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Macrophages in intestinal homeostasis and inflammation

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Summary: The intestine contains the largest pool of macrophages in the body which are essential for maintaining mucosal homeostasis in the face of the microbiota and the constant need for epithelial renewal but are also important components of protective immunity and are involved in the pathology of inflammatory bowel disease (IBD). However, defining the biological roles of intestinal macrophages has been impeded by problems in defining the phenotype and origins of different populations of myeloid cells in the mucosa. Here, we discuss how multiple parameters can be used in combination to discriminate between functionally distinct myeloid cells and discuss the roles of macrophages during homeostasis and how these may change when inflammation ensues. We also discuss the evidence that intestinal macrophages do not fit the current paradigm that tissue-resident macrophages are derived from embryonic precursors that self-renew *in situ*, but require constant replenishment by blood monocytes. We describe our recent work demonstrating that classical monocytes constantly enter the intestinal mucosa and how the environment dictates their subsequent fate. We believe that understanding the factors that drive intestinal macrophage development in the steady state and how these may change in response to pathogens or inflammation could provide important insights into the treatment of IBD.

Keywords: *intestine, macrophages, monocytes, homeostasis, inflammation*

Introduction

As discussed elsewhere in this volume, the intestine is the largest mucosal surface of the body and is the single biggest compartment of the immune system. It is exposed constantly to an array of foreign antigens and must discriminate between harmful and harmless antigens to ensure that the appropriate response is mounted to each. Robust protective immune responses must be generated in response to pathogenic insult, but similar responses mounted against innocuous dietary proteins or commensal bacteria can lead to the development of chronic inflammatory disorders such as celiac disease and Crohn's disease, respectively.

Mononuclear phagocytes (MPs) comprising both macrophages (mϕ) and dendritic cells (DCs) are central to this discrimination process and therefore hold promise as targets for potential novel therapies to treat IBD. In this article, we

discuss the role of mφ in gut homeostasis and describe how they change when inflammation or infection is present. Furthermore, we discuss the ontogeny of intestinal mφ and highlight that unlike many other tissue mφ, those resident in the gut wall must be replenished continually by blood monocytes, whose fate is ultimately dictated by the environment into which they arrive. These unusual mφ are ideally adapted to their surroundings and raise many questions about current views of the MP system and its functions.

The challenges of identifying intestinal macrophages

MPs are one of the most abundant populations of leukocytes in the healthy intestinal mucosa, and this is the largest population of mφ in the body (1, 2). However, our understanding of the roles that individual subsets of DCs and macrophages play in maintaining homeostasis, and how they change when protective immune responses or inflammation occur has been stifled by the inability to identify these cells accurately. In large part, this is due to the substantial overlap in surface phenotype of intestinal DCs, mφ, and other myeloid cells in the mucosa. For instance, although DCs in mice are traditionally identified by their co-expression of CD11c and major histocompatibility complex class II (MHCII), it is clear that mφ of the gut wall express both these markers at high levels (3). Furthermore, CD11c has been shown to be expressed by plasma cells (4) and intestinal eosinophils in mice (5, 6), with the latter also expressing the pan- mφ marker F4/80 (7, 8). Expression of the fractalkine receptor CX3CR1 has also been used extensively to distinguish murine DCs from mφ, with the proposal that these cells exhibited mutually exclusive expression of CD103, the α_E integrin, and CX3CR1, respectively (9–11). However, it is now clear that both mφ and some DCs can express CX3CR1, albeit at different levels, while not all bona fide mucosal DCs are CD103$^+$ (12–14). Thus, many of the surface markers regarded as 'lineage-specific' are now known to be expressed by multiple cell types, which in the past has led to the mischaracterization of mucosal MP. Some studies still use CD11c and MHCII co-expression alone to identify DC in non-lymphoid tissues, which makes the interpretation of these studies complex (15). Therefore, it is clear that unambiguous identification of intestinal mφ and DC requires thorough multi-parameter analysis, with several surface markers needing to be examined in combination.

A useful approach to this comes from recent work that has demonstrated that expression of CD64 (the high affinity FcRγ1), F4/80 and MerTK (12, 16, 17), can be used in combination with CD11c and MHCII to define functionally distinct MP populations in mice. In the intestine, we have shown that CD11c$^+$ MHCII$^+$ MPs expressing CD64 and/or F4/80 are highly phagocytic cells with abundant foamy cytoplasm (12). These cells do not migrate to the mesenteric lymph nodes (MLNs) (13, 14), cannot prime naive T cells (14), and their development in vivo requires the CSF-1R (18, 19), but not the DC-specific growth factor flt3 ligand (flt3L) (12, 13, 16). Together, these features confirm the classification of CD64$^+$ MPs as mφ.

When identified appropriately, murine intestinal mφ can be shown to express a number of characteristic surface molecules in addition to F4/80 and CD64, including the hemoglobin scavenger receptor CD163 and the mannose receptor (CD206) (12). Mature intestinal mφ also express high levels of CX3CR1, although as discussed below, this appears to be acquired during local differentiation in situ and some cells of the mφ lineage express lower levels of CX3CR1 (12). It is important to note that CX3CR1 expression is not a characteristic shared by all tissue mφ. For instance, lung alveolar mφ, splenic red pulp mφ, liver Kupffer cells, epidermal Langerhans cells, and mφ of the peritoneal cavity all lack CX3CR1 expression (20), although fate mapping studies suggest they derive from a CX3CR1-expressing progenitor (20). Retention and/or upregulation of CX3CR1 expression only occurs in some mφ. As well as those in the intestine, this includes microglia of the CNS and mφ resident in the kidney (20). It seems likely that this is driven by signals from the environment (see below).

In contrast, CD64$^-$ F4/80$^-$ CD11c$^+$ MHCII$^+$ MPs display poor phagocytic activity, expand markedly in response to exogenous flt3L administration, and express the DC-specific transcription factor Zbtb46 (12, 13, 21). As these CD64$^-$ F4/80$^-$ MPs also migrate constitutively to the MLNs in a CCR7-dependent manner and excel at priming naive T cells (13, 14), they appear to represent classical migratory DCs. As discussed in more detail by Agace and colleagues elsewhere in this volume, it is important to mention that the intestinal DC compartment is itself heterogeneous, with discrete subsets identifiable on the basis of CD103 and CD11b expression (13, 21, 22). Although it was once thought that all mucosal DC expressed CD103, it is now clear that bona fide CD103$^-$ DCs exist (13, 21) and that CD103 cannot be used as a de facto marker of mucosal DCs. Importantly, CD103$^-$ CD11b$^+$ DCs also express intermediate levels of CX3CR1, making them almost identical to mφ in terms of their surface phenotype. This emphasizes the need to use multiple markers to distinguish intestinal DCs from mφ, using markers such as CD64, as well as CD26 and

CD272, recently identified by the Immunological Genome Project as DC-specific markers (23).

Characterization of mϕ in the human gut can also prove difficult, with investigators using different panels of pan-myeloid cell markers, including CD33, CD14, and CD13 (24–26). Interestingly, more powerful multi-parameter analysis has shown recently that there is conservation of surface marker expression between mouse and human which may prove useful. For instance, expression of CD64 also appears to allow accurate identification of mϕ in human gut and, like those found in the mouse gut, they express high levels of MHCII, CD163, and CD68 (12, 26, 27). Unlike murine intestinal mϕ, those in the human mucosa essentially lack CD11c expression, except for a small population that also expresses high levels of the LPS co-receptor CD14, normally found at low levels on mature macrophages (12). As discussed below, these CD11c$^+$ CD14^{hi} cells may represent recently arrived monocytes from blood. Importantly, CX3CR1 has also been shown to be expressed by intestinal macrophages in humans (28), although it is unclear whether levels of CX3CR1 expression identify different subsets of MP in man.

It has become commonplace to classify mϕ into one of two major subtypes based on their surface receptor expression and which mediators they produce – M1 and M2 (29). However, gut-resident mϕ do not fit readily into this 'M1-M2 paradigm', having some of hallmarks of both M1 and M2 mϕ. For instance, they express high levels of MHCII and produce TNFα constitutively (12, 30), features normally associated with M1 or 'classically activated' mϕ (31). However as discussed above, they also express CD206, CD163, and produce interleukin-10 (IL-10), features associated with M2 or M2-like mϕ (32). However, they fail to express arginase which is a cardinal feature of M2 mϕ (12). Thus, like most tissue mϕ *in vivo*, those resident in the gut wall adapt to their local environment in complex and specific ways that may not be reflected by the rigid classification of the M1-M2 paradigm.

### Anatomical distribution of intestinal macrophages

Mϕ are found in the mucosa throughout the entire GI tract and are located mostly in the *lamina propria* (LP) in close proximity to the epithelial monolayer (2). A discrete population is also present in the smooth muscle layers of the gut wall, playing important roles in regulating intestinal motility (33, 34). The number of mϕ in the LP varies between the different parts of the GI tract, with more being found in the colon than the small intestine of humans and rodents (35, 36, authors' unpublished observations). Although it has been reported that there is a continuous gradient in mϕ numbers from proximal to distal ends of the entire mouse intestine (36), this may not be the case in humans, where the colon appears to have similar numbers of macrophages throughout its length (35).

Functions of macrophages in the steady state mucosa

Like most tissue mϕ, those resident in the normal gut wall play essential housekeeping functions, such as the clearance of apoptotic or senescent cells, and tissue remodeling (35, 37, 38) (Fig. 1). This would be consistent with their expression of scavenger receptors such as CD36 that can bind and engulf apoptotic cells (39). Furthermore, intestinal mϕ are known to produce a variety of cytokines and other soluble factors that help maintain tissue homeostasis. One such factor is PGE2 (40), which allows local mϕ to stimulate the proliferation of epithelial progenitors in intestinal crypts (41), thereby regulating the integrity of the epithelial barrier. Reflecting the importance of these homeostatic functions, selective depletion of mϕ by targeting the CSF1R leads to enhanced susceptibility to dextran sodium sulfate (DSS)-induced colitis (42) and epithelial repair in this model requires MyD88 signaling in myeloid cells (43).

However, it is in their role as innate immune effector cells that mϕ are best known. Resident intestinal mϕ are highly phagocytic and are actively bactericidal (44), which together with their positioning immediately under the epithelial monolayer, means they are ideally located to capture and destroy any material breaching the epithelial barrier (Fig. 1). They also express high levels of the TREM2 phagocytic receptor (30), which enhances their ability to engulf bacteria (45). However, one of the most characteristic features that distinguishes intestinal mϕ from their counterparts elsewhere in the body is that exposure to bacteria or their products does not trigger pro-inflammatory responses by mucosal mϕ (12, 44). Similarly, ingestion of bacteria does not stimulate respiratory burst activity (46) or the generation of nitric oxide (47) by intestinal mϕ. There is also no enhanced production of TNFα, IL-1, IL-6, or other inflammatory cytokines and chemokines in response to ligation of TLR or intracellular NOD receptors (44, 48). However, recent studies in mice indicate that intestinal mϕ are not completely anergic in terms of cytokine production, as they show constitutive production of substantial amounts of IL-10, as well as low levels of TNFα (12). Although it may
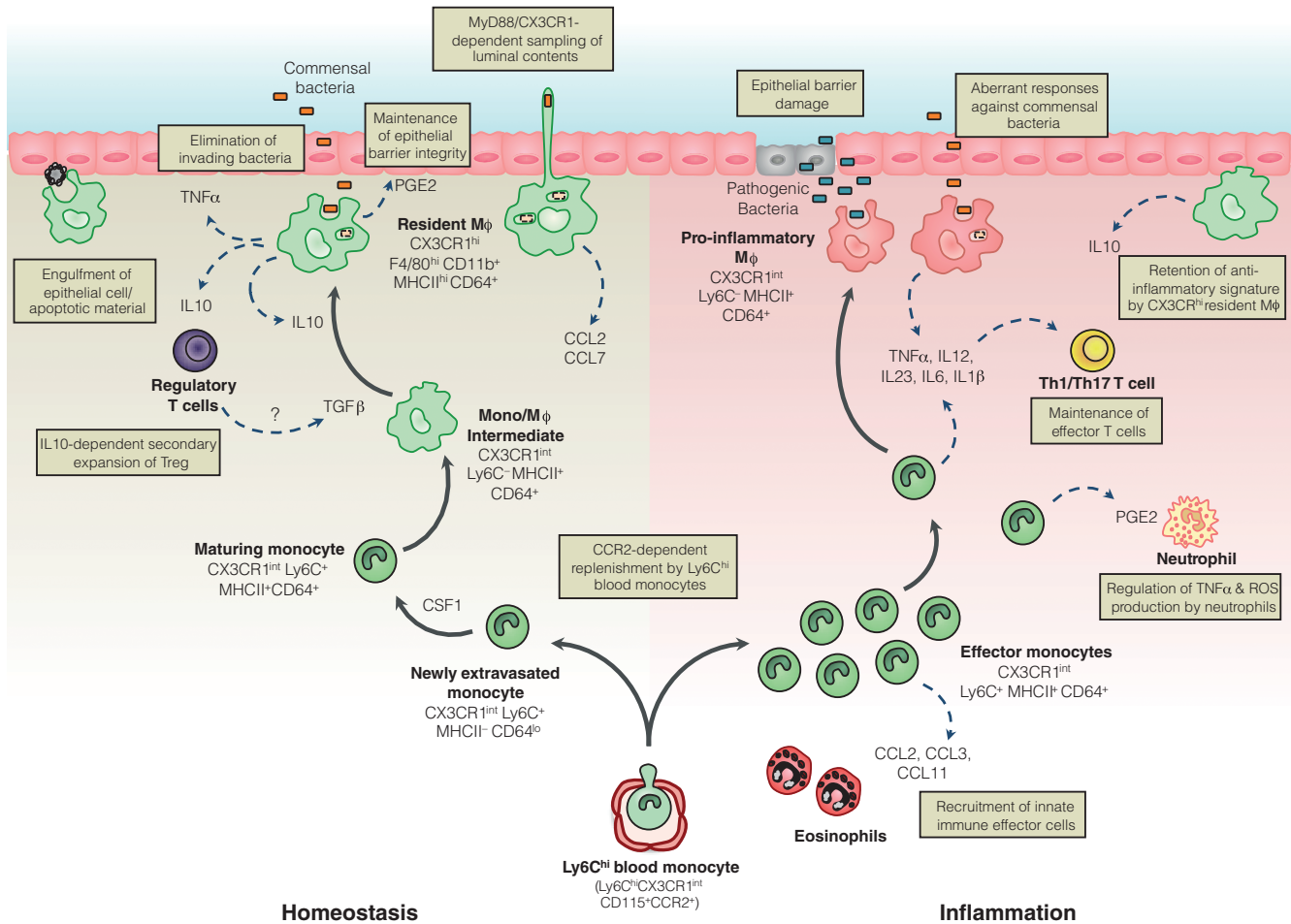
Fig. 1. Under steady state conditions, Ly6C^{hi} monocytes constitutively enter the intestinal mucosa and differentiate into mature CX3CR1^{hi} F4/80$^+$ macrophages (mϕ) through a series of short-lived CX3CR1^{int} intermediaries. These CX3CR1^{hi} mϕ are positioned immediately beneath the epithelial barrier and contribute to its integrity by secreting PGE2 which stimulates proliferation of epithelial progenitors. This positioning coupled with their high phagocytic capacity means they are poised to capture and destroy any invading commensals or pathogens, as well as clearing apoptotic or senescent cells. They may also be able to directly sample the luminal contents by the extending processes between the cells of the intestinal epithelial barrier. CX3CR1^{hi} mϕ also produce interleukin-10 (IL-10) constitutively, which facilitates secondary expansion of regulatory T cells in the mucosa and may also condition newly arrived monocytes. Regulatory T cells may also contribute to the conditioning of newly extravasated monocytes through their production of TGFβ. When homeostasis is perturbed by inflammation or infection, Ly6C^{hi} monocytes and their CX3CR1^{int} derivatives accumulate in large numbers and display enhanced pro-inflammatory characteristics. They produce pro-inflammatory cytokines which may support the maintenance of other effector cells such as IFNγ producing and IFNγ$^+$ IL-17 double producing T cells. They also orchestrate the recruitment of other innate effector cells such as eosinophils through secretion of inflammatory chemokines. Importantly, during inflammation, CX3CR1^{hi} mϕ retain their anti-inflammatory signature, e.g. IL-10 production. Elicited classical monocytes may also play a regulatory role by controlling the production of TNFα and ROS by neutrophils.

seem surprising that mϕ constitutively produce this prototypical pro-inflammatory cytokine, TNFα may have much wider effects. For instance, TNFα can regulate enterocyte growth and alter the permeability of the epithelial barrier (49). Furthermore, TNFα stimulates production of matrix metalloproteinases and other tissue remodeling enzymes in the intestinal mesenchymal cells that are central to regulating epithelial cell function (50).

Given their high expression of MHCII and their ability to take up orally administered protein antigens (51–53) and bacteria (37), intestinal mϕ may also serve as antigen-presenting cells, interacting with and influencing the differentiation of CD4$^+$ T cells (Fig. 1). It has also been suggested that mϕ may play a selective role in initiating the differentiation of FoxP3$^+$ regulatory T cells in the intestine (54). However, as mϕ do not migrate to the MLN (14) and the intestinal mucosa is essentially devoid of naive CD4$^+$ T cells (55), they are unlikely to be involved in the initial priming of naive CD4$^+$ T cells. Furthermore, LP mϕ are extremely poor at activating naive CD4$^+$ T cells in vitro (14). Thus if mucosal mϕ do present antigens to CD4$^+$ T cells, it seems more likely that this involves lymphocytes

that have returned to the mucosa after being first primed in the MLN. Consistent with this idea, Hadis *et al.* (56) have recently shown that mφ facilitate the secondary expansion and maintenance of antigen-specific FoxP3^{+} Treg in the intestinal mucosa through their production of IL-10 (Fig. 1). The importance of this local fine tuning of T-cell responses by mφ is highlighted by the loss of FoxP3 expression and suppressive capacity that occurs when T cells are rendered unresponsive to IL-10 through deletion of the IL-10R (57). Further evidence that intestinal mφ may contribute to the generation of homeostatic Treg comes from work showing that mice lacking TRAF6 expression in CD11c^{+} MP develop a spontaneous Th2 cell-mediated enteritis. This is associated with a defective ability of mucosal MP to induce FoxP3^{+} T cells *in vitro* and reduced numbers of Treg in the LP (58). Although interpreted as reflecting a role for 'DCs', the relevant myeloid cells were defined only as CD11c^{+} and as we have noted, most of these cells in the normal LP are in fact mφ. Further work is needed to define the precise cellular basis of TRAF6-mediated generation of Treg in the LP.

Intestinal mφ may also contribute to the maintenance of other T cells in the mucosa. For instance, the production of IL1β in response to TLR stimulation may be relatively preserved in intestinal mφ and this has been reported to help support the development of Th17 cells in the steady state intestine (59). It remains to be determined whether these effects of intestinal mφ on T cells require cognate interactions via peptide-MHC complexes and the TCR, or whether it involves non-specific factors such as polarizing cytokines alone, as has been suggested for the generation of Th17 responses by mucosal CD103^{+} CD11b^{+} DCs (60).

Recent studies have proposed a further way in which mucosal mφ may contribute to indirectly to T-cell priming, through Connexin 43-mediated transfer of soluble antigen obtained from the lumen to neighboring CD103^{+} DCs (52). This cooperative process has been suggested to be important for the development of oral tolerance and if confirmed, these findings could indicate an important new role for mφ in the steady state intestine.

Exactly how mφ in the LP acquire luminal antigen remains to be established with certainty. One possibility is that they may extend transepithelial dendrites (TED) across the epithelium to capture soluble antigen in the lumen (Fig. 1). Originally thought to be derived from DCs on the basis of the expression of MHCII and CD11c (61, 62), it now seems clear that these TED originate from CX3CR1[+] mφ. Their formation requires CX3CR1 (61, 63) and indeed,

the induction of oral tolerance to protein antigens is impaired in CX3CR1-deficient mice (52, 56), consistent with a requirement for CX3CR1-mediated uptake of luminal antigen in this process. However, the physiological significance of such TED remains unclear. Not only did the original reports disagree on which part of the small intestine they were present in and if they were dependent on TLR signaling (61, 62), but more recent studies have failed to find TED anywhere in the steady state intestine (64). Furthermore, earlier studies on the uptake of soluble protein from the intestinal lumen reported that this process was not dependent on the CX3CL1–CX3CR1 axis (14). Finally, as we have noted, other work has also proposed that the defect in oral tolerance in CX3CR1-deficient mice reflects an absence of IL-10 production by intestinal mφ (56). If and how LP mφ contribute to the uptake of soluble antigen and then help shape subsequent immune responses clearly warrants further investigation.

Despite their phagocytic activity and morphological appearance, there is similar uncertainty about whether LP mφ play a role in the capture of and induction of immunity or tolerance to local bacteria. Although it is well established that CD103^{+} DCs can present bacterial antigen to naive T cells in the MLN (53), initial reports indicated that the TED formed by MP in the mucosa could capture *Salmonella* organisms in the lumen (61, 62). Furthermore, a very recent study showing that CX3CR1^{+} cells may transport *Salmonella* in lymph to the MLN was interpreted as showing a role for migratory mφ in this process (65). However, this phenomenon was only revealed after depletion of the normal microbiota with antibiotics and the study did not exclude the possibility that the antigen loaded CX3CR1[+] MP belonged to the population of bona fide CX3CR1 expressing DC that we and others have observed in intestinal lymph (13, 14). Again, these findings reveal how much is still to be understood about how mφ contribute to the induction and shaping of mucosal immune responses.

Ontogeny of intestinal macrophages in the steady state

The mononuclear phagocyte system

Tissue mφ are traditionally viewed as part of the mononuclear phagocyte system (MPS). First described in 1972 (66), the concept of the MPS proposes that all tissue mφ are the terminally differentiated progeny of blood monocytes, which constitutively enter tissues under steady state conditions, and are themselves replaced by rapidly dividing

precursors in the bone marrow (BM). Originating from the common myeloid progenitor, which also generates DCs and granulocytes, a common macrophage and dendritic cell progenitor gives rise to both committed DC precursors (pre-DCs) and to monocytes (67, 68), with the latter passing through a recently identified common monocyte progenitor (69). The development of monocytes via these processes is dependent on the transcription factor PU.1 and the growth factors CSF1 (M-CSF), CSF2 (GM-CSF), and IL-3 (69).

It is now known that the monocyte pool is heterogeneous and, in mice, $CSF1R^+$ (CD115) monocyte subsets can be distinguished by their expression of Ly6C/Gr1 and the chemokine receptors CCR2 and CX3CR1 (70). The majority of blood monocytes expresses high levels of Ly6C/Gr-1, CCR2 and CD62L, but low levels of CX3CR1 (so-called $Ly6C^{hi}$ monocytes). The entry of $Ly6C^{hi}$ monocytes into the circulation from the BM is dependent on the CCL2-CCR2 chemokine axis and as a result, mice with genetic deletion of either of these molecules have markedly fewer circulating monocytes (71). Although initially referred to as 'inflammatory' monocytes because of an apparent preference to migrate to inflamed tissues, these $Ly6C^{hi}$ monocytes are now classified as 'classical' monocytes, as they fulfill all the roles previously attributed to generic monocytes in the MPS. In human, an analogous population of classical monocytes is identified as $CD14^{hi}$ $CD16^-$ and these also express CCR2 (72).

A smaller population of murine monocytes expressing lower levels of Ly6C and CCR2, but higher amounts of CX3CR1 ($Ly6C^{lo}$ monocytes) were initially proposed to be the precursors of steady state tissue mφ (70). However, little direct evidence exists to support this idea, and more recently it has become clear that the primary function of $Ly6C^{lo}$ monocytes is to act as phagocytes in the bloodstream, patrolling and maintaining the vasculature (73, 74). As such, they are now commonly referred to as 'patrolling' or non-classical monocytes (75), and there is accumulating evidence that there is a developmental relationship between classical and non-classical blood monocytes, with the latter being the CSF1R-dependent progeny of the former (20, 76). The equivalents of these non-classical $Ly6C^{lo}$ monocytes in human are identified as $CD14^{lo}$ $CD16^+$, and these express relatively high levels of MHCII (70). Whether they exert similar patrolling functions to their murine counterparts is not known, although gene expression analysis has revealed many parallels between the monocyte subsets in mouse and human (72).

Macrophage development independent of the MPS

The validity of the MPS has been questioned recently, with multiple reports from independent groups proposing that the majority of tissue mφ exists independently of blood monocytes (77–79). Using a variety of techniques including fate mapping, parabiosis, and analysis of proliferative capacity, it has been shown that epidermal Langerhans cells (79, 80), lung alveolar mφ (20, 81), liver Kupffer cells (20, 77), microglia of the CNS (20, 77, 82, 83), and mφ of the peritoneal cavity (78) derive from embryonic precursors that seed tissues early in development and maintain themselves through in situ proliferation. Although there is debate about whether these precursors originate in the mesenchyme of the yolk sac, or from the fetal liver, an assumption has come about that all tissue mφ populations may be derived from these early progenitors (84).

Our recent work shows that the intestine is an exception to this paradigm, as mφ in the adult steady state mucosa require continuous replenishment by $Ly6C^{hi}$ blood monocytes (12). Although earlier studies had shown that $Ly6C^{hi}$ monocytes could enter the colonic mucosa following intense depletion of resident MP (9), it was unclear whether such a mechanism operated in the unmanipulated mucosa and if the progeny represented mφ or DCs (10, 85). Using an adoptive transfer approach, we could demonstrate that $Ly6C^{hi}$ monocytes constantly enter the normal colonic mucosa and mature into $CD64^+$ $F4/80^{hi}$ $CX3CR1^{hi}$ $MHCII^+$ cells (12, 16), indistinguishable from endogenous resident mφ (Fig. 1). The development of mature mφ involves a local differentiation process, in which a series of intermediaries lose Ly6C and upregulate F4/80, CX3CR1, CD163, and CD206, as well as acquiring CD11c and MHCII expression (12), features that account for their previous misclassification as DCs (9, 10). Full maturation appears to take around 5 days and as well as adopting the phenotypic signature of mature colonic mφ, the differentiating monocytes begin to acquire the characteristic functional signature of intestinal mφ. Thus they become increasingly phagocytic and begin to produce IL-10, as well as developing resistance to TLR stimulation (12).

There are multiple lines of evidence to support the idea that monocyte recruitment is the principal mechanism of replenishment of intestinal mφ. Firstly, mφ in the adult mucosa have very poor proliferative capacity (12, 86), suggesting in situ self-renewal plays little or no role under normal physiological conditions. Secondly, mice in which the CCL2–CCR2 axis has been disrupted by genetic deletion of

either molecule have significantly fewer colonic mφ (12, 87). Next, the intestinal mφ pool derives almost exclusively from WT BM in WT:CCR2$^{-/-}$ mixed BM chimeric mice (16). Finally, cells matching the phenotype of intestinal mφ are completely eliminated by DT-mediated depletion in CCR2-DTR mice (88). This requirement for the CCL2-CCR2 axis does not simply reflect its role in BM egress of monocytes, as CCR2-sufficient Ly6C^{hi} monocytes fail to enter the CCL2-deficient gut mucosa in WT-CCL2$^{-/-}$ parabiotic mice (87), demonstrating that the CCL2-CCR2 axis is needed for entry of monocytes into the intestinal LP. Although the source of CCL2 in the intestine is unclear, CX3CR1^{hi} resident mφ in the normal mucosa express high levels of the CCR2 ligands CCL2 and CCL7 (87, 89) and therefore they may control their own replenishment. This mechanism has been shown to occur in the mouse uterus, where CSF1R-signaling results in production of CCR2 ligands by uterine mφ (90). Intestinal mφ are all labeled in MacGreen mice in which GFP is expressed under control of the CSF1R promoter (91, 92) and thus it is intriguing to speculate that rather than being required for their local proliferation/survival, CSF1R-signalling in gut mφ may be required to elicit monocyte recruitment to the mucosa, as has been suggested in other tissues (90, 93).

As CCR2- and CCL2-deficient mice are not completely devoid of intestinal mφ (12, 87), there may be other mechanisms of replenishment in addition to the CCL2-CCR2 axis. This is unlikely to involve non-classical Ly6C^{lo} monocytes, as these fail to migrate into non-lymphoid tissues when adoptively transferred (9, 12), and as discussed above, they seem to have a distinct effector role patrolling the vasculature (73). In addition, although CX3CR1-deficient mice have markedly fewer circulating Ly6C^{lo} monocytes due to impaired survival (63, 94), the intestinal mφ compartment of these mice remains unaffected in size (56). In vitro studies have also implicated TGFβ and IL-8 in the recruitment of monocytes to the intestinal mucosa (27, 86), but the role of these and other mediators has not been addressed in vivo.

Why the gut mucosa should need continuous recruitment and differentiation of Ly6C^{hi} monocytes is not completely understood. An obvious possibility is that this is required to monitor the constant exposure to the commensal microbiota and other environmental agents found at barrier surfaces. Although the abundance of differentiating monocytes is reduced in the dermis of germ free (GF) mice (16), different studies mice have reached conflicting conclusions on the size of colonic mφ compartment in GF mice (11, 95, 96).

Furthermore, whether GF conditions or antibiotic treatment alter monocyte recruitment to the gut mucosa and/or their subsequent differentiation has never been formally examined.

An analogous monocyte differentiation process seems to exist in human intestine. We recently showed that CD14^{hi} monocytes appear to differentiate into mature mφ through a series of intermediaries which involves downregulation of CD14 and CD11c, and upregulation of MHCII, CD209 and CD163 (12). Together these findings indicate that classical monocytes are the principal source of the resident intestinal mφ pool in mouse and human.

Monocyte education in the mucosa

The environmental cues that shape monocyte differentiation in the intestinal mucosa remain to be identified with certainty, and it is likely that multiple factors are responsible for the imprinting of the phenotypic and functional characteristics adopted by maturing monocytes (Fig. 1). These factors are likely to be specific to the intestinal mucosa, as monocytes do not acquire the same characteristics after entering other mucosal sites such as the lung (97). Multiple growth factors are responsible for the maturation of cells of the MPS, including flt3L, CSF1, and CSF2. As noted above, intestinal mφ do not expand in response to exogenous flt3L and remain equally abundant in flt3L-deficient mice (16, authors' unpublished data). Similarly, although some mφ such as lung alveolar mφ have been shown to depend on the CSF2R for their development (78, 98), it is unlikely that this applies to intestinal mφ, as cells matching their phenotype are derived equally from CSF2R-deficient BM in WT:CSF2R-deficient BM chimeras (99). The development of intestinal mφ requires signaling through the CSF1R and as a result, they are derived almost exclusively from WT BM in WT:CSF1R$^{-/-}$ mixed BM chimeric mice (19). There are currently two identified ligands of the CSF1R: CSF1 and IL-34. To date, only microglia and Langerhans cells of the epidermis have been reported to depend on IL-34 and in these cases, this appears to be the driver of in situ self-renewal (100, 101), making it seem unlikely that IL-34 will play a role in intestinal mφ homeostasis. However this has never been examined directly. It is likely that CSF1 is the predominant ligand required in the gut as evidenced by a marked reduction in intestinal mφ in osteopetrotic Csf1$^{op/op}$ mice, which have a mutation in the gene encoding CSF1 (102). It also remains to be determined at which stage of intestinal mφ development CSF1R signalling is involved and specifically whether it is important simply for the generation

of progenitors, or also for the differentiation of monocytes that enter the mucosa. The loss of Ly6C and upregulation of F4/80 that occurs during the transition of Ly6C^{hi} to Ly6C^{lo} monocytes in blood is dependent on CSF1-CSF1R interactions (18, 20), as is the development of monocyte-derived mϕ in other tissues (18, 19). However, it seems unlikely that CSF1R signalling alone can account for all aspects of intestinal mϕ development given the fact that adoptively transferred monocytes generate entirely distinctive mϕ in this tissue. Thus, the local environment of the intestinal mucosa must play the definitive role in local mϕ development.

A distinctive feature of intestinal mϕ is their expression of MHCII at levels even greater than those seen on mucosal DCs. Indeed, acquisition of MHCII is one of the first phenotypic changes to occur during monocyte maturation in the intestine, although its functional significance remains unclear (12). This does not appear to be a default property of monocyte maturation, as it is not a universal feature of other monocyte-derived tissue mϕ (90, 103) or of the blood Ly6C^{lo} monocytes, which mature from Ly6C^{hi} monocytes (97). IFNγR signaling is known to induce MHCII on mϕ (104), but MHCII expression by intestinal mϕ is normal in IFNγR-deficient mice (our unpublished observations). It also appears to be independent of CSF2, IL-10, and lymphocytes or their products (our unpublished observations). Recently, the Randolph laboratory has suggested that signals from vascular endothelial cells may instruct upregulation of MHCII by extravasating monocytes (97). However, whether this occurs as monocytes enter the gut mucosa remains unclear.

As noted above, acquisition of the CX3CR1^{hi} phenotype appears to be associated with the development of an anti-inflammatory signature in fully differentiated intestinal mϕ. Given that the majority of mϕ in other tissues lack CX3CR1, it is clear that the local environment of the gut must instruct developing monocytes to upregulate this molecule. The factor responsible has not been identified, although TGFβ is a clear candidate, as it is known to induce CX3CR1 expression by microglia in the brain (105). There is accumulating evidence that CX3CR1 itself may play a role in controlling the differentiation and function of intestinal mϕ. Firstly, as discussed above, the CX3CL1–CX3CR1 axis has been shown to control the ability of CX3CR1$^+$ MPs to extend cellular protrusions into the intestinal lumen (61, 63, 106). Although this remains an area of contention, it is conceivable that the expression of CX3CL1 by enterocytes (63) could influence the anatomical positioning of mϕ under the epithelial layer. However to date, it has not been reported that CX3CR1-deficient mϕ

inhabit different anatomical locales than their WT counterparts. Next, the CX3CL1–CX3CR1 axis has been shown to be required for optimal production of IL-10 by intestinal mϕ (56) and finally, CX3CR1-deficiency has been reported to lead to decreased numbers of intestinal mϕ (107, 108) as well as altering susceptibility to chemically induced colitis. However, given that CX3CR1-deficient mice have been shown both to be protected (11, 63) and more susceptible to experimental colitis (107), further investigation of the CX3CR1–CX3CL1 axis is required to elucidate its involvement in the control of mϕ function in the intestine.

The IL-10–IL-10R axis plays a crucial role in conditioning the behavior of mϕ in the mucosa. One such effect is the upregulation of scavenger receptors such as CD206 and CD163, which is reduced in IL-10-deficient mice (96, 109). Even more marked is the requirement for IL-10 in the hyporesponsiveness of intestinal mϕ to pro-inflammatory stimuli (44) (see below). The cellular source of the IL-10 remains unclear. Although mϕ themselves produce IL-10 constitutively and this could act in an autocrine manner, selective knockout of IL-10 in LysM$^+$ cells appears not to lead to spontaneous IBD in mice (110), suggesting other cells are more important. This is likely to be IL-10-producing FoxP3$^+$ CD4$^+$ T cells, as these are abundant in the normal intestine and IBD occurs after specific deletion of IL-10 in these cells (111, 112). The induction of IL-10 in maturing mϕ may be a response to colonization of the intestinal tract by the commensal microbiota, as colonic mϕ from mice reared in GF conditions produce less IL-10 (95, 96). However given that this failure to upregulate IL-10 production cannot be recapitulated in MyD88$^{-/-}$ mice (95), the mechanisms underlying this phenomenon require further investigation. Interestingly, the production of protective IL-10 by CD4$^+$ T cells is also dependent on the microbiota (113). While it has been reported that IL-10 may be important for more general aspects of maturation of newly recruited monocytes in the peritoneum, including upregulation of MHCII (114), our work on inflamed intestine suggests that the lack of MHCII may reflect the failure of monocytes to differentiate fully due to the enhanced inflammation found in the absence of IL-10, rather than a direct effect of IL-10 (see below).

Mechanisms of inflammatory anergy in intestinal macrophages

Hyporesponsiveness to activation via TLRs and other stimuli is a cardinal feature of resident intestinal mϕ. Early work suggested that this was the result of a failure to

express pattern recognition receptors. However, it is now clear that gut-resident mφ in mice and human express a full range of TLR (12, 27), and it is now believed that regulation of adapter molecules downstream of the TLR may instead be responsible for the hyporesponsiveness. For instance, signaling molecules such as CD14, MyD88, TRAF-6, MD2, TRIF, and IRAK1 appear to be downregulated in mature intestinal mφ (27, 44, 89). In parallel, mechanisms that inhibit TLR signaling and/or NF-κB activation (e.g. IRAK-M and IkBNS) appear to overexpressed in gut-resident mφ (27, 89, 109), suggesting that molecules that propagate TLR signaling are targeted rather than the TLR expression itself.

At least some of these inhibitory processes may be driven by IL-10 (109). In mice, the hyporesponsiveness develops progressively as monocytes mature in the mucosa and it correlates with increasing IL-10 production (12). As a result, colonic mφ from IL-10$^{-/-}$ mice or mice with LysM-mediated deletion of the IL-10R signaling molecule STAT3 display exaggerated pro-inflammatory responses to bacterial products (95, 96, 109, 115), and the animals develop spontaneous colitis (116, 117). Importantly, IL-10R deficiency is also associated with an early onset form of severe IBD in human (118, 119). Thus, it is clear that IL-10 is one of the most important factors ensuring mφ quiescence in the face of environmental stimuli. However, other mechanisms are likely to be involved. For instance, TGFβ has been shown to render blood monocytes hyporesponsive to a variety TLR ligands (27) and active TGFβ is abundant in the steady state LP (120). The spontaneous, microbiota-dependent enteritis that occurs in mice lacking expression of TRAF6 in CD11c$^+$ MP could also reflect defective TGFβR-dependent signaling in mφ, as TRAF6 and its downstream partner TAK1 are critical components in this pathway (58).

The control of mφ activity may also involve dedicated inhibitory receptors including SIRPα and CD200R1. Indeed, the CD200–CD200R1 axis appears to play an important role in regulating alveolar mφ activity (121). However, our recent work suggests that the same regulatory loop is dispensable in the intestinal mucosa, with CD200R1-deficient colonic mφ showing no signs of dysregulation (8). Furthermore, deletion of CD200R1 or its only ligand, CD200 does not render mice more susceptible to DSS-induced colitis, as would be expected if mφ activity was not controlled appropriately (8). Although resident mφ express high levels of SIRPα, whether this plays any role in controlling intestinal mφ activity through interaction with its ligand CD47 has never been formally tested.

The development of the hyporesponsive state appears to involve recognition of microbial signals, as colonic MP from GF mice respond robustly to stimulation with the TLR4 agonist LPS (96). However, it must be noted that in this study mφ were identified only as CD11b$^+$ CD11c$^-$ cells, which will include many cell types and exclude the majority of resident mφ which express CD11c. Therefore, the full impact of the microbiota on the behavior of resident colonic mφ remains to be elucidated.

Together, these findings demonstrate that fully responsive Ly6C^{hi} monocytes continuously enter the steady state intestinal mucosa as a surveillance measure. In the absence of any threat, the monocytes adopt an anti-inflammatory phenotype which is imprinted by local factors in the mucosa. The nature and mechanisms of action of these mediators remain to be defined fully.

Monocytes and macrophages in intestinal inflammation

It is well known that the composition of the human intestinal mφ pool changes considerably when there is perturbation of homeostasis. For instance, in the mucosa of Crohn's disease and ulcerative colitis patients, there is an accumulation of pro-inflammatory macrophages. These can be identified by their high expression of CD14, in contrast to the CD14$^{lo/-}$ population of resident mφ (12, 24, 122, 123). The ability of these cells to produce large amounts of mediators such as IL1, IL6, TNFα, reactive oxygen intermediaries and nitric oxide makes them quite distinct from the mφ found in healthy intestine and has led to interest in the idea of targeting the monocyte-macrophage lineage for therapeutic purposes. For these reasons, it has become important to establish whether the inflammatory cells represent newly arrived monocyte-derived cells, or are resident cells that have altered their behavior in the presence of inflammation.

Mouse models of intestinal inflammation

There is similar infiltration of pro-inflammatory monocytes and mφ in animal models of intestinal inflammation such as colitis induced by administration of DSS (12, 89), by the adoptive transfer of naive CD4$^+$ T cells into lymphopenic hosts (16, 30), or after infection with *Helicobacter hepaticus* coupled with neutralization of IL-10 (our unpublished observations). In all these cases, the infiltrate is characterized by a reversal in the ratio of CX3CR1^{hi} and CX3CR1^{int} cells, caused by the accumulation of Ly6C^{hi} monocytes and their CX3CR1^{int} progeny (Fig. 1). The infiltrating CX3CR1^{int} cells display typical pro-inflammatory characteristics, including

the production of TNFα, IL-6, IL-1β, IL-12, IL-23, and expression of iNOS. In contrast, the remaining CX3CR1^{hi} mφ retain their anti-inflammatory signature, continuing to produce high levels of IL-10 and being TLR hyporesponsive, suggesting that resident mφ do not turn rogue during inflammation (12, 16, 30, 89). Not unexpectedly, CCR2 is required for the entry of pro-inflammatory cells into the inflamed colonic mucosa, with considerably fewer CCR2$^{-/-}$ cells entering the mucosa when co-transferred with their WT counterparts (89, 124). A pathological role for these elicited Ly6C^{hi} monocytes is evidenced by amelioration of colitis in monocytopenic CCR2-deficient mice (124) and in mice depleted of CCR2-expressing cells (89). Furthermore, TNFα production by these cells appears to be central to disease progression, as the severity of DSS-induced colitis is reduced in mice in which Ly6C^{hi} monocytes are deficient in TNFα production (9). On the basis of our studies of DSS colitis, we have proposed that the normal monocyte differentiation process is arrested during inflammation, resulting in the retention of cells that remain responsive to TLR stimulation and produce copious amounts of pro-inflammatory cytokines and chemokines (12, 30, 89). Why these processes are disrupted in inflammation remains to be determined, but presumably reflects alterations in the local microenvironment, leading to defects in the conditioning mechanisms that normally specify maturation of monocytes into anti-inflammatory mφ. Clearly however, the resident mφ themselves cannot be influenced by these changes, indicating these are likely to be terminally differentiated cells and raising the possibility that homeostasis may be restored relatively quickly if the inflammatory environment can be modified.

Although Ly6C^{hi} monocytes and their immediate progeny appear to be detrimental during sterile models of intestinal inflammation (12, 124), they are also indispensable for the eradication of certain enteric pathogens. This is consistent with findings that elicited CX3CR1^{int} cells, many of which are likely to be derivatives of Ly6C^{hi} monocytes, have been shown to take up *Salmonella* organisms as efficiently as resident CX3CR1^{hi} mφ (37). During the acute ileitis caused by oral inoculation of certain mouse strains with the protozoan parasite *Toxoplasma gondii*, there is accumulation of Ly6C^{hi} monocytes, which display the same behavior as those arriving into the DSS-inflamed colon, including the production of the pro-inflammatory cytokines TNFα and IL-12, and reactive nitrogen species (125). The protective role of these monocytes is demonstrated by the fact that both CCR2- and CCL2-deficient mice succumb to lethal toxoplasmosis, and

that the adoptive transfer of CCR2-competent Ly6C^{hi} monocytes can rescue this lethality (125). Interestingly, the CCL3–CCR1 chemokine axis driven by innate lymphoid cells is also involved in the recruitment of pro-inflammatory Ly6C^{hi} monocytes and protective immunity in this model of infection, indicating that CCR2 may not be the only mechanism responsible for accumulation of monocytes during inflammation (126). However, no defects in steady state intestinal mφ have been reported in mice lacking CCR1, perhaps suggesting distinct roles for individual chemokine receptors under different conditions.

Ly6C^{hi} monocytes have also been implicated in the protective immune response to *Citrobacter rodentium*, a mouse model of enteropathogenic and enterohaemorrhagic *E. coli* infection in human, with delayed clearance in CCL2- and CCR2-deficient mice (127). However, recent elegant work has questioned this conclusion, by showing that ablation of the entire monocyte-macrophage compartment in mice with LysM-driven expression of DTR by CSF1R-expressing cells has no effect on susceptibility to *C. rodentium* infection (128). In contrast, the robust Th17 response needed to clear this organism (127) is absolutely dependent on classical migratory DC expressing zbtb46 (21). Interestingly however, monocyte-derived macrophages may make an additional contribution to the adaptive immune response under these conditions, by producing IL12, which maintains IFNγ$^+$ and IFNγ$^+$ IL17$^+$ effector T cells in the mucosa (128). Thus, whereas resident mφ maintain regulatory T cells via IL-10 production, recently elicited monocytes may maintain effector T cells through the production of pro-inflammatory cytokines (Fig. 1).

Contrasting with their role as inflammatory effector cells, recent studies suggest that Ly6C^{hi} monocytes may have a wider ability to regulate other aspects of intestinal immunity (Fig. 1). For instance, in the DSS colitis model, Ly6C^{hi} monocytes mediate the recruitment of eosinophils through their production of CCL11 (eotaxin) (129). In *T. gondii*-induced ileitis, elicited Ly6C^{hi} monocytes may actually prevent immunopathology by inhibiting the ability of local neutrophils to produce tissue damaging TNFα and ROI. This PGE2-dependent process involves production of IL-10 by monocytes (40) and may go some way to explain earlier observations of enhanced neutrophil accumulation in the mucosa of *T. gondii* infected CCR2-deficient animals (125). These results support other reports that monocytes are not intrinsically inflammatory in nature, but are highly plastic cells that coordinate the first stages of tissue repair in response to damage, infection, or inflammation (130).

Furthermore, the regulatory properties of elicited Ly6C^{hi} monocytes are diminished in GF mice, underlining how the local microenvironment determines the fate of monocytes in the mucosa (40).

Mφ have also been implicated in Th2 cell-mediated resistance to intestinal helminth infections (131). Their numbers are expanded during infection by organisms such as *Trichuris muris*, *Nippostrongylus brasiliensis*, *Heligmosomoides polygyrus*, *Brugia malayi*, and *Litomosiodes sigmodontis* (132–134), and under these conditions, 'alternative' activation of mφ by the Th2 products IL-4 and IL-13 leads to the production anti-parasitic mediators such as arginase and RELM-α (133, 135). However, the exact role of mφ in these infections is uncertain. The expulsion of these parasites is compromised in mice with mφ depletion secondary to absence of CCL2 (132) or administration of clodronate-containing liposomes (136, 137). Alternatively activated mφ are also partly responsible for the increased smooth muscle contractility which contributes to parasite expulsion (136). However, the protective effects of mφ do not require them to produce arginase-1 (138) or even to be polarized via the IL-4 receptor (139). Thus, mφ may play an indirect role in protective immunity, perhaps by producing mediators that recruit other effector cells such as eosinophils and mast cells. Alternatively, they may be involved in the anti-inflammatory and tissue repair processes that are crucial components of the host response to multicellular pathogens and that are important properties of mediators such as arginase (140).

The origin of the alternatively activated macrophages in helminth infection is also uncertain. Although the requirement for CCL2 in parasite expulsion could indicate they are derived from recruited Ly6C^{hi} monocytes as in the models of inflammation discussed above, this has never been investigated directly. In addition, recent studies of macrophage expansion during helminth infection of the peritoneal and pleural cavities suggest that this may be driven by IL4- and CSF-1-dependent proliferation of the pre-existing resident population (141). The relative roles of *in situ* expansion of mφ and monocyte recruitment during intestinal helminth infection could generate important insights into the plasticity and functional properties of mucosal macrophages.

Monocytes and macrophages in human IBD

As discussed above, CD14^{hi} MP accumulate in the mucosa of IBD patients (12, 24), and CD14^{hi} cells in human are the equivalent of Ly6C^{hi} monocytes in the mouse. As in mice, the CD14^{hi} cells that appear in human intestine display enhanced production of TNFα, IL-1β, and IL-6 (123) and respiratory burst activity (46). They also retain their responsiveness to microbial products (24, 122). Using the transfer of radiolabelled autologous blood monocytes, it has been demonstrated that the CD14^{hi} cells in the inflamed mucosa derive from circulating monocytes (142), likely in response to elevated levels of the monocyte chemoattractants CCL2 and CCL4 in the inflamed mucosa (39). In addition to their own pro-inflammatory effects, elicited monocytes in human have also been shown to orchestrate the recruitment of other innate effector cells, such as CCR3$^+$ eosinophils through their production of CCL11 (eotaxin-1) (25, 129). Furthermore, CD68$^+$ mφ in the inflamed mucosa have enhanced expression of CD40 (143), which may facilitate their local interaction with effector T cells, analogous to elicited monocytes in mice. Thus, monocytes and mφ appear to behave similarly in the inflamed intestine of humans and mice. Furthermore, our findings that analogous processes may govern mφ differentiation in the two species intestine which seem to follow analogous processes in both steady state and in inflammation, suggest animal models remain a valuable means of exploring these processes.

A role for elicited monocytes in T-cell priming?

An area of controversy in myeloid cell biology is whether monocytes can generate APCs that can contribute to the initiation of adaptive immune responses. Under the influence of IFNγ, monocytes in inflamed tissues acquire MHCII and are proposed to migrate to draining LN in a CCR7-dependent manner, where they may prime naive CD4$^+$ T cells. By their ability to produce large amounts of cytokines, these monocyte-derived 'DCs' (mo-DCs) may complement and shape the generation of effector T cells by conventional, flt3L-dependent DCs (144). It has been suggested that Ly6C^{hi} monocytes entering the inflamed mucosa may behave in this manner, acquiring migratory capacity and transiting to the draining MLN (89, 95). In support of this idea, Zigmond *et al.* (89) reported that a population of zbtb46$^+$ CD11b$^+$ Ly6C$^-$ DCs in the colonic mucosa was depleted by administration of anti-CCR2 antibody and concluded that these were derived from Ly6C^{hi} CCR2$^+$ monocytes (89). However, it is clear that some bona fide mature CD11b$^+$ DCs can express CCR2 (21, 145), and pre-DC themselves may express CCR2 (21), suggesting that *in vivo* depletion of CCR2$^+$ cells may not be specific to monocytes and their progeny. Importantly, in our hands, CCR2$^+$ CD11b$^+$ DCs are derived from committed DC

precursors and Ly6C^{hi} monocytes cannot repopulate any DC population in either steady state or inflamed intestine (12). Monocytes and mϕ also cannot be found in the pseudo-afferent intestinal lymph of mice with colitis (authors' unpublished observations), and although monocyte-derived MHCII$^+$ APC do accumulate in the MLN during experimental colitis, the vast majority of these arrive via the bloodstream in a CCR7-independent manner (16, 144). Thus, while it seems likely that inflammatory monocytes may help shape effector T-cell responses in the mucosa itself, it remains unclear whether they can also act as migratory APCs and initiate T-cell responses in the MLN.

Monocytes/macrophages during resolution of inflammation

Following the clearance of an infectious or inflammatory agent, the intestine must restore homeostasis so that chronic inflammation does not ensue. This is accompanied by major changes in the mϕ pool. During the resolution of colitis in mice, the expanded population of CX3CR1^{int} cells returns to normal size, paralleled by a reduction in granulocyte numbers (89). The mechanisms responsible for the contraction in inflammatory cells is unclear, although it seems likely that most elicited CX3CR1^{int} cells are cleared by apoptosis, as has been shown for the monocyte-derived cells recruited to the inflamed peritoneum (146). On the other hand, given that Ly6C^{hi} monocytes replenish CX3CR1^{hi} macrophages in the steady state, it is also conceivable that some of the elicited Ly6C^{hi} cells may convert into anti-inflammatory 'resident' mϕ once the inflammation begins to resolve, although this has not been shown directly. Even if this does not occur, we have already noted that the resident population of CX3CR1^{hi} mϕ appears to persist during mucosal inflammation and irrespective of their origin, these anti-inflammatory cells may play an active role in resolution of tissue damage (Fig. 1). This is supported by the evidence discussed above that deletion of mϕ or MyD88 signaling in myeloid cells leads to increased susceptibility to experimental colitis and impaired tissue repair (42, 43). Furthermore there is delayed resolution of DSS-induced colitis in CD68-dnTGFβRII mice which lack TGFβ signaling on mature mϕ (38). This is suggested to result in part from reduced IL-10 and enhanced IL-33 production by mucosal mϕ. The ability of anti-TNFα antibodies to promote mucosal healing in patients with IBD also correlates with the generation of 'regulatory' mϕ expressing the mannose receptor and able to suppress T-cell proliferation in vitro (147). Whether the promotion of tissue repair by mucosal mϕ reflects the mϕ-derived mediators, such as arginase-1 and PGE-2 that can influence mesenchyme function or epithelial stem cell renewal directly, or if downstream effects on other immune cells are involved remains to be determined.

Concluding remarks

There have been significant advances recently in our understanding of how mϕ contribute to the maintenance of homeostasis in the intestine, largely due to improvements in methods for identifying them more precisely. Furthermore, we and others have established approaches which help explore the origins and developmental stages of intestinal mϕ. In particular, our findings show that the current paradigm that tissue-resident mϕ are derived from fetal precursors that self-renew in situ, does not apply to the intestine. Rather, the mucosal mϕ compartment requires continuous replenishment by blood monocytes that are Ly6C^{hi} in mice and CD14^{hi} in human and once considered to be inflammatory in nature. Under steady state conditions, these monocytes differentiate locally into anti-inflammatory mϕ that express scavenger receptors, MHCII, are highly phagocytic and hyporesponsive to pro-inflammatory stimuli, but produce large amounts of IL-10. This process is dictated by cues received from the immediate environment and ensures that mucosal mϕ can ingest and degrade dying tissue cells and any commensal bacteria that penetrate across the epithelial barrier without provoking inflammation. They can also help maintain epithelial integrity and contribute to physiological tissue remodeling. Interruption of the mechanisms that promote the development and/or function of anti-inflammatory mϕ leads to inflammation in response to luminal materials such as the microbiota. During inflammation, or in response to invading pathogens, the normal pattern of monocyte differentiation is disrupted, leading to the accumulation of potent pro-inflammatory effector monocyte/mϕ. Although these clearly play important roles in tissue pathology and protective immunity, they also may be needed for controlling harmful neutrophil activity and for generating the inert mϕ required for resolution of inflammation and tissue repair. These properties underline the plasticity of this population of 'classical' monocytes and understanding the local factors that determine their various fates after arrival in the mucosa under different conditions could hold promise as new therapeutic targets for the treatment of IBD.

References

1. Lee SH, Starkey PM, Gordon S. Quantitative analysis of total macrophage content in adult mouse tissues. Immunochemical studies with monoclonal antibody F4/80. J Exp Med 1985;**161**:475–489.

2. Hume DA, Perry VH, Gordon S. The mononuclear phagocyte system of the mouse defined by immunohistochemical localisation of antigen F4/80: macrophages associated with epithelia. Anat Rec 1984;**210**:503–512.

3. Bain CC, Mowat AM. Intestinal macrophages – specialised adaptation to a unique environment. Eur J Immunol 2011;**41**:2494–2498.

4. Racine R, Chatterjee M, Winslow GM. CD11c expression identifies a population of extrafollicular antigen-specific splenic plasmablasts responsible for CD4 T-independent antibody responses during intracellular bacterial infection. J Immunol 2008;**181**:1375–1385.

5. Carlens J, Wahl B, Ballmaier M, Bulfone-Paus S, Forster R, Pabst O. Common gamma-chain-dependent signals confer selective survival of eosinophils in the murine small intestine. J Immunol 2009;**183**:5600–5607.

6. Uematsu S, et al. Regulation of humoral and cellular gut immunity by lamina propria dendritic cells expressing Toll-like receptor 5. Nat Immunol 2008;**9**:769–776.

7. McGarry MP, Stewart CC. Murine eosinophil granulocytes bind the murine macrophage-monocyte specific monoclonal antibody F4/80. J Leukoc Biol 1991;**50**:471–478.

8. Bain CC, Mowat AM. CD200 receptor and macrophage function in the intestine. Immunobiology 2012;**217**:643–651.

9. Varol C, et al. Intestinal lamina propria dendritic cell subsets have different origin and functions. Immunity 2009;**31**:502–512.

10. Bogunovic M, et al. Origin of the lamina propria dendritic cell network. Immunity 2009;**31**:513–525.

11. Niess JH, Adler G. Enteric flora expands gut lamina propria CX3CR1+ dendritic cells supporting inflammatory immune responses under normal and inflammatory conditions. J Immunol 2010;**184**:2026–2037.

12. Bain CC, et al. Resident and pro-inflammatory macrophages in the colon represent alternative context-dependent fates of the same Ly6Chi monocyte precursors. Mucosal Immunol 2013;**6**:498–510.

13. Cerovic V, et al. Intestinal CD103(-) dendritic cells migrate in lymph and prime effector T cells. Mucosal Immunol 2013;**6**:104–113.

14. Schulz O, et al. Intestinal CD103+, but not CX3CR1+, antigen sampling cells migrate in lymph and serve classical dendritic cell functions. J Exp Med 2009;**206**:3101–3114.

15. Wumesh KC, et al. L-Myc expression by dendritic cells is required for optimal T-cell priming. Nature 2014;**507**:243–247.

16. Tamoutounour S, et al. CD64 distinguishes macrophages from dendritic cells in the gut and reveals the Th1-inducing role of mesenteric lymph node macrophages during colitis. Eur J Immunol 2012;**42**:3150–3166.

17. Gautier EL, et al. Gene-expression profiles and transcriptional regulatory pathways that underlie the identity and diversity of mouse tissue macrophages. Nat Immunol 2012;**13**:1118–1128.

18. MacDonald KP, et al. An antibody against the colony-stimulating factor 1 receptor depletes the resident subset of monocytes and tissue- and tumor-associated macrophages but does not inhibit inflammation. Blood 2010;**116**:3955–3963.

19. Schlitzer A, et al. IRF4 transcription factor-dependent CD11b+ dendritic cells in human and mouse control mucosal IL-17 cytokine responses. Immunity 2013;**38**:970–983.

20. Yona S, et al. Fate mapping reveals origins and dynamics of monocytes and tissue macrophages under homeostasis. Immunity 2013;**38**:79–91.

21. Satpathy AT, et al. Notch2-dependent classical dendritic cells orchestrate intestinal immunity to attaching-and-effacing bacterial pathogens. Nat Immunol 2013;**14**:937–948.

22. Schraml BU, et al. Genetic tracing via DNGR-1 expression history defines dendritic cells as a hematopoietic lineage. Cell 2013;**154**:843–858.

23. Miller JC, et al. Deciphering the transcriptional network of the dendritic cell lineage. Nat Immunol 2013;**13**:888–899.

24. Kamada N, et al. Unique CD14 intestinal macrophages contribute to the pathogenesis of Crohn disease via IL-23/IFN-gamma axis. J Clin Invest 2008;**118**:2269–2280.

25. Lampinen M, Waddell A, Ahrens R, Carlson M, Hogan SP. CD14+CD33+ myeloid cell-CCL11-eosinophil signature in ulcerative colitis. J Leukoc Biol 2013;**94**:1061–1070.

26. Rogler G, et al. Isolation and phenotypic characterization of colonic macrophages. Clin Exp Immunol 1998;**112**:205–215.

27. Smythies LE, et al. Inflammation anergy in human intestinal macrophages is due to Smad-induced IkappaBalpha expression and NF-kappaB inactivation. J Biol Chem 2010;**285**:19593–19604.

28. Mann ER, et al. Intestinal dendritic cells: their role in intestinal inflammation, manipulation by the gut microbiota and differences between mice and men. Immunol Lett 2013;**150**:30–40.

29. Mantovani A, Sica A, Locati M. Macrophage polarization comes of age. Immunity 2005;**23**:344–346.

30. Weber B, Saurer L, Schenk M, Dickgreber N, Mueller C. CX3CR1 defines functionally distinct intestinal mononuclear phagocyte subsets which maintain their respective functions during homeostatic and inflammatory conditions. Eur J Immunol 2011;**41**:773–779.

31. Mosser DM, Edwards JP. Exploring the full spectrum of macrophage activation. Nat Rev 2008;**8**:958–969.

32. Biswas SK, Mantovani A. Macrophage plasticity and interaction with lymphocyte subsets: cancer as a paradigm. Nat Immunol 2010;**11**:889–896.

33. Mikkelsen HB, Rumessen JJ. Characterization of macrophage-like cells in the external layers of human small and large intestine. Cell Tissue Res 1992;**270**:273–279.

34. Tajima T, et al. EP2 and EP4 receptors on muscularis resident macrophages mediate LPS-induced intestinal dysmotility via iNOS upregulation through cAMP/ERK signals. Am J Physiol Gastrointest Liver Physiol 2012;**302**: G524–G534.

35. Nagashima R, Maeda K, Imai Y, Takahashi T. Lamina propria macrophages in the human gastrointestinal mucosa: their distribution, immunohistological phenotype, and function. J Histochem Cytochem 1996;**44**: 721–731.

36. Denning TL, et al. Functional specializations of intestinal dendritic cell and macrophage subsets that control Th17 and regulatory T cell responses are dependent on the T cell/APC ratio, source of mouse strain, and regional localization. J Immunol 2011;**187**:733–747.

37. Muller AJ, et al. Salmonella gut invasion involves TTSS-2-dependent epithelial traversal, basolateral exit, and uptake by epithelium-sampling lamina propria phagocytes. Cell Host Microbe 2012;**11**:19–32.

38. Rani R, Smulian AG, Greaves DR, Hogan SP, Herbert DR. TGF-beta limits IL-33 production and promotes the resolution of colitis through regulation of macrophage function. Eur J Immunol 2011;**41**:2000–2009.

39. Smith PD, Smythies LE, Shen R, Greenwell-Wild T, Gliozzi M, Wahl SM. Intestinal macrophages and response to microbial encroachment. Mucosal Immunol 2010;**4**:31–42.

40. Grainger JR, et al. Inflammatory monocytes regulate pathologic responses to commensals during acute gastrointestinal infection. Nat Med 2013;**19**:713–721.

41. Pull SL, Doherty JM, Mills JC, Gordon JI, Stappenbeck TS. Activated macrophages are an adaptive element of the colonic epithelial progenitor niche necessary for regenerative responses to injury. Proc Natl Acad Sci USA 2005;**102**:99–104.

42. Qualls JE, Kaplan AM, Van Rooijen N, Cohen DA. Suppression of experimental colitis by intestinal mononuclear phagocytes. J Leukoc Biol 2006;**80**:802–815.

43. Malvin NP, Seno H, Stappenbeck TS. Colonic epithelial response to injury requires Myd88 signaling in myeloid cells. Mucosal Immunol 2012;**5**:194–206.

44. Smythies LE, et al. Human intestinal macrophages display profound inflammatory anergy despite avid phagocytic and bacteriocidal activity. J Clin Invest 2005;**115**:66–75.

45. N'Diaye E-N, et al. TREM-2 (triggering receptor expressed on myeloid cells 2) is a phagocytic receptor for bacteria. J Cell Biol 2009;**184**:215–223.

46. Rugtveit J, Haraldsen G, Høgåsen AK, Bakka A, Brandtzaeg P, Scott H. Respiratory burst of intestinal macrophages in inflammatory bowel

disease is mainly caused by CD14+L1+ monocyte derived cells. Gut 1995;**37**:367–373.

47. Roberts PJ, Riley GP, Morgan K, Miller R, Hunter JO, Middleton SJ. The physiological expression of inducible nitric oxide synthase (iNOS) in the human colon. J Clin Pathol 2001;**54**:293–297.

48. Hedl M, Li J, Cho JH, Abraham C. Chronic stimulation of Nod2 mediates tolerance to bacterial products. Proc Natl Acad Sci USA 2007;**104**:19440–19445.

49. Ma TY, et al. TNF-alpha-induced increase in intestinal epithelial tight junction permeability requires NF-kappa B activation. Am J Physiol Gastrointest Liver Physiol 2004;**286**:G367–G376.

50. Pender SL, Quinn JJ, Sanderson IR, MacDonald TT. Butyrate upregulates stromelysin-1 production by intestinal mesenchymal cells. Am J Physiol Gastrointest Liver Physiol 2000;**279**: G918–G924.

51. Chang S-Y, et al. Circulatory antigen processing by mucosal dendritic cells controls CD8(+) T cell activation. Immunity 2013;**38**:153–165.

52. Mazzini E, Massimiliano L, Penna G, Rescigno M. Oral tolerance can be established via gap junction transfer of fed antigens from CX3CR1(+) macrophages to CD103(+) dendritic cells. Immunity 2014;**40**:248–261.

53. Farache J, et al. Luminal bacteria recruit CD103+ dendritic cells into the intestinal epithelium to sample bacterial antigens for presentation. Immunity 2013;**38**:581–595.

54. Denning TL, Wang YC, Patel SR, Williams IR, Pulendran B. Lamina propria macrophages and dendritic cells differentially induce regulatory and interleukin 17-producing T cell responses. Nat Immunol 2007;**8**:1086–1094.

55. MacDonald TT, Pender SL. Lamina propria T cells. Chem Immunol 1998;**71**:103–117.

56. Hadis U, et al. Intestinal tolerance requires gut homing and expansion of FoxP3+ regulatory T cells in the lamina propria. Immunity 2011;**34**:237–246.

57. Murai M, et al. Interleukin 10 acts on regulatory T cells to maintain expression of the transcription factor Foxp3 and suppressive function in mice with colitis. Nat Immunol 2009;**10**:1178–1184.

58. Han D, et al. Dendritic cell expression of the signaling molecule TRAF6 is critical for gut microbiota-dependent immune tolerance. Immunity 2013;**38**:1211–1222.

59. Shaw MH, Kamada N, Kim Y-G, Núñez G. Microbiota-induced IL-1β, but not IL-6, is critical for the development of steady-state TH17 cells in the intestine. J Exp Med 2012;**209**:251–258.

60. Welty NE, Staley C, Ghilardi N, Sadowsky MJ, Igyártó BZ, Kaplan DH. Intestinal lamina propria dendritic cells maintain T cell homeostasis but do not affect commensalism. J Exp Med 2013;**210**:2011–2024.

61. Niess JH, et al. CX3CR1-mediated dendritic cell access to the intestinal lumen and bacterial clearance. Science 2005;**307**:254–258.

62. Chieppa M, Rescigno M, Huang AY, Germain RN. Dynamic imaging of dendritic cell extension into the small bowel lumen in response to

epithelial cell TLR engagement. J Exp Med 2006;**203**:2841–2852.

63. Kim KW, et al. In vivo structure/function and expression analysis of the CX3C chemokine fractalkine. Blood 2011;**118**:e156–e167.

64. McDole JR, et al. Goblet cells deliver luminal antigen to CD103+ dendritic cells in the small intestine. Nature 2012;**483**:345–349.

65. Diehl GE, et al. Microbiota restricts trafficking of bacteria to mesenteric lymph nodes by CX(3)CR1 (hi) cells. Nature 2013;**494**:116–120.

66. van Furth R, Cohn ZA, Hirsch JG, Humphrey JH, Spector WG, Langevoort HL. The mononuclear phagocyte system: a new classification of macrophages, monocytes, and their precursor cells. Bull World Health Organ 1972;**46**:845–852.

67. Fogg DK, et al. A clonogenic bone marrow progenitor specific for macrophages and dendritic cells. Science 2006;**311**:83–87.

68. Auffray C, et al. CX3CR1+ CD115+ CD135+ common macrophage/DC precursors and the role of CX3CR1 in their response to inflammation. J Exp Med 2009;**206**:595–606.

69. Hettinger J, et al. Origin of monocytes and macrophages in a committed progenitor. Nat Immunol 2013;**14**:821–830.

70. Geissmann F, Jung S, Littman DR. Blood monocytes consist of two principal subsets with distinct migratory properties. Immunity 2003;**19**:71–82.

71. Serbina NV, Pamer EG. Monocyte emigration from bone marrow during bacterial infection requires signals mediated by chemokine receptor CCR2. Nat Immunol 2006;**7**:311–317.

72. Ingersoll MA, et al. Comparison of gene expression profiles between human and mouse monocyte subsets. Blood 2010;**115**:e10–e19.

73. Auffray C, et al. Monitoring of blood vessels and tissues by a population of monocytes with patrolling behavior. Science 2007;**317**:666–670.

74. Carlin LM, et al. Nr4a1-dependent Ly6C(low) monocytes monitor endothelial cells and orchestrate their disposal. Cell 2013;**153**:362–375.

75. Ziegler-Heitbrock L, et al. Nomenclature of monocytes and dendritic cells in blood. Blood 2010;**116**:e74–e80.

76. Sunderkotter C, et al. Subpopulations of mouse blood monocytes differ in maturation stage and inflammatory response. J Immunol 2004;**172**:4410–4417.

77. Schulz C, et al. A lineage of myeloid cells independent of Myb and hematopoietic stem cells. Science 2012;**336**:86–90.

78. Hashimoto D, et al. Tissue-resident macrophages self-maintain locally throughout adult life with minimal contribution from circulating monocytes. Immunity 2013;**38**:792–804.

79. Hoeffel G, et al. Adult Langerhans cells derive predominantly from embryonic fetal liver monocytes with a minor contribution of yolk sac-derived macrophages. J Exp Med 2012;**209**:1167–1181.

80. Chorro L, et al. Langerhans cell (LC) proliferation mediates neonatal development, homeostasis, and inflammation-associated

expansion of the epidermal LC network. J Exp Med 2009;**206**:3089–3100.

81. Tarling JD, Lin HS, Hsu S. Self-renewal of pulmonary alveolar macrophages: evidence from radiation chimera studies. J Leukoc Biol 1987;**42**:443–446.

82. Ajami B, Bennett JL, Krieger C, Tetzlaff W, Rossi FM. Local self-renewal can sustain CNS microglia maintenance and function throughout adult life. Nat Neurosci 2007;**10**:1538–1543.

83. Kierdorf K, et al. Microglia emerge from erythromyeloid precursors via Pu.1- and Irf8-dependent pathways. Nat Neurosci 2013;**16**:273–280.

84. Satpathy AT, Wu X, Albring JC, Murphy KM. Re (de)fining the dendritic cell lineage. Nat Immunol 2012;**13**:1145–1154.

85. Bogunovic M, Mortha A, Muller PA, Merad M. Mononuclear phagocyte diversity in the intestine. Immunol Res 2012;**54**:37–49.

86. Smythies LE, et al. Mucosal IL-8 and TGF-beta recruit blood monocytes: evidence for cross-talk between the lamina propria stroma and myeloid cells. J Leukoc Biol 2006;**80**:492–499.

87. Takada Y, et al. Monocyte chemoattractant protein-1 contributes to gut homeostasis and intestinal inflammation by composition of IL-10-producing regulatory macrophage subset. J Immunol 2010;**184**:2671–2676.

88. Kinnebrew MA, et al. Interleukin 23 production by intestinal CD103(+)CD11b(+) dendritic cells in response to bacterial flagellin enhances mucosal innate immune defense. Immunity 2012;**36**:276–287.

89. Zigmond E, et al. Ly6C hi monocytes in the inflamed colon give rise to proinflammatory effector cells and migratory antigen-presenting cells. Immunity 2012;**37**:1076–1090.

90. Tagliani E, Shi C, Nancy P, Tay CS, Pamer EG, Erlebacher A. Coordinate regulation of tissue macrophage and dendritic cell population dynamics by CSF-1. J Exp Med 2011;**208**:1901–1916.

91. Sasmono RT, et al. A macrophage colony-stimulating factor receptor-green fluorescent protein transgene is expressed throughout the mononuclear phagocyte system of the mouse. Blood 2003;**101**:1155–1163.

92. Bradford BM, Sester DP, Hume DA, Mabbott NA. Defining the anatomical localisation of subsets of the murine mononuclear phagocyte system using integrin alpha X (Itgax, CD11c) and colony stimulating factor 1 receptor (Csf1r, CD115) expression fails to discriminate dendritic cells from macrophages. Immunobiology 2011;**216**:1228–1237.

93. Pierce JH, et al. Macrophage-colony-stimulating factor (CSF-1) induces proliferation, chemotaxis, and reversible monocytic differentiation in myeloid progenitor cells transfected with the human c-fms/CSF-1 receptor cDNA. Proc Natl Acad Sci USA 1990;**87**:5613–5617.

94. Landsman L, et al. CX3CR1 is required for monocyte homeostasis and atherogenesis by promoting cell survival. Blood 2009;**113**:963–972.

95. Rivollier A, He J, Kole A, Valatas V, Kelsall BL. Inflammation switches the differentiation

program of Ly6Chi monocytes from antiinflammatory macrophages to inflammatory dendritic cells in the colon. J Exp Med 2012;**209**:139–155.

96. Ueda Y, et al. Commensal microbiota induce LPS hyporesponsiveness in colonic macrophages via the production of IL-10. Int Immunol 2010;**22**:953–962.

97. Jakubzick C, et al. Minimal differentiation of classical monocytes as they survey steady-state tissues and transport antigen to lymph nodes. Immunity 2013;**39**:599–610.

98. Guilliams M, et al. Alveolar macrophages develop from fetal monocytes that differentiate into long-lived cells in the first week of life via GM-CSF. J Exp Med 2013;**210**:1977–1992.

99. Greter M, et al. GM-CSF controls nonlymphoid tissue dendritic cell homeostasis but is dispensable for the differentiation of inflammatory dendritic cells. Immunity 2012;**36**:1031–1046.

100. Wang Y, et al. IL-34 is a tissue-restricted ligand of CSF1R required for the development of Langerhans cells and microglia. Nat Immunol 2012;**13**:753–760.

101. Greter M, et al. Stroma-derived interleukin-34 controls the development and maintenance of langerhans cells and the maintenance of microglia. Immunity 2012;**37**:1050–1060.

102. Ryan GR, et al. Rescue of the colony-stimulating factor 1 (CSF-1)-nullizygous mouse (Csf1(op)/Csf1(op)) phenotype with a CSF-1 transgene and identification of sites of local CSF-1 synthesis. Blood 2001;**98**:74–84.

103. Tamoutounour S, et al. Origins and functional specialization of macrophages and of conventional and monocyte-derived dendritic cells in mouse skin. Immunity 2013;**39**:925–938.

104. Steimle V, Siegrist CA, Mottet A, Lisowska-Grospierre B, Mach B. Regulation of MHC class II expression by interferon-gamma mediated by the transactivator gene CIITA. Science 1994;**265**:106–109.

105. Chen S, Luo D, Streit WJ, Harrison JK. TGF-beta1 upregulates CX3CR1 expression and inhibits fractalkine-stimulated signaling in rat microglia. J Neuroimmunol 2002;**133**:46–55.

106. Rescigno M, et al. Dendritic cells express tight junction proteins and penetrate gut epithelial monolayers to sample bacteria. Nat Immunol 2001;**2**:361–367.

107. Medina-Contreras O, et al. CX3CR1 regulates intestinal macrophage homeostasis, bacterial translocation, and colitogenic Th17 responses in mice. J Clin Invest 2011;**121**:4787–4795.

108. Hochheiser K, et al. Exclusive CX3CR1 dependence of kidney DCs impacts glomerulonephritis progression. J Clin Invest 2013;**123**:4242–4254.

109. Hirotani T, et al. The nuclear IkappaB protein IkappaBNS selectively inhibits lipopolysaccharide-induced IL-6 production in macrophages of the colonic lamina propria. J Immunol 2005;**174**:3650–3657.

110. Schwarz T, et al. T cell-derived IL-10 determines leishmaniasis disease outcome and is suppressed by a dendritic cell based vaccine. PLoS Pathog 2013;**9**:e1003476.

111. Rubtsov YP, et al. Regulatory T cell-derived interleukin-10 limits inflammation at environmental interfaces. Immunity 2008;**28**:546–558.

112. Roers A, et al. T cell-specific inactivation of the interleukin 10 gene in mice results in enhanced T cell responses but normal innate responses to lipopolysaccharide or skin irritation. J Exp Med 2004;**200**:1289–1297.

113. Pils MC, et al. Commensal gut flora reduces susceptibility to experimentally induced colitis via T-cell-derived interleukin-10. Inflamm Bowel Dis 2011;**17**:2038–2046.

114. Nguyen H-H, Tran B-T, Muller W, Jack RS. IL-10 acts as a developmental switch guiding monocyte differentiation to macrophages during a murine peritoneal infection. J Immunol 2012;**189**:3112–3120.

115. Hoshi N, et al. MyD88 signalling in colonic mononuclear phagocytes drives colitis in IL-10-deficient mice. Nat Commun 2012;**3**:1120.

116. Takeda K, et al. Enhanced Th1 activity and development of chronic enterocolitis in mice devoid of Stat3 in macrophages and neutrophils. Immunity 1999;**10**:39–49.

117. Kuhn R, Lohler J, Rennick D, Rajewsky K, Muller W. Interleukin-10-deficient mice develop chronic enterocolitis. Cell 1993;**75**:263–274.

118. Glocker EO, et al. Inflammatory bowel disease and mutations affecting the interleukin-10 receptor. N Engl J Med 2009;**361**:2033–2045.

119. Murugan D, et al. Very early onset inflammatory bowel disease associated with aberrant trafficking of IL-10R1 and cure by T cell replete haploidentical. Bone Marrow Transplantation. J Clin Immunol 2014;**34**:331–339.

120. Maheshwari A, et al. TGF-beta2 suppresses macrophage cytokine production and mucosal inflammatory responses in the developing intestine. Gastroenterology 2011;**140**:242–253.

121. Snelgrove RJ, et al. A critical function for CD200 in lung immune homeostasis and the severity of influenza infection. Nat Immunol 2008;**9**:1074–1083.

122. Thiesen S, et al. CD14hiHLA-DRdim macrophages, with a resemblance to classical blood monocytes, dominate inflamed mucosa in Crohn's disease. J Leukoc Biol 2013;**95**:531–541.

123. Rugtveit J, Nilsen EM, Bakka A, Carlsen H, Brandtzaeg P, Scott H. Cytokine profiles differ in newly recruited and resident subsets of mucosal macrophages from inflammatory bowel disease. Gastroenterology 1997;**112**:1493–1505.

124. Platt AM, Bain CC, Bordon Y, Sester DP, Mowat AM. An independent subset of TLR expressing CCR2-dependent macrophages promotes colonic inflammation. J Immunol 2010;**184**:6843–6854.

125. Dunay IR, et al. Gr1(+) inflammatory monocytes are required for mucosal resistance to the pathogen Toxoplasma gondii. Immunity 2008;**29**:306–317.

126. Schulthess J, et al. Interleukin-15-dependent NKp46+ innate lymphoid cells control intestinal inflammation by recruiting inflammatory monocytes. Immunity 2012;**37**:108–121.

127. Kim YG, et al. The Nod2 sensor promotes intestinal pathogen eradication via the chemokine CCL2-dependent recruitment of inflammatory monocytes. Immunity 2011;**34**:769–780.

128. Schreiber HA, et al. Intestinal monocytes and macrophages are required for T cell polarization in response to Citrobacter rodentium. J Exp Med 2013;**210**:2025–2039.

129. Waddell A, et al. Colonic eosinophilic inflammation in experimental colitis is mediated by Ly6C(high) CCR2(+) inflammatory monocyte/macrophage-derived CCL11. J Immunol 2011;**186**:5993–6003.

130. Arnold L, et al. Inflammatory monocytes recruited after skeletal muscle injury switch into antiinflammatory macrophages to support myogenesis. J Exp Med 2007;**204**:1057–1069.

131. Kreider T, Anthony RM, Urban JF, Gause WC. Alternatively activated macrophages in helminth infections. Curr Opin Immunol 2007;**19**:448–453.

132. de Schoolmeester ML, Little MC, Rollins BJ, Else KJ. Absence of CC chemokine ligand 2 results in an altered Th1/Th2 cytokine balance and failure to expel Trichuris muris infection. J Immunol 2003;**170**:4693–4700.

133. Filbey KJ, et al. Innate and adaptive type 2 immune cell responses in genetically controlled resistance to intestinal helminth infection. Immunol Cell Biol 2014. doi: 10.1038/icb.2013.109 [Epub ahead of print].

134. Schmidt S, et al. Nippostrongylus-induced intestinal hypercontractility requires IL-4 receptor alpha-responsiveness by T cells in mice. PLoS ONE 2012;**7**:e52211.

135. Reyes JL, Terrazas LI. The divergent roles of alternatively activated macrophages in helminthic infections. Parasite Immunol 2007;**29**:609–619.

136. Zhao A, et al. Th2 cytokine-induced alterations in intestinal smooth muscle function depend on alternatively activated macrophages. Gastroenterology 2008;**135**:217–225 e1.

137. Anthony RM, et al. Memory T(H)2 cells induce alternatively activated macrophages to mediate protection against nematode parasites. Nat Med 2006;**12**:955–960.

138. Bowcutt R, et al. Arginase-1-expressing macrophages are dispensable for resistance to infection with the gastrointestinal helminth Trichuris muris. Parasite Immunol 2011;**33**:411–420.

139. Herbert DR, et al. Alternative macrophage activation is essential for survival during schistosomiasis and downmodulates T helper 1 responses and immunopathology. Immunity 2004;**20**:623–635.

140. Herbert DR, et al. Arginase I suppresses IL-12/IL-23p40-driven intestinal inflammation during acute schistosomiasis. J Immunol 2010;**184**:6438–6446.

141. Jenkins SJ, et al. Local macrophage proliferation, rather than recruitment from the blood, is a signature of TH2 inflammation. Science 2011;**332**:1284–1288.

142. Grimm MC, Pullman WE, Bennett GM, Sullivan PJ, Pavli P, Doe WF. Direct evidence of monocyte recruitment to inflammatory bowel disease mucosa. J Gastroenterol Hepatol 1995;**10**:387–395.

143. Carlsen HS, Yamanaka T, Scott H, Rugtveit J, Brandtzaeg P. The proportion of CD40+ mucosal macrophages is increased in inflammatory bowel disease whereas CD40 ligand (CD154)+ T cells are relatively decreased, suggesting differential modulation of these costimulatory molecules in human gut lamina propria. Inflamm Bowel Dis 2006;**12**:1013–1024.

144. Siddiqui KR, Laffont S, Powrie F. E-cadherin marks a subset of inflammatory dendritic cells that promote T cell-mediated colitis. Immunity 2010;**32**:557–567.

145. Lewis KL, et al. Notch2 receptor signaling controls functional differentiation of dendritic cells in the spleen and intestine. Immunity 2011;**35**:780–791.

146. Gautier EL, Ivanov S, Lesnik P, Randolph GJ. Local apoptosis mediates clearance of macrophages from resolving inflammation in mice. Blood 2013;**122**:2714–2722.

147. Vos ACW, et al. Regulatory macrophages induced by infliximab are involved in healing in vivo and in vitro. Inflamm Bowel Dis 2012;**18**:401–408.