MONOCYTE AND MACROPHAGE HETEROGENEITY

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Abstract | Heterogeneity of the macrophage lineage has long been recognized and, in part, is a result of the specialization of tissue macrophages in particular microenvironments. Circulating monocytes give rise to mature macrophages and are also heterogeneous themselves, although the physiological relevance of this is not completely understood. However, as we discuss here, recent studies have shown that monocyte heterogeneity is conserved in humans and mice, allowing dissection of its functional relevance: the different monocyte subsets seem to reflect developmental stages with distinct physiological roles, such as recruitment to inflammatory lesions or entry to normal tissues. These advances in our understanding have implications for the development of therapeutic strategies that are targeted to modify particular subpopulations of monocytes.

OSTEOCLAST
A multinucleate cell that resorbs bone.

Circulating monocytes give rise to a variety of tissue-resident macrophages throughout the body, as well as to specialized cells such as dendritic cells (DCs) and OSTEOCLASTS. Monocytes are known to originate in the bone marrow from a common myeloid progenitor that is shared with neutrophils, and they are then released into the peripheral blood, where they circulate for several days before entering tissues and replenishing the tissue macrophage populations[1]. The morphology of mature monocytes in the peripheral circulation is heterogeneous, and these cells constitute ~5–10% of peripheral-blood leukocytes in humans. They vary in size and have different degrees of granularity and varied nuclear morphology, which at the extremes of variation can lead to confusion with granulocytes, lymphocytes, natural killer cells and DCs. The basic features of the mononuclear-phagocyte system (which includes macrophages and their monocyte precursors and lineage-committed bone-marrow precursors, as well as all other cells that are derived from this lineage[2]) are summarized in BOX 1.

As long ago as 1939, Ebert and Florey[3] reported the observation that monocytes emigrated from blood vessels and developed into macrophages in the tissues. Pro-inflammatory, metabolic and immune stimuli all elicit increased recruitment of monocytes to peripheral sites[4], where differentiation into macrophages and DCs occurs, contributing to host defence, and tissue remodelling and repair. Studies of the mononuclear-phagocyte system, using monoclonal antibodies specific for various cell-surface receptors and differentiation antigens, have shown that there is substantial heterogeneity of phenotype, which most probably reflects the specialization of individual macrophage populations within their microenvironments. Although it is clear that monocytes are precursors of both macrophage and DC lineages, this development and differentiation pathway is still relatively poorly studied *in vivo*. However, the identification of heterogeneity among peripheral-blood monocytes — first, in humans, and more recently, in mice — has provided a powerful insight into the nature of myeloid-cell heterogeneity and has provided novel ways to assess cell fate and function *in vivo*.

The cellular and molecular adhesion mechanisms that are used by migrating monocytes have recently been reviewed[5] and are not discussed here. Instead, we briefly summarize the current understanding of human monocyte heterogeneity and then describe recent advances in the study of mouse monocyte heterogeneity, to highlight the different physiological

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roles of the subsets *in vivo*, and we discuss the relevance of heterogeneity to the study of human monocyte biology. We also discuss the origins of tissue-resident macrophage populations, and we describe how knowledge of their origins might be pertinent to tissue homeostasis at times when it could be advantageous to modulate monocyte function as part of a therapeutic strategy.

Monocyte heterogeneity in humans

Peripheral-blood monocytes show morphological heterogeneity, such as variability of size, granularity and nuclear morphology. Monocytes were initially identified by their expression of large amounts of CD14 (which is part of the receptor for lipopolysaccharide). However, the subsequent identification of differential expression of antigenic markers showed that monocytes in human peripheral blood are heterogeneous, and this provided the first clues to the differential physiological activities of monocyte subsets. Differential expression of CD14 and CD16 (also known as FcγRIII) allowed monocytes to be divided into two subsets: $CD14^{hi}CD16^-$ cells, which are often called classic monocytes, because this phenotype resembles the original description of monocytes; and $CD14^+CD16^+$ cells[6]. It was subsequently shown that the $CD14^+CD16^+$ monocytes expressed higher amounts of MHC class II molecules and CD32 (also known as FcγRII), and it was suggested that these cells resemble mature tissue macrophages[7]. Distinct chemokine-receptor expression profiles were also among the phenotypic differences that were recognized between these subsets: for example, $CD14^+CD16^+$ monocytes expressed CC-chemokine receptor 5 (CCR5), whereas $CD14^{hi}CD16^-$ monocytes expressed CCR2 (REF. 8). A summary of the cell-surface-marker phenotype of these human monocyte subsets is presented in TABLE 1.

When cultured, both human monocyte subsets can differentiate into DCs in the presence of granulocyte/macrophage colony-stimulating factor (GM-CSF) and interleukin-4 (IL-4)[9,10]. Furthermore, using an *in vitro* transendothelial-migration model, in which freshly isolated peripheral-blood mononuclear cells are incubated with human umbilical-vein endothelial-cell monolayers grown on an endotoxin-free collagenous matrix, it has been shown that monocytes can migrate across an endothelial barrier *in vitro* and differentiate either into macrophages, which remain in the sub-endothelial matrix, or into DCs, which then migrate back across the endothelial layer[11]. In this model, the $CD14^+CD16^+$ monocyte subset was found to be more likely to become DCs and reverse transmigrate than was the $CD14^{hi}CD16^-$ monocyte subset[12], indicating that the $CD14^+CD16^+$ cells might be precursors of DCs, which can pass through tissues and then migrate to the lymph nodes through the AFFERENT LYMPHATIC VESSELS. However, these observations do not preclude an *in vivo* role for $CD14^{hi}CD16^-$ monocytes in contributing to the DC pool. Importantly, the extent to which monocytes differentiate into macrophages or DCs in this model depends on both the phenotype of the cultured monocyte population and the factors that are present in the culture. For example, transendothelial migration of $CD14^+CD16^+$ monocytes can be induced with soluble CX_3C-chemokine ligand 1 (CX_3CL1; also

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Box 1 | The mononuclear-phagocyte system
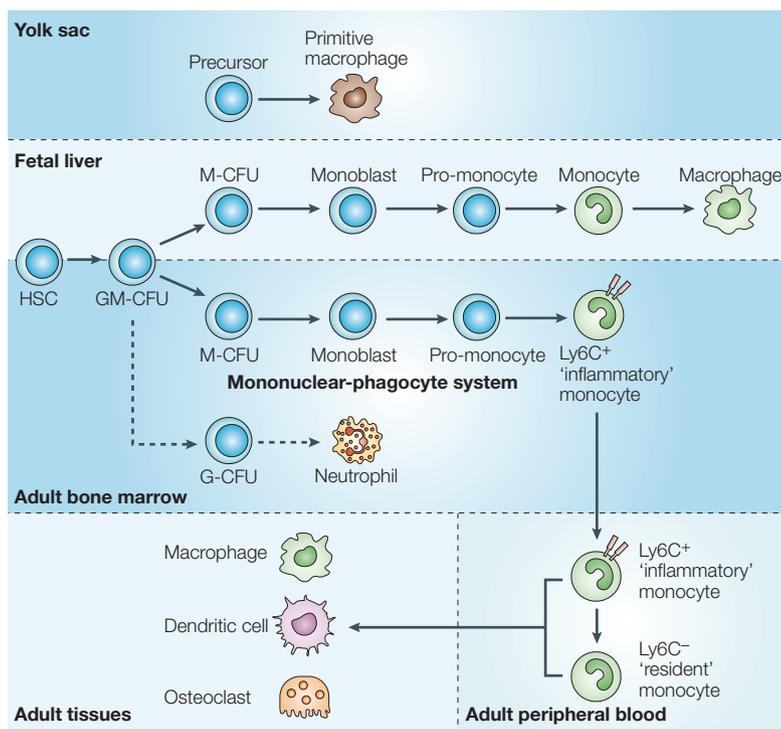
Phagocytic cells were initially classified as the reticuloendothelial system[87]; however, this classification failed to distinguish between 'true' sinusoidal endothelial cells and sinus-lining macrophages. As a consequence, the classification of the mononuclear-phagocyte system was altered to include only macrophages and their monocyte precursors and lineage-committed bone-marrow precursors[2]. However, this classification might require further refinement as the origins of fetal macrophages become clearer and as unanswered questions about the common precursors of macrophages and lymphocytes are addressed.

During development, the origins of cells from the yolk sac that have macrophage-like phenotypes might be distinct from the origins of these cells in adults and after haematopoiesis properly begins in the fetal liver[88,89] (see figure). Developing macrophages are first found in the yolk sac, as identified by morphological characteristics, as well as by expression of macrophage markers such as FMS, CD11b and the mannose receptor[90–92]. Studies in zebrafish, in which cell lineage can more easily be followed because of the transparency of the embryos, have shown population of the yolk sac with macrophage precursors; these cells then differentiate and emigrate into the head mesenchyme and its circulation[93]. Later in development, haematopoiesis in the fetal liver becomes a source of macrophages that resemble those that are present in adults. Initially, haematopoiesis in the liver generates large numbers of macrophages, which are present in most organs. Population of the organs of the embryo with phagocytes is discussed elsewhere[88,89]. Furthermore, many recent studies (for further details, see the main text) indicate that, although monocytes can be precursors for the replenishment of tissue-resident macrophage populations, many of these macrophages might be derived from local proliferation in the adult rather than from recruited peripheral monocytes. When bone-marrow monocytes are released into the peripheral blood (having a Ly6C⁺ phenotype), they are thought to differentiate into a phenotypically distinct (Ly6C⁻) cell subset (for further details, see the main text).

G-CFU, granulocyte colony-forming unit; GM-CFU, granulocyte/macrophage colony-forming unit; HSC, haematopoietic stem cell; M-CFU, macrophage colony-forming unit.

known as fractalkine) or CXC-chemokine ligand 12 (CXCL12; also known as SDF1α), the receptors for which are preferentially expressed by these cells[13].

An additional monocyte subset that is defined by the expression of CD14, CD16 and CD64 (also known as FcγRI) has been reported more recently[14]. These cells seem to combine characteristics of monocytes and DCs, with high expression of CD86 and HLA-DR and high T-cell-stimulatory activity. Compared with CD14hiCD16– (classic) monocytes (which are also CD64+), these CD14+CD16+CD64+ cells have a similarly high phagocytic activity and produce similarly

Table 1 | Phenotype of the two best-characterized monocyte subsets in various mammals*

Antigen	Human CD14hi CD16– 'inflammatory' monocytes	Human CD14+CD16+ 'resident' monocytes	Mouse CCR2+ CX₃CR1low 'inflammatory' monocytes	Mouse CCR2– CX₃CR1hi 'resident' monocytes	Rat CD43low 'inflammatory' monocytes	Rat CD43hi 'resident' monocytes	Pig CD163– 'inflammatory' monocytes	Pig CD163+ 'resident' monocytes
Chemokine receptors								
CCR1	+	–	ND	ND	ND	ND	ND	ND
CCR2‡	+	–	+	–	+	–	ND	ND
CCR4	+	–	ND	ND	ND	ND	ND	ND
CCR5	–	+	ND	ND	ND	ND	ND	ND
CCR7	+	–	ND	ND	+	–	ND	ND
CXCR1	+	–	ND	ND	ND	ND	ND	ND
CXCR2	+	–	ND	ND	ND	ND	ND	ND
CXCR4	+	++	ND	ND	ND	ND	ND	ND
CX₃CR1‡	+	++	+	++	–	+	ND	ND
Other receptors								
CD4	+	+	ND	ND	+/–	++	ND	ND
CD11a	ND	ND	+	++	ND	ND	+	++
CD11b	++	++	++	++	++	++	ND	ND
CD11c‡	++	+++	–	+	–	+	+	++
CD14	+++	+	ND	ND	ND	ND	++	+
CD31	+++	+++	++	+	ND	ND	ND	ND
CD32	+++	+	ND	ND	+++	+	ND	ND
CD33	+++	+	ND	ND	ND	ND	ND	ND
CD43	ND	ND	–	+	–	+	ND	ND
CD49b	ND	ND	+	–	ND	ND	ND	ND
CD62L‡	++	–	+	–	+	–	ND	ND
CD80	ND	ND	ND	ND	ND	ND	+	++
CD86	+	++	ND	ND	ND	ND	+	++
CD115	++	++	++	++	ND	ND	ND	ND
CD116	++	++	++§	++§	ND	ND	ND	ND
CD200R	ND	ND	ND	ND	+	–	ND	ND
F4/80	ND	ND	+	+	ND	ND	ND	ND
Ly6C	ND	ND	+	–	ND	ND	ND	ND
7/4	ND	ND	+	–	ND	ND	ND	ND
MHC class II	+	++	–	–	ND	ND	+	++

*'Inflammatory' and 'resident' nomenclature is based on studies carried out in mice and extrapolated to other species. Data have been assigned arbitrary symbols that represent no expression (–), marginal expression (+/–) and increasing amounts of expression (+, ++, +++). Mouse monocyte-subset phenotypic data are derived from REFS 16,22,25,35,94,95. Rat monocyte data were kindly provided by U. Yrlid (personal communication). Other data are taken from the references cited in the main text. For a more detailed definition of the cell-surface receptor and functional heterogeneity of human monocytes, see REF. 15. ‡These receptors show good conservation of expression-pattern differences between the subsets of at least three species. §There is no available commercial reagent that recognizes the extracellular domain of mouse CD116 (also known as granulocyte/macrophage colony-stimulating-factor receptor α-chain). 7/4, an unidentified mouse antigen recognized by monoclonal antibody 7/4; CCR, CC-chemokine receptor; CD200R, CD200 receptor; CXCR, CXC-chemokine receptor; CX₃CR1, CX₃C-chemokine receptor 1; EMR1, epidermal-growth-factor-module-containing mucin-like hormone receptor 1; F4/80, monoclonal antibody that recognizes the mouse homologue of the human protein EMR1; ND, either differences between the subsets have not been determined or, in some cases, there is no clear species conservation of the antigens.

large amounts of cytokines (such as tumour-necrosis factor (TNF) and IL-6), and these phenotypes are not shared with the $CD14^+CD16^+CD64^-$ subset. However, the $CD14^+CD16^+CD64^+$ cells share with the $CD14^+CD16^+CD64^-$ subset a greater stimulatory activity in MIXED LEUKOCYTE REACTIONS than $CD14^{hi}CD16^-$ monocytes. A summary of the phenotypic properties of human monocyte subsets, such as the reduced phagocytic activity and increased stimulatory capacity in mixed leukocyte reactions has been compiled by Grage-Griebenow et al.[15] The authors speculated that, although the origins of this $CD14^+CD16^+CD64^+$ subset are not known, it could be an immunoregulatory monocyte phenotype or, possibly (because of the similar characteristics of these cells to both monocytes and DCs), an intermediate phenotype between monocytes and DCs[14].

So, although these observations from the past 20 years have provided insights into the fate and function of human monocyte subsets, the restriction of the study of monocyte heterogeneity to *in vitro* analyses of human cells and the initial failure to recognize the mouse counterparts of these monocyte subsets hampered determination of the functional roles of the monocyte subsets in a physiological context.

Monocyte heterogeneity in mice

Antigenic differentiation of two monocyte subsets in mice was first achieved after the observation that monocytes (identified in mice by their $F4/80^+CD11b^+$ phenotype) could be subdivided according to their expression of CCR2, CD62L (also known as L-selectin) and CX_3C-chemokine receptor 1 (CX_3CR1; measured by expression of green fluorescent protein (GFP) in cells from mice in which GFP had been 'knocked-in' to one of the Cx_3cr1 alleles)[16]. One monocyte subset expressed CCR2, CD62L and only moderate amounts of CX_3CR1, whereas the second did not express CCR2 or CD62L but expressed higher amounts of CX_3CR1. The $CCR2^+$ monocyte subset was, as expected, found to migrate towards the CCR2 ligand CC-chemokine ligand 2 (CCL2; also known as MCP1)[16]. The expression of CCR2 and the capacity to migrate towards CCL2 is consistent with the important role of this chemokine and its receptor in the recruitment of monocytes to inflammatory lesions, so the subset of monocytes that expresses CCR2 in mice is known as the 'inflammatory' subset[17–21]. Dan Littman's research group extended our knowledge of the phenotypic differences between the two monocyte subsets that have been described for mice and humans, and they paid particular attention to the expression pattern of chemokine receptors (TABLE 1). As can be seen from TABLE 1, the receptors that are differentially expressed by the inflammatory-monocyte subset are broadly considered to be chemokine and adhesion receptors that are involved in the recruitment of leukocytes to an inflammatory lesion. In addition, Geissmann et al.[22] identified Ly6C (which is part of the epitope of GR1) as an additional marker of $CCR2^+$ monocytes

in mice (TABLE 1). These studies indicated that $CCR2^+$ $CD62L^+CX_3CR1^{low}Ly6C^+$ mouse monocytes correspond to $CD14^{hi}CD16^-$ (classic) human monocytes, which are also $CCR2^+CX_3CR1^{low}$ and that $CCR2^-$ $CD62L^-CX_3CR1^{hi}Ly6C^-$ mouse monocytes correspond to $CD14^+CD16^+CD64^-$ human monocytes, which also express large amounts of CX_3CR1. These observations were the first to indicate that it would be possible to address the *in vivo* relevance of human monocyte heterogeneity by studying mice.

Functional characterization of mouse monocyte subsets. To distinguish functionally between the two mouse monocyte subsets, Geissmann and colleagues[22] adoptively transferred GFP-expressing monocytes from the Cx_3cr1-knock-in mice and studied cell fate and function in naive and immunologically challenged recipient mice. They found that the $CCR2^+CX_3CR1^{low}$ monocytes were short-lived after adoptive transfer and were difficult to detect in the tissues of naive recipients. However, as anticipated by their expression of CCR2 and CD62L (molecules that are known to be involved in inflammatory-cell recruitment[17–21,23,24]), these cells were rapidly recruited to sites of experimentally induced inflammation[22] (the reason for the term inflammatory monocyte). After migration into the inflamed site, the $CCR2^+$ monocytes upregulated expression of CD11c and MHC class II molecules, and some $CD11c^+MHC$ class II^+ cells were recovered from the draining lymph nodes, indicating that they might have differentiated into DCs. Experimental evidence for the ability of $CCR2^+$ monocytes to differentiate into DCs was also obtained by transferring $CCR2^+$ monocytes into MHC-class-I-deficient mice and showing that the transferred cells could prime naive $CD8^+$ T cells[22].

By contrast, the $CCR2^-CX_3CR1^{hi}$ monocyte population was found to persist for longer in animals after adoptive transfer, and it was possible to recover GFP-marked cells from the blood, spleen, lungs, liver and brain of recipients for several days after transfer. Some of the donor cells that were recovered from the spleen had acquired a DC-like phenotype (that is, $CD11c^+MHC$ class II^+) following transfer. Similar to their human counterparts[9,10], both mouse monocyte subsets have been reported to differentiate into DCs *in vitro* when cultured with GM-CSF and IL-4 (REF. 22). However, the observation that the $CCR2^-$ monocytes can enter tissues and acquire a DC-like phenotype under steady-state conditions is consistent with the finding that their human counterparts ($CD14^+CD16^+$ monocytes) preferentially differentiate into DCs in the *in vitro* transendothelial-migration model[12].

It was not clear at which point the division in lineage between the two monocyte subsets occurred. Sunderkotter and colleagues[25] attempted to address this question with a series of experiments based on *in vivo* depletion of all monocytes with CLODRONATE-LOADED LIPOSOMES and *in vivo* labelling with DIL-LABELLED LIPOSOSOMES, followed by study of the repopulation kinetics of both monocyte subsets. After depletion, the first monocytes

MIXED LEUKOCYTE REACTION
A tissue-culture technique for testing T-cell reactivity. The proliferation of one population of T cells, induced by exposure to inactivated MHC-mismatched stimulator cells, is determined by measuring the incorporation of ${}^3H$-thymidine into the DNA of dividing cells.

CLODRONATE-LOADED LIPOSOME
A liposome that contains the drug dichloromethylene diphosphonate. These liposomes are ingested by macrophages, resulting in cell death.

DIL-LABELLED LIPOSOSOME
A liposome that is labelled with the fluorochrome Dil (1,1′-dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine perchlorate). These liposomes are internalized by phagocytic cells, rendering the cells fluorescent.
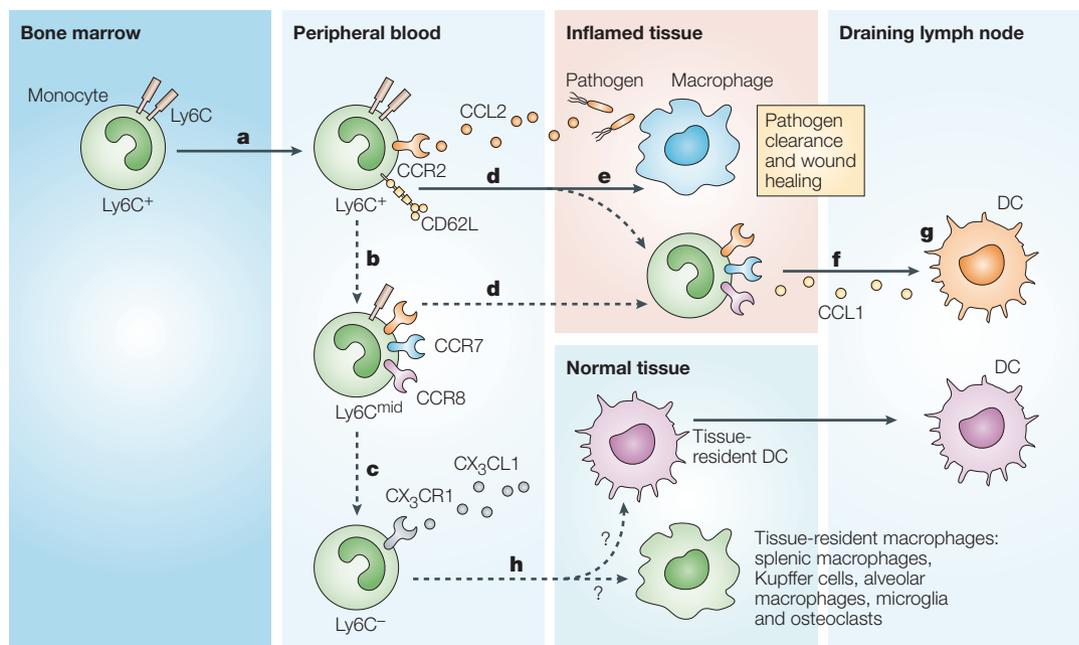
Figure 1 | **Development and function of monocyte subsets in mice.** Ly6C^{+} bone-marrow monocytes are released into the peripheral blood (**a**) and are thought to adopt a Ly6C[mid] phenotype (**b**), which is associated with selective expression of CC-chemokine receptor 7 (CCR7) and CCR8 and retention of CCR2, before (under steady-state conditions) they form CCR2^{–}Ly6C^{–} monocytes (**c**) that are characterized by high CX$_3$C-chemokine receptor 1 (CX$_3$CR1) expression[25,28]. Both Ly6C^{+} and Ly6C[mid] monocytes respond to pro-inflammatory cues, such as the CCR2 ligand CC-chemokine ligand 2 (CCL2), and are recruited to inflammatory lesions[22,28] (**d**). Most 'inflammatory' monocytes are thought to differentiate into macrophages, which are important for clearance of pathogens and for the resolution of inflammation (**e**). Some monocytes emigrate from the tissues to the draining lymph nodes, a process that uses CCR7 and CCR8 receptor–ligand interactions[28] (**f**). CCR7^{+}CCR8^{+} monocytes that are present in the tissue must be recruited directly from the peripheral blood to the inflammatory site or must differentiate from Ly6C^{+} monocytes *in situ* (or must arise from a combination of both of these mechanisms). The expression of CCR7 and CCR8 by these cells makes them uniquely disposed to emigrate into the lymphatic vessels. In the draining lymph nodes, these monocytes acquire dendritic cell (DC)-like characteristics (**g**), which they do not obtain if they are retained in the tissue by selective chemokine-receptor deficiency[28]. In the absence of inflammation, CX$_3$CR1^{hi}Ly6C^{–} monocytes enter the tissues and replenish the tissue-resident macrophage and DC populations[22] (**h**). Solid arrows represent pathways that are supported by established data, whereas dashed arrows represent pathways that are indicated from a compilation of more recent data and speculation. CX$_3$CL1, CX$_3$C-chemokine ligand 1.

to reappear in the circulation were the CCR2^{+} (inflammatory) subset. The phenotype of these cells resembles that of monocytes found in the bone marrow[26], and their appearance at this stage is consistent with the hypothesis that these monocytes precede the CCR2^{–} subset in the developmental pathway. Unfortunately, the adoptive transfer of purified CCR2^{+} monocytes did not formally confirm this hypothesis, although this was attributed to inappropriate manipulation of the monocyte subset during purification, because the transferred cells could not be recovered from the circulation[25]. In addition, Sunderkotter and colleagues[25] identified a rare population of monocytes that were characterized by intermediate expression of Ly6C. Assuming that the proposed pathway of CCR2^{–}Ly6C^{–} monocyte derivation from CCR2^{+}Ly6C^{+} monocytes is true, this population might be an intermediate phenotypic state between the two subsets (FIG. 1).

It had already been shown that inflammatory monocytes recruited to the skin after injection of fluorescent latex beads could ingest the beads, and although most of these cells remained in the tissue as macrophages, others could migrate to the T-cell area

of the draining lymph node and acquire a DC-like phenotype (that is, CD11c^{+}MHC class II^{+})[27]. While investigating the possible role of CCR2 and CX$_3$CR1 in regulating the migration of latex-bead-carrying monocytes from the skin to the draining lymph nodes, proliferation of Ly6C[mid] monocytes, associated with a relative reduction in the size of the Ly6C^{+} monocyte subset, was observed in the peripheral blood of CCR2-deficient mice[28]. Genetic deficiency in CCR2 or CX$_3$CR1 did not reduce the migration of the latex-bead-carrying DC-like cells to the lymph nodes; however, an unexpected increase in migrating latex-bead-carrying cells was observed in CCR2-deficient animals. This was reflected by an increase in the proportion of latex-bead-carrying Ly6C[mid] cells in the skin of CCR2-deficient animals after the injection of latex beads, indicating that the recruitment of these cells to the skin did not depend on CCR2 in this model. The authors speculated that the increase in the proportion of these cells in the peripheral blood and inflamed skin of CCR2-deficient mice and in the number of monocyte-derived DC-like cells in the draining lymph node might be linked and that the

Ly6C^{mid} monocytes could be a subset of monocytes that is predisposed to differentiate into DCs[28]. Ly6C^{mid} monocytes have also been suggested to resemble Ly6C$^-$ monocytes rather than Ly6C$^+$ monocytes in terms of their capacity to stimulate allogeneic cells[28]. Reverse-transcription-PCR analysis of the three subsets indicated that Ly6C^{mid} monocytes express higher amounts of the mRNAs that encode CCR7 and CCR8 than do the other two monocyte subsets but similar amounts of mRNA that encodes CCR2 to that of Ly6C$^+$ monocytes. Furthermore, analysis of mice that are deficient in CCR8 or the ligands of CCR7 showed a reduction in the number of latex-bead-carrying cells with DC-like phenotype that migrated to the draining lymph node, supporting a role for these molecules in this process. Parallel *in vitro* studies with human monocytes showed that blockade of CCR8 considerably reduced the reverse transmigration of human monocytes without affecting the initial migration across the endothelial layer[28].

The results of these studies of the heterogeneity of mouse (and human) monocytes are summarized in FIG. 1. Briefly, by extrapolation from the current data, a picture is emerging in which bone-marrow-derived monocytes (with the phenotype CCR2$^+$CX$_3$CR1^{low}Ly6C$^+$) are released into the circulation and, in the absence of inflammation, alter their functional and phenotypic characteristics, passing through an intermediate phenotype (CCR2$^+$CCR7$^+$CCR8$^+$Ly6C^{mid}). Both the bone-marrow-derived monocytes and the monocytes of intermediate phenotype can respond to pro-inflammatory cues, migrate to inflamed tissues and differentiate into macrophages and DCs. The monocytes with an intermediate phenotype might be particularly predisposed to migrate to the draining lymph nodes and differentiate into DCs. In the absence of inflammation, a switch in monocyte phenotype, the mechanism of regulation of which is unknown, generates monocytes that are postulated to enter the tissues and replenish the tissue-resident macrophage and DC populations; these are known as the 'resident' monocyte population (which has the phenotype CCR2$^-$CX$_3$CR1^{hi}Ly6C$^-$). The similarities between human and mouse monocyte subsets indicate that this is a conserved system, and further analysis of the mouse system and other mammalian systems will be helpful for understanding human monocyte biology.

Monocyte heterogeneity in other mammals
The study of monocyte heterogeneity in mice, in the context of transgenic and gene-knockout technology, is a powerful tool to investigate the mechanisms of monocyte function and fate, but it also has limitations. Constraints in working with mice, such as the limited availability of cells and the limited accessibility of physiological systems to physical manipulation and/or intervention, merit the extension of these studies to larger mammalian models. In this context, heterogeneity of monocytes has been reported in rats and in pigs, with notable parallels evident between these species and the observations that have been made for mice and humans (TABLE 1).

Rats. Monocyte heterogeneity was first shown in rats by using CD43 as a differential marker[29]. In rats, CD43^{hi} monocytes express higher amounts of CD4 than do CD43^{low} monocytes, and the CCR2$^-$CX$_3$CR1^{hi} monocyte subset in mice has also been shown to express CD43 (REF. 25). Recently, rat monocytes have been further characterized antigenically and functionally (U. Yrlid, personal communication). CD43^{low} monocytes express higher amounts of CCR2, CCR7, CD32 and CD62L than do CD43^{hi} monocytes, and they migrate to the PERITONEUM during experimental STERILE PERITONITIS, confirming their similarity to CCR2$^+$Ly6C$^+$ (inflammatory) mouse monocytes. By contrast, CD43^{hi} monocytes express higher amounts of CX$_3$CR1 and CD11c than do CD43^{low} monocytes, so this population is analogous to the Ly6C$^-$ (resident) monocyte population in mice.

The use of rats has facilitated two important experiments that were technically more demanding in mice. First, when adoptively transferred, purified CCR2$^+$CX$_3$CR1^{low} rat monocytes intravenously acquired the phenotype of CCR2$^-$CX$_3$CR1^{hi} monocytes, strongly supporting the lineage development that was previously proposed to occur in mice[25]. Second, a model of mesenteric lymphadenectomy and thoracic-duct cannulation, in which afferent and EFFERENT LYMPHATIC VESSELS fuse, allowed the collection of migrating DCs. Following adoptive transfer of CCR2$^-$CX$_3$CR1^{hi} monocytes, some donor cells acquired the phenotype of intestinal-lymph DCs without the requirement for additional stimulation, indicating that the transferred resident-monocyte population could differentiate into DCs under steady-state conditions. Importantly, both the conversion of CCR2$^+$CX$_3$CR1^{low} monocytes into CCR2$^-$CX$_3$CR1^{hi} monocytes and the differentiation of CCR2$^-$CX$_3$CR1^{hi} monocytes into cells with the phenotype of intestinal-lymph DCs occurred without cell division.

Pigs. Monocyte heterogeneity has also been described in pigs[30]. CD163$^-$ monocytes express higher amounts of CD14 than do their CD163$^+$ counterparts, which in turn express higher levels of MHC class II molecules, several adhesion molecules (such as CD11a and CD11c) and the co-stimulatory molecules CD80 and CD86; CD163$^+$ monocytes also have a higher allostimulatory activity and a greater ability to present soluble antigen to T cells than do CD163$^-$ monocytes[30,31]. Both subsets of monocyte can also differentiate into DCs when cultured in the presence of GM-CSF and IL-4 (REF. 32). The greater allostimulatory activity and co-stimulatory molecule expression by the CD163$^+$ monocyte subset indicates that these cells might correspond to Ly6C$^-$ (resident) monocytes in mice.

Origins of macrophages and DCs
Tissue macrophages have a broad role in the maintenance of tissue homeostasis, through the clearance of senescent cells and the remodelling and repair of tissues after inflammation[33,34]. They are generally considered to be derived from circulating monocytes and show a

PERITONEUM
The membrane that lines the abdominal cavity.

STERILE PERITONITIS
Inflammation of the peritoneum that is induced by sterile injection of an irritant, such as thioglycollate broth. This results in the sequential recruitment of granulocytes, monocytes and lymphocytes. It is widely used to study acute inflammation.

EFFERENT LYMPHATIC VESSEL
A vessel that carries lymph out of a lymph node.

high degree of heterogeneity, which has largely been uncovered through studies with monoclonal antibodies[35–37]. The heterogeneity reflects the specialization of function that is adopted by macrophages in different anatomical locations, including the following: the ability of osteoclasts to remodel bone[38]; the high expression of PATTERN-RECOGNITION RECEPTORS and SCAVENGER RECEPTORS by alveolar macrophages[39–41], which are involved in clearing microorganisms, viruses and environmental particles in the lungs; and the positioning of thymic macrophages[42] and TINGIBLE-BODY MACROPHAGES[43] in the germinal centre for clearance of apoptotic lymphocytes that are generated during the development of an acquired immune response. The gut is one of the richest sources of macrophages in the body, and isolation of macrophages from the LAMINA PROPRIA has highlighted a unique macrophage phenotype that is characterized by high phagocytic and bactericidal activity but weak production of pro-inflammatory cytokines. This phenotype can be induced in peripheral-blood-derived macrophages by intestinal stromal-cell products, indicating that the tissue microenvironment can markedly influence the phenotype of tissue-resident macrophages[44]. In addition to macrophage heterogeneity in different organs, macrophage heterogeneity can be observed in a single organ, and the mouse spleen, which is discussed briefly in this section, is a particularly good example of this.

Since the recent unravelling of monocyte heterogeneity, immediate attention has focused on studies of monocyte fate, mainly regarding the question of the potential of monocytes to form DCs *in vitro* and *in vivo* and the possible effect of this on the immune response. By contrast, relatively little attention has been given to the perhaps more challenging question of the role of monocyte heterogeneity in the generation of macrophage populations in mice and the nature of the circulating precursors of macrophage populations. For example, it is still not clear whether tissue macrophages are derived from particular lineage-committed precursors or whether they are derived randomly from the monocyte pool. In addition (and particularly in the case of inflammation-elicited macrophages), after cells have 'differentiated' in a microenvironment, are the cells then terminally differentiated, or are they functionally flexible and able to alter their phenotype in response to changes in their location? As previously mentioned, most macrophages in the tissues of an adult are considered to be derived from circulating monocytes, which constitutively replenish tissue-resident macrophage populations. However, studies of the origins of many tissue-resident macrophage populations have shown that local proliferation has a considerable role in the renewal and maintenance of many macrophage types (particularly under steady-state conditions), with the recruitment of circulating precursors having little, if any, role in this process in some cases. But inflammatory insults, such as trauma or infection, can lead to an increased dependence on the recruitment of blood-borne precursors to aid repopulation of the tissue-resident populations in many of these

inflamed tissues. Van Furth et al.[45] summarized the results of the initial studies in this area, although these questions have been re-investigated more recently, leading to the need to modify some of the older ideas about the cellular origins of macrophages.

A good understanding of the origins of these cells, as well as the timing and context of their recruitment, will be essential not only to understand how tissues recover from exposure to pro-inflammatory stimuli but also to determine how these cells help to restore the status quo ante, which is important for diseases in which a loss of tissue homeostasis might result from dysfunction of the tissue-resident macrophage population(s). Examples in which the origins of tissue-resident macrophage populations have been investigated are discussed in this section and are illustrated in FIG. 2. We have concentrated on the adult because of the substantial differences in the development of phagocytes in the adult and the embryo, which are briefly summarized in BOX 1. One aspect that is clear in many of these examples is that the nature of the circulating precursor that is involved in the renewal of tissue-resident populations remains poorly defined. Studies such as those that were outlined in previous sections for determining monocyte fate during inflammation might be useful for studying the potential involvement of individual monocyte subsets in the renewal of tissue-resident cells.

Langerhans cells. LANGERHANS CELLS populate the epidermis during ontogeny and were originally thought to be constantly replenished by bone-marrow-derived cells. However, it has recently been shown that Langerhans cells are self-renewing[46]. Using BONE-MARROW CHIMERAS made with congenic cells (which were followed for 18 months) and PARABIOTIC MICE (which were followed for 6 months), it was shown that Langerhans cells were solely of recipient origin for the duration of these studies[46]. When Langerhans cells were depleted by irradiation with ultraviolet light, they were replenished from circulating bone-marrow-derived precursors in a CCR2-dependent manner, indicating that a circulating CCR2+ precursor is only utilized when the system is under stress[46]. Similar observations have been made in a case of human hand-allograft transplantation. At 4.5 years after transplantation, the Langerhans cells were solely of donor origin, supporting the idea that replacement of Langerhans cells by recipient bone-marrow precursors is rare under steady-state conditions[47]. Whether the CCR2-dependent recruitment of the Langerhans-cell precursor involves recruitment of an inflammatory monocyte or a lineage-committed precursor is unclear.

Osteoclasts. Osteoclast precursors are found in the GRANULOCYTE/MACROPHAGE COLONY-FORMING UNIT (GM-CFU PRECURSOR population and can be derived from unfractionated, mature monocytes from peripheral blood[48–50]. Osteoclast precursors express the receptor for macrophage colony-stimulating factor (M-CSF; also known as CSF1) and depend on it for their development.

OSTEOPETROTIC MICE (*Mcsf op/Mcsf op* mice) have a defect in osteoclast development[51], and this has been shown to result from a naturally occurring mutation in the gene that encodes M-CSF[52], underlining the importance of M-CSF in osteoclast development. Mice with a disrupted *Rankl* (receptor-activator-of-nuclear-factor-κB ligand) gene also have osteopetrosis[53], and this is consistent with the proposed role of RANKL in osteoclast development[54]. Culture of unfractionated peripheral-blood monocytes with M-CSF and RANKL is sufficient to induce their differentiation

into osteoclasts, and it has been assumed that osteoclast precursors are monocytes, although this has not been shown *in vivo*.

Alveolar macrophages. Alveolar macrophages have been reported to be derived both from precursors in peripheral blood and from local proliferation of precursors. Early experiments in which radiosensitive precursors of monocytes in mouse bone marrow were transiently depleted with [89]Sr — resulting in the loss of peripheral-blood monocytes without affecting the
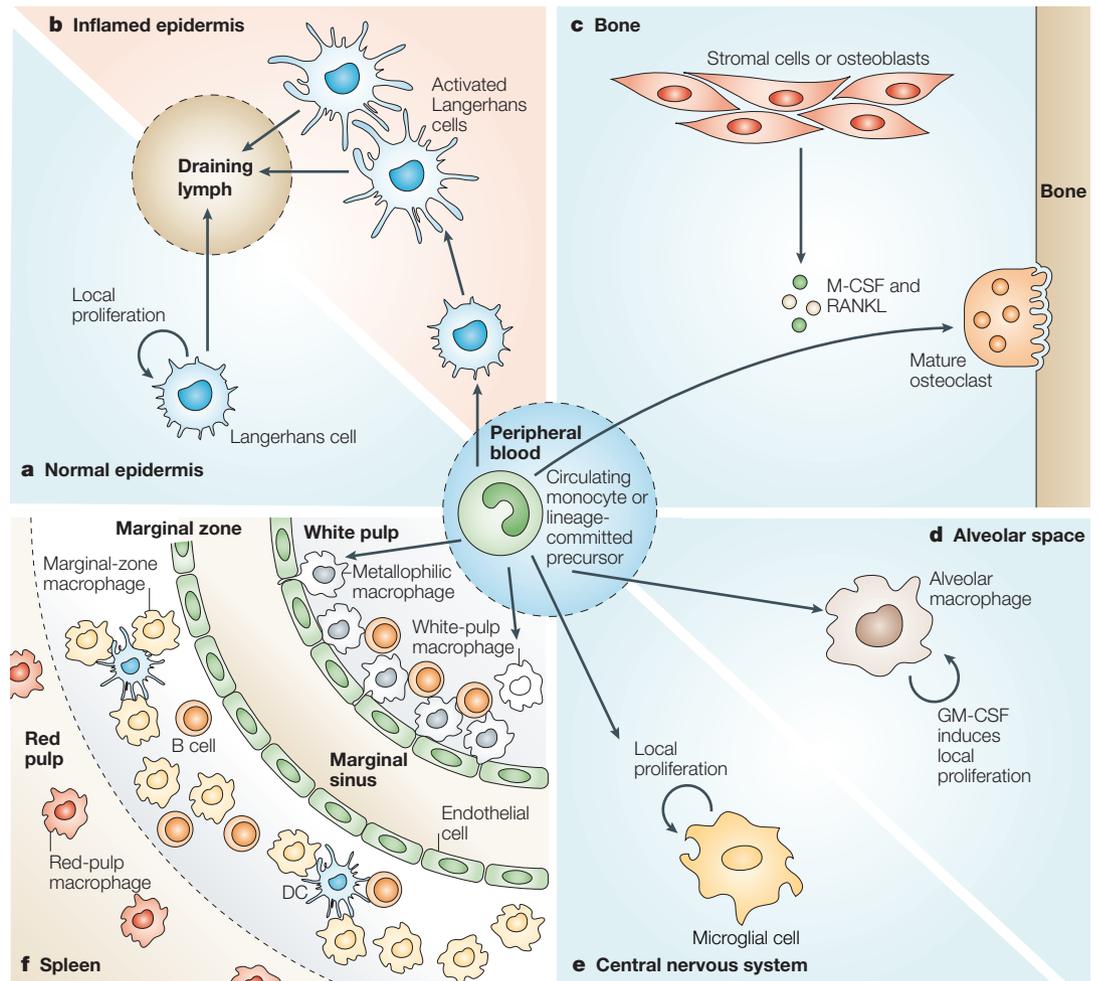

Figure 2 | **Origins of mature cells in the periphery in adult mice.** The importance of circulating precursors in the repopulation of tissue-resident macrophage and dendritic cell (DC) populations is not fully understood. Furthermore, the nature of the precursor — that is, whether it is a peripheral-blood monocyte or a circulating lineage-committed precursor — is unclear. In the case of Langerhans cells, local proliferation in the normal epidermis (**a**) is adequate to maintain Langerhans-cell numbers without the requirement for replenishment by circulating precursors; however, after inflammation (**b**), the CC-chemokine receptor 2 (CCR2)-dependent recruitment of precursors to the epidermis allows repopulation of the tissue-resident populations. In bone (**c**), it is thought that circulating precursors are recruited to the bone surface, where — under the influence of macrophage colony-stimulating factor (M-CSF) and receptor-activator-of-nuclear-factor-κB ligand (RANKL) — they differentiate into mature osteoclasts, which are multinucleate and resorb bone. In the lungs (**d**), alveolar macrophages can be sustained for long periods by local proliferation; however, bone-marrow-transplantation experiments show that blood-borne precursors can repopulate and divide in the lungs. In the central nervous system (**e**), microglia are similarly maintained by local proliferation after an initial embryonic phase that populates the central nervous system, but peripheral-blood monocytes also contribute to this pool. In the spleen (**f**), there is marked heterogeneity of macrophages, with red-pulp, white-pulp, marginal-zone and metallophilic macrophages occupying specific anatomical niches. A simplified schema is shown, which also indicates B cells, DCs and endothelial cells. At present, splenic macrophages are thought to be replenished by a mixture of emigration from the circulating monocyte pool and local proliferation. GM-CSF, granulocyte/macrophage colony-stimulating factor.

OSTEOPETROTIC MICE (*Mcsf op/Mcsf op* mice). An inbred strain of mice that suffers from osteopetrosis (stony bones) as a result of deficient function of osteoclasts. The defect has been localized to the gene that encodes macrophage colony-stimulating factor (M-CSF; also known as CSF1).

number of alveolar macrophages — supported a role for local proliferation; however, these experiments allowed only short-term studies[55]. Further support for local proliferation of precursors being the main source of alveolar macrophages under normal conditions came from radiation-chimera studies in mice: if the lungs were protected from radiation, then most alveolar macrophages were of recipient origin almost 1 year after treatment[56]. However, after whole-body irradiation and transfer of GFP-labelled bone marrow, host alveolar macrophages were replaced with cells of donor origin over a long period, indicating that alveolar macrophages can be replenished from the bone marrow[57]. Studies in humans who have received allogeneic bone-marrow transplants support this argument and indicate that this replenishment occurs by recruitment of precursors, followed by proliferation of these cells *in situ*[58,59]. The importance of GM-CSF in lung physiology indicates that this growth factor has a crucial role in the maintenance of alveolar-macrophage populations, the maturation and/or activity of which are impaired in GM-CSF-deficient mice, which suffer from an ALVEOLAR-PROTEINOSIS-like disease[60,61]. Although it is assumed that a monocyte subset might be the precursor of alveolar macrophages, this has not been directly tested.

Macrophages in the central nervous system. The central nervous system (CNS) contains various macrophage subsets, including MICROGLIA, PERIVASCULAR MACROPHAGES, MENINGEAL MACROPHAGES and CHOROID-PLEXUS MACROPHAGES. Meningeal macrophages are thought to be rapidly replaced by cells of bone-marrow origin, whereas the replacement of perivascular and choroid-plexus macrophages is slower, which indicates that replacement of the individual populations might occur through different mechanisms or might rely on a shared mechanism to differing extents[62]. For example, microglial cells seem to persist much longer than do other macrophages in the CNS, and their origins have been investigated by a combination of *in vivo* labelling of dividing cells and bone-marrow-transplantation experiments. Microglial cells can proliferate *in situ*, and this might be one of the main sources of microglia in adults; however, bone-marrow-derived cells can enter the CNS across the blood–brain barrier and populate the microglial-cell compartment[63,64]. Monocytes are assumed to be capable of entering the CNS and differentiating into microglia.

Splenic macrophages. Aspects of the heterogeneity of splenic macrophages have been summarized elsewhere[65,66], but we briefly highlight, in FIG. 2, the discrete anatomical localization of the different macrophage populations. Macrophages in the white pulp include tingible-body macrophages. MARGINAL-ZONE MACROPHAGES are found adjacent to the marginal sinus (through which the circulation passes), and they express pattern-recognition receptors and scavenger receptors, which aid in the clearance of blood-borne pathogens[67–69]. METALLOPHILIC MACROPHAGES are found adjacent to the white pulp and marginal sinus and

can sample the circulation[70]; although their function is unknown, they might be important during viral infections[71] and other infections. Several studies have attempted to address the question of the origins of these different macrophage populations, both under steady-state conditions and after experimental depletion of the tissue-resident cells[72–75], which is important because (as mentioned previously) repopulation after experimental insult might differ from repopulation that occurs in the steady state. It seems that local proliferation does occur under steady-state conditions, at least with respect to some macrophage subsets, such as white-pulp macrophages and metallophilic macrophages. In addition, it seems that circulating precursors also contribute, but the nature of these precursors is unknown. Unlike in humans, the spleen of adult mice is considered to be a haematopoietic organ, and it differs structurally from the human spleen. This might mean that the mouse is not an ideal model for the study of cellular origins for comparison with humans. Studies with the M-CSF-deficient mice (*Mcsf*[op]/*Mcsf*[op] mice) highlight the different dependencies of certain macrophage subsets on M-CSF for their survival, because these mice lack metallophilic macrophages while maintaining reasonable numbers of most other splenic macrophage subsets[76–78].

Kupffer cells. Kupffer cells are an important component of the mononuclear-phagocyte system that is present in the liver. The origin of Kupffer cells has been speculated to involve two mechanisms: replenishment by local proliferation, and recruitment of circulating precursors. Twelve hours after administration of ^{3}H-thymidine, ~1.5% of Kupffer cells incorporate ^{3}H-thymidine, giving an indication of the low number of cells that are proliferating under steady-state conditions in the adult[79]. The proportion of Kupffer cells that incorporate ^{3}H-thymidine increases if mice are exposed to whole-body irradiation; however, shielding of the hind legs of the animals during whole-body irradiation indicates that the increase in ^{3}H-thymidine incorporation depends on bone-marrow precursors[79]. In mouse bone-marrow transplants, donor-derived cells rapidly populate the liver with Kupffer cells (within 3 weeks), and donor Kupffer cells in liver transplants are replaced with similar kinetics[80]. However, experiments in rats indicate that Kupffer cells might be long-lived[81], and temporary depletion of peripheral-blood monocytes with ^{89}Sr had little effect on Kupffer-cell numbers[80]. So, Kupffer cells, similar to many other macrophage populations, can be replenished by distinct mechanisms, and it is probable that the mechanisms used are affected by inflammation and other factors.

Inflammatory-monocyte-derived macrophages. It has long been recognized that inflammatory monocytes (now defined as CCR2^{+}Ly6C^{+} monocytes) are recruited and differentiate into macrophages at the site of the inflammatory lesion[4]. A series of *in vitro* experiments has led to macrophages often being ascribed
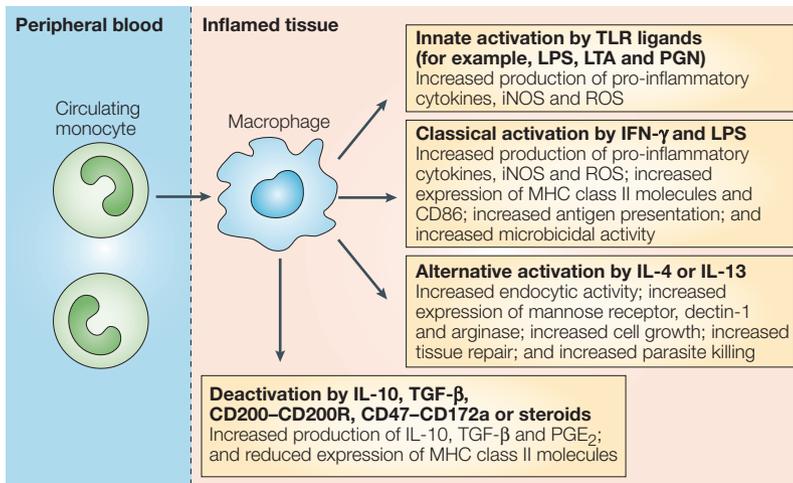
Figure 3 | **Macrophage heterogeneity during inflammation.** In inflamed tissues, the precursors of the elicited macrophages — that is, 'inflammatory' peripheral-blood monocytes — are now better understood than was previously the case but are still not completely understood. However, the ability of these elicited macrophages to acquire distinct phenotypes and physiological activities has been observed *in vitro*. For example, when stimulated with interferon-γ (IFN-γ), macrophages show high microbicidal activity and produce reactive oxygen species (ROS). By contrast, when cultured with interleukin-4 (IL-4), IL-10, IL-13 or transforming growth factor-β (TGF-β), a phenotype is generated that promotes tissue repair and suppresses inflammation. Whether these phenotypes are distinct or whether they indicate a continuum of physiological responsiveness remains unclear. CD200R, CD200 receptor; iNOS, inducible nitric-oxide synthase; LPS, lipopolysaccharide; LTA, lipoteichoic acid; PGE$_2$, prostaglandin E$_2$; PGN, peptidoglycan; TLR, Toll-like receptor; TNF, tumour-necrosis factor.

activation states[66,82–84] (FIG. 3). These include the following: classical activation, which can be induced by *in vitro* culture of macrophages with interferon-γ and lipopolysaccharide (which induces TNF production) and is associated with high microbicidal activity, pro-inflammatory cytokine production and cellular immunity; alternative activation, which results from culture in IL-4 or IL-13 and is associated with tissue repair and humoral immunity; innate activation, which is mediated in culture by ligation of receptors such as Toll-like receptors (most of which are expressed by cells of the monocyte–macrophage lineage[85]) and is associated with microbicidal activity and pro-inflammatory cytokine production; deactivation, which is induced by culture in the presence of cytokines such as IL-10 or transforming growth factor-β, or by ligation of inhibitory receptors such as CD200 receptor or CD172a, and is associated with anti-inflammatory cytokine production and reduced MHC class II expression. Despite these classifications, the extent of plasticity in this system is unclear: that is, it is unclear whether macrophage fate is determined once or whether it is constantly malleable, and it is also unclear whether distinct 'activation states' exist *in vivo* or whether macrophages, instead, show a broad range of phenotypes. It is probable that, in most situations, an inflammatory environment leads to the exposure of macrophages to multiple stimuli, with complex phenotypic consequences. The recent advances in our ability to follow the fate of the monocyte lineage *in vivo* (such as the identification and adoptive transfer of monocyte subsets),

in conjunction with single-cell analysis, could be applied to these fundamental questions of macrophage behaviour and fate during inflammation. It would be particularly useful to study situations such as granuloma formation and parasitic infections, in which polarization of macrophage activation has been implicated in disease pathology and in which a strong bias in the nature of the immune response has been observed *in vivo*.

Concluding remarks

The study of animal models has led to a rapid advance in our understanding of the functional consequences of monocyte heterogeneity, and this knowledge can be directly applied to the study of human biology. The ever-expanding pool of genetically manipulated models will accelerate the progression of knowledge in this field. The initial similarities between the species that have been studied reinforce that there is a conserved commonality in the function of these systems, thereby validating their use. Further study will be important to understand how monocytes are recruited to particular inflammatory sites and what determines their differentiation into DCs or macrophages, cell populations that regulate acquired immune responses and/or carry out surveillance of normal and abnormal tissues.

Until now, the characterization of monocyte heterogeneity has largely been led by hypothesis-driven investigations and has been restricted to candidate approaches, such as the extensive use of chemokine-receptor-deficient mice in experimental models of inflammation. Advances in the isolation of monocyte subsets, as well as the increasing availability of improved DNA-microarray and proteomic analysis, should provide important and more objective insights into the extent of the cellular diversification of function that is present in the monocyte lineage.

Another important issue will be how this current research translates into the advancement of medical knowledge. Techniques for imaging macrophage recruitment and accumulation in humans (for example, in patients with atherosclerosis) have involved transfer of labelled autologous monocytes or *in vivo* uptake of fluorodeoxyglucose by metabolically active cells, which (although not specific) correlates with macrophage numbers and plaque stability[86]. More selective strategies have been adopted in animal models, such as the *in vivo* administration of radiolabelled chemokines, which would be anticipated to bind selectively to certain subsets of monocytes (among other cells)[86].

Perhaps a more challenging aspect of these studies is to determine the contribution of monocytes, or alternative, lineage-committed precursors, to the macrophage and DC populations of the body. It is clear that, for the development of appropriate therapeutic interventions, a better understanding of the natural cell lineage of monocytes is required, because long-term modulation of monocyte activities could have implications for the maintenance of tissue populations of macrophages and DCs and therefore could affect homeostasis, immunity and tolerance.

1. Volkman, A. & Gowans, J. L. The origin of macrophages from human bone marrow in the rat. *Br. J. Exp. Pathol.* **46**, 62–70 (1965).
2. van Furth, R. & Cohn, Z. A. The origin and kinetics of mononuclear phagocytes. *J. Exp. Med.* **128**, 415–435 (1968).
3. Ebert, R. H. & Florey, H. W. The extravascular development of the monocyte observed *in vivo. Brit. J. Exp. Pathol.* **20**, 342–356 (1939).
4. van Furth, R., Diesselhoff-den Dulk, M. M. & Mattie, H. Quantitative study on the production and kinetics of mononuclear phagocytes during an acute inflammatory reaction. *J. Exp. Med.* **138**, 1314–1330 (1973).
5. Imhof, B. A. & Aurrand-Lions, M. Adhesion mechanisms regulating the migration of monocytes. *Nature Rev. Immunol.* **4**, 432–444 (2004).
6. Passlick, B., Flieger, D. & Ziegler-Heitbrock, H. W. Identification and characterization of a novel monocyte subpopulation in human peripheral blood. *Blood* **74**, 2527–2534 (1989).
   Characterization of antigenically distinct monocyte subsets in human peripheral blood.
7. Ziegler-Heitbrock, H. W. *et al*. The novel subset of CD14+/CD16+ blood monocytes exhibits features of tissue macrophages. *Eur. J. Immunol.* **23**, 2053–2058 (1993).
8. Weber, C. *et al*. Differential chemokine receptor expression and function in human monocyte subpopulations. *J. Leukoc. Biol.* **67**, 699–704 (2000).
9. Sallusto, F. & Lanzavecchia, A. Efficient presentation of soluble antigen by cultured human dendritic cells is maintained by granulocyte/macrophage colony-stimulating factor plus interleukin 4 and downregulated by tumor necrosis factor α. *J. Exp. Med.* **179**, 1109–1118 (1994).
10. Sanchez-Torres, C., Garcia-Romo, G. S., Cornejo-Cortes, M. A., Rivas-Carvalho, A. & Sanchez-Schmitz, G. CD16+ and CD16– human blood monocyte subsets differentiate *in vitro* to dendritic cells with different abilities to stimulate CD4+ T cells. *Int. Immunol.* **13**, 1571–1581 (2001).
11. Randolph, G. J., Beaulieu, S., Lebecque, S., Steinman, R. M. & Muller, W. A. Differentiation of monocytes into dendritic cells in a model of transendothelial trafficking. *Science* **282**, 480–483 (1998).
12. Randolph, G. J., Sanchez-Schmitz, G., Liebman, R. M. & Schakel, K. The CD16+ (FcγRIII+) subset of human monocytes preferentially becomes migratory dendritic cells in a model tissue setting. *J. Exp. Med.* **196**, 517–527 (2002).
    Identification that the CD16+ human peripheral-blood monocyte subset preferentially differentiates into DCs after endothelial reverse transmigration.
13. Ancuta, P. *et al*. Fractalkine preferentially mediates arrest and migration of CD16+ monocytes. *J. Exp. Med.* **197**, 1701–1707 (2003).
14. Grage-Griebenow, E. *et al*. Identification of a novel dendritic cell-like subset of CD64+/CD16+ blood monocytes. *Eur. J. Immunol.* **31**, 48–56 (2001).
15. Grage-Griebenow, E., Flad, H. D. & Ernst, M. Heterogeneity of human peripheral blood monocyte subsets. *J. Leukoc. Biol.* **69**, 11–20 (2001).
16. Palframan, R. T. *et al*. Inflammatory chemokine transport and presentation in HEV: a remote control mechanism for monocyte recruitment to lymph nodes in inflamed tissues. *J. Exp. Med.* **194**, 1361–1373 (2001).
17. Kuziel, W. A. *et al*. Severe reduction in leukocyte adhesion and monocyte extravasation in mice deficient in CC chemokine receptor 2. *Proc. Natl Acad. Sci. USA* **94**, 12053–12058 (1997).
18. Kurihara, T., Warr, G., Loy, J. & Bravo, R. Defects in macrophage recruitment and host defense in mice lacking the CCR2 chemokine receptor. *J. Exp. Med.* **186**, 1757–1762 (1997).
19. Lu, B. *et al*. Abnormalities in monocyte recruitment and cytokine expression in monocyte chemoattractant protein 1-deficient mice. *J. Exp. Med.* **187**, 601–608 (1998).
20. Gu, L. *et al*. Absence of monocyte chemoattractant protein-1 reduces atherosclerosis in low density lipoprotein receptor-deficient mice. *Mol. Cell* **2**, 275–281 (1998).
21. Serbina, N. V., Salazar-Mather, T. P., Biron, C. A., Kuziel, W. A. & Pamer, E. G. TNF/iNOS-producing dendritic cells mediate innate immune defense against bacterial infection. *Immunity* **19**, 59–70 (2003).
22. Geissmann, F., Jung, S. & Littman, D. R. Blood monocytes consist of two principal subsets with distinct migratory properties. *Immunity* **19**, 71–82 (2003).
    The first modern functional characterization and cell-fate studies of mouse peripheral-blood monocyte subsets during steady-state conditions and inflammation.

23. Tedder, T. F., Steeber, D. A. & Pizcueta, P. L-selectin-deficient mice have impaired leukocyte recruitment into inflammatory sites. *J. Exp. Med.* **181**, 2259–2264 (1995).
24. Rosen, S. D. Ligands for L-selectin: homing, inflammation, and beyond. *Annu. Rev. Immunol.* **22**, 129–156 (2004).
25. Sunderkotter, C. *et al*. Subpopulations of mouse blood monocytes differ in maturation stage and inflammatory response. *J. Immunol.* **172**, 4410–4417 (2004).
26. de Bruijn, M. F. *et al*. Distinct mouse bone marrow macrophage precursors identified by differential expression of ER-MP12 and ER-MP20 antigens. *Eur. J. Immunol.* **24**, 2279–2284 (1994).
27. Randolph, G. J., Inaba, K., Robbiani, D. F., Steinman, R. M. & Muller, W. A. Differentiation of phagocytic monocytes into lymph node dendritic cells *in vivo. Immunity* **11**, 753–761 (1999).
28. Qu, C. *et al*. Role of CCR8 and other chemokine pathways in the migration of monocyte-derived dendritic cells to lymph nodes. *J. Exp. Med.* **200**, 1231–1241 (2004).
    Suggestion that a third, intermediate monocyte phenotype in mouse peripheral blood might be an inflammatory monocyte that is prone to differentiate into DCs.
29. Ahuja, V., Miller, S. E. & Howell, D. N. Identification of two subpopulations of rat monocytes expressing disparate molecular forms and quantities of CD43. *Cell. Immunol.* **163**, 59–69 (1995).
30. Chamorro, S. *et al*. Phenotypic characterization of monocyte subpopulations in the pig. *Immunobiology* **202**, 82–93 (2000).
31. Chamorro, S. *et al*. Phenotypic and functional heterogeneity of porcine blood monocytes and its relation with maturation. *Immunology* **114**, 63–71 (2005).
32. Chamorro, S. *et al*. *In vitro* differentiation of porcine blood CD163– and CD163+ monocytes into functional dendritic cells. *Immunobiology* **209**, 57–65 (2004).
33. Gordon, S. Biology of the macrophage. *J. Cell Sci. Suppl.* **4**, 267–286 (1986).
34. Gordon, S. The role of the macrophage in immune regulation. *Res. Immunol.* **149**, 685–688 (1998).
35. Austyn, J. M. & Gordon, S. F4/80, a monoclonal antibody directed specifically against the mouse macrophage. *Eur. J. Immunol.* **11**, 805–815 (1981).
36. Dijkstra, C. D., Van Vliet, E., Dopp, E. A., van der Lelij, A. A. & Kraal, G. Marginal zone macrophages identified by a monoclonal antibody: characterization of immuno- and enzyme-histochemical properties and functional capacities. *Immunology* **55**, 23–30 (1985).
37. Kraal, G. & Janse, M. Marginal metallophilic cells of the mouse spleen identified by a monoclonal antibody. *Immunology* **58**, 665–669 (1986).
38. Quinn, J. M. & Gillespie, M. T. Modulation of osteoclast formation. *Biochem. Biophys. Res. Commun.* **328**, 739–745 (2005).
39. McCusker, K. & Hoidal, J. Characterization of scavenger receptor activity in resident human lung macrophages. *Exp. Lung Res.* **15**, 651–661 (1989).
40. Palecanda, A. *et al*. Role of the scavenger receptor MARCO in alveolar macrophage binding of unopsonized environmental particles. *J. Exp. Med.* **189**, 1497–1506 (1999).
41. Taylor, P. R. *et al*. The β-glucan receptor, dectin-1, is predominantly expressed on the surface of cells of the monocyte/macrophage and neutrophil lineages. *J. Immunol.* **169**, 3876–3882 (2002).
42. Surh, C. D. & Sprent, J. T-cell apoptosis detected *in situ* during positive and negative selection in the thymus. *Nature* **372**, 100–103 (1994).
43. Tabe, H., Kawabata, I., Koba, R. & Homma, T. Cell dynamics in the germinal center of the human tonsil. *Acta Otolaryngol. Suppl.* **523**, 64–67 (1996).
44. Smythies, L. E. *et al*. Human intestinal macrophages display profound inflammatory anergy despite avid phagocytic and bactericidal activity. *J. Clin. Invest.* **115**, 66–75 (2005).
45. van Furth, R., Diesselhoff-den Dulk, M. M., Sluiter, W. & Van Dissel, J. T. in *Mononuclear Phagocytes: Characteristics*, *Physiology and Function* (ed. van Furth, R.) 201–210 (Martinus Nijhoff, Dordrecht, 1985).
46. Merad, M. *et al*. Langerhans cells renew in the skin throughout life under steady-state conditions. *Nature Immunol.* **3**, 1135–1141 (2002).
    Interesting demonstration of the differences in requirement for circulating precursors or local proliferation in the renewal of Langerhans cells during steady-state conditions and inflammation.
47. Kanitakis, J., Petruzzo, P. & Dubernard, J. M. Turnover of epidermal Langerhans' cells. *N. Engl. J. Med.* **351**, 2661–2662 (2004).

48. Kurihara, N., Chenu, C., Miller, M., Civin, C. & Roodman, G. D. Identification of committed mononuclear precursors for osteoclast-like cells formed in long term human marrow cultures. *Endocrinology* **126**, 2733–2741 (1990).
49. Udagawa, N. *et al*. Origin of osteoclasts: mature monocytes and macrophages are capable of differentiating into osteoclasts under a suitable microenvironment prepared by bone marrow-derived stromal cells. *Proc. Natl Acad. Sci. USA* **87**, 7260–7264 (1990).
50. Matsuzaki, K. *et al*. Osteoclast differentiation factor (ODF) induces osteoclast-like cell formation in human peripheral blood mononuclear cell cultures. *Biochem. Biophys. Res. Commun.* **246**, 199–204 (1998).
51. Marks, S. C. Jr & Lane, P. W. Osteopetrosis, a new recessive skeletal mutation on chromosome 12 of the mouse. *J. Hered.* **67**, 11–18 (1976).
52. Yoshida, H. *et al*. The murine mutation osteopetrosis is in the coding region of the macrophage colony stimulating factor gene. *Nature* **345**, 442–444 (1990).
53. Kong, Y. Y. *et al*. OPGL is a key regulator of osteoclastogenesis, lymphocyte development and lymph-node organogenesis. *Nature* **397**, 315–323 (1999).
54. Arai, F. *et al*. Commitment and differentiation of osteoclast precursor cells by the sequential expression of c-Fms and receptor activator of nuclear factor κB (RANK) receptors. *J. Exp. Med.* **190**, 1741–1754 (1999).
55. Sawyer, R. T., Strausbauch, P. H. & Volkman, A. Resident macrophage proliferation in mice depleted of blood monocytes by strontium-89. *Lab. Invest.* **46**, 165–170 (1982).
56. Tarling, J. D., Lin, H. S. & Hsu, S. Self-renewal of pulmonary alveolar macrophages: evidence from radiation chimera studies. *J. Leukoc. Biol.* **42**, 443–446 (1987).
57. Matute-Bello, G. *et al*. Optimal timing to repopulation of resident alveolar macrophages with donor cells following total body irradiation and bone marrow transplantation in mice. *J. Immunol. Methods* **292**, 25–34 (2004).
58. Thomas, E. D., Ramberg, R. E., Sale, G. E., Sparkes, R. S. & Golde, D. W. Direct evidence for a bone marrow origin of the alveolar macrophage in man. *Science* **192**, 1016–1018 (1976).
59. Nakata, K. *et al*. Augmented proliferation of human alveolar macrophages after allogeneic bone marrow transplantation. *Blood* **93**, 667–673 (1999).
60. Stanley, E. *et al*. Granulocyte/macrophage colony-stimulating factor-deficient mice show no major perturbation of hematopoiesis but develop a characteristic pulmonary pathology. *Proc. Natl Acad. Sci. USA* **91**, 5592–5596 (1994).
61. Hamilton, J. A. GM-CSF in inflammation and autoimmunity. *Trends Immunol.* **23**, 403–408 (2002).
62. Hickey, W. F. Leukocyte traffic in the central nervous system: the participants and their roles. *Semin. Immunol.* **11**, 125–137 (1999).
63. Lawson, L. J., Perry, V. H. & Gordon, S. Turnover of resident microglia in the normal adult mouse brain. *Neuroscience* **48**, 405–415 (1992).
64. de Groot, C. J., Huppes, W., Sminia, T., Kraal, G. & Dijkstra, C. D. Determination of the origin and nature of brain macrophages and microglial cells in mouse central nervous system, using non-radioactive *in situ* hybridization and immunoperoxidase techniques. *Glia* **6**, 301–309 (1992).
65. Kraal, G. Cells in the marginal zone of the spleen. *Int. Rev. Cytol.* **132**, 31–74 (1992).
66. Taylor, P. R. *et al*. Macrophage receptors and immune recognition. *Annu. Rev. Immunol.* **23**, 901–944 (2005).
67. van der Laan, L. J. *et al*. Regulation and functional involvement of macrophage scavenger receptor MARCO in clearance of bacteria *in vivo. J. Immunol.* **162**, 939–947 (1999).
68. Kang, Y. S. *et al*. SIGN-R1, a novel C-type lectin expressed by marginal zone macrophages in spleen, mediates uptake of the polysaccharide dextran. *Int. Immunol.* **15**, 177–186 (2003).
69. Geijtenbeek, T. B. *et al*. Marginal zone macrophages express a murine homologue of DC-SIGN that captures blood-borne antigens *in vivo. Blood* **100**, 2908–2916 (2002).
70. Taylor, P. R. *et al*. Development of a specific system for targeting protein to metallophilic macrophages. *Proc. Natl Acad. Sci. USA* **101**, 1963–1968 (2004).
71. Eloranta, M. L. & Alm, G. V. Splenic marginal metallophilic macrophages and marginal zone macrophages are the major interferon-α/β producers in mice upon intravenous challenge with herpes simplex virus. *Scand. J. Immunol.* **49**, 391–394 (1999).
72. van Furth, R. & Diesselhoff-den Dulk, M. M. Dual origin of mouse spleen macrophages. *J. Exp. Med.* **160**, 1273–1283 (1984).

73. van Rooijen, N., Kors, N. & Kraal, G. Macrophage subset repopulation in the spleen: differential kinetics after liposome-mediated elimination. *J. Leukoc. Biol.* **45**, 97–104 (1989).

74. Van Rooijen, N., Kors, N., van de Ende, M. & Dijkstra, C. D. Depletion and repopulation of macrophages in spleen and liver of rat after intravenous treatment with liposome-encapsulated dichloromethylene diphosphonate. *Cell Tissue Res.* **260**, 215–222 (1990).

75. Wijffels, J. F., de Rover, Z., Beelen, R. H., Kraal, G. & van Rooijen, N. Macrophage subpopulations in the mouse spleen renewed by local proliferation. *Immunobiology* **191**, 52–64 (1994).

76. Witmer-Pack, M. D. *et al*. Identification of macrophages and dendritic cells in the osteopetrotic (op/op) mouse. *J. Cell Sci.* **104**, 1021–1029 (1993).

77. Takahashi, K., Umeda, S., Shultz, L. D., Hayashi, S. & Nishikawa, S. Effects of macrophage colony-stimulating factor (M-CSF) on the development, differentiation, and maturation of marginal metallophilic macrophages and marginal zone macrophages in the spleen of osteopetrosis (op) mutant mice lacking functional M-CSF activity. *J. Leukoc. Biol.* **55**, 581–588 (1994).

78. Cecchini, M. G. *et al*. Role of colony stimulating factor-1 in the establishment and regulation of tissue macrophages during postnatal development of the mouse. *Development* **120**, 1357–1372 (1994).

79. Crofton, R. W., Diesselhoff-den Dulk, M. M. & van Furth, R. The origin, kinetics, and characteristics of the Kupffer cells in the normal steady state. *J. Exp. Med.* **148**, 1–17 (1978).

80. Naito, M., Hasegawa, G. & Takahashi, K. Development, differentiation, and maturation of Kupffer cells. *Microsc. Res. Tech.* **39**, 350–364 (1997).

81. Bouwens, L., Baekeland, M., De Zanger, R. & Wisse, E. Quantitation, tissue distribution and proliferation kinetics of Kupffer cells in normal rat liver. *Hepatology* **6**, 718–722 (1986).

82. Gordon, S. Alternative activation of macrophages. *Nature Rev. Immunol.* **3**, 23–35 (2003).

83. Mosser, D. M. The many faces of macrophage activation. *J. Leukoc. Biol.* **73**, 209–212 (2003).

84. Goerdt, S. & Orfanos, C. E. Other functions, other genes: alternative activation of antigen-presenting cells. *Immunity* **10**, 137–142 (1999).

85. Takeda, K., Kaisho, T. & Akira, S. Toll-like receptors. *Annu. Rev. Immunol.* **21**, 335–376 (2003).

86. Davies, J. R., Rudd, J. F., Fryer, T. D. & Weissberg, P. L. Targeting the vulnerable plaque: the evolving role of nuclear imaging. *J. Nucl. Cardiol.* **12**, 234–246 (2005).

87. Aschoff, L. Das Reticulo-endotheliale System. *Ergeb. Inn. Med. Kinderheilkd.* **26**, 1–118 (1924) (in German).

88. Lichanska, A. M. & Hume, D. A. Origins and functions of phagocytes in the embryo. *Exp. Hematol.* **28**, 601–611 (2000).

89. Shepard, J. L. & Zon, L. I. Developmental derivation of embryonic and adult macrophages. *Curr. Opin. Hematol.* **7**, 3–8 (2000).

90. Takahashi, K., Donovan, M. J., Rogers, R. A. & Ezekowitz, R. A. Distribution of murine mannose receptor expression from early embryogenesis through to adulthood. *Cell Tissue Res.* **292**, 311–323 (1998).

91. Hughes, D. A. & Gordon, S. Expression and function of the type 3 complement receptor in tissues of the developing mouse. *J. Immunol.* **160**, 4543–4552 (1998).

92. Hume, D. A., Monkley, S. J. & Wainwright, B. J. Detection of *c-fms* protooncogene in early mouse embryos by whole mount *in situ* hybridization indicates roles for macrophages in tissue remodelling. *Br. J. Haematol.* **90**, 939–942 (1995).

93. Herbomel, P., Thisse, B. & Thisse, C. Ontogeny and behaviour of early macrophages in the zebrafish embryo. *Development* **126**, 3735–3745 (1999).

94. Henderson, R. B., Hobbs, J. A., Mathies, M. & Hogg, N. Rapid recruitment of inflammatory monocytes is independent of neutrophil migration. *Blood* **102**, 328–335 (2003).

95. Taylor, P. R., Brown, G. D., Geldhof, A. B., Martinez-Pomares, L. & Gordon, S. Pattern recognition receptors and differentiation antigens define murine myeloid cell heterogeneity *ex vivo*. *Eur. J. Immunol.* **33**, 2090–2097 (2003).

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