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Gut Macrophages: Key Players in Intestinal Immunity and Tissue Physiology

Paul A Muller^{1,‡,*}, Fanny Matheis^{1,‡}, Daniel Mucida^{1,*}

¹Laboratory of Mucosal Immunology, The Rockefeller University, New York, NY, USA

Abstract

The mammalian gastrointestinal tract harbors a large reservoir of tissue macrophages, which, in concert with other immune cells, help to maintain a delicate balance between tolerance to commensal microbes and food antigens, and resistance to potentially harmful microbes or toxins. Beyond their roles in resistance and tolerance, recent studies have uncovered novel roles played by tissue-, including intestinal-, resident macrophages in organ physiology. Here, we will discuss recent advances in the understanding of the origin, phenotype and function of macrophages residing in the different layers of the intestine during homeostasis and under pathological conditions.

Keywords

innate; immunity; macrophage; intestine; intestinal; muscularis; lamina propria; neuro-immune

Introduction

Macrophages are known to quickly adapt to the tissue environment in which they reside, but initial descriptions of intestinal macrophages were the result of whole tissue preparations where physical, or phenotypical, separation between macrophage populations residing in the different layers of the intestinal wall was not routinely performed. Recent work has shown that within the human and murine intestine, macrophages can be defined by their anatomical location, broadly divided into mucosal or lamina propria macrophages (LpM) and muscularis macrophages (MM) [1–4]. Further functionally distinct populations likely exist in the epithelium, submucosal and serosal regions [2,3]. To provide a comprehensive and concise overview of the current understanding of intestinal macrophages, our discussion of inflammatory and steady state functions of these cells will take this anatomical distinction into account.

*Correspondence: pmuller@rockefeller.edu (P.A.M.), mucida@rockefeller.edu (D.M.).

‡These authors contributed equally to this work.

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Conflicts of interest

The authors declare no conflict of interest

Origin and Maintenance of Intestinal Macrophages

Intestinal macrophages, in contrast to other murine tissue-resident macrophages, are thought to be continuously replaced by circulating monocytes through a process known as the “monocyte waterfall”, and evidence has recently emerged that this step-wise differentiation is also present in humans [5–7]. As an early population of embryo-derived macrophages are turned over during development, monocytes, defined as Ly6C^{hi} CD64-CX3CR1^{int} MHCII⁻ (P1), enter the gut where they begin to transition through three subsequent stages of differentiation, P2-P4, becoming mature CD64+ CX3CR1^{hi} MHCII^{hi} macrophages [5,6]. While the fractalkine receptor (CX3CR1) is a critical mediator of murine intestinal macrophage function, macrophages in the intestine of humans do not express significant levels during homeostasis [7]. However, recent work by two different groups has challenged the concept of continuous replenishment by identifying a population of early-seeded, long-lived tissue-resident intestinal macrophages not replaced by circulating monocytes [2,8]. Flow cytometric phenotyping and fate mapping experiments in mice indicated the existence of macrophages that were present at birth and displayed little to no turnover as the animals aged [2,8]. Observations from human patients receiving intestinal transplants also identified a population of long-lived macrophages that differ from those readily replaced by circulating monocytes [3]. Other departures from the monocyte waterfall can occur in the context of inflammation induced by intestinal manipulation in mice or inflammatory bowel disease in humans, as it was recently suggested that recruited monocytes can directly transition into mature macrophages, effectively bypassing the intermediate stages of differentiation [7,9].

Macrophages in the intestine express high levels of colony stimulating factor receptor (CSF1R), and targeting of CSF1 or CSF1R by genetic or pharmacologic approaches results in a complete lack of macrophages in the gut [4,10,11]. Among the sources of CSF1, studies have reported a role for nestin+ stem cells in the bone marrow systemically [12], or non-hematopoietic compartments within the intestine, including endothelial cells and interstitial cells of Cajal in developing mice, or intestinal crypts and enteric neurons in the adult, the latter of which is postulated to be age- and partially microbiota-dependent [4,10]. Additional factors have been reported to play a role for macrophage recruitment and differentiation in different segments of the GI tract, including CD11c+-cell-intrinsic Notch signaling in the small intestine, macrophage-secreted monocyte chemoattractants, and TGF- β signaling in the colon [13,14]. Beyond the role for local growth factors and recruitment cytokines, the presence of the microbiota is a critical mediator of intestinal macrophage recruitment and differentiation. Both monocyte-derived and tissue-resident macrophages are decreased in number in mice that lack a microbiota, or in mice that are acutely depleted of a microbiota with antibiotics [4,5,8].

The basic mechanisms by which LpM and MM seed the tissue are likely similar, however there appears to be a clear difference in the balance of macrophage composition, as MM were recently described to primarily originate from a long-lived subset rather than being monocyte-derived [2]. This, coupled with the fact that the lamina propria and muscularis comprise very distinct niches, has led to the recent recategorization of intestinal macrophages based upon their location within the tissue.

Mucosal Macrophages

In the initial description of the intestinal villus as a unit in 1959 by electron microscopy, Karlin and colleagues first described the presence of LpM [15]. These macrophages line the intestinal epithelium and are thus strategically positioned to sample luminal antigens, phagocytose dead cells, and eliminate pathogens that succeed in crossing the epithelial layer. LpM are mostly composed of macrophages derived from the monocyte waterfall, with a smaller contribution of long-lived macrophages compared to deeper layers of the intestine. They are classically defined as CD64+ CD11c+ MHCII^{hi}, and in mice are CX3CR1^{hi}. Intravital imaging showed that these sessile macrophages have rapid and dynamic membrane ruffling and pseudopod extension [1]. LpM pseudopods can form transepithelial dendrites (TEDs) that efficiently cross the epithelium to sample the intestinal lumen and capture potential pathogens, a process that is dependent on CX3CR1 expression in mice, but may be antigen-dependent [16–18]. Due to their unique positioning in a tissue continuously exposed to foreign antigen, LpM possess a more pro-inflammatory transcriptional profile than their muscularis counterparts [1]. Regardless, LpM are also precisely equipped to be tolerogenic in nature. While seemingly contradictory, this allows for balancing pathogen clearance, while simultaneously regulating local immune cell populations, and maintaining basic tissue integrity.

Infection

Due to the positioning of a large number of LpM immediately beneath the intestinal epithelium, there is a high probability of them encountering or directly sampling pathogens. These macrophages are programmed such that they can contribute to the immediate antibacterial or -parasitic response. Current work has shown that in addition to TED-mediated sampling of luminal stimuli that may contribute to IL-10 production [19], CX3CR1+ LpM can extravasate from the lamina propria to the lumen of the intestine during *Salmonella* infection, a process that is also dependent on toll-like receptor signaling in intestinal epithelial cells [20]. Once in the lumen, they can phagocytose pathogens to prevent entry to the intestine early in infection and decrease pathogen load. While LpM can play a role in barrier function, it is also clear that they possess antibacterial machinery to respond to bacterial invasion, which, in some cases, can lead to tissue pathology if left unchecked. In mouse models of *Helicobacter pylori* and *Citrobacter rodentium* infection, LpM-specific epidermal growth factor receptor signaling (EGFR) was found to be responsible for induction of cytokine production and macrophage activation, which directly contributes to tissue inflammation [21]. In another study by the same group using identical infection models, ODC1, the rate-limiting enzyme of polyamine synthesis, and its product putrescine, were described as critical regulators of proinflammatory LpM activation. This was proposed to be mediated via direct alteration of histone modifications of proinflammatory genes such as IL-1 β , IL-6 and TNF- α [22]. Again, as was the case with EGFR, LpM-specific loss of ODC1 enhanced antibacterial properties, but at the expense of increased tissue inflammation. In line with polyamine histone modification, a role for the microbial-derived short chain fatty acid (SCFA) butyrate as an HDAC inhibitor was recently investigated. Butyrate was shown to induce lasting metabolic and transcriptional changes in colonic macrophages, which led to increased potential to ramp up ROS production as well as

upregulation of the antimicrobial proteins lysozyme and calprotectin. These effects were mediated by inhibition of HDAC3, which led to increased macrophage antimicrobial activity, and resistance to enteric pathogens *in vivo* [23]. The ability of LpM to properly balance resistance with limiting tissue damage is a key feature of these macrophages, and this tolerogenic programming is reflected in their role in maintaining homeostasis in the inflammatory local environment.

Tissue tolerance and homeostasis

The intestinal lamina propria comprises an inflammatory milieu due to the constant exposure to luminal commensal bacterial and diet-derived antigens. Despite this reactive environment, the immune system is tightly regulated to avoid inadvertent harm to self, and LpM are key mediators in generating a tolerogenic environment [24]. The most studied LpM tolerogenic factor is IL-10, the total lack of which leads to spontaneous enterocolitis [25]. Production of IL-10 by LpM is important for the balance of T cell populations in the gut. A recent study identified that epithelial-adherent bacteria were required to induce IL-10 production of CX3CR1⁺ LpM, which in turn increased intestinal Treg numbers while dampening the expansion of Th1 cells. Conversely, during antibiotic-mediated microbiota depletion, LpM no longer produced IL-10, and instead promoted antigen-specific Th1 cell expansion [26]. These findings align with previous work showing that the adherent bacterium *Clostridium butyricum* was sufficient to induce IL-10 production by LpM, and suppress an antimicrobial program [27]. Another new study reported that a common bacterial mouse pathobiont, *H. hepaticus*, induces a tolerogenic response in mucosal macrophages triggered by TLR2 stimulation. This was mediated by *H. hepaticus* secretion of a soluble polysaccharide, SNH₁, and involved MSK/CREB-dependent induction of an anti-inflammatory macrophage profile, including IL-10 production [28]. Thus, it is becoming evident that interactions with resident microbial species play a key role in balancing proinflammatory and tolerogenic responses in LpM. Further, while an anti-inflammatory role of IL-10 in LpM has been established, other work has demonstrated that IL-10R α signaling in CX3CR1^{hi} macrophages is crucial for their tolerogenic profile during homeostasis. The failure of macrophages to respond to, rather than produce, IL-10 results in their expression of an array of proinflammatory cytokines driving deleterious T cell responses and the development of spontaneous colitis in mice [29]. Current work from the same group showed that IL-23 production from LpM lacking IL10-R α was the primary driver of colitis, leading to IL-22 release from Th17 T cells and ILC3s [30], suggesting IL-10 sensing as a major LpM-intrinsic tolerogenic axis.

Beyond a role for the microbiota in imprinting an anti-inflammatory program in LpM, how else do these macrophages maintain homeostasis? LpM are critically involved in controlling the maintenance and activation of intestinal ILCs and T, including Treg cells. For example, tissue-resident CX3CR1⁺ LpM have been implicated as the main producers of proinflammatory cytokines such as IL-23 and IL-1 β in the intestine, thereby increasing IL-22-production by ILC3s [31,32]. LpM production of IL-23 and IL-1 β in turn is kept under control by MHCII modulation of LAG3⁺ Treg cells [33]. Other recent work demonstrated that IL-1 β production by LpM selectively induces ILC3s to produce IL-2, which in turn was required for Treg maintenance [32]. Together, these findings establish a

feedback loop in which proinflammatory LpM induce Treg cells to contribute to a tolerogenic milieu. Other neighboring cell-LpM interactions can contribute to the anti-inflammatory status of LpM. In concert with dendritic cells, two distinct CD11b+ LpM subsets were shown to sample and clear apoptotic intestinal epithelial cells (IECs) via efferocytosis. Uptake of apoptotic IECs induced a cell-type specific transcriptional program associated with dead cell clearance along with immunosuppression, including TLR2-downmodulation. These adaptations may likely be coupled to prevent unwanted inflammatory or autoimmune responses [34]. Additional input may also come from neuronal interactions, as LpM are in close apposition with nerve fibers in the intestinal villi and mucosa [2,15] and they have been shown to express receptors for neurotransmitters or neuropeptides, such as vasoactive intestinal peptide (VIP) [1,35]. A recent report showed that in the context of experimental food allergy, stimulation of the vagus nerve significantly improved intestinal inflammation, and this was associated with an increase in allergen uptake by CX3CR1^{hi} LpM, suggesting a role for a neuron-macrophage axis to dampen tissue damage during food allergy [36]. Despite these descriptions and literature suggesting a role for neuropeptides in general modulation of macrophage inflammatory responses [37–39], a specific function for neuron-LpM interactions is largely lacking in experimental evidence, as opposed to their muscularis counterparts (discussed below, *Muscularis Macrophages*). Recent advances in neuronal mapping within the intestine [40,41] coupled with high-resolution single cell macrophage sequencing [2] is likely to uncover the true potential for neuron-LpM crosstalk.

Basic tissue physiology

Given the unique challenges of the intestinal environment, the majority of studies on LpM function has focused on their specific roles in inflammatory settings and protection from invading pathogens. Yet, LpM, like their counterparts in other tissue sites, also exert functions more classically associated with macrophages, including phagocytosis of cell debris (see above, Tissue tolerance), and mechanical support of surrounding tissue. A study using single-cell RNA sequencing coupled with fate-mapping strategies recently identified a distinct population of tissue-resident CX3CR1+ LpM associated with the vasculature. Targeted depletion of tissue-resident macrophages led to overt restructuring of blood vessels in the mucosa and submucosa, and functionally, to leaky vasculature as evidenced by increased presence of intravenously delivered microspheres [2]. A role for LpM has also been described in the maintenance of the intestinal stem cell niche. Here, antibody-mediated depletion of CSF1R-dependent LpM led to impairment in differentiation of Paneth cells and a reduction in *Lgr5*+ stem cells, which in turn affected the differentiation and replenishment of further IECs, including Goblet and M cells [42]. These findings suggest that a population CSF1R-dependent LpM is crucial for intestinal crypt homeostasis. Indeed, effects on crypt IEC development may be mediated by long-lived LpM that appear to associate with Paneth cells [2]. Given the highly heterogeneous numbers of cell types and structures in the mucosa and lamina propria that LpM associate with, it is likely that we have only scratched the surface of their ability to support basic intestinal physiology.

Muscularis Macrophages

Macrophage-like cells were first observed in the muscularis externa of the intestine by Mikkelsen and colleagues in 1982 through analysis of electron microscopy images [43,44]. Following this initial observation, these cells were defined to be macrophages present in the serosal, circular, and longitudinal muscle layers with a significant proportion in the myenteric or Auerbach's plexus containing intrinsic enteric-associated neurons (iEAN). Muscularis macrophages (MM) display two distinct morphologies, bipolar and stellate, and are intimately associated with cell bodies and processes of both glia and neurons [1,44]. Phenotypically, they can be defined by their high expression of MHCII, CD163, and CX3CR1 [4]. In the steady state, they do not migrate through the tissue and display slow continuous remodeling of their dendritic-like pseudopod processes [1]. In comparison to LpM, a large proportion of MM are composed of early-seeded macrophages of both bone marrow and embryonic origin [2]. MM are skewed towards an anti-inflammatory profile, suggestive of roles in tissue homeostasis and repair [1]. Despite their description close to four decades ago, the function of MM remained relatively unknown until recent work that has established these macrophages as key players in gut function and tissue homeostasis, with a particular emphasis on gut motility.

Infection and Inflammation

Bauer and colleagues in the late 1990s focused on clinical conditions associated with GI dysmotility, sepsis and post-operative ileus (POI), to better understand possible functions of MM. The release of LPS in sepsis resulted in MM activation and upregulation of lymphocyte activation marker 1 (LFA-1), and correlated with a profound decrease in intestinal motility [45]. An increase in LFA-1+ MM was also correlated with POI in humans [46], which is characterized by a large influx of immune cells, predominantly neutrophils [47,48]. In turn, a recent study suggested that the immune infiltrate in POI is driven by activation of MM, as depletion of macrophages improve the symptoms in murine models [48]. However, the resolution of POI has also been attributed to an increase in monocyte-derived macrophages, a process that may be partially driven by the activation of local dendritic cells [47,48]. Additionally, cholinergic stimulation of macrophages, in part derived from vagal efferent input to enteric neurons, has been described to reduce neutrophil and additional macrophage recruitment, and ameliorate symptoms in POI [49–51]. The understanding of possible effects of MM activation on gut motility is not limited to POI. In the context of diabetes induced gastroparesis, MM-derived inflammatory cytokines, including IL-6, TNF- α and IL-1 β , seem to contribute to the loss of the pacemaker cells in the muscularis, the interstitial cells of Cajal (ICC) [11]. These findings clearly implicate the pro-inflammatory potential of MM in dictating subsequent inflammation-induced dysmotility.

In contrast to the aforementioned pro-inflammatory roles for MM in specific GI disorders, recent studies have also begun to elucidate a possible anti-inflammatory, tissue protective function for these cells. A recent study investigating the distribution of intestinal macrophages in chemical-induced colitis found an increase in mannose receptor (CD206)+ MM during the resolution of inflammation, suggesting a potential tissue protective role for

MM [52]. During enteric infections, the gut-sympathetic nervous system has been shown to further skew this macrophage population towards a tissue-protective profile [1]. Rapid changes in MM gene expression, in particular the gene *Arg1*, were found to be mediated by stimulation of MM adrenergic receptor beta 2 (ADRB2) through sympathetic, extrinsic EAN activation. Current work started to ascribe a role to this sympathetic-MM-iEAN circuit during the course of inflammation. Although different enteric pathogens were shown to induce significant iEAN loss, this effect was limited by MM, which responded to luminal infection by upregulation of a neuro-protective program involving β_2 -adrenergic signaling and stimulation of an arginase-polyamine axis [53]. In combination with steady state control of neuronal differentiation and survival, these studies point to MM as key regulators of the enteric nervous system, perhaps with parallels to microglial function in the central nervous system.

Steady State

The use of anatomical dissection to study layer-specificity in intestinal macrophages revealed that MM are transcriptionally distinct from their LpM counterparts, with particular enrichment for receptors and molecules that can contribute to neuronal interaction [1,2,4]. While the original concept of macrophage contribution to GI motility was put forth by groups studying models of sepsis and POI, high resolution light-microscopy at steady state depicted the extent to which MM are positioned within the smooth muscle and in close apposition to EAN [54]. Additional studies established that MM are in close contact with enteric neurons within the myenteric and submucosal plexuses [1,2]. A direct role for MM in steady state peristalsis was demonstrated by an antibody-mediated MM-preferential depletion, which led to significant changes in colonic motility [4]. Among the most differentially expressed genes, bone morphogenetic protein 2 (BMP2) was found to be one of the main drivers of steady state MM control of GI motility [4]. BMP2 was shown to impact the generation of colonic migrating motor complexes in the large intestine, an effect that was mediated by BMP2 signaling through intrinsic EANs expressing BMPRII α . The role of macrophages in setting GI motility does not seem to be only a result of interactions with neuronal networks. Macrophages in the small and large intestines were found to express transient receptor potential cation channel 4 (TRPV4) [55]. Specific loss of *Trpv4* in CX3CR1+ macrophages led to deficits in colonic motility, an effect that was attributed to TRPV4-stimulation of prostaglandin E production by MM, which in turn directly modulates smooth muscle contractility. In conjunction with observations of inflammation-associated dysmotility, these studies establish a role for MM in the regulation of peristaltic activity.

Macrophage deficiency during development, as observed for instance in *Csf1^{op/op}* mice, leads to an increase in numbers of enteric neurons, possibly due to a reduction in MM-derived BMP2 [4,56]. Developmental control of neuronal numbers also appears specific to particular subpopulations of enteric neurons. For example, in the case of the stomach, the number of nNOS-expressing neurons are preferentially controlled by the presence of MM [11]. Recent work has challenged the idea that enteric neuronal numbers are set developmentally, rather it has now been proposed that enteric neurons may be continuously turned over during adulthood. MM were shown to continuously phagocytose dying neurons, a process that may be necessary to maintain appropriate numbers of enteric neurons [57].

During aging, the neuro-supportive role of MM switches as they upregulate pro-inflammatory markers. This phenotypic change in MM during aging was linked to a macrophage-intrinsic decrease in the immune-regulatory forkhead transcription factor FoxO3, resulting in a decline in enteric neural stem cell populations [58]. However, the exact nature of MM contribution to neuronal numbers in the adult remains unclear, as ablation of resident neuron-associated macrophages has been reported to either have no effect on neuronal populations or to be associated with rapid neuronal degeneration [2,53]. These findings may be particularly relevant in clinical cases of hypo-, hyper-, or agangliosis, where large changes in enteric neuronal numbers can lead to intestinal pathology, as observed in pseudo-obstruction or even irritable bowel syndrome [59]. Although further work is needed to link the above-mentioned studies that span a wide range of experimental contexts, a multifaceted role for MM in enteric neuronal differentiation and support is becoming evident.

Conclusions

The development of high-resolution imaging and transcriptomic tools, sophisticated mouse genetics and more detailed observations in clinical GI disorders allowed a rapid development in the understanding of gut macrophage biology. The continuing development of techniques for both model organisms and human studies will allow the dissection of the next series of unanswered questions in this field. Beyond small and large intestine distinction, how are macrophages tuned to different compartments of the intestine, for example are there unique macrophage properties in the highly absorptive duodenum versus the bacterial rich cecum? What further role do both LpM and MM play in crosstalk not only with neurons, but with additional non-hematopoietic cells such as stromal, enteric glia and interstitial cells of Cajal? How does the microbiota influence the development of both resident and replenished LpM or MM populations? Is there a role for macrophages, particularly locally maintained populations, in mediating tissue memory of past inflammatory insults within the gut? Based on recent findings of systemic effects of inflammation-induced macrophage-derived GDF15 [60], are there similar parallels for gut macrophages under steady-state or inflammatory conditions?

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Highlights

- Distinct populations of intestinal macrophages segregated both transcriptionally and anatomically, exist in the mouse and human.
- The intestine hosts long-lived macrophage populations, primarily closer to the serosa, that are not replenished by circulating monocytes in the steady state.
- Macrophages in the intestine lamina propria are critical mediators of tissue tolerance under steady state and inflammatory conditions.
- Macrophages in the intestinal muscularis are transcriptionally, morphologically, and topographically specialized to interact with the enteric-associated nervous system.
- Muscularis and lamina propria macrophages in the intestine have also co-opted additional roles for regulation of intestinal physiology including vasculature integrity, smooth muscle contraction, and neuronal maintenance.