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The monocyte-macrophage axis in the intestine

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ABSTRACT

Macrophages are one of the most abundant leucocytes in the intestinal mucosa where they are essential for maintaining homeostasis. However, they are also implicated in the pathogenesis of disorders such as inflammatory bowel disease (IBD), offering potential targets for novel therapies. Here we discuss the function of intestinal monocytes and macrophages during homeostasis and describe how these populations and their functions change during infection and inflammation. Furthermore, we review the current evidence that the intestinal macrophage pool requires continual renewal from circulating blood monocytes, unlike most other tissue macrophages which appear to derive from primitive precursors that subsequently self-renew.

1. Introduction

The intestine encounters more antigen than any other part of the body and therefore it is no surprise that it is home to the largest compartment of the immune system. Mononuclear phagocytes (MPs), including both dendritic cells (DC) and macrophages (mϕ), play a central role in discriminating harmful from harmless antigens. They initiate and sustain protective immune responses mounted towards pathogenic organisms, but also ensure that local and systemic tolerance is generated in response to innocuous antigens. When tolerance against dietary proteins and the resident commensal microbiota breaks down, this can lead to chronic inflammatory disorders such as coeliac disease and Crohn's disease; MPs are also implicated in these processes [1]. Understanding whether these somewhat paradoxical roles are carried out by the same MP, or if independent, functionally distinct subsets of MP exist is important for the development of new therapies for the treatment of inflammatory bowel disease (IBD) and other conditions.

In this review, we will discuss how intestinal macrophages (mϕ) contribute to these processes, highlighting how they develop from classical monocytes and showing how their function and fate alters depending on the presence or absence of inflammation.

2. Mononuclear phagocyte heterogeneity in the intestine

Defining the nature and origins of individual subsets of intestinal MPs has been complicated by the fact that functionally distinct populations have overlapping phenotypes. For instance, although DC are identified traditionally by their expression of CD11c and MHCII, it is now clear that multiple cell types express CD11c in the mucosa, including mϕ and eosinophils [2]. Furthermore, resident gut mϕ express high levels of MHCII [3–5], meaning that a panel of different markers is needed to discriminate these cells properly (see below). The importance of identifying these cells precisely is underlined by the fact that DC and mϕ fulfil quite distinct functions in intestinal immune responses. By definition, DCs migrate constitutively in a CCR7-dependent manner to the draining lymph node, where they interact with and cause differentiation of recirculating naïve T cells. On the other hand, mϕ are sessile, tissue resident cells whose principal role is to clear and degrade debris or pathogens, with little or no ability to prime naïve T cells [6].

Recent work from ourselves and others has shown that a combination of surface markers is needed to tease apart DC and mϕ amongst the MP populations found in normal intestinal lamina propria (LP) (see Table 1). F4/80 and CD64 (the high affinity FcRγ1) are particularly useful in this respect, as $CD11c^{+}MHCII^{+}$ MPs expressing these markers have been shown to be resident mϕ, with high phagocytic activity, little ability to prime naïve T cells and being absent from intestinal lymph [6–9]. In contrast, $CD11c^{+-}MHCII^{+}$ MPs that lack expression of F4/80 and CD64 are found in afferent lymph, prime naïve T cells and are non-phagocytic [7–9]. Their identity as *bona fide* DC is confirmed by their expression of the DC-specific transcription factor Zbtb46 and their dependence

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Table 1
Surface marker expression by monocytes, macrophages and dendritic cells in the intestinal mucosa.

	Newly extravasated monocytes	Mature macrophages	Dendritic cells
CD11b	+	+	+/–*
CD11c	–	++	+++
CD14	+	++	–
CD64	Low	+++	–
CD103	–	–	+/–*
CD172a	+	+	+/–*
F4/80	Low	+++	–
MHCII	–	+++	+++
Ly6C	+++	–	–
CX3CR1	++	+++	+/–*

* These markers define functionally distinct dendritic cell subsets with specific transcription factor requirements.

on flt3L for their development, properties not shared by the CD64$^+$ mϕ [6,9,10]. The migratory F4/80/CD64$^-$ MPs can be further subdivided into four subsets on the basis of CD103 and CD11b expression, with each subset displaying functional specialisation in terms of T cell polarisation [9] (reviewed in [11]). Importantly, these studies also show that CD103 expression alone cannot be used to distinguish DC from mϕ, as has sometimes been assumed. Similar cautions must be applied to the interpretation of CX3CR1 expression. Although it is now clear that all intestinal MPs expressing high levels of CX3CR1 are F4/80$^+$CD64$^+$ mϕ and most mature intestinal mϕ are CX3CR1^{hi}, intermediate levels of CX3CR1 (CX3CR1^{int}) can be found on cells of both the DC and mϕ lineage [6,7,9], emphasising the need for additional markers in distinguishing these cell types (see Table 1).

3. Functions of macrophages in the steady state mucosa

The primary function of intestinal mϕ is to act as effector cells of the innate immune system. Their close association with the epithelial monolayer, coupled with their high phagocytic and bactericidal activity, means they are ideally positioned to capture and destroy any material breaching the epithelial barrier. They may also be involved in clearing effete epithelial cells [12] and in tissue remodelling [13]. However, unlike mϕ in other tissues, ingestion of bacteria or exposure to stimuli such as TLR ligands does not trigger pro-inflammatory responses by mucosal mϕ, which display remarkable anergy to such stimulation [7,14]. Despite being avidly phagocytic, they also lack respiratory burst activity [15] or generation of nitric oxide [16]. Nevertheless, these cells are not completely inert and indeed, they show features of having been activated by their local environment, including high levels of MHCII expression, constitutive production of TNFα and a foamy, vacuolated cytoplasm [7,17–19]. Intestinal mϕ also play an active role in maintaining the integrity of the epithelial barrier through the production of prostaglandin E2, which promotes the proliferation and survival of epithelial progenitors [20]. Thus, they appear to exist in a balanced state in which partial activation has occurred, but inflammation is prevented.

4. Intestinal macrophages and adaptive immunity

CX3CR1^{hi} mϕ in LP take up orally administered protein antigens efficiently [21] and because of their expression of high levels of MHCII, intestinal mϕ are frequently included amongst the "antigen presenting cells" (APC) of the mucosa [22]. Indeed it has been proposed that they play a specific role in driving the differentiation of antigen-specific FoxP3$^+$ regulatory T cells (Treg) from naïve CD4$^+$ T cells in the intestine [23] and anatomical differences in the numbers of Treg in different parts of the intestine correlate with the numbers of mϕ [24]. However under steady state conditions, intestinal mϕ cannot activate naïve CD4$^+$ T cells *in vitro* and do not migrate to the MLN [6]; furthermore naïve CD4$^+$ T cells are essentially absent from the normal mucosa [25]. Thus it is unlikely that mϕ can be involved in the initial priming of Treg. Instead IL10 production by gut resident mϕ may facilitate the secondary expansion and maintenance of FoxP3$^+$ Treg that have migrated there after initial priming in the MLN [26,27]. Intestinal mϕ may play a similar role in the maintenance of other types of mucosal T cells, with microbiota-driven production of IL1β by mucosal mϕ having been shown to assist the development of Th17 cells [28]. Whether these processes require cognate interactions between antigen specific T cells and mϕ *in vivo* remains to be determined.

5. Origins of steady state intestinal macrophages

5.1. Circulating monocytes and tissue macrophage homeostasis

The traditional view of the mononuclear phagocyte system (MPS) is that monocyte precursors develop in the bone marrow (BM), with mature monocytes then entering the circulation and migrating into the organs of the body to replenish tissue macrophages [29]. In mice, monocytes arise from the common macrophage and dendritic cell progenitor (MDP) [30,31] through a common monocyte progenitor (cMoP) [32]. They express the CSF1R (CD115) and two subsets can be identified on the basis of Ly6C (or Gr-1) expression [33]. The larger subset is Ly6C^{hi} (Gr-1$^+$) and expresses high levels of CCR2 and CD62L, but low levels of CX3CR1 [33]. Although these Ly6C^{hi} monocytes were originally termed "inflammatory", given their readiness to enter inflamed tissues, they are now referred to as 'classical' monocytes [34]. Ly6C^{hi} monocyte egress from the BM is dependent on the CCR2 chemokine receptor and mice deficient in CCL2 or CCR2 have essentially no circulating monocytes, as well as showing defective recruitment of mϕ during inflammation [35,36]. The smaller subset of Ly6C^{lo} CX3CR1$^+$ monocytes expresses lower levels of CCR2 and CD62L and was originally proposed to be a distinct lineage of monocytes that replenished steady state macrophage populations [33]. However current evidence indicates that Ly6C^{lo} monocytes are the progeny of CSF-1 dependent maturation of Ly6C^{hi} monocytes and they have little or no ability to emigrate from the bloodstream into tissues [37,38]. As such, their primary function is now believed to be in the maintenance of the vasculature, including the disposal of apoptotic endothelial cells through the recruitment of neutrophils [39,40]; they are now referred to as 'patrolling' or 'non-classical' monocytes [34].

Human CD115$^+$ monocytes have also been segregated into subsets based on their expression of the LPS co-receptor CD14 and CD16 (FcγRIII) [41]. Whereas CD14^{hi} CD16$^-$ monocytes express CCR2 and are the equivalent of classical Ly6C^{hi} murine monocytes, CD14^{lo}CD16$^+$ monocytes lack CCR2 expression and are homologous to non-classical monocytes [34]. As in mice, the human monocyte subsets appear to be related to each another developmentally, with CD14^{hi}CD16$^-$ monocytes maturing into CD14^{lo}CD16$^+$ 'non-classical' monocytes through a CD14$^+$CD16$^+$ intermediary. Gene expression analysis has revealed that there is a high degree of conservation between the homologous subsets in mouse and man [42].

5.2. Generation of tissue macrophages from foetal precursors

More recently, it has been suggested that blood monocytes play little or no role in the homeostasis of resident tissue mϕ. Rather, it