

REVIEW

The Role of Intestinal Macrophages in Gastrointestinal Homeostasis: Heterogeneity and Implications in Disease

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SUMMARY

Intestinal macrophages are the most abundant immune cells in our gut but understanding of their function is impeded by their heterogeneity. In this review we dissect different subtypes of intestinal macrophages and highlight their roles in several gastrointestinal (GI) disorders.

Intestinal macrophages play a key role in the gut immune system and the regulation of gastrointestinal physiology, including gut motility and secretion. Their ability to keep the gut from chronic inflammation despite constantly facing foreign antigens has been an important focus in gastrointestinal research. However, the heterogeneity of intestinal macrophages has impeded our understanding of their specific roles. It is now becoming clear that subsets of intestinal macrophages play diverse roles in various gastrointestinal diseases. This occurs through a complex interplay between cytokine production and enteric nervous system activation that differs for each pathologic condition. Key diseases and disorders in which intestinal macrophages play a role include postoperative ileus, inflammatory bowel disease, necrotizing enterocolitis, as well as gastrointestinal disorders associated with human immunodeficiency virus and Parkinson's disease. Here, we review the identification of intestinal macrophage subsets based on their origins and functions, how specific subsets regulate gut physiology, and the potential for these heterogeneous subpopulations to contribute to disease states. Furthermore, we outline the potential for these subpopulations to provide unique targets for the development of novel therapies for these disorders. (*Cell Mol Gastroenterol Hepatol* 2021;12:1701-1718; <https://doi.org/10.1016/j.jcmgh.2021.08.021>)

Keywords: Enteric nervous system (ENS); gastrointestinal (GI) disorders; gut inflammation; intestinal macrophages.

Intestinal macrophages are key resident immune cells in the gastrointestinal (GI) tract. Although they play a macrophage-conventional role in recognizing and combating pathogenic bacteria, unlike macrophages in other organs they also reduce systemic inflammation in the gut lumen. This role is crucial given their proximity to the interface between the GI tract and myriad microbiota.

Intestinal macrophages therefore are widely studied for their ability to maintain gut homeostasis while simultaneously sampling both harmful and commensal bacteria in the lumen. Recent work has suggested that disruptions to macrophage abundance and function contribute to altered gut physiological homeostasis and chronic hyperinflammation associated with many GI diseases.

The mucosal barrier of the GI tract is constantly exposed to foreign antigens and thus requires precise functioning of the immune system to prevent systemic infection. Gut homeostasis is orchestrated mainly by the interplay between the immune system, the enteric nervous system (ENS), and intestinal microbiota that include bacteria, viruses, fungi, and archaea.¹⁻⁴ Within the immune system, macrophages, dendritic cells, and innate lymphoid cells also regulate gut function.⁵⁻⁹ Macrophages play a prominent role in combating pathogens by phagocytosis as well as presenting antigens to activate the adaptive immune response. Conventional macrophages are activated upon bacterial stimulation and subsequently induce an inflammatory response via the release of proinflammatory cytokines.¹⁰ Intestinal macrophages differ from conventional macrophages in terms of phenotypic profiles, cytokine production, and their role in repressing responses to bacterial infection to protect the gut from excessive inflammation.¹¹ In addition to their

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Abbreviations used in this paper: $\alpha 7$ nAChR, $\alpha 7$ nicotinic acetylcholine receptors; AH, afterhyperpolarization; AIDS, acquired immunodeficiency syndrome; $\beta 2$ -AR, $\beta 2$ -adrenergic receptor; BMP2, bone morphogenetic protein 2; CCR2, C-C motif chemokine receptor 2; CD, cluster of differentiation; CNS, central nervous system; CSF1, colony-stimulating factor 1; CX3CR1^{int}, intermediate levels of the CX3C chemokine receptor 1; DSS, dextran sulfate sodium; ENS, enteric nervous system; GI, gastrointestinal; HIV, human immunodeficiency virus; IBD, inflammatory bowel disease; IBS, irritable bowel syndrome; IEC, intestinal epithelial cell; IL, interleukin; IL10R, interleukin 10 receptor; LPS, lipopolysaccharide; Ly6C^{hi}, high levels of the circulating lymphocyte antigen 6 complex; MHCII, major histocompatibility complex class II; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; mRNA, messenger RNA; MyD88, Myeloid differentiation primary response 88; NEC, necrotizing enterocolitis; NF- κ B, nuclear factor- κ B; NO, nitric oxide; P, population; PD, Parkinson's disease; POI, postoperative ileus; SIV, simian immunodeficiency virus; Smad7, mothers against decapentaplegic homolog 7; TGF, transforming growth factor; TNBS, 2,4,6-trinitrobenzene sulfonic acid; TNF α , tissue necrosis factor α ; TRPV4, transient receptor potential vanilloid 4; WT, wild-type.

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role in gut immunity, intestinal macrophages interact with the ENS to regulate gut secretion and motility. Thus, changes in the function and abundance of intestinal macrophages potentially contribute to different diseases associated with gastrointestinal tract.

Intestinal macrophages are highly heterogeneous in terms of their function and localization within the different gut layers. They are conventionally classified into 3 main groups: monocyte-derived mature macrophages, monocyte-derived inflammatory macrophages, and self-maintaining macrophages. Deciphering the precise mechanisms by which these subtypes of intestinal macrophages contribute to maintaining homeostasis or exacerbate pathology in different diseases may provide avenues for subtype-specific therapeutic targets. Most current research has used transgenic and chemically induced mouse models of disease to investigate macrophage activity in pathologic conditions.¹²⁻¹⁹ In mice, multiple intestinal macrophage subtypes are present, identified by the expression of surface proteins and categorized according to their cell lineage, localization, and characteristics of their inflammatory response to infection.²⁰ It is therefore important to dissect this heterogeneity of intestinal macrophages and their functions (such as interactions with the ENS), however, the complexity in identifying macrophage subtypes has to date impeded research into their potential contribution to disease.

Here, we outline the characteristics of known subtypes of intestinal macrophages and their interactions with the ENS. We then highlight the roles of these macrophages in gastrointestinal physiology, including in the regulation of inflammatory responses, gut secretion, and motility under healthy conditions. Finally, based on disease-specific evidence, we describe the potential roles of intestinal macrophage subsets in inflammatory diseases impacting the GI tract such as inflammatory bowel disease (IBD), postoperative ileus (POI), necrotizing enterocolitis (NEC) and gastrointestinal disorder associated with human immunodeficiency virus (HIV) and Parkinson's disease (PD).

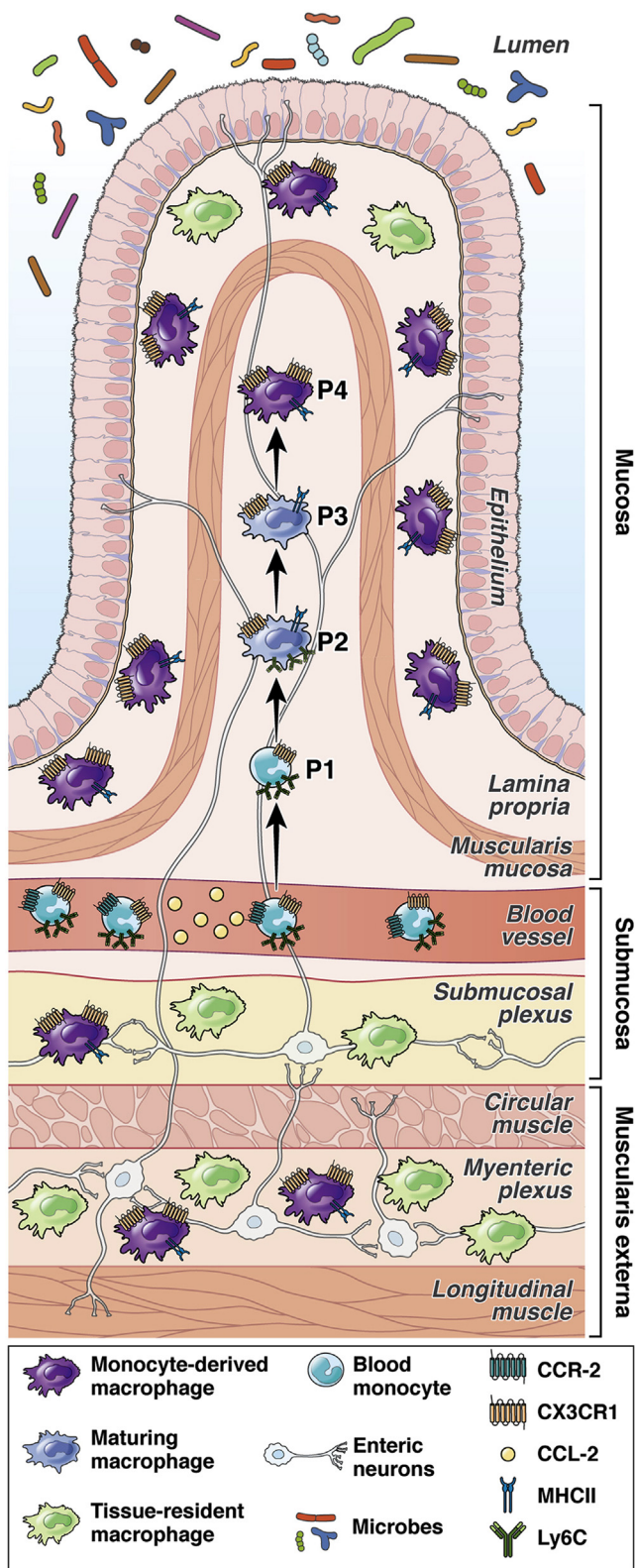


Figure 1. (See previous column) Differentiation, maturation, and layer-specific localization of intestinal macrophages. In steady-state conditions, circulating blood monocytes extravasate into the gut in a C-C motif chemokine ligand 2 (CCL-2)/CCR2-dependent manner. Newly extravasated monocytes (P1) undergo differentiation via 2 phases (P2 and P3) into mature intestinal macrophages (P4). During P1, monocytes express Ly6C^{hi} and CX3CR1^{int} first acquire MHCII expression. Subsequently, these Ly6C^{hi} CX3CR1^{int} MHCII⁺ maturing macrophages represent the P2 differentiation phase. Ly6C expression then is down-regulated, forming Ly6C⁻ CX3CR1^{int} MHCII⁺ macrophages (P3) as the differentiation process continues. Finally, with the up-regulation of CX3CR1, Ly6C⁻ CX3CR1^{hi} MHCII⁺ macrophages complete the maturation process to reach P4. At P4, although macrophages reside mainly in the lamina propria of the gut, they also are present in the submucosal plexus and muscularis externa (subtype 1). Apart from these monocyte-derived intestinal macrophages, self-maintaining resident macrophages (subtype 3) originating from embryonic precursors and bone-marrow-derived monocytes are present predominantly in the submucosa and muscularis externa, but also are found within the lamina propria.

Ontogeny and Heterogeneity of Intestinal Macrophages

Intestinal macrophages are derived from continuous replenishment of monocytes that express high levels of the circulating lymphocyte antigen 6 complex (Ly6C^{hi}). These (Ly6C^{hi}) monocytes extravasate into the GI tissue in both healthy and inflamed gut in a C-C motif chemokine ligand 2/C-C motif chemokine receptor 2 (CCR2)-dependent manner.^{14,21} Under steady-state conditions, mouse intestinal macrophages undergo a cascade of differentiation from population 1 (P1; newly extravasated monocytes) to P4 (fully differentiated resident macrophages). Similar to the general population of blood monocytes, newly extravasated P1 monocytes express high levels of the Ly6C (Ly6C^{hi}) surface markers and intermediate levels of the Cysteine-X3-Cysteine chemokine receptor 1 (CX3CR1^{int}). By approximately P2, maturing macrophages acquire major histocompatibility complex class II (MHCII) expression. This is followed by down-regulation of Ly6C expression by P3, and, finally, high levels of CX3CR1 expression occurs in the mature form (P4) of intestinal macrophages (Figure 1). During inflammation, P1 monocytes differentiate into proinflammatory (subtype 2) macrophages that lack up-regulated CX3CR1 expression. Taken together, subtypes 1 and 2 intestinal macrophages are both derived from circulating monocytes and reside predominantly in the lamina propria, but respond differently according to inflammation state.

In addition to mature and proinflammatory macrophages that continuously are replenished from blood circulating monocytes, a third major type of intestinal macrophage (subtype 3) is derived from either embryonic precursors or bone marrow monocytes, and resides specifically in the lamina propria and muscularis externa layer of the gut.²² In adult mice, intestinal macrophage populations in these specific regions consist of both tissue resident and circulating monocyte-derived macrophages. Recent work has identified a self-maintaining population of macrophages that originates from both embryonic precursors and adult bone marrow-derived monocytes. These macrophages are identified by high expression levels of cluster of differentiation 4 (CD4) and T-cell immunoglobulin and mucin-domain containing 4 expression.²³ However, they have a relatively low replenishment rate from blood monocytes compared with Ly6C^{hi} MHCII⁺ CX3CR1^{hi} macrophages, suggesting that this type of intestinal macrophage is maintained locally. Collectively, intestinal macrophages are classified into these 3 subtypes (Table 1).

The 3 intestinal macrophage subtypes also show functional heterogeneity. Subtype i macrophages show increased tolerance to microbial stimulation while, during inflammation, P1 monocytes differentiate into proinflammatory (subtype 2) macrophages, which produce proinflammatory cytokines such as tissue necrosis factor α (TNF α), interleukin 1 β (IL1 β), and IL6.²⁴ Subtype iii macrophages are reported to be involved in interactions with ENS, which regulates gut motility and secretion.²²

Because the tissue localization of macrophages often correlates with functional attributes, isolating macrophages from specific layers of the gut can provide a better understanding of their specific roles. Two of the 3 macrophage subsets (monocyte-derived mature macrophages, subtype 1; and monocyte-derived inflammatory macrophages, subtype 2) that are replenished by blood monocytes accumulate mainly in the lamina propria but also are present in other compartments of the gut.^{20,25} Of note, Honda et al²⁶ discovered that CX3CR1⁺CCR2^{lo/-} macrophages (subtype 1) form a lattice network that is in close contact with the lamina propria vasculature. Interestingly, these perivascular macrophages are regulated by the gut microbiome and are crucial in vascular repair. In contrast, self-maintaining resident macrophages (subtype 3) are found predominantly within the muscularis externa, although they also are present in the lamina propria. In particular, resident macrophages are located in close proximity to enteric neurons, Peyer's patches, Paneth cells, and blood vessels, and therefore are poised to facilitate neuroimmune interactions. Both their specific localization plus findings from transcriptional profile analysis imply a unique role for this subset of self-maintaining macrophages in gut homeostasis.^{22,27} In terms of gene expression, profiles of lamina propria macrophages include more genes responsible for initiating an immune response than muscularis macrophages, whereas genes involved in tissue structural support and wound healing are more prominent in muscularis macrophages.²⁷

Given the difference in origin and functions of the 3 subtypes of intestinal macrophages, it is crucial to carefully identify cellular markers and isolate each group of intestinal macrophages to fully understand their roles in gut homeostasis. However, the overlapping surface markers within each macrophage subtype and with other immune cells hinders our ability to distinguish between them. For example, although intestinal macrophages and dendritic cells share similar phenotypes as the 2 main mononuclear phagocytes in the gut, they also have contrasting functionality.⁹ In contrast with intestinal macrophages, dendritic cells play an important role in initiating the adaptive immune response by priming T cells owing to their ability to migrate to mesenteric lymph nodes.⁹ Despite significant functional differences, the overlapping surface protein expression between these cell types makes it difficult to isolate intestinal macrophages. Therefore, it has been debatable whether studies using protein markers such as CD11c, MHCII, and CX3CR1, which are expressed in both dendritic cells and intestinal macrophages, are specific enough to characterize the roles of either of these immune cells in gut homeostasis. Nevertheless, in the past decade it has been shown that CD64 expression can differentiate intestinal macrophages from dendritic cells in both mice and human tissue.^{9,28} Together with the expression of CD11c and MHCII, it therefore is possible to distinguish intestinal macrophages from dendritic cells within the gut wall in mice.²⁸ Clearly, however, these markers alone are insufficient to distinguish intestinal macrophage subtypes. To overcome this difficulty, it is recommended to use a combination of markers in routine assays involving intestinal macrophages in different layers of the gut.

Table 1. Classification of Subtypes of Intestinal Macrophages in Mice

Subtype of intestinal macrophage	Major surface molecules	Origin	Location	Function	Relevance to disease	References
1) Monocyte-derived mature macrophages	Ly6C ⁺ CD64 ⁺ MHCII ⁺ CX3CR1 ^{hi}	Circulating monocytes	Lamina propria (++)	Secrete anti-inflammatory cytokines	Inflammation-related: IBD, necrotizing enterocolitis, GI disorder associated with HIV	19, 21
2) Monocyte-derived inflammatory macrophages	CD64 ⁺ MHCII ⁺ CX3CR1 ^{int}	Circulating monocytes	Lamina propria (++)	Secrete proinflammatory cytokines	Inflammation-related: IBD, necrotizing enterocolitis, GI disorder associated with HIV	19, 21
3) Self-maintaining macrophages	Tim-4 ⁺ CD4 ⁺	Bone marrow-derived monocytes/embryonic precursors	Lamina propria (+), muscularis externa (++)	Interaction with ENS	Motility/secretion related: POI, GI disorders associated with PD	22, 23

Tim-4, T-cell immunoglobulin and mucin-domain containing 4.

Because macrophages from different origins reside in a single layer of the gut (eg, in the case of the lamina propria), it is challenging to differentiate resident macrophages from monocyte-derived macrophages. However, these cell types can be differentiated based on gene expression and protein markers. Based on transcriptome analysis of monocyte-derived macrophages and resident macrophages, resident macrophages in the lamina propria show higher levels of gene transcripts involved in development and tissue support, suggesting roles in angiogenesis and epithelial cell differentiation. In the muscularis externa, resident macrophages show increased expression of transcripts implicated in cell-cell adhesion and neuronal development relative to monocyte-derived macrophages.²² In lamina propria resident macrophages, up-regulated expression levels of T-cell immunoglobulin and mucin-domain containing 4, CD4, and CD63 compared with their monocyte-derived counterparts also have been reported. Importantly, these differences in expression profiles allow these 2 macrophage classes to be distinguished.²² However, the surface protein expression of self-maintaining macrophages and monocyte-derived macrophages located within the muscularis externa is similar. Currently, only differential gene expression of (ie, protocadherin β 16 and insulin-like growth factor 2 messenger RNA (mRNA) binding protein 3) has been used to distinguish these 2 macrophage subtypes.²² Given the diverse roles and heterogeneity of the 3 subtypes of intestinal macrophages, dissecting the specific localization and cellular subtype present in each gut region will allow these cells and their potential roles in disease to be targeted.

Intestinal macrophages and central nervous system (CNS) microglia share similar functional traits and are derived from the same cell lineage. Microglia are early responders in inflammation and phagocytose cellular debris in the brain and spinal cord. In addition, microglia modify neuronal morphology and activity via synaptic pruning in the CNS.^{29,30} Notably, some microglia-specific genes such as *Cx3cr1*, *Fcrls*, and *Mertk* also are expressed by resident macrophages of the intestine, but not by macrophages from other organs.³¹ Based on the phenotypic and functional similarities between intestinal macrophages and microglia, intestinal macrophages are proposed to contribute significantly to neuroimmune interactions and gut homeostasis.³¹ Recently, it was reported that immune cells in the gut such as macrophages, mast cells, and innate lymphoid cells are capable of communicating with the ENS.^{32,33} However, unlike the substantial evidence for microglial-neuronal communication, little is known about interactions between intestinal macrophages and the ENS.

It is important to bear in mind that despite the extensive work to characterize intestinal macrophages in mice, this characterization cannot fully represent the macrophage populations in human beings. One of the main differences between mouse intestinal macrophages and their human counterparts is in the expression of CX3CR1. In human beings, monocyte-derived gastrointestinal inflammatory macrophages are CD11c^{hi}CCR2⁺CX3CR1⁺ and resident macrophages are CD11c⁺CCR2⁺CX3CR1⁻.³⁴ In contrast, CX3CR1 is

highly expressed in resident macrophages in mouse.¹³ Nevertheless, mouse models remain as powerful investigative tools to characterize these cells while controlling environmental factors, genetic modification, and enabling the use of invasive techniques for understanding disease processes.

Understanding the ontogeny and heterogeneity of intestinal macrophages allows us to design transgenic experimental models to target specific subsets of intestinal macrophages and investigate different signaling pathways and pathologic conditions. For example, Koscsó et al^{35,36} described several mouse models targeting CX3CR1⁺ intestinal macrophages and showed differential immune responses generated by macrophage subsets during *Salmonella* infection. CX3CR1 knockout mice also have been used successfully to decipher the novel signaling pathway of CX3CR1⁺ macrophage-induced IgA production that is independent of Toll-like receptor-mediated microbial recognition.³⁷ This research highlights the importance of using highly specific animal models to understand the specificity of intestinal macrophages.

Interactions of Macrophages With the ENS

Previous reports have shown that intestinal macrophages are in close contact with nitrergic and cholinergic neurons in the myenteric plexus.³⁸ Interestingly, these reports also identified filopodia-like extensions originating from MHCII-expressing intestinal macrophages that extend into α -synuclein aggregates in the myenteric plexus of healthy aged rats, suggesting a phagocytic morphology.³⁸ In addition, intestinal macrophages also form synapse-like structures with neuronal somata and processes in the muscularis layer.³⁸ The synapse-like structure formed between intestinal macrophages and myenteric neurons also has been observed under a transmission electronic microscope, showing that they do not make physical contact with each other but maintain a close distance (200–300 nm) to allow signal transduction.³⁹ Despite this morphologic and structural evidence for close interaction with enteric neurons, it remains unclear how intestinal macrophages communicate with the ENS on a molecular level and in turn regulate gut activity. Notably, Muller et al⁴⁰ reported that a distinct population of macrophages resides in the muscularis externa layer of the gut wall. These muscularis macrophages regulate peristalsis in the steady-state by secreting bone morphogenetic protein 2 (BMP2) to activate BMP receptors expressed in enteric neurons.⁴⁰ Interactions between macrophages and enteric neurons are critical because BMP2 is vital for enteric neuron survival and enteric neurons secrete colony stimulating factor 1 (CSF1), which is essential for macrophage development.⁴⁰ Furthermore, evidence suggests that the distinctive muscularis externa macrophage population, which is essential for the survival of enteric neurons, consists of self-maintaining resident macrophages.²² Whether the mechanism of this supportive function is via CSF1/BMP2 has yet to be deciphered.²²

Intestinal macrophages also exert neuroprotective effects during bacterial infection. The β 2-adrenergic receptor

(β 2-AR) is a key mediator in communication between muscularis macrophages and enteric neurons.²⁷ During bacterial infections, extrinsic sympathetic innervation onto myenteric neurons triggers norepinephrine release, which in turn activates the β 2-AR on muscularis macrophages and causes up-regulation of arginase 1, a prominent M2-associated gene, rendering this cellular population to polarize toward tissue-repairing M2 macrophages. Recently, it also was reported that the neuroprotective role of intestinal macrophages is mediated through the β 2-AR signaling pathway.⁴¹ During bacterial infections, β 2-AR signaling via the arginase 1–polyamine axis provides neuronal protection by limiting caspase-11-dependent neuronal cell death.⁴¹ These studies show direct evidence of the importance of intestinal macrophage communication with enteric neurons.

Microglia, the resident macrophages in the CNS, are engaged in bidirectional cross-talk with neurons in different regions of the brain under healthy and diseased conditions.⁴² Microglia and intestinal macrophages share similarities in surface protein expression, such as the high expression of CX3CR1, and their anti-inflammatory profile.^{31,43,44} More importantly, communication between immune cells and neurons in the CNS has been studied extensively and these mechanisms and pathways show similarities with those occurring in the gastrointestinal system,^{31,45–47} suggesting that intestinal macrophages may play a similar role in the ENS. In this regard, there is evidence showing that some of the tissue-resident macrophages are originated from yolk-sac precursors—the same lineage as microglia.^{22,48} The ENS regulates 2 critical gastrointestinal functions: gut secretion and motility. There is increasing evidence that intestinal macrophages also regulate gut homeostasis through interaction with the ENS.^{40,49} Muscularis macrophages have been shown to regulate intestinal peristalsis in steady-state conditions.⁴⁰ To further investigate neuroimmune interactions in the gut, existing animal models that previously have been used to study microglia could be used to provide a better understanding of macrophage function in the gut–brain axis.⁵⁰

Intestinal Macrophages Influence Gastrointestinal Physiology

Maintaining Gastrointestinal Homeostasis via Regulation of Inflammatory Responses

Intestinal macrophages are capable of protecting the gut from a hyperinflammatory response upon bacterial stimulation through the regulation of surface-receptor expression and cytokine secretion.¹¹ In particular, when encountering gram-negative bacteria, intestinal macrophages down-regulate expression of CD14, a glycoprotein receptor for lipopolysaccharide (LPS), compared with conventional macrophages, to reduce the inflammation response.⁵¹ Intestinal macrophages also can produce IL10 and transforming growth factor β (TGF- β),²¹ 2 prominent cytokines that predominantly contribute to reduced inflammation and homeostasis of the GI tract.

Intestinal macrophages constitutively produce IL10 as well as sensing IL10 via the IL10 receptor (IL10R) to inhibit

the synthesis of proinflammatory cytokines.²¹ IL10R is a heterotetramer consisting of 2 IL10R α and 2 IL10R β subunits. *Il10rb*^{-/-} mice spontaneously develop severe colitis, but in mice with deletion of both *Il10rb* and *Rag2*, a gene exclusively expressed in mature B and T lymphocytes, colitis does not result. These findings indicate that colitis driven by IL10R is lymphocyte-dependent.⁵² The transfer of wild-type (WT) CD4⁺ T cells to *Rag2*^{-/-} *Il10rb*^{-/-} mice leads to a drastic increase in the production of inflammatory cytokines compared with *Rag2*^{-/-} mice, suggesting that deletion of IL10R is the cause of the inflammatory response.⁵² Apart from cytokine production, IL10R signaling also affects the generation and function of regulatory T cells. These are crucial in resolving colitis, as evidenced by the significantly reduced population of forkhead box P3⁺ regulatory T cells in *Rag2*^{-/-} *Il10rb*^{-/-} mice compared with *Rag2*^{-/-} mice.⁵²

The expression of IL10R by macrophages is essential in maintaining an anti-inflammatory response to spontaneous colitis. Of note, the deletion of IL10R impairs the differentiation of blood monocytes to anti-inflammatory macrophages. As a result, *Il10rb*^{-/-} mice show a significant increase in proinflammatory macrophages. *CX3CR1*^{cre/cre} *Il10*^{fl/fl} mice do not show spontaneous colitis, indicating that IL10 derived from CX3CR1^{hi} resident macrophages is not required to regulate the gut anergic phenotype. *CX3CR1*^{cre/cre} *Il10rd*^{fl/fl} mice with loss of IL10R, however, show a proinflammatory profile and develop colitis.⁵³ These findings imply that it is crucial for intestinal macrophages to be able to respond to IL10 through IL10R, which regulates proinflammatory mediators through downstream signaling pathways.⁵⁴

TGF- β also plays an important role in achieving gut homeostasis. For example, upon LPS stimulation the production of proinflammatory cytokines by intestinal macrophages is down-regulated via TGF- β release from intestinal stromal cells.¹¹ TGF- β stimulates blood monocytes to acquire an anti-inflammatory phenotype, with decreased expression of the proinflammatory CD14 receptor that binds directly to LPS. It also has been suggested that the TGF- β receptor plays an important role in regulating monocyte differentiation into homeostatic intestinal macrophages.⁵⁵ Although IL10 similarly induces blood monocytes to shift toward anti-inflammatory macrophages, transcriptome analysis between TGF- β receptor-deficient and IL10R-deficient macrophages show that more than 300 genes are differentially expressed when compared with controls, suggesting that TGF- β and IL10 signaling pathways may work concomitantly to achieve gut homeostasis.⁵⁵ Collectively, these findings show that IL10 and TGF- β signaling in intestinal macrophages plays a central role in maintaining hyporesponsiveness to microbial stimulation, a function that is essential in preventing inflammatory responses to microorganisms at the interface between the lumen and the mucosal epithelium of the gastrointestinal tract.

Involvement of Intestinal Macrophages in Gut Secretion

Gastrointestinal secretion includes the movement of fluid and ions across intestinal epithelial cells (IECs) and

is essential for homeostasis of the body.^{56–58} The submucosal plexus mainly is responsible for regulating gut secretion in the intestinal tract.^{59–61} Despite limited understanding of how intestinal macrophages regulate gut secretion through interactions with submucosal neurons, IL6, a proinflammatory cytokine secreted by intestinal macrophages, is capable of exciting submucosal neurons.²⁸ In addition, cytokines secreted from intestinal immune cells significantly influence IEC activity,⁶² which can impact epithelial integrity and permeability of the mucosal wall. Previously, it was shown that cross-talk between intestinal macrophages and IECs through Toll-like receptor-4 signaling up-regulates expression of the anti-inflammatory cytokine IL10 in these cells.⁶³ Intestinal macrophages also are responsible for small intestine epithelial recovery from nonsteroidal anti-inflammatory drug-induced injury in an IL10-dependent manner.⁶⁴ In addition, macrophages belonging to the muscularis macrophage subgroup are in close contact with submucosal plexus neurons, which contribute to the neural circuitry responsible for gut secretion. Therefore, intestinal macrophages influence gut secretion through interactions with IECs and submucosal neurons.

Fluid movement across IECs is regulated by ion transport and mucosal integrity. Previous studies have shown that both processes are regulated through interactions between the submucosal plexus and intestinal macrophages.^{22,65} Administration of the voltage-gated sodium channel agonist veratridine to intestinal muscle strips containing the submucosal plexus typically increases short-circuit current (as a measure of electrogenic ion movement) across the epithelial membrane. However, this increase of short-circuit current is reduced significantly when self-maintaining macrophages associated with submucosal neurons are depleted using a conditional CX3CR1 knock-out model, implying a role for these cells in regulating intestinal secretion via submucosal plexus.²² Mucosal integrity usually is disrupted during inflammation, enabling toxins to cross the mucosal barrier, which can result in apoptosis of IECs. IL6 produced by proinflammatory macrophages regulates claudin-2 expression, a channel-forming protein vital for controlling ion and fluid secretion along the gut via regulation of tight junction permeability.⁶⁶ Interestingly, a recent study showed that macrophages located within the distal colon form balloon-like protrusions into the epithelium, which prevent the absorption of fungal toxins and thus protect mucosal integrity.⁶⁷ These data illustrate that intestinal macrophages are capable of governing gut secretion by interacting with submucosal neurons via the secretion of cytokines.

Gut Motility Is Regulated by Intestinal Macrophages

Gastrointestinal motility facilitates the mixing of luminal content for digestion in the small intestine, ensures directional movement of stool in the colon, and regulates the frequency of defecation.^{68,69} Intestinal macrophages influence gut motility in both direct and indirect manners by

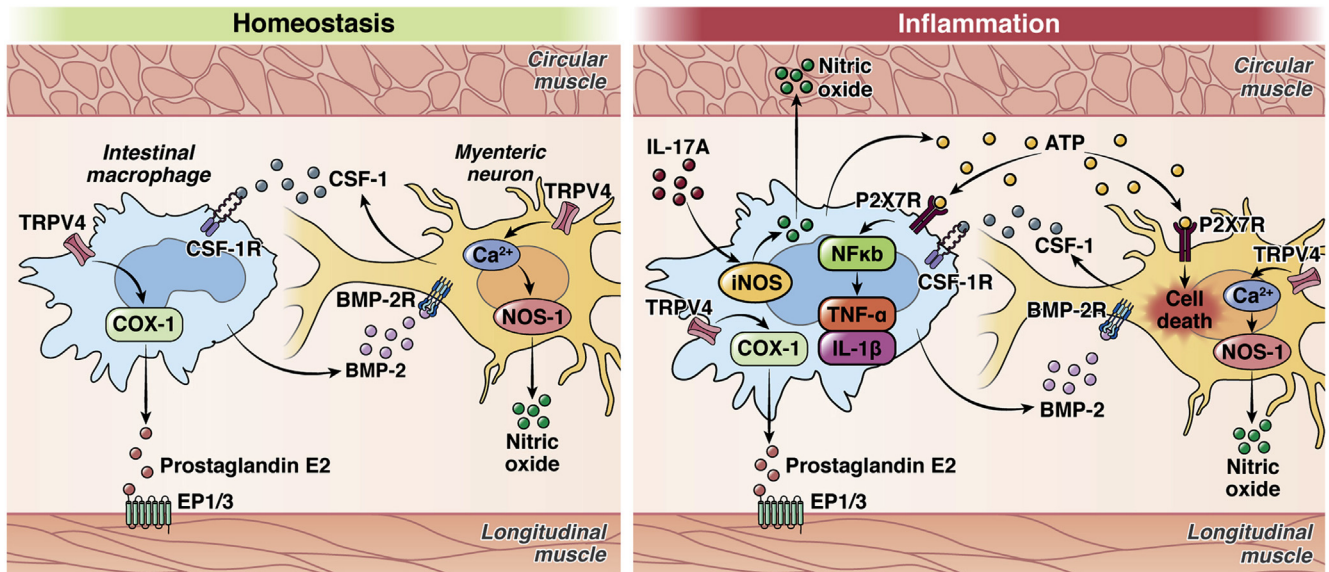


Figure 2. Interaction between intestinal macrophages, smooth muscle cells, and myenteric neurons in homeostasis and inflammation. During homeostasis, intestinal macrophages communicate with myenteric neurons by secreting BMP-2 to activate BMP-2-receptor (BMP-2R) signaling in neurons. Myenteric neurons produce CSF-1, which acts on intestinal macrophages via the CSF-1 receptor. This bidirectional communication is crucial in regulating gut motility in homeostasis. Intestinal macrophages also directly influence circular/longitudinal smooth muscle to regulate gastrointestinal motility. When TRPV4 channels on intestinal macrophages are activated, prostaglandin E2 is generated by cyclooxygenase 1 (COX-1). Prostaglandin E2 activates the G-protein-coupled E-prostanoid receptor 1 or 3 (EP1/3) on smooth muscle cells, which leads to muscle contraction. However, activating TRPV4 channels on enteric neurons induces the NO synthase-1 (NOS-1) via intracellular Ca^{2+} , leading to decreased muscle contractility. Under conditions of inflammation, IL17A is released by infiltrating monocytes and neutrophils. It induces expression of inducible NO synthase (iNOS) in intestinal macrophages, which in turn leads to the production of NO, resulting in circular muscle relaxation. In addition, adenosine triphosphate (ATP) is released by intestinal macrophages and other immune cells during inflammation. Extracellular ATP causes the production of proinflammatory cytokines $TNF\alpha$ and $IL1\beta$ through activating the purinergic receptor P2X7 on macrophages and subsequent NF- κ B activation. P2X7 receptors on enteric neurons also sense high extracellular ATP levels, which lead to neuronal cell death.

interacting with smooth muscle cells as well as via cross-talk with enteric neurons, and therefore can contribute to the disruption of healthy motility patterns in diarrhea and constipation.^{40,70,71} It has been well established that both cholinergic and noncholinergic neurons, and the neuro-modulator nitric oxide (NO), regulate circular and longitudinal muscle to influence gut motility.⁷² Given that muscularis macrophages are in close proximity to nitrergic neurons, a role for intestinal macrophages in gut motility has been proposed.³⁸ In addition, mice lacking muscularis macrophages have increased proportions of nitrergic but not cholinergic neurons in the myenteric plexus, suggesting that macrophage populations impact neurochemical coding of enteric neurons.^{73,74} Muscularis macrophages express inducible NO synthase, and, during inflammation, NO suppresses gastrointestinal smooth muscle activity through activation of guanylyl cyclase.⁷⁵⁻⁷⁷ In fact, IL-17A secreted by infiltrating monocytes can reduce smooth muscle contractions by inducing iNOS expression in muscularis macrophages.⁷⁸ Despite being identified more than 30 years ago,⁷⁹ Luo et al⁸⁰ only recently discovered a mechanism by which muscularis macrophages affect smooth muscle cells in an ENS-independent manner. This group showed that muscularis macrophages interact directly with intestinal smooth muscle cells through transient receptor potential

vanilloid 4 (TRPV4) channels. Activation of this mechanically sensitive channel leads to release of prostaglandin E2, which then triggers muscle contraction by prostaglandin E-receptor signaling.⁸⁰ The exertion of these effects on intestinal motility via TRPV4 was shown by using macrophage-specific TRPV4 knock-out mice, which have reduced GI transit and impaired gut motility relative to WT.⁸⁰ However, it is important to note an opposing effect of activation of the TRPV4 channel on gut motility via a different mechanism also has been reported.⁸¹ Specifically, Fichna et al⁸¹ showed increased NO production from NO synthase-1 after activation of TRPV4 channels expressed on enteric neurons. Given that NO is a major inhibitory neurotransmitter in the GI tract, it is anticipated that activation of this TRPV4 pathway reduces gut contractility. Therefore, intestinal macrophages as well as enteric neurons potentially regulate gut motility by interacting with smooth muscle cells. Opposing effects from intestinal macrophages and enteric neurons via activation of TRPV4 channels can occur. Thus, dysregulation of the TRPV4 signaling pathway within intestinal macrophages during inflammation is likely to affect gut motility.

In addition to exerting direct effects on smooth muscle, intestinal macrophages regulate gut motility by communicating with enteric neurons via BMP2 and CSF-1⁴⁰

(Figure 2). BMP2 is expressed in MHCII⁺ muscularis macrophages but not in lamina propria macrophages. In addition, muscularis macrophages regulate gut motility via the BMP receptor located on enteric neurons.⁴⁰ Administration of the BMP-receptor antagonist dorsomorphin causes inefficient gastrointestinal contractions and longer gastrointestinal transit time in mice.⁴⁰ Activation of BMP receptor signaling also may induce the production of neurotransmitters that regulate intestinal muscle contraction. Moreover, both BMP2 and CSF-1, secreted by muscularis macrophages and enteric neurons, respectively, are essential for the survival of both cell types. Together with evidence that muscularis macrophages phagocytose dying enteric neurons and neuronal debris,⁸² this suggests that intestinal macrophages are indispensable for the maintenance of the ENS. Further clarification of the interactions between intestinal macrophages and myenteric neurons therefore will assist in understanding the roles of intestinal macrophages in GI motility disorders.

The Role of Intestinal Macrophages in Disease

Intestinal macrophages are widely studied for their ability to maintain gut homeostasis while sampling both harmful and commensal bacteria in the lumen. Disruption of gut physiological homeostasis results in chronic- and hyperinflammation, which leads to many GI diseases such as colitis and Crohn's disease. In this section, we explore the role of intestinal macrophages in key GI diseases.

Postoperative Ileus

POI is defined as the cessation or reduction in GI motility after surgery.⁸³ There remains no cure for this disease.⁸³ People with POI have significantly increased hospitalization time, morbidity, and mortality after surgery than those without.⁸⁴ POI also causes great discomfort and includes symptoms such as nausea and abdominal distension for a prolonged period of time owing to reduced gut motility caused by inflammation.^{83–85} Recent research has cast light on the influence of intestinal macrophages, predominantly muscularis macrophages, on gut motility, making them a potential therapeutic target for POI. For example, local inflammation initiated by macrophages residing in the muscularis layer leads to the initial onset of POI through the production of inflammatory cytokines.⁸⁶

Intestinal macrophages located in the muscularis layer are heavily involved in the pathogenesis of POI, particularly via the cholinergic anti-inflammatory pathway. The cholinergic anti-inflammatory pathway modulates the immune response through the stimulation of the vagus nerve via $\alpha 7$ nicotinic acetylcholine receptors ($\alpha 7$ nAChR) on immune cells.⁸⁷ In the gut, vagus nerve stimulation can reduce intestinal inflammation and eventually ameliorate POI in mice through intestinal macrophage-mediated mechanisms.⁸⁸ Anterograde tracing provides evidence that the vagus nerve interacts directly with enteric neurons that are in

close contact with resident muscularis macrophages.⁸⁹ Further evidence of the importance of the cholinergic anti-inflammatory pathway was observed after injection of $\alpha 7$ nAChR into the bone marrow of $\alpha 7$ nAChR knockout mice, which rescued a phenotype of intestinal inflammation and proinflammatory cytokine expression. These findings suggest that $\alpha 7$ nAChR in muscularis macrophages are the ultimate effector of this anti-inflammatory circuitry.⁸⁹

Monocytes also contribute to neuromuscular dysfunction that leads to POI.⁹⁰ CCR2 knock-out mice have impaired monocyte migration and persistent muscular dysfunction. Specifically, blocking the infiltration of circulating monocytes in *Ccr2*^{-/-} mice leads to fewer monocyte-derived macrophages in the muscularis externa, which in turn leads to increased neutrophil-mediated immunopathology.^{86,91} Overall, these mice also show delayed resolution of muscularis inflammation and GI function compared with WT mice upon intestinal manipulation-induced POI (a surgical method of rolling 2 cotton applicators on the gut). Therefore, circulating monocytes are a potential target for reversing the symptoms of POI.

IL10 is proposed to play a role in POI pathophysiology based on its role as a major anti-inflammatory mediator secreted mainly by infiltrating monocyte-derived macrophages during the course of this disease.⁹¹ IL10 is responsible for directing M2 polarization of macrophages, which leads to a tissue repair phenotype that typically is implicated in resolution of inflammation.⁹² However, opposing evidence suggests that IL10 aggravates POI by regulating neutrophil chemokine expression. In particular, IL10 is thought to promote neutrophil transmigration to the injured muscularis externa, causing further inflammation.⁹¹ In *IL10*^{-/-} mice, the transmigration of neutrophils to the traumatized muscularis externa site is reduced compared with WT mice, and this is accompanied by a decrease in levels of the neutrophil-attracting chemokines CXCL1 and CXCL2.⁹¹ Taken together, these findings suggest that IL10 secreted by monocyte-derived macrophages contributes to the pathogenesis of POI, instead of contributing to the resolution of this disorder.

Muscularis macrophages play a key role in the pathogenesis of POI, but the ambiguity in characterizing subsets of muscularis macrophages and the controversial role of IL10 make it difficult to target them as potential therapy of POI. In the future, research should focus on the interactions between muscularis macrophages and myenteric neurons to better understand mechanisms underlying altered gut motility in POI.

Inflammatory Bowel Disease

Intestinal macrophages are implicated in the pathogenesis of IBD, which includes Crohn's disease and ulcerative colitis. IBD is characterized by an excessive inflammatory response to the luminal bacteria, which is caused by the secretion of proinflammatory cytokines from intestinal macrophages. In Crohn's disease, inflammation can occur in any part of the GI system, whereas ulcerative colitis, by

definition, mainly affects the colon.⁹³ These diseases affect approximately 1 in 300 people globally and the prevalence is increasing.⁹⁴ In response to microbial stimulation, the general pathology of IBD includes the recruitment of proinflammatory blood monocytes that extravasate to the intestinal mucosa, but instead of undergoing differentiation into tolerogenic resident macrophages, these monocytes develop into proinflammatory macrophages producing inflammatory cytokines that cause an excessive inflammatory response.^{95,96}

The role of macrophages in colitis has been examined using 2 main experimental rodent models.⁹⁷ One approach uses a 2,4,6-trinitrobenzene sulfonic acid (TNBS)-ethanol enema, the other, dextran sulfate sodium (DSS) in the drinking water. Colitis is induced through aberrant T-cell or B-cell responses in the TNBS model whereas the DSS approach does not involve the adaptive immune response.⁹⁷⁻⁹⁹ DSS is an anticoagulant that damages the epithelial monolayer lining of the large intestine.¹⁰⁰ Both of these colitis models resemble human colitis in terms of histopathologic characteristics.¹⁰⁰

Intestinal macrophages contribute directly to the onset of colitis through cytokine production. In T-cell-mediated colitis, as in the TNBS preclinical model, IL23 (a cytokine produced by intestinal macrophages) plays a central role in pathogenesis by activating T cells at the mesenteric lymph node. Specifically, IL23 exacerbates colitis via generating the inflammatory cytokines IL17 and IL6.^{101,102} Moreover, inflammatory intestinal macrophages at the mesenteric lymph node are differentiated directly from extravasated monocytes¹⁰³ because monocyte-derived macrophages at the lamina propria do not migrate to the mesenteric lymph node.¹⁰³ These macrophages also induce the production of proinflammatory T cells, which contribute to T-cell-mediated colitis.²⁸ Therefore, intestinal macrophages contribute to the pathogenesis of colitis by inducing the adaptive immune response.

In mouse models of colitis, circulating monocytes invade the lamina propria and differentiate into CX3CR1^{int} proinflammatory macrophages (subtype 2) rather than tolerogenic CX3CR1^{hi} resident macrophages (subtype 1), leading to the development of colitis.²¹ In addition, the anti-inflammatory cytokine IL10, which is secreted constitutively by resident macrophages, can induce autophagy in dysfunctional mitochondria in a mouse model of colitis and IBD patients by constitutively expressing the anti-inflammatory cytokine IL10. IL10 expression also inhibits the nucleotide-binding oligomerization domain-like receptor protein 3 inflammasome activation through mammalian target of rapamycin signaling. As a result, IL10-deficient mice develop spontaneous colitis.¹⁵

Gut inflammation during colitis increases excitability in myenteric neurons, suggesting that intestinal macrophages also may impact ENS function during IBD.¹⁰⁴ Myenteric neurons are classified according to their morphology and are designated as Dogiel type I to VII neurons.¹⁰⁵ Functionally, these neurons also are categorized based on electrophysiological properties. The 2 main functional neuronal types are S neurons, which show spontaneous activity and

afterhyperpolarization (AH) neurons, which show a long duration hyperpolarization profile after the firing of an action potential.¹⁰⁶ The excitability of colonic myenteric AH neurons is increased during TNBS-induced colonic inflammation in guinea pig distal colon.¹⁰⁷ S neurons with ascending projections also show increased excitability in the inflamed colon.¹⁰⁸ It also has been reported that in the inflamed ileum in guinea pigs, Dogiel type I neurons undergo electrophysiological changes from showing traits relevant to S neurons in healthy conditions to having similar functional characteristics to AH neurons.¹⁰⁸ Furthermore, in inflamed regions of the colon of a guinea pig model of colitis, circular muscle inhibitory purinergic neuromuscular transmission is reduced.¹⁰⁹ In addition to changes in enteric neuronal electrical properties at the cellular level, inflammation-induced neuroplasticity also may affect gut motility during and after colitis.¹¹⁰ Although it is unclear whether macrophages have a direct influence on myenteric neurons in these preclinical models of inflammation, there is evidence that these 2 cell types affect each other during inflammation at the cellular level. High levels of extracellular adenosine triphosphate released by intestinal macrophages during inflammation can activate the purinergic receptor P2X7 on both intestinal macrophages themselves and enteric neurons. Such changes in purinergic signaling can trigger the production of proinflammatory cytokines such as TNF α and IL1 β by intestinal macrophages, as well as neuronal cell death in the mouse myenteric plexus.^{111,112} Overall, interactions between intestinal macrophages and enteric neurons have been shown to exacerbate inflammatory effects in IBD models.

Individuals who have fully recovered from IBD commonly continue to experience stool irregularities and abdominal pain, collectively known as irritable bowel syndrome (IBS).^{113,114} Based on the expression profiles of anti-inflammatory (M2-polarized) macrophages and mast cells identified via mannose receptor and tryptase labeling, respectively, it is evident that the localization of macrophages and mast cells is altered after IBD in patients.¹¹⁵ Macrophage/mast cell density is increased significantly in the intestinal smooth muscle layer of the intestine between 2 and 24 weeks after DSS-induced colitis in rats. Interestingly, alongside an increase in macrophage numbers, GI transit time is shortened whereas an increase in mast cell numbers increases transit times in this model. Based on these changes in GI transit time, alterations in these immune cell populations may contribute to gut dysmotility in IBS. Although the precise mechanisms by which intestinal macrophages may affect gut motility in IBS are unknown, the close proximity and interactions of intestinal macrophages with enteric neurons support a role for such neuroimmune modulation in IBS. Further research is needed to better understand interactive mechanisms between intestinal macrophages and enteric neurons to identify potential treatment targets for IBD and IBS.

Similar to colitis, Crohn's disease is characterized by excessive inflammation in the gut, however, the macrophage subsets involved in these disorders differ.^{116,117} During the course of Crohn's disease, CD68⁺ monocytes accumulate at

the lamina propria and subsequently shift intestinal macrophages at the subepithelium toward a proinflammatory M1 polarization phenotype. M1 macrophages show up-regulated TNF α expression that contributes to the disruption of epithelial barrier integrity via alteration of expression levels of the gap junction protein Claudin-2, and eventually epithelial cell apoptosis, changes that are characteristics of Crohn's disease.¹¹⁷ It also has been identified that a unique subset of CD14⁺ intestinal macrophages is exclusively expanded in Crohn's disease patients.¹¹⁶ CD14⁺ macrophages secrete inflammatory cytokines such as IL23 and TNF α , which are largely up-regulated in patients with Crohn's disease. In addition, up-regulated IL23 and TNF α further induce the production of interferon γ , an inflammatory cytokine produced by T cells in Crohn's disease patients. This inflammatory condition further directs macrophages to differentiate into an IL23 hyperproducing phenotype, forming a positive feedback loop that leads to the pathogenesis of Crohn's disease. Thus, accumulation of specific inflammatory CD68- and CD14-expressing macrophage subtypes and the resulting increases in cytokine production are major contributors to intestinal inflammation in Crohn's disease patients.

GI Disorders Associated With HIV

HIV causes acquired immunodeficiency syndrome (AIDS), which renders the immune system susceptible to opportunistic infections.¹¹⁸ AIDS has been regarded as a global epidemic and caused more than 700,000 deaths in 2018. Despite improved treatment regimens, almost all HIV-infected populations still experience at least 1 GI abnormality, with more than half presenting with diarrhea.¹¹⁹ Other GI symptoms associated with HIV include the following: malabsorption, weight loss, and abdominal pain caused by increased GI inflammation and permeability.¹¹⁹ Intestinal macrophages play a crucial role in GI disorders associated with HIV. The GI tract is believed to be the main target organ during HIV infection owing to the large number of resident lymphocytes, especially CD4⁺ T cells.^{120–122} Apart from the loss of CD4⁺ T cells, HIV infection also induces the release of the transactivator of transcription protein. The transactivator of transcription directly increases enteric neuron excitability, proinflammatory cytokine levels, and GI transit time,¹²³ indicating interactions between HIV and GI dysfunction. In a recent African study, increased mRNA expression of macrophage-related proinflammatory cytokines (TNF α and IL1 β) and genes involved in macrophage activation (C-C motif chemokine ligand 2 and TNF Receptor Associated Factor 6) was identified in infected patients relative to a healthy population.¹²⁴ This study also unveiled that CD14⁺ macrophages are up-regulated in patients with AIDS. Interestingly, this finding is in line with the pathophysiology of IBD in which CD14⁺ macrophages are up-regulated in response to plasma LPS levels (an indication of microbial translocation as a result of disruption of epithelial barrier integrity).¹²⁴ In addition, the first-in-class anti-HIV drug candidate ABX464 induces production of

IL22, a cytokine involved in tissue repair in DSS-induced colitis in mice, as well as increasing the population of LPS-stimulated bone marrow-derived macrophages.¹²⁵ The increased levels of both CD14⁺ macrophages and IL22 in HIV suggest similarities in the inflammatory and immunometabolic response of HIV infection and IBD.¹²⁶

The involvement of intestinal macrophages in HIV infection also has been implicated in simian immunodeficiency virus (SIV), an HIV-like virus that infects primates. CD163⁺ intestinal macrophages secrete low levels of cytokines irrespective of disease stage. In SIV, the presence of CD163⁺ intestinal macrophages and sensitized mesenteric lymph node macrophages (which recruit additional monocytes to extravasate into the gut) lead to accumulation of noninflammatory CD163⁺ macrophages with impaired phagocytic function.¹²⁷ In addition, it is observed that there is a shift in macrophage functional dynamics during infection. CD163⁺CD206⁺ (double-positive) macrophages within the lamina propria are outnumbered by short-lived CD163⁺CD206⁻ (single-positive) macrophages upon SIV infection.¹²⁸ This shift is owing to the swift targeting and killing of double-positive macrophages during SIV infection and their subsequent constitutive replenishment by the single-positive, short-lived macrophages (derived from recruited monocytes). Within the submucosa, in contrast with the lamina propria, double-positive macrophages survive longer, meaning that they potentially contribute to the SIV reservoir.¹²⁸ Change of surface marker expression on intestinal macrophages therefore is crucial for detecting virus reservoir in monitoring the progression of HIV infection.

In summary, altered levels of macrophage subsets are characteristic of individuals with HIV. With enhanced understanding of the distinct molecular profiles and roles of macrophage subsets in HIV, the development of therapeutic strategies to treat HIV-associated GI disorders is anticipated.

GI Disorders Associated With PD

PD is a motor dysfunction disorder caused by progressive degeneration of dopaminergic neurons in the nigrostriatal pathway.¹²⁹ A PD diagnosis is based on 4 dysfunctional motor features, namely: tremor at rest, bradykinesia, postural instability, and muscle rigidity, however, gastrointestinal symptoms such as constipation and delayed gastric emptying frequently occur before motor symptoms become evident.^{130–132}

Similar to the CNS, a loss of dopaminergic neurons also has been observed in the myenteric plexus in a mouse model of PD.¹³³ These findings suggest that alterations in the ENS may contribute to gastrointestinal dysfunction in PD.¹³³ In fact, it has been hypothesized that PD originates in the intestines where α -synuclein aggregates in submucosal and myenteric neurons are transported retrogradely to the CNS via neuronal pathways.^{134–137} A potential role for intestinal macrophages in phagocytosing α -synuclein aggregates has been suggested based on the activated morphology and close proximity of MHCII-positive intestinal macrophages to α -synuclein aggregates in PD.^{38,134,138}

Moreover, it has been observed that IBD patients are more susceptible to PD than non-IBD individuals.¹³⁹ Given these findings, intestinal macrophages are ideal candidates to play a key role in GI disorders associated with PD.

In mice, intestinal macrophages have a neuroprotective role after neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced parkinsonism. Myeloid differentiation primary response 88 (MyD88), an important adaptor in the inflammatory signaling pathway that activates the transcription factor nuclear factor- κ B (NF- κ B), regulates the infiltration of monocytes to tissue surrounding the myenteric plexus, as well as directs macrophages toward M2 polarization.¹⁴⁰ After MPTP induction of Parkinson-relevant traits, total macrophage numbers in myenteric tissue remain largely unchanged in MyD88 knockout mice when compared with WT. This is owing to the infiltration of circulating monocytes, which compensates for the cell death of resident macrophages upon the introduction of the MPTP toxin. However, deletion of MyD88 shifts resident macrophages toward M2 polarization, resulting in a tissue-repair phenotype that protects the gut from inflammation. Thus, shifting intestinal macrophages toward M2 polarization through MyD88 shows the potential of intestinal macrophages to be the therapeutic target of PD.

Several neuroprotective agents administered to reduce PD traits in preclinical models exert effects by mediating an anti-inflammatory response. For example, 17 β -estradiol (E2), an estrogen receptor α agonist, the G-protein-coupled estrogen receptor 1 agonist G1, and the selective estrogen-receptor modulator raloxifene prevent dopaminergic neuron damage upon MPTP administration, as well as reduce immune responses.^{141,142} Specifically, these compounds reduce the response of NF- κ B and NO via G-protein-coupled estrogen receptor 1 to result in M2 polarization and fewer proinflammatory macrophages accumulating within the GI tract at the site of inflammation.^{141,142} Based on these findings, treatments that reduce the number of proinflammatory intestinal macrophages that accumulate during inflammation and induce M2 polarization may be useful in ameliorating intestinal inflammation involved in PD.

Intestinal macrophages also may impact gut dysfunction in PD through actions on ghrelin, an orexigenic hormone secreted by enteroendocrine cells in the epithelium of the GI tract. For example, administration of ghrelin rescues delayed gastric emptying symptoms in the 6-hydroxydopamine Parkinsonism model in rats.^{143,144} Ghrelin's actions likely occur via the receptor for the acylated form of ghrelin (the growth hormone secretagogue receptor 1a), which is expressed by intestinal macrophages. Relevant to a potential role of inflammation in PD, via this pathway, ghrelin induces macrophages to acquire an anti-inflammatory phenotype.¹⁴⁵ Taken together, these findings suggest that promoting anti-inflammatory pathways to achieve neuroprotective function may be beneficial in PD, however, it also will be of interest to clarify how specific immunomodulatory agents affect gut physiology including motility and permeability.

NEC

NEC is a leading cause of mortality and the most common life-threatening GI malfunction in premature infants.¹⁴⁶ Although the cause is unknown, NEC is characterized by atypical bacterial colonization of the immature gut and a dysregulated inflammatory response that leads to mucosal disruption¹⁴⁷ in which intestinal macrophages have been implicated.^{146,148} In particular, intestinal macrophages are postulated to alter cytokine production, resulting in TGF- β 2 deficiency and other abnormalities in inflammatory responses that commonly are observed in NEC.

Macrophage-depleted mice infected with *Cronobacter sakazakii*, a gram-negative opportunistic bacterium often present at high abundance in NEC, show significantly increased proinflammatory cytokines including TNF α , IL1 β , IL6, and IL12, suggesting that intestinal macrophages are vital in preventing hyperinflammation during NEC.¹⁴⁹ In a baboon model of NEC, TGF- β 2 deficiency is caused by an increased expression of mothers against decapentaplegic homolog 7 (Smad7), which inhibits autocrine expression of TGF- β 2 through transcriptional silencing.¹⁵⁰ Smad7 mRNA expression is increased significantly in both baboon and human NEC intestine samples, especially in lesion sites within the G tract. Because TGF- β 2 suppresses macrophage cytokine production and the mucosal inflammatory response in the developing intestine,¹⁵¹ decreased levels of TGF- β 2 levels may contribute to the development of NEC.

Relevant to a role for reduced TGF- β 2 in NEC, Smad7 disrupts TGF- β signaling in intestinal macrophages and activates the proinflammatory response during NEC.¹⁵² Specifically, Smad7 activates the inflammatory NF- κ B pathway by inducing the inhibitor of the nuclear factor κ B kinase through binding to its promoter. Interestingly, deletion of inhibitor of the nuclear factor κ B kinase in blood Ly6C⁺ monocytes prevents the NEC-induced recruitment and infiltration of monocytes as well as their subsequent differentiation into intestinal macrophages.¹⁵³ Because the NF- κ B pathway plays an important role in the pathogenesis of NEC, components of the NF- κ B pathway could be targeted to combat hyperinflammation in NEC.

Impaired vascular development in the small intestine has been shown to increase susceptibility to NEC. In addition to regulating inflammatory responses, depletion of intestinal macrophages is implicated in impaired small intestinal vascular development in infants.¹⁵⁴ Specifically, depletion of CX3CR1⁺ embryonic intestinal macrophages but not adult intestinal macrophages results in disorganized vasculature. These studies show that intestinal macrophages play a decisive role in NEC and modulation of these cells could provide a promising therapeutic target to treat this currently incurable disease.

Summary and Conclusions

Understanding the roles of different intestinal macrophage subtypes will provide valuable insights for the design of therapeutics for GI disorders. The heterogeneity of intestinal macrophages is shown by the classification of 3 described macrophage subsets, each with a different role

with respect to GI function. Subtypes 1 and 3 intestinal macrophages originate from blood monocyte and bone marrow-derived monocytes, respectively, which largely are responsible for maintaining the anti-inflammatory environment in our gut. Subtype 2 intestinal macrophages are implicated mainly in inflammation-related diseases such as IBD. In particular, subtype iii intestinal macrophages are responsible for interaction with ENS, which enable them to be targeted in motility/secretion-related GI disorders. Here, we outline evidence showing the dysregulation of intestinal macrophages in terms of polarization, cytokine production, and interactions with the ENS in different GI disease conditions. Although the mechanisms by which changes in intestinal macrophages lead to pathogenesis in these diseases remain to be fully defined, further functional characterization of subtypes of intestinal macrophages in different layers of the gut will provide the groundwork for future research to exploit these immune cells as therapeutic targets. Potential treatment targets for diseases involving intestinal macrophages may include specific receptors/channels expressed on particular subsets of these macrophages.

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Conflicts of interest

The authors disclose no conflicts.

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