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REVIEW

# Role of Macrophages and Mast Cells as Key Players in the Maintenance of Gastrointestinal Smooth Muscle Homeostasis and Disease

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**SUMMARY**

This review describes the factors contributing to muscularis macrophage heterogeneity and the nature of muscularis macrophage's interactions with cells that regulate gastrointestinal function. The emerging role of mast cells in gastrointestinal homeostasis and diseases also is described.

**The gut contains the largest macrophage pool in the body, with populations of macrophages residing in the mucosa and muscularis propria of the gastrointestinal (GI) tract. Muscularis macrophages (MMs), which are located within the muscularis propria, interact with cells essential for GI function, such as interstitial cells of Cajal, enteric neurons, smooth muscle cells, enteric glia, and fibroblast-like cells, suggesting that these immune cells contribute to several aspects of GI function. This review focuses on the latest insights on the factors contributing to MM heterogeneity and the functional interaction of MMs with other cell types essential for GI function. This review integrates the latest findings on macrophages in other organs with increasing knowledge of MMs to better understand their role in a healthy and diseased gut. We describe the factors that contribute to (muscularis macrophage) MM heterogeneity, and the nature of MM interactions with cells regulating GI function. Finally, we also describe the increasing evidence suggesting a critical role of another immune cell type, the mast cell, in normal and diseased GI physiology. *(Cell Mol Gastroenterol Hepatol 2022;13:1849–1862; https://doi.org/10.1016/j.jcmgh.2022.02.017)***

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Early studies based on electron microscopy and immunohistochemistry identified "macrophage-like cells" in the muscularis propria of the gut.[1-5] From their first identification, the progression of technology led to a more complex understanding of the role of muscularis macrophages (MMs) in gastrointestinal (GI) homeostasis and disease development and their heterogeneity. For years, the macrophage's phenotype relied on the classic vs alternative activation nomenclature based primarily on macrophage activation to in vitro signals.[6,7] After treatment with lipopolysaccharide (LPS) and interferon-γ (IFN-γ), macrophages acquire a proinflammatory phenotype called "classically activated."[8] On the other side of the spectrum, the treatment of macrophages with interleukin (IL)4 or IL13 promotes an anti-inflammatory phenotype called "alternative activated."[9]

Recently, this phenotypic dichotomy has been surpassed and replaced by a more complex network model that considers the cellular changes within a specific tissue and the numerous stimuli macrophages undergo, resulting in a continuum of phenotypes and more plasticity than previously recognized[10,11]

MMs express multiple markers as microglia, such as major histocompatibility complex II (MHCII), cluster of differentiation 11c (CD11c), cluster of differentiation 103 (CD103), and cluster of differentiation ($CD11b^{+}$).[11,12] As in other tissues, most MMs at steady-state conditions express a high level of C-X3-C Motif Chemokine Receptor 1 (CX3CR1) combined with colony-stimulating factor 1 (CSF1) and Fcγ receptor. The importance and role of CSF1 in the development and maintenance of MMs is highlighted by the near-complete absence of MMs in $Csf1^{op/op}$ mice, lacking the gene encoding for CSF1 from birth.[13,14] MMs share a general anti-inflammatory phenotype at steady-state conditions compared with mucosal macrophages, which, in contrast, have an overall inflammatory phenotype.[15,16] This may be a consequence of the location of MMs because the mucosa is

***Abbreviations used in this paper:*** α-Syn, α-synuclein; CB, cannabinoid; CCR2, C-C Motif Chemokine Receptor 2; CD, cluster of differentiation; CGRP, calcitonin gene–related peptide; CNS, central nervous system; CSF1, colony-stimulating factor 1; Cx, connexin; CX3CR1, C-X3-C Motif Chemokine Receptor 1; EGC, enteric glial cell; EN, enteric neuron; GFAP, Glial Fibrillary Acidic Protein; GI, gastrointestinal; IBD, inflammatory bowel disease; IBS, irritable bowel syndrome; ICC, interstitial cells of Cajal; IFN-γ, interferon γ; IL, interleukin; LPS, lipopolysaccharide; MC, mast cell; MHCII, major histocompatibility complex II; MM, muscularis macrophage; POI, postoperative ileus; SP, substance P; TNF-α, tumor necrosis factor α; WT, wild-type.

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constantly exposed to external stimuli. In addition, MMs share wound healing and tissue-protective genes such as *Rental* (encoding Fizz1), *Mrc1*, *Cd163*, and *Il10*.[17,18] Contrary to the increasing understanding of the murine MM phenotype complexity, human MM information is limited and relies heavily on immunohistochemistry and morphology-based studies. Similar to the observations obtained in mice, transition of monocytes to a tissue-resident phenotype has been described in mucosal macrophages, where a population of monocytes sharing $CD14^{high}$ C-C Motif Chemokine Receptor 2 $(CCR2^{+})CD11c^{high}$ markers is transitioning to a $CD14^{low}CCR2^{-}CD11c^{high}$ macrophage phenotype.[19]

Further investigations are needed to provide information about the composition and distribution of human MMs to understand the degree of similarities with murine MMs. Overall, the heterogeneity of the MM phenotype (Figure 1) depends mostly on the origin and location in different regions of the gut (interdiversity) and between different regions across the muscularis propria (intradiversity).

## Origin

For a long time, it has been thought that resident macrophages were continuously replenished by circulating monocytes.[20] In the past decades, investigators have challenged this concept, suggesting that macrophage heterogeneity within tissues depends on different cell origins. It is now clear that resident macrophages are established before birth in several organs and can maintain their number by cell division without monocyte recruitment.[21,22] Recently, an elegant report[23] identified a population of MMs in the small intestine that engrafted the GI tract during the embryonic state. This population of embryonic origin is known as long-lived MMs because it persists in the muscularis propria depending exclusively on cell proliferation. Besides this population, throughout life, the muscularis propria is populated primarily by monocyte-derived MMs that continuously replenish tissue-resident MMs. Monocyte- and embryonic-derived MMs represent the entire pool of tissue-resident MMs. In the same study, the investigators provided evidence that this population of embryonic origin clusters near enteric neurons (ENs) and regulates their number. Pieces of evidence suggest the possible contribution of monocyte-derived MMs to GI dysfunction. CCR2-dependent monocyte-derived macrophages play a role in resolving and restoring GI motility in postoperative ileus.[24]

In diabetic mice with gastroparesis, an increased number of MMs[25] coincides with a higher level of MMs expressing proinflammatory markers,[26] which can depend on increased recruitment of monocytes. A recent study[27] identified a population of MMs closely associated with blood vessels in the GI muscularis called perivascular MMs. This population of MMs is regulated by the transcription factor Maf, which also controls the expression of several genes associated with the anti-inflammatory MMs. This population of MMs related to adipose tissue overlap with CD206 MMs. Interestingly, using a lineage tracing mouse model, the investigators clearly showed that this population is not dependent on circulating monocytes and primarily of embryonic origin. Conditional removal of the transcription factor c-MAF from MMs leads to CD206 macrophage loss.

Immune cell diversity has been described in 3 commonly used murine strains (C57BL/6NCr, 129/SvHsd, and BALB/cAnNCr).[28] In this study, the investigators clearly showed a different macrophage phenotype in the spleen of the 3 different strains. In addition, transcriptomic and genetic analysis of macrophages from 5 different strains identified different levels of transcription factors leading to differences in the expression of genes[29] driving macrophage phenotype. In addition, macrophages from BALB/cAnNCr and C57BL/6NCr respond differently to corneal transplantation.[30] Almost all the information relative to MMs has been produced using C57BL/6NCr mice except for some studies on nonobese diabetic/ShiLtJ. Notably, as an indication of variability in MM phenotype owing to mouse strain, at steady-state conditions, nonobese diabetic mice do not express[25] *CD206*, which otherwise is highly expressed in tissue-resident MMs in C57BL/6NCr mice.

## Interdiversity and Intradiversity

As microglia in the central nervous system (CNS), MM phenotype depends on regional distribution across the smooth muscle layers and their interaction with other cell types populating the same environment (intradiversity). MMs are diverse and dynamic,[31,32] and show a different morphology depending on their location. MMs can be divided into 3 distinct populations based on their distribution within the smooth muscle layers. MMs located in the myenteric plexus and serosal regions are multipolar, with many branches originating from the main body. MMs located within the muscular layers have a bipolar morphology following the muscle cell orientation. Further

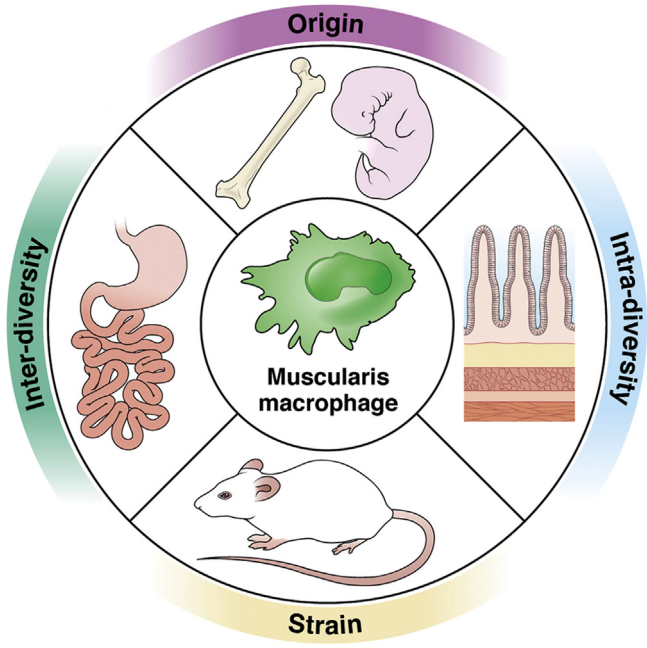



**Figure 1. MM heterogeneity.** Overview of the different factors contributing to MM diversity.

data are needed to understand if the morphologic differences between these diverse MM populations translate into functional changes.

A recent study[23] associated a MM population with a specific area of the small intestine muscularis propria. De Schepper et al[23] identified a MM population essential for EN maintenance, located within the myenteric plexus region where they interact with ENs. Although we have a clearer picture of MM distribution in different smooth muscle layers at steady-state conditions, we only have partial information about their distribution in states of altered homeostasis and disease. In aging,[31] clusters of CD163-IR immune cells were visualized in proximity to sympathetic hyperinnervation of the jejunum of rats. In a mouse model of diabetic gastroparesis, an increase in MM number was described[25] with the onset of diabetes, but no changes in the distribution of MMs has been reported. As briefly mentioned in the previous section, perivascular MMs[27], associated with an anti-inflammatory and protective phenotype, are localized within the myenteric plexus region, where the blood vessels are located in the GI muscularis propria.

Another factor contributing to MM heterogeneity is the location of MMs within the different regions of the GI tract (interdiversity). We recently showed that MM distribution within the stomach and the small intestine presents some differences that require more attention in the future.[32] MM distribution within the myenteric plexus and the smooth muscle layers were more homogenous in the stomach than in the small intestine. Gastric MMs are distributed evenly between the myenteric plexus and the smooth muscle layers, whereas small intestine MMs are distributed primarily in the myenteric plexus. Further studies are needed to understand if this critical difference in MM distribution also is responsible for functional changes. Phenotypically, at resting, gastric MMs do not express *CD206* as MMs from the small intestine do. It also appeared that MMs in the different gut regions respond to external stimuli differently, suggesting a possible intrinsic phenotypic difference. For example, in diabetes, gastric MMs change their phenotype, leading to gastric dysfunction, whereas MMs in the small intestine appeared to be unchanged. More studies are needed to understand the differences between the populations of MMs residing in the different gut regions looking at the following: (1) phenotypic changes, (2) changes in response to inflammation/stimuli, and (3) origin.

## Proximity Defines Functionality

As previously described, one of the most critical factors contributing to MM heterogeneity is the tissue cue where MMs reside and interact with different cell types. MMs share the muscularis propria environment with ENs, interstitial cells of Cajal (ICC), glial cells, smooth muscle cells, and fibroblast-like cells, contributing to GI physiology (Figure 2). This type of interaction also has been described in the CNS, where microglia functionally interact with neurons and astrocytes, representing a multicellular system close to that observed in the enteric nervous system. Therefore, we will use this parallelism with microglia to describe the latest information about MMs' interaction with important cells for GI physiology and to underline areas that could be investigated further in the future

### MMs/Enteric Glia

In the CNS, astrocytes and oligodendrocytes are macroglia, and both maintain the neuron's work environment by keeping neurotransmitters around synapses and providing support to axons.[33,34] Enteric glial cells (EGCs) are found in the enteric nervous system surrounding the ENs[35-40] and share similar features with the brain macroglia,[41] represented by astroglia and oligodendrocytes. As a consequence of their proximity to ENs, enteric glia contribute significantly to EN maintenance, survival, and function. Several studies in the CNS have described microglia–macroglia interaction. Activated microglia change astrocyte phenotype,[41] providing the basis for developing new therapeutic strategies to target microglia-astrocytes in degenerative brain disease. Astrocyte–microglial interaction is required for synapse homeostasis, and the release of IL33 from astrocytes to microglia is required for maintaining neuron number and function.[42] Different nerve injury types are associated with the activation of astrocytes and altered microglia phenotype.[43] MMs lie in the proximity of enteric glial cells, suggesting a possible contribution of this interaction to GI physiology.

Dogiel[44] described enteric glia in 1949, but only lately have been defined as peripheral glia thanks to the accurate morphologic analysis conducted by Gabella.[45] Enteric glia modulates neuronal circuits by providing neurotransmitter precursors and generating neuroactive substances.[46] Because of this interaction, enteric glia's ablation leads to EN death.[47] Similar studies to those in the CNS have suggested that enteric glial cells can change their phenotype to respond to the environment. Enteric glial cells respond to different cytokines such as IL4 and tumor necrosis factor $\alpha$ (TNF-$\alpha$).[48] Notably, incubation of cultured LPS-activated glial cells leads to increased expression of Glial Fibrillary Acidic Protein (*GFAP*$^{+}$), which is independent of proliferation. The inhibition of the nuclear factor-$\kappa$B pathway in glial cells can effectively ameliorate colonic inflammation in mice and cultured human biopsy specimens.[48] In addition, enteric glia become activated during acute inflammation in vitro and in vivo.[48] The increased number of CD68 MMs during inflammation, partially owing to circulating monocyte extravasation, is reduced after conditional depletion of connexin (Cx)43, a gene that encodes for gap junctions, from enteric glial cells (EGCs).[49] IL1$\beta$ level, which is increased in inflammation, binds to its receptor on EGCs and regulates *CSF1* expression by a Cx43-dependent mechanism.[50]

A similar mechanism involving EGC-MM crosstalk also was observed in postoperative ileus (POI), which is associated with an increased level of *IL1β*. Intraperitoneal injection of IL1$\beta$ promoted the expression of proinflammatory genes, and mice deficient for IL1R1, a receptor for IL1$\beta$, are protected from POI. Interestingly, immunohistochemistry study showed that Interleukin 1 Receptor Type 1 (IL1R1) in POI co-labeled with EGC labeled with GFAP. EGCs stimulated

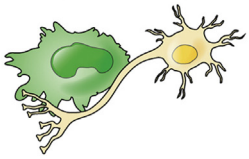
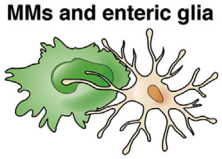
| Cell to cell | Homeostasis | Disease |
|---|---|---|
| MMs and ICCs  | • Needs to be further elucidated | • Anti-inflammatory MMs protect ICCs<br>• Reduction of anti-inflammatory MMs and increased number of proinflammatory MMs is associated with ICC loss in diabetic gastroparesis |
| MMs and ENs  | • Maintenance of ENs<br>• ENs promote MMs activation<br>• B2AR-mediated sympathetic innervation of MMs induces neuroprotection | • Vagal nerve perturbation from surgery or inflammation leads to ENs loss from proinflammatory MMs<br>• ENs promote MMs activation<br>• B2AR-mediated sympathetic innervation of MMs induces neuroprotection |
| MMs, SMC, FLC  | • MMs expressing TRPV4 are responsible for colonic contractions<br>• SMCs/FLC and MMs establish peg and socket type connections | • POI caused by MMs recruiting monocytes, neutrophils, and other lymphocytes<br>• In Crohn's, differentiation of monocytes in tissue is halted in proinflammatory state |
| MMs and enteric glia  | • Release of TNF-α and IL-1β by MMs promotes NGF expression<br>• Need to be further elucidated | • Enteric glia mediate chronic and acute inflammation by regulating monocyte extravasation<br>• MMs promote activation of enteric glia |

**Figure 2. Cell-to-cell interaction between MMs and cells required for GI contractility.** MMs establish multiple functional interactions with cells important for GI function. B2AR, beta-2 adrenergic receptor ; FLC, Fibroblast like cell; NGF, nerve growth factor; SMC, smooth muscle cell; TRPV4, transient receptor potential vanilloid-type 4.

with 10 ng/mL IL1$\beta$ *in-vitro* for 24 hours expressed a high level of monocyte chemoattractant protein-1 (*MCP-1*), suggesting the possible involvement of these cells on circulating monocyte recruitment.[51] Another study showed that EGCs, after inflammation, express *CCL2*, which promotes monocyte recruitment upon binding with its receptor CCR2.[52]

In colitis induced by *Heligmosomoides polygyrus* infection, transcriptome analysis of isolated MMs showed the enrichment of IFN-$\gamma$ in the colon. These data are consistent with an enrichment of IFN-$\gamma$ from EGCs from patients undergoing inflammation. Interestingly, IFN-$\gamma$ drives a feedback effect on EGCs by eliciting chemokine IFN-$\gamma$–inducible protein 10 kilodalton (CXCL10) and guanylate-binding protein 10 expression through signal transducer and activator of transcription 1, leading to reduced proliferation of EGCs. CXCL10 and guanylate-binding protein 10 are involved in host defense and mediate immune responses with regard to antibacterial immunity and cancer, respectively.[53] In addition to directly impacting EGCs, the IFN-$\gamma$–EGC–Cxcl10 signaling axis regulates tissue repair after helminth infection through MMs via CCL8, CCL7, CXCL2, and CCL2 activation.

$\alpha$-synuclein ($\alpha$-Syn) aggregates are found in the brain of patients with Alzheimer's disease. Most patients with this disease suffer from GI functional disorders, however, the mechanism is not understood. Application of $\alpha$-Syn aggregates into the muscularis propria promotes the expansion of EGCs and overall tissue inflammation. Although it is not directly tested, it is possible that in this context, EGCs orchestrate the overall $\alpha$-Syn–mediated inflammation by talking with MMs because multiple genes expressed after EGC expansion are associated with MMs.[54]

Proinflammatory MM products, such as IL1, IL4, and TNF-$\alpha$, promote EGC activation such as reactive gliosis in the CNS. It also is evident that MM products can affect EGC phenotype during inflammation. Esposito et al[48] showed that upon LPS treatment, EGCs acquire an activated phenotype that coordinates the inflammatory response in the enteric nervous system. Inhibiting the nuclear factor-$\kappa$B pathway on EGCs ameliorated the overall inflammatory response in colitis and in vitro models. On the other hand, treatment of EGCs in vitro with LPS promoted the expression of genes associated with an anti-inflammatory response.

## MMs/ENs

*Intrinsic innervation.* A microglia/neuron interaction has been described in the healthy and diseased brain. CX3CR1 is present in neurons' membrane, and its expression level is associated with stroke pathophysiology. The CX3CL1/CX3CR1 axis mediates microglial activation and plays a detrimental role in mice with ischemia.[55] Because of this interaction, CX3CR1 null mice show an increased number of immature and dendritic spines as a result of microglia absence.[56] Microglia modify neuronal membrane properties and functionality.[57]

The role of MM–EN functional interactions in the GI tract has been studied extensively. One of the oldest dogmas in the neurogastroenterology field on the stability of the

neuronal circuits was challenged recently by Kulkarni et al.[58] In this study, the investigators suggested that the number of ENs in adults results from a dynamic balance between EN death by apoptosis and the continuous production of ENs by neurogenesis. Interestingly, MMs, which are near ENs, phagocyte ENs that undergo apoptosis, as MHCII co-labeled with ENs by flow cytometry, and portions of apoptotic ENs are found within the MHCII$^{+}$ nuclei.

In the colon and small intestine, MMs interact with ENs, releasing bone morphogenetic protein 2 (BMP2) upon release of CSF1 from ENs, a system regulated by the microbiome.[13] Depletion of MMs results in poorly coordinated colonic contractions in an ex vivo model and abnormal colonic transit time in vivo. The addition of exogenous bone morphogenetic protein 2 to the colonic rings of MM-deficient mice decreases stretch-induced contractions. CSf1$^{op/op}$ mice, which do not have MMs from birth, have an abnormal myenteric plexus and more ENs than control mice.

Although the number of nitrergic ENs is increased in CSf1$^{op/op}$ mice, the number of cholinergic ENs is not altered, suggesting that MMs may regulate different subtypes of ENs.[59] In the same animal model, we also showed that the absence of MMs from birth is associated with more ENs sharing cholinergic and nitrergic phenotypes, indicative of a more undifferentiated population of ENs. A reduced number of anti-inflammatory MMs in aged mice is linked to EN loss.[60,61] In another report, the investigators tested the association of MMs with ENs during development using a double-transgenic zebrafish model. This study provided evidence of the intimate proximity between MMs and ENs starting at 3 days after fertilization. The number of total MMs that colonize the tissue increased, and more MMs associate with ENs. Furthermore, conditional depletion of Interferon Regulatory Factor 8 (*irf8)* from MMs reduced the total number of MMs during development, leading to faster intestinal transit.[62]

*Extrinsic innervation.* The amount of data describing the functional interaction between tissue macrophages and peripheral nerves are limited, mainly because the number of nerve-associated macrophages is minimal compared with the total number of tissue macrophages. For example, macrophages expressing *CX3CR1* are closely associated with sympathetic nerve fibers of adipose tissue.[63,64] A population of macrophages also has been described in the proximity of dermal sensory nerves in the skin.[64] Precise extrinsic afferent (visceral sensory) and efferent (sympathetic and parasympathetic) innervation of the gut is fundamental for gut–brain crosstalk. Although the extrinsic nerves are not directly modulating gut motility, they can affect it by regulating other cell types within the enteric nervous system. Interactions between MMs and extrinsic innervation[65] and the effect of sympathetic and catecholaminergic signaling in the immune cells' modulation have been studied extensively in the past. However, their possible involvement in modulating MM polarization phenotype in the gut has been described only recently.

In their study, Gabanyi et al[15] suggest the regulation of MM activation by the beta-2 adrenergic receptor ($\beta$2AR). $\beta$2AR$^{+}$ MMs reside near neuronal cell bodies or processes of the myenteric ganglia. Because of this interaction, MMs express higher levels of $\beta$2AR, a neuropeptide receptor, than lamina propria macrophages. Notably, adrenergic signaling through this receptor reduces ENs loss after infection.[66] Acetylcholine represents the primary parasympathetic neurotransmitter released by preganglionic nerve fibers and the vagus nerve. Acetylcholine (ACh) has been studied for its anti-inflammatory effects in the periphery, where its stimulation was sufficient to suppress systemic inflammation in response to endotoxin.[67] With this discovery, mice lacking $\alpha$7 nicotinic acetylcholine ($\alpha$7 nAChR) have increased systemic levels of TNF-$\alpha$, IL1$\beta$, and Il6 in the skin.[68] Cholinergic neuronal release of acetylcholine during vagal nerve stimulation promotes an anti-inflammatory macrophage phenotype via the $\alpha$7 nAChR. It results in the amelioration of muscular inflammation.[69,70] In addition, vagal manipulation promotes an increased number of gastric MHCII MMs, resulting in delayed gastric emptying.[71] In the CNS, the type of communication microglia establishes with nerve fibers or the cell body is different. Recently, a beautiful study suggested that microglia monitor and protect neuronal function through specialized somatic purinergic junctions when communicating with cell bodies.[72]

Because of their location, MMs establish interaction with both nerve fibers and neuronal bodies across the smooth muscle layers; however, we still are far from understanding if, as described in the CNS, the type of communication between nerve fibers and cell bodies is anatomically and functionally different. The nature of communication between MMs and ENs has been the most described in the field, but we still are far from deciphering MM–EN communication in homeostasis and diseases. Because many functional diseases have been associated with EN number reduction, understanding the contribution of MMs to this phenomenon represents a fundamental step to build future and promising therapeutic strategies to regulate and maintain ENs in functional diseases characterized by EN loss.

## MMs/ICC

For many years, ICC were characterized only by nonspecific histologic stains, and, later, more reliably, by electron microscopy. The ultrastructural features and the ICC anatomic distributions suggested their critical physiological roles: (1) they are pacemaker cells and propagate slow waves,[73,74] (2) they mediate both inhibitory and excitatory neurotransmission,[75-77] and (3) they work as mechanosensors. A similar cell type is absent in the CNS, making this parallelism impossible. ICC role and function are similar to cells in the heart that represent the heart's pacemaker cells as the ICC for the gut. In a recent publication, Hulsmans et al[78] described the functional interaction between macrophages and pacemaker cells in the heart. The study described an abundance of macrophages closely associated with the heart's AV nodes in homeostasis. Macrophages couple electrically with pacemaker cells through Cx43-containing gap junctions. The absence of this

interaction (Cx43 knockout mice) results in impaired AV node conductance, suggesting the macrophages' critical role in facilitating the heart's electrical conduction. In the enteric nervous system, limited data describe MM–ICC interactions in homeostatic conditions. Electron microscopy and immunofluorescence analysis showed that MM populations are closely associated with ICC, suggesting a potential functional role for this type of interaction.[1,2] In CSf1$^{op/op}$ mice that do not have macrophages from birth, ICC appeared to have a normal distribution,[14] and the level of expression of *Kit*, a specific ICC marker, did not differ in comparison with controls. However, gastric emptying, which partly depends on ICC function, is faster than in control mice. The information relative to MM–ICC functional interactions in GI disease is more extensive. Blockade of IL17A signal reduced ICC loss in sepsis because of the reduction of proinflammatory MMs.[79] In an inflammation model, numerous MMs were observed near ICC compared with controls at the ultrastructural level.[80] In the same model with the progression of inflammation, macrophages presented large phagosomes and lysosomes in the proximity of injured ICC, indicating an ongoing phagocytic activity. During development, ICC release CSF1 in the small intestine, suggesting their possible contribution to MM migration and survival during this period.[81] The bidirectional communication between ICC and MMs still needs to be elucidated. IL6 released by MMs during GI surgery promotes up-regulation of microRNA-19a responsible for ICC depletion.[82] An increase of proinflammatory cytokines produced by MMs is associated with decreased c-kit–positive ICC in the dilated colon of Hirschsprung disease, associated with enterocolitis.[83] Almost all of the information acquired in recent years describing a potential functional interaction between MMs and ICC has been produced in the context of diabetic gastroparesis, a functional disease affecting the stomach.

Conditioned media from proinflammatory activated macrophages reduces kit-positive ICC in vitro.[84] The ineffectiveness of the same conditional media in the presence of a TNF-$\alpha$ neutralizing antibody implies this cytokine's implication in regulating kit expression.[84] MM phenotype changes are associated with ICC loss and delayed gastric emptying in animal models and human beings.[85,86] Patients with diabetic gastroparesis have fewer CD206$^{+}$ MMs, a marker of anti-inflammatory macrophages. This reduction correlates with ICC loss,[87,88] suggesting a protective role of anti-inflammatory MMs for ICC that is absent in diabetic gastroparesis. Anti-inflammatory MMs also secrete IL10 to induce heme oxygenase 1 expression, an enzyme that has a protective effect on ICC.[87] Mice with delayed gastric emptying given exogenous IL10 return to regular gastric emptying with higher levels of heme oxygenase 1 and better-connected, more organized, and evenly distributed ICC networks.[88] MM polarization to an anti-inflammatory phenotype may be a viable treatment for diabetic gastroparesis. Recently, we showed that the development of diabetes was associated with an increased level of proinflammatory MMs and reduced anti-inflammatory MMs. Notably, delayed gastric emptying and loss of ICC are related to lower protection exerted by the anti-inflammatory component and the increased level of expression of *IL6* and inducible nitric oxide synthase (*iNOS*). A small number of data suggest the importance of MM–ICC functional interactions with other GI diseases. Chron's disease is associated with ICC injury and changes to MM morphology.[89] In achalasia, ICC closely related to immune cells are preserved.[90] Cytokines released by MMs have been considered responsible for ICC network disruption in the endothelin-B–receptor null rat, representing a model for long-segment Hirschsprung's disease.[91]

### MMs/Smooth Muscle Cells and Fibroblast-Like Cells

Macrophages interact with smooth muscle cells in various organs. Conditioned medium from the macrophage cell line promotes differentiation of human adipose tissue–derived mesenchymal stem cells to smooth muscle cells.[92] The interaction of macrophages with smooth muscle cells is responsible for extracellular matrix deposition during plaque progression. In addition, macrophages regulate vessel tone and diameters of blood pressure while interacting with vascular smooth muscle cells. However, the information of a potential interaction between MMs and smooth muscle cells in the gastrointestinal tract is, at best, limited. MMs expressed the transient receptor potential vanilloid 4 channel, and MMs' stimulation via transient receptor potential vanilloid 4 promotes prostaglandin E2 release, leading to colon contraction.[93]

LPS treatment, which induces MM polarization to a proinflammatory phenotype, reduces smooth muscle contractility.[94,95] Treatment with a monocyte chemoattractant protein-1 reduces monocyte infiltration and leads to intestinal smooth muscle dysfunction.[96] Notably, IL1$\beta$ inhibits intestinal smooth muscle proliferation.[97] Another type of interstitial cell in the gut that differs from ICC has been identified using antibodies against platelet-derived growth factor receptor-$\alpha$.[98,99] These cells are located between smooth muscle cells and intramural nerve fibers and play an essential role in neurotransmission.[100] In addition to the expression of platelet-derived growth factor receptor-$\alpha$, these cells differ from ICC at the ultrastructural level. These cells show a well-developed rough endoplasmic reticulum but do not have caveolae.[101] In a recent publication,[31] electron microscopy and immunohistochemistry showed that MMs establish cell-to-cell contact with fibroblast-like cells. The nature and the functional translation of these types of interaction need to be addressed in the future.

## Interaction of Mast Cells With Enteric Neurons and Other Cells Required for Gastrointestinal Motility

As described earlier, the GI tract contains the largest pool of macrophages in the body. In addition, different types of immune cells are essential for developing normal

intestinal morphology and function. Among that, mast cells (MCs) are certainly one of the most significant. MCs are principally localized in tissues that represent a barrier such as skin and respiratory and intestinal mucosa. At the same time, in the GI tract, their number is small, principally located in the mucosa (2%–3%) and the submucosa (approximately 1%) layers.[102] However, MCs also can be recruited (mastocytosis) in response to specific stimuli.[103] In addition, MCs contribute to intestinal functions (host defense, vascular permeability, peristalsis, and so forth).[104,105] Thus, changes in their number or activity may lead to several pathologic states such as inflammatory bowel disease (IBD) or can impact gut functions, modifying sensory nociception, intestinal permeability, or altering motility and secretion.[106]

MCs are incredibly plastic and exert their functions almost exclusively by releasing mediators after activation. As reported by Buhner and Schemann,[107] mediators released by MCs can be subdivided into 2 classes: preformed molecules, such as histamine and proteases that are stored as granules and therefore can be released within seconds; and mediators newly synthesized, produced after MC activation, such as lipid mediators and cytokines. Early anatomic studies described the nonrandom spatial association and membrane-to-membrane contacts between MCs and nerves in the human gut.[108] Stead et al[109,110] reported that an estimated 70% of MCs are in direct contact with nerves, and another 20% are within 2 $\mu$m. This anatomic association has functional relevance because communication between nerves and MCs is crucial for maintaining mucosal homeostasis and ensuring an appropriate response to injury, thanks to the expression of neurotransmitter and neuropeptide receptors by MCs and several receptors expressed by neurons.[111] In guinea pig and human small intestine, the communication and interaction between MCs and peripheral nerves were meticulously described by Wang et al.[112] As reported by the investigators, the relationship between spinal afferents and enteric MCs and their activation can be resumed at 5 pivotal points: (1) MCs expressed receptors for the neurotransmitters substance P (SP) and calcitonin gene–related peptide (CGRP); (2) after exposure to SP and CGRP, MCs released protease II and histamine; (3) histamine and protease II act in a diffuse paracrine manner to influence the behavior of ENs and spinal afferent terminals; (4) sensitization of the afferent terminals amplify MC release; and (5) MC-stabilizing drugs such as cromolyn and doxantrazole or neural blockade with (tetrodotoxin) TTX, suppressed the release of MC protease II and histamine. Furthermore, Buhner and Schemann[107] recently showed that the mediators involved in nerve–MC interactions were CGRP and (vasoactive intestinal polypeptide) VIP rather than SP. Interestingly, Buhner and Schemann,[107] divided the MC–nerve axis in the human gut, into MCs to nerve and nervetoMC signaling, underlining how activation of MCs causes mostly nerve sensitization, while nerves can activate or inhibit MC mediator release.

The reciprocal relationship between MCs and glial cells was first studied by Shalit et al[113] in MCs co-cultured with confluent glial cell monolayers. In this experimental model, in the presence of IL3 (an important cytokine for the regulation of MC growth and differentiation),[114] glial cells do not interfere with MC viability, function, and biochemical properties and these cells maintain their functional activity as shown with the release of histamine. In a model of stress owing to neonatal maternal separation and early weaning, linked to IBS,[115] MCs were increased at the level of the myenteric plexus and produced more histamine than controls and were closely associated with enteric glia. The involvement of histamine in the glia–MC communication also was confirmed in an in vitro study on human EGCs obtained from surgical patients. The contribution of the histaminergic pathway in the pathogenesis of IBS is further pointed out by the observation that in the small intestine of IBS patients, the histamine N-methyltransferase is altered, producing an increased amount of histamine owing to lack of is degradation. MCs can influence and affect the numbers and the function of ICC. Reduced ICC are associated with more MCs in several pathologic states such as diabetic gastroparesis,[116] Crohn's disease,[89] and ulcerative colitis (UC)[117]

## Mast Cells and Functional Diseases

There is strong evidence supporting nerve–MC interaction in diseases such as IBD and irritable bowel syndrome (IBS). In colon samples from dextran sulfate sodium (DSS)-treated rats, 5-hydroxytryptamine–containing MCs were close to CGRP-immunoreactive fibers in inflamed serosa, suggesting a functional association in the diseased state.[118] Several studies have shown increased MC density in both IBD and IBS patients, and these cells could play a role in developing strictures and fistulas, irreversible complications of IBD.[119] Studies on IBS colonic mucosa showed increased *GFAP* expression, indicating an enteric glial activation that was correlated negatively with MC density, suggesting altered EGC-MC interactions.[120] Inflammation, even if mild, leads to persistent GI nerve changes, and these inflammation-induced changes persist after recovery. These changes could affect structural morphology (nerve bundles, hypertrophy, and/or hyperplasia) and neurotransmitter expression or receptor, such as a decreased release of acetylcholine and increased secretion of 5-hydroxytryptamine. Increased SP and neurokinin 1 (NK-1) receptors were reported in IBD patients and blocking of NK-1 receptors resulted in reductions of experimental colitis. Treatment with VIP after the onset of 2,4,6-Trinitrobenzenesulfonic acid (TNBS)-induced colitis in mice reduces colitis's clinical and histopathologic severity.[121] In TNBS-induced colitis in rats, no differences in colonic damage, weight, and adhesion scores between wild-type (WT) and MC-deficient rats were observed.[122] In the same model, nedocromil sodium, a MC stabilizer, reduced inflammation and fibrosis, possibly by decreasing MC numbers and activation and consequent collagen production. Compound 48/80, a MC activator, slightly enhanced the severity of the disease. In vitro, rat peritoneal MC increased fibroblast proliferation, collagen synthesis, and contractile activity, all hallmarks of fibrosis. These properties have been attributed to mediators such as

tryptase, histamine, and TNF-$\alpha$.[123] On DSS-induced colitis and acute otitis media (AOM)/DSS-induced colorectal cancer, peptide F991, a functional inhibitor of free Ig light chain, significantly attenuated colitis progression and carcinogenesis, reduced the infiltration of inflammatory cells, and blocked inflammasome activation by inhibiting the cleavage of caspase-1 and the activation of IL1$\beta$ and IL18.[124] The severity of IBD triggered by exposure to piroxicam was similar in Il10-/- mice, in dystrophin-utrophin double knockout (DKO) mice with no MCs owing to the KitW-sh/KitW-sh mutation, and in DKO mice reconstituted with WT or Il10-/- bone marrow–derived MCs. However, DKO mice had significantly increased TNF-$\alpha$ and IFN-$\gamma$ release, showing that MCs play a role in down-regulating proinflammatory cytokines within the inflamed colon.[125] Thus, differences in results from different experimental models could be partly owing to differences between species (eg, rat vs mice), both in terms of localization and number of MCs.

## MCs and POI

POI is a relatively common postoperative disorder. Although most patients recover in 2–3 days without medical intervention, in some cases, GI motility disorders last for a long time and prolong hospitalization, increasing medical costs. Surgical trauma and gut manipulation trigger an inflammatory response in the muscularis externa involving inflammatory cells, reactive enteric glia, neurons, smooth muscle cells, ICCs, epithelial cells, and the microbiome in the lumen of the gut.[126] The recruitment of inflammatory cells is mediated by cellular adhesion molecules, including selectins, integrins, and adhesion molecules. Surgical manipulation causes a significant up-regulation of intercellular adhesion molecule-1 (*ICAM-1) and P-selectin*, and massive extravasation of leukocytes; the administration of adhesion molecule antibodies prevented the recruitment of monocytes and neutrophils, resulting in a significant reduction of all 3 populations of infiltrating leukocytes and prevented muscle dysfunction[127] As reported earlier, complex communication pathways exist among neurons, glia, immune cells, and other cells, which may contribute to the pathogenesis of POI. Vagus nerve stimulation prevents POI in preclinical models by reducing nicotinic-receptor activation, and 5-Hydroxytryptamine Receptor 4 (5HT4R) agonists reduce the activation of MMs. Cholinergic neurons express cannabinoid (CB) receptors, and their activation inhibits the release of acetylcholine with subsequent slowing of intestinal motility and a delay in gastric emptying. Endogenous ligands of CB1 receptors attenuate inflammation by decreasing proinflammatory cytokine release and inhibiting MC degranulation. However, decreased GI transit and marked intestinal and systematic inflammatory responses were observed in both WT and $CB1^{-/-}$ mice after surgery. However, $CB1^{-/-}$ mice showed higher plasma levels of IL6, cytokine-induced neutrophil chemoattractant-1 (KC/CINC1)expression in mucosal epithelium and submucosa compared with WT mice.[128] The contribution of MCs to POI was highlighted by experimental and clinical studies. Mechanical stretch results in the local release of sensory neurotransmitters, especially SP and CGRP, which can directly activate mast cells. Intestinal manipulation also elicited a significant increase in MC degranulation and gastroparesis, prevented by MC stabilizing agents.[129] The involvement of MCs is shown further using the MC secretagogue C48/80. Moreover, intestinal manipulation in MC-deficient Kit/Kitv mice did not elicit significant leukocyte recruitment. Still, the reconstitution of the MC population in these animals restored the manipulation-induced inflammatory response to WT levels.[130] Mouse models with abnormal Kit signaling also lack ICC, resulting in aberrant GI motility even before surgery. In a mouse model with selective depletion of MCs and typical ICC network, Cpa3Cre/+ mice, intestinal manipulation induced inflammation and delayed gut motility as in WT mice, which strongly argues against MCs as a crucial player in the development of POI, at least in mice.[131] These investigators underlined that several issues such as nonspecific effects of MC degranulation or stabilization agents used in other studies may have led to misinterpretation of experimental data and incorrect conclusions. The et al[131] reported that tryptase release in peritoneal lavage during abdominal surgery increased from 5.2 (basal level) to 23.1 in early samples and 51.7 mg/L at the end of surgery. In contrast, neither laparoscopic nor transvaginal intraperitoneal surgery elicited a significant MC response during 2 minimally invasive surgical procedures. Moreover, at the end of the surgical procedure, *IL6* and *IL8* increased while, in laparoscopy, only intraperitoneal *IL8* was increased, but not as profoundly as in the laparotomy group. These findings in human beings support the hypothesis that the degree of intestinal handling, MC degranulation, and subsequent inflammation determines POI.

## MCs and Achalasia

Achalasia is a motility disorder characterized by the absence of coordinated peristalsis and incomplete relaxation of the lower esophageal sphincter of unknown etiology. Liu et al[132] determined the number of MCs, ICC, neuronal nitric oxides synthase (nNOS)-positive, and S-100–positive cells in lower esophageal sphincter muscle biopsy specimens from 116 patients with achalasia, observing an increased MC infiltration, significantly associated with decreased ICC, nNOS-positive, and S-100–positive cells.

Nelson et al[133] did not observe differences in the lower esophageal sphincter MC density between patients and controls. However, almost all patients showed profound MC degranulation involving perimysium and myenteric plexus nerves. Moreover, patients with different manometric phenotypes showed different expression profiles: achalasia type II patients showed an up-regulation of genes that might increase cytosolic calcium and smooth muscle contraction. Both achalasia type I and type III (only 1) patients had up-regulated genes to replenish calcium stores in the sarcoplasmic reticulum. The aganglionic segment of the colon of Hirschsprung's disease patients showed an increased number of MCs, correlated with the number and perimeter of

nerve fibers, suggesting a possible role of these cells in Hirschsprung's disease pathogenesis.[134]

## Conclusion and Future Directions

MMs are specialized phagocytic cells that fulfill an important role in regulating GI homeostasis and disease. They contain different subpopulations whose phenotype depends on their GI tract location and origin. These unique MMs, compared with mucosal Ms, share an anti-inflammatory phenotype. It is evident now that MMs have a dual origin. The MM pool is maintained by both monocyte-derived and embryonic-derived MMs. Although there is evidence suggesting the involvement of embryonic-derived MMs in regulating ENs, no information supports the possible contribution of circulating monocytes to tissue homeostasis. Depending on their location, MMs can interact functionally with cells required for GI physiology. Further studies are needed to elucidate the underlying mechanisms regulating this type of interaction.

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