Diversity and functions of intestinal mononuclear phagocytes

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The intestinal lamina propria (LP) contains a diverse array of mononuclear phagocyte (MNP) subsets, including conventional dendritic cells (cDC), monocytes and tissue-resident macrophages (mϕ) that collectively play an essential role in mucosal homeostasis, infection and inflammation. In the current review we discuss the function of intestinal cDC and monocyte-derived MNP, highlighting how these subsets play several non-redundant roles in the regulation of intestinal immune responses. While much remains to be learnt, recent findings also underline how the various populations of MNP adapt to deal with the challenges specific to their environment. Understanding these processes should help target individual subsets for 'fine tuning' immunological responses within the intestine, a process that may be of relevance both for the treatment of inflammatory bowel disease (IBD) and for optimized vaccine design.

DEFINING MURINE INTESTINAL MONONUCLEAR PHAGOCYTE SUBSETS

Conventional dendritic cells (cDC) and macrophages (mϕ) are found throughout the intestinal LP, but much remains to be understood about the precise function of each cell type. In part, this is because they are difficult to isolate, but to a great extent it reflects the imprecise ways in which they were identified until recently.[1–3] Thus it is now clear that markers such as CD11c, CD11b and major histocompatability complex II (MHCII) are not sufficient to distinguish between cDC and mϕ in many tissues, including the intestine. This is because most mature intestinal mϕ express as high levels of all these molecules as cDC and distinguishing these cells correctly requires the additional use of markers that are specific to mϕ, such as CD64 (FcγRI), F4/80 or Mer tyrosine kinase (MerTK).[2,4–6] Initial ideas that CD103 (integrin α_E) and CX3CR1 (fractalkine receptor) could be used to identify intestinal cDC and mϕ respectively, have also proved incorrect, as it is now clear that not all cDC in the intestine express CD103 and that some also express CX3CR1.

Intestinal LP cDC can be identified as lineage (CD3, CD19, B220, NK1.1, CD64)-negative cells that express CD11c and MHCII (**Table 1**; **Figure 1a,b**).[7,8] In compliance with the current definition of cDC these cells express FMS-like tyrosine kinase 3 (FLT3/CD135), the receptor for the cDC-specific growth factor FLT3 ligand (FLT3L) and show dependence on this receptor–ligand pathway for their development from cDC precursors in the bone marrow (BM).[8,9] They also express the zinc finger and BTB domain containing 46 (Zbtb46) transcription factor (TF),[8,10] have a lifespan in the tissue of only a few days, and have the capacity to migrate in afferent lymph to the draining lymph nodes, where they can prime naive T cells.[2,3,5] In contrast, cells of the monocyte-mϕ lineage are CD64$^+$ CD11b$^+$ (**Table 2**; **Figure 1a,d**), independent of FLT3 signaling, but require the colony-stimulating factor 1 receptor (CSF-1R/CD115) for development.[11] They are derived from monocytes or embryonic stem cells, and in steady state they have a lifespan of several weeks in tissues, do not migrate in lymph and have little or no ability to prime naive T cells.[2,4] Wherever possible, we will discuss intestinal cDC and mϕ that have been identified based on these parameters, but caution needs to be used when interpreting the many studies in the field that have not applied such rigorous strategies.

PART A. INTESTINAL cDC

While Peyer's patches (PP) contain a sizeable population of cDC, the current review focuses on those cDC that are diffusely

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Table 1 Conventional DC subsets in the intestine

Phenotype		Ontogeny		Function		Abundance	
Common	Subset-specific	GF	TF Involvement	Common Functions	Specialized Functions	siLP	coLP
CD11c⁺ MHC II⁺ CD64⁻ F4/80⁻ MerTK⁻ Ly6C⁻ CD3⁻ CD19⁻ B220⁻ NK1.1⁻	XCR1⁺ **CD103⁺** CD11b⁻ CX3CR1ⁿᵉᵍ	FLT3L-dependent	ZBTB46 **IRF8 BatF3 ID2**	• CCR7-dependent migration to intestine draining lymph nodes • Presentation of soluble protein antigens	• Th1 • Cross presentation • Induction of CD8αβ⁺ and CTL-like CD8αα⁺ CD4⁺ IEL • Predominant in CCR9/α₄β₇ induction	+	++
	SIRPα⁺ **CD103⁺ CD11b⁺** CX3CR1ˡᵒʷ		**IRF4 KLF4 Notch2**		• Th17 • Th2 • ILC3 activation	+++	+
	CD103⁻ **CD11b⁺ CX3CR1ⁱⁿᵗ**		**Zeb2** IRF4 Notch2? KLF4?		• Th1 priming • Th2 priming • Th17 priming • ILC3 activation?	+	+++

Abbreviation: coLP, colonic lamina propria; GF, growth factors; siLP, small intestinal lamina propria; TF, transcription factors.
Summary of the phenotypic markers, ontogeny, key functions and relative abundance of intestinal cDC subsets.

distributed throughout the intestinal LP. It should, however, be noted that intestinal LP cell preparations will contain cDC from 'contaminating' isolated lymphoid follicles. As the major antigen-presenting cells of the immune system, intestinal cDC continually sample their environment for foreign (food proteins, microbes) and self-antigens.[12–18] In addition to uptake of apoptotic or damaged cells, several mechanisms have been proposed for how intestinal cDC acquire antigen from the lumen including the extension of trans-epithelial dendrites into the lumen,[19] via goblet cell-associated antigen-passages,[20,21] indirectly via villus M cells,[22] or after transfer from tissue-resident mϕ.[23] Some soluble materials may also be taken up by cDC after crossing the epithelium by paracellular or transcellular mechanisms.[24] After acquiring antigen, cDC migrate in a chemokine receptor 7 (CCR7) dependent manner via afferent lymphatics to intestinal-draining mesenteric lymph nodes (MLN), where they present processed antigen to cells of the adaptive immune system.[15,17,18,25–26] T cells activated in MLN are induced to express a 'gut homing' CCR9⁺α₄β₇⁺ phenotype,[27,28] in part through the action of retinoic acid (RA) derived from migratory intestinal cDC and/or local stromal cells.[25,29–31] In the steady state, intestinal cDC are considered to be tolerogenic, a property that appears to be maintained, at least in part through MAP kinase p38α,[32] transforming growth factor β receptor II (TGFβRII) signaling,[33] tumor necrosis factor receptor-associated factor 6 (TRAF6),[34] β-catenin signaling,[35] as well as the signaling regulator A20 (TNFAIP3).[36,37] In this state, presentation of innocuous antigens by cDC promotes the generation of forkhead box P3⁺ (FoxP3⁺) inducible regulatory T cells (iT_reg) in MLN and is key to the development of oral tolerance to dietary proteins and for maintaining the symbiotic relationship with the microbiota.[18,38–40] Intestinal cDC also express a wide array of cytokine and pattern recognition receptors, allowing them to

respond to local inflammatory cues and microbial products and so to initiate active immunity when necessary. Notably the various individual subsets of intestinal cDC (see below) are equipped with distinct combinations of pattern recognition receptors, and thus may respond to microbes in different ways.[41–47]

PHENOTYPE AND ONTOGENY OF INTESTINAL cDC SUBSETS

Intestinal cDC are highly heterogeneous and recent years have seen great progress in our understanding of intestinal cDC subset development and functions. Although early work assumed that intestinal cDC comprised a homogeneous population of CD103⁺ cells, this is now known to be an over-simplification and most recent studies have divided them on the basis of CD103 and CD11b expression. This reveals three major (CD103⁺CD11b⁻, CD103⁺CD11b⁺ and CD103⁻CD11b⁺) and one minor (CD103⁻CD11b⁻) cDC subset, all of which express the TF Zbtb46, are dependent on FLT3L for development *in vivo* and are derived from a pre-cDC precursor.[8,10,13] Importantly all four populations of CD103⁺CD11b⁻, CD103⁺CD11b⁺, CD103⁻CD11b⁺, CD103⁻CD11b⁻ intestinal cDC can be found in the migratory compartment of intestine draining LN, as well as in pseudo-afferent lymph draining the steady state intestine.[8,10,13,14] Thus each has the potential of initiating and regulating adaptive immune responses within LN.

Apart from CD103⁻CD11b⁻ cDC, all the other cDC subsets are present in the intestinal-draining lymph of RA-receptor related orphan receptor γt deficient mice (Rorc⁻/⁻), which lack isolated lymphoid follicles and PP,[13] suggesting that they derive from the LP. Since the minor population of CD103⁻CD11b⁻ DC appears heterogeneous and its functions are unknown, it will not be discussed further here.
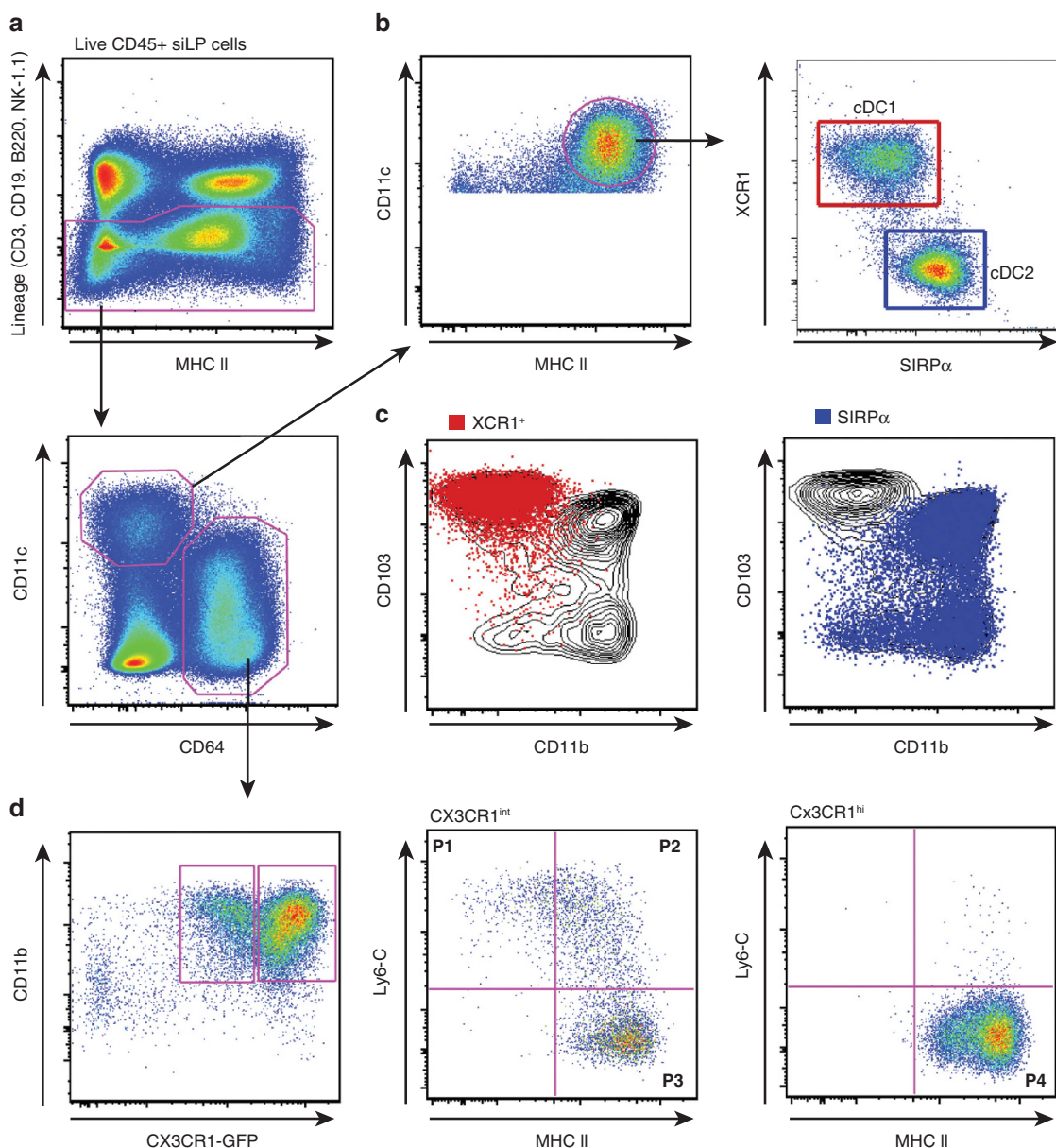
Figure 1 Identification of intestinal MNP subsets by flow cytometry. Representative analysis of small intestinal LP cells pre-gated on live CD45$^+$ cells. (**a**) Following exclusion of lineage (CD3, CD19, B220, NK-1.1), cells of the monocyte/mϕ lineage are identified as CD64$^+$, whereas cDC fall into the CD11c^{hi}CD64$^-$ fraction. (**b**) *Bona fide* cDC are further characterized by high expression of MHCII and can be divided into XCR1$^+$ and SIRPα$^+$ subsets. (**c**) XCR1$^+$ cDC are almost entirely CD103$^+$CD11b$^-$, whereas SIRPα$^+$ cDC are heterogeneous and comprise CD103$^+$CD11b$^+$ and CD103$^-$CD11b$^+$ cDC, together with a minor population of CD103$^-$CD11b$^-$ cDC. (**d**) Using *Cx3cr1*$^{GFP/+}$ reporter mice, the monocyte/mϕ lineage can be subdivided into CD11b$^+$CX3CR1^{int} cells and CD11b$^+$CX3CR1^{hi} cells. The CD11b$^+$CX3CR1^{int} cells contain a mixture of monocytes and mϕ precursors, which can be further classified into a "monocyte waterfall" as Ly6C^{hi}MHCII$^-$ recently recruited monocytes (P1), Ly6C^{hi}MHCII$^+$ maturing monocytes (P2) and Ly6C$^-$MHCII$^+$ monocyte/mϕ intermediates (P3); the CD11b$^+$CX3CR1^{hi} cells consist exclusively of Ly6C$^-$MHCII$^+$ tissue-resident mϕ. cDC, conventional dendritic cells; LP, lamina propria; MNP, mononuclear phagocyte; SIRPα, signal regulatory protein α; XCR1, X-C motif chemokine receptor 1.

The proportions of CD103$^+$CD11b$^-$, CD103$^+$CD11b$^+$, CD103$^-$CD11b$^+$ LP cDC differ markedly along the length of the intestine. For example, CD103$^+$CD11b$^+$ cDC make up the major proportion of cDC in the small intestine, but constitute only a minor fraction of cDC in the colon, where CD103$^+$CD11b$^-$ cDC predominate.[48,49] While these differences may reflect the distinct physiological requirements of these tissues and variations in the environmental conditioning factors present in each site (see below), the functional consequences remain to be explored.

Parallel studies have demonstrated that cDC across tissues can be split into two major branches of X-C motif chemokine receptor 1 (XCR1) or signal regulatory protein α (SIRPα/CD172a) expressing cDC. Such studies have resulted in a unifying nomenclature for XCR1$^+$ cDC as cDC1 and SIRPα$^+$ cDC as cDC2.[9,41,50] Murine cDC1, but not cDC2, are dependent

on the TFs interferon regulatory factor 8 (IRF8), basic leucine rich zipper transcription factor ATF-like 3 (BATF3) and inhibitor of DNA-binding 2 (ID2) for their development, while absence of the TFs IRF4, neurogenic locus notch homolog protein 2 (NOTCH2), RelB and Krüppel-like factor 4 (KLF4) impacts negatively on the development of cDC2 (see article by Sichien et al.[51]). XCR1 and SIRPα expression also splits the murine intestinal cDC compartment into two major populations. Intestinal XCR1$^+$ cDC are almost entirely CD103$^+$ CD11b$^-$, with a very minor population being CD103$^-$ CD11b$^-$ (**Figure 1b** and **c**). As with extra-intestinal XCR1$^+$ cDC, intestinal CD103$^+$CD11b$^-$ cDC express IRF8,[7] and their generation is dependent on the TFs IRF8, ID2 and BATF3.[7,12,52,53] Of note, mice that are haplo-insufficient in *Irf8* have normal numbers of intestinal and migratory CD103$^+$CD11b$^-$ cDC in MLN, but severely reduced numbers of MLN resident XCR1$^+$ cDC, indicating some differences in IRF8 dependency between XCR1$^+$ cDC populations.[7] Intestinal SIRPα$^+$ cDC are more heterogeneous and include major populations of CD103$^+$CD11b$^+$ and CD103$^-$CD11b$^+$ cDC, together with a small group of CD103$^-$CD11b$^-$ cDC (**Figure 1b** and **c**). CD11c-targeted deletion in *Irf4* leads to a partial reduction of CD103$^+$CD11b$^+$ cDC in the small intestinal LP and an absence of these cells in the MLN[54] as well as reduced numbers of CCR2 expressing CD103$^-$CD11b$^+$ DCs.[8] Further, mice whose CD11c$^+$ cells are deficient in *Notch2* display reduced numbers of CD103$^+$ CD11b$^+$ cDC in the small intestine and MLN,[10,55] while deficiency in *Klf4* results in reduced proportions of small intestinal CD103$^+$CD11b$^+$ cDC, as well as both migratory and LN-resident SIRPα$^+$ cDC in MLN.[56] Whether intestinal CD103$^-$CD11b$^+$ cDC numbers are also altered in the absence of *Klf4* or *Notch2* remains to be determined using rigorous gating strategies.[10,55] It also remains unclear whether, and at what point, these TFs interact to regulate CD103$^+$CD11b$^+$ cDC development. Finally mice with deficiency of Zinc finger E-box-binding homeobox TF 2 (*Zeb2*) in CD11c$^+$ cells show a reduced proportion of CD103$^-$CD11b$^+$ cDC within the intestine but not MLN, together with normal proportions of intestinal and MLN CD103$^+$CD11b$^+$ cDC, although the absolute number of each of these SIRPα$^+$ cDC subsets was not assessed.[57]

In preliminary studies we have found that the transcriptomes of small intestinal CD103$^-$CD11b$^+$ and CD103$^+$CD11b$^+$ cDC differ by only 100–200 genes and that absence of TGFβRI signaling in CD11c$^+$ cells leads to a marked reduction in small intestinal CD103$^+$CD11b$^+$ cDC and a concomitant accumulation of CD103$^-$CD11b$^+$ cDC (Montgomery, C.C. Bain, J. Montgomery, C.L. Scott, J.M. Kel, M.J.H. Girard-Madoux, L. Marten et al., unpublished data). Collectively these findings indicate that these subsets are closely related to one another and that steady state TGFβR signaling drives the differentiation of at least some CD103$^-$ CD11b$^+$ cDC into CD103$^+$CD11b$^+$ cDC. Nevertheless given the heterogeneity in phenotype, TF requirements and function of intestinal SIRPα$^+$ cDC, we refer to these intestinal cDC

populations below as XCR1$^+$ or SIRPα$^+$ cDC, rather than cDC1 and cDC2.

FUNCTIONS OF INTESTINAL cDC SUBSETS
Non-redundant functions of intestinal XCR1$^+$ cDC
Several mouse models have been utilized to draw conclusions on the *in vivo* function of intestinal XCR1$^+$ cDC, including *Batf3*$^{-/-}$, and more recently *Cd11c-cre.Irf8*$^{fl/fl}$, *Zbtb46-cre.Irf8*$^{fl/fl}$, *Xcr1*-DTA and *Clec9a*-DTR mice.[7,38,45,52,58] While each of these models results in a marked or complete depletion of intestinal XCR1$^+$ cDC, findings from these studies should be taken with a degree of caution, as direct effects on additional cell types have often not been ruled out. It can also be unclear whether the consequences of deletion reflect absence of XCR1$^+$ cDC in the intestine or other tissues. Nevertheless, these models have greatly extended our understanding of the biological function of XCR1$^+$ cDC and demonstrate that they play key non-redundant roles in regulating multiple aspects of the intestinal immune response.

Cd11c-cre.Irf8$^{fl/fl}$ and *Xcr1*-DTA mice have reduced numbers of CD4$^+$ and CD8$^+$ LP T cells and conventional CD8αβ$^+$TCRαβ$^+$ and unconventional CD8αα$^+$TCRαβ$^+$ and CD8αα$^+$TCRγδ$^+$ intra-epithelial lymphocytes in the small intestine.[7,58] Several mechanisms by which XCR1$^+$ cDC potentially support small intestinal T-cell homeostasis have been identified. First small intestine-derived CD103$^+$CD11b$^-$ cDC in MLN appear to be the major source of cDC-derived RA,[7] a key inducer of CCR9 and α$_4$β$_7$ on responding T cells, and their absence results in a reduced generation of CCR9$^+$ α$_4$β$_7^+$ small intestinal homing T cells.[7] Second, XCR1$^+$ DC play a dominant role in cross-presenting intestinal epithelium-derived cell-associated antigen to CD8$^+$ T cells in steady state,[14] and are required for optimal CD8$^+$ T-cell responses to orally administered protein antigen and rotavirus infection.[7,59] Thus reduced CD8$^+$ T-cell priming may contribute to reduced numbers of small intestinal CD8αβ$^+$ T cells. Finally, XCR1 and XCL1 deficient mice also display a selective reduction in small intestinal TCRαβ$^+$ T cells. Here, crosstalk between XCR1 on intestinal CD103$^+$CD11b$^-$ cDC- and XCL1-expressing T cells appears to promote intestinal T-cell survival as well as modulate CD103$^+$CD11b$^-$ cDC maturation, promoting their migration to the MLN.[58]

Intestinal XCR1$^+$ cDC are also required for the generation and maintenance of particular intestinal CD4$^+$ T-cell subsets. Small intestinal CD4$^+$ T cells in *Xcr1*-DTA mice have reduced levels of IFN-γ mRNA[58] and the small intestine and colon LP of *Cd11c-cre.Irf8*$^{fl/fl}$ mice have a dramatic reduction in Th1 cells.[7] These mice also fail to generate colonic Th1 responses to the colonic-dwelling protozoan *Trichomonas musculis*.[60] One explanation for the reduction in intestinal Th1 cells in the absence of XCR1$^+$ cDC is defective Th1 cell generation in MLN.[7] While the mechanisms underlying this defect remain to be determined, one possibility is that a loss of XCR1$^+$ cDC results in reduced levels of IL-12. Consistent with this idea, CD103$^+$CD11b$^-$ cDC are the major cDC source of IL-12 in the MLN in the steady state[61] and during acute graft versus host

disease.[62] Further IL-12 production by CD103^{+}CD11b^{−} cDC is required for driving IFN-γ production in T and NK cells during *Toxoplasma gondii* infection,[63] while IL-12 and IL-15 production by colonic CD103^{+}CD11b^{−} cDC has been implicated in driving tonic release of IFN-γ by intestinal T cells, promoting epithelial resistance to dextran sodium sulphate (DSS)-induced colitis.[45] Of note, deletion of the A20 inhibitor of nuclear factor-κB activation in CD11c-expressing cells leads to upregulation of the co-stimulatory molecules CD80 and CD86 on CD103^{+}CD11b^{−} cDC and unleashes their ability to drive massive IFN-γ production by CD4^{+} T cells. Therefore in the steady state this signal attenuator may normally limit the capacity of XCR1^{+} cDC to drive Th1 cell differentiation.[37]

Cd11c-cre.Irf8^{fl/fl}, *Xcr1*-DTA and *Batf3*^{−/−} mice also lack CD4^{+}CD8αα^{+} intra-epithelial lymphocytes,[7,58,64] a population of MHCII-restricted cytotoxic T cells whose development from conventional CD4^{+} T cells requires TGFβ and RA.[65,66] We recently demonstrated that this process also requires CD103^{+}CD11b^{−} cDC expression of the α_vβ_8 integrin,[7] involved in the activation of the latent TGFβ complex.[67] Interaction between cell adhesion molecule 1 (CADM1) on intestinal CD103^{+}CD11b^{−} cDC and class 1-restricted T-cell adhesion molecule (CRTAM) on intestinal CD4^{+} T cells has also been implicated in the generation of CD4^{+}CD8αα^{+} intra-epithelial lymphocytes,[64] although intestinal mφ also express CADM1 (based on IMMGEN.org database and authors unpublished observation) and so could also contribute to local differentiation of this population.

Finally, CD103^{+}CD11b^{−} cDC are required for the optimal generation of iT_reg in MLN following oral administration of protein antigen.[38] This could reflect the high capacity of CD103^{+}CD11b^{−} cDC to generate RA[7,68] and TGFβ2 mRNA,[38] as well as their expression of the highest levels of α_vβ_8 integrin amongst intestinal cDC,[7,38,69] all factors that are associated with iT_reg conversion. However the overall significance of these findings remains to be elucidated, as mice deficient in XCR1^{+} cDC display normal proportions of endogenous FoxP3^{+} T_reg in the intestine[7,52,58,70,71] and develop oral tolerance normally.[38]

Non-redundant functions of SIRPα[+] cDC subsets

Models that have been utilized to draw conclusions on the *in vivo* functions of SIRPα[+] cDC include *Cd11c-cre.Irf4*[fl/fl,54,72], *Cd11c-cre.Notch2*[fl/fl,10,55], *Cd11c-cre.Klf4*[fl/fl,56], *Clec4a4*-DTR[45] and *Sirpα*^{−/−} mice[73]; as well as *huLangerin*-DTA mice that express Diphtheria toxin A (DTA) under the control of the human Langerin promoter, which is primarily active in intestinal CD103^{+}CD11b^{+} cDC.[71] Notably, none of the above models show depletion in all intestinal SIRPα^{+} cDC. Further it is important to bear in mind that changes in immunological responses observed in mice with CD11c-Cre-mediated deletion of *Irf4*, *Notch2* or *Klf4* may not necessarily be due to depletion of a particular SIRPα[+] cDC subset, but could reflect an impact on cDC function. Of further note, as our understanding of MNP subsets has advanced, it remains possible that functions ascribed previously to cells of the monocyte-mφ lineage through the use of for example *Ccr2*^{−/−} and *Cx3cr1*-DTR mice may in fact be a function of SIRPα[+]

cDC, as some of these cells express CCR2 and intermediate levels of CX3CR1; the numbers of several SIRPα[+] cDC subsets are also markedly reduced in these animals.[8,74]

Cd11c-cre.Irf4^{fl/fl}, *Cd11c-cre.Notch2*^{fl/fl} and *Sirpα*^{−/−} mice display a selective reduction in intestinal Th17 cells,[54,55,72–73] collectively suggesting a key role for SIRPα[+] cDC in intestinal Th17 cell homeostasis. *Cd11c-cre.Irf4*^{fl/fl} mice also fail to generate colonic Th17 responses to *Trichomonas musculis*.[60] Further, *huLangerin*-DTA mice have reduced numbers of small intestinal Th17 cells, suggesting that loss of CD103^{+}CD11b^{+} cDC underlies Th17 deficiency at this site, although MHCII expression by these cells may not be required.[71]

While the mechanisms underlying reduced intestinal Th17 cell numbers in the above models remains unclear, one likely contributing factor is reduced Th17 differentiation in intestinal-draining lymph nodes. Thus *Cd11c-cre.Irf4*^{fl/fl} mice fail to support Th17 differentiation in MLN following immunization with antigen and adjuvant.[54] Mechanistically, Th17 priming under these conditions is IL-6 dependent and absence of small intestinal-derived migratory CD103^{+}CD11b^{+} cDC in MLN of *Cd11c-cre.Irf4*^{fl/fl} mice leads to reduced levels of cDC-derived IL-6.[54] Consistent with these findings small intestinal LP CD103^{+}CD11b^{+} cDC but not CD103^{+}CD11b^{−} cDC from *Cd11c-cre.A20*^{fl/fl} mice spontaneously produce large amounts of IL-6 and drive IL-6-dependent Th17 differentiation *in vitro*.[37]

Recent studies have also shown that CD103^{−}CD11b^{+} cDC, and in particular CCR2^{+}CD103^{−}CD11b^{+} cDC, are capable of driving Th17 cell differentiation *in vitro*.[8,75] Since CD103^{−}CD11b^{+} cDC are the dominant SIRPα^{+} cDC in the colon and colon-draining nodes,[49] it remains plausible that these cells play an important role in colonic Th17 cell homeostasis, although this remains to be addressed experimentally. Interestingly *Cd11c-cre.Klf4*^{fl/fl} mice, that also have reduced numbers of intestinal SIRPα[+] cDC appear capable of mounting normal intestinal Th17 cell responses,[56] suggesting that KLF4 may be involved in the development of a distinct SIRPα[+] cDC subset or regulate distinct functional modules in these cells.

Despite the clear role for SIRPα[+] cDC in intestinal Th17 cells homeostasis, some Th17 cells remain in the LP in all the models above. This may be because SIRPα[+] cDC are not completely depleted in these models, or that additional MNP subsets contribute to driving intestinal Th17 responses. Consistent with this latter possibility, Th17 responses to the intestinal commensal organism, segmented filamentous bacteria (SFB) require input from intestinal mφ.[76]

In addition to their role in Th17 homeostasis, IRF4-dependent SIRPα[+] cDC are key for the development of Th2 responses. While studies on intestinal Th2 responses remain limited, *Cd11c-cre.Irf4*^{fl/fl} mice do not develop a protective Th2 response to the parasitic worms *Nippostrongylus brasiliensis*[77] or *Trichuris muris* (J.U. Mayer, M. Demiri, W.W. Agace, A.S. MacDonald, M. Svensson-Frej, S.W. Milling, unpublished data). Further, in collaboration with the group of Dr. S. Milling (University of Glasgow), we recently identified

a key role for migratory CD103$^+$CD11b$^+$ cDC in initiating Th2 responses in small intestinal-draining MLN in response to subserosal injection of *Schistosoma mansoni* eggs into the small intestine. In contrast, migratory CD103$^-$CD11b$^+$ cDC drove Th2 responses in colonic draining MLN after subserosal egg injection into the colon (J.U. Mayer, M. Demiri, W.W. Agace, A.S.MacDonald, M.Svensson-Frej, S.W. Milling unpublished data). Thus the SIRPα$^+$ cDC subset responsible for driving Th2 immunity may depend on where in the intestine the infection takes place. Notably, *Cd11c-cre.Klf4*$^{fl/fl}$ mice also have impaired Th2 responses,[56] collectively suggesting that KLF4 expression in IRF4-dependent SIRPα$^+$ cDC is required for the development of Th2 immunity.

Finally, several reports have suggested a role for intestinal SIRPα$^+$ cDC in regulating innate lymphoid cell (ILC) function. Intestinal CD103$^+$CD11b$^+$ cDC produce IL-23 following challenge with flagellin or in response to *Citrobacter rodentium* (*C.rodentium*) infection, driving IL-22 expression by intestinal resident type 3 ILCs (ILC3).[10,78] However CD103$^-$CD11b$^+$ cDC and intestinal mϕ can also produce IL-23 and the relative importance of these MNP subsets as a source of IL-23 may depend on the model and the intestinal location under study.[71,74,78–81]

Cooperation and antagonism between intestinal cDC subsets

Although XCR1$^+$ and SIRPα$^+$ cDC have non-redundant functions in intestinal immune homeostasis, there is also evidence of cooperation between these subsets. For example, while intestinal FoxP3$^+$ T$_{reg}$ are found in normal numbers in *Cd11c-cre.Irf4*$^{fl/fl}$ and *huLangerin*-DTA mice,[54,71] mice lacking XCR1$^+$ cDC and CD103$^+$CD11b$^+$ cDC have decreased numbers of T$_{reg}$ in the small intestine.[71] Further, iT$_{reg}$ generation in MLN in response to orally administered soluble protein antigen is reduced in *Zbtb46-cre.Irf8*$^{fl/fl}$ mice that lack XCR1$^+$ cDC, while almost absent in diphtheria toxin-treated *Zbtb46*-DTR mice that lack all cDC; however, only *Zbtb46*-DTR mice fail to develop oral tolerance.[38] iT$_{reg}$ generation to commensal antigens is also dependent on cDC and here, NOTCH2-dependent SIRPα$^+$ cDC rather than XCR1$^+$ cDC appear to play a dominant role.[82] Finally while XCR1$^+$ cDC are the superior cross-presenting antigen-presenting cell subset, there may also be redundancy in the induction of CTL responses in a setting of infection. Thus CD8$^+$ T-cell responses to rotavirus are only partially reduced in *Batf3*$^{-/-}$ mice lacking XCR1$^+$ cDC, but are completely ablated in *Zbtb46*-DTR mice depleted of all cDC.[59]

Two recent studies have also provided evidence of antagonism between intestinal XCR1$^+$ and SIRPα$^+$ cDC. First, IL-12 production by XCR1$^+$ cDC appears to reduce the Th2 response to *Heligmosomoides polygyrus* infection[61] and secondly, IL-23 production by CX3CR1$^+$ cDC and mϕ reduces IL-12 production by XCR1$^+$ cDC during *C.rodentium* infection, protecting mice from Th1 cell-mediated fatal immunopathology.[74] These results suggest that cDC subsets can mutually dampen each other's responses, probably to help prevent excessive immune activation and to maintain a balanced immune response. A summary of the reported functions of intestinal XCR1$^+$ and SIRPα$^+$ cDC can be seen in **Figure 2** and **Table 1**.

HUMAN cDC

Three putative cDC subsets have been identified in the human small intestine that can be distinguished based on differential expression of CD141, CD103 and SIRPα, together with a lack of the monocyte-macrophage markers CD64 and/or CD14.[3,42,83] Comparative transcriptome profiling revealed close similarity between the two major human and murine cDC subsets; human small intestinal CD103$^+$SIRPα$^-$ cDC resemble CD141$^+$ cDC in other human tissues and murine intestinal CD103$^+$CD11b$^-$ cDC, while human small intestinal CD103$^+$SIRPα$^+$ cDC resemble human blood-/tissue-derived CD1c$^+$ cDC and murine CD103$^+$CD11b$^+$ cDC. As in the mouse, CD103$^-$SIRPα$^+$ cells are also present in the human intestine, the majority of which express IRF4, CD11b and intermediate levels of CX3CR1.[8,42] They are also heterogeneous for CCR2 expression, suggesting these may be equivalent to murine CD103$^-$CD11b$^+$ cDC.[8] Surprisingly, however, at the transcriptional level, these cells appeared to be more related to monocytes,[42] indicating that they may represent a mixed population of cells due to insufficient gating criteria to exclude all monocyte-derived populations. Consistent with this possibility, Guilliams *et al.* recently provided evidence that CD14$^-$SIRPα^{hi} cells in human lung and intestine contain both CD1c$^-$IRF4^{lo}IRF8^{lo} monocyte-derived cells, as well as *bona fide* CD1c$^+$IRF4^{hi} cDC.[84] Because of these findings we confine our discussion on the function of human intestinal cDC below to the CD103$^+$SIRPα$^+$ and CD103$^+$SIRPα$^-$ cDC subsets.

As in mice, human intestinal CD103$^+$SIRPα$^+$ and CD103$^+$SIRPα$^-$ cDC express CCR7, indicating they have the potential to migrate to draining LN.[42,83] In line with this, CD103$^+$CD141$^+$ and CD103$^+$CD1c$^+$ cDC can both be found within the migratory compartment of human MLN.[85] The few studies that have assessed the *in vitro* functions of human intestinal cDC subsets thus far have highlighted both similarities and differences compared with their mouse homologs. As in mice, human small intestinal-derived cDC induce RA-dependent expression of α$_4$β$_7$ and CCR9 on responding T cells and those from the small intestine imprint CCR9 expression on responding T cells more efficiently than colonic cDC.[31,83] Human small intestinal CD103$^+$SIRPα$^+$ cDC display higher aldehyde dehydrogenase activity and induce CCR9 on responding T-cells *in vitro* more efficiently than CD103$^+$SIRPα$^-$ cDC.[42] This former population also has the highest capacity to induce FoxP3 expression on CD4$^+$ T cells, whereas CD103$^+$SIRPα$^-$ cDC appear more efficient at inducing Th1 differentiation.[42] While the role of human cDC subsets in intestinal inflammation and infection remains to be determined, perturbations in the cDC compartment in inflamed mucosa and MLN have been observed in patients with Crohn's disease and ulcerative colitis.[85] Altered aldehyde dehydrogenase activity in intestinal cDC has also been reported
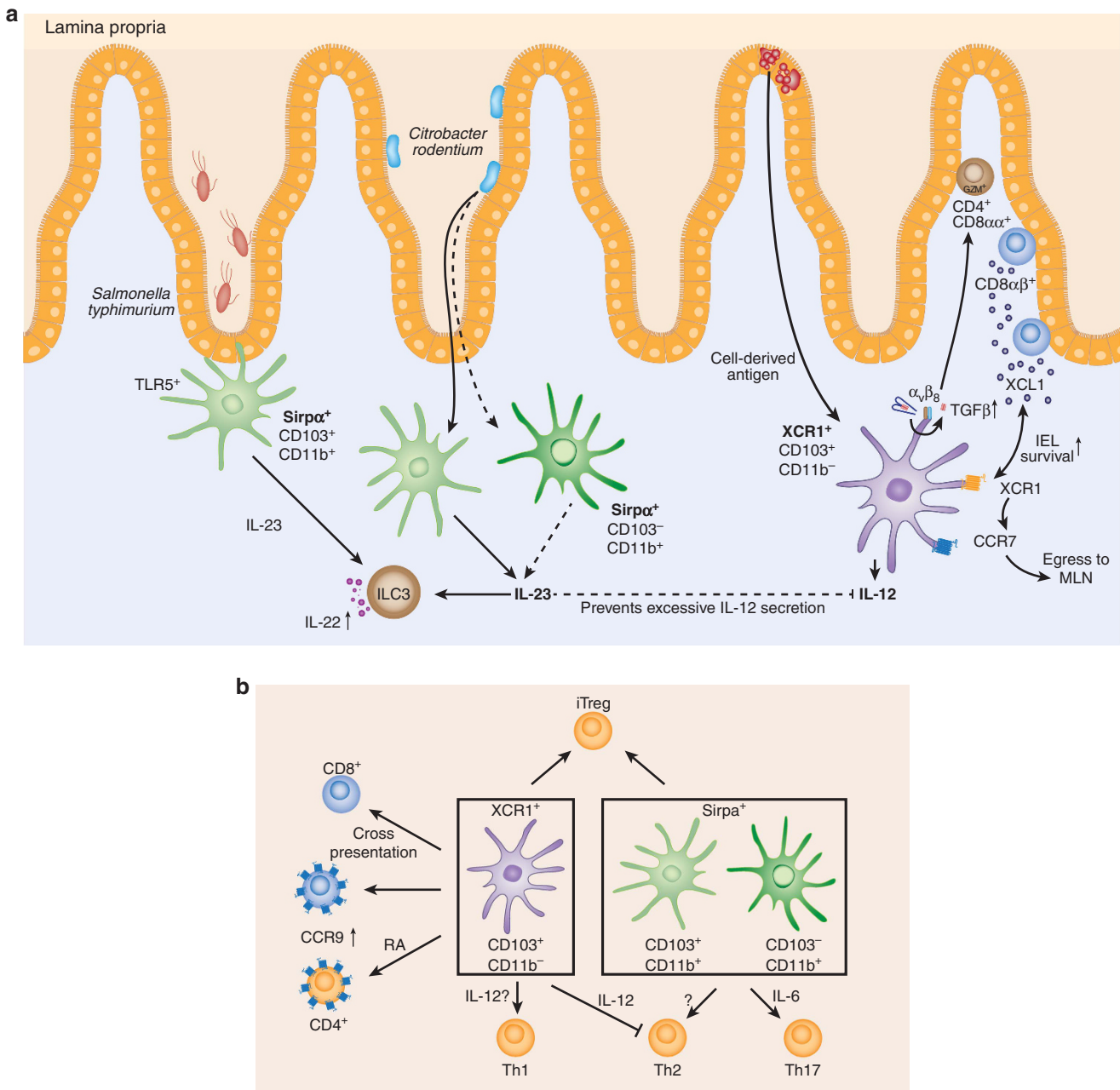
Figure 2 Functions of intestinal cDC. (**a**) cDC functions in the small intestinal LP. Sensing of flagellin via TLR5 or *Citrobacter rodentium* by SIRPα+CD103+CD11b+ cDC and potentially SIRPα+CD103−CD11b+ cDC may induce IL-23 secretion, leading to ILC3 activation and IL-22 secretion. IL-23 production by SIRPα+ cDC has also been described to limit IL-12 secretion by XCR1+ cDC. XCR1+ cDC are the predominant APC that takes up cell-derived antigen for cross-presentation to CD8+ T cells. In addition they have been shown to promote the development of CD8αα+CD4+ cytotoxic IEL via the generation of active TGFβ through integrin $\alpha_v\beta_8$ and to sustain the survival of intestinal T cells via the XCR1/XCL1 axis. Finally XCL1 production by intestinal lymphocytes has been implicated in inducing CCR7 expression in XCR1+ cDC, promoting their migration to MLN. (**b**) cDC functions in the intestinal lymph nodes. XCR1+ cDC are superior at inducing CD8+ T-cell responses to cross-presented antigen and are the major cDC subset that produces RA, inducing CCR9 expression on responding CD4+ (orange cells) and CD8+ (blue cells) T cells. They also promote Th1 induction, but dampen the development of Th2 responses via release of IL-12. SIRPα+ cDC are specialized in the induction of Th2 and Th17 responses. Both XCR1+ and SIRPα+ cDC have been implicated in iTreg conversion. Similar DC dependent processes are likely to occur in the colon and its draining lymph nodes, although many of these have not been explored directly in this tissue. APC, antigen-presenting cell; cDC, conventional dendritic cells; IEL, intra-epithelial lymphocytes; LP, lamina propria; MLN, mesenteric lymph nodes; RA, retinoic acid; SIRPα, signal regulatory protein α; TFGβ, transforming growth factor β; XCR1, X-C motif chemokine receptor 1.

in human IBD, although there is contradictory evidence about whether it is increased or reduced under these circumstances.[85,86] A recent study identified a panel of lineage-specific markers suggested to identify XCR1+ cDC (CADM1hi SIRPαlo CD11cint − hi CD26hi IRF8hi IRF4lo) and SIRPα+ cDC (CADMlo SIRPαhi CD1chi CD11chi IRF4hi IRF8lo) across

species and tissues that should prove useful in comparative phenotypic and functional studies between human and murine intestinal cDC subsets.[84]

ENVIRONMENTAL CONDITIONING OF INTESTINAL cDC

Intestinal cDC have many characteristics which set them apart from their counterparts in other tissues, indicating that their development and functional specializations may involve tissue-specific factors. The same cDC subsets also display distinct characteristics depending on their location within the intestine, probably reflecting the differences in anatomy, function, microbial composition and nature of micronutrients that are found along the length of the intestine.[87]

The presence of large numbers of CD103$^+$CD11b$^+$ cDC in the murine small intestine is perhaps the most obvious example of how the cDC lineages differ between the intestine and other tissues. As we have discussed, this appears to be at least partly due to tonic TGFβ signals, although the source and molecular basis of this effect are unclear (Montgomery *et al.*, submitted for publication). Several dietary and bacterial metabolites have also been implicated in regulating intestinal cDC development and function. For example, RA has a major impact on the function of murine intestinal cDC, particularly in the small intestine. Tissue retinol levels are highest in the small intestine, due to uptake from the diet and potentially through delivery of liver-stored retinol via the bile, resulting in an increase in RA signaling in small intestinal cDC.[88–90] Such signaling induces aldehyde dehydrogenase 1a2 (*Aldh1a2*) expression in small intestinal cDC endowing them with the capacity to generate CCR9$^+$α$_4$β$_7^+$ small intestinal homing T cells.[30,31,91–92] Both epithelial cells and local stromal cells in the small intestinal LP have been implicated in the conversion of retinol to RA for delivery to local cDC.[89,93] Vitamin A deficient mice, or mice whose CD11c$^+$ cells are unable to respond to RA, also display reduced numbers of intestinal CD103$^+$CD11b$^+$ cDC[94]. Intestinal CD103$^+$CD11b$^-$ cDC in these mice start to express langerin,[94,95] indicating an additional role for RA in regulating intestinal cDC development. Consistent with this, RA has been proposed to promote the development of 'gut homing' pre-cDC in the BM,[96] and to participate in the imprinting of an 'intestine-like' phenotype on pre-cDC subsequent to their entry into the intestine.[94,96] Additional metabolites implicated in modulating intestinal cDC function include AhR ligands and short chain fatty acids. For example mice lacking AhR on CD11c$^+$ cells display minor alterations in intestinal cDC subset composition and their cDC display altered expression of Wnt signaling components,[97] while butyrate signaling through the G-protein-coupled receptor GPR109A may impart an anti-inflammatory profile on colonic cDC, promoting their ability to generate iT$_{reg}$ and IL-10 producing T cells.[98]

PART B. INTESTINAL MACROPHAGES

Macrophages defined using the phenotypic and functional criteria discussed above are found throughout the gastrointestinal tract from before birth onwards.[6,99,100] Most intestinal mϕ are found in the LP in close proximity to the epithelium and some may extend processes into the epithelial layer itself (see below). Discrete populations of mϕ are also present within the muscularis externa and serosa, where they engage in two-way interactions with the enteric nervous system,[101–104] but here we will confine our discussion to the mϕ present in the intestinal mucosa. Mϕ are relatively more abundant in the colon than small intestine,[87] while those expressing high levels of CD11c may be found closer to the epithelium than the CD11c$^-$ subset.[105] A specific population expressing CD169 (sialoadhesin) is typically found near the crypt base, close to the submucosa and may have distinct functions and developmental requirements[106,107] (and see below).

Ontogeny of intestinal macrophages

Like resident mϕ in other tissues, mature mϕ in the intestinal mucosa are large, vacuolated cells with abundant cytoplasm containing lysosomes and other granules.[4] In mice they express classical mϕ markers including CD11b, CD64, MERTK and F4/80, but unlike mϕ in many other tissues, virtually all those in the intestine also express high levels of MHCII and CX3CR1. Most are also CD11c$^+$ (**Table 2**).[4,108,109]

Tissue-resident mϕ derive from a number of different precursors, including mesenchymal stem cells in the embryonic yolk sac (YS), fetal liver monocytes and adult BM-derived monocytes, with the relative contribution of each precursor source varying from organ to organ (see ref. 109 for review). Although embryonically derived mϕ are present in the mouse intestine at birth, BM-derived Ly6C^{hi} ("classical") monocytes appear soon afterwards. Using a variety of approaches, we and others have shown that adult intestinal mϕ are entirely of haematopoietic origin and require constant replenishment by Ly6C^{hi} monocytes.[4,6,108] This process is dependent on CCR2, which is not only needed for emigration of Ly6C^{hi} monocytes from the BM,[4,78,108,111] but also for their recruitment to the intestine.[112] Thus while Ly6C^{hi} monocytes were originally considered to be involved only in inflammatory processes, it is now clear that they can also give rise to tissue mϕ under either steady state or inflammatory conditions.[108]

In contrast to these studies, it has been reported that a subpopulation of CD11c$^-$ mature intestinal mϕ cannot be replenished by CCR2-dependent monocytes, although the exact origin of these mϕ was not examined directly.[81,105] It has also been reported that intestinal mϕ may be dependent on CX3CR1,[113,114] whose function is required for the survival of the alternative group of Ly6C^{lo} monocytes.[115] However in our hands, this population of monocytes could not be found entering the intestine and *Cx3cr1*$^{-/-}$ mice had normal numbers of intestinal mϕ.[108] The reasons for these discrepant results are unclear, but they could indicate that the relative contribution of different precursors to the intestinal mϕ pool may vary depending on factors in the environment, such as the nature of the microbiota.

Table 2   Monocytes and mϕ in the intestine

Phenotype		Ontogeny & Differentiation			Function
Common	Subset Specific	GF	TF Involvement	Other Factors	
CD11b⁺ **CD64⁺** **F4/80⁺** **MerTK⁺** Ly6G⁻ Siglec-F⁻ CD3⁻ CD19⁻ B220⁻ NK1.1⁻	CX3CR1ⁱⁿᵗ · **Ly6Cʰⁱ** MHC IIⁿᵉᵍ CD11cⁿᵉᵍ **"P1"** / **Ly6Cʰⁱ⁻ⁱⁿᵗ** MHC II⁺ CD11c⁺/⁻ **"P2"** / **Ly6Cⁿᵉᵍ** MHC II⁺ CD11c⁺/⁻ **"P3"**	CFS1-dependent	Runx 3 PU.1	TGFβR-signalling IL-10R-signalling	• Functional plasticity • Production of pro-inflammatory mediators in response to TLR ligands etc
	CX3CR1ʰⁱ · **Ly6Cⁿᵉᵍ** MHC IIʰⁱ CD11cˡᵒ⁻ʰⁱ **"P4"**				• Scavenging, destruction of apoptotic cells, microbes • Tissue remodelling, epithelial renewal • IL-10 production • Local maintenance of T_reg

Abbreviation: GF, growth factors; siLP, small intestinal lamina propria; TF, transcription factors.
Summary of the phenotypic markers, ontogeny and key functions of intestinal mf and their monocyte precursors. See main text for detail.

An important point to note here is that the embryonic mϕ present at birth share many of the characteristic properties of their monocyte-derived counterparts in adult intestine including high-phagocytic activity, production of IL-10 and expression of scavenger receptors.[116] Furthermore adult CD11c⁺ and CD11c⁻ mϕ differ by expression of only a few genes.[105] These findings are consistent with the emerging view that location and not origin defines the functions of tissue-resident mϕ.[110,117]

After arrival in the mucosa, monocytes undergo a process of local differentiation through a series of phenotypic intermediaries known as the monocyte "waterfall".[6,108] In mice this can be followed phenotypically, with CX3CR1ⁱⁿᵗLy6CʰⁱMHCII⁻ monocytes ("P1") first acquiring MHCII ("P2"), before losing Ly6C expression ("P3") and finally acquiring the CX3CR1ʰⁱLy6C⁻MHCIIʰⁱ phenotype of mature resident mϕ ("P4"; **Figure 1d**). In mouse colon this differentiation process takes around 5–6 days from when the monocytes first enter, with the mature mϕ having an estimated $t_{1/2}$ of 6–8 weeks.[4,118] As with most resident tissue mϕ, this developmental process in the intestine requires the CSF-1R.[11]

The properties of fully mature intestinal mϕ are acquired progressively as they develop through the local differentiation process described above (**Table 2**; **Figure 3**). Consistent with their morphological appearance, they express high levels of receptors associated with the recognition and uptake of apoptotic cells or opsonised organisms, including CD163 (heme/haptoglobin scavenger receptor), CD206 (mannose receptor), CD36, scavenger receptor A, the receptor tyrosine kinases Axl and MerTK, the complement component

C1Q, T-cell immunoglobulin mucin protein 4 (TIM4) and $\alpha_v\beta_5$ integrin (refs 107, 118, 119 and A.K. Kumawat & A.McI. Mowat, unpublished data). In parallel they are avidly phagocytic and bactericidal even without additional stimulation.[4,119–122] Under steady state conditions they also produce mediators such as TNFα, IL-1β and IL-10, with up to 50% of mature intestinal mϕ producing IL-10 constitutively.[108,118,123–125]

Despite this evidence of being activated *in situ*, intestinal mϕ fail to respond to agents that normally activate mϕ by producing pro-inflammatory cytokines, nitric oxide (NO) or reactive oxygen intermediaries. This unresponsiveness is global in extent, as it also applies to non-Toll-like receptor (TLR) stimuli such as IFNγ and NOD2 ligands, or when mϕ have taken up pathogens.[108,122,126–129] These properties allow intestinal mϕ to act as efficient scavengers without generating the inflammation that normally ensues upon encounter of microbes and would compromise intestinal homeostasis.

A variety of factors in the local environment have been implicated in maintaining the anergy of intestinal mϕ, including downregulation and/or active inhibition of TLR signaling pathways, despite normal expression of the TLRs[122,130,131] and other pattern recognition receptors.[108,122] Mice lacking IL-10 or its receptor develop microbiota-dependent spontaneous IBD, associated with hyperactivity of intestinal mϕ[105,123,132–135] and lack of signaling via the IL-10R on mϕ leads to spontaneous colitis.[123,135,136] Mutations in this pathway are associated with severe, early onset IBD in humans.[137–139] IL-10 has many anti-inflammatory effects on myeloid cells, including epigenetic silencing of inflammatory gene loci via the activation of histone deacetylase.[140,141]

Whether the epigenome of resident intestinal mφ shows evidence of this gene silencing remains to be determined.

TGFβ has been implicated in the TLR unresponsiveness of human intestinal mφ,[142,143] but in our hands, mouse intestinal mφ that lacked TGFβR signaling did not show enhanced responses to TLR ligation.[144] Therefore there may be species differences in how intestinal mφ reactivity is controlled. One possibility is that the IL-10 and TGFβ pathways play complementary roles and that these may vary with age. This is supported by findings that the effects of TGFβ can be observed from late in gestation onwards,[142] while those of IL-10 appear to develop in a narrow time window in early neonatal
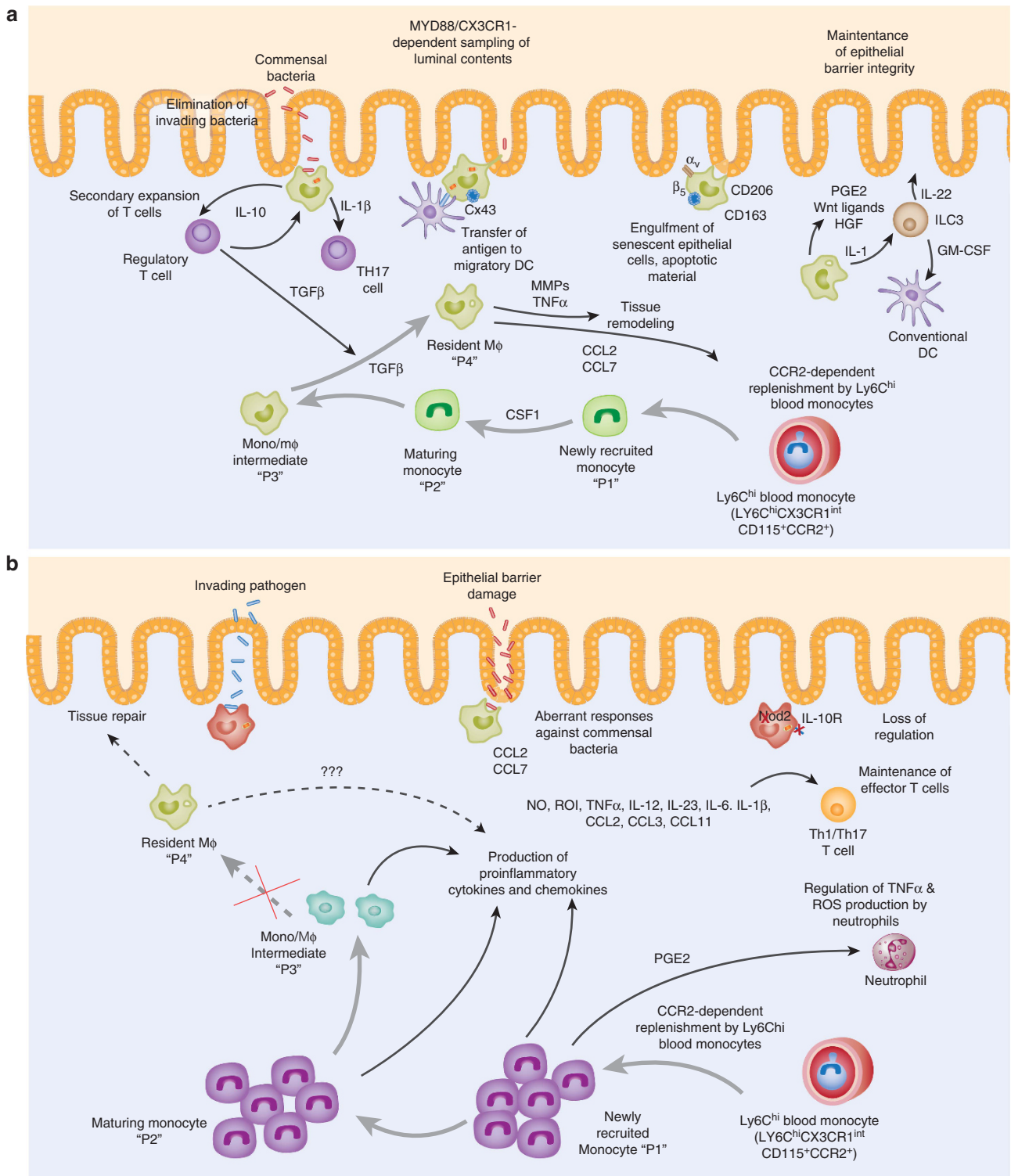
life.[141] In parallel, IBD in *IL-10*^{−/−} mice can be prevented by exogenous replacement of the cytokine in neonatal, but not in adult animals, consistent with there being a limited period during which IL-10 can cause epigenetic silencing of mϕ genes.[141]

Finally, resident intestinal mϕ express a variety of inhibitory receptors with the potential for restricting their activation in a cell intrinsic manner, such as CD200R, SIRPα and Axl. Intestinal mϕ from mice lacking Axl show evidence of an increased inflammatory profile,[145] but there are contradictory findings on whether absence of CD200–CD200 receptor signaling pathway leads to mϕ activation or inflammation in the intestine.[146,147] In contrast to what is observed with intestinal cDC (see above), mice with a mutant form of SIRPα that lacks the signaling domain do not have alterations in the intestinal monocyte/mϕ compartment in steady state intestine and are also not susceptible to inflammation (ref. 72) and C.L. Scott & A.McI. Mowat, unpublished data).

Perhaps surprisingly, intestinal mϕ retain their global hyporesponsiveness to TLR stimulation even when the microbiota is entirely lacking in germ free and embryonic mice (refs 115,139) and our unpublished observations).

PHYSIOLOGICAL FUNCTIONS OF INTESTINAL MACROPHAGES
Symbiosis with the microbiota
As mucosal mϕ are typically found in close proximity to the epithelium and are more abundant in colon compared with small intestine, it seems reasonable to assume that one of their main roles *in vivo* is the uptake and clearance of microbes that have crossed the epithelial barrier (**Figure 3**). Indeed bacteria have been detected within lamina propria mϕ from steady state intestine[100] and CX3CR1[+] mϕ account for most of the uptake of organisms when *Escheria coli* are administered orally.[148] In this regard CX3CR1[+] phagocytes have been observed to send processes from the LP across the epithelium to capture luminal bacteria in a CX3CL1-dependent manner and although originally thought to be cDC,[149] it now seems likely that these were mϕ.[109] As noted above, intestinal mϕ are actively bactericidal, possibly reflecting the constitutive expression of mediators such as TNFα and metallothionein-dependent Zn^{2+} cations.[150] Recent work also suggests that CCR2-dependent mϕ may be required for the induction of Th17 cell responses to segmented filamentous bacteria in steady state intestine.[76] Because these functions are achieved without causing inflammation, mϕ are one component of the host response that ensures a healthy symbiotic relationship with the commensal microbiota.

Maintenance of epithelial homeostasis
The presence of mϕ in the intestine from before birth and in germ free mice indicates they are likely to have physiological functions in addition to scavenging bacteria. The intestinal epithelium is one of the most dynamic and rapidly dividing tissues in the body and this constant movement and cell loss requires continuous remodeling of the underlying extracellular matrix, as well as disposal of effete cells. The high phagocytic activity of intestinal mϕ is ideally suited to these processes and indeed, cytokeratin[+] inclusions have been observed within mϕ from steady state intestine (**Figure 3**).[121,144] Resting intestinal mϕ also express a variety of mediators that can promote tissue remodeling such as metalloproteinases and TNFα (**Figure 3**).[151,152]

That these functions of intestinal mϕ are necessary to maintain epithelial integrity is suggested by studies showing increased epithelial susceptibility to inflammatory insults in animals lacking mϕ themselves or with loss of TLR or AhR signaling on myeloid cells.[97,153–155] Furthermore, loss of mϕ in *Csf1*^{−/−} mice or following depletion with anti-CSF-1R antibody was found to result in dysregulated enterocyte differentiation and loss of Paneth cells.[156,157] A similar phenotype has been reported in mice lacking AhR expression on CD11c^{+} cells. However these experiments did not define whether the effects reflected a property of cDC, mϕ or both.[97] In this respect, mϕ have been reported to produce several mediators, that could drive intestinal epithelial cell renewal and differentiation, including prostaglandin

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Figure 3 Intestinal mϕ in health and inflammation. (**a**) In steady state adult intestine, Ly6C^{hi} monocytes are recruited continuously from the bloodstream in a CCR2-dependent manner. After arrival in the mucosa, they develop through the "monocyte waterfall", in which LyC[hi]MHCII[−]CX3CR1[int]F4/80[lo] monocytes ("P1") acquire MHCII ("P2"), before losing Ly6C to become Ly6C^{−}MHCII[+]F4/80[+] monocyte/macrophage intermediaries ("P3") and finally acquiring high levels of CX3CR1 as they terminally differentiate into the majority population of resident macrophages ("P4"). This process is determined by factors specific to the intestinal microenvironment, with the final step being at least partly dependent on TGFβ. The resident macrophages engage in important homeostatic functions, as they have avid phagocytic activity, express scavenger receptors, metalloproteases, and produce cytokines and chemokines. As a result, the macrophages can clear effete tissue cells and bacteria that cross the epithelium, contribute to tissue remodeling, recruit further monocytes and interact with local immune cells such as T cells and ILC. They also send processes through the epithelium to capture luminal antigens and can transfer antigen to neighboring migratory cDC via Connexin 43 (Cx43). All these activities take place without inflammation occurring, due to IL-10 from T cells and macrophages preventing activation and also because of intrinsic mechanisms that inhibit PRR signaling in macrophages. IL-10 produced by resident macrophages also drives secondary expansion and survival of FoxP3^{+} T[reg] that have been primed in lymph nodes. (**b**) Mucosal inflammation can be caused by invasive pathogens, damage to the epithelial barrier leading to increased exposure to commensal bacteria, or to defects in macrophage control pathways such as NOD2 or IL-10 function. Under these circumstances, there is enhanced recruitment of Ly6C^{hi} monocytes and the normal differentiation process is disrupted, allowing accumulation of pro-inflammatory monocytes and early intermediary macrophages. These release inflammatory mediators such as NO, ROI, TNFα, IL-12, IL-23, IL-6 and IL-1β which cause tissue damage, as well as activating other inflammatory cells including Th1 or Th17 effector cells. Pre-existing resident macrophages may contribute to these inflammatory events, or may remain poised to initiate tissue repair when required. Monocytes can also help limit pathology by releasing PGE$_2$, which inhibits the harmful effects of neutrophils. NO, nitric oxide; PGE$_2$, prostaglandin E$_2$; ROI, reactive oxygen intermediaries.

E_2 (PGE_2), hepatocyte growth factor and Wnt signaling ligands.[97,158–160]

Maintenance of immunological homeostasis in the mucosa

The numbers of endogenous FoxP3$^+$ T cells are higher in the colon than small intestine, correlating with the relative abundance of mφ in each site and mucosal mφ have been found to stimulate the differentiation of T_{reg} *in vitro*.[48] In part, this may reflect their constitutive production of IL-10 and indeed, the fact that a higher proportion of mφ produces IL-10 in the colon than in small intestine is consistent with the relative abundance of T_{reg} at these sites.[123] Intestinal mφ-derived IL-10 has also been implicated in the local expansion of antigen-specific iT_{reg} that have been primed in MLN by orally administered protein. In parallel, impaired IL-10 production by mφ in *Cx3cr1*$^{-/-}$ mice results in a failure to induce oral tolerance.[39] In the context of inflammation, IL-10 from intestinal CD11b$^+$ cells considered to be mφ, has been shown to stabilize the function of iT_{reg} by maintaining the expression of FoxP3.[124] TRAF6 expression in CD11c$^+$ MNPs has also been implicated in the generation of iT_{reg} *in vitro* and mice lacking this pathway develop spontaneous Th2 cell-mediated intestinal inflammation,[34] although it remains to be proven whether the relevant CD11c$^+$ cells are mφ or cDC in this case. Despite these findings, IL-10 production from mφ themselves appears not to be essential for preventing spontaneous IBD in response to the microbiota.[135,161] Thus although prevention of mφ activation via IL-10R signaling is crucial for preventing inflammatory reactions to the microbiota, cells such as CD4$^+$ T cells may be more important sources of the IL-10.

Intestinal mφ may also be involved in the survival and/or maintenance of other immune cells in the mucosa. For instance, IL-1 produced by LP mφ in response to the microbiota has been reported to drive the production of granulocyte macrophage CSF (GM-CSF) from ILC3, which in turn promotes intestinal cDC development.[162] IL-1β produced by intestinal mφ in response to the microbiota can also sustain the expansion and survival of IL-17-producing Th17 cells in the mucosa.[125]

Mature intestinal mφ also produce monocyte-specific chemokines such as CCL2, CCL7, CCL11 and CXCL16, as well as chemokines that can recruit other immune cells.[112,163] Thus resident mφ may also regulate their own replenishment and maintain a dynamic *status quo*.

Macrophages and induction of adaptive immune responses

Intestinal mφ are poor professional antigen-presenting cells *in vitro* and do not express CCR7 or migrate in afferent lymph to MLN,[2,13,109] indicating they are unlikely to play a major role in the initiation of adaptive immune responses. However, evidence from other systems suggests that antigen-specific T cells require a second stimulus from an antigen-presenting cell in non-lymphoid tissues to acquire full effector functions.[164] Although MHCII$^+$ mφ would be well placed to serve this function in the mucosa, a role of this kind remains to be assessed.

One way in which mφ might contribute indirectly to the priming of mucosal immunity comes from evidence that orally administered proteins or bacteria taken up by CX3CR1^{hi} mφ in LP can be passed to migratory CD103$^+$ cDC.[148,165] In the case of protein antigen, this transfer process has been suggested to involve Connexin 43 (Cx43)- mediated gap junctions formed between mφ and cDC and this leads to the induction of oral tolerance.[165] Division of labor amongst intestinal mφ and CD103$^+$ cDC of this kind has also been suggested to be involved in the induction of local Th17 responses to segmented filamentous bacteria.[76]

Macrophages in intestinal inflammation

Disorders such as IBD and infection are accompanied by infiltration with mφ and monocytes.[4] Our own and other work indicates that Ly6C^{hi} monocytes entering the intestine fail to differentiate fully to become mature mφ in the presence of inflammation, failing to upregulate IL-10 or to become unresponsive to stimulation.[6,105,108] In addition, there is enhanced recruitment of monocytes above the baseline levels seen in steady state intestine and as a result, the early stages of the monocyte waterfall (CX3CR1^{int}Ly6C^{hi}MHCII$^{+/-}$) dominate the inflammatory infiltrate.[166] Although predominantly dependent on the CCL2-CCR2 axis,[81,127,163,167–169] other chemokine receptors can also come into play in inflammation, including CCR1 and its ligand CCL3.[170] Contradictory findings have been reported on whether the CX3CR1 chemokine receptor plays a role in recruiting inflammatory mφ in experimental IBD, with both increased and decreased susceptibility having been observed in *Cx3cr1*$^{-/-}$ mice.[114,171,172]

It is not known why monocytes that arrive in the inflamed mucosa do not go through the full differentiation process seen in normal intestine. One possibility is that they simply do not survive long enough to be able to progress through the entire waterfall, which normally takes up to a week to complete.[108] Alternatively there may be loss of the factors that usually drive mφ survival and development in the healthy mucosa. Interestingly, the fate of monocytes may also be determined before they arrive in the mucosa, as reported in mice with intestinal toxoplasmosis, where IL-12 produced by local cDC induces NK cells in the BM to produce IFNγ, leading to altered differentiation of monocytes or their precursors.[173]

Monocytes and immature mφ are the main producers of pro-inflammatory mediators such as IL-1, IL-6, TNFα, IL-23, NO and reactive oxygen intermediates that are found in inflamed intestine.[6,81,105,118,120,135,146,154,167,169,170,174–178] As well as contributing to tissue damage directly, these mediators can also recruit and activate additional immune effector cells. IL-23 from monocyte-derived cells is critical for protective Th17 in infections by bacteria such as *C.rodentium* or *Helicobacter hepaticus*[74,174] and together with IL-1β it induces the production of antimicrobial and cyto-protective IL-22 by stimulating ILC3.[74,81] Inflammatory monocytes can also promote IFNγ- and IL-17-producing Th1 and Th17 cells via the production of IL-12 and IL-6, respectively.[133,177] As in the steady state, these effects on T cells are likely to involve a role in local maintenance

of previously primed effector T cells, rather than in initiation of the immune response. Pro-inflammatory monocytes/mϕ also produce chemokines such as CCL2, CCL3, CCL4, CCL5, CCL8 and CCL11, which recruit monocytes, eosinophils and neutrophils.[106,167,178]

It is not clear if pre-existing resident mϕ can also contribute to these inflammatory processes. The resident mϕ population does not expand markedly in chemically induced or T-cell-dependent colitis and there is little or no change in function or gene expression under these conditions.[6,108,120] Indeed IL-10 secretion by intestinal mϕ is important in limiting IL-23-mediated pathology during experimental infection with *C.rodentium*.[123] Nevertheless, resident mϕ can produce pro-inflammatory mediators when colitis develops in the absence of IL-10 signaling,[135,174] perhaps reflecting retained functional plasticity when IL-10 driven epigenetic silencing is lacking.[141] It is also possible that there is heterogeneity amongst resident mϕ in their ability to respond to inflammatory stimuli. Indeed the small subset of CD169$^+$ mucosal mϕ expands during inflammation and recruits neutrophils via the production of CCL8.[106]

Intestinal macrophages and Th2-mediated immune responses

Mϕ are also important in the Th2 driven immune responses involved in resistance to infection by intestinal helminths, when there is infiltration by monocytes and expansion of the local mϕ population.[179–183] Under these conditions, production of IL-4 and IL-13, together with activation of peroxisome proliferator-activated receptor γ (PPARγ) by cyclooxygenase-2 (COX2), generates genuine alternatively activated mϕ, with release of typical mediators such as arginase and resistin-like molecule α (RELM-α).[180,182,184,185] Depletion of monocytes or mature mϕ inhibits the expulsion of helminths,[179,186–188] which may be because mϕ and their products can attack the parasites directly. Alternatively activated mϕ of this kind also help generate a hostile environment by promoting smooth muscle contractility, depriving metabolites such as glucose[187,188] and by producing chemokines that recruit additional anti-parasite effector cells such as eosinophils.[169,178]

In contrast to what has been described in the pleural or peritoneal cavities,[189,190] IL-4- and CSF-1-driven proliferation of resident mϕ does not seem to occur to any significant extent during intestinal helminth infection. Under these conditions, most mϕ are derived from Ly6C^{hi} monocytes and only low amounts of local proliferation can be observed.[182]

Monocytes and macrophages and resolution of intestinal inflammation

Mϕ play central roles in tissue repair and restoration of homeostasis in intestinal inflammation, as depletion of mϕ delays recovery from experimental colitis.[153,155,191] A variety of mechanisms have been implicated in the repair function of intestinal mϕ, including TLR/MyD88-dependent restoration of the epithelial stem cell niche via stimulation of PGE$_2$ production from stromal cells.[155,192] Recovery from *Heligmosomoides polygyrus* induced intestinal pathology also involves

mϕ producing chemokine ligands for CXCR2 on fibroblasts, driven in part via CD64.[193] The ability of mϕ to induce IL-22 production by ILC3 also favours restitution of a damaged epithelial barrier,[81,194] while TGFβR-mediated suppression of IL-33 production by mϕ has been proposed to mediate resolution of chemical colitis.[191] Arginase produced by alternatively activated mϕ in parasite infections is a further important tissue repair mechanism under these conditions.[195,196] Although generally thought of as a pro-inflammatory mediator, IL-1β production by myeloid cells has also been shown to promote epithelial repair after acute colitis,[197] while IL-36 produced by both mϕ and epithelial cells may contribute to enhanced turnover of enterocytes as a reparative response to inflammation.[198]

As many of the properties of resident mϕ in the healthy intestine would be ideally suited to help in tissue repair, cells of this kind that have survived the initial inflammatory insult may be important in this process. Alternatively, some recruited monocytes may differentiate into more mature mϕ with repair functions, as has been described for monocytes infiltrating the damaged heart and after depletion of liver Kupffer cells.[199,200] Inflammatory monocytes themselves have been shown to inhibit the pathological effects of neutrophils during the intestinal inflammation induced by dextran sodium sulfate or *Toxoplasma gondii*.[155,175] In the latter case, this reflected synthesis of PGE$_2$ by monocytes[175] and other work has confirmed enhanced PGE$_2$ production by monocytes during colitis.[135] Production of GM-CSF and VEGF-C during inflammation may reprogramme the differentiation of monocytes into mϕ that produce reparative mediators such as arginase, prostaglandins and IL-13.[201,202] Thus the overall role of the monocyte-mϕ lineage in intestinal inflammation may reflect a balance between local pathogenic and regulatory mechanisms.

HUMAN INTESTINAL MACROPHAGES

Mϕ show a similar distribution along the human gastrointestinal tract and they share the morphological features of their mouse counterparts, as well as being refractory to stimulation and expressing MHCII and phagocytic receptors.[86,108,119,203–206] However, many phenotypic markers of the myeloid lineage including CD11b, F4/80 and CD11c are not conserved between the species and a variety of different markers need to be used to distinguish human intestinal mϕ from cDC, including CD14, CD33, CD64 and CD68.[5] CD163 or CD206 are also expressed by most mature mϕ in humans, but they express low amounts of CD14, which is found at high levels on classical monocytes and immature mϕ.[86,108,119,203–206]

Using markers of this kind, it can be shown that a monocyte-mϕ "waterfall" exists in the normal human intestine, with some CD14^{hi} monocytes being present, together with intermediary stages and a major population of CD14^{lo}CD163$^+$CD206$^+$ mϕ.[5,108] Interestingly, recent studies in human BM transplantation have suggested that as in mice, human intestinal mϕ are continuously replenished by circulating precursors (Dr M. Haniffa, University of Newcastle, Personal Communication).

Nevertheless, there may be some differences between mφ in steady state human and mouse intestine. Thus human intestinal mφ are capable of producing RA via expression of the *Aldh1a1* gene whereas in mice, RA production appears limited to cDC.[85,86] Furthermore, as we have noted earlier, TGFβ may play a more important role in the hyporesponsiveness of human intestinal mφ to TLR stimulation than it does in mice.[143] Indeed, there appear to be several differences in how responsiveness to pattern recognition receptors is regulated in human and murine mφ in general.[207] As in mice however, IL-10 plays an essential role in controlling the pro-inflammatory potential of human intestinal mφ and non-functional mutations in the IL-10R lead to severe, early onset IBD.[137–139] The mucosa in Crohn's disease also shows evidence of open chromatin in the pro-inflammatory gene loci normally silenced by IL-10.[141]

As in mice, studies of human inflammatory bowel disease have highlighted the presence of large numbers of classical (CD14^{hi}) monocytes and a relatively decreased frequency of mature mφ.[85,86,108,176,208–210] Furthermore, these immature cells are responsible for producing large amounts of the pro-inflammatory mediators that typify these disorders, including TNFα, IL-1, IL-18, IL-23, NO and reactive oxygen intermediates.[129,203,204,208,211–212] Production of RA by infiltrating monocytes and mφ may also be enhanced during IBD, perhaps secondary to increased levels of GM-CSF.[86] Interestingly, autocrine signaling via the RA receptor has been suggested to contribute to the production of TNFα by mφ.[86]

ENVIRONMENTAL CONDITIONING OF INTESTINAL MONOCYTES AND MACROPHAGES

As with cDC, the characteristics of intestinal mφ set them apart from their counterparts in other tissues and reveal a role for the local environment in imprinting their properties. Thus we have found that although mφ in the dermis and intestine are both derived from classical monocytes,[116,213] these cells show transcriptional differences very soon after arriving in the two tissues, becoming increasingly distinct as they develop into mature mφ.[144] TGFβ again may play a central role in the terminal differentiation of monocytes into intestinal mφ. TGFβ is known to upregulate CX3CR1 on other mφ[214] and mice lacking KLF10, a transcription factor involved in the TGFβR pathway, have reduced numbers of intestinal CX3CR1[hi] mφ.[215] TGFβ does not seem to be required for the initial stages of monocyte recruitment and maturation, consistent with the findings from other tissues that a number of different factors need to act in concert to specify resident mφ differentiation.[216] Active epigenetic marking of the enhancer of the Runt-related transcription factor 3 (Runx-3) was also recently shown to be a selective feature of resident intestinal mφ[217] and Runx-3 is known to interact with TGFβ signaling in other cells including Langerhans cells.[218] However, its role in intestinal mφ development has not been explored directly. RA is required for the development of the CD169^{+} subset of intestinal mφ, but not of other intestinal mφ[94,107,219] and dietary amino acids

such as glutamine have also been proposed to be important for mφ development in the small intestine.[220]

A number of mechanisms may help drive the production of IL-10 by intestinal mφ, including products of the microbiota such as the short chain fatty acid butyrate[98,221] or ligands for the MyD88 signaling pathway,[105,134,140,222] as well as for the AhR[97] and PPAR receptor pathways.[223,224] Intestinal mφ in *Cx3cr1*^{−/−} mice show defective IL-10 production[39] and fractalkine (CX3CL1), the only ligand for CX3CR1 down-regulates nuclear factor-κB and pro-inflammatory cytokine production in BM-derived mφ via PPARγ signaling.[225] Thus there could be links between these pathways in the regulatory functions of intestinal mφ. IL-10 itself can also drive some of the properties of intestinal mφ in addition to their hypor-esponsiveness to stimulation, including the expression of scavenger receptors such as CD163.[123,134]

Finally, although there is much reduced recruitment of monocytes to the colon of germ free mice compared with conventionally housed controls,[116] mature mφ remain present in germ free colon, indicating the microbiota does not play an essential role in their development. However, the production of IL-1β by intestinal mφ is dependent on the presence of the microbiota[125,162] and exposure to the microbiota can also act as a feedback mechanism by limiting the generation of active IL-1β from pro-IL-1β.[226] Whether there are additional effects on mφ differentiation and function remains to be determined.

SUMMARY

The normal intestinal mucosa contains large populations of cDC and mφ which play distinct, but complementary roles in local immune responses and tissue homeostasis. Whereas cDC are specialised in sampling the microenvironment, carrying antigens to the draining LN and shaping the nature and location of subsequent T-cell responses, mφ are sessile scavengers that clear microbes and contribute to tissue remodeling, as well as helping to maintain an anti-inflammatory milieu in the mucosa. Both populations have properties that make them distinct from their counterparts in other tissues, reflecting adaptation to the special demands and characteristics of the intestine. As well as containing a unique subset of IRF4-dependent SIRPα[+] DC that expresses both CD103 and CD11b, cDC from the small intestine have an enhanced ability to produce RA and promote the generation of gut-homing T cells. Intestinal SIRPα[+] cDC have been associated with the generation of mucosal Th17 and Th2 responses, while XCR1^{+} cDC are involved in the induction of Th1 immunity, cross-priming of CD8^{+} T cells, generation of gut tropic T cells and establishment/maintenance of the intestinal intra-epithelial lymphocyte compartment. In contrast both cDC branches appear to contribute to mucosal iT$_{reg}$ induction. Whether these functions are hard-wired or vary depending on context remains to be fully explored.

Unlike resident mφ in many other tissues, those in the intestine require constant replenishment from circulating classical monocytes that differentiate locally after their arrival in the mucosa, under tissue-specific control. This allows

constant surveillance of the intestine and the opportunity to react rapidly and flexibly to changes in its environment. Under inflammatory conditions, the local differentiation process that normally generates mature, anti-inflammatory mϕ from the same monocyte precursors is dysregulated, allowing accumulation of pro-inflammatory cells with pathological functions.

Understanding more about the factors that determine the tissue-specific properties of intestinal cDC and mϕ and how these cells change in response to inflammation or infection will be important challenges for the future.

AUTHORS CONTRIBUTIONS
All authors have contributed equally to the writing and editing of this review.

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The authors declared no conflict of interest.

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