

The multiple roles of monocyte subsets in steady state and inflammation

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Abstract Monocytes participate importantly in immunity. Produced in the bone marrow and released into the blood, they circulate in blood or reside in a spleen reservoir before entering tissue and giving rise to macrophages or dendritic cells. Monocytes are more than transitional cells that adapt to a particular tissue environment indiscriminately. Accumulating evidence now indicates that monocytes are heterogeneous in several species and are themselves predetermined for particular function in the steady state and inflammation. Future therapeutics may harness this heterogeneity to target harmful functions while sparing those that are beneficial. Here, we review recent advances on the ontogeny and function of monocytes and their subsets in humans and mice.

Keywords Monocyte subsets · Inflammation · Hematopoietic system · Macrophage · Dendritic cell

Introduction

Monocytes are large circulating leukocytes of the myeloid lineage that mediate essential functions of innate immunity including phagocytosis and cytokine production [1, 2]. Generated in the bone marrow, monocytes enter blood and circulate briefly before extravasating to tissue. Upon tissue infiltration, they are believed to differentiate to macrophages or dendritic cells. Thus, they have often been

regarded as transitional cells whose eventual phenotypes depend on the particular environment in which they accumulate; in vitro, they are impossible to maintain and either differentiate rapidly or die.

Over the last several years, renewed interest in monocytes has revealed them to be more than simply transitional and reactive. Monocytes are functionally heterogeneous in several species. They can be inflammatory and anti-inflammatory, and they need not differentiate to macrophages or dendritic cells in order to participate in immunity. In sum, monocytes are active constituents of the immune system. In this review, we will discuss the major findings that have enriched our understanding of monocyte subset biology. We will highlight the unanswered pressing questions and discuss some of the lingering controversies.

Human monocyte subsets

The blood of a healthy human adult contains approximately 450 monocytes/ μ l, which amounts to over 2 billion cells in a 5-l volume. Monocytes are the largest of the circulating leukocytes and can be distinguished morphologically by their characteristic kidney or horse-shoe-shaped nucleus, although their variable nucleus to cytoplasm ratio renders monocytes sometimes difficult to distinguish from other leukocytes. It has been known for a long time that monocytes exist [3, 4], but, until recently, they have been regarded primarily as transitional cells whose main function is to repopulate various tissue macrophage populations.

Relying on gradient centrifugation and counterflow centrifugation (elutriation), numerous studies from the early 1980s postulated the existence of morphological and

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functional monocyte heterogeneity. Collectively, this work showed the presence of a dominant, large and dense “classical” subset that expressed the LPS co-receptor CD14, was phagocytic, produced cytokines including IL-1 and colony-stimulating factor (CSF), had high MPO activity, high antibody-dependent cell-mediated cytotoxicity (ADCC), and suppressed antigen-activated lymphocytes. The second, minor, subset was smaller and less dense, less phagocytic, expressed lower levels of CD14 and higher levels of HLA-DR, exhibited greater capacity to present soluble tetanus toxoid (TT) and particulate antigens, and produced high levels of interferon-alpha [5–12].

A series of studies conducted in the late 1980s and 1990s focussed on defining human monocyte subsets according to their cell surface antigen expression [13]. One line of inquiry differentiated subsets on the basis of Fc γ R-I (CD64) expression. Two major subsets were identified. The majority of monocytes were CD64⁺; these were large, phagocytic, and inflammatory. In contrast, CD64[−] were rare, smaller, had higher capacity for antigen presentation, and had low cytostatic effects against tumors. In general, then, characterization of monocytes according to CD64 expression recapitulated the earlier studies in which monocyte subsets were separated by size and density [14–20].

A second line of inquiry focused on the expression of Fc γ R-III (CD16) [21, 22]. It was shown that the majority of monocytes were CD16[−], had low expression of HLA-DR, were highly phagocytic and inflammatory, and expressed the chemokine receptor CCR2. A minor subset was CD16⁺, HLA-DR⁺, CCR2[−], and CCR5⁺. Given the finding that CD16[−] monocytes can upregulate CD16 and that macrophages express CD16, it was postulated that the CD16⁺ subset was a more mature version of CD16[−] monocytes [23]. CD16⁺ monocytes were termed pro-inflammatory because of their capacity to produce TNF- α in response to LPS [24, 25], and subsequent studies have shown that this subset can differentiate to dendritic cells, respond to fractalkine [26–28], and increase under various stress or disease conditions [29–33].

Grage-Griebenow and colleagues [13] proposed the existence of at least three subsets of human monocytes, two of them within the CD16⁺ population. It was shown, for example, that CD16⁺CD14^{high} monocytes respond differentially to LPS, zymosan, and *Staphylococcus aureus* compared to CD16⁺CD14^{dim} monocytes. Whereas CD16⁺CD14^{high} monocytes are the main producers of IL-10, the CD16⁺CD14^{dim} monocytes are major producers of TNF- α , but only in response to LPS and not to zymosan or *S. aureus* [34].

Accumulating evidence nevertheless suggests that it is the CD16[−] monocyte subset that is more inflammatory:

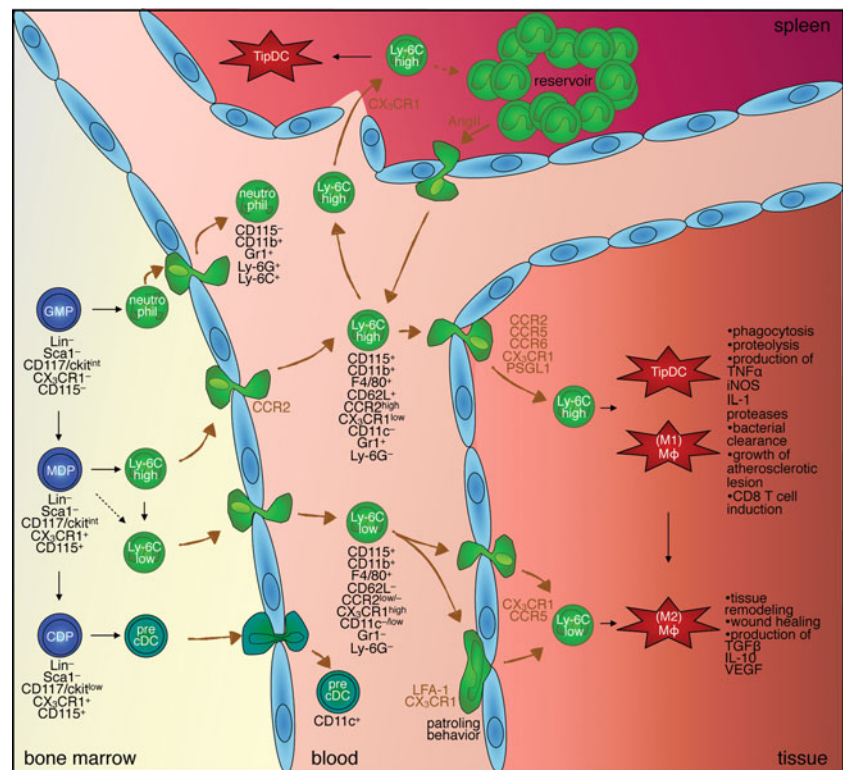
CD16[−] monocytes express CCR2 and MPO at higher levels [35], phenotypically resemble inflammatory monocytes in mice (see below) [2], rise in the blood shortly after myocardial infarction [36], and upregulate PD-1, an anti-inflammatory co-stimulatory molecule, slightly less than CD16⁺ monocytes [37]. Perhaps the more important question, then, is not which subset is inflammatory and which is anti-inflammatory, but how a particular subset responds to a given stimulus.

Demarcation of human monocyte subsets according to the expression of CD16 has endured, but, in the absence of correlates in animal models, insight into the functional consequence of monocyte heterogeneity was limited to ex vivo and in vitro approaches. With the discovery of mouse monocyte subsets, however, it became possible to investigate monocyte heterogeneity in vivo.

Mouse monocyte subsets

In contrast to humans, mouse monocytes represent about 1.5% of the total peripheral blood leukocyte pool, which corresponds to fewer than 100 cells/ μ l blood volume. This rarity, combined with the difficulty in maintaining them in culture, has made it difficult to study monocytes in the mouse. In 2000, Jung et al. [38] reported the generation of a transgenic mouse that expresses GFP under the CX₃CR1 promoter. Cells that typically express the chemokine receptor could now be visualized microscopically or by flow cytometry. Phenotypic characterization of either homozygous GFP⁺ mice (and hence CX₃CR1-deficient) or heterozygous GFP⁺ CX₃CR1⁺ mice revealed that GFP⁺ cells were mostly monocytes and their tissue descendants. A study by von Andrian and colleagues [39] in 2001 that utilized these mice revealed two CD11b^{high}F4/80⁺ populations that differed according to GFP expression: GFP^{high} cells were CCR2^{low}CD62L^{low} while GFP^{med} cells were CCR2^{high}CD62L^{high}. The existence of two distinct subsets was postulated, but functional studies were not pursued. In 2003, Geissmann et al. [40] described the phenotype and functional relevance of monocyte subsets. In a series of profiling and fate mapping studies, it was shown that the main subset is large, Ly-6C^{high}(Gr-1⁺) CX₃CR1^{low}CCR2^{high}CD62L⁺, has a short half-life, and migrates to inflamed tissues. A second subset is smaller in size, Ly-6C^{low}(Gr-1[−]) CX₃CR1^{high}CCR2^{low/neg}CD62L^{neg} and is found in inflamed and resting tissue. Accordingly, Ly-6C^{high} monocytes were called ‘inflammatory’ while Ly-6C^{low} monocytes were termed ‘resident’. Collectively, these studies provided the tools and stimulated further interest into how monocyte subsets uniquely influence the immune response (Fig. 1).

Fig. 1 The development, phenotype, differentiation, and distribution of mouse monocyte subsets and allied myeloid cells. *Cartoon* depicts the development of monocyte subsets in the bone marrow, their entrance into the blood and accumulation in tissue. *Brown arrows* depict migration and *black arrows* depict differentiation. *Markers listed in brown* depict molecules by which movement is mediated. *Ly-6C^{high}* *Ly-6C^{high}* monocyte, *Ly-6C^{low}* *Ly-6C^{low}* monocyte, *GMP* granulocyte and macrophage progenitor, *MDP* macrophage and dendritic cell progenitor, *CDP* common dendritic cell progenitor, *preDC* pre-dendritic cell, *TipDC* TNF α and iNOS-producing dendritic cell, *M ϕ* macrophage



The origin of mouse monocytes and their subsets

Monocytes belong to the myeloid lineage and develop along a path of increasing phenotypic restriction and commitment starting with the earliest progenitor, the hematopoietic stem cell (HSC). Downstream, monocyte restriction follows along the common myeloid progenitor (CMP) and the granulocyte and macrophage progenitor (GMP). GMPs can give rise to monocytes and neutrophils but not megakaryocytes, erythrocytes, or lymphocytes [41, 42]. Monocyte commitment depends on growth factors such as M-CSF acting via its ligand M-CSFR, and involves transcription factors such as PU.1, KLF-4, MafB, and c-Maf [1].

Recently, several groups have characterized the developmental pathways of monocytes, macrophages, and dendritic cells downstream of the GMP. Geissmann and colleagues described progenitors that express the fractal-kine receptor CX₃CR1, c-kit (CD117) [43] and CD115 [44] downstream of the GMP. Termed macrophage and dendritic cell progenitors (MDP), they give rise to monocytes, macrophages, and classical dendritic cell subsets, but not neutrophils. Separate groups have described dedicated dendritic cell precursors, termed common dendritic cell precursors (CDP) that express Flt-3 (CD135) and give rise to classical dendritic cells (both CD8⁺ and CD8⁻) as well as plasmacytoid dendritic cells (pDC), but not monocytes [45, 46]. Phenotypically, MDPs and CDPs differ according

to c-kit expression: MDPs express c-kit at higher levels than CDPs. Fate-mapping experiments have determined that these precursors fall on a continuum of increased phenotypic restriction: CDPs are most restricted because they give rise to DCs at the exclusion of monocytes or macrophages; MDPs give rise to DCs, monocytes and macrophages; GMPs give rise to all these phenotypes as well as to neutrophils [47].

Overall, these studies have been insightful about the nature of monocytes and their relationship to dendritic cells and macrophages. A salient observation is that monocytes do not give rise to classical dendritic cells. Classical dendritic cells (sometimes called “conventional” dendritic cells) were discovered by Steinman and Cohn [48–50] more than 30 years ago. They were first shown to reside in secondary lymphoid organs such as the spleen. Today, we know that there are many dendritic cell subsets found in various organs that are specialized for different immune functions [51]. The fact that many of these subsets do not derive from monocytes but from dedicated precursors challenges our assumptions that monocytes’ main role is to repopulate tissue dendritic cells and macrophages.

A lingering ontogeny issue of some contention is the relationship between subsets. Is one subset a more mature version of another or do subsets derive from separate precursors? As mentioned above, it is believed that the CD16⁺ human monocyte subset is a more mature form of the CD16⁻ subset. Likewise in the mouse, a series of

experiments have argued that Ly-6C^{high} monocytes convert to Ly-6C^{low} monocytes. In support of this, Sunderkotter et al. [52] depleted peripheral blood monocytes by clodronate-rich liposomes and showed sequential repopulation of subsets: Ly-6C^{high} monocytes appeared in the circulation first; Ly-6C^{low} monocytes emerged next. Fate-mapping experiments by Varol et al. [53] argued that Ly-6C^{high} accumulate in the bone marrow and convert to Ly-6C^{low} monocytes before re-entering the blood. Given that Ly-6C^{low} monocytes circulate longer than Ly-6C^{high} monocytes [54], it is possible that the sequential appearance of Ly-6C^{high} and Ly-6C^{low} monocytes indicates separate and differently-timed differentiation of either subset rather than conversion. However, a dedicated precursor that gives rise to Ly-6C^{low} monocytes in the absence of a Ly-6C^{high} intermediate has not been identified and therefore conversion remains a plausible mechanism by which the two subsets are linked.

The location where monocytes originate has also received attention lately. It has been believed that monocytes are exclusively generated and stored in the bone marrow. It is a paradigm first proposed in 1968 [4] and supported by many studies, including those investigating monocyte progenitors [1]. It has recently been shown, however, that bona fide monocytes also reside in a splenic reservoir in large numbers [55]. Splenic monocytes cluster in the subcapsular red pulp and are distinct from other macrophage and dendritic cell populations in the spleen. Unlike bone marrow monocytes, which require CCR2 to enter the circulation [56, 57], splenic monocyte exit is independent of CCR2. Signals such as angiotensin II mobilize splenic monocytes en masse, which then enter the circulation and mediate immunity in other tissue sites.

The function of mouse monocyte subsets

Monocyte accumulation in the blood

A growing body of literature indicates that Ly-6C^{high} monocytes preferentially accumulate in the circulation during acute and chronic inflammation. Bone marrow-derived Ly-6C^{high} monocytes enter the blood via CCR2, a chemokine receptor they express at high levels [56–58]. In response to acute injury or infection, the number of Ly-6C^{high} monocytes rapidly and dramatically increases in the circulation [59, 60]. This increase is conferred not only by a heightened production and release of monocytes from the bone marrow, but also by their mobilization from a splenic monocyte reservoir [55]. The presence of large numbers of Ly-6C^{high} monocytes in the circulation likely confers a stochastic advantage for their eventual accumulation in the affected site. As injury wanes or infection is resolved, Ly-6C^{high} monocyte numbers wane in the

circulation by mechanisms that are still poorly understood but almost certainly involve a decrease in the rate of cell production. During chronic inflammation in which the inflammatory insult persists, such as atherosclerosis, the number of circulating Ly-6C^{high} monocytes increases progressively [61, 62]. Consequently, monocytes themselves contribute to the pathology and, by extension, represent potential therapeutic targets. To what extent Ly-6C^{low} monocytes increase in the circulation is a matter of some debate. In models of acute inflammation such as myocardial infarction, Ly-6C^{low} monocyte numbers tend to remain relatively unchanged [60]. In chronic inflammation, such as during atherosclerosis, different groups have observed a range of Ly-6C^{low} monocyte levels [62–65]. It has also been argued that Ly-6C^{low} monocytes selectively accumulate oxidized lipoproteins, raising the intriguing possibility that, even if less numerous, Ly-6C^{low} monocytes contribute importantly to atherosclerosis by acting as Trojan horses and bringing lipids to the growing atheroma. Clearly, more research is required to address how monocyte numbers are controlled in the steady state and during inflammation.

Monocyte accumulation in tissue depends on chemokines and their receptors

Accumulation of monocyte subsets into peripheral tissues depends on a myriad of adhesion molecules and chemokines/chemokine receptors. Deficiency of adhesion molecules such as VCAM-1, ICAM-1, or CD18/ β 2 is known to inhibit atherosclerosis, a disease that depends on monocyte accumulation in the growing atheromata [66]. Chemokines and their receptors have received considerable attention in the context of monocyte subsets because subsets are typically differentiated according to their chemokine receptor expression profile [67, 68]. In experimentally-induced myocardial infarction, CCR2/MCP1 signaling drives the early accumulation of Ly-6C^{high} monocytes to the infarcted myocardium while CX₃CR1/fractalkine signaling is more important to the later accumulation of Ly-6C^{low} monocytes [60]. In infection, CCR2-mediated recruitment of Ly-6C^{high} monocytes from the bone marrow to the circulation depends on MCP-1 and MCP-3, although CCR2 is not required for the accumulation of these cells to the spleen [69]. CCR6/CCL20 have also been implicated in the accumulation of monocytes to tissue [70]. In models of atherosclerosis, a number of chemokine/chemokine receptor pairs relevant to monocyte recruitment have been implicated and include CCR2/MCP-1, CX₃CR1/fractalkine, and CCR5/MIP-1 α [71]. Atherosclerosis is markedly reduced when CCR2/MCP-1 and CX₃CR1/fractalkine signaling is inhibited, and is almost absent through additional inhibition of CCR5 [63, 72–74]. These studies indicate that in atherosclerosis both subsets

accumulate to promote disease, despite the observation that Ly-6C^{high} monocytes accumulate more frequently [62, 65, 75–78]. It is important to note, however, that chemokines and their receptors are not engaged exclusively in chemotaxis. For example, CX₃CR1 has been implicated in cell survival [79]. Future studies will need to investigate the contribution of these molecules to such processes as proliferation, differentiation, and survival, in addition to their reported role in mobilization from the bone marrow and accumulation in tissue.

Monocytes are functionally heterogeneous upon accumulation

Several groups have shown that, upon accumulation, monocyte subsets and their progeny are functionally heterogeneous. In myocardial infarction, early-accumulating Ly-6C^{high} monocytes scavenge necrotic debris and promote inflammation through the production of TNF- α and proteolytic enzymes [60]. Likewise, in response to bacterial, viral, or parasitic infection, Ly-6C^{high} monocytes are inflammatory and produce a combination of TNF- α , iNOS, IL-12, and type 1 interferon [80–86]. It has also been shown in a model of skeletal muscle injury that Ly-6C^{high} monocytes acquire a less inflammatory profile in tissue characterized by reduced Ly-6C expression and upregulation of CX₃CR1, CD11c, and IL-10 [87]. Ly-6C^{low} monocytes, on the other hand, contribute to wound healing in the infarcted myocardium rather than inflammation: they are a source of VEGF and their accumulation coincides with angiogenesis and increased collagen deposition. Ly-6C^{low} monocytes may also be the first to respond following injury or infection. In the steady state, they crawl along the luminal side of blood vessels and patrol the vasculature [88], a behavior that is probably not unique to monocytes but may be essential to how these cells sense their environment [89, 90]. Within the first couple of hours following infection they produce TNF α transiently [88].

Differentiation potential of monocytes in tissue

Differential accumulation and function of monocyte subsets in tissue may also indicate that monocyte subsets give rise to different progeny. One idea is that monocyte subsets differ in their capacity to give rise to either dendritic cells or macrophages. Given that Ly-6C^{low} monocytes express CD11c at low levels, and that they upregulate CD11c upon accumulation to atherosclerotic lesions, it was postulated that Ly-6C^{low} monocytes are precursors of dendritic cells while Ly-6C^{high} monocytes preferentially give rise to macrophages. Evaluation of transcription factors such as Pu.1, RelB, cMaf, and Mafk involved in dendritic cell and macrophage differentiation has led some to conclude,

however, that Ly-6C^{high} monocytes accumulating in the peritoneum are more prone toward dendritic cell differentiation while Ly-6C^{low} monocytes become macrophages [88, 91]. Pamer and colleagues [82] have described TNF α and iNOS-producing dendritic cells, termed TipDC, that differentiate from Ly-6C^{high} monocytes in response to *Listeria* infection. The process of differentiation is MyD88-dependent [85, 92], and studies now suggest Ly-6C^{high} monocyte-derived DCs may be important in cross-priming [70, 93, 94]. Jung and colleagues have explored the differentiation potential of blood monocytes in the lung. They reported that Ly-6C^{low} monocytes give rise to lung macrophages [95] while Ly-6C^{high} monocytes differentiate to dendritic cells in the lung but not in the spleen [53]. The authors further state, however, that Ly-6C^{high} monocytes can convert to Ly-6C^{low} monocytes in the bone marrow and, therefore, also differentiate to macrophages.

A second possibility is that monocyte subsets can be grouped according to the particular macrophage or dendritic cell phenotype they eventually acquire. M1 macrophages, or classically-activated macrophages, arise *in vitro* when bone marrow-derived cells are cultured with inflammatory triggers such as IFN γ or LPS, whereas M2 macrophages, or alternatively-activated macrophages, arise when cells are cultured with cytokines typically associated with the resolution of inflammation, such as IL-10. M1 macrophages typically express inflammatory mediators such as IL-1, iNOS, ICAM, and MMP9 while M2 macrophages express arginase, CCR7, IL-10, and Fizz, among others. Polarization of these macrophage populations represents an extreme, and there is likely a continuum of phenotypes *in vivo* [96, 97]. It is possible therefore, that Ly-6C^{high} monocytes give rise to M1 macrophages while Ly-6C^{low} monocytes give rise to M2 macrophages. This is supported by studies in a model of myocardial infarction in which Ly-6C^{high} monocytes that accumulated early were inflammatory whereas Ly-6C^{low} monocytes that accumulated later mediated granulation tissue formation and resolved inflammation, a finding that was consistent in blood of patients with primary acute myocardial infarction [36]. Demarcation of monocyte subsets as M1 and M2 has also been observed in kidney injury [98], and in response to *Toxoplasma gondii* infection where it was shown that Ly-6C^{high} monocytes migrated to the infected ileum where they produced TNF α and iNOS, and upregulated F4/80, but not CD11c [83].

Subsets may also be grouped according to the type of DC phenotype they acquire [99]. Randolph and colleagues have shown that Ly-6C^{high} monocytes preferentially give rise to CD103⁺ DCs in the lung whereas Ly-6C^{low} monocytes give rise to CD103[−] DCs [100]. In the lamina propria, Jung and colleagues [101] have shown that Ly-6C^{high} monocytes preferentially give rise to CD103[−] DCs while CD103⁺ DCs arise from MDPs without a

monocyte intermediate. In the skin, Merad and colleagues [102] have shown that Langerhans cells may arise from Ly-6C^{high} monocytes, although recent studies indicate that the contribution of Ly-6C^{high} monocyte-derived Langerhans cells may be less numerous than previously believed [103]. Ly-6C^{high} monocytes may also give rise to microglia during inflammation [104]. Finally, studies in tumor-bearing mice reveal that Ly-6C^{high} monocytes may be the precursors of myeloid-derived suppressor cells (MDSC) [105–108]. Studies are needed, however, to investigate the relationship between monocytes and MDSC in more detail.

Concluding remarks

The discovery that monocyte subset heterogeneity is conserved between species is important to the study of functional differences between monocyte populations in human health and disease. Today, we are, however, learning that human monocyte subsets and mouse monocyte subsets are not fully equivalent [109], and a number of conflicting data and controversies still need to be addressed. Despite this, renewed interest in monocytes over the last several years has revealed some fundamental mechanisms by which these cells participate in immunity. Ly6C^{high} and Ly6C^{low} monocytes function in a highly orchestrated and complementary manner during the development of the immune response. Monocytes are not simply intermediates transitioning to tissue macrophages and dendritic cells, but function as effectors of the immune system. Continued study of functional differences between monocyte subsets will increase our understanding of the underlying processes involved in the initiation and resolution of inflammatory processes.

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