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Research paper

Muscularis macrophages: Key players in intestinal homeostasis and disease

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ABSTRACT

Macrophages residing in the muscularis externa of the gastrointestinal tract are highly specialized cells that are essential for tissue homeostasis during steady-state conditions as well as during disease. They are characterized by their unique protective functional phenotype that is undoubtedly a consequence of the reciprocal interaction with their environment, including the enteric nervous system. This muscularis macrophage-neuron interaction dictates intestinal motility and promotes tissue-protection during injury and infection, but can also contribute to tissue damage in gastrointestinal disorders such as post-operative ileus and gastroparesis. Although the importance of muscularis macrophages is clearly recognized, different aspects of these cells remain largely unexplored such their origin, longevity and instructive signals that determine their function and phenotype. In this review, we will discuss the phenotype, functions and origin of muscularis macrophages during steady-state and disease conditions. We will highlight the bidirectional crosstalk with neurons and potential therapeutic strategies that target and manipulate muscularis macrophages to restore their protective signature as a treatment for disease.

Tissue-resident macrophages (Mφ) are highly specialized phagocytes that actively contribute to organ homeostasis. This delicate task relies on their ability to sense and respond to challenges including metabolic changes, tissue damage and microbial insults, while performing tissue-specific functions to support surrounding cells and structures [1]. Depending on the tissue in which they reside, Mφ may have to fulfill completely different tasks. For example, lung alveolar Mφ are specialized in removal and recycling of surfactant molecules produced by alveolar epithelial cells, while in the intestinal lamina propria, Mφ contribute to the local tolerogenic milieu [2,3]. In the brain, resident Mφ, i.e. microglia, assist in synaptic pruning and provide neurotrophic factors such as brain-derived neurotrophic factor [4]. Yet, resident Mφ populations are highly heterogeneous and can acquire distinct phenotypes in response to the dynamic environment within different tissues, paralleled by distinct gene-expression programs [5]. Indeed, even within the same organ, a variety of different Mφ subtypes can be identified. In the intestine, Mφ residing in the muscular layer have a different gene-expression profile compared to those residing in the lamina propria [6], while the genetic signature of lung alveolar Mφ differs from that of interstitial Mφ residing in the lung parenchyma [7]. Also in the brain, different subpopulations of Mφ subtypes can be identified, including microglia, perivascular, meningeal and choroid plexus Mφ, undoubtedly each with a different and specific function [8].

In the gastrointestinal (GI) tract, most studies have focused on the Mφ population present in the lamina propria (LpMφ). These immune cells play a crucial role in protecting the host against harmful microorganisms and continuously phagocytose and clear luminal antigens that occasionally breach the epithelial layer. Furthermore, LpMφ express receptors for anti-inflammatory cytokines such as IL-10 that prevent unnecessary inflammation towards harmless commensal bacteria and install tolerance to harmless dietary antigens [3,9]. Hence, loss of tolerance towards commensal bacteria or food antigens is believed to underlie chronic inflammation of the intestine, which can lead to inflammatory bowel diseases. However, the GI tract contains another important yet largely understudied subpopulation of resident Mφ that resides within the muscularis externa (MMφ). The recent awareness

Abbreviations: Mφ, Macrophage; LpMφ, Lamina propria macrophage; MMφ, Muscularis macrophage; ENS, Enteric nervous system; DCs, Dendritic cells; β2-AR, ß2-adrenergic receptors; POI, Postoperative ileus; α7nAChR, α7 nicotinic receptor; CNS, Central nervous system; I/R, Ischemia-reperfusion; ICC, Interstitial cells of Cajal; VNS, Vagus nerve stimulation; CAIP, Cholinergic anti-inflammatory pathway

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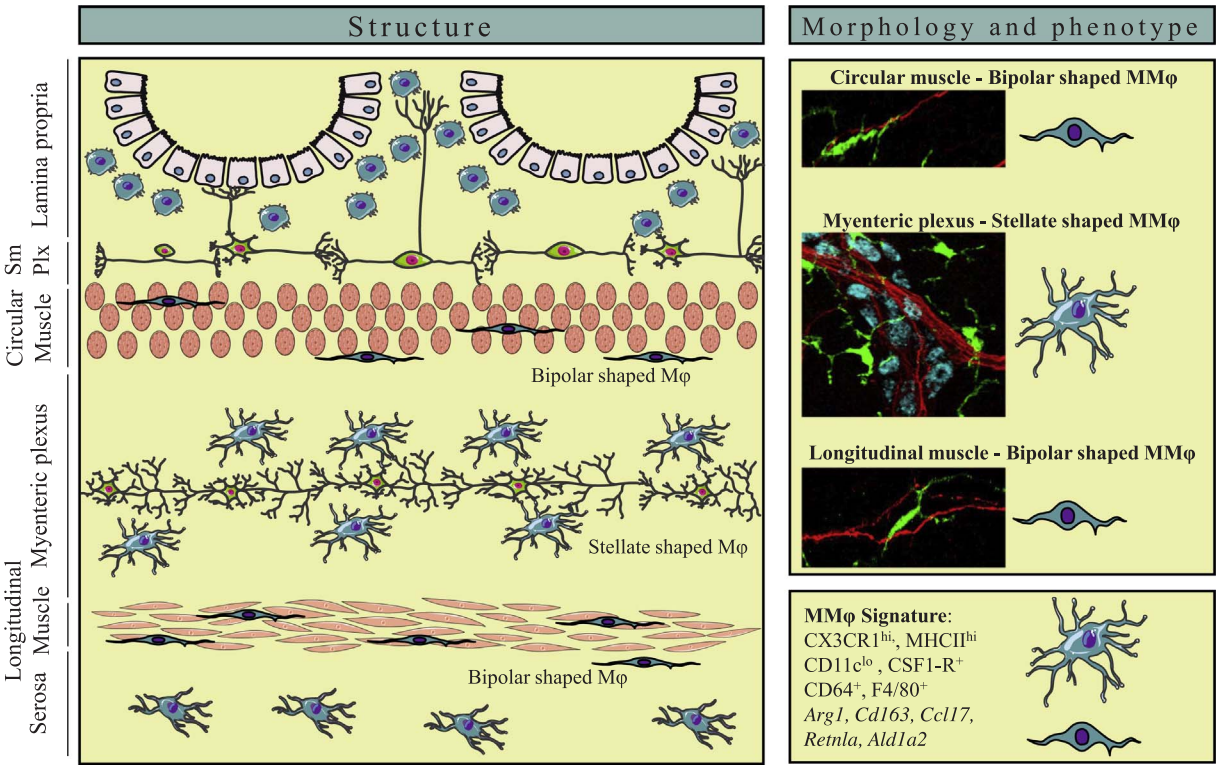

Fig. 1. The localization and phenotype of muscularis macrophages. (Left panel) Anatomical overview of macrophage (Mφ) distribution in different layers of the gastrointestinal tract. (Upper right panel) Mφ (green, CX3CR1) located in the muscularis externa (MMφ) have a distinct morphology dependent on their position within circular and longitudinal muscle layers or myenteric plexus. MMφ in the myenteric plexus resemble microglia with a ramified, stellate shaped morphology and closely contact enteric neurons (red, TUBB3) and myenteric ganglia (blue, HUC/D). (Lower right panel) MMφ express typical Mφ-specific surface markers such as CX3CR1, CD64 and F4/80. They are characterized by their tissue-protective genetic signature, such as the expression of *Arg1*, *Cd163*, *Ccl17*, *Retnla* and *Ald1a2*. SmPlx = submucosal plexus. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

that these MMφ have a distinct gene expression profile and morphology compared to LpMφ provided evidence of the strong heterogeneity among intestinal Mφ [6]. Although MMφ have important functions in GI motility during homeostasis and disease, they are largely neglected and far less defined compared to their lamina propria counterparts. In this review, we will focus on the current knowledge of MMφ and discuss their functions and phenotypes during homeostasis and pathological conditions. We will speculate on the unexplored topics of origin and longevity of MMφ, which are highly relevant factors to understand the complexity and the heterogeneity of the intestinal Mφ compartment.

1. Phenotypic characterization of muscularis macrophages

Intestinal Mφ are highly heterogeneous cells that are abundantly present in different layers of the GI tract as shown in Fig. 1. The lamina propria contains the largest number of Mφ within the intestine and are mostly found in close proximity to the intestinal epithelium, where they phagocytose bacterial antigens and produce mediators that drive epithelial cell renewal [10]. In contrast, Mφs in the muscularis externa are located distant from the intestinal lumen and are found in a dense network within the myenteric plexus, part of the enteric nervous system (ENS). Mφs are also present in lower numbers within the circular and longitudinal muscle layers of the muscularis externa and within the serosal layer, that separates the intestine from the peritoneum [6]. Of note, a CX3CR1^{hi} Mφ population is also positioned within the submucosal plexus, located immediately below the lamina propria [6]. Earlier histological studies confirmed the distribution of “macrophage-like” cells that were able to endocytose FITC-conjugated dextran particles in the muscularis externa of both mice and human [11]. Depending on the position within the muscularis externa, CX3CR1^{hi} MMφ indeed display either a bipolar or stellate morphology, what could suggest that these Mφ represent at least two phenotypically different subsets (Fig. 1) [6]. Interestingly, a similar stellate morphology is seen by microglia in the brain, that use their highly ramified filopodia to survey the brain parenchyma and actively communicate with surrounding neurons [12].

Phagocytes in the muscularis externa were initially identified based on their high expression levels of MHC class II and together with their antigen-presenting functions, they were originally classified as dendritic cells (DCs) [13]. Defining the exact nature of these cells in the muscularis externa was stifled by the lack of a clear panel of surface markers and the phenotypic similarities with LpMφ in whole-intestinal tissue preparations. To date, MMφ are typically identified based on flow cytometric analysis. Single cell suspensions can be obtained from the muscularis externa by using a protocol to mechanically separate and enzymatically digest the different layers of the intestinal tract [14]. This approach demonstrated that high expression levels of CX3CR1, F4/80 and CSF1-R and the absence of CD103 comprehensively distinguish Mφ from DCs in separate layers of the intestinal tract, including the muscularis externa. In contrast to the lamina propria, mainly populated by MHCII^{hi}CX3CR1^{hi}CD11c^{hi} Mφ, the muscularis externa is exclusively populated by MHCII^{hi} CX3CR1^{hi} Mφ that express low levels of CD11c [14]. However, the presence of a minor population of CD11c$^{-/low}$ cells within the lamina propria does not allow the use of CD11c to distinguish MMφ in whole-intestinal tissue preparations (own observations). Of note, MHCII^{hi}CX3CR1^{hi} cells within the muscularis externa also express the Mφ-specific gene marker *Fcgr1* (encoding for CD64). CSF-1 is crucial for the differentiation and maintenance of MMφ, which seem to be more dependent on the CSF-1R than LpMφ as indicated by the nearly absence of MMφ compared to the significant reduction in LpMφ in *Csf1r*$^{-/-}$ mice [14,15]. Finally, together with their characteristic large