REVIEW

Intrinsic Gastrointestinal Macrophages: Their Phenotype and Role in Gastrointestinal Motility

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SUMMARY

This review focuses on the role of muscularis propria macrophages in gastrointestinal tract motility under both normal and pathologic conditions.

There is an increasing awareness of the role of macrophages in the regulation and maintenance of gastrointestinal function in health and disease. This work has proceeded in the context of an increased understanding of the complex phenotypic variation in macrophages throughout the body and has shown previously unidentified roles for macrophages in diseases such as gastroparesis, postoperative ileus, and inflammatory bowel disease. Opportunities for exploiting the phenotypic modulation of tissue resident macrophages have been identified as possible therapies for some of these diseases. In addition, macrophages are an established component of the innate immune system and can respond to variations and changes in the intestinal microbiome and potentially mediate part of the impact of the microbiota on intestinal health. We reviewed the latest work on novel concepts in defining macrophage phenotype, discuss possible mechanisms of action for tissue-resident macrophages in the gut, address the significance of microbiome effects on macrophage phenotype, and review the known and possible roles of macrophages in motility disorders of the gastrointestinal tract. (*Cell Mol Gastroenterol Hepatol 2016;2:120–130;* http://dx.doi.org/10.1016/j.jcmgh.2016.01.003)

The extraordinary cellular complexity of the gastrointestinal (GI) tract is a reflection of the diverse roles of the organ. From an immune perspective, this means that there are complex interactions between multiple cell types that differ along the length as well as across the thickness of the wall of the GI tract. These interactions are constantly changing with development, age, alterations to the luminal content, and disease. This review is about one cell type, the macrophages, that within the GI tract represent the largest population of macrophages in the body[1,2] and can be involved in multiple GI functions. We review recent discoveries with respect to the complex phenotypic variations in macrophages and how these are modified in health, development, and disease. We refer to cells in all layers of the wall of the GI tract as shown in Figure 1, which shows the sources and destinations of macrophages in the GI tract. The primary focus is on macrophages residing in the muscularis propria and the consequences of their phenotypic changes on GI motility. Because there already are extensive reviews in the literature on the origins and roles of macrophages in the mucosa and submucosa,[3,4] the functions and properties of those macrophages are discussed in less detail and in the context of disturbances to GI motility or in comparison with the functions and properties of muscularis propria macrophages.

Macrophages in the Gastrointestinal Tract: Where Do They Come From?

Many types of cells of myeloid lineage including macrophages, but also microglia in the brain, Kupffer cells in the liver, and bone osteoclasts, are considered to be tissue resident.[5] The source and maintenance of these cells are either ongoing population from circulating monocytes with varying rates of turnover or in some cases by population from yolk sac–derived progenitors and maintenance by self-renewal. Microglia represent a well-defined example of these self-renewing, stable, tissue resident cells.[6] Renewal of resident macrophages is also from macrophage precursors that develop in the bone marrow, then enter into the blood stream as mature monocytes, and, finally, migrate into a specific tissue where they develop into a resident macrophage population.[7] In many tissues they develop from primitive macrophages existing in the yolk sac or fetal liver and are maintained independently from bone marrow–derived monocytes in the steady-state condition.[4,8–10] However, by
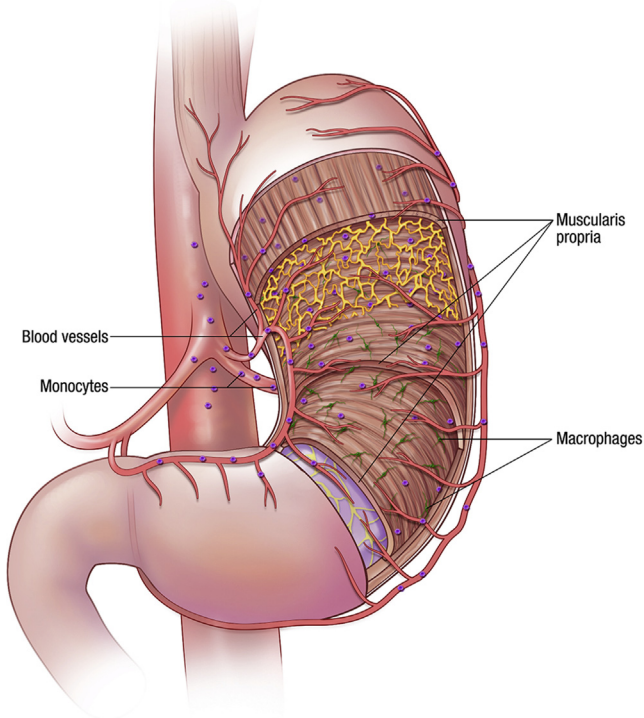
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Figure 1. Distribution of resident macrophages in the gastric muscularis propria. Circulating monocytes are recruited to the tissue where they become macrophages influenced by the local environment.

tracking macrophage development from birth to adulthood in the mucosa and lamina propria, it was observed that although primitive macrophages are present in the neonatal intestinal mucosa and lamina propria they are not maintained in adulthood.[11,12] This important finding suggests that in contrast to other tissues, macrophages that reside in the GI mucosa and lamina propria of adult mice are replaced continuously by blood monocytes and that yolk sac–derived macrophages are short-lived and/or rare. This is reasonable given that mucosal and lamina propria macrophages are thought to monitor and respond to multiple factors at the interface between the organism and the luminal environment, and that these factors and responses are variable and can change rapidly. The resident macrophages of the muscle layers have not been studied in as much detail. These cells are abundant throughout the GI tract and express a limited number of phenotypic markers of activation.[13–17] It is clear that monocytes can be detected in the muscularis propria layers of control mice, an immune cell infiltrate derived from monocytes can be detected after inflammation in animal models,[18,19] and that varying numbers of macrophages can be identified by mannose receptor, C Type 1 (CD206, MRC1) immunoreactivity in human gastric muscularis propria.[20] Our recent data have indicated that population of the gastric muscularis propria in macrophage-depleted, osteopetrotic (op/op) mice can occur by monocyte invasion and can produce both CD206-positive and CD206-negative macrophages. These studies all identified macrophages that express markers that are not present on resident macrophages

in healthy, GI smooth muscle, such as those in the GI tract of nonobese diabetic (NOD) mice when they are not diabetic.[21] Thus, muscularis propria macrophages are derived at least partially from monocytes and it remains to be determined whether some resident macrophages are the equivalent of microglia in the brain, derived from yolk sac progenitors and sustained by self-renewal. Determining the fate and source of all macrophages in the muscularis propria is an important research opportunity for the future.

Macrophage Phenotypes in Healthy Tissues

The phenotype of macrophages in healthy tissues appears to be determined in part by the original monocyte progenitor from which the macrophages were derived as shown in Figure 2 and Supplemental Poster. This is shown most clearly in op/op mice, which show reduced numbers of macrophages in most tissues. Monocyte development and survival depends on colony-stimulating factor 1 (macrophage) (CSF1), and op/op mice have an inactivating mutation in the gene encoding CSF1.[15,22]

In mice, monocytes can be identified by CD115 immunoreactivity and can be divided into 2 different groups according to differences in lymphocyte antigen 6C (Ly6C) expression. $CD115^+$ $Ly6C^+$ monocytes are associated with an inflammatory phenotype and differentiation into conventionally activated, proinflammatory M1 macrophages or inflammatory dendritic cells when they enter tissues. In the GI mucosa and lamina propria, $Ly6c^{hi}$ monocytes develop an intermediate phenotype once inside the tissue, defined as chemokine receptor 1^{int} ($CX_3CR_1^{int}$) before they differentiate into mature macrophages.[12,23] It takes 4–5 days for monocytes to acquire the phenotype of resident mucosal and lamina propria macrophages characterized by the expression of F4/80, CD64, major histocompatibility complex II, CD11c, and CX_3CR_1, and are associated with a slower rate of cellular turnover.[12] CX_3CR_1 expression is associated with a signature cytokine profile. $CX_3CR_1^-$ macrophages are characterized by high levels of proinflammatory cytokines such as interleukin (IL)6, inducible nitric oxide synthase (iNOS, NOS2), IL1β, and tumor necrosis factor (TNF)α.[12,24] In contrast, $CD115^+$, $Ly6C^-$ monocytes do not express inflammatory markers, are long-lived, and contribute to the resident macrophage population in GI mucosa.[25,26] $Ly6C^{lo}$ monocytes appear to be a precursor of a subset of resident $CX_3CR_1^+$ macrophages that are characterized by lower levels of C-C chemokine receptor type 2 (CCR2) and CD62L (L-selectin), and have an anti-inflammatory signature with increased levels of IL10 and heme oxygenase-1 (HO-1) and CD206.[12] These $Ly6C^{lo}$ monocytes are a possible alternative to yolk sac progenitors as a source of resident macrophages in GI muscularis propria, but this has not been tested and it should be noted that the resident macrophages in the healthy muscularis propria express very low levels of HO-1 and CD206 in, for example, nondiabetic NOD mice.[21] Furthermore, flow cytometry and immunohistochemistry indicate that macrophages in healthy mouse intestinal muscularis propria are a homogeneous population of major histocompatibility complex II^{hi}, $CD11c^{lo}CD103^+$ $CD11b^+$
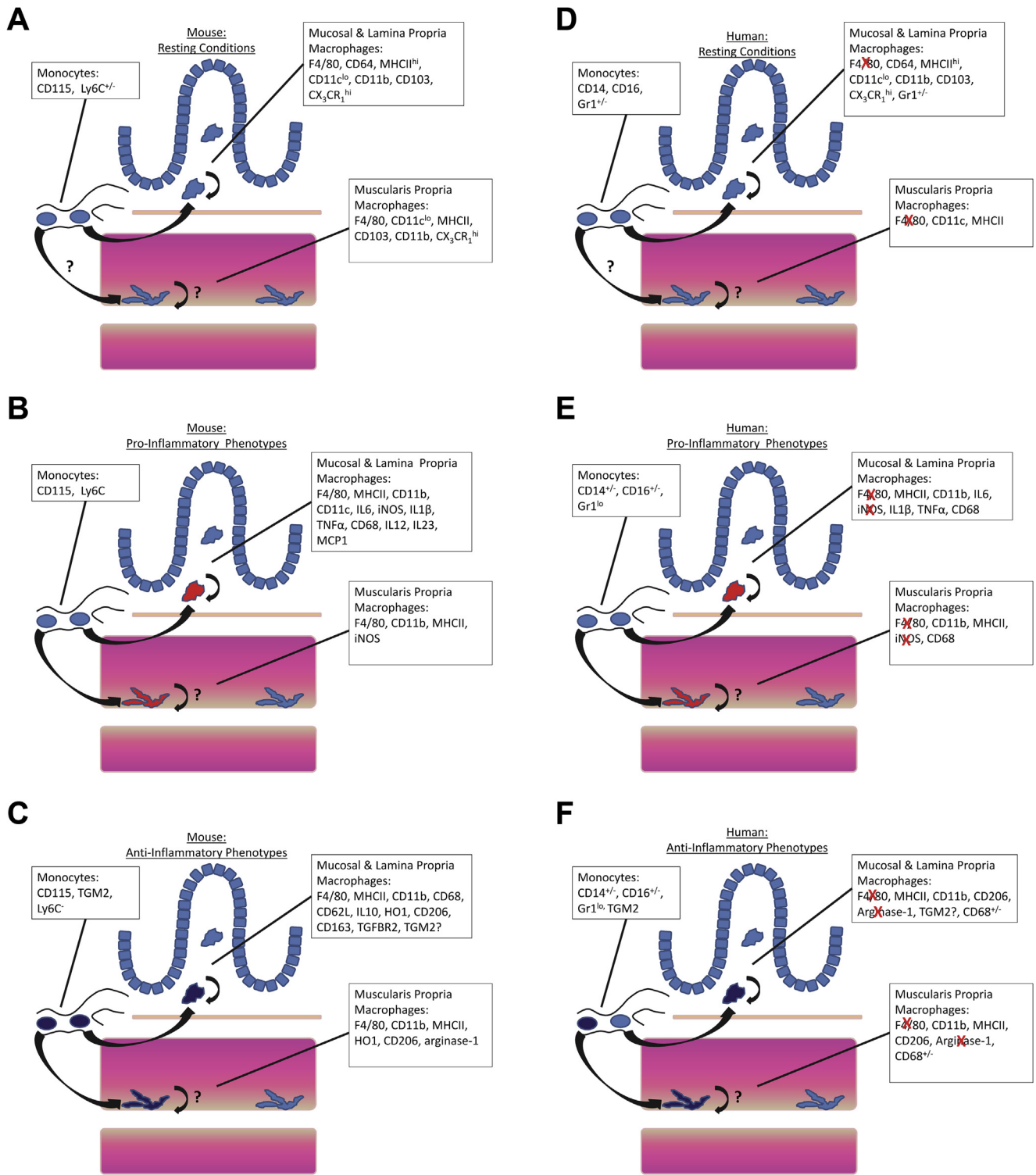
Figure 2. Summary of the published markers of macrophage and monocyte phenotype in (A–C) mouse and (D–F) human gastrointestinal tract. Note that differing expression patterns likely are dependent on the disease state, stimulus, or genetic background of the tissue under study (see text and Table 1 for more detail). Red "X" indicates genes that are expressed in mouse but not human macrophages.

expressing high levels of CX_3CR_1.[27] Further research is necessary in understanding the source and fate of muscularis propria macrophages, with close attention to the strain of the mice under study. The immune systems in mice do

vary widely by strain[28] and some of the differences in macrophage phenotype and markers identified by different studies likely can be ascribed to differences in the genetic background strains used.

In human beings, 3 monocyte populations have been defined based on CD14 and CD16 expression with distinct differences from mice in the markers and likely fate of those cells. For example, the equivalent of the mouse marker Ly6C is the Gr1 epitope in human monocytes, but although mouse $Ly6C^+$ monocytes are proinflammatory, Gr1+ human monocytes release IL10 and do not express proinflammatory markers.[26] These differences are of great importance because to translate the large body of work performed in mice to understanding human health and disease, it is necessary to fully characterize human macrophages. One effort to achieve this was a comprehensive survey of markers conserved on macrophages cultured and differentiated from human and mouse blood monocytes and then validated in human lung tissue. An 87-gene transcriptome was identified that can be used as a basis for characterizing human macrophages in other tissues[23] including the GI mucosa, lamina propria, and muscularis propria. In particular, transglutaminase 2 (TGM2) is identified as a novel marker of alternatively activated, anti-inflammatory M2 macrophages that is as reliable as CD206 for tissue macrophages, but that also labels circulating monocytes with an anti-inflammatory phenotype, unlike CD206/mannose receptor C1. It will be very interesting to see how TGM2 expression correlates with disorders of gastrointestinal function, especially if it proves that the levels of circulating TGM2-positive monocytes correlate with the numbers of TGM2 or CD206-positive tissue macrophages.

Macrophage Phenotypes: What Happens in Disease and Injury?

Changes in macrophage phenotype or activation of macrophages frequently are evident in response to disease or injury as discussed later for GI disorders. A variety of factors initiate signaling cascades that alter the expression of phenotypic markers in tissue macrophages in human beings and in animal models of disease. Recent position pieces and reviews have moved beyond a strict division of macrophages into defined *inflammatory vs anti-inflammatory, M1 vs M2, or conventionally vs alternatively activated* phenotypes.[29,30] It is clear that the tissue, strain, and species under study and the injury or disease that activated the macrophages can produce many different outcomes. In the GI tract, specific enzymes and cytokines have been identified when the tissues are challenged by injury or disease; these include iNOS, TNFα, various interleukins and cytokines, HO-1, arginase-1, or CD206. The association of these molecules with inflammation, repair, or suppression of inflammation is summarized in Table 1. Some of these markers are reliable when studied in some species but not others; for example, iNOS is expressed robustly in mouse macrophages in inflammation but is repressed epigenetically in human macrophages.[31] Arginase-1 and Ym1 are examples of other proteins that are expressed in mouse but not human M2 macrophages.[32] Many of the markers are autocrine factors that feed back to increase or decrease the level of inflammation in injured tissues, or paracrine factors that support

Table 1. Protein Markers Used to Study and Characterize Macrophages, Monocytes, and Dendritic Cells in the GI Tract, Whether Associated With Proinflammatory or Anti-inflammatory States of the Cells or as Markers of Phenotype and Cell Type

Proinflammatory markers		Anti-inflammatory markers		Other markers	
Marker	Notes	Marker	Notes	Marker	Notes
iNOS	Not in human	CD206	Classic M2 marker	MCP1	Monocyte recruitment
IL1β		HO-1	Produces CO	CSF1	Monocyte differentiation
IL6		Arginase-1	Lowers substrate for iNOS activity, not human	BMP2	Cellular signaling
IL12		TGFBR2	Receptor of TGFβ	CD115	CSF1 receptor
IL23		CD163	Heme transport	CX3CR1	Also on lymphocytes
TNFα	Target of infliximab, adalimumab, and so forth	IL10		MHCII	Antigen-presenting cells
CCR2	Receptor for MCP1	5-HT2B	Receptor for serotonin	CD68	Phagocytic cells
Ly6C	High on proinflammatory monocytes	5-HT7	Receptor for serotonin	F4/80	Epitope on EMR1 (mouse only)
CD11B	Also known as integrin αM, MAC1, CR3	Nicotinic α7R	Receptor for acetylcholine	CD64	Also known as FcγR1
SMAD7	Suppressor of TGFβ signaling	CD103	Dendritic cells	CD11c	High on dendritic cells
E-cadherin	Dendritic cells			CD62L	Also known as L-selectin. Also in lymphocytes
				Gr1	Epitope on human Ly6G
				MyD88	Toll-like receptor adapter protein

NOTE. For details and references see text. Figure 2 provides additional information.
CD62L, L-selectin; MHCII, major histocompatibility complex II; MyD88, myeloid differentiation primary response gene 88; TGFBR2, transforming growth factor-β receptor II.

or repress the invasion and differentiation of cells in the affected tissue. IL10 is an example of an autocrine factor that is released from and promotes CD206-positive, anti-inflammatory macrophages, while suppressing proinflammatory markers in other macrophages and immune cells.[33,34] For example, in *Toxoplasma gondii* ileitis in the mouse, prostaglandin E2–dependent IL10 production after accumulation of Ly6C^{hi} monocytes inhibits production of tissue-damaging TNFα by neutrophils.[35] We discuss later the changing balance in proinflammatory and anti-inflammatory markers in macrophages of the mouse gastric muscularis propria that leads to discrete temporal patterns of cellular injury during diabetes and the development of gastroparesis.

The eventual phenotype of activated macrophages in disease also is determined significantly by the cellular origin of the cells. The underlying mechanisms can involve activation of monocyte invasion, expansion of the resulting macrophages in situ after monocyte invasion, or expansion of resident macrophages. For muscularis propria macrophages, these mechanisms have not been studied extensively. On the other hand, there have been many studies on mucosal and lamina propria macrophages in animal models of inflammation and human tissues. In the mucosa and lamina propria of C57Bl6 mouse colon, dextran sulfate sodium–induced inflammation initiates a tissue invasion of Ly6C^{hi} monocytes that expand into CX3CR1[int] macrophages, characterized by high levels of IL6, TNFα, CCR2, IL1β, IL12, and IL23.[12,36] At the same time, the CX3CR1[hi] population, representing the resident macrophages, does not change because it is the infiltrating monocytes that develop into a CX3CR1[int] phenotype. In fact, during the same period of time, the CX3CR1[hi] population maintains its *anti-inflammatory* phenotype, characterized by high production of CD206, CD163, and transforming growth factor-β receptor II.[12] CCR2 activation by monocyte chemoattractive protein-1 (MCP-1) has an important role in Ly6C^{hi} monocyte accumulation in inflamed tissues as indicated in studies on the CCR2 knockout mouse showing reduced systemic inflammation[37] and low levels of monocyte invasion in atherosclerosis,[38] type II diabetes, and obesity.[39] In the GI tract, CCR2 knockout mice have reduced mucosal inflammation and damage after dextran sulfate sodium colitis[40] and in rats, 2,4,6-trinitrobenzene sulfonic acid–induced injury to the muscularis propria is reduced by MCP-1–neutralizing antibodies.[18] In surveying the complexities of macrophage activation, it is evident that the mechanisms and regulation of activation are not worked out in sufficient detail and certainly not for the muscularis propria. However, there are real opportunities for understanding and treating disease by examining these important cellular processes.

Potential Roles of Macrophages
Regulation of Excitable Cells in the Muscularis Propria

Macrophages have been observed in the muscularis propria in proximity to neuronal processes and interstitial cells of Cajal (ICC), cells that are fundamental for the maintenance of normal GI function.[13,16,41] In this context, these muscularis macrophages have been proposed not only to be immune regulators, but also to play a housekeeping role and be involved in the maintenance of normal motility in the healthy gut. With respect to ICCs, muscularis macrophages are observed to be enveloped by and in close association with ICC in healthy mouse and human GI tract,[42] suggesting a possible role for them in the regulation of ICC network organization and integrity. In disease, the expression of proinflammatory markers such as iNOS is associated with ICC network damage, whereas expression of anti-inflammatory markers is associated with preservation of ICC networks in diabetic mice with delayed gastric emptying[21] (see later). In disease, although macrophages clearly are activated or polarized in some manner, it is not clear whether unpolarized, resident macrophages also have a role in ICC network maintenance. The interaction of macrophages with neurons is indicated by the variety of neurotransmitter receptors that are present on macrophages that functionally regulate macrophage phenotype including neurokinin receptors, glycine receptors, nicotinic α7 acetylcholine receptors, and P2 purine receptors.[43–46] The converse also is true, neurons express receptors for macrophage-derived signaling molecules that alter neuronal activity and survival including bone morphogenetic protein (BMP)2[27] and TNFα.[47] Nitric oxide produced by iNOS in mouse (but not human) macrophages[31] suppresses neuronal[48] and smooth muscle excitability.[49] Thus, neuronal excitability can be impaired significantly when adjacent macrophages in the muscularis propria are activated, especially in disease; as discussed in the section below on known roles of macrophages in gastrointestinal function and disease.

Muscularis propria macrophages seem to interact preferentially with only specific subtypes of neurons, namely those expressing choline acetyltransferase and neuronal NO.[41] Because those subtypes of neurons appear to be reduced with age,[50–52] as well as in particular pathologies affecting GI function, it has been proposed that macrophages play a potential role in the regulation of the enteric nervous system plasticity and development.[27] In support of this conclusion, op/op mice that lack muscularis macrophages have more total enteric neurons compared with wild-type littermates,[27] although it has been not reported whether this phenomenon affects all neurons or only specific subtypes. It will be important to further elucidate the role of macrophages in pathologies affecting the GI tract and characterized by neuronal loss.[53]

Reciprocal Interaction With Microbiome

Gut microbiota and their products can affect GI motility by interacting with key cells that regulate GI motility including enteric neurons, glial cells, enterochromaffin cells, and muscularis propria macrophages.[27,54,55] The ability of different microbes and their products to modulate immune responses in the lamina propria of the gut has been studied extensively.[56] Intestinal macrophages play an important role in sensing microbial signals and produce IL1β,

required for RAR-related orphan receptor γ-positive innate lymphoid cell–driven granulocyte monocyte colony stimulating factor (aka CSF2) production, which in turn regulate regulatory T-cell numbers.[57] In addition to the effect on immune homeostasis, recently the mechanisms by which gut microbiota interact with muscularis propria macrophages and potentially influence gastrointestinal motility have been identified.[27] Muscularis macrophages secrete BMP2, which signals constitutively to enteric neurons through the BMP receptor. In turn, enteric neurons contribute to the maintenance of muscularis macrophages through secretion of the macrophage growth factor, CSF1. Antibiotic treatment diminishes the expression of BMP2 and the number of macrophages, as well as signaling via the BMP receptor and expression of CSF1 in neurons, and results in changes in GI transit.[27] These results strongly suggest a regulatory role for gut microbiota on the enteric neurons by affecting the muscularis macrophages, however, further work is needed to elucidate the specific signaling pathways by which microbes or their specific products mediate this effect.

Gut microbiota also can influence gastrointestinal motility by signaling the host to increase serotonin (5-hydroxytryptamine [5-HT]) biosynthesis in enterochromaffin cells via fermentative end products such as short-chain fatty acids and bile acids.[55,58] 5-HT is a neurotransmitter that activates a large variety of receptors on cells in the muscularis propria and plays an important role in gastrointestinal motility. Macrophages as a target of serotonin have not been explored in the GI tract but human alveolar macrophages express both 5-HT2B and 5-HT7 serotonin receptors and activation of those receptors increases the expression of anti-inflammatory markers and reduces the expression of proinflammatory markers.[59] We are not aware of any data indicating expression of serotonin receptors on GI tract macrophages and this serves to emphasize that a detailed transcriptional profiling of subsets of muscularis propria macrophages such as that performed by Martinez et al[23] is necessary and would be revealing. Although the earlier-described studies highlight the effect of gut microbiota on intestinal macrophages, future work will shed light on the role of gut microbiota/microbial products on potential cross-talk between macrophages and other key cell types involved in gastrointestinal motility such as enteric glia and ICCs.

Known Roles of Macrophages in Gastrointestinal Function and Disease

Studies on a variety of GI diseases and disorders have indicated that the activation of specific macrophage-mediated pathways in different subsets of cells results in either sustained impairment of tissue function and morphology or activation of potential mechanisms for repair and recovery of function. The extreme heterogeneity of tissue-resident macrophages during homeostasis and inflammation shows that a macrophage often is not just *M1* or *M2* when residing in a specific tissue,[29,60,61] therefore it is important to characterize these signatures, determine the important effector molecules, their mechanism of effect, and

the principal cellular targets. Progress has been made by the study of several conditions in which a contribution of the innate immune system is either a part of, or the major mediator of, alterations in function. In this section we discuss those conditions in pathologies affecting the GI tract.

Ileus

Ileus is a prolonged decrease in the rate of movement of intestinal contents that can be induced after sepsis or after abdominal surgery. The bacterial product, lipopolysaccharide, is sufficient to reproduce the symptoms of sepsis-induced ileus including macrophage activation and reduced GI transit and motility.[62,63] Cannon and Murphy[64] identified that it is handling of the intestine that results in postoperative ileus, and although modern surgical techniques have reduced the likelihood of the development of severe ileus, it continues to be a complication of intestinal surgery that significantly extends hospital stays for the affected patients and can be fatal.[65,66] The underlying mechanisms for the development of ileus have been studied extensively because of the existence of reliable animal models that recapitulate the pathophysiological changes observed for ileus in human beings. For postoperative ileus, these animal studies have shown that an initial, neuronally mediated inhibition of motility[67,68] and also mast cell degranulation is followed by a late inflammatory phase caused by activation of resident macrophages in the muscularis externa, which release chemoattractant molecules that in turn stimulate the population of the tissue with large numbers of monocyte-derived macrophages.[14] TNFα, IL6, MCP-1 CCL2, IL1β, and other cytokines and chemokines produced by these macrophages suppress motility by effects on smooth muscle and both intrinsic and extrinsic nerves.[69]

Neurons are both the targets and the mediators of the macrophage response in postoperative ileus. Stimulation of the vagal nerve after induction of ileus has been shown to reduce the release of proinflammatory cytokines and, consequently, inflammation as a result of the effect of released acetylcholine on α7 nicotinic receptors expressed on macrophages.[70,71] This effect can be reproduced by using selective α7 nicotinic receptors agonists to treat gastric ileus.[72] Because vagal innervation is most dense in the stomach and proximal small intestine,[73] vagal nerve activity is most likely to regulate inflammation in those regions. In the distal GI tract, the interaction of other cholinergic nerves with macrophages, including possibly intrinsic enteric motor neurons, is plausible but has not been shown. It also is not clear whether release of anti-inflammatory cytokines from M2-like macrophages are affected by vagal nerve stimulation in the GI tract, and it is also not clear whether all macrophages express α7 nicotinic receptors. This requires further research because it was reported that IL10, a potent cytokine derived from M2-like macrophages, which suppresses expression of inflammatory cytokines by M1-like macrophages,[33] is necessary for recovery from postoperative ileus.[74] Thus, parallel mechanisms may be exploited for suppression of proinflammatory macrophages and up-regulation of anti-inflammatory macrophages in

treatment of ileus. Up-regulation of HO-1 in macrophages of the intestinal muscularis externa is one effective treatment for ileus[74] that exploits the potent cytoprotective effects of HO-1 and its active product, carbon monoxide.[75] Indeed, inhaled carbon monoxide is effective in the treatment of postoperative ileus in mice.[76] Activation of glycine receptors to reduce proinflammatory cytokine production by macrophages and the resulting impairment of smooth muscle contractility is another effective way to reduce the degree of ileus after surgery in rats.[77]

The animal studies have shown that macrophage activation is central in the cascade of cellular and molecular events that result in ileus, and that modifying macrophage phenotype can ameliorate or treat the symptoms of ileus. Furthermore, the process of studying postoperative ileus in animal models has identified interactions between macrophages and the other cells in the GI tract that are relevant to other gastroenteropathies as well as normal physiology and the development of the GI tract. These observations in animal models should be translatable to treatment of ileus in affected patients, but at present this has not yet been exploited fully.

Inflammatory Bowel Disease

In inflammatory bowel disease (IBD), macrophages and dendritic cells have been identified as homeostatic regulators of mucosal integrity and function by interacting with T cells and responding to T-cell activation.[78] Because inflammation in the intestinal mucosa represents one of the defining pathologies of IBD, the mucosa is the focus of research into understanding the causes and opportunities for treatment of these diseases. Genetic risk factors that impair the mucosal barrier and exposure to microbes appear to determine whether inflammation occurs at the mucosal level, but how this results in changes of the macrophage activation in the muscularis, as well as changes in motility, have received limited attention.[18]

Inflammatory changes in the mucosa are a consequence of activation of T cells with lamina propria dendritic cells and CX_3CR_1-positive macrophages playing a central role in these responses.[79] The effectiveness of TNFα-neutralizing antibodies (infliximab, adalimumab, dolimumab, and so forth) in the treatment of IBD[80] indicates that proinflammatory cytokines released from macrophages, such as TNFα, contribute to the disease. In addition, TNFα neutralization induces CD206-positive, regulatory macrophages,[81] which can increase T-cell apoptosis[82] and contribute to mucosal healing.[83] Several powerful anti-inflammatory molecules are generated by regulatory or alternatively activated macrophages including transforming growth factor (TGF)-β and IL10.[84,85] TGF-β signaling is tightly linked to the development of colitis. For example, in patients with IBD, the absence of an anti-inflammatory response to TGF-β was associated with up-regulation of SMAD7, a suppressor of TGF-β signaling.[86] IL10 promotes immune tolerance, suppresses Th1 immune responses, and appears to be a central determinant or gatekeeper of the pattern of immune responses.[85] The importance of IL10 signaling in the mucosa is indicated clearly by the severe colitis that develops in IL10-receptor knockout mice.[87] However, administration of recombinant IL10 to patients with inflammatory bowel disease has not proved effective in treating the disease,[88,89] possibly because IL10 signaling is most important in preventing the initiation of inflammation rather than resolving established responses. In IL10-receptor knockout mice, knockout of Toll-like receptor signaling in myeloid differentiation primary response gene 88–deficient mice prevents colitis,[90] which supports the suggestion that IL10 mediates initiation of inflammation in response to Toll-like receptors. Interactions between dendritic cells and T cells also depend on which subtypes of dendritic cells are examined because, similar to macrophages, dendritic cells can show diverse phenotypes. CD103-positive dendritic cells suppress colitis,[91] whereas E-cadherin–positive dendritic cells appear to promote inflammation via increased Th17 responses.[92]

The persistence of GI dysmotility during and after infection or inflammation is a well recognized but incompletely understood phenomenon.[93] It may be mediated partly by molecules generated by immune cells. In this respect, persistent symptoms during remission from IBD are similar to postinfectious irritable bowel syndromes in which symptoms persist after an infection and the accompanying macroinflammation has resolved.[94,95] Changes to enteric neurons, glia, ICC, and smooth muscle have been reported,[96,97] as well as effects on extrinsic nerves.[98] However, although macrophage activation in the muscularis is known to occur after immune cell infiltration in both type 1 and type 2 immune responses,[99] the role of cytokines that are derived from macrophages and persistently up-regulated postinflammation is not well defined. For example, there may be a residual maintenance of changes to cellular function and activity mediated by macrophage-derived factors.

Gastroparesis

Gastroparesis is defined as delayed gastric emptying that is not accompanied by mechanical obstruction. Symptoms usually include early satiety, nausea, vomiting, and abdominal pain.[100–102] Gastroparesis affects people with diabetes, but also can be idiopathic or iatrogenic. Because of the development of animal models of diabetic gastroparesis[53,103] in which delayed gastric emptying developed in a subset of diabetic mice,[104] it has been possible to explore the cellular changes in gastroparesis and translate those observations into studies on human tissues. As a result, there is a growing body of evidence that macrophages are involved in the cellular changes that are evident in gastroparesis.[20,21,105] Changes in CD206^{+} macrophage populations have been correlated directly with the development of delayed gastric emptying in diabetic NOD mice[21] with the expression of HO-1 in those cells appearing to provide a mechanism for protecting ICC when diabetes develops and neuronal NO activity from neurons has been down-regulated.[104] In human subjects with diabetic gastroparesis, CD206^{+} cell numbers in the muscularis propria of the gastric body correlated with ICC numbers,[20,106] and

ICC loss is the cellular defect that most strongly associates with delayed gastric emptying.[106] Loss of the anti-inflammatory CD206+ cells causes the cellular injury in diabetic gastroparesis because the direct damaging effects of M1-like macrophage cytokines, oxidative stress, high glucose, and insulin resistance were not being negated by the cytoprotective effects of anti-inflammatory cytokines and the HO-1 metabolite carbon monoxide generated by the CD206+ cells.[104,107] However, studies in the op/op, macrophage-depleted mouse also have determined that in the absence of any macrophages, diabetic op/op mice do not develop delayed gastric emptying and ICC networks are preserved.[108] Thus, the CD206- macrophages that appear to express iNOS and are abundant in diabetic NOD mice with delayed gastric emptying[104] must be up-regulated to result in cellular damage and delayed gastric emptying. The source of these cells and mechanisms for up-regulation require further investigation and the results will need translating into studies in human gastroparesis.

There are few effective treatments for gastroparesis but the evidence that active regulation of macrophage phenotype contributes to the development of delayed gastric emptying in mice and ICC loss in human beings suggests that targeting the polarization of macrophages might be an effective therapy. Indeed, in mice, up-regulating HO-1 in CD206+ gastric muscularis macrophages reverses delayed gastric emptying in diabetic NOD mice[104] and delivering the product of HO-1 activity, which is CO, has the same effect.[107] IL10 also has shown promise in this animal model by increasing the expression of HO-1 in the gastric body of diabetic NOD mice and returning delayed gastric emptying, electrical slow-wave abnormalities, and ICC network changes to normal.[109] This is consistent with the known effects of IL10 in suppressing proinflammatory macrophages and increasing anti-inflammatory macrophages.[33] IL10 is known to be safe for use in human beings and could be more effective in treating a disorder such as diabetic gastroparesis, in which macrophage changes appear to be a cause of the damage and functional changes to the tissue as opposed to inflammatory bowel diseases such as colitis in which a large variety of immune responses are causing the disease and IL10 did not produce a clear clinical response.[110] The recent demonstration that proinflammatory macrophages are necessary for the development of delayed gastric emptying in diabetic mice[108] also suggests that neutralizing the proinflammatory cytokines generated by conventionally activated, M1-like macrophages may be another opportunity for treatment.

In conclusion, macrophages are involved in many processes that control gastrointestinal motility in health and disease. In addition to being the mediators of responses to injury and disease, they appear to have roles in the development and regulation of cells in the healthy GI muscularis propria. This diversity of roles is reflected in many mechanisms for macrophage activation and up-regulation of various phenotypic markers. The responses and phenotypes are tissue- and disease-dependent and further understanding of these intricacies represents opportunities for better understanding of GI motility disorders and potentially treating the disorders.

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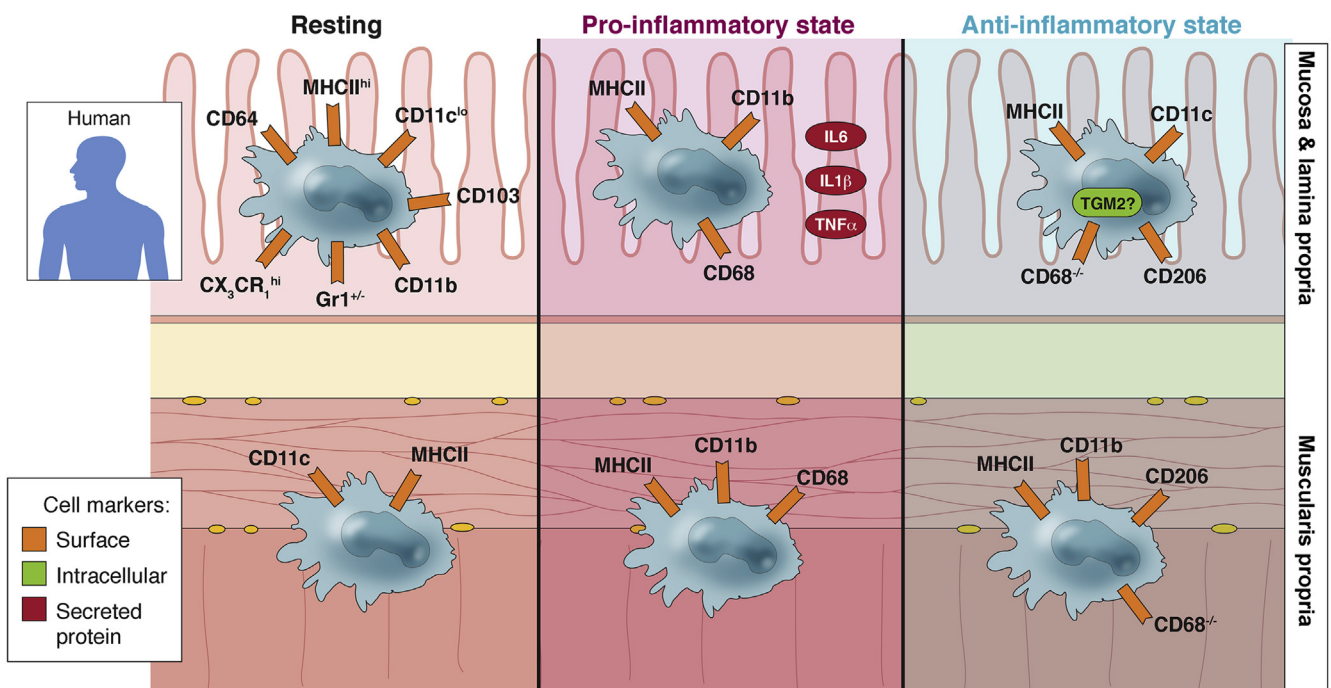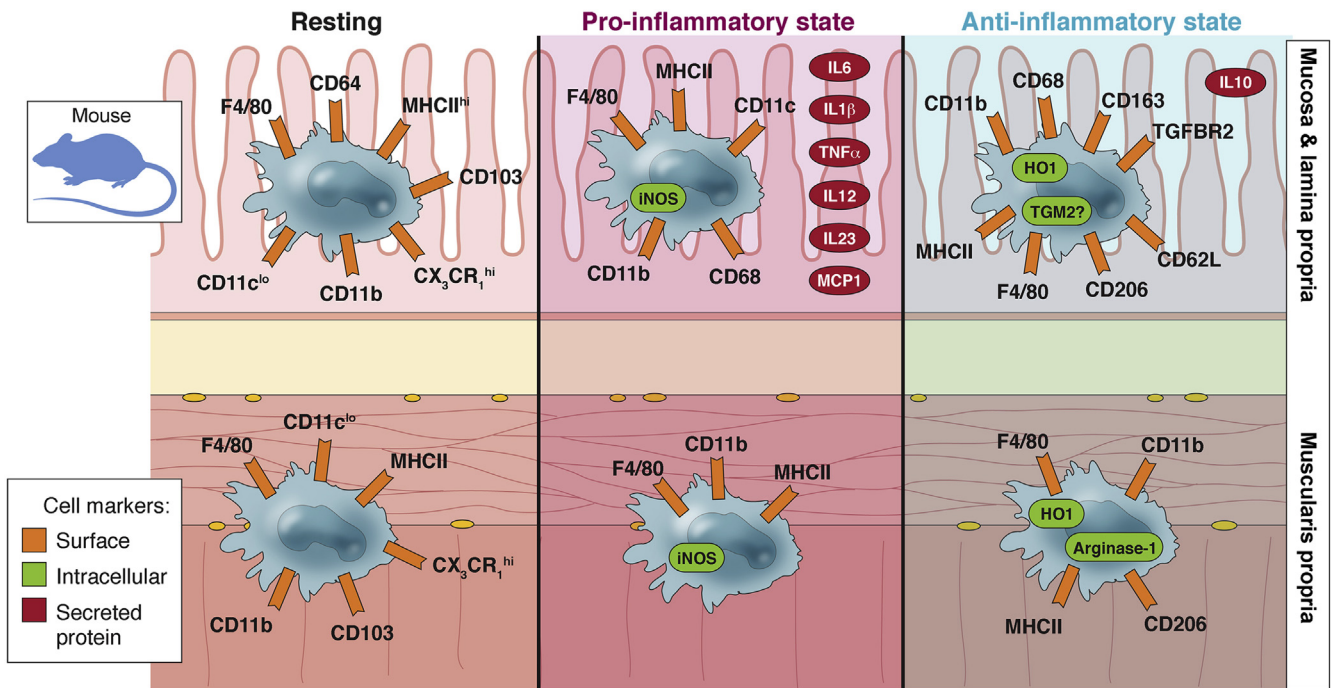
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Supplemental Poster. **Tissue resident macrophages in the gastrointestinal wall express a variety of cell-surface and intracellular markers as well as secreted cytokines and chemokines.** These markers change in response to injury or disease. If the macrophages polarize towards a pro-inflammatory state then the cells contribute towards tissue injury and death and increase inflammation, if the macrophages polarize towards to an anti-inflammatory state then the cells release molecules that can reverse inflammation and promote tissue healing and repair. Phenotypic characterization of macrophages is more thorough in the mucosa and lamina propria of both humans and rodents. Data on the source and mechanism of activation of macrophages in the muscularis propria is less well characterized. It should also be noted that certain proteins including iNOS and arginase-1 are expressed in rodent macrophages but not in human macrophages. (Cell Mol Gastroenterol Hepatol 2016;2:120–130;http://dx.doi.org/10.1016/j.jcmgh.2016.01.003)