Monocytes are the primary inflammatory cell type that infiltrates early atherosclerotic plaques,1 and considerable evidence implicates monocytes as critical to the development of atherosclerosis.2–7 Atherosclerotic lesion size correlates strongly with the number of circulating monocytes, and combined inhibition of the monocyte recruitment factors chemokine (C-C motif) ligand 2 (CCL2), CX3C chemokine receptor 1 (CX3CR1), and C-C chemokine receptor 5 (CCR5) nearly abolishes atherosclerotic development in hypercholesterolemic mice.5 In mouse models of cardiac injury, the depletion of monocytes leads to higher mortality and reduced ventricular function, suggesting that monocytes are necessary for proper wound healing and rebuilding of heart tissue.8 Thus, understanding the role of monocyte activities in the vasculature, cardiac injury, and inflammatory disease is important to understanding the cause of cardiovascular disease and potential therapeutic targeting of monocyte activities.

Nonclassical Patrolling Monocytes Function in the Vasculature

Graham Thomas, Robert Tacke, Catherine C. Hedrick, Richard N. Hanna

Abstract—Nonclassical patrolling monocytes are characterized by their unique ability to actively patrol the vascular endothelium under homeostatic and inflammatory conditions. Patrolling monocyte subsets (CX3CR1highLy6C− in mouse and CX3CR1highCD14dimCD16+ in humans) are distinct from the classical monocyte subsets (CCR2highLy6C+ in mouse and CCR2highCD14+CD16− in humans) and exhibit unique functions in the vasculature and inflammatory disease. Patrolling monocytes function in several disease settings to remove damaged cells and debris from the vasculature and have been associated with wound healing and the resolution of inflammation in damaged tissues. This review highlights the unique functions of these patrolling monocytes in the vasculature and during inflammation. (Arterioscler Thromb Vasc Biol. 2015;35:1306-1316. DOI: 10.1161/ATVBAHA.114.304650.)

Key Words: atherosclerosis ■ inflammation ■ macrophages ■ monocytes

Monocytes are short-lived mononuclear phagocytes that are continuously replaced throughout life from a common committed...
followed by a gradual displacement of the Ly6C+ population by Ly6C− monocytes independent of Ly6C+ monocytes, however, this does not exclude the existence of an alternative route for Ly6C− monocytes development. Several studies support the notion that Ly6C+ monocytes give rise to Ly6C− monocytes in vivo, although studies of nonclassical monocyte differentiation have yet to be conducted. In mice, monocytes mostly live only 2 days in circulation in the absence of inflammation, but a small fraction can live ≥7 days. Nonclassical Ly6C− monocytes are generally longer lived than classical Ly6C+ monocytes. In the absence of classical Ly6C+ monocytes, the half-life of nonclassical Ly6C− monocytes is increased from 2.5 to 11 days suggesting an increased surveillance function of nonclassical patrolling monocytes in the vasculature when the classical monocyte population has been disrupted or recruited to inflammatory sites.

Monocytes develop predominantly in the bone marrow from a CD117+CD115+CX3CR1+ monocyte dendritic cell precursor that also gives rise to dendritic cells. Recently, a common monocyte progenitor subset of the monocyte dendritic cell precursor was described that has potential to produce both monocyte subsets but not dendritic cells, and are defined as CD117+CD115+Ly6C+Flt3−. Several studies support the notion that Ly6C+ monocytes give rise to Ly6C− monocytes. BrdU pulse chase studies have shown rapid incorporation of the thymidine analogue into the DNA of Ly6C+ monocytes followed by a gradual displacement of the Ly6C+ population by Ly6C− monocytes in the BrdU-labeled fraction. Adoptive transfer studies have shown that congenically labeled Ly6C+ monocytes give rise to Ly6C− monocytes 1 to 3 days post transfer. Corresponding studies of human monocyte subset differentiation and lifespan have yet to be conducted. Ly6C+ monocytes can give rise to Ly6C− monocytes in vivo, however, this does not exclude the existence of an alternative route for Ly6C− monocytes development independent of the Ly6C+ population. Indeed, genetic evidence for this proposal exists. Two myeloid determining transcription factors, interferon regulatory factor 8 (IRF8) or Kruppel-like factor 4 (KLF4), both specifically regulate Ly6C+ monocyte production without affecting Ly6C− monocyte numbers. Studies of either global IRF8−/− mice or fetal liver transplant of KLF4−/− marrow. Consequently, CCR2−/− mice have reduced Ly6C+ monocytes in the periphery, but an increased frequency of Ly6C− monocytes in bone marrow, whereas the distribution of Ly6C− monocytes in these mice is relatively normal.

Less is known about the molecular pathways governing the emigration of nonclassical monocytes from the bone marrow and their recruitment to sites of vascular damage or inflammation (Figure). Ly6C− monocytes are recruited to sites of atherosclerosis, which was reduced ≈40% with CCR5 blockade, suggesting that CCR5 expression is at least partially responsible for nonclassical monocyte recruitment. However, CCR5 expression is found at low levels on patrolling monocytes putting into question whether this recruitment is directly acting on patrolling monocytes. Recent insight on patrolling monocyte recruitment has been gained from functional studies of sphingosine-1-phosphate (S1P) receptors, which are major regulators of leukocyte activation and trafficking. Administration of the S1P agonist FTY720 facilitates the internalization and degradation of S1P receptors and leads to a reduction in monocyte egress from the BM and spleen.

include the enhanced survival of Ly6C− monocytes in these models. Therefore, it remains possible that Ly6C− monocytes derive from either blood or bone marrow Ly6C+ monocytes, from an independent bone marrow monocyte progenitor, or from a combination of all 3 of these scenarios. To resolve these issues, a detailed understanding of the factors and pathways regulating the development and survival of both Ly6C+ and Ly6C− monocyte populations will be required.

A major advance in our understanding of patrolling nonclassical monocyte differentiation was made with the discovery that the transcription factor Nur77, encoded for by the gene NR4A1, is absolutely required for Ly6C− monocyte development. Nur77 is highly expressed in patrolling monocytes, and patrolling monocytes are specifically missing in the blood, spleen, and bone marrow of Nur77 knockout mice. The few patrolling monocytes remaining in the bone marrow of Nur77 knockout mice are arrested in S phase of the cell cycle and undergo apoptosis, implying that Nur77 functions as a master regulator of the differentiation and survival of nonclassical patrolling monocytes from myeloid progenitors in the bone marrow. Unlike the chemokine receptor-deficient models used to study monocyte function (discussed below), Nur77-deficient mice lack Ly6C− monocytes both in the bone marrow and periphery. In the homologous human CD14dimCD16+ population of patrolling monocytes, there is also high Nur77 expression, and likely common function.

Mechanisms of Monocyte Recruitment, Adherence, Patrolling, and Survival in the Vasculature

Mature classical and nonclassical monocytes must exit the bone marrow into the peripheral circulation to perform their duties. The migratory properties of monocytes are facilitated by the actions of chemokines, and both classical and nonclassical monocytes express a different repertoire of chemokine receptors enabling their differential mobilization from the BM into tissues. Classical monocytes express high levels of CCR2 and migrate from the BM into the vascular circulation in response to its ligands CCL2, CCL7, and CCL12. Consequently, CCR2−/− mice have reduced Ly6C+ monocytes in the periphery, but an increased frequency of Ly6C− monocytes in bone marrow, whereas the distribution of Ly6C− monocytes in these mice is relatively normal.

Nonstandard Abbreviations and Acronyms

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>CCL</td>
<td>chemokine (C-C motif) ligand</td>
</tr>
<tr>
<td>CCR</td>
<td>C-C chemokine receptor</td>
</tr>
<tr>
<td>CX3CL1</td>
<td>chemokine (C-X3-C motif) ligand 1</td>
</tr>
<tr>
<td>CX3CR1</td>
<td>CX3C chemokine receptor 1</td>
</tr>
<tr>
<td>ICAM</td>
<td>intercellular adhesion molecule</td>
</tr>
<tr>
<td>KLF</td>
<td>Kruppel-like factor</td>
</tr>
<tr>
<td>LFA1</td>
<td>lymphocyte function–associated antigen-1</td>
</tr>
<tr>
<td>IL</td>
<td>interleukin</td>
</tr>
<tr>
<td>IRF</td>
<td>interferon regulatory factor</td>
</tr>
<tr>
<td>MI</td>
<td>myocardial infarction</td>
</tr>
<tr>
<td>S1P</td>
<td>sphingosine-1-phosphate</td>
</tr>
<tr>
<td>TLR</td>
<td>toll-like receptor</td>
</tr>
<tr>
<td>TNF</td>
<td>tumor necrosis factor</td>
</tr>
</tbody>
</table>
Localized FTY720 application can selectively enhance recruitment of patrolling monocytes to ischemic vessels in a S1PR3-dependent manner, suggesting S1P can regulate recruitment of patrolling monocytes. Furthermore, S1PR5 is highly expressed on patrolling monocytes and is important for their homeostasis. S1PR5−/− mice have greatly reduced blood-circulating and splenic Ly6C− monocytes, but have normal numbers in the bone marrow. This effect was attributed to defective cell intrinsic emigration of S1PR5-deficient monocytes from bone marrow because the survival of intravenously transferred S1PR5-deficient Ly6C− monocytes was comparable with wild-type controls.

Patrolling monocytes interact with the vascular endothelium via the β2 integrin, lymphocyte function–associated antigen-1 (LFA-1), and the chemokine receptor CX3CR1. A pioneering study by Auffray et al described the characteristic patrolling behavior that nonclassical monocytes display on the luminal side of the vascular endothelium. Human CD14dim monocytes have also demonstrated patrolling behavior when infused into mice. This patrolling is a much slower process (≈12 μm/min) than the rolling process of classical monocytes and is independent of the direction of blood flow. Because in vivo analysis of patrolling function of monocytes is difficult to assess in humans, the majority of studies examining nonclassical monocyte patrolling function and activities have used mouse models. Patrolling is in part dependent on the expression of LFA-1, which is highly expressed by nonclassical monocytes. Antibody blockade of either subunit of the LFA-1 complex, CD11a or CD18, leads to the rapid and prolonged disassociation of nonclassical monocytes from the endothelium in vivo and abolishes patrolling behavior. The LFA-1 ligands, intercellular adhesion molecule 1 (ICAM1) and ICAM2, act in a redundant manner to facilitate LFA-1–dependent adhesion. ICAM1/2−/− mice have a greatly reduced frequency of nonclassical monocyte adhesion to the vasculature, which is a phenocopy of the CD11a−/− (ITGAL) mice. The capacity for other integrin complexes to mediate patrolling has not been shown; however, studies to date cannot rule this out. Interestingly, neutrophil arrest in the mouse is entirely dependent on LFA-1, whereas human neutrophils use both LFA-1 and MAC-1 (macrophage-1 antigen) (ITGAM/CD11b). Whether this is also the case in monocyte patrolling is not known. Furthermore, neutrophil arrest requires integrin conformational changes that allow for high affinity binding. This process involves cytoskeletal adaptor proteins, Talin-1 and Kindlin-3. The requirement for integrin high affinity binding conformational changes in monocyte patrolling, as well as the requirement for integrin activating adaptor proteins, is not known and will be important to understanding how the process of patrolling takes place.

Both classical and nonclassical monocyte subsets express the chemokine receptor CX3CR1, although patrolling monocytes express it at much higher levels. CX3CR1high patrolling monocytes respond to chemokine (C-X3-C motif) ligand 1 (CX3CL1; also known as fractalkine), a chemokine that is expressed in tissues and can be used as an apoptotic cell recognition receptor. CX3CR1 deletion reduces the patrolling of Ly6C− monocytes and recruitment to the spleen during bacterial infection. CX3CR1 also provides a survival signal to Ly6C− monocytes. CX3CR1−/− mice have fewer circulating Ly6C− monocytes, which has been attributed to reduced monocyte survival resulting from CX3CR1-dependent expression of the antiapoptotic protein BCL2. In this context, it is interesting to note that global interleukin (IL) 17 receptor A deficiency leads to a similar reduction in Ly6C− monocyte survival, and that BCL2 is an IL17 target gene in fibroblast-like synoviocytes. However, a causal link between interleukin 17 receptor A–dependent signaling and BCL2 expression in patrolling monocytes has not been established. Although CX3CR1 is dispensable for the initiation of monocyte patrolling, CX3CR1–CX3CL1 interactions stabilize patrolling in a CD11b-dependent manner. Specifically, toll-like receptor 7 (TLR7) agonists mediate an increase in epithelial CX3CL1 expression that led to a direct increase in the retention time of patrolling monocytes at the epithelial surface.

Infection and tissue damage are not the only environmental cues that result in monocyte mobilization. Other physical factors, such as exercise and stress, have been demonstrated to recruit monocytes into circulation. Exercise leads to more than a 4-fold increase of nonclassical monocytes in the blood. This exercise-induced increase can be inhibited by β-adrenergic receptors blockade suggesting a catecholamine-dependent mechanism of recruitment. Studies have shown
that monocytes express β-adrenergic receptors and rapidly appear in circulation from vascular marginal pools, a process called demargination, in response to catecholamine release. Chronic stress also induces a β3-adrenergic receptor–dependent increase in monocyte progenitors in bone marrow, and classical monocytes in circulation, promoting atherosclerotic development. In addition to their progenitors, monocytes themselves also express β-adrenergic receptors, thus future studies to learn how stress and exercise-induced β-adrenergic receptor signaling directly affects monocyte function in the vasculature and atherosclerotic development should be of interest.

**Activation Activities of Patrolling Monocytes**

Classical monocytes circulate in the bloodstream and respond to inflammatory cues, such as microbial products, cytokines, and chemokines, produced by infected or damaged tissue. In the presence of inflammatory stimuli, circulating inflammatory monocytes can quickly extravasate into affected nonlymphoid tissues in a CCR2–CCL2–dependent manner, where they differentiate into macrophage and dendritic cell subsets. On stimulation, these monocytes produce inflammatory cytokines, such as IL12, nitric oxide, tumor necrosis factor-α (TNF-α), and IL1, and can differentiate into TNF-α–producing inflammatory macrophages and dendritic cells. These responses result in not only increased immune activation and bacterial clearance, but also significant tissue damage.

Patrolling monocytes, however, produce only low levels of proinflammatory cytokines in response to bacteria-derived stimuli, such as lipopolysaccharides and PAM3C.K4, but high levels of anti-inflammatory and wound healing factors, such as IL1 receptor antagonist, IL10R, and ApoA. These expression studies identify several protein expression between monocyte subsets in humans and mouse. Patrolling monocytes express lower levels of CD14 (a TLR4 coreceptor), suggesting a weaker lipopolysaccharides response. However, little is known about the ability of patrolling monocytes to extravasate and differentiate into effector macrophage populations in nonhomeostatic conditions. Interestingly, murine patrolling monocytes are the first immune cell detected to extravasate into the peritoneum following listeria monocytogenes infection, and produce high levels of TNF-α showing that these cells are not always anti-inflammatory counterparts to the proinflammatory classical monocytes. In addition, CD16+ human monocyte stimulated with tumor cells showed enhanced production of TNF-α, IL12, and nitric oxide compared with classical monocytes, suggesting that nonclassical monocytes may be an important subpopulation of monocytes involved in antitumor response. Furthermore, both human and murine patrolling monocytes strongly respond to viruses and nucleic acids via a TLR7/8-MEK pathway by producing proinflammatory cytokines like TNF-α and IL1β, as well as chemokines CCL3, CCL5, and CXCL10. Nonclassical monocytes can recruit neutrophils that function to kill damaged or infected cells in response to nucleic acid–derived TLR7-mediated danger signals within the vasculature. TLR7 activation results in intravascular retention of Ly6C+ monocytes and subsequent removal of cellular debris by the retained monocyte. It is not known if other cell types are directly recruited by patrolling monocytes in response to CCL3, CCL5, and CCL10 production, though this is of high interest to the field. In the absence of inflammatory cues, patrolling monocytes scan the vasculature and uptake microparticles along the endothelium. Thus, these cells function as intravascular housekeepers that scavenge microparticles, remove cellular debris from the vasculature, and can vigorously respond to cell damage and infection. Therefore, it is possible that nonclassical monocytes function as terminally differentiated cells.

In addition to uptake of cellular debris, patrolling monocytes engulf apoptotic cells and can then cross present antigen from the engulfed cell to T cells in the spleen. Interestingly, these antigens presenting Ly6C+ monocytes express high levels of PDL1 following apoptotic cell engulfment and suppress endogenous antigen-specific T cell responses. Thus, Ly6C+ monocytes may contribute to self-antigen tolerance. Studies in human monocytes show that CD16+ nonclassical monocytes express ILT4, a receptor that binds HLA-G. The ILT4/HLA-G interaction is associated with fetal tolerance during pregnancy. Taken together these studies in mouse and man suggest that patrolling monocytes function in some capacity in antigen presentation to T cells and may aid in eliciting a tolerogenic response.

**Comparisons of Human and Mouse Patrolling Monocytes**

Much of our knowledge about the functions of human nonclassical monocytes is derived from comparative transcriptomic analyses. Several studies have compared gene and protein expression between monocyte subsets in humans and mouse. These expression studies identify several unique genes and activities in the patrolling monocyte population (Table 1). Two independent studies have provided transcriptional array data of all 3 human monocyte subsets and show that the intermediate (CD14+CD16+) monocytes share a transcriptional repertoire that is in between that of both the

**Table 1. Genes Upregulated in Nonclassical Monocytes in Both Humans and Mice Grouped by Function**

<table>
<thead>
<tr>
<th>Cellular Function</th>
<th>Gene Name</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lineage-determining receptor</td>
<td>CSFRI</td>
<td>50</td>
</tr>
<tr>
<td>Survival/differentiation</td>
<td>Nur77 (NR4A1), BCL2A1A, DUSP5, HES1, OCT2 (POU2F2), TCF7L2</td>
<td>29,50,57</td>
</tr>
<tr>
<td>Adhesion and patrolling</td>
<td>CX3CR1, CD43 (SPN), CD11a (ITGAL/LFA-1), CD11c (ITGAX), CD31 (PECAM1), RRAS</td>
<td>16,50</td>
</tr>
<tr>
<td>Immune regulation</td>
<td>CD16 (FCGR3), TNF, IL10R, IL1B, IL6, IL1RA, TGFBR3, LAR1, LTB, KLRE1, GZMA</td>
<td>10,16,50,57</td>
</tr>
<tr>
<td>Viral immunity</td>
<td>TL7R, IFITM1, IFITM2, IFITM3</td>
<td>10,54,57</td>
</tr>
</tbody>
</table>

*In response to toll-like receptor 7 (TLR7) stimulation.
nonclassical (CD14+CD16−) and classical (CD14+CD16+) subsets.10,50 Although there is disagreement as to which subset the intermediate monocyte is more closely related, this might simply reflect differences in placement of the gating used to sort this population by flow cytometry. Nevertheless, the intermediate monocyte subset only represents a minor frequency (5% to 10%) of the total nonclassical subset,59 thus the inclusion of this population should have little confounding influence on the interpretation of data comparing the mouse and human classical and nonclassical subsets.

Ingersoll et al50 published a detailed comparative analysis between the human classical CD14+CD16− and nonclassical CD14+CD16+ subsets and the mouse Ly6C− and Ly6C+ populations. This analysis demonstrated statistically significant similarities in the global gene expression profiles of the mouse and human classical and nonclassical subsets alike.50 Cros et al50 independently confirmed these findings and went on to show that human CD14+CD16+ monocytes patrol the vasculature of lymphoid deficient RAG1−/−IL2RG−/−CX3CR1GFP mice. Genomic studies also verify that nonclassical monocytes have increased metabolic activities and efficient cytoskeletal dynamics that are indicative of actively patrolling cells.54,55 These data provide compelling evidence that the human CD14+CD16+ population represent a functionally orthologous cell subset to the mouse Ly6C− patrolling monocyte.

Conserved upregulated genes encode many of the proteins that impart the key mechanistic features of the nonclassical subset, these include the patrolling-associated integrin LFA-1, the chemokine receptor CX3CR1, as well as the inflammatory cytokine TNF. In total, 63 genes were more abundant in both human and mouse nonclassical monocytes relative to their classical counterparts.50 The roles of most of these genes in monocyte biology are yet to be determined, but future studies will likely provide significant new insight into the functions of these cells. Other functional similarities between the mouse and human nonclassical monocytes include an attenuated response to stimulation with the TLR4 agonist lipopolysaccharide (because of relatively low lipopolysaccharide/TLR4 coreceptor CD14 expression) and a heightened response to the viral DNA sensor TLR7 (because of relatively high TLR7 expression).10,16

Although global similarities have been drawn between the human CD14+CD16+ monocytes and Ly6C+ monocytes, there are notable differences. For example, the transcription factor PPARγ (peroxisome proliferator-activated receptor gamma) and known target genes, including CD36 and FABP4, are higher in murine Ly6C+ monocytes, but not observed in human CD16+ monocytes. Importantly, mouse nonclassical monocytes also expressed higher levels of genes involved in apoptotic cell uptake, including Tgm2, Trem14, CD36, and CD51, whereas these genes were more highly expressed by human CD14+ classical monocytes, suggesting potential differences in apoptotic cell recognition.50 Major histocompatibility complex class II expression is also low/absent on mouse Ly6C+ patrolling monocytes, but high on human patrolling monocytes, which may suggest better presentation of certain antigens by the human subset.16,50 In total, Ingersoll et al50 found 33 genes to be reciprocally regulated between the human and mouse subsets. Thus, some caution must be made in making direct comparisons in patrolling monocyte function between species.

A recent ChIP-Seq (chromatin immunoprecipitation sequencing) analysis of primary human classical and nonclassical monocytes has provided mechanistic insight into transcriptional differences between these cell types.54 Transcription factor-binding motifs for the CEBP (CCAAT-enhancer-binding proteins) and ETS (E26 transformation-specific) factors are enriched in the enhancers of all monocytes. This result is expected as these motifs are bound by the myeloid pioneer transcription factors, CEBP and PU.1, which collaborate to drive the myeloid gene expression program.58 Transcription factor-binding sites for KLF, IRF, and NR4A transcription factors were more abundant in the nonclassical subset. Currently, no obligatory roles for KLF or IRF transcription factors have been implicated in nonclassical monocyte function or development although KLF2 is more abundant in CD16+ monocytes.57 However, the nuclear receptor transcription factor NR4A1 is crucial for Ly6C+ monocyte development.59 Studies of mouse monocyte enhancer activity will inform whether or not the same enrichment for IRF and KLF factors is conserved between species.

Roles in Disease
The unique ability of nonclassical monocytes to actively patrol the vasculature and potential capacity to resolve inflammation make them attractive targets for disease therapy. Generally, nonclassical monocytes are thought to be involved in the resolution of inflammation and differentiate into resident macrophage populations that work to heal wounding and resolve inflammation.17 Studies have suggested that patrolling monocytes can preferentially differentiate into CD11c+ resident lung macrophages, implying a specialized role for these effector cells in the lung.20,42 There is also some evidence that patrolling monocytes can differentiate into resident or anti-inflammatory M2-like macrophages, but the evidence for this is far from definitive and may be specific for the tissue or stimulus. Others have found that recruitment of patrolling monocytes and subsequent monocyte-mediated inflammatory responses (including TNF and reactive oxygen species production) in certain tissues may potentially aggravate autoimmune diseases, such as lupus nephritis and arthritis-induced joint inflammation. These findings are summarized below and in Table 2.

Atherosclerosis
Currently, little is known about the importance of nonclassical monocytes and their role in atherosclerotic development. Tracking studies have demonstrated that both classical and nonclassical subsets can enter atherosclerotic plaques in mice.53 Atherosclerotic lesion size has been shown to positively correlate with the number of both Ly6C+ and Ly6C− circulating monocyte subsets.5 Quantitative intravital imaging has also demonstrated a substantial accumulation of nonclassical mouse monocytes in lesions that can be reduced with statin treatment.50 In blood draws from APOE-deficient mice on high fat diet, there is an increase in the percentage of
classical monocytes and decrease in the nonclassical population. However, low numbers of patrolling monocytes analyzed in blood draws could be because of increased adherence and patrolling of nonclassical monocytes in the vasculature and not because of a true reduction in cell number, although this has yet to be properly examined.

Experiments suggest that the Ly6C⁺ and Ly6C⁻ monocyte subsets use different chemokines for recruitment to atherosclerotic lesions. Ly6C⁻ monocyte recruitment to sites of atherosclerosis was reduced ≈40% with CCR5 blockade, whereas Ly6C⁺ monocyte recruitment was reduced ≈50% with either CCR5, CCR2, or CX3CR1 blockade. These findings suggest that CCR5 expression is at least partially responsible for nonclassical monocyte recruitment, but there are likely other recruitment factors yet to be identified.

Nonclassical monocytes actively patrol the vasculature and have been shown to phagocytize microparticles and mediate the removal of damaged endothelial cells in the vasculature. Nonclassical Ly6C⁺ monocytes can preferentially scavenge and accumulate lipids, including oxidized low-density lipoprotein, from the vasculature and increase in numbers in response to oxidized low-density lipoprotein treatment. Likewise in patients with familial hypercholesteremia, CD16⁺ monocytes preferentially uptake oxidized low-density lipoprotein. Therefore, it is easy to speculate that nonclassical monocytes may work to repair the vasculature or remove lipids from circulation under damaging inflammatory atherosclerotic conditions.

It has also been suggested that nonclassical monocytes may differentiate into resident anti-inflammatory M2-like macrophage populations that could suppress inflammatory conditions in atherosclerotic plaques. However, a recent study suggests that although monocyte recruitment is important for early plaque development, it is local macrophage proliferation and not continual monocyte recruitment that dominate atherosclerotic plaque growth. This provocative possibility, in its early stage of investigation, merits closer study.

### Table 2. Observed Activities of Nonclassical Monocytes in Disease

<table>
<thead>
<tr>
<th>Disease</th>
<th>Observed Activity</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atherosclerosis</td>
<td>CCR5 mediated recruitment to atherosclerotic plaques and reduced accumulation with statin treatment</td>
<td>19,59</td>
</tr>
<tr>
<td></td>
<td>Number of circulating cells positively correlated with atherosclerotic lesion size</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>Regulated survival and recruitment to atherosclerotic plaques by CX3CR1</td>
<td>17,42</td>
</tr>
<tr>
<td></td>
<td>Nur77 hematopoietic knockout mice with absence of patrolling monocytes develop increased atherosclerosis and inflammatory macrophage content</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>Remove lipids including oxLDL from circulation, and increased numbers in circulation in response to oxLDL treatment</td>
<td>60</td>
</tr>
<tr>
<td></td>
<td>Preferential uptake of oxLDL by CD16⁺ monocytes in hypercholesterolemic patients</td>
<td>61</td>
</tr>
<tr>
<td></td>
<td>Inversely correlated with HDL cholesterol levels and associated with increased APOE4 expression in hypercholesterolemic patients</td>
<td>62</td>
</tr>
<tr>
<td></td>
<td>Percentage of cells in circulation correlated with small HDL levels</td>
<td>63</td>
</tr>
<tr>
<td></td>
<td>Circulating CD16⁺ monocyte levels positively correlated with vulnerable plaques in patients with coronary heart disease</td>
<td>64–66</td>
</tr>
<tr>
<td>MI and vascular wounding</td>
<td>Recruitment during the reparative phase of MI</td>
<td>67,68</td>
</tr>
<tr>
<td></td>
<td>Higher numbers in circulation of MI patients that did not develop ventricular thrombus formations</td>
<td>69</td>
</tr>
<tr>
<td></td>
<td>Nur77 hematopoietic knockout mice have adverse cardiac remodeling post MI</td>
<td>70</td>
</tr>
<tr>
<td></td>
<td>Recruitment associated with the recovery of vascular flow and a regenerative phenotype in a hindlimb ischemia model</td>
<td>71</td>
</tr>
<tr>
<td></td>
<td>S1PR3 mediated recruitment and correlation with arteriogenesis in ischemic microvessels</td>
<td>36</td>
</tr>
<tr>
<td></td>
<td>Redundant role in progression and recovery of ischemic stroke</td>
<td>72</td>
</tr>
<tr>
<td>Neurological disease and damage</td>
<td>Attracted to and actively remove amyloid-β peptides from brain vasculature</td>
<td>73</td>
</tr>
<tr>
<td></td>
<td>Differentiate into perivascular macrophages and important role in maintaining blood–brain barrier</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td>Prevent excitotoxity and neuronal cell death</td>
<td>74</td>
</tr>
<tr>
<td></td>
<td>Beneficial recruitment to the injured spinal cord</td>
<td>75</td>
</tr>
<tr>
<td>Lupus and kidney disease</td>
<td>Accumulation of nonclassical monocytes to glomerular vessels</td>
<td>10,76</td>
</tr>
<tr>
<td></td>
<td>Accumulation associated with elevated levels of CX3CL1, proliferative glomerular lupus nephritis lesions, and disease activity</td>
<td>76</td>
</tr>
<tr>
<td></td>
<td>Accumulate, recruit neutrophils, and remove damaged endothelial cell in the kidney vasculature in response to a TLR7-induced danger signal</td>
<td>16</td>
</tr>
<tr>
<td>Arthritis</td>
<td>Critical for the initiation and progression of sterile joint inflammation, but derived macrophages may also be important for arthritic resolution</td>
<td>77</td>
</tr>
<tr>
<td></td>
<td>Increased circulating levels in patients with rheumatoid arthritis associated with elevated levels of C-reactive protein, rheumatoid factor, and more active disease</td>
<td>78,79</td>
</tr>
</tbody>
</table>

CCR5 indicates C-C chemokine receptor 5; CX3CL1, chemokine (C-X3-C motif) ligand 1; CX3CR1, CX3C chemokine receptor 1; HDL, high-density lipoprotein, MI, myocardial infarction; oxLDL, oxidized low-density lipoprotein; and TLR7, toll-like receptor 7.
Work in our laboratory has identified that in the absence of Nur77 and patrolling monocytes, the remaining macrophage populations are polarized to a more inflammatory phenotype leading to increased atherosclerotic development.30 Macrophages from Nur77−/− mice express relatively high levels of TNF-α and nitric oxide, and low expression of arginase-I in response to inflammatory stimuli. Three independent studies have confirmed a direct role of Nur77 in resolving myeloid cell–mediated inflammation and suppressing atherosclerotic development.30,82,83 A conflicting study found no role of Nur77 in atherosclerotic development and may represent an outlier in the literature.84 In the majority of studies linking Nur77 deficiency to atherosclerotic development, Nur77-deficient macrophages are polarized toward a proinflammatory phenotype, exhibiting increased IL-12 and nitric oxide synthesis in response to activation by TLR agonists. This increase in macrophage activation is driven by enhanced NF-kB activity and TLR expression in the absence of Nur77.30,85 These results suggest that Nur77-dependent monocytes may be atheroprotective, and that the nonclassical monocytes themselves, or their derived effector cells, may suppress inflammatory macrophage activity. We are currently investigating if these differences in macrophage activity are related to the absence of a patrolling monocyte derived anti-inflammatory macrophage subset or if these effects are related to intrinsic Nur77 activity in macrophages.

In humans, increased numbers of circulating CD16+ monocytes are associated with increased coronary heart disease.64 CD16+ monocyte levels are also positively correlated with vulnerable plaques in patients with coronary heart disease, and levels of CD16+ monocytes are significantly decreased in patients receiving statin treatment.65,66 Unfortunately, these human studies did not distinguish between CD16+CD14dim nonclassical and CD16+CD14+ generally more inflammatory intermediate monocyte populations. A recent study of >900 patients has suggested that it is mainly the CD16+CD14+ intermediate monocytes that are positive predictors for cardiovascular events, whereas the CD16+CD14dim nonclassical monocyte subset showed no correlation.67

In hypercholesterolemic patients, the number of nonclassical monocytes is inversely correlated with high-density lipoprotein cholesterol levels and is associated with increased APOE4 expression, a factor related to higher plasma cholesterol.62 Percentage of nonclassical monocytes is also positively associated with small high-density lipoprotein levels.63 Nevertheless, the association between nonclassical monocytes and atherosclerosis does not necessarily imply causality. Research into the functional relationships between patrolling monocytes, production of inflammatory or anti-inflammatory factors, and ability to remove lipids or repair the vasculature in cardiovascular disease is needed to establish positive effector cell associations.

Myocardial Infarction and Wounding
With respect to myocardial infarction (MI) and wound healing, patrolling monocytes have been associated with reparative and proangiogenic effects.57,71,87 In mouse models of MI, the depletion of monocytes leads to higher mortality and reduced ventricular function suggesting that monocytes facilitate wound healing and cardiac remodeling.8 Classical monocytes migrate into infarcted heart tissue during the early inflammatory phase of injury, and nonclassical monocytes are sequentially recruited at day 5 during the reparative phase.67 Similar findings were observed in patients where nonclassical monocytes peaked at 5 days post MI in the reparative phase, suggesting a possible reparative role of nonclassical monocytes.68 MI patients who did not develop ventricular thrombus formations had higher circulating nonclassical monocytes further suggesting a protective function of the subset.69 Mice in which bone marrow–derived cells lack Nur77 expression serve as a model for the absence of patrolling monocytes. These mice display adverse cardiac remodeling post MI, again highlighting a role for patrolling monocyte in post MI wound healing.70 However, other anti-inflammatory effects of Nur77 on classical monocyte–derived macrophages may also affect the MI outcome in this model. Further research is needed to examine the mechanisms by which patrolling monocytes may respond and repair damaged cardiac tissue. For example, it will be interesting to learn whether patrolling monocytes contribute to the local cardiac macrophage pool or if they function as terminally differentiated cells within the myocardium.

In a hindlimb ischemia model, proangiogenic nonclassical monocytes were associated with recovery of vascular flow and a more regenerative phenotype.71 Similarly, S1PR3-recruited nonclassical monocytes were correlated with arteriogenesis in ischemic microvessels.36 With laser-induced focal tissue damage of the ear dermis in mice, CX3CR1high nonclassical monocytes quickly align along collagen fibers at the outer edges of the wounding, in a prime location for repairing tissue.88 However, another study concluded that in a cerebral hypoxia–ischemia model patrolling monocytes were redundant in the progression and recovery of ischemic stroke.72 Additional research is needed to determine if these conflicting findings can be attributed to patrolling monocyte function in specific tissues or general differences in ischemic models. Combined these data generally demonstrate a beneficial effect of nonclassical monocytes in vascular repair and restoring organ function.

Neurological Diseases and Damage
Nonclassical Ly6C− monocytes patrol the brain and nervous system vasculature and have been associated with a variety of generally protective activities.10,73 Nonclassical patrolling monocytes are attracted to amyloid-β peptides and have been observed actively removing amyloid-β from the brain vasculature.73 This suggests that patrolling monocytes may be potential therapeutic targets for reducing amyloid-β deposits associated with Alzheimer’s disease. Live imaging of the brain has also revealed Ly6C− CX3CR1high monocyte differentiation into perivascular macrophages, a cell that is important for maintaining the blood–brain barrier and preventing damaging inflammatory cell influx into nervous tissue.10 Nonclassical monocytes were attracted during endotoxemia by TNF-α, IL1β, and angiopoietin-2.

Other possible roles of patrolling monocytes in the nervous system include preventing excito-toxicity and beneficial...
recruitment to the injured spinal cord. Absence of patrolling monocytes using hematopoietic deletions of Nur77 or CX3CR1 exacerbated excitotoxicity and neuronal cell death.74 Another study demonstrated that Ly6C+ CX3CR1high monocytes are actively recruited via the choroid plexus to help recovery of spinal cord injuries.75 Taken together, these findings reveal multiple protective roles of patrolling monocytes in the vasculature of the brain and nervous system.

Lupus and Kidney Disease
Glomerulonephritis is the leading cause of kidney failure and death in patients with lupus. Kidney glomerulus inflammation results from an increased duration and retention of migratory leukocytes and patrolling monocytes in a CD11b-dependent manner.16,89,90 Accumulation of nonclassical monocytes to glomerular vessels in the kidneys of lupus patients has been documented.10,76 Recruitment to the kidney vasculature is at least partially mediated by CX3CL1 (fractalkine) expression in the damaged kidney. Elevated levels of CX3CL1 in the glomerular vessels of lupus patients are associated with recruitment of CD16+ monocytes, proliferative glomerular lupus nephritis lesions, and disease activity.76 Interestingly, glucocorticoid therapy had a tendency to decrease both glomerular fractalkine expression and CD16+ monocyte numbers. Nonclassical human monocytes can preferentially make TNF and CCL3 in response to serum from patients with lupus, suggesting an inflammatory and active state of these cells under lupus-like conditions.10 The pattern recognition receptor TLR7 plays important roles in autoimmune responses directed against DNA- and RNA-containing nuclear antigens and the pathogenesis of lupus in susceptible hosts.91–94 TLR7 agonists can specifically induce nonclassical monocytes to produce proinflammatory cytokines, including TNF, CCL3, IL6, IL1β, and CXCL1.16 In response to TLR7-induced danger signals patrolling monocytes accumulate in the kidney microvasculature which recruit neutrophils and work to remove damaged endothelial cells.16 These findings suggest that although patrolling monocytes may initially be recruited to protect the kidney vascular endothelium, they could also contribute to tissue damage in susceptible individuals.

Arthritis
Nonclassical monocytes have also been associated with both the initiation and resolution of autoimmune joint inflammation.77 An elegant study by Misharin et al77 demonstrated that Ly6C+ monocytes are actively recruited to injured joints from the vasculature, can differentiate to inflammatory macrophages in the joint, and are critical for the initiation and progression of sterile joint inflammation in mouse models. However, with the development of arthritis Ly6C+ monocyte–derived macrophages shift to an alternatively activated phenotype, which promotes the resolution of joint inflammation.

In humans, nonclassical monocyte recruitment is likewise associated with rheumatoid arthritis. Nonclassical monocytes in blood were significantly increased in patients with rheumatoid arthritis compared with healthy controls.78,79 Patients with elevated nonclassical monocytes were associated with more active disease and elevated levels of erythrocyte sedimentation rates, C-reactive protein, and rheumatoid factor.79 Patients responding to therapy developed lower nonclassical monocyte frequencies, but nonresponders increased their frequency. In addition, the expression of the chemokine receptors CCR5, CCR1, and ICAM-1 was higher on nonclassical monocytes from patient with active arthritis.79 These findings show that nonclassical monocytes infiltrate inflamed joints and may contribute to both the induction and resolution of joint inflammation.

Potential Targeting and Future Research
Although many interesting functions have been associated with patrolling monocytes, the kinetics of their recruitment and capacity to differentiate into effector macrophage populations in inflammatory disease are still uncertain. Furthermore, the extent to which patrolling monocytes directly participate in the resolution of inflammation and the specific populations of macrophages/myeloid effector cells (if any) that derive from patrolling monocytes is currently unknown. Better in situ techniques and models to distinguish monocyte and derived macrophage populations in tissues are needed to verify the extent of which nonclassical patrolling monocytes contribute to the myeloid compartment in inflammatory disease. Other functional characteristics of both monocyte subsets including differences in their respective capacities to proliferate, particularly in response to insult, are still unknown and critical to our understanding of these cells.

Specifically, targeting nonclassical monocyte production and activities via Nur77-mediated regulation or liposomes may be of therapeutic benefit. Directed recruitment of nonclassical monocytes by S1P, TLR7 agonists, CX3CL1, or CCL5 may also be of interest to enhancing repair of damaged vascular or tissues. Interestingly, for possible targeting of aberrant nonclassical monocyte activities, it has been shown that glucocorticoid treatment can deplete nonclassical monocyte populations within a few days of treatment and may contribute to the mechanism of glucocorticoid mediated immune suppression.95,96 Additional research is needed to delineate the inherent function of patrolling monocyte subsets in inflammatory diseases.

Acknowledgments
We would like to thank Julie Larson for assistance with the artistic rendering of the figure.

Sources of Funding
This work is supported by National Institutes of Health R01 HL118765 (to C.C. Hedrick), American Heart Association Postdoctoral Fellowship 13POST16990029 (to R. Tacke), American Heart Association Scientist Development Grant 12SDG12070005 (to R.N. Hanna), and LJI Board of Director’s Fellowship (to R.N. Hanna).

Disclosures
None.

References


40. Auffray C, Fogg DK, Nam-Mancinelli E, et al. CX3CR1+ CD11b+ CD135+ common macrophage/DC precursors and the role of CX3CR1


inflammatory disease. This review summarizes the current knowledge and unique functions of nonclassical patrolling monocytes in the vasculature and during inflammatory diseases, comparatively few have assessed the unique activities of patrolling monocytes. Patrolling monocytes function to remove damaged cells and debris from the vasculature and have been associated with wound healing and the resolution of inflammation in damaged tissues.

Recently studies have identified at least 2 distinct monocyte subpopulations within the circulation—classical and nonclassical patrolling monocytes. Although many studies have examined the role of classical monocytes within the vasculature and in response to inflammatory diseases, comparatively few have assessed the unique activities of patrolling monocytes. Patrolling monocytes function to remove damaged cells and debris from the vasculature and have been associated with wound healing and the resolution of inflammation in damaged tissues. This review summarizes the current knowledge and unique functions of nonclassical patrolling monocyte in the vasculature and during inflammatory disease.
Nonclassical Patrolling Monocyte Function in the Vasculature
Graham Thomas, Robert Tacke, Catherine C. Hedrick and Richard N. Hanna

Arterioscler Thromb Vasc Biol. 2015;35:1306-1316; originally published online April 2, 2015;
doi: 10.1161/ATVBAHA.114.304650
Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272
Greenville Avenue, Dallas, TX 75231
Copyright © 2015 American Heart Association, Inc. All rights reserved.
Print ISSN: 1079-5642. Online ISSN: 1524-4636

The online version of this article, along with updated information and services, is located on the
World Wide Web at:
http://atvb.ahajournals.org/content/35/6/1306

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published
in Arteriosclerosis, Thrombosis, and Vascular Biology can be obtained via RightsLink, a service of the
Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for
which permission is being requested is located, click Request Permissions in the middle column of the Web
page under Services. Further information about this process is available in the Permissions and Rights
Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Arteriosclerosis, Thrombosis, and Vascular Biology is online
at:
http://atvb.ahajournals.org//subscriptions/