Intestinal mast cells in gut inflammation and motility disturbances

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1. Mast cells and gut functions: scope of this review

Inflammation of the gut wall as observed in postoperative ileus (POI) or in inflammatory bowel diseases (IBD) can involve a complex interplay between neurons, smooth muscle cells, interstitial cells of Cajal, enteric glial cells, vascular tissue, mucosal epithelial cells, mast cells, enteroendocrine cells and immunocytes [11,21,77,86]. In this process mast cells are proposed to act as sentinels at the host–environment interface, responding to allergens, bacteria, toxins, parasites, neuropeptides and stress by initiating enhanced epithelial secretion, peristalsis and alarm programs by releasing proinflammatory mediators [3,8,9,60]. Mast cells in the gut can be sensitized against foreign antigens but also play an important role in the innate and adaptive immune responses that are very relevant to human disease, such as in oral vaccination strategies, or snake and honeybee responses [59]. Other more specific examples thereof are POI and IBD, which are described in the current review. Elsewhere in this special issue a critical role for mast cell activation in for instance the pathogenesis of functional GI disorders such as IBS and eosinophilic esophagitis is highlighted.

2. Mast cells in the gastrointestinal tract

Progenitor mast cells are derived from myeloid pluripotent hematopoietic progenitor cells in bone marrow; they circulate in the blood flow and migrate into peripheral tissues where they further differentiate towards different subtypes of mature mast cells depending on the local microenvironment [67,86]. Mast cells are derived from hematopoietic Thy1+cKit+high mast cell-committed precursors [68], and their growth and proliferation is regulated by growth factors such as the cKit ligand stem cell factor (SCF), nerve growth factor (NGF) and interleukins (IL3, IL4, IL9, and IL10) [36]. There are large species differences in mast cell distribution as well as density, where especially the mouse GI tract is generally low in intestinal mast cells. In other rodents, mast cells can be found in the lamina propria of the intestine, but also associated with the epithelium, the submuccosa and the serosa, where subsets of mast cells exist divided in different classes such as connective tissue mast cells, located mainly in connective tissue around blood vessels and in the peritoneal cavity and mucosal mast cells found in the intestinal mucosa. Important differences exist in these subpopulations of mast cells for instance regarding their activation and responses to basic secretagogues, such as compound 48/80 and bee venom peptide 401 [5,6,27]. Specific differences in mast cell subpopulations are highlighted more extensively in this review series and elsewhere, i.e. see [36] and for recent reviews [35] and [9] amongst others.

In humans, mast cell contents are used to describe different classes: tryptase and chymase containing mast cells (closely resemble
connective tissue mast cells) and only tryptase containing mast cells (closely resembling the rodent mucosal mast cells) [9,10,67]. Mast cell regulators such as SCF and IL4 promote mast cell development and/or regulate mediator release [9]. Mast cells can be activated via the classical IgE-mediated pathway but can also be activated by a variety of substances such as cytokines, hormones, immunoglobulins, neuropeptides, and complement components. TLR triggering also activates mast cells and activates cytokine production most likely via mechanisms that require new protein synthesis [87]. Hence, functional activation of mast cells leads to degranulation of their mediators preformed and stored in secretory granules or to de novo synthesis of mediators. Piecemeal degranulation is an alternative form of secretion involving the selective secretion of certain mediators (and therefore not the whole content) of the secretory granules. The preformed mediators include tryptase, histamine, serotonin (5-hydroxytryptamine), serine proteases, proteoglycans and cytokines such as tumor necrosis factor α (TNFα). Arachidonic acid metabolites (prostaglandins and leukotrienes), platelet activating factor and several chemokines and cytokines (IL-1β) and IL-6 can be synthesized de novo. Cytokines also change the cytokine profile released by mast cells: IL4 decreases the amount of proinflammatory cytokines and increases the release of Th2 cytokines [35,89].

3. Neurogenic control of mast cells in the GI tract

Mast cells function as intermediaries between the inflammatory cells and their mediators and the neuroenteric system. Both cell types are affected by the inflammatory environment and upon activation release mediators which affect the gastrointestinal neuromuscular and secretory functions [7,8,15,75,78]. These mediators can stimulate epithelial cells, residential macrophages and intrinsic and extrinsic neurons amongst others. The close morphological relationship between mast cells and afferent nerve endings both in human and animal studies supports the latter notion [78]. There is early evidence for bidirectional communication between mast cells and neurons in the gastrointestinal tract and the mast cell may be regarded as the classical immune cell that is activated by neuronal factors and neurotransmitters. MacQueen et al. demonstrated in 1989 that rats sensitized to a protein antigen in combination to an audiovisual cue, and then re-exposed to an audiovisual cue alone released a quantity of protease that was not significantly different from animals re-exposed to both the cue and the antigen [54]. These results support a role for the central nervous system as a functional effector of mast cell function [7,19,75]. In conjunction, a positive feedback loop is described during which mast cell mediators activate nerves that on their turn release neurotransmitters able to activate nerves that on their turn release neurotransmitters affecting the gastrointestinal neuromuscular and secretory functions [7,8,15,75,78]. These effects might influence the secretory response for instance in allergic or inflammatory conditions. Together with the close morphological relationship between mast cells and their mediators and the neuroenteric system. Both cell types are affected by the inflammatory environment and upon activation release mediators which affect the gastrointestinal neuromuscular and secretory functions [7,8,15,75,78]. These mediators can stimulate epithelial cells, residential macrophages and intrinsic and extrinsic neurons amongst others. The close morphological relationship between mast cells and afferent nerve endings both in human and animal studies supports the latter notion [78]. There is early evidence for bidirectional communication between mast cells and neurons in the gastrointestinal tract and the mast cell may be regarded as the classical immune cell that is activated by neuronal factors and neurotransmitters. MacQueen et al. demonstrated in 1989 that rats sensitized to a protein antigen in combination to an audiovisual cue, and then re-exposed to an audiovisual cue alone released a quantity of protease that was not significantly different from animals re-exposed to both the cue and the antigen [54]. These results support a role for the central nervous system as a functional effector of mast cell function [7,19,75]. In conjunction, a positive feedback loop is described during which mast cell mediators activate nerves that on their turn release neurotransmitters able to activate nerves that on their turn release neurotransmitters affecting the gastrointestinal neuromuscular and secretory functions [7,8,15,75,78]. These effects might influence the secretory response for instance in allergic or inflammatory conditions.

4. The clinical features of postoperative ileus

Postoperative ileus (POI) is an almost inevitable phenomenon occurring after each abdominal surgical procedure that includes bowel manipulation, although POI may sometimes also be associated with extra-abdominal operations. It clinically presents as the inability to tolerate food with abdominal distension, absence of bowel sounds and lack of flatus and defecation. Nausea and vomiting, pain and postoperative fatigue further contribute to the morbidity and prolonged hospitalization of patients. On average, this period lasts 2–4 days for conventional abdominal procedures, but decreases to as little as 2 days or less in case of laparoscopic surgery [22]. Some surgeons consider the inability to tolerate food and absence of bowel sounds during the first few postoperative days as a normal phenomenon, and only consider “prolonged” or “pathologic paralytic ileus,” which lasts more than 3 days after surgery, as clinically relevant. Others propose to prolong this period to more than 6 days [1]. Transient inhibition of gastrointestinal motility is well documented as an underlying mechanism and involves the entire gastrointestinal tract. It is established that not all segments are equally affected; small intestinal motility is on average disturbed for approximately 24 h, gastric motility between 24 and 48 h, whereas colonic motility is impaired between 48 and 72 h (reviewed for instance in [21,22,46]). It should be emphasized though that normalization of motility, for example the return of the migrating motor complex in the small intestine, does not necessarily imply that normal function and transit have returned. Nevertheless, these studies underscore that colonic motility is the main determinant of clinical recovery.

5. The pathophysiology of POI

Over the past decade, our insight in the pathophysiology has increased exponentially. The general paralysis of the entire GI tract, including the un-manipulated segments, is a commonly seen characteristic of POI. This clinically important aspect of POI involves the activation of an inhibitory neural reflex arch by local inflammatory infiltrates [18], and was recently also shown to involve the production of IFNγ by CD8+ T-cells that are activated at the site of manipulation [28]. Although a role of gut-homing inflammatory cells triggered by handling of the intestine is now put forward as the key event in the widespread inflammatory response seen after local intestinal manipulation in POI, see for instance [28], insight in the bidirectional interaction between the immune system (mast cells, macrophages and other leukocytes) and the autonomic nervous system (afferents and efferents) has significantly contributed to a better understanding of its pathophysiology [4,11]. Moreover, it has become clear that inflammatory mediators released by leukocytes within the gut wall also directly impair smooth muscle contractility [4,48,83].

The intestinal mucosa, submucosa, and muscularis externa are densely populated with several subsets of resident phagocytes and antigen presenting cells (APCs) of haematopoietic origin [32]. Under healthy conditions, such resident macrophages are organized into a network of intramuscular antigen presenting macrophage-like cells, that reside at the level of the myenteric plexus (between the longitudinal and circular muscle layer) and in the intestinal serosa [60,61]. Most of these cells possess phagocytic properties, express LPS binding receptor CD14 [32], express TLRs and are activated by LPS [11,21,29,32,61]. The latter distinguishes these muscularis phagocytes from those found in the lamina propria that are—at least in human—generally negative for CD14 and most TLRs and display a surprising tolerance towards TLR ligation [76]. Moreover, muscularis macrophages stain for macrophage scavenger receptor CD163, that has been shown to possess bacteria binding and sensing capacities [31]. Thus, this phagocyte population in the muscularis externa has an interesting nature and most likely consists of different subsets of APCs, including macrophage-like cells expressing F4/80 and dendritic cell-like cells expressing most common DC markers such as CD11c and DEC205 [32]. However in mouse bowel wall, MHCII-positive cells outnumber F4/80- cells indicating that the majority of these resident muscularis macrophages function as APC. Hence, the exact cellular constituent of the phagocyte population is yet to be defined but their importance in the development of the intestinal inflammation following intestinal manipulation was first demonstrated in earlier studies done by Kalff et al. [43,44]. In a rodent model, surgical manipulation caused an increased expression of intergrins on muscularis macrophages, as well as an increase in resident phagocytes that stained for the activation
The intestine is also demonstrated in human tissue. Intestinal tissue removed during surgery shows activation of resident macrophages and time-dependent induction of IL6, IL1β, TNFα, iNOS, COX-2, ICAM-1 and LFA-1 [43–45,80]. In line with this, increased levels of the cytokines TNFα, IL6, IL8 and IL10 have been documented in the peritoneal fluid and blood of patients undergoing abdominal surgery [80,81,85]. In conjunction, influx of leukocytes was clearly demonstrated in intestinal tissue removed at the end of the surgical procedure and in tissue obtained from re-operated patients [80,81]. In addition, in vivo recruitment of radio-labelled leukocytes to the intestine was demonstrated in patients undergoing conventional abdominal surgery, but not in patients undergoing a laparoscopic procedure [80].

The importance of mast cells in the pathogenesis of POI could be verified in human studies as well. Mast cell mediators are detected in peritoneal lavage fluid very early during surgery that involved bowel inspection and handling. Intestinal manipulation during abdominal hysterectomy resulted in an immediate release of tryptase in the peritoneal fluid followed by an increase of IL6 and IL8 [80]. The degree of intestinal handling correlated with the degree of mast cell activation and the subsequent inflammatory response. Even gentle inspection of the intestines at the beginning of the abdominal procedure increased the level of peritoneal mast cell-derived tryptase in this study [80,81]. Similar data were obtained by others that showed that intestinal handling triggered mast cell activation [80] as well as leukocyte infiltration in large bowel resection material [45].

Stabilization of mast cells has been proven successful in our mouse model of POI [17]. Pre-treatment with the mast cell stabilizers ketotifen and doxantrazole significantly reduced the release of mast cell mediators in the peritoneal cavity, impaired the inflammatory response to intestinal handling and prevented POI. Based on these findings, we conducted a dose-finding pilot study on 60 patients undergoing major abdominal surgery for gynaecological malignancy with standardized anaesthesia, randomized to oral treatment with ketotifen (4 or 12 mg) or placebo [81]. Gastric retention 1 h after liquid intake was significantly reduced by the highest dose compared to placebo. Abdominal cramps improved significantly in patients treated with 12 mg ketotifen, whereas other clinical parameters were unaffected. This study indirectly supports the mast cell-inflammation cascade as key event in postoperative ileus, providing proof-of-concept for further investigation of mast cell stabilizers as a therapeutic approach for postoperative ileus.

8. Mast cells affecting barrier function in POI

Intestinal manipulation induces barrier dysfunction that is likely to perpetuate the inflammatory response, acting via a mechanism that is likely to be dependent on mast cells. In patients, barrier dysfunction frequently occurs during abdominal surgery and has been associated with increased postoperative septic morbidity in surgical patients undergoing laparotomy [52,53,63,66]. In addition to a key role for mast cells in intestinal manipulation (IM) induced inflammation in this model of POI [17], as well as in humans [80,81], mast cell activation has been associated with disturbed intestinal barrier function in several disease entities such as stress-induced hypersensitivity of the bowel [69] and endotoxemia [62]. Hence, mast cell activation may be a contributing factor in the pathogenesis of POI by inducing barrier disturbances after IM. Mast cells degranulate within seconds to a few minutes upon chemical physical and pathogenic stimuli. The quick release of mediators is responsible for a rapid loss of barrier function in in vivo models of helminth infection [56], stress [69] and endotoxemia [23].

The exact mechanistic role mast cells have in POI pathogenesis remains to be demonstrated but in the above mentioned models, the release of serine proteases, including tryptase, following triggering of mast cells is responsible for an increase in epithelial permeability,
As such, mast cell activation could represent transportation to the lymph nodes via the lymphatics and travel back to the intestinal wall, they are phagocytosed by the resident macrophages or through the mucosa into the intestinal wall. Once the beads enter the intestinal wall, often in pairs or threes, and particularly closely associated with afferent nerve fibers (~25 μm) [13]. Mast cells are vital for the recruitment of neutrophils and the elimination of bacteria from the peritoneal cavity, which is exemplified by the fact that mast cell deficient mice show a significantly increased mortality and impaired bacterial clearance in a model of acute septic peritonitis [24].

As indicated, the exact nature of the mast cell mediator that affects barrier function in POI remains to be elucidated, but it likely involves similar rapid mechanisms and neuromediator release. Neuropeptides such as substance P or CGRP released from activated afferent nerves could be involved and once activated, vasoactive and proinflammatory substances such as histamine, and proteases are released by mast cells in the peritoneal cavity. As described above, both in rodents and human, these agents can indeed be detected in the peritoneal fluid immediately after intestinal manipulation. Other mediators, such as IL1β, are presently considered as candidate mast cell-derived cytokines involved in the pathogenesis of POI. Given the anatomical location of mesenteric mast cells, i.e. adjacent to the mesenteric blood vessels where these enter the intestinal wall [13], mast cell mediators will easily diffuse into the mesenteric blood vessels. Despite the involvement of T cell responses in POI [28], mast cell activity could explain the diffuse increase in mucosal permeability observed after intestinal manipulation [84]. When fluorescent LPS and fluorescent microbeads are introduced into the intestine prior to surgery, intestinal handling results in translocation of fluorescent material through the mucosa into the intestinal wall. Once the beads enter the intestinal wall, they are phagocytosed by the resident macrophages or transported to the lymph nodes via the lymphatics and travel back to the gut tissue [71,72,84]. As such, mast cell activation could represent a key event that triggers the next stage of the inflammatory cascade, i.e. activation of muscularis APCs and the subsequent widespread inflammatory response [28]. Mast cells therefore should be considered as sentinels of the peritoneal cavity providing protection against potential threats.

**9. The option of mast cells inhibition measured against current treatment of human POI**

The preventive techniques and treatment of POI are reviewed elsewhere [46]. Important to stress however is that for new drugs to enter the clinic, they will have to prove their clinical benefit against or in combination with the current new and exciting initiatives in peri-operative patient care. In particular the fast track program, a multimodal approach for patients undergoing colonic surgery, has proven to significantly reduce the rate of peri-operative morbidity, hospital stay and costs [46]. In this program, several peri-operative measures, i.e. restricted fluid management, optimised analgesia, forced patient mobilisation and early oral feeding, are introduced into patient management with impressive results. Most likely fluid restriction and an effective epidural analgesia are the key factors determining the outcome. To what extent a similar improvement is achieved in other types of surgery and whether the fast track program can easily be implemented in a general surgical ward remains to be awaited. Hence, it should be noted that that postsurgical recovery can be significantly improved with relatively simple and cheap measures rather than pharmacological intervention.

**10. The role of mast cells in GI disease**

**10.1. Inflammatory bowel diseases**

Inflammatory bowel diseases (IBD) includes ulcerative colitis (UC), Crohn's disease (CD) and indeterminate colitis. It is generally assumed that IBD results from an uncontrolled immune response in genetically predisposed subjects towards a normal microbial gut flora [64,91]. There is substantial evidence for the involvement of mast cells and a mast cell-mediated neuroimmune interaction during IBD as demonstrated both by experimental animal studies and human data a.o.
Mast cells

Activation triggers:
neuromediators,
inflammatory mediators and cytokines

Release via granules

- Proteases
- Histamine
- Cytokines
  (i.e. IL-1β, TNFα)

De novo synthesis

Concerning evidence for mast cells in human IBD, data are reviewed the last years [19,38,67], showing an increased number of mast cells in the colorectal mucosa, in the lamina propria and in the submucosa from patients with CD and UC. Next, not only the mast cell content but also the degranulation of mast cells was markedly altered in the mucosa of IBD patients as evidenced by an increased expression of TTNFα, IL6, substance P and elevated histamine, prostaglandins, leukotrienes and tryptase levels [2,6,70]. Together, these studies suggest that mast cells are involved in chronic inflammation of the gut. Also in IBD, the bidirectional communication between mast cells and nerves stands as mediators from both cell types are capable of stimulating or inhibiting each other’s function. Mast cell and neuronal mediators can increase vascular permeability, influx of inflammatory cells, gut motility and hyperalgesia [67]. Nevertheless, direct evidence for the interaction between mast cells and nerves is limited in the pathogenesis of IBD as it is the case for ileus. Limitations are the difficulty of studying this interaction directly in human colon and the search for the first trigger for activation of mast cells or nerves also remains a key question.

The therapeutic potential of mast cell stabilizers in the treatment of IBD merits further investigations. This potential is supported by the following evidence. On the one hand some of the drugs currently used in the treatment of IBD possess properties affecting mast cell activities such as 5-aminosalicylic acid, steroid therapy and methotrexate as evidenced by in vitro research [38]. 5-Aminosalicylic acid inhibits histamine and prostaglandin D2 release in stimulated intestinal mast cells in vitro [33]. And it has been known for decades that corticosteroids reduce the number of mast cells in rectal biopsies of IBD patients treated with corticosteroids independent of the degree of inflammation [37], whereas dexamethasone was shown to affect the growth and differentiation of bone marrow derived mucosal mast cells in vitro [57].

On the other hand some preliminary data on ketotifen in the treatment of IBD are available [38]. Marshall and Irvine describe 3 case reports with a benefit from ketotifen in the treatment of respectively a patient with Crohn’s disease, colitis ulcerosa and collagenous colitis [55], whereas Jones et al. report the beneficial effect of ketotifen in 5 out of 10 children with mild to moderate ulcerative colitis [41]. Although this evidence is rather anecdotal, a more recent open-label phase II multicentre pilot study in 56 patients with mild to moderate active ulcerative colitis showed a beneficial effect of APC 2059, a
trypstatine inhibitor, as evidenced by an improvement or normalization of the disease activity index in more than half of the patients with continuing symptoms despite oral 5-aminosalicylate therapy [82]. The trypstatine inhibitor proved to be safe with as much common adverse events reported in more than 10% of the patients: headache (19.6%), injection site erythema (12.5%), and injection site burning (10.7%) [82].

11. Concluding remarks

Both the experimental data and the clinical data support further research on mast cell-related therapy either by stabilizing the mast cells or by blocking specific mast cell mediators as adjunctive therapy in IBD or motility disturbances associated with intestinal inflammation. In accepting that the “inflammatory” or “secondary prolonged” phase of POI is clinically most relevant, therapy should preferentially aim to prevent or reduce the inflammatory response to intestinal handling. Needless to stress though that interference with the immune response—or mast cell stabilization—may have devastating effects on the first defense against bacterial infection and perhaps even more importantly on wound healing. The latter is of clinical importance as increased risk on anastomotic leak is the most feared consequence of any immune modulating therapy. Even if drugs prove to be safe, ideally, handling of the intestine should be prevented or minimized, most likely explaining the shortened POI reported after minimal invasive or laparoscopic procedures. Moreover, one would rather like to prevent than to treat inflammation, again provided that treatment does not interfere with the healing process or does not lead to an increased risk of infectious complications. Interference early in the inflammatory cascade may also be more effective compared to drugs administered at a later stage when inflammation is well established and a variety of inflammatory mediators are released. Therefore, given the fact that mast cells and macrophages initiate and to a large extent orchestrate the cascade of events, these immune cells seem to be the most interesting targets for treatment.

It remains an intriguing question whether the main initiators of the inflammatory cascade are residential macrophages or mast cells. The role of residential APCs (dendritic cells or macrophages) is undoubtedly proven [18,28–30,44,48] and some authors support their role as first responders and conductors orchestrating the inflammatory events after surgical manipulation or endotoxin exposure [4]. Recently, a hypothesis was proposed suggesting a first responder role for peritoneal mast cells adjacent to mesenteric blood vessels. According to this hypothesis neuropeptides, such as substance P and calcitonin gene-related peptide (CGRP), released from the adjacent afferent neurons activate mast cells that on their turn release proinflammatory mediators into the peritoneal cavity. Via the blood circulation, these mediators increase mucosal permeability, allowing luminal bacterial and/or bacterial products to enter the gastrointestinal wall and activate the resident macrophages triggering intracellular signalling pathways, leading to transcription of inflammatory molecules, cytokines, chemokines and adhesion molecules. This hypothesis needs to be proven experimentally and meanwhile it remains necessary to further investigate the interplay between these initiating cells and the nervous system as both the mediators released from mast cells and from residential macrophages are able to affect neuronal pathways within the gastrointestinal wall and from the gastrointestinal wall towards higher brain centres.

Also in IBD mast cells might participate in the neuroimmune interactions leading to visceral sensitivity and motility disturbances. Although it is generally believed that mast cells might not be the crucial initiators of pathology in IBD, mast cell stabilizing drugs or drugs interfering with mast cell mediator activity might be an adjunctive therapeutic possibility in the treatment of IBD. These hypotheses support further research in the interplay between mast cells, immunological cells and the neuronal pathways.

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