Regulation of the Migration and Survival of Monocyte Subsets by Chemokine Receptors and Its Relevance to Atherosclerosis

Emmanuel L. Gautier, Claudia Jakubzick, Gwendalyn J. Randolph

Abstract—Monocytes are central mediators in the advance of atherosclerotic plaque, making them a natural therapeutic target for reducing disease burden. Here, we highlight recent advances in our current understanding of monocyte heterogeneity and its relevance to regulation of monocyte accumulation and function within atherosclerotic plaques. Differences that distinguish monocyte subsets include differential expression of chemokine receptors, especially CCR2 and CX3CR1. Ablation of expression of these 2 receptors (or their ligands) in mice has an additive inhibition on monocyte recruitment to atherosclerotic plaques. Moreover, simultaneously interfering with 3 key pathways—CCR2, CX3CR1, and CCR5—essentially abolishes atherosclerosis in mice. Here, we discuss how these chemokine receptors act at multiple points on at least 1 monocyte subset, regulating their mobilization from bone marrow, survival, or recruitment to plaques. Finally, we discuss how this knowledge may be useful clinically, emphasizing that CX3CR1 may in particular be a viable target for therapeutic manipulation of monocyte-derived cell fate in cardiovascular disease. (Arterioscler Thromb Vasc Biol. 2009;29:1412-1418.)

Key Words: chronic inflammation ■ arteriosclerosis ■ macrophage ■ chemotaxis ■ diapedesis

Atherosclerosis is both a metabolic and an immunoinflammatory disease. Whereas dyslipidemia is required for the initiation and progression of atherosclerotic lesions, the role of inflammatory cells, especially monocytes, is central to plaque development. Indeed, reduction in circulating monocytes limits plaque development in animal models of atherosclerosis,1,2 and blood monocyte counts are an independent risk factor for coronary artery disease in humans.3–5 Moreover, simultaneously interfering with 3 key pathways—CCR2, CX3CR1, and CCR5—essentially abolishes atherosclerosis in mice. Here, we discuss how these chemokine receptors act at multiple points on at least 1 monocyte subset, regulating their mobilization from bone marrow, survival, or recruitment to plaques. Finally, we discuss how this knowledge may be useful clinically, emphasizing that CX3CR1 may in particular be a viable target for therapeutic manipulation of monocyte-derived cell fate in cardiovascular disease.

Classical and Nonclassical Monocyte Subsets

There are 2 major subsets of monocytes that are recognized in humans, mice, and other species (rat, pig)7,8 (Figure 1). They are distinguished by distinct chemokine receptor expression patterns, especially differential expression of CCR2 and CX3CR1.9–11 as well as conservation of other surface markers.7,8,12,13 A recent gene expression analysis comparing human and mouse monocytes reveals further conservation between monocyte subsets, though it also identifies key differences between the species, including patterns of CD36 expression (unpublished observations; M.A. Ingersoll et al, unpublished observations, 2009, M.A. Ingersoll, R. Spanbroek, C. Lottoz, M. Frankenberger, R. Hoffmann, R. Lang, M. Collin, A.J.R. Habenicht, L. Zeisler-Heitbrock, G.J. Randolph).

The various terms used in the literature to describe mouse monocyte subsets, and their analogous populations in human, can be confusing (Table), with references to different combinations of surface markers for different species, or use of terms like “inflammatory monocytes” that do not refer to the same populations in mice and humans (Table). To use unifying terms that cross species, we refer to CCR2+ monocytes in all species as “classical monocytes.”14 CCR2+ monocytes comprise the vast majority of human monocytes (≥ 92%), and we regard their ability to respond to the chemokine monocyte chemotactant protein 1 (MCP-1; CCL215) as a classical characteristic. Conversely, CD14+CD16+ human and Ly-6C+ mouse monocytes, low in CCR2 expression16–18 and relatively rare among monocytes in humans, are “nonclassical monocytes.” In mice, classical and nonclassical monocyte frequency is approximately 1:1,11,12 compared with the less than 10% of human monocytes that bear the nonclassical phenotype. There is evidence that nonclassical mono-
cytes derive from classical monocytes,\textsuperscript{12,19,20} but it remains possible that some nonclassical monocytes arise independently.\textsuperscript{21}

Functionally, a number of differences between circulating monocyte subsets has been identified, and some of these differences are summarized in the Table. For example, nonclassical monocytes in mouse and man produce more TNF than classical monocytes\textsuperscript{22,23} and are more active as antigen-presenting cells in assays measuring T cell stimulation.\textsuperscript{24–27} The role of nonclassical monocytes in regulating T cell responses may be more for the promotion of tolerance than priming, as recently suggested in one study of mouse nonclassical monocytes.\textsuperscript{28} In humans, nonclassical monocytes include cells that express ILT4, a ligand for HLA-G implicated in fetal tolerance during pregnancy.\textsuperscript{29}

In line with their differential chemokine receptor profiles, the trafficking patterns of the 2 monocyte subsets are distinct, at least in mice. Classical mouse monocytes are recruited to sites of inflammation in a “classical” time course (following the typical wave of neutrophils) during acute inflammation.\textsuperscript{14} The migratory behavior of nonclassical monocytes is far less clear, with different studies on them reaching quite different conclusions, raising the possibility that their behavior is context-dependent. Nonclassical mouse monocytes migrate less abundantly than classical monocytes in acute peritonitis.

Table. Classification of Human and Mouse Monocyte Subsets

<table>
<thead>
<tr>
<th></th>
<th>Classical Human</th>
<th>Classical Mouse</th>
<th>Nonclassical Human</th>
<th>Nonclassical Mouse</th>
<th>Refs.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Common phenotypic identifiers</td>
<td>CD14\textsuperscript{++} CD16\textsuperscript{−}</td>
<td>CD115\textsuperscript{Gr1\textsuperscript{−}/Ly6C\textsuperscript{−}CD16\textsuperscript{+}}</td>
<td>CD14\textsuperscript{+}/CD14\textsuperscript{hi} CD16\textsuperscript{+}</td>
<td>CD115\textsuperscript{Gr1\textsuperscript{−}/Ly6C\textsuperscript{−}CD16\textsuperscript{+}}</td>
<td>\textsuperscript{9,11,12,17,27}</td>
</tr>
<tr>
<td>Chemokine receptors differentially expressed</td>
<td>CCR2\textsuperscript{+} CCR1\textsuperscript{+} CXCR1\textsuperscript{+} CXCR2\textsuperscript{+} CX3CR1\textsuperscript{int}</td>
<td>CCR2\textsuperscript{+} CCR1\textsuperscript{+} CXCR1\textsuperscript{+} CX3CR1\textsuperscript{int}</td>
<td>CCR2\textsuperscript{−} CCR1\textsuperscript{−} CX3CR1\textsuperscript{hi}</td>
<td>CCR2\textsuperscript{10} CCR1\textsuperscript{−} CX3CR1\textsuperscript{hi}</td>
<td>\textsuperscript{10,11,17,18,30}</td>
</tr>
<tr>
<td>Other differentially expressed markers</td>
<td>CD62L\textsuperscript{+} CD43\textsuperscript{+} CD11a\textsuperscript{+} CD11c\textsuperscript{+}</td>
<td>CD62L\textsuperscript{−} CD43\textsuperscript{−} CD11a\textsuperscript{−} CD11c\textsuperscript{−}</td>
<td>CD62L\textsuperscript{+} CD43\textsuperscript{+} CD11a\textsuperscript{+} CD11c\textsuperscript{+}</td>
<td>CD62L\textsuperscript{−} CD43\textsuperscript{−} CD11a\textsuperscript{−} CD11c\textsuperscript{−}</td>
<td>\textsuperscript{7,11,12,22,30}</td>
</tr>
<tr>
<td>Functional Assessments</td>
<td>Highly phagocytic relative to nonclassical counterparts; High SR-A and CD36 expression</td>
<td>Not known to differ in phagocytosis relative to nonclassical counterparts</td>
<td>Signaling cascades analyzed by proteomics suggest a phagocytic past for nonclassical monocytes; preference for recognition of oxidized LDL, despite lower scavenger receptor (SR-A, CD36) expression; High TNF secretion; Superior in T cell activation</td>
<td>May be more likely to take up oxidized LDL; high scavenger receptor (SR-A) expression; High TNF secretion; Superior in T cell activation (mixed lymphocyte reaction)</td>
<td>\textsuperscript{22–25,37,39,84–87}</td>
</tr>
<tr>
<td>Alternative names in the literature</td>
<td>Inflammatory monocytes</td>
<td>Proinflammatory monocytes</td>
<td>Resident monocytes; stationary monocytes</td>
<td></td>
<td>\textsuperscript{11,22,88}</td>
</tr>
</tbody>
</table>

Classical human and mouse populations share overlapping features, as do nonclassical human and mouse populations. Bold indicates terms used to describe monocyte subsets that do not correspond to populations with overlapping features between species.
In contrast, in the steady state, they accumulate readily in spleen, liver, brain, and lung, but not in the peritoneum. The cell types they differentiate into in these organs and their roles therein remain unclear. In spleen, they likely participate in T-dependent antibody production by innate B cells (for review and more extensive discussion on this topic, see11), and, as mentioned above, participate in cross-tolerance.28 A role for these monocytes in facilitating natural antibody production by innate B cells may be particularly relevant to atherosclerosis, because many natural antibodies react with oxidized LDL and serve atheroprotective roles in mouse models of atherosclerosis.22

After injection of Listeria monocytogenes into the peritoneal cavity, nonclassical mouse monocytes appear to accumulate very early,23 earlier than neutrophils. By contrast, after myocardial infarction, nonclassical monocyte entry into the injured heart occurs mainly during later stages of inflammation (day 3 and beyond), where they are thought to coordinate tissue repair, including angiogenesis.31 On the other hand, nonclassical monocytes are not obviously recruited to sites of mild or severe skin inflammation, nor at sites of skeletal muscle injury.35 In these sites, however, there is evidence that robustly recruited classical monocytes acquire phenotypic features of nonclassical monocytes (upregulation of CX3CR1, induction of CD11c, loss of Gr-1/Ly-6C expression) and these cells in turn promote wound healing35 or egress to lymph nodes.27 Both the myocardial infarction study and the skeletal injury study proposed that classical monocytes were critical to setting the stage for resolution by clearing dead cell debris and that the nonclassical monocyte phenotype mediated the next stages in healing after debridement of the injured tissue.33,35

Both classical and nonclassical monocytes enter mouse atherosclerotic lesions,30 but the entry of classical monocytes into plaques occurs in greater magnitude,30,36 making classical monocytes a natural focal point as major mediators of atherosclerotic disease. It remains to be determined whether these populations maintain distinct phenotypes within plaques or whether one population is more injurious to plaque stability than the other. In general, numbers of nonclassical human monocytes rise in the context of inflammatory disease.14 However, it is not known whether this is true in peripheral arterial disease and whether elevations in this typically rare subset clinically correlate with coronary disease as well as or better than total monocyte counts do.4

Recent studies indicate that nonclassical monocytes, in particular, can become loaded with cholesterol by binding oxidized LDL in the circulation. Nonclassical mouse monocytes express CD11c, in contrast to their classical counterparts.30 Wu et al recently identified CD11c− monocytes in apoE knockout mice as the most prone among circulating monocytes to take up oxidized LDL.37 This uptake was limited to conditions of high-fat diet feeding but not observed during low-fat chow feeding. This finding is consistent with another recent study in which a significant portion, but not all mouse monocytes, took up minimally modified LDL but not native LDL in the circulation.38 Mosig et al studied the uptake of oxidatively modified LDL by human monocytes from patients with familial hypercholesterolemia ex vivo.39 In agreement with the mouse studies, human nonclassical monocytes from a highly hypercholesterolemic environment were selectively able to become cholesterol-loaded after incubation with oxidized LDL.39 These data raise the intriguing possibility that nonclassical monocytes, though they enter plaques at a reduced rate compared with classical monocytes, may be especially important as a source of cholesterol that is brought to plaques from the bloodstream under conditions of extreme hypercholesterolemia. Overall, however, much remains to be studied on the fate of monocyte-derived cell subsets in atherosclerosis, and it even remains possible that the 2 blood subsets do not maintain distinct functional roles or phenotypes (such as dendritic cell versus macrophage) within plaques. Even if they do maintain separate fates within plaque, their proportion relative to the differential degree to which they are recruited might shift, for example, if the subsets have differing half-lives or if they proliferate. More research in this area is required to address these possibilities.

Chemokine Receptors in the Mobilization, Recruitment, and Survival of Monocyte-Derived Cells and Its Relevance to Atherosclerosis

Since the central role of monocytes in atherosclerosis was recognized more than a decade ago, major efforts have focused on molecules involved in their recruitment and persistence within plaques. Deficiency or blockade of adhesion molecules including ICAM-1, VCAM-1, CD18/b2 integrin, and P-selectin slow down murine plaque development.40–43 Chemokine or chemokine receptors implicated in monocyte recruitment to plaques, typically studied by genetic deletion in mice, are numerous and include CCR2, its ligand CCL2/MCP-1, CXCR2, its ligand CXCL1/KC/Groα, CCR5, its ligand CCL5/RANTES, CXCL4/platelet factor 4, CX3CR1/fractalkine receptor, and its ligand CX3CL1/fractalkine.44–48,49–51 When 3 chemokine/chemokine receptor pairs—CCL2, CX3CR1, and CCR5—are simultaneously targeted, murine atherosclerosis is nearly absent,52 fitting with the importance and coordinated roles of these 3 molecules in the recruitment of monocyte subsets to atherosclerosis plaques (Figure 2). Reduction of mouse atherosclerosis is in fact markedly reduced even when only 2 of these pathways (CCL2 and CX3CR1 or CCR2 and CX3CL1) are genetically deleted,52,53 though additional inhibition is observed by interfering with CCR5.52

Deficiency in these molecules can alter accumulation of mouse monocyte-derived cells within plaques by means other than controlling their recruitment across the arterial endothelium overlying lesions. For example, deficiency in CCR2 has a profound impact on monocyte homeostasis. Indeed, classical monocyte mobilization from the bone marrow is dramatically impaired in the absence of CCR2 in mice (Figure 2).17,54 As a consequence, CCR2−/− mice have a marked decrease in the number of circulating classical monocytes,17,27,54 whereas nonclassical monocyte numbers appear relatively normal.27 Thus, the effect of CCR2 deficiency in ameliorating plaque growth is, at best, likely only partly a consequence of a role for CCR2 is direct recruitment of monocytes into plaques,17,30,52 As another example, many chemokines act as scavenger receptors with the capacity to bind oxidized LDL.
In turn, such binding interferes with their function. Interestingly, neither CCL-2 nor CX3CL1, the ligands of CCR2 and CX3CR1, bind oxidized LDL, possibly allowing unabated recruitment of monocyte subsets into plaques while the function of other chemokines, such as CCR7 ligands that may mediate migration of monocyte-derived cells out of plaques, are impaired. The CXCR6 ligand CXCL16 is a rare cleavable transmembrane chemokine, and was the first chemokine shown to function as a scavenger receptor. In mice, CXCL16 deficiency leads to heightened atherosclerosis. Its expression on human cultured macrophages allows it to induce cholesterol efflux genes in response to oxidized LDL binding. Thus, the concept emerges that CXCL16 on macrophages generates an atheroprotective response after recognition of oxidized LDL.

The Multiple Roles of CX3CR1 in Monocyte Biology and Atherosclerosis

The homeostasis of nonclassical monocytes, which express approximately 2-fold higher levels of surface CX3CR1 than classical monocytes, is altered in CX3CR1-deficient mice. Their number in the peripheral blood is mildly decreased in CX3CR1 wild-type (WT) vs. CX3CR1-deficient mice. This decreased survival stemming from a lack of CX3CR1 and CXCR5, abated recruitment of monocyte subsets into plaques while the function of other chemokines, such as CCR7 ligands that may mediate migration of monocyte-derived cells out of plaques, are impaired. The CXCR6 ligand CXCL16 is a rare cleavable transmembrane chemokine, and was the first chemokine shown to function as a scavenger receptor. In mice, CXCL16 deficiency leads to heightened atherosclerosis. Its expression on human cultured macrophages allows it to induce cholesterol efflux genes in response to oxidized LDL binding. Thus, the concept emerges that CXCL16 on macrophages generates an atheroprotective response after recognition of oxidized LDL.

This suppression may be related to impaired survival as much as or more than a role for CX3CR1 in monocyte migration to plaques, because introduction of a hBcl-2 expression vector regulated by the myeloid-restricted MRP8 promoter corrected the decreased survival stemming from a lack of CX3CR1 and abolished the antiatherogenic effect of CX3CR1 deficiency. However, hBcl-2 overexpression increased plaque development in CX3CR1-/-/ control mice as substantially as it did in CX3CR1-/-/ mice. Thus, it remains possible that enhanced survival independently influences plaque development so greatly that other effects of CX3CR1, such as roles in adhesion and migration, are hard to discern. In any case, regulation of monocyte-derived cell survival during or on recruitment to tissues is generally relevant to atherosclerosis progression.

The consideration of an important role for CX3CR1 in monocyte and monocyte-derived cell survival adds one more function of this molecule to its well-established roles in adhesion or migration (its ligand CX3CL1 is the only other known transmembrane chemokine besides CXCL16), chemotaxis, and intercellular interactions between cells within plaques. Indeed, it is likely that classical monocytes, the major subset entering plaques, require CX3CR1 for interacting with or traversing the endothelium. Nonetheless, it is often assumed that the dominant role of CX3CR1 is in adhesion or chemotaxis, when in fact its role is not always clear. For example, Nahrendorf et al argued that absence of Ly6C

Figure 2. Cartoon depicts the life cycle of monocyte subsets and their recruitment to atherosclerotic plaques, with an emphasis on the role of chemokine receptors in these processes. Classical monocytes leave the bone marrow in a CCR2-dependent manner. It remains unknown whether the development of nonclassical monocytes requires incubation in the bone marrow and mobilization into the bloodstream thereafter. In the bloodstream, the chemokine receptor CX3CR1 regulates the survival of nonclassical monocytes selectively, whereas the cytokine M-CSF, also a critical factor in atherosclerotic plaque development, controls survival of monocytes generally. Chemokine receptors CCR2, CX3CR1, and CXCR5 are differentially involved in monocyte subset recruitment into plaques. Once in plaques, the persistence of monocyte-derived cells stemming from either subset appears to be regulated by CX3CR1, and it may also control their retention.
nonclassical monocyte-like cells in infarcted hearts of CX3CR1-deficient mice 7 days after infarction was attributable to failure of nonclassical monocytes to be recruited to the heart in the absence of CX3CR1.33 Another viable possibility, however, is that these cells did not survive, or that Ly6C<hi> classical monocytes, recruited earlier to infarcted hearts,33 differentiate into nonclassical monocyte-like cells in situ and need CX3CR1 to survive during this differentiation.

The role of CX3CR1 in affecting the net accumulation of monocyte-derived cells, whether acting at the level of recruitment, retention, or survival, is hard to predict, as it appears to vary depending on the context. Absence of one copy of CX3CR1 is sufficient to impair macrophage accumulation in atherosclerotic plaques50 and also suppresses the accumulation of some populations in the steady state, such as CD11b<sup>+</sup> pulmonary DCs, but leaves many others intact (Jakubzick and Randolph, unpublished observations, 2004). Furthermore, net accumulation of monocyte-derived cells in sterile peritonitis is unaffected between 12 hours and 3 days,64 even though early recruitment of nonclassical monocytes after instillation of Listeria into the peritoneum appears suppressed.23 Deficiency in CX3CR1 ligand, CX3CL1/fractalkine, also does not lead to major reductions in macrophage accumulation in a variety of inflammatory insults including peritonitis, enterocolitis, and Listeria challenge, even though the role of fractalkine in blood monocyte survival is likely similar to that of its receptor CX3CR1.65

**Targeting Chemokines to Control Monocyte Accumulation in Human Atherosclerosis**

Because compound deficiency of 2 or more distinct chemokine/chemokine receptor pairs produces a stronger impact on plaque development than targeting only one chemokine/chemokine receptor pair,22,53 targeting multiple chemokine pathways may be ideal in atherosclerosis. One risk, of course, is the enhanced probability of side effects resulting from impaired monocyte recruitment in other organs and during other important inflammatory responses. The fact that lack of CX3CR1 gives rise to subtle changes systemically with more dramatic effects in mouse atherosclerotic plaques, as discussed above, suggests that its antagonism may not be dangerously disruptive to innate or adaptive responses. In contrast, CCR2 deficiency reveals a much greater impact on systemic innate and adaptive responses.66–68 Another risk is the potential for lack of efficacy. It is remarkable that attempts to prevent disease by eliminating monocytes2 or abrogate pathways like CCR2-mediated mobilization and recruitment69 once atherosclerosis has reached a highly progressed state are not markedly successful. Because treating advanced disease in humans is more practical at present than treating with compounds to prevent disease, more research effort is needed on how to modify monocyte-derived cell accumulation within advanced plaques. Rather than preventing recruitment of monocytes, therapies that focus on causing monocyte-derived cells to avoid retention in plaque and instead emigrate out of lesions50,71 or those that appropriately modulate survival within plaques62 may be more beneficial. Besides potentially participating in recruitment, CX3CR1 has been implicated in both retention72 and survival of monocytes and monocyte-derived cells within plaques.18,60 Moreover, CX3CR1 is one of the few targets already validated in humans, because the M280 polymorphism in the human CX3CR1 gene clearly protects against cardiovascular events.73–75 Polymorphisms in other chemokine receptors, such as CCR5,63 have also been implicated in affecting human atherosclerosis, but the data are strongest for CX3CR1. Inhibition of CX3CR1, therefore, may be a suitable clinical target in humans. A human CX3CR1 antagonist was recently described,76 opening the door to clinical testing. On the other hand, the negative consequences of the CX3CR1 polymorphism, including increased progression of HIV77–79 and enhanced macular degeneration and retinal vasculitis,80–82 serve as reminders of the considerable challenges in therapeutically targeting chemokines that control human monocyte migration and survival in atherosclerosis. One way around the unwanted systemic side effect of chemokine antagonists may be to target chemokine complexes that may be more uniquely localized to plaques48 or to develop delivery methods that would restrict therapeutic availability and action to plaques. Altogether, at present, it remains unclear whether targeting chemokines to treat atherosclerosis can limit monocyte accumulation in plaques at stages relevant for treatment in humans and do so without impairing immunity during acute inflammatory reactions or infections.

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**Disclosures**

None.

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mice is at the brachiocephalic artery, not the aortic root. Proc Natl Acad Sci U S A. 2004;101:17795–17800.


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