IBS supernatants, but not controls, caused increased nociception in response to colorectal distention in mice, an effect that could be prevented by a serine protease inhibitor and was absent in neurons lacking functional protease-activated receptor-2 (PAR2)[79]. More recently, Cenac et al. showed that colonic biopsies from IBS patients contain increased levels of PUFA metabolites, these are endogenous TRPV4 agonists, compared to healthy subjects and these increases correlated with pain and bloating scores[86]. PUFA metabolites extracted from IBS biopsies or colons of mice with visceral hypersensitivity activated mouse sensory neurons in vitro, by activating TRPV4, an effect that could be prevented by siRNA knockdown of TRPV4[86]. Finally,

application of supernatants on muscle strips evoked excitatory cholinergic longitudinal muscle contractions of the guinea pig ileum, an effect that was not dependent on serotonin, proteases or histamine but was (partially) mediated by TRPV1, purinergic P2X receptors and prostanoid receptors[87]. Ballestra et al speculate that afferent nerve activation may induce myenteric cholinergic depolarizations, leading to altered motor function **(Figure 3)**.

Besides increased neuronal activation, supernatant of biopsies from IBS patients also has the capacity to potentiate sensory nerves. In a recent, elegant study, murine DRG neurons were incubated overnight with supernatants of submucosal colonic biopsies of IBS. Patch clamp recordings the next day revealed that the intrinsic excitability of the colonic nociceptive DRG neurons was increased by IBS-D supernatants. This increased excitability was not observed in DRG neurons lacking PAR-2[88]. Finally, incubation of a neuronal cell line or rat primary myenteric neuron cultures with mucosal biopsy supernatants from IBS also induced long-lasting neuroplastic changes as reflected by increased NGF-dependent neuronal sprouting[77].

Together, these preclinical data consistently indicate that the mucosa and submucosa of IBS patients contains increased levels of various MC mediators that have the potential to activate and potentiate intrinsic and afferent neurons, thereby leading to increased visceral pain perception and altered motor function that may cause diarrhea or constipation as a result of excessive segmental contractile colonic motor activity (**Table 4**). Of note, the use of human supernatants on animal models or isolated neurons may not completely reflect human physiology as MCs and enteric neurons exhibit species specificity in mediator release mechanisms and receptor profile[16]. To further assess the functional relevance and specificity of supernatant-mediated activation of nerve endings in the gut, it may be of great interest to perform live-imaging of MC-nerve signaling in human preparations or to

perform confocal endomicroscopy. The latter has recently been used to identify suspected food intolerance in IBS patients[23].

Role of MCs in the regulation of intestinal barrier function: secretion and permeability

MCs contribution to barrier function was first described in animal studies in which increased ion secretion and transepithelial transport of macromolecules was reversed with a MC stabilizer[89]. In humans, stress induces the release of MC mediators (tryptase, histamine) to the intestinal lumen[39] and increases intestinal permeability, which can be reversed by oral DSCG[42]. Stress can severely impact on barrier function and favour intestinal disease, as might be the case for FGIDs. IBS and FD patients experience high levels of anxiety, depression and stress[3] and intestinal permeability, as measured by probe excretion assays, has been found altered, primarily in PI-IBS and IBS-D[90]. The mechanisms underlying epithelial barrier alterations are not fully understood, but disruption of the proteins that seal the paracellular space seems to play a role. Actually, in IBS, the expression of several tight junctions (TJ) proteins is reduced compared to controls and, in IBS-D, this reduction correlates with MC activation and with common clinical symptoms[91]. In FD, the altered expression of cell-to-cell adhesion proteins also correlates with impaired duodenal integrity and with mucosal inflammation[9]. MCs proximity to the epithelium facilitates tryptase activation of PAR-2 receptors on the basolateral side of enterocytes, leading to redistribution of TJ and increased paracellular permeability to macromolecules[92]. Other mediators released by MCs upon activation, such as histamine, chymase and prostaglandin D2, regulate epithelial chloride and water secretion and permeability[93,94]. MC-mediated intestinal barrier alterations have been also related to neuropeptides, neurotransmitters, hormones (vasoactive intestinal peptide, SP, NGF, estrogen, estradiol),

and inflammatory mediators (tumor necrosis factor-α, interferon-γ and cytokines) released by other immunocytes[93] (**Figure 4**).

Role of MCs in IBS cardinal manifestations

MCs in close proximity to nerves in the descending colon were significantly correlated with severity and frequency of abdominal pain/discomfort[7]. In another study, mucosal MC infiltration was significantly associated with abdominal bloating frequency and with symptoms of dysmotility-like dyspepsia[74]. In contrast, in IBS, there was no correlation between severity or frequency of abdominal pain/discomfort and lamina propria area occupied by MCs, release of tryptase and histamine, and number of degranulated MCs per field[7,57]. IBS-D patients with rectal hypersensitivity, according to the maximally tolerable pressure to barostat distention, showed significantly lower counts of MCs in the terminal ileum, ascending colon and rectum in one study[57]. Park et al speculated that this counterintuitive finding was related to tissue desensitization by MCs mediators[57]. More recently, Braak et al found no correlation between the sensory thresholds to barostat distention, abdominal pain, bloating, urgency, incomplete evacuation, hard stools, loose stools, frequent and decreased bowel moments and flatulence and MCs counts in the colon[51]. An association between duodenal and antral MCs with pain, and postprandial distress syndrome, respectively, has been shown in children with FD[67].

Impaired intestinal permeability, and the expression of TJ proteins has been shown to correlate with pain/discomfort and/or bowel habit[90,91,95,96]. Interestingly, tryptase mRNA and protein expression in the jejunum of IBS-D patients correlated with stool frequency and consistency but not with abdominal pain, whereas the correlation with MC number was poor[91].

Cecum MC counts correlated significantly with the fatigue and depression scores in IBS[60,] and in some studies there is a tendency or an association between depression and state of anxiety scores and the number of MCs in patients with IBS-D[57]. A significant correlation with antral mast cell densities with anxiety, depression and somatization has been reported in children with FD[67]. Moreover, the degranulation of MCs in the duodenum appears to be highly sensitive and specific for the identification of adult FD patients as shown by 100% sensitivity and specificity indicated by an area under the ROC curve of 1.0 for the optimal degranulation rate cutoff values of 30.2% at the duodenal bulb and 36.8% at the descending part of the duodenum[66].

Taken together, these findings suggest that interactions between the MCs and the enteric and brain-gut neural networks could be of importance in symptom perception in at least a subgroup of patients with FGIDs.

Targeting MCs: implications for treatment of FGIDs

The MC stabilizer DSCG abolished the effect of acute psychological stress on small bowel permeability in human subjects[42]. Aside from experimental studies demonstrating the efficacy of several MC inhibitors to decrease colonic hypersensitivity, in humans, a number of uncontrolled observations[56], and open clinical studies with DSCG, in doses between 600-1800g/day, suggest its clinical benefit for chronic persistent diarrhea[97], allergic enteritis[98], FD[99], and IBS[100,101,102]. However, these studies had several limitations including poor design, small sample size, and selection bias. Likewise, ketotifen has been recently proven to increase the sensory threshold, leading to improved visceral perception, especially in the hypersensitivity IBS group[103]. Although preliminary, there is some indication of the clinical benefit of ketotifen and the tryptase inhibitor APC 2059 in ulcerative colitis[104,105]. Our group has recently finished an open trial (awaiting publication)

and a consecutive double-blind, placebo controlled, clinical assay, with prolonged (6 months) oral administration of DSCG, with promising results in the control of main clinical manifestations in IBS-D patients (Gastroenterology 2015;148 (Supl 1):S-494). In addition, small studies have shown improvement in gastrointestinal symptoms with DSCG therapy in systemic mastocytosis[106]. However, the mechanisms by which MC stabilization could interfere with IBS clinical response have not been clearly delineated.

Other interventions that block the effects of MC mediators and improve GI symptoms should be considered. In this sense, anti-inflammatory treatment with mesalazine appeared to show improvement in symptom perception in unselected IBS patients in a small proof-of-concept randomized, double-blind, placebo-controlled trial, in which, in addition, a 36% decrease in MC numbers, and a reduction of the number of total immune cells, and T cells was observed in the colonic mucosa[107]. However, two subsequent large clinical trials differ in the clinical benefit of mesalazine in IBS[108,109], and the effect of mesalazine on MC counts and degranulation not confirmed[109]. Furthermore, there was no effect of mesalazine on 5-HT containing enterochromaffin and CD68 cell numbers, although, there was significant increase in CD3 count in the mesalazine group[109]. In further reinforcing the role of MC activation in the origin of FGIDs manifestations, it is important to note that heartburn, cramping, nausea, abdominal pain and diarrhea are the second most common complaint of patients with mastocytosis, and that H2-histamine receptor antagonists have been quite effective in controlling these symptoms[56,98,110]. A recent proof-of-principle clinical trial confirmed the clinical relevance of these findings showing improvement of abdominal pain and global relief by the H1R antagonist ebastin in IBS patients (Gastroenterology 2013;144 (Supl 1):S-160). Palmitoylethanolamide and other inhibitors of cannabinoid receptors seem efficacious in controlling pain, motor disturbances and

inflammation in animal models through modulation of neuronal and non-neuronal cells, including MCs[111,112]. Slow-release of vitamin C may be also helpful as it increases degradation of histamine; and inhibits MC degranulation; in doses not superior to 750 mg/day[110]. Natural flavonoids (fisetin, kaempferol, quercetin, rutin, luteolin,..) and the active alkaloid berberine inhibit the mediator release of MCs in vitro[113] and protect intestinal epithelial barrier[114]. While some of these products have shown to be useful in cardiovascular health[115] and cancer[116], their clinical efficacy in FGIDs has not been established. There is some evidence of symptomatic response to specific diets in FGIDs, such as low FODMAP and gluten-depleted food[20]. However, there is no support for the role of MCs in this symptomatic response with the exception of the benefit after individualized exclusion of foods in FGIDs suffering food allergy.

Finally, the development of more specific and safe blockers or modulators of IgE, IgG or other activation pathways of MC activation, including pathways involved in the selective release of mediators, may offer therapeutic advantages, although their benefit remains to be established.

Conclusion

Current evidence implicating MCs in the pathogenesis and pathophysiology of FGIDs, particularly in IBS, and the contribution of their activation and released mediators to the development of cardinal manifestations, such as epigastric and abdominal pain, and altered defecation is robust, and supports the targeting of MCs in the management in FGIDs.

Key points

- Mast cells play a central pathophysiological role in IBS and possibly in FD, although not well defined.
- Increased mast cell activation is a common finding in the mucosa of patients with FGIDs.
- There is a need to implement standardized methods to count mast cells in the gastrointestinal mucosa, and to establish reference ranges of normality.
- Evaluation of spontaneous and stimulated mast cell function and activity on gastrointestinal samples is recommended when available.
- More studies are required to fully understand the implication of mast cells in the origin of clinical manifestations of these disorders.
- Treatment with mast cell stabilizers offers a reasonably safe and promising option for the management of those IBS patients non-responding to conventional approaches, though future studies are warranted to evaluate efficacy and indications.

Table 1. Triggers of mast cell activation (modified from[117])

Type of stimuli	Type of molecule	Molecule/stimuli
Immune	Immunoglobulins	IgE, IgG, free light chain-Ig (+antigen)
	Other	C3a, C5a, IL-4, IL-6, IL-9, IL-10, TNF-α, IFN-γ
Non-immune	Neurotransmitters	Acetylcholine, Dopamine, Serotonin, Epinephrine, Histamine
	Neuropeptides	SP, VIP, HRP, CGRP, SS, NT, Bradykinin
	Hormones	ACTH, CRF, PTH, Ucn, Estradiol
	Growth factors	NGF, SCF, TGF-β, FGF-2, VEGF, PD-ECGF
	Biological	LPS, Peptidoglican, Micobacterium
	Physico-chemical	NO, osmotic, thermal, pH, humidity, trauma, pressure, hypoxia, radiation, free radicals

Ig: immunoglobulin; IL: interleukin; C3a: complement component 3a; TNF-α: tumor necrosis factor alpha; IFN-γ: interferon gamma; SP: substance P; VIP: vasoactive intestinal peptide; HRP: histamine-releasing peptide; CGRP: calcitonin gene-related peptide; SS: somatostatin; NT: neurotensin; ACTH: adenocorticotropin hormone; CRF: corticotropin releasing factor; PTH: parathormone; Ucn: urocortin; NGF: nerve growth factor; SCF: stem cell factor; TGF-β: transforming growth factor beta; FGF-2: fibroblast growth factor-2; VEGF: vascular endothelial growth factor; PD-ECGF: platelet-derived endothelial cell growth factor; LPS: Lipopolysaccharide; NO: nitric oxide.

Table 2. Studies describing mast cell infiltration and activation in FD and IBS and potential correlation with symptoms.

Condition and number of subjects	Site of biopsy	Mast cell numbers	Mast cell detection	Mast cell mediators	Correlation with symptoms	Reference
141 FD and 39 controls	Duodenum	Increased counts	Toluidine blue staining	NA	NA	66
15 FD and 15 controls	Duodenum	Increased	Anti-tryptase	NA	NA	9
19 FD and 19 controls (pediatric)		No change	Anti-tryptase	NA	No correlation between permeability and mast cell density	118
65 H. PYLORI - negative FD (pediatric)	Gastric body and duodenum		Anti-tryptase	NA	Headache was associated with high mast cell counts in the gastric body and duodenum	65
51 FD, 20 IBS-D and 21 IBS-C and 48 controls	Duodenum	Increased counts in IBS-C and IBS-D and trend for increase in FD	Anti-CD-117	NA	NA	61
62 FD (33 Helicobacter pylori positive, 29 H. pylori negative and 29 H. pylori positive inflammatory control subjects and 20 controls,	Antrum and corpus	Increased in H. pylori negative and positive FD samples in antrum and corpus	Anti-tryptase	NA	NA	64
225 patients with non-ulcer dyspepsia	Antrum	31 (13%) were found to have 11 or greater mast cells per high-	Alcian blue staining	NA	NA	119

		power field				
Total of 101 IBS and 23 controls, for IHC: 15 controls; 15 IBS-C; 14 IBS-D	Descending colon	Increased counts	Anti-tryptase	NA	NA	77
13 IBS-D, 8 IBS-C and 10 controls (pediatric)	Ileum, right colon and left colon	No change in numbers but mast cells in closer proximity to nerves in IBS (MC-NF/mm2)	Anti-tryptase	NA	Abdominal pain correlated with MC/mm2 in the ileum and MC-NF/mm2 in the right colon	120
49 IBS-D and 30 controls	Jejunum	Increased counts in non-atopic IBS	Anti-CD-117	NA	NA	35
100 IBS and 100 controls	Colon, ileum, duodenum and stomach	No change	Anti-CD-117 and anti-CD-25	NA	NA	52
55 IBS-D and 18 controls with lactase deficiency	Sigmoid colon, ascending colon and terminal ileum	increased counts in the terminal ileum, ascending and sigmoid colon	Anti-tryptase	NA	Anxiety scores were associated with mast cell counts in sigmoid colon, ascending colon and terminal ileum. Visceral sensitivity (i.e. decrease in urgency, discomfort/pain threshold) was increased in patients with high mast cell density in the terminal ileum	121
22 IBS-D and 21 controls	Rectum	Increased counts in IBS-D	Anti-tryptase	NA	Mast cell counts did not correlate with	122

					IBS symptoms including abdominal pain; Mast cell counts correlated with substance P and VIP in women but not in men	
83 D-IBS, 49 UC (28 in remission and 21 mildly active UC), and 25 controls	Ascending, transverse, descending, and sigmoid colon	Increased counts in patients with D-IBS, UC in remission, and mildly active UC	Anti-tryptase	NA	NA	123
51 IBS, 49 quiescent IBD (31 CD and 18 UC) and 27 controls	Caecum	increased in patients with IBS, CD or UC (no difference between patients between patients with or without IBS-like symptoms	Anti-CD-117	NA	NA	124
16 IBS-D and 7 controls	Rectum	No change	Anti-tryptase	Increased tryptase release	Mast cell counts correlated with intestinal permeability	125
45 IBS-D and 30 controls	Jejunum	Increased counts	Anti-CD-117	Increased tryptase mRNA and protein	Tryptase mRNA expression but not mast cell counts correlated with stool frequency and consistency in IBS-D patients; tryptase protein expression correlated CLDN2 protein overexpression and increased	8

					OCLN cytoplasmic staining	
16 IBS-D, 21 C-IBS and 11 controls	Descending colon	Increased counts in IBS-C but not IBS-D	Anti-tryptase	NA	Mast cell counts of IBS but not controls correlated with the twitch enhancement evoked by biopsy supernatants	87
34 IBS and 15 controls	Rectum	Increased counts	Anti-CD-117	Increased tryptase release	IBS severity correlated with colonic permeability, mast cell counts and tryptase	95
4 IBS-C, 11 IBS-D, 8 IBS-A and 15 controls	Colorectum	No change	Anti-tryptase	NA	NA	126
11 IBS-D and 14 controls (pediatric)	Rectum	No change	Anti-tryptase and anti-CD-117	NA	NA	127
15 IBS-D, 15 IBS-C, 36 IBS-A and 20 controls	Descending colon	decreased mast cell counts	Anti-CD-117	NA	No correlation between the the number of mast cells and abdominal pain or sensory thresholds of first sensation, urge or discomfort and	51
25 IBS-D and 23 controls	Jejunum	Increased counts	Anti-CD-117	Increased tryptase mRNA	Tryptase and SCF correlated with tight junction ZO protein expression. Bowel frequency and	91

					stool consistency correlated with both the number of mast cells and tryptase mRNA expression, and with the expression of ZO proteins	
12 IBS-C, 13 IBS-D and 12 controls	Descending colon	Increased counts	Anti-tryptase	Increased serotonin, histamine and tryptase release irrespective of bowel habit	5-HT release correlated with mast cell counts and the severity of abdominal pain	128
60 IBS and 22 controls	Rectum and descending colon	decreased mast cell counts in rectal biopsies	Anti-tryptase and anti-CD117	Lower release of tryptase, slight increase in histamine release	The severity of abdominal pain was not correlated with mast cell counts; no correlation between abdominal pain and spontaneous histamine or tryptase release	103
13 IBS-D, 8 IBS-C, 4 IBS-A, 10 active CD and 18 controls	Descending colon	Increased counts in IBS-D but not in IBS-C	Anti-CD-117	Increased trypsin-like protein	NA	129
27 IBS-D, 21 IBS-C, 12 MC 20 UC, and 24 controls	Descending colon	Increased counts	Anti-tryptase	NA	Mast cell counts in IBS patients was associated with abdominal bloating frequency and with symptoms of dysmotility-like dyspepsia, but	74

					not ulcer-like dyspepsia	
7 IBS-D, 4 IBS-C and 4 controls	Descending colon	Increased counts	Anti-tryptase	Increased tryptase and histamine release but not serotonin	Association between the number of mast cells and the corresponding supernatant-evoked spike frequency. Tryptase, histamine and serotonin concentrations all correlated with the supernatant-evoked action potential discharges.	80
8 IBS-D, 8 IBS-C, 7 IBS-A and 22 controls	Rectosigmoid	Increased counts	Anti-CD-117	NA	c-kit^{+} cells correlated with maximal VAS pain score	28
50 IBS, 21 controls, 11 depressed/fatigued patients without IBS	Caecum	Increased in IBS, unchanged in depressed/fatigued patients w/o IBS	Anti-CD-117	NA	In IBS, but not in controls or depressed patients, mast cell counts correlated with the severity of fatigue and depression	60
29 IBS and 15 controls	Descending colon	Increased counts	Anti-tryptase	Increased tryptase, histamine and PGE2 release	NA	78
18 IBS and 12 controls and 4 UC and 1 CD	Rectum and ascending colon	No change	Alcian blue staining and anti-tryptase	Increased trypsin and tryptase protein	NA	79
20 IBS-D and 14 controls	Jejunum	Increased counts	CD-117	Increased tryptase release	No correlation between mast cell counts	62

					and gender or stress levels	
20 IBS-D, 18 IBS-C, and 20 controls	Descending part of the duodenum, proximal end of jejunum and terminal ileum	Increased counts in IBS-C and IBS-D in ileum but not duodenum or jejeunum	Anti-tryptase	Decreased 5-HT contents at the jejunum in IBS-C patients	NA	130
18 IBS-D and 15 controls	Terminal ileum, ascending colon and rectum	Increased in the terminal ileum, ascending colon and rectum	Anti-tryptase Electron microscopy	NA	Activated mast cells were significantly closer to the nerves in IBS No correlation between mast cell counts and abdominal pain, urgency, depression scores and STAI-S/T; The increase in mucosal mast cell count in the terminal ileum was significantly associated with that in the ascending colon and rectum	57
44 IBS and 22 controls	Descending colon	Increased counts	Anti-tryptase	Increased tryptase and histamine release	Vicinity of mast cells to nerves correlated with both severity and frequency of abdominal pain/discomfort	7
28 PI-IBS, 28 patient controls and 34 healthy volunteers	Rectum	No change	Anti-tryptase	NA	NA	63

42 IBS-D, 11 IBS-C, 20 IBS-A, 4 unknown IBS subtype and 28 controls	Ascending colon, transverse colon, descending colon, and rectum	No change	Anti-tryptase	NA	NA	48
10 IBS and 15 controls	Jejunum (full thickness)	No change	Giemsa staining	NA	NA	131
21 PI-IBS and 12 controls	Rectum	No change	Anti-tryptase	NA	NA	132
14 IBS, 7 normal controls and 7 inflammatory controls	Caecum, ascending colon, descending colon and rectum	Increased numbers in caecum but not at other sites	Anti-tryptase	NA	NA	50

FD: functional dyspepsia, IBS: irritable bowel syndrome, IBD: inflammatory bowel disease, UC: ulcerative colitis, CD: Crohn's Disease, MC: microscopic colitis, VIP: Vasoactive intestinal peptide, NA: Not assessed

Table 3. Conditions that may alter, commonly increase, the number of mast cell counts in the gastrointestinal tract

Allergic diseases: chronic urticaria, food allergy, atopy, hereditary angioedema[11,98]

Mastocytosis and mast cell activation syndrome[52]

Celiac disease: increased in initial stages and decreased in later stages[133]

Neuroendocrine cancer, lymphoma, epithelial cancers, carcinoid syndrome

H. Pylori gastritis, infectious and parasitic enteritis,

Inflammatory bowel disease[134], lymphocytic colitis[48]

Intestinal pseudo-obstruction, diverticulitis[135]

Vasculitis, amiloidosis, drugs

Table 4. Effect of mast cell mediators on gastrointestinal function

Mediator	Receptor	IBS/cell type	Effect	Referenc
Histamine	H1R	IBS-C; IBS-D	Excite rat mesenteric afferents	78
			Excite murine DRG neurons	78
	H1-H3R	IBS-C; IBS-D	Excite human submucosal neurons	80
	-	-	Epithelial secretion of Cl^- and H_2O	136
Tryptase	PAR2	IBS-C; IBS-D	Sensitize/activate murine DRG	78
		IBS-C; IBS-D	Excite human submucosal neurons	80
		IBS-D but not	Sensitization murine colonic DRG	88
		IBS-D, IBS-A	Increase epithelial permeability	137
Serotonin	5HT3R	IBS-C; IBS-D	Excite human submucosal neurons	80
		T84 cells	Secretory response	138
PGD2	DP1	IBS-C and IBS-D	Excite guinea pig longitudina	87
	-	-	Epithelial secretion of Cl^- and H_2O	136
Chymase	PAR2	Caco BBe	Increase epithelial permeability	94

IBS-C: constipation-predominant irritable bowel syndrome; IBS-D: diarrhea-predominant IBS; IBS-A: alternating subtype of IBS; H1R: Histamine receptor 1; PAR2: proteinase-activated receptor 2; 5HT3R: 5-hydroxytriptamine receptor 3; PGD2: prostaglandin D2; DP1: PGD2 receptor;

Figure legends

Figure 1. Ultrastructure of human mucosal mast cell. (**A**) Ultrastructure of an activated mast cell in the intestinal mucosa, with irregular plasma membrane and numerous lipid bodies (arrow) and cytoplasmic granules, displaying piecemeal degranulation. Intact (white arrowhead) and degranulated (black arrowhead) granules are identified. (**B,C**) High-magnification micrographs of cytoplasmic granules from a mucosal mast cell. Different granule patterns are observed, with crystalloid structure (B) and scrolls (arrow, C). Enlarged empty and partially empty granule containers (black arrowhead) are typical of piecemeal degranulation. Bars: 1µm (A) and 0.5µm (B, C).

Figure 2. Schematic representation of the experimental procedure to assess mucosal mast cell activation. Mast cell activation can be measured in intestinal samples. Luminal content can be obtained by aspiration, before biopsies are collected, and tryptase content can be quantified. Different mucosal biopsies can be processed for: histological examination, including mast cell counting after immunohistochemistry (tryptase and/or c-kit staining) and laser microdisection for ulterior gene expression analysis; ultrastructure analysis, to assess the type and degree of degranulation and to identify granule pattern; gene expression analysis of specific mediators synthesized and released by mast cells (tryptase, carboxypeptidase, chymase); quantification of mediators that are spontaneously released from biopsies and/or performing functional studies in vitro (muscle/nervous cells) or in vivo (mice/rats); and electrophysiology experiments in Ussing chambers for identification of mast cell-dependent changes in barrier function. Finally, analysis of the possible association between clinical manifestations and mast cell activation can be performed.

Figure 3. Schematic illustration of mast cell-nerve interactions in human gut.

MCs and nerves communicate bidirectionally, thereby modulating peristalsis and pain signalling. The release of bioactive, pro-inflammatory, mediators by mast cells results in a variety of neuronal effects including activation, sensitization and recruitment of nociceptors to the cell membrane, neurogenic inflammation and neural sprouting, ultimately leading to visceral hypersensitivity. On the other hand, neuronal activation triggers the release of neuropeptides and neurotransmitters, thereby further activating mast cells.

Ig, immunoglobulins; TLR, toll-like receptor; NK1, neurokinin 1 receptor, PGs, prostaglandins; NGF, neuronal growth factor, H1R, histamine receptor 1; TRPV1, transient receptor potential vanilloid 1; 5-HT3, 5-hydroxytryptamine receptor 3; PAR2, proteinase-activated receptor-2; TrkA, receptor for nerve growth factor; SP, substance P; CRGP, calcitonin-related gene peptide.

Figure 4. Intestinal barrier function elements and mast cell interactions in the intestinal mucosa. Illustration of the potential mast cell interactions in the regulation of barrier function, including epithelial permeability (through TJ modulation and secretory response), recruitment and activation of other immunocytes, endothelial functions (vascular permeability and blood flow), peristalsis, and pain signalling through bidirectional communication with the nervous system. TJ, tight junction; AJ, adherens junction; D, desmosome; PAR2, proteinase-activated receptor-2; $5HT_3R$, 5-hydroxytryptamine receptor; TNFα, tumor necrosis factor alpha; ILs, interleukins; SCF, stem cell factor; GM-CSF, granulocyte and monocyte colony stimulating factor; IFNγ, interferon gamma; Igs, immunoglobulins; LT, leukotrienes; PGD2, prostaglandin D-2; CRF, corticotropin-releasing factor; CRFR1/2, CRF receptors 1 and 2; IgE, Immunoglobulin E; IgG,

Immunoglobulin G; IgLC, immunoglobulin free-light chains; TLR, toll-like receptor; CNS, central nervous system; ENS, enteric nervous system.

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The role of mast cells in functional gastrointestinal disorders

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Abbreviations used in this paper: FGIDs, functional gastrointestinal disorders; FD, Functional dyspepsia; IBS, irritable bowel syndrome; MCs, mast cells; GI, gastrointestinal; IL, interleukin; IgLC, immunoglobulin free-light chains; SP, substance P; NGF, nerve growth factor; TRPV, transient receptor potential vanilloid; PI, post infection; IBS-D,

diarrhea predominant IBS; IBD, inflammatory bowel disease; CRF, corticotropin releasing hormone; DSCG, disodium cromoglycate; DRG, dorsal root ganglia; PAR, protease-activated receptor; TJ, tight junctions; DSCG, disodium cromoglycate; TLR, toll-like receptor.

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Introduction

Functional gastrointestinal disorders (FGIDs) are characterized by chronic complaints arising from disorganized brain-gut interactions leading to dysmotility and hypersensitivity. FGIDs diagnosis is made by symptom-based approach using the corresponding Rome criteria. Functional dyspepsia (FD) and irritable bowel syndrome (IBS) are the two most prevalent FGIDs, affecting up to 16-26% of worldwide population[1,2]. However, despite these figures, their etiopathogenic mechanisms remain unclear, accounting for the lack of diagnostic biomarkers and the paucity of therapeutic options providing satisfactory long-standing clinical remission[3].

FGIDs are associated with a high prevalence of psychiatric comorbidities, chronic fatigue and chronic somatic and visceral pain disorders, rendering substantial social, humanistic and direct and indirect health care costs[4]. Recent observations revealing the presence of low-grade mucosal inflammation and immune activation, in association with impaired epithelial barrier function[5,6] and aberrant neuronal sensitivity come to challenge the traditional view of FGIDs as pure functional disorders, and relate the origin to a tangible organic substrate that stimulates the search for innovative diagnostic and therapeutic approaches. Mast cells (MCs), eosinophils and intraepithelial lymphocytes dominate the inflammatory infiltrate in the intestine of FGIDs. MC activation can generate epithelial and neuro-muscular dysfunction and promote visceral hypersensitivity and altered motility patterns in FGIDs[7,8,9], postoperative ileus, food allergy and inflammatory bowel disease (IBD)[10]. This review will discuss the role of mucosal MCs in the gastrointestinal (GI) tract with a specific focus on recent advances in disease mechanisms and management in IBS and FD.

The origin, phenotypes, and function of gastrointestinal MCs

MCs are long-lived granulated cells derived from bone marrow myeloid-cell progenitors ($CD34^+$), under the influence of stem cell factor and interleukin (IL)-4, cytokines that also regulate the development of MCs subtypes[11]. MC progenitor cells ($CD34^+$, $CD13^+$, c-kit^+, $Fc\varepsilon RI^-$) circulate in low numbers in the blood and migrate to locate in close proximity to blood and lymphatic vessels, glands, smooth muscle, and nerves. In the tissue, they remain as a homeostatic pool or they complete their differentiation process into mature MCs, as a direct consequence of genetic background, and inflammatory or bacterial-derived molecules released in the local micro-environment including IL-3, IL-4, IL-9, IL-10, IL-33, CXCL12, transforming growth factor-β, nerve growth factor (NGF), and stem cell factor[12]. Intestinal homing of MCs progenitor cells depends mostly on the binding of α4β7 integrin with their corresponding adhesion molecules such as cell adhesion molecule-1 or vascular cell adhesion molecule-1 on the endothelium, although the CXC chemokine receptor 2, expressed on MC progenitors, has been also implicated[13]. Mature MCs are particularly abundant in body barriers, ready for optimal interaction with the local environment. In the GI tract, MCs comprise 1-5% of mononuclear cells in the lamina propria and the submucosa, and are also found intraepithelial and deep in the muscle and serosal layers. Based on the anatomical location, human MCs are classified into mucosal MCs and connective tissue MCs, while depending on protease content, MCs are divided in two large subsets: MC_T, containing tryptase but little or no chymase, and MC_{TC}, containing tryptase, chymase, and carboxypeptidase[12,13]. MC_C, which express chymase but little or no tryptase also have been described, but they appear to be infrequent[12,13]. MC_T prevail in the intestinal and pulmonary mucosa, near T cells, whereas MC_{TC} are found in the skin and lymph nodes, in addition to the lung and the gut submucosa[11]. In the human small intestine, MC_T

represent ~98% of all MCs in the mucosa and ~13% of MCs in the submucosa are MC_T[12]. Recently, a new phenotype of MCs expressing tryptase and carboxypeptidase A3, but not chymase, has been described in the airway epithelium in asthmatic subjects and in esophageal samples of patients with eosinophilic esophagitis[14]. Heterogeneity of MCs also includes differential content in heparin, cytokines, and the receptor for the complement C5a, and the trans-differentiation between subtypes[12,13]. Therefore, location and granule content will determine the nature of mediators released to the extracellular milieu, accounting for modulation of specific functions in the GI tract[11]

MCs have been viewed, for the most part, as effectors of allergy and anaphylaxis and are best known for their association with pathological conditions such as asthma. However, the advent of MC lines, mouse strains deficient in MCs, and the reconstitution of these strains with bone marrow-derived MCs, has greatly facilitated the characterization of various aspects of MC function in vivo and their involvement in several disease states by interacting with a variety of other cells implicated in physiological and immunological responses. In the GI tract, MCs regulate vascular and epithelial permeability, ion secretion, angiogenesis, peristalsis, fibrosis and tissue repair, innate and adaptive immunity, bacterial defence, chemotaxis, and nociception[11]. Hence, uncontrolled or dysregulated MC activation may interfere with gut homeostasis and generate tissue dysfunction and promote inflammation in diverse GI diseases such as food allergy, IBD, postoperative ileus,, autoimmune disorders, cancer, and FGIDs[11]. However, at the same time, MCs are indispensable for controlling a wide range of pathogenic infections, and for modulation innate and adaptive immune responses[15]. Indeed, MCs can be intentionally activated to enhance protective host responses, including the production of high-affinity antibodies and

immunological memory, raising the possibility of incorporating MC activators in vaccine formulations to harness the inherent adjuvant activity of MC activation[15].

Regulation and activation of MCs

The classical and most effective stimulus for MC activation is crosslinking of cell surface-bound IgE to its high-affinity receptor (FcεRI) by allergen in sensitized individuals[16]. This results in a sequence of phosphorylation cascades and activation motifs that leads to intracellular calcium flux, activation of certain transcription factors such as AP-1 (c-FOS, v-Jun), MITF and STAT-5, and MC degranulation and cytokine production[17]. MCs also express receptors for IgG (FcγRI), immunoglobulin free-light chains (IgLC), other Ig-associated receptors, complement fractions, and toll-like receptors (TLRs). Moreover, MCs can be activated by neurotransmitters, neuropeptides, growth factors, and hormones (**Table 1**), accounting for MC versatility. Upon activation, MCs release newly synthesized (lipid mediators and cytokines) and stored (histamine, heparin, proteases) bioactive substances contained in cytoplasmic lipid bodies and granules (**Figure 1**). Secretion is achieved by IgE-mediated rapid release of all granule contents by fusion of granules and extrusion (anaphylactic degranulation) or by partial or total granule emptying without inter-granule fusion (piecemeal degranulation)[18]. Neuropeptides, cytokines, and microbial products induce piecemeal degranulation as frequently seen in diverse diseases, including IBD, IBS and FD[19].

Factors and mechanisms underlying MC activation in the gut

Food antigens as trigger for MC activation

The majority of FGIDs patients consider their symptoms to be related to meals. For example, more than 60% of patients with IBS report the onset or worsening of symptoms after meals, within 15 min in 28% and within 3 h in 93% of these patients[20,21]. Classically,

in food allergy, MCs are activated by food antigen-dependent cross-linking of antigen-specific IgE to FcЄRI. Although some patients with IBS have a higher incidence of atopy[22], food allergy has not convincingly been associated to FGIDs pathogenesis. Of note, adverse reactions to food, including some types of food intolerance, may occur through IgG-mediated sensitization of MCs but the role for these IgG-mediated immune reactions remains to be established[20,22]. When candidate food antigens are directly applied to the duodenal mucosa of IBS patients with suspected food intolerance through an endoscope it caused immediate epithelial breaks, increased intervillous spaces, and increased IEL numbers in the intestinal mucosa[23], and an individualized exclusion diet improved symptoms in 74% of patients at 1 year follow-up. The underlying mechanism and the potential role for mast cells, requires further study. On the other hand, the response to food is also partly regulated by neuroendocrine factors including peripheral serotonergic responses[24]. Although MCs can secrete and synthesize serotonin from tryptophan and serotonin is a chemotactic molecule for MCs[25], and some adverse reactions to diet in FGIDs involve foods containing serotonin, including cheese, meat, soya beans, cereals, nuts and vegetables[26], the role of MCs in such responses, if any, is mostly ignored. Finally, spice intake correlates directly with the likelihood of developing IBS in females[27]. Spicy foods contain capsaicin, the natural ligand of transient receptor potential vanilloid 1 (TRPV1) receptors on nociceptive afferent C-fibers. The increased density of sensory fibers expressing TRPV1 receptors reported in patients with FGIDs and visceral hypersensitivity[28], the genetic polymorphism of TRPV1 gene in FD[29], the potential TRPV1 sensitization in IBS patients[30], the close proximity of MCs to TRPV1 expressing sensory nerve fibers, and the ability of capsaicin to modulate MCs[31] all suggest that transmission of pain signals, including those generated by spicy foods, may be enhanced in FGIDs. In

contrast, desensitization of afferent terminals by a high capsaicin diet seems also plausible, as one study reported beneficial effects on abdominal bloating and pain in response to the ingestion of encapsulated red pepper for 6 weeks in IBS[32].

The role of infections

Post-infectious (PI)-FGIDs represent common entities in daily clinical practice. Infectious gastroenteritis is associated with an increased risk for FD and IBS, however the mechanisms leading to chronicity remain unknown[33]. MCs are potential regulatory linkers between innate and adaptive immunity and have been demonstrated to play critical roles in host defense, participating in effective immune responses to a number of bacterial, parasitic, viral and fungal pathogen products[15]. Antibody titers against bacterial flagellin are increased in IBS patients and are even higher in PI-IBS[34]. Recently, increased mucosal Ig production and up-regulation of germline transcripts and Ig genes have been identified in diarrhea predominant IBS (IBS-D) together with increased proximity between MC and plasma cell, suggesting MC activation by Ig[35]. Whether FGIDs individuals may become sensitized to food and microbial antigens during an acute infection and subsequently develop antibodies that will activate MCs upon antigen exposure remains to be established.

The role of stress

Chronic stress may also lead to MC activation. In preclinical studies, several types of stresses and stress mediators such as corticotropin releasing hormone (CRF) and related peptides have been shown to modulate ion and water secretion as well as intestinal and colonic paracellular and transcellular permeability, primarily via nerve-MC interactions[36,37]. Similarly, stress-induced rectal hyperalgesia could be prevented and reversed by administration of a MC stabilizer[38]. Other studies have confirmed and extended this paradigm to the human intestine. Santos et al. showed that a cold stress increased jejunal

MC tryptase and histamine release along with intestinal water secretion[39], and intestinal permeability, with larger responses in women with moderate levels of background stress[40]. CRF has been shown to enhance transcellular uptake of macromolecules in human colonic mucosa via CRF-R1 and CRF-R2 receptors located on subepithelial MCs[41]. More recently, acute psychological stress (public speech) has been shown to increase small intestinal permeability in humans[42]. This effect could be reproduced by peripheral administration of CRF, and blocked by the MC stabilizer disodium cromoglycate (DSCG). Preclinical models showed that chronic stress can induce substance P (SP) release by efferent nerves in the periphery, leading to CRF expression and release by intestinal eosinophils. Eosinophil-derived CRF was then capable of activating MCs resulting in jejunal epithelial barrier dysfunction[43]. SP, NGF and sex steroids also induce the release of vasoactive mediators from MCs, contributing to chloride secretion, barrier dysfunction, hyperalgesia, diarrhea, inflammation and motility changes[44,45].

MC infiltration in the GI tract in FGIDs

Since the description by Weston et al in 1993 on the infiltration of the terminal ileum by MCs in IBS[46], numerous studies evaluated MC numbers in the gastrointestinal mucosa of FGIDs (**Table 2**). It is interesting to note here that the presence of low-grade intestinal inflammation in the gut of these patients also involves an increase in intraepithelial T lymphocytes, and less consistently, enterochromaffin cells, plasma cells, B lymphocytes, neutrophils, and other immunocytes[47,48].

MCs have been identified by metachromatic stains such as Giemsa or toluidine blue, but these methods have been replaced by immunohistochemistry (antibodies for *c-kit* (CD117) or tryptase)[49,50,51] because it is more sensitive and specific. MC counts are comparable with both stains, yet $CD117^+$ cells display a more stable membranous staining whereas $tryptase^+$

cells display cytoplasmic staining that could be influenced by cell degranulation[52]. FGIDs biopsies contain singly dispersed MCs with no aggregates[52]. When elevated MC counts are detected, it may be helpful to exclude systemic mastocytosis by staining for the low-affinity receptor for interleukin-2 (CD25)[52]. A reference range for significant increased MC counts is still lacking. This is partly due to the absence of agreement and standardization on the methodology used to count MCs, to differences in patient and control selection, inter-individual variation, location of the biopsy, the relatively small cohort numbers for the majority of individual studies, and to other uncontrolled potential confounding factors (**Table 3**) [see Nasser et al for a detailed review]. The great variation in reporting mean mucosal MC numbers in the GI tract makes the interpretation of discriminatory cutoff values very complicated and currently un-interpretable according to some pathologists[53]. MC counts have been found to be normal, increased or decreased in IBS (**Table 2**). However, although the numbers vary across studies and segments, the analysis of more than one thousand IBS biopsies detects a mean, modest 1.2-2.5 fold increase in MC numbers throughout the entire gastrointestinal tract[54,55]. This is also true for cases of chronic undefined diarrhea, mostly studied in the upper small bowel and left colon, to the point that some pathologists debate the convenience of coining the term mastocytic enterocolitis for this clinical-pathological association[56]. A significant finding is that mucosal MC "hyperplasia", when present, is not limited to the lower small intestine[57-58] and colon[59,60] but also involves the duodenum[61], the jejunum[62], and the rectum[63]. While there is discrepancy in IBS, available studies in FD reveal that MC numbers are significantly increased in the antrum and corpus of H. pylori negative FD[64,65], and in the duodenum of FD patients (**Table 2**)[9,61,66,67]. Moreover, increased MCs have been recently reported in the esophagus of patients suffering noncardiac chest pain[68]. Even so, it is hard to dismiss the

physiological relevance of such "modest" increases because, on one side, similar incremental changes in leukocyte counts in circulating blood occur in infectious and inflammatory conditions, and on the other side, the magnitude of cell change is enormous if we consider the total mucosal surface of the gastrointestinal tract.

When evaluating MCs in IBS subtypes, some studies show that MC hyperplasia is more common in IBS-D[69,70] and in non-PI IBS[71] than in other subtypes, though in many other studies this is not the case[72,73,74]. In contrast, MCs are increased similarly in gastric biopsies in PI-FD and nonspecific FD[75]. Moreover, others found MC numbers decreased in the descending colon of diarrhea and alternating predominant IBS, but not constipation predominant IBS compared to health[51]. There is also some indication that MC numbers remain increased compared to both non-PI IBS and controls, three years after Shigella infection[73]. Although not the scope of this review, an increased number of MCs has been reported in the colorectal mucosa, in the lamina propria and in the submucosa from patients with Crohn's disease and ulcerative colitis[10].

The role of gender differences in MC number is unclear. Several lines of evidence indicate that gonadal steroids are involved in gender-related differences in tissue MC infiltration in the colon. This difference in the number of MCs has been described in a variety of tissues from rodents, such as skin, myocardium and rat colon. When specifically analyzed, some authors found increased MC counts in the terminal ileum, ascending and descending colon, and rectum of female vs male controls[57,60,74] , with females showing 43% increase in the area occupied by MCs[7], similar to observations in patients with chronic undefined diarrhea[53], while others do not[51,60,63]. These data raise the hypothesis that gender-dependent differences in immune responses are involved in the observed higher prevalence of IBS in

females, in the described gender-related differences in IBS pathophysiology, and in the known effects of the menstrual cycle in the modulation of rectal sensitivity[76].

Differences in MC numbers in the jejunum, cecum, colon, or rectum of IBS are not attributable to age, stress and cortisol levels, anxiety or depression, or duration of the disease[51,60,62,69]. Although disputed, it seems that changes in MC counts cannot easily be explained by differences in bowel preparation[7,48]. The role of diet on MC counts remains to be established. Thus, the diagnostic utility of routine MC stains in gastrointestinal biopsies remains unclear and requires further investigation.

MC activation in the gastrointestinal tract in FGIDs

MC activation in the gastrointestinal tract may be evaluated by: 1-Morphological analysis, most commonly by checking ultrastructural characteristics of piecemeal or anaphylactic degranulation on transmission electron microscopy (TEM); 2-Measuring the spontaneous or stimulated release of mediators in tissue, intestinal fluid, and blood, most commonly tryptase and histamine, and less often hexosaminidase, carboxypeptidase A, heparin, chromogranin A, leukotriene E4, prostaglandin D2, and prostaglandin 9α,11βPGF2, and methylhistamine in urine, and; 3-The expression of related genes and proteins in the mucosa (**Figure 2**).

Based on TEM studies, it has been shown that MCs display higher activation rates in the cecum and rectum in IBS-D, and that activation rates increase even more when nerve-MC distance is less than 2 μm[59]. Moreover, MCs located within 5 μm of nerve fibers were 3.1-times more frequent in the descending colon of IBS than in controls, and there was a 150% increase in the number of degranulating MCs[7]. Furthermore, the ileal and colonic density of neuronal specific enolase, SP, and 5-hydroxytryptamine positively stained nerve fibres

increased and appeared in clusters, surrounding an increased number of MCs with no differences between PI and non-post-infection IBS patients[73,77].

Supernatants of mucosal biopsies of IBS patients contain increased concentrations of histamine, serotonin, trypsin, tryptase, prostaglandin E2, other proteases, and cytokines[7,78,79,80]. Moreover, jejunal luminal tryptase release was 5-times higher[62] and the expression of both tryptase mRNA and protein enhanced in jejunal tissue[8] in IBS-D, while serum tryptase remained unaltered. Tryptase protein expression was also higher in both postinfectious FD and nonspecific FD gastric biopsies[64].

It is interesting that λIgLC$^+$ MCs but not IgE or IgG$^+$ MCs are reduced in the colon of IBS[51]. This finding, together with the description of elevated serum concentrations of λ and κ IgFLC in IBS[81], suggests that Ig light chain-mediated MC activation may be associated with IBS.

Taken together, evidence indicates that the activity of MCs rather than an increased number is essential in the pathophysiology of FGIDs, a point that has been recently raised by several experts in the field.

Linking MC infiltration and activation in the gastrointestinal tract with clinical manifestations in FGIDs

Role of MCs in visceral hypersensitivity and motility changes: motor and neuronal activation and sensitization

In the human gut, MCs lie in close proximity to gastrointestinal mucosal sensory nerve fibers containing neuropeptides, including visceral afferents expressing TRPV1 receptors[82]. This close spatial association, when coupled with MC activation, has been suggested to be of functional relevance for neuromuscular function and altered pain perception in response to insults such as infections, stress, and emotions in FGIDs[47,83]. Indeed, afferent innervation

of enteric MCs can trigger the release of histamine and mast cell protease II, mediators that act in a paracrine manner to elevate the sensitivity of spinal afferent terminals[84]. The use of supernatants obtained from biopsies allows studying the effect of these mediators on neuronal activation and sensitization. Injection of IBS-derived supernatants into rat mesenteric arteries evoked a marked increase in afferent nerve discharge, whereas injection of control supernatants had no effect[79]. In addition, IBS-dependent excitation of dorsal root ganglia (DRG) was inhibited by histamine H1 receptor blockade and serine protease inactivation[78], underscoring the role of MC mediators in neuronal activation. These findings were confirmed by Buhner et al. who reported that IBS biopsy supernatants, but not those of healthy controls, significantly increased the spike discharge of human submucosal neurons. This effect was inhibited by histamine receptor (H1-H3) antagonists, 5-HT3 receptor antagonist and protease inhibition[80]. Moreover, supernatants from hypersensitive IBS patients caused stronger activation of guinea pig enteric and mouse DRG neurons compared to supernatants of normosensitive patients[85], indicating that neuronal activation responses *in vitro* correlate with the individual pain threshold pressure values. Others showed that intracolonic infusion of IBS supernatants, but not controls, caused increased nociception in response to colorectal distention in mice, an effect that could be prevented by a serine protease inhibitor and was absent in neurons lacking functional protease-activated receptor-2 (PAR2)[79]. More recently, Cenac et al. showed that colonic biopsies from IBS patients contain increased levels of PUFA metabolites, these are endogenous TRPV4 agonists, compared to healthy subjects and these increases correlated with pain and bloating scores[86]. PUFA metabolites extracted from IBS biopsies or colons of mice with visceral hypersensitivity activated mouse sensory neurons in vitro, by activating TRPV4, an effect that could be prevented by siRNA knockdown of TRPV4[86]. Finally,

application of supernatants on muscle strips evoked excitatory cholinergic longitudinal muscle contractions of the guinea pig ileum, an effect that was not dependent on serotonin, proteases or histamine but was (partially) mediated by TRPV1, purinergic P2X receptors and prostanoid receptors[87]. Ballestra et al speculate that afferent nerve activation may induce myenteric cholinergic depolarizations, leading to altered motor function **(Figure 3)**.

Besides increased neuronal activation, supernatant of biopsies from IBS patients also has the capacity to potentiate sensory nerves. In a recent, elegant study, murine DRG neurons were incubated overnight with supernatants of submucosal colonic biopsies of IBS. Patch clamp recordings the next day revealed that the intrinsic excitability of the colonic nociceptive DRG neurons was increased by IBS-D supernatants. This increased excitability was not observed in DRG neurons lacking PAR-2[88]. Finally, incubation of a neuronal cell line or rat primary myenteric neuron cultures with mucosal biopsy supernatants from IBS also induced long-lasting neuroplastic changes as reflected by increased NGF-dependent neuronal sprouting[77].

Together, these preclinical data consistently indicate that the mucosa and submucosa of IBS patients contains increased levels of various MC mediators that have the potential to activate and potentiate intrinsic and afferent neurons, thereby leading to increased visceral pain perception and altered motor function that may cause diarrhea or constipation as a result of excessive segmental contractile colonic motor activity (**Table 4**). Of note, the use of human supernatants on animal models or isolated neurons may not completely reflect human physiology as MCs and enteric neurons exhibit species specificity in mediator release mechanisms and receptor profile[16]. To further assess the functional relevance and specificity of supernatant-mediated activation of nerve endings in the gut, it may be of great interest to perform live-imaging of MC-nerve signaling in human preparations or to

perform confocal endomicroscopy. The latter has recently been used to identify suspected food intolerance in IBS patients[23].

Role of MCs in the regulation of intestinal barrier function: secretion and permeability

MCs contribution to barrier function was first described in animal studies in which increased ion secretion and transepithelial transport of macromolecules was reversed with a MC stabilizer[89]. In humans, stress induces the release of MC mediators (tryptase, histamine) to the intestinal lumen[39] and increases intestinal permeability, which can be reversed by oral DSCG[42]. Stress can severely impact on barrier function and favour intestinal disease, as might be the case for FGIDs. IBS and FD patients experience high levels of anxiety, depression and stress[3] and intestinal permeability, as measured by probe excretion assays, has been found altered, primarily in PI-IBS and IBS-D[90]. The mechanisms underlying epithelial barrier alterations are not fully understood, but disruption of the proteins that seal the paracellular space seems to play a role. Actually, in IBS, the expression of several tight junctions (TJ) proteins is reduced compared to controls and, in IBS-D, this reduction correlates with MC activation and with common clinical symptoms[91]. In FD, the altered expression of cell-to-cell adhesion proteins also correlates with impaired duodenal integrity and with mucosal inflammation[9]. MCs proximity to the epithelium facilitates tryptase activation of PAR-2 receptors on the basolateral side of enterocytes, leading to redistribution of TJ and increased paracellular permeability to macromolecules[92]. Other mediators released by MCs upon activation, such as histamine, chymase and prostaglandin D2, regulate epithelial chloride and water secretion and permeability[93,94]. MC-mediated intestinal barrier alterations have been also related to neuropeptides, neurotransmitters, hormones (vasoactive intestinal peptide, SP, NGF, estrogen, estradiol),

and inflammatory mediators (tumor necrosis factor-α, interferon-γ and cytokines) released by other immunocytes[93] (**Figure 4**).

Role of MCs in IBS cardinal manifestations

MCs in close proximity to nerves in the descending colon were significantly correlated with severity and frequency of abdominal pain/discomfort[7]. In another study, mucosal MC infiltration was significantly associated with abdominal bloating frequency and with symptoms of dysmotility-like dyspepsia[74]. In contrast, in IBS, there was no correlation between severity or frequency of abdominal pain/discomfort and lamina propria area occupied by MCs, release of tryptase and histamine, and number of degranulated MCs per field[7,57]. IBS-D patients with rectal hypersensitivity, according to the maximally tolerable pressure to barostat distention, showed significantly lower counts of MCs in the terminal ileum, ascending colon and rectum in one study[57]. Park et al speculated that this counterintuitive finding was related to tissue desensitization by MCs mediators[57]. More recently, Braak et al found no correlation between the sensory thresholds to barostat distention, abdominal pain, bloating, urgency, incomplete evacuation, hard stools, loose stools, frequent and decreased bowel moments and flatulence and MCs counts in the colon[51]. An association between duodenal and antral MCs with pain, and postprandial distress syndrome, respectively, has been shown in children with FD[67].

Impaired intestinal permeability, and the expression of TJ proteins has been shown to correlate with pain/discomfort and/or bowel habit[90,91,95,96]. Interestingly, tryptase mRNA and protein expression in the jejunum of IBS-D patients correlated with stool frequency and consistency but not with abdominal pain, whereas the correlation with MC number was poor[91].

Cecum MC counts correlated significantly with the fatigue and depression scores in IBS[60], and, in some studies, there is a tendency or an association between depression and state of anxiety scores and the number of MCs in patients with IBS-D[57]. A significant correlation with antral mast cell densities with anxiety, depression and somatization has been reported in children with FD[67]. Moreover, the degranulation of MCs in the duodenum appears to be highly sensitive and specific for the identification of adult FD patients as shown by 100% sensitivity and specificity indicated by an area under the ROC curve of 1.0 for the optimal degranulation rate cutoff values of 30.2% at the duodenal bulb and 36.8% at the descending part of the duodenum[66].

Taken together, these findings suggest that interactions between the MCs and the enteric and brain-gut neural networks could be of importance in symptom perception in at least a subgroup of patients with FGIDs.

Targeting MCs: implications for treatment of FGIDs

The MC stabilizer DSCG abolished the effect of acute psychological stress on small bowel permeability in human subjects[42]. Aside from experimental studies demonstrating the efficacy of several MC inhibitors to decrease colonic hypersensitivity, in humans, a number of uncontrolled observations[56], and open clinical studies with DSCG, in doses between 600-1800g/day, suggest its clinical benefit for chronic persistent diarrhea[97], allergic enteritis[98], FD[99], and IBS[100,101,102]. However, these studies had several limitations including poor design, small sample size, and selection bias. Likewise, ketotifen has been recently proven to increase the sensory threshold, leading to improved visceral perception, especially in the hypersensitivity IBS group[103]. Although preliminary, there is some indication of the clinical benefit of ketotifen and the tryptase inhibitor APC 2059 in ulcerative colitis[104,105]. Our group has recently finished an open trial (awaiting publication)

and a consecutive double-blind, placebo controlled, clinical assay, with prolonged (6 months) oral administration of DSCG, with promising results in the control of main clinical manifestations in IBS-D patients (Gastroenterology 2015;148 (Supl 1):S-494). In addition, small studies have shown improvement in gastrointestinal symptoms with DSCG therapy in systemic mastocytosis[106]. However, the mechanisms by which MC stabilization could interfere with IBS clinical response have not been clearly delineated.

Other interventions that block the effects of MC mediators and improve GI symptoms should be considered. In this sense, anti-inflammatory treatment with mesalazine appeared to show improvement in symptom perception in unselected IBS patients in a small proof-of-concept randomized, double-blind, placebo-controlled trial, in which, in addition, a 36% decrease in MC numbers, and a reduction of the number of total immune cells, and T cells was observed in the colonic mucosa[107]. However, two subsequent large clinical trials differ in the clinical benefit of mesalazine in IBS[108,109], and the effect of mesalazine on MC counts and degranulation not confirmed[109]. Furthermore, there was no effect of mesalazine on 5-HT containing enterochromaffin and CD68 cell numbers, although, there was significant increase in CD3 count in the mesalazine group[109]. In further reinforcing the role of MC activation in the origin of FGIDs manifestations, it is important to note that heartburn, cramping, nausea, abdominal pain and diarrhea are the second most common complaint of patients with mastocytosis, and that H2-histamine receptor antagonists have been quite effective in controlling these symptoms[56,98,110]. A recent proof-of-principle clinical trial confirmed the clinical relevance of these findings showing improvement of abdominal pain and global relief by the H1R antagonist ebastin in IBS patients (Gastroenterology 2013;144 (Supl 1):S-160). Palmitoylethanolamide and other inhibitors of cannabinoid receptors seem efficacious in controlling pain, motor disturbances and

inflammation in animal models through modulation of neuronal and non-neuronal cells, including MCs[111,112]. Slow-release of vitamin C may be also helpful as it increases degradation of histamine; and inhibits MC degranulation; in doses not superior to 750 mg/day[110]. Natural flavonoids (fisetin, kaempferol, quercetin, rutin, luteolin,..) and the active alkaloid berberine inhibit the mediator release of MCs in vitro[113] and protect intestinal epithelial barrier[114]. While some of these products have shown to be useful in cardiovascular health[115] and cancer[116], their clinical efficacy in FGIDs has not been established. There is some evidence of symptomatic response to specific diets in FGIDs, such as low FODMAP and gluten-depleted food[20]. However, there is no support for the role of MCs in this symptomatic response with the exception of the benefit after individualized exclusion of foods in FGIDs suffering food allergy.

Finally, the development of more specific and safe blockers or modulators of IgE, IgG or other activation pathways of MC activation, including pathways involved in the selective release of mediators, may offer therapeutic advantages, although their benefit remains to be established.

Conclusion

Current evidence implicating MCs in the pathogenesis and pathophysiology of FGIDs, particularly in IBS, and the contribution of their activation and released mediators to the development of cardinal manifestations, such as epigastric and abdominal pain, and altered defecation is robust, and supports the targeting of MCs in the management in FGIDs.

Key points

- Mast cells play a central pathophysiological role in IBS and possibly in FD, although not well defined.
- Increased mast cell activation is a common finding in the mucosa of patients with FGIDs.
- There is a need to implement standardized methods to count mast cells in the gastrointestinal mucosa, and to establish reference ranges of normality.
- Evaluation of spontaneous and stimulated mast cell function and activity on gastrointestinal samples is recommended when available.
- More studies are recommended to fully understand the implication of mast cells in the origin of clinical manifestations of these disorders, and to develop new management protocols.
- Treatment with mast cell stabilizers offers a reasonably safe and promising option for the management of those IBS patients non-responding to conventional approaches, though future studies are warranted to evaluate efficacy and indications.

Table 1. Triggers of mast cell activation (modified from[117])

Type of stimuli	Type of molecule	Molecule/stimuli
Immune	Immunoglobulins	IgE, IgG, free light chain-Ig (+antigen)
	Other	C3a, C5a, IL-4, IL-6, IL-9, IL-10, TNF-α, IFN-γ
Non-immune	Neurotransmitters	Acetylcholine, Dopamine, Serotonin, Epinephrine, Histamine
	Neuropeptides	SP, VIP, HRP, CGRP, SS, NT, Bradykinin
	Hormones	ACTH, CRF, PTH, Ucn, Estradiol
	Growth factors	NGF, SCF, TGF-β, FGF-2, VEGF, PD-ECGF
	Biological	LPS, Peptidoglican, Micobacterium
	Physico-chemical	NO, osmotic, thermal, pH, humidity, trauma, pressure, hypoxia, radiation, free radicals

Ig: immunoglobulin; IL: interleukin; C3a: complement component 3a; TNF-α: tumor necrosis factor alpha; IFN-γ: interferon gamma; SP: substance P; VIP: vasoactive intestinal peptide; HRP: histamine-releasing peptide; CGRP: calcitonin gene-related peptide; SS: somatostatin; NT: neurotensin; ACTH: adenocorticotropin hormone; CRF: corticotropin releasing factor; PTH: parathormone; Ucn: urocortin; NGF: nerve growth factor; SCF: stem cell factor; TGF-β: transforming growth factor beta; FGF-2: fibroblast growth factor-2; VEGF: vascular endothelial growth factor; PD-ECGF: platelet-derived endothelial cell growth factor; LPS: Lipopolysaccharide; NO: nitric oxide.

Table 2. Studies describing mast cell infiltration and activation in FD and IBS and potential correlation with symptoms.

Condition and number of subjects	Site of biopsy	Mast cell numbers	Mast cell detection	Mast cell mediators	Correlation with symptoms	Reference
141 FD and 39 controls	Duodenum	Increased counts	Toluidine blue staining	NA	NA	66
15 FD and 15 controls	Duodenum	Increased	Anti-tryptase	NA	NA	9
19 FD and 19 controls (pediatric)		No change	Anti-tryptase	NA	No correlation between permeability and mast cell density	118
65 H. PYLORI - negative FD (pediatric)	Gastric body and duodenum		Anti-tryptase	NA	Headache was associated with high mast cell counts in the gastric body and duodenum	65
51 FD, 20 IBS-D and 21 IBS-C and 48 controls	Duodenum	Increased counts in IBS-C and IBS-D and trend for increase in FD	Anti-CD-117	NA	NA	61
62 FD (33 Helicobacter pylori positive, 29 H. pylori negative and 29 H. pylori positive inflammatory control subjects and 20 controls,	Antrum and corpus	Increased in H. pylori negative and positive FD samples in antrum and corpus	Anti-tryptase	NA	NA	64
225 patients with non-ulcer dyspepsia	Antrum	31 (13%) were found to have 11 or greater mast cells per high-	Alcian blue staining	NA	NA	119

		power field				
Total of 101 IBS and 23 controls, for IHC: 15 controls; 15 IBS-C; 14 IBS-D	Descending colon	Increased counts	Anti-tryptase	NA	NA	77
13 IBS-D, 8 IBS-C and 10 controls (pediatric)	Ileum, right colon and left colon	No change in numbers but mast cells in closer proximity to nerves in IBS (MC-NF/mm2)	Anti-tryptase	NA	Abdominal pain correlated with MC/mm2 in the ileum and MC-NF/mm2 in the right colon	120
49 IBS-D and 30 controls	Jejunum	Increased counts in non-atopic IBS	Anti-CD-117	NA	NA	35
100 IBS and 100 controls	Colon, ileum, duodenum and stomach	No change	Anti-CD-117 and anti-CD-25	NA	NA	52
55 IBS-D and 18 controls with lactase deficiency	Sigmoid colon, ascending colon and terminal ileum	increased counts in the terminal ileum, ascending and sigmoid colon	Anti-tryptase	NA	Anxiety scores were associated with mast cell counts in sigmoid colon, ascending colon and terminal ileum. Visceral sensitivity (i.e. decrease in urgency, discomfort/pain threshold) was increased in patients with high mast cell density in the terminal ileum	121
22 IBS-D and 21 controls	Rectum	Increased counts in IBS-D	Anti-tryptase	NA	Mast cell counts did not correlate with	122

					IBS symptoms including abdominal pain; Mast cell counts correlated with substance P and VIP in women but not in men	
83 D-IBS, 49 UC (28 in remission and 21 mildly active UC), and 25 controls	Ascending, transverse, descending, and sigmoid colon	Increased counts in patients with D-IBS, UC in remission, and mildly active UC	Anti-tryptase	NA	NA	123
51 IBS, 49 quiescent IBD (31 CD and 18 UC) and 27 controls	Caecum	increased in patients with IBS, CD or UC (no difference between patients between patients with or without IBS-like symptoms	Anti-CD-117	NA	NA	124
16 IBS-D and 7 controls	Rectum	No change	Anti-tryptase	Increased tryptase release	Mast cell counts correlated with intestinal permeability	125
45 IBS-D and 30 controls	Jejunum	Increased counts	Anti-CD-117	Increased tryptase mRNA and protein	Tryptase mRNA expression but not mast cell counts correlated with stool frequency and consistency in IBS-D patients; tryptase protein expression correlated CLDN2 protein overexpression and increased	8

					OCLN cytoplasmic staining	
16 IBS-D, 21 C-IBS and 11 controls	Descending colon	Increased counts in IBS-C but not IBS-D	Anti-tryptase	NA	Mast cell counts of IBS but not controls correlated with the twitch enhancement evoked by biopsy supernatants	87
34 IBS and 15 controls	Rectum	Increased counts	Anti-CD-117	Increased tryptase release	IBS severity correlated with colonic permeability, mast cell counts and tryptase	95
4 IBS-C, 11 IBS-D, 8 IBS-A and 15 controls	Colorectum	No change	Anti-tryptase	NA	NA	126
11 IBS-D and 14 controls (pediatric)	Rectum	No change	Anti-tryptase and anti-CD-117	NA	NA	127
15 IBS-D, 15 IBS-C, 36 IBS-A and 20 controls	Descending colon	decreased mast cell counts	Anti-CD-117	NA	No correlation between the the number of mast cells and abdominal pain or sensory thresholds of first sensation, urge or discomfort and	51
25 IBS-D and 23 controls	Jejunum	Increased counts	Anti-CD-117	Increased tryptase mRNA	Tryptase and SCF correlated with tight junction ZO protein expression. Bowel frequency and	91

					stool consistency correlated with both the number of mast cells and tryptase mRNA expression, and with the expression of ZO proteins	
12 IBS-C, 13 IBS-D and 12 controls	Descending colon	Increased counts	Anti-tryptase	Increased serotonin, histamine and tryptase release irrespective of bowel habit	5-HT release correlated with mast cell counts and the severity of abdominal pain	128
60 IBS and 22 controls	Rectum and descending colon	decreased mast cell counts in rectal biopsies	Anti-tryptase and anti-CD117	Lower release of tryptase, slight increase in histamine release	The severity of abdominal pain was not correlated with mast cell counts; no correlation between abdominal pain and spontaneous histamine or tryptase release	103
13 IBS-D, 8 IBS-C, 4 IBS-A, 10 active CD and 18 controls	Descending colon	Increased counts in IBS-D but not in IBS-C	Anti-CD-117	Increased trypsin-like protein	NA	129
27 IBS-D, 21 IBS-C, 12 MC 20 UC, and 24 controls	Descending colon	Increased counts	Anti-tryptase	NA	Mast cell counts in IBS patients was associated with abdominal bloating frequency and with symptoms of dysmotility-like dyspepsia, but	74

					not ulcer-like dyspepsia	
7 IBS-D, 4 IBS-C and 4 controls	Descending colon	Increased counts	Anti-tryptase	Increased tryptase and histamine release but not serotonin	Association between the number of mast cells and the corresponding supernatant-evoked spike frequency. Tryptase, histamine and serotonin concentrations all correlated with the supernatant-evoked action potential discharges.	80
8 IBS-D, 8 IBS-C, 7 IBS-A and 22 controls	Rectosigmoid	Increased counts	Anti-CD-117	NA	c-kit^{+} cells correlated with maximal VAS pain score	28
50 IBS, 21 controls, 11 depressed/fatigued patients without IBS	Caecum	Increased in IBS, unchanged in depressed/fatigued patients w/o IBS	Anti-CD-117	NA	In IBS, but not in controls or depressed patients, mast cell counts correlated with the severity of fatigue and depression	60
29 IBS and 15 controls	Descending colon	Increased counts	Anti-tryptase	Increased tryptase, histamine and PGE2 release	NA	78
18 IBS and 12 controls and 4 UC and 1 CD	Rectum and ascending colon	No change	Alcian blue staining and anti-tryptase	Increased trypsin and tryptase protein	NA	79
20 IBS-D and 14 controls	Jejunum	Increased counts	CD-117	Increased tryptase release	No correlation between mast cell counts	62

					and gender or stress levels	
20 IBS-D, 18 IBS-C, and 20 controls	Descending part of the duodenum, proximal end of jejunum and terminal ileum	Increased counts in IBS-C and IBS-D in ileum but not duodenum or jejeunum	Anti-tryptase	Decreased 5-HT contents at the jejunum in IBS-C patients	NA	130
18 IBS-D and 15 controls	Terminal ileum, ascending colon and rectum	Increased in the terminal ileum, ascending colon and rectum	Anti-tryptase Electron microscopy	NA	Activated mast cells were significantly closer to the nerves in IBS No correlation between mast cell counts and abdominal pain, urgency, depression scores and STAI-S/T; The increase in mucosal mast cell count in the terminal ileum was significantly associated with that in the ascending colon and rectum	57
44 IBS and 22 controls	Descending colon	Increased counts	Anti-tryptase	Increased tryptase and histamine release	Vicinity of mast cells to nerves correlated with both severity and frequency of abdominal pain/discomfort	7
28 PI-IBS, 28 patient controls and 34 healthy volunteers	Rectum	No change	Anti-tryptase	NA	NA	63

42 IBS-D, 11 IBS-C, 20 IBS-A, 4 unknown IBS subtype and 28 controls	Ascending colon, transverse colon, descending colon, and rectum	No change	Anti-tryptase	NA	NA	48
10 IBS and 15 controls	Jejunum (full thickness)	No change	Giemsa staining	NA	NA	[131]
21 PI-IBS and 12 controls	Rectum	No change	Anti-tryptase	NA	NA	[132]
14 IBS, 7 normal controls and 7 inflammatory controls	Caecum, ascending colon, descending colon and rectum	Increased numbers in caecum but not at other sites	Anti-tryptase	NA	NA	50

FD: functional dyspepsia, IBS: irritable bowel syndrome, IBD: inflammatory bowel disease, UC: ulcerative colitis, CD: Crohn's Disease, MC: microscopic colitis, VIP: Vasoactive intestinal peptide, NA: Not assessed

Table 3. Conditions that may alter, commonly increase, the number of mast cell counts in the gastrointestinal tract

Allergic diseases: chronic urticaria, food allergy, atopy, hereditary angioedema[11,98]

Mastocytosis and mast cell activation syndrome[52]

Celiac disease: increased in initial stages and decreased in later stages[133]

Neuroendocrine cancer, lymphoma, epithelial cancers, carcinoid syndrome

H. Pylori gastritis, infectious and parasitic enteritis,

Inflammatory bowel disease[134], lymphocytic colitis[48]

Intestinal pseudo-obstruction, diverticulitis[135]

Vasculitis, amiloidosis, drugs

Table 4. Effect of mast cell mediators on gastrointestinal function

Mediator	Receptor	IBS/cell type	Effect	Referenc
Histamine	H1R	IBS-C; IBS-D	Excite rat mesenteric afferents	78
			Excite murine DRG neurons	78
	H1-H3R	IBS-C; IBS-D	Excite human submucosal neurons	80
	-	-	Epithelial secretion of Cl^- and H_2O	136
Tryptase	PAR2	IBS-C; IBS-D	Sensitize/activate murine DRG	78
		IBS-C; IBS-D	Excite human submucosal neurons	80
		IBS-D but not	Sensitization murine colonic DRG	88
		IBS-D, IBS-A	Increase epithelial permeability	137
Serotonin	5HT3R	IBS-C; IBS-D	Excite human submucosal neurons	80
		T84 cells	Secretory response	138
PGD2	DP1	IBS-C and IBS-D	Excite guinea pig longitudina	87
	-	-	Epithelial secretion of Cl^- and H_2O	136
Chymase	PAR2	Caco BBe	Increase epithelial permeability	94

IBS-C: constipation-predominant irritable bowel syndrome; IBS-D: diarrhea-predominant IBS; IBS-A: alternating subtype of IBS; H1R: Histamine receptor 1; PAR2: proteinase-activated receptor 2; 5HT3R: 5-hydroxytriptamine receptor 3; PGD2: prostaglandin D2; DP1: PGD2 receptor;

Figure legends

Figure 1. Ultrastructure of human mucosal mast cell. (**A**) Ultrastructure of an activated mast cell in the intestinal mucosa, with irregular plasma membrane and numerous lipid bodies (arrow) and cytoplasmic granules, displaying piecemeal degranulation. Intact (white arrowhead) and degranulated (black arrowhead) granules are identified. (**B,C**) High-magnification micrographs of cytoplasmic granules from a mucosal mast cell. Different granule patterns are observed, with crystalloid structure (B) and scrolls (arrow, C). Enlarged empty and partially empty granule containers (black arrowhead) are typical of piecemeal degranulation. Bars: 1µm (A) and 0.5µm (B, C).

Figure 2. Schematic representation of the experimental procedure to assess mucosal mast cell activation. Mast cell activation can be measured in intestinal samples. Luminal content can be obtained by aspiration, before biopsies are collected, and tryptase content can be quantified. Different mucosal biopsies can be processed for: histological examination, including mast cell counting after immunohistochemistry (tryptase and/or c-kit staining) and laser microdisection for ulterior gene expression analysis; ultrastructure analysis, to assess the type and degree of degranulation and to identify granule pattern; gene expression analysis of specific mediators synthesized and released by mast cells (tryptase, carboxypeptidase, chymase); quantification of mediators that are spontaneously released from biopsies and/or performing functional studies in vitro (muscle/nervous cells) or in vivo (mice/rats); and electrophysiology experiments in Ussing chambers for identification of mast cell-dependent changes in barrier function. Finally, analysis of the possible association between clinical manifestations and mast cell activation can be performed.

Figure 3. Schematic illustration of mast cell-nerve interactions in human gut.

MCs and nerves communicate bidirectionally, thereby modulating peristalsis and pain signalling. The release of bioactive, pro-inflammatory, mediators by mast cells results in a variety of neuronal effects including activation, sensitization and recruitment of nociceptors to the cell membrane, neurogenic inflammation and neural sprouting, ultimately leading to visceral hypersensitivity. On the other hand, neuronal activation triggers the release of neuropeptides and neurotransmitters, thereby further activating mast cells.

Ig, immunoglobulins; TLR, toll-like receptor; NK1, neurokinin 1 receptor, PGs, prostaglandins; NGF, neuronal growth factor, H1R, histamine receptor 1; TRPV1, transient receptor potential vanilloid 1; 5-HT3, 5-hydroxytryptamine receptor 3; PAR2, proteinase-activated receptor-2; TrkA, receptor for nerve growth factor; SP, substance P; CRGP, calcitonin-related gene peptide.

Figure 4. Intestinal barrier function elements and mast cell interactions in the intestinal mucosa. Illustration of the potential mast cell interactions in the regulation of barrier function, including epithelial permeability (through TJ modulation and secretory response), recruitment and activation of other immunocytes, endothelial functions (vascular permeability and blood flow), peristalsis, and pain signalling through bidirectional communication with the nervous system. TJ, tight junction; AJ, adherens junction; D, desmosome; PAR2, proteinase-activated receptor-2; $5HT_3R$, 5-hydroxytryptamine receptor; TNFα, tumor necrosis factor alpha; ILs, interleukins; SCF, stem cell factor; GM-CSF, granulocyte and monocyte colony stimulating factor; IFNγ, interferon gamma; Igs, immunoglobulins; LT, leukotrienes; PGD2, prostaglandin D-2; CRF, corticotropin-releasing factor; CRFR1/2, CRF receptors 1 and 2; IgE, Immunoglobulin E; IgG,

Immunoglobulin G; IgLC, immunoglobulin free-light chains; TLR, toll-like receptor; CNS, central nervous system; ENS, enteric nervous system.

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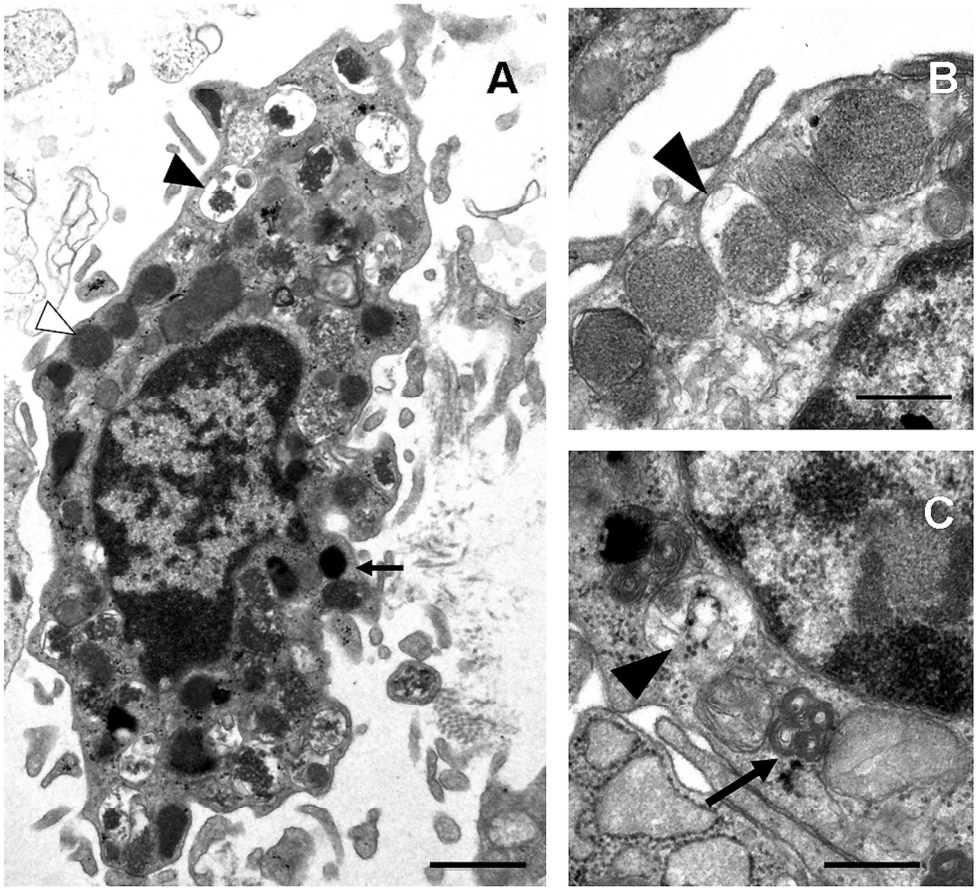

Figure 1. Ultrastructure of human mucosal mast cell. (A) Ultrastructure of an activated mast cell in the intestinal mucosa, with irregular plasma membrane and numerous lipid bodies (arrow) and cytoplasmic granules, displaying piecemeal degranulation. Intact (white arrowhead) and degranulated (black arrowhead) granules are identified. (B,C) High-magnification micrographs of cytoplasmic granules from a mucosal mast cell. Different granule patterns are observed, with crystalloid structure (B) and scrolls (arrow, C). Enlarged empty and partially empty granule containers (black arrowhead) are typical of piecemeal degranulation. Bars: 1µm (A) and 0.5µm (B, C).
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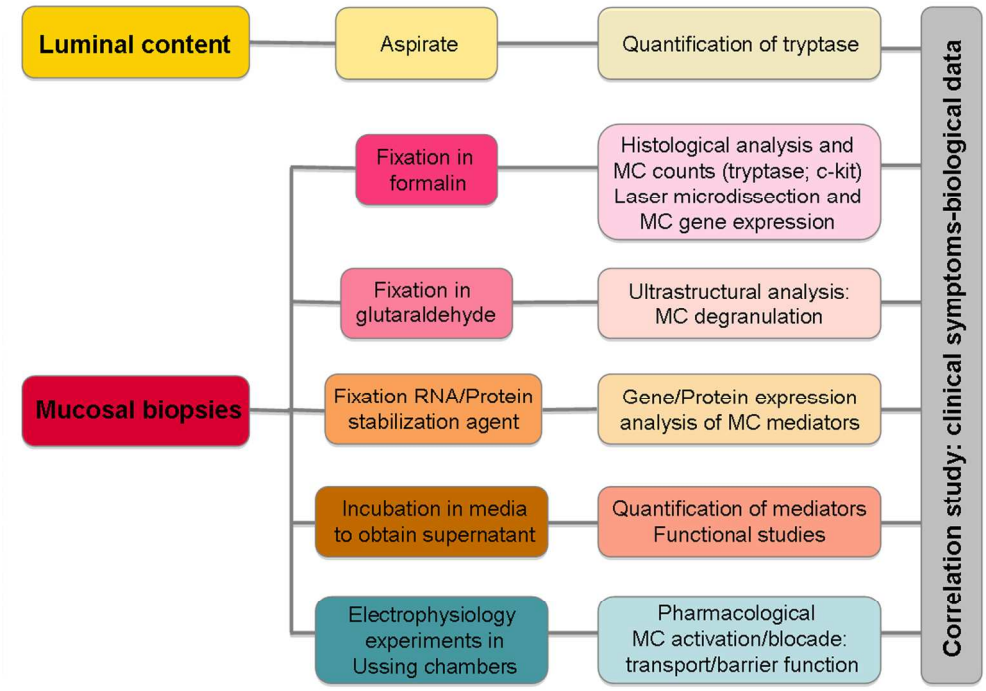

Figure 2. Schematic representation of the experimental procedure to assess mucosal mast cell activation. Mast cell activation can be measured in intestinal samples. Luminal content can be obtained by aspiration, before biopsies are collected, and tryptase content can be quantified. Different mucosal biopsies can be processed for: histological examination, including mast cell counting after immunohistochemistry (tryptase and/or c-kit staining) and laser microdisection for ulterior gene expression analysis; ultrastructure analysis, to assess the type and degree of degranulation and to identify granule pattern; gene expression analysis of specific mediators synthesized and released by mast cells (tryptase, carboxypeptidase, chymase); quantification of mediators that are spontaneously released from biopsies and/or performing functional studies in vitro (muscle/nervous cells) or in vivo (mice/rats); and electrophysiology experiments in Ussing chambers for identification of mast cell-dependent changes in barrier function. Finally, analysis of the possible association between clinical manifestations and mast cell activation can be performed.
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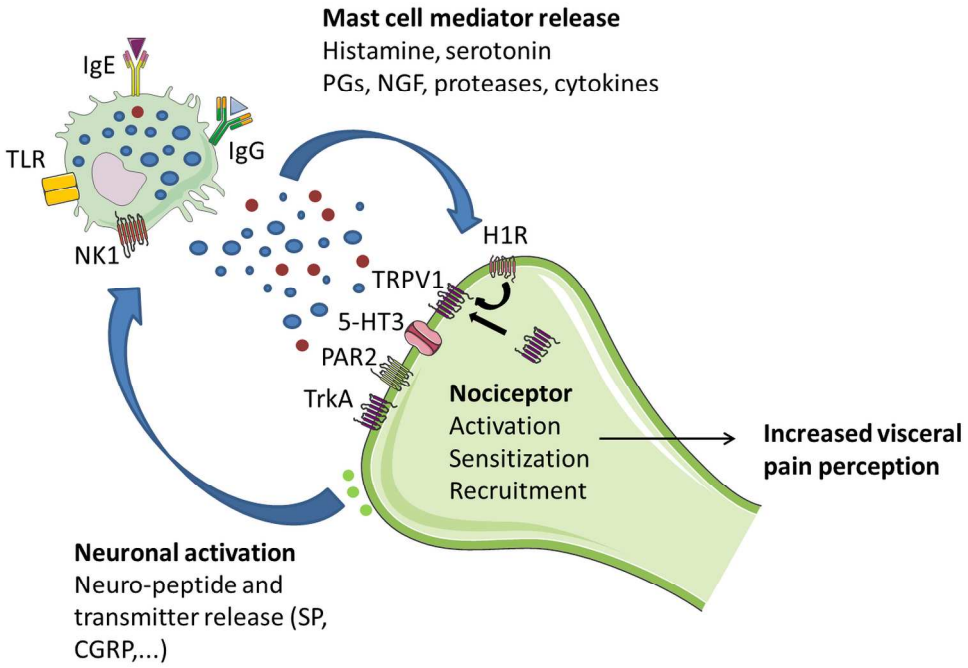

Figure 3. Schematic illustration of mast cell-nerve interactions in human gut.
MCs and nerves communicate bidirectionally, thereby modulating peristalsis and pain signalling. The release of bioactive, pro-inflammatory, mediators by mast cells results in a variety of neuronal effects including activation, sensitization and recruitment of nociceptors to the cell membrane, neurogenic inflammation and neural sprouting, ultimately leading to visceral hypersensitivity. On the other hand, neuronal activation triggers the release of neuropeptides and neurotransmitters, thereby further activating mast cells.
Ig, immunoglobulins; TLR, toll-like receptor; NK1, neurokinin 1 receptor, PGs, prostaglandins; NGF, neuronal growth factor, H1R, histamine receptor 1; TRPV1, transient receptor potential vanilloid 1; 5-HT3, 5-hydroxytryptamine receptor 3; PAR2, proteinase-activated receptor-2; TrkA, receptor for nerve growth factor; SP, substance P; CRGP, calcitonin-related gene peptide.

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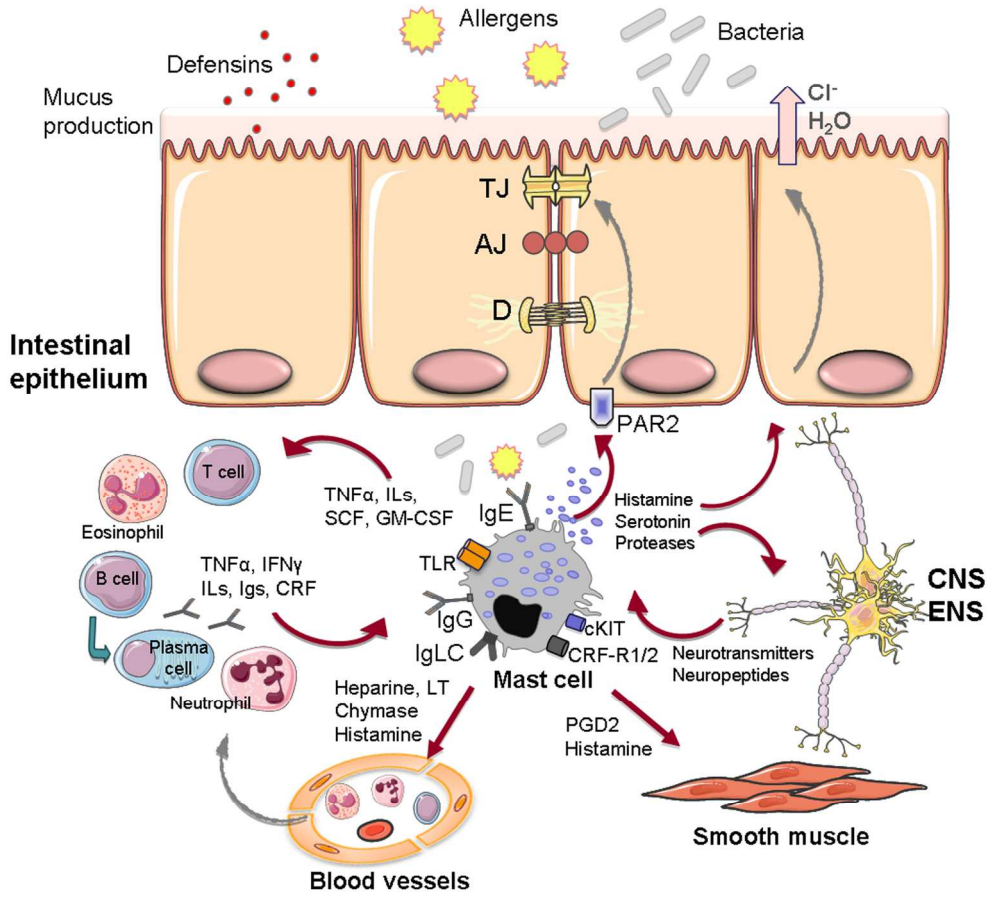

Figure 4. Intestinal barrier function elements and mast cell interactions in the intestinal mucosa. Illustration of the potential mast cell interactions in the regulation of barrier function, including epithelial permeability (through TJ modulation and secretory response), recruitment and activation of other immunocytes, endothelial functions (vascular permeability and blood flow), peristalsis, and pain signalling through bidirectional communication with the nervous system. TJ, tight junction; AJ, adherens junction; D, desmosome; PAR2, proteinase-activated receptor-2; 5HT3R, 5-hydroxytryptamine receptor; TNFα, tumor necrosis factor alpha; ILs, interleukins; SCF, stem cell factor; GM-CSF, granulocyte and monocyte colony stimulating factor; IFNγ, interferon gamma; Igs, immunoglobulins; LT, leukotrienes; PGD2, prostaglandin D-2; CRF, corticotropin-releasing factor; CRFR1/2, CRF receptors 1 and 2; IgE, Immunoglobulin E; IgG, Immunoglobulin G; IgLC, immunoglobulin free-light chains; TLR, toll-like receptor; CNS, central nervous system; ENS, enteric nervous system.
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