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The many flavors of monocyte / macrophage-endothelial cell interactions

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Abstract

Purpose of review—The well-recognized plasticity and diversity typical of monocytes and macrophages have recently been expanded by the knowledge that additional macrophage lineages originated directly from embryonic progenitors, populate and establish residency in all tissues examined so far. This review aims to summarize our current understanding on the diversity of monocyte/macrophage subtypes associated with the vasculature, their specific origins, and nature of their cross-talk with the endothelium.

Recent findings—Taking stock of the many interactions between the endothelium and monocytes / macrophages reveals a far more intricate and ever-growing depth. In addition to circulating and surveilling the endothelium, monocytes can specifically be differentiated into patrolling cells that crawl on the surface of the endothelium and promote homeostasis. The conversion of classical to patrolling is endothelium-dependent uncovering an important functional link. In addition to patrolling cells, the endothelium also recruits and harbor an intimal-resident myeloid population that resides in the tunica intima in the absence of pathological insults. Moreover, the adventitia is populated with resident macrophages that support blood vessel integrity and prevent fibrosis.

Summary—The last few years have witnessed a significant expansion in our knowledge of the many subtypes of monocytes and macrophages and their corresponding functional interactions with the vascular wall. In addition to surveying the endothelium for opportunities of diapedeses, monocyte and macrophages take residence in both the intima (as patrolling or resident) and in the adventitia. Their contributions to vascular function are broad and critical to homeostasis, regeneration, and expansion.

Keywords

Blood vessels; collaterals; perivascular; tunica adventitia; tunica intima; vascular inflammation

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CONFLICTS OF INTEREST

There are no conflicts of interests.

INTRODUCTION

As the selective gatekeeper between blood and tissues, the endothelium specifically interacts and activates immune cells to facilitate their trafficking. These heterotypic cell interactions are usually brief and are meant to gain access to specific tissue/organ sites. Nonetheless, in contrast to other immune cells, the interactions between endothelium and monocytes/macrophages appear to be far more diverse, interdependent, and specialized.

During developmental angiogenesis, macrophages establish bridges that bring tip cells from adjacent sprouts in proximity facilitating the formation of a new vascular branch [1]. In this manner, macrophages have been acknowledged to actively participate in vascular expansion. Monocyte / macrophages also contribute to angiogenesis through a variety of mechanisms including secretion of VEGF and other cytokines [2], promote intima regeneration [3*], and contribute to collateral growth under hypoxic conditions [2,4]. In small vessels, perivascular macrophages can also regulate permeability responses [5*,6**]. Clearly amongst all bone-marrow derived cells, heterotypic associations between endothelial and monocytes / macrophages are versatile and broad. This wide functional diversity has uncovered the realization of multiple subtypes of monocytes and macrophages that interact with the vascular wall and that are derived from distinct precursors. In fact, blood vessels, particularly in the adventitia are initially populated by macrophages directly derived from embryonic progenitors and supplemented by infiltrating monocytes that contribute to the resident pool of macrophages post-birth [7**]. How this distinct origin and long-term residency impacts their function in comparison to monocyte-derived macrophages is yet to be understood.

Here we examine a fast-growing body of new information on monocyte / macrophage interactions with the vascular wall. Our objective is to offer a timely and informed summary of the current knowledge on the subject, bringing to light differences in origin, molecular cross-talk with the endothelium and functional relationships.

PATROLLING MONOCYTES

Monocytes circulate and constantly survey the endothelium, in some cases this surveillance results in extravasation. Once in the extravascular space, these highly plastic cells can display wide functional diversity in response to their environment [8]. However, in other cases, interaction with the endothelium does not result in vascular extravasation instead, monocytes constantly crawl on the endothelial surface. These two major monocyte subpopulations can be molecularly identified by their cell surface profile which is shared by both mouse and human [9]. Specifically: Ly6c^{hi}Ccr2^{hi}Cx3cr1^{lo} (CD14⁺CD16⁻ in humans) defines “**inflammatory**” or “**classical**” monocytes that are abundant and highly responsive to inflammatory signals, these cells can infiltrate tissues and differentiate into macrophages [8]. In contrast, Ly6c^{lo}Ccr2^{lo}Cx3cr1^{hi} (CD14⁺CD16⁺ in humans), also referred as “**non-classical**” or “**patrolling**” monocytes patrol the endothelium by crawling along the luminal aspect of blood vessels in both the micro- and macro-vasculature (Fig. 1a). This second monocyte subtype is thought to support the endothelium and maintain its homeostasis

[10,11]. Patrolling monocytes are generally long-lived and constantly attaching to the tunica intima of the circulatory system and interacting with the endothelium [9].

During steady-state, Ly6c^{hi} classical monocytes are the precursors to Ly6c^{low} patrolling monocytes [12]. The process of conversion from classical to patrolling requires contact with the endothelium and activation of the Notch pathway [13*]. Specifically, activation of Notch2 in monocytes by Dll1 in endothelial cells effectively mediates the transition from classical to patrolling in several vascular beds [13*]. In addition to Notch, the transcription factor *Nr4a1* (Nur77) is a critical regulator of patrolling, while not affecting classical monocytes [14]. Several studies have demonstrated that depletion in *Nr4a1* impairs differentiation and survival of patrolling monocytes [14–16]. A caveat of these studies is the broad expression of *Nr4a1* which could impact related cell subtypes. Recently, two studies further refined the link between *Nr4a1* and monocytes. One study performed targeted inactivation of a sub-domain within the *Nr4a1* enhancer that is exclusively utilized by patrolling but not other monocytes (Fig. 1a) [15**]. The other showed a critical role for C/EBP β in the regulation of *Nr4a1* expression during monocyte conversion using a single cell transcriptomics approach [17*]. Both studies confirmed the absolute requirement of *Nr4a1* in the conversion of classical to patrolling monocytes. Furthermore, myeloid depletion of *Klf2*, an upstream regulator of *Nr4a1*, explicitly reduced patrolling monocytes [15**].

In the microvasculature of the dermis, kidney, and mesentery, patrolling monocytes crawl along the endothelium regardless of the direction of blood flow [16]. However, in large arteries like the carotid, patrolling monocytes preferentially migrate in the direction of blood flow [18**]. The crawling process requires $\alpha_L\beta_2$ integrin (or LFA-1), a highly expressed receptor in patrolling monocytes in all healthy tissues [10]. Intracellular adhesion molecule-1 (Icam-1) is the major endothelial ligand for $\alpha_L\beta_2$, while Icam-2 enhances, but it is dispensable for adhesion [16]. Besides $\alpha_L\beta_2$, the patrolling function of monocytes is thought to require $\alpha_4\beta_1$ (or very-late antigen 4, VLA-4), but alone, $\alpha_4\beta_1$ is unable to fully support this function (Fig. 1a) [18**]. Recently, $\alpha_4\beta_1$ has been implicated in patrolling of renal glomeruli [19].

Patrolling monocytes express high levels of *Cx3cr1*. Consequently, monoallelic *Cx3cr1*-GFP knock-in mice (*Cx3cr1*^{GFP/+}) are the most commonly used labeling strategy to distinguish patrolling versus other monocytes in vivo, although this reporter mouse also labels other immune cell populations (i.e. tissue-resident macrophages, dendritic cells, and subsets of NK and T cells) [20]. Deficiency of *Cx3cr1* results in an overall reduction of patrolling monocytes; however, motility of these monocytes in the microvasculature and in large arteries is independent of *Cx3cr1* [16,18**].

On the endothelial side, in addition to Icam-1/2, patrolling monocyte-endothelial interactions also require the cystein-rich angiogenic inducer 61 (Cyr61 or *Ccn1*), a matricellular protein with chemoattractant properties for monocytes that is produced and secreted by endothelial cells, fibroblasts, and other cell types [21]. Endothelium bound-*Ccn1* promotes efficient surveillance of patrolling monocytes at steady-state, but this molecule is not required for adhesion to the endothelium [21].

Functionally, patrolling monocytes, which express high levels of toll-like receptors (TLRs), behave as the “housekeepers” of the vasculature [16,22]. They have phagocytosis capabilities that allows them to scavenge microparticles and remove cellular debris from the endothelial surface at sites of necrosis in a TLR-7-dependent manner [10,16] (Fig. 1b). In addition, patrolling monocytes have proangiogenic properties and aide in organ repair [3*,23,24*-26].

Recently, Gitzin et al. showed that following carotid injury, patrolling monocytes are recruited to the endothelium at wound sites to promote endothelial cell proliferation and tissue repair [3*]. In this study, the authors showed that carotid injury induces endothelial cell expression of Cx3cr1 at the wound site which results in the recruitment of patrolling monocytes. Depletion of patrolling monocytes using *Cx3cr1* loss-of-function (*Cx3cr1*^{GFP/GFP}) mice impaired endothelial regeneration. Lastly, *in-vitro* experiments suggest patrolling monocytes mediate endothelial cell proliferation through secretion of Vefg-a [3*].

In disease, patrolling monocytes largely play protective roles and contribute to the resolution of inflammation. During atherosclerosis, patrolling monocytes are functionally atheroprotective [18**,27,28]. Monocyte crawling is increased at early stages of the disease, through CD36-mediated uptake of oxidized lipids (OxLDL) [29*]. Depletion of patrolling monocytes in atherosclerotic models results in aggravated atherosclerosis [18**,28]. Following myocardium infarction, patrolling monocytes are part of a second-wave of inflammatory cell recruitment that aids to repair, promote angiogenesis and improve myofibroblast accumulation, as well as deposition of collagen necessary for granulation tissue formation [23,25]. In renal ischemia reperfusion injury CD169+ monocytes, which include both classical and patrolling, play an anti-inflammatory role by regulating Icam-1 expression on endothelial cells [26]. Mice depleted of Cd169 had exacerbated renal reperfusion injury [26]. To clarify the subset of CD169+ monocytes contributing to the resolution of the pathology, the authors performed adoptive transfer experiments. Transfer of Ly6c^{lo} patrolling monocytes into CD169-depleted mice rescued the mice from lethal renal injury and normalized renal endothelial cell Icam-1 expression levels. These findings indicate that CD169+ Ly6c^{lo} patrolling monocytes play a major role in regulating inflammation in renal reperfusion injury [26]. Additionally, patrolling monocytes contribute to cancer immune-surveillance by orchestrating an antitumor response through recruitment of NK cells, and thus, controlling lung metastasis in the mouse [30]. In other diseases, like systemic lupus erythematosus and arthritis, patrolling monocytes have been implicated in the pathogenesis of disease [31,32].

MONOCYTES/MACROPHAGE IN COLLATERAL ARTERIAL REMODELING

In adults, arteriogenesis refers to the growth or remodeling of arteries (collaterals) triggered by the occlusion of an upstream vascular branch. Although the mechanisms of collateral growth are still unclear [4], monocytes and macrophages have been identified as important players in the process.

Studies suggesting the importance of monocyte/macrophages in collateral arteriogenesis can be traced back to the 1970s, when Schaper et al. [33] showed a large number of monocytes adhering to the surface of endothelial cells following canine chronic coronary artery occlusion. Subsequently, monocyte/macrophage accumulation was also noted around collateral arteries in rabbit and murine hindlimb ischemia models [34,35]. Monocyte recruitment requires the upregulation of chemoattractant or activating cytokines and adhesion molecules [36]. During collateral arteriogenesis, the usually quiescent endothelium becomes activated, resulting in the upregulation of Icam-1, vascular cell adhesion molecule-1 (Vcam-1) and chemokines such as monocyte chemoattractant protein 1 (Mcp-1 or Ccl2), amongst others [2,4]. Mcp-1 is the ligand for C-C chemokine receptor (Ccr2), which is required for classical monocyte recruitment [37]. Mcp-1 plays a significant and beneficial role during collateral arteriogenesis. Mice depleted of Mcp-1 (Ccl2^{-/-}) had lower monocyte recruitment, reduced collateral artery regeneration, and low perfusion recovery [37,38]. Furthermore, administration of Mcp-1 during hindlimb ischemia increased collateral artery regeneration [34,39]. Recently, it was shown that nuclear factor of activated T cells 5 (Nfat5) controls Mcp-1 release in endothelial cells during hindlimb ischemia [40*]. Specifically, reduction of Nfat5 by shRNA adenovirus resulted in attenuated arteriogenesis in rat hindlimb ischemia [40*].

Experiments using Ccr2-depleted (Ccr2^{-/-}) mice, where classical monocyte infiltration into damaged tissues is abrogated has also supported a critical role for monocytes in collateral formation [37,41]. Bone-marrow transplantations of Ccr2^{-/-} mice into wild-type recipients (Ccr2-WT) has revealed that both arteriolar remodeling and monocyte/macrophage recruitment are significantly impaired in a dorsal skinfold model of injury [42]. Importantly, not all macrophages are derived from Ccr2 classical monocytes. As previously discussed, a major subset of adventitia-resident macrophages originate from embryonic precursors and proliferate to maintain the lineage through self-renewal [7**]. This subtype of macrophage (tissue-resident) is not affected by Ccr2 depletion and abundantly populate the perivascular wall. It is also possible that these cells, in addition to monocyte-derived macrophages, contribute to collateral formation.

In myocardial infarction and hindlimb ischemia, early invading Ly6c^{hi} monocytes give rise to ischemic macrophages that undergo phenotypic changes overtime [25,43*]. What exactly promotes macrophage differentiation and maturation in ischemic tissue? Previously it has been shown that endothelial cells can promote macrophage polarization [5*]. Further supporting this concept, Krishnasamy et al. [43*] recently showed that macrophage maturation from Ly6c^{hi} monocytes and inflammatory polarization after hindlimb ischemia is regulated by endothelial Dll1. Mice with either endothelial-specific deletion of Dll1 (*Dll1^{i EC}*) or myeloid cell-specific Notch-deficiency not only exhibited a low perfusion recovery and a reduction in collateral branch formation, but also in low macrophage numbers and macrophage activation state [43*]. Therefore, monocyte-derived macrophage maturation is dependent on canonical Notch signaling, which is essential for collateral arteriogenesis following ischemia. Additionally, monocytes/macrophages are believed to be the major source of matrix metalloproteinases (Mmps) and other growth factors like vascular endothelial growth factor (Vegf), which leads to the rearrangement of extracellular matrix and remodeling of arterioles [37,44,45]. Whether monocyte-derived macrophages or

adventitia-resident macrophages are the most critical source of these secreted proteins for collateral formation is not yet fully understood. Defined lineage-tracing experiments could help clarify the individual and overlapping contributions of these two subpopulations associated with the vascular wall.

ARTERIAL “ADVENTITIA-RESIDENT” MACROPHAGES

Genetic tracing studies and others have shown that arterial “adventitia-resident” macrophages densely populate the tunica adventitia: the outermost layer of connective tissue common to all blood vessels [6**,7**,46**]. These cells can be recognized by expression of Lyve-1, as well as Csf1r (CD115), CD11b, F4/80, and CD64 (FcγR1) [6**,7**,46**]. As other macrophages, adventitia-resident macrophages are dependent on Csf1r-Csf1 signaling for survival and their maintenance requires Cx3cr1 activation, where endothelial cells and Pdgfra+ mesenchymal cells constitute the primary source of Cx3c1 [7**,46**].

In recent years, our knowledge of the ontogeny of tissue-resident macrophages has changed drastically. It is now understood that most tissue-resident macrophages arise from embryonic precursors, that seed tissues during development. These cells are capable of self-renewal and fully independent of bone marrow-derived cells [12,47,48] (Fig. 1a). Two waves of precursors have been reported for adventitial macrophages. Tracing studies identified Cx3cr1+ embryonic progenitors as a definitive source of adventitial macrophages. These cells are observed by embryonic day 16.5 (E16.5) in the mouse aorta [7**]. Subsequently, the adventitia receives a second wave of precursors, this time originated from circulating monocytes. In adulthood, adventitia-resident macrophages are maintained via self-renewal, independent of circulating monocytes [7**]. In addition, following bacterial (LPS) infection, adventitia-resident macrophages are severely depleted, but eventually reach steady-state levels through self-renewal [7**].

In terms of cell-surface profile, a recent study has identified two independent populations of monocyte-derived tissue-resident macrophages characterized by distinct gene expression profiles: Lyve^{lo}MHCII^{hi}Cx3cr1^{hi} (Lyve^{lo}MHCII^{hi}) and Lyve^{hi}MHCII^{lo}Cx3cr1^{lo} (Lyve^{hi}MHCII^{lo}), the latter speaks to adventitia-resident macrophages [6**]. These parallel populations were found in the heart, fat, dermis, and lung, as well as in human lung and fat. This study elegantly showed specific niches to these two macrophage populations, concluding that Lyve^{lo}MHCII^{hi} macrophages preferentially reside adjacent to nerve bundles and fibers whereas Lyve^{hi}MHCII^{lo} macrophages are prevalent to blood vessels across tissues. Specific depletion of Lyve^{hi}MHCII^{lo} macrophages in a mouse model resulted in exacerbated experimental lung and heart fibrosis. The authors conclude that Lyve^{hi}MHCII^{lo} macrophages support blood vessel integrity at steady-state since depletion of these macrophages leads to better infiltration of monocytes and other inflammatory cells upon fibrosis.

In general, tissue-resident macrophages have a diverse array of functions; in addition to their conventional immune regulation, they also participate in homeostasis, repair and angiogenesis [5,47,48]. For example, embryonic tissue macrophages in the brain act as fusion cells that physically bridge tip cells of sprouting vessels to promote vascular

anastomosis [1]. Specifically in relation to adventitial-resident macrophages, depletion studies using Csf1r inhibitors or *Lyve1^{wt/cre};Csf1l^{lox/lox}* mice resulted in an increase in collagen deposition within the arterial wall and arterial stiffness [46**]. *In vitro* co-culture experiments further suggested that adventitia-resident macrophages regulate collagen content via Mmp9-dependent proteolysis [46**]. Since Lyve-1 is the receptor for the extracellular matrix glycosaminoglycan hyaluronan (HA), blocking antibodies were used against the Lyve-1 binding site, which resulted in the reduction of collagen degradation *in vitro* [46**]. Therefore, the authors speculate that adventitia-resident macrophages regulate collagen levels through HA-dependent smooth muscle cell adhesion and thus, modulate smooth muscle cell function.

INTIMAL CD11C+ MYELOID CELLS

Endothelial cells provide an antithrombotic surface by forming tight barriers through homotypic cell interactions that effectively separate the blood from the surrounding tissues. In general, only under inflammatory conditions are endothelial cells capable of recruiting immune cells and form transient heterotypic interactions that promote their extravasation at sites of inflammation. However, in large arteries there appears to be an exception to the transient nature of these interactions. Early morphological observations have suggested that a **resident myeloid population** might occupy the intimal layer of healthy (uninflamed) vessels in regions of turbulent flow like the lesser curvature of aortic arch, bifurcation, and branch openings [49,50].

In healthy C57BL/6 mice, an intimal CD11c+ myeloid cell population was also observed in the aorta at regions that experience disturbed blood flow (Fig. 2a) [51,52]. Very rarely were these CD11c+ myeloid cells described in the intima of the descending aorta, which experiences uniform laminar flow, suggesting that their recruitment and/or residency might be flow-type dependent [52]. Intimal CD11c+ myeloid cells increase with age in the ascending aorta and were found to populate the thoracic aorta of 60wk old mice [53]. These myeloid cells reside in the subintimal space with long cellular processes projecting into the lumen [51,52]. Collectively, these intimal cells are CD11c+, MHCII+, CD68+, Cx3cr1+ as validated by immunofluorescence and reporter mice [51–53]. Additionally, they express the dendritic markers $\alpha E\beta 7$ (CD103) [54] and 33d1, although the latter remains controversial [54–56]. A previous study showed that FACS sorted CD11c+MHCII+ aortic cells have low phagocytic activity and are strong stimulators of T-cell proliferation, functionally supporting their classification as dendritic cells [54]. However, their identity as *dendritic* or *macrophage* is still in under debate. Using single-cell RNAseq Chakarov et al. [6**] recently characterized a tissue-resident macrophage population with a conserved phenotype across tissues. The profile identified by the authors matches the aortic intimal CD11c+ myeloid population. This finding prompts the question: Are the aortic intimal immune cells actually macrophages? Detailed transcriptomic profiling using single cell RNA-sequencing combined with precise immunofluorescence could help elucidate the cellular identity of the CD11c+ myeloid cells.

The accumulation of intimal CD11c+ myeloid cells seems to be partially dependent on Cx3cr1. In Cx3cr1-deficient mice, there is 40% less CD11c+ myeloid cells in the ascending

aorta [53]. Furthermore, intimal CD11c⁺ myeloid cells are believed to be Flt3L-dependent [54]. Treatment of Flt3L increases intimal CD11c⁺ cells by 2.5 fold and the number of these intimal myeloid cells were remarkably reduced in Flt3^{-/-} mice [54]. Using BrdU labeling experiments, it is believed that there is a low-grade contribution of recruited blood monocytes to the normal intima that serve as the predominant source of intimal CD11c⁺ myeloid cells [52]. However, additional studies involving parabiosis, transplants, and lineage tracing are needed to further advance our understanding on how intimal CD11c⁺ myeloid cells are maintained throughout adulthood. Moreover, at what age do CD11c⁺ myeloid cells seed the intima of the aorta? Understanding their origin could shed light into their physiological roles.

The function of intimal CD11c⁺ myeloid cells remains unknown. What is the relationship between these myeloid cells and endothelial cells? Are these myeloid cells necessary to maintain vascular homeostasis? Paulson et al. [55] showed that a single injection of tamoxifen to mice bearing the CD11c promoter-DTR transgene (CD11c-DTR mice) depletes the intimal myeloid cells by approximately 98% after 24hrs post-injection. Interestingly, approximately 75% of intimal CD11c⁺ myeloid cells are recovered by 21-days post-depletion and are re-localized to the lesser curvature of the aortic arch [55]. This implies the necessity for these cells by the endothelium in this region. Further studies to define the consequences of depletion of intimal CD11c⁺ cells in vascular homeostasis are still needed.

Recently, Roufaiel et al., [57*] showed that intimal CD11c⁺ myeloid cells have the potential to clear *chlamydia muridarum* in the arterial wall through reverse transendothelial migration (Fig. 2b). Upon infection of *chlamydia muridarum* (or with injection of LPS and PolyIC), there is a decrease in the number of intimal CD11c⁺ myeloid cells in the ascending aortic arch. Consequently, clearance of *chlamydia muridarum* 16S rRNA in the intima correlated with the reduction of CD11c⁺ myeloid cells present in the intima [57*]. These findings indicate that intimal CD11c⁺ myeloid cells have important innate-like functions in the normal artery.

The location of intimal CD11c⁺ myeloid cell makes them likely suspects in the initiation of atherosclerosis. Intimal CD11c⁺ myeloid cells are capable of uptaking neutral lipids just after 5 days of high-fat diet in Ldlr-depleted (Ldlr^{-/-}) mice, a popular atherosclerotic model (Fig. 2c) [55]. Depleting these cells using CD11c-DTR mice crossed to Ldlr^{-/-} mice prior to the induction of hypercholesterolemia resulted in the overall reduction of early lipid accumulation in the aortic wall [55]. In addition, lipid accumulation was found only in extracellular spaces, suggesting intimal CD11c⁺ myeloid cells play a role in lipid endocytosis during the earliest stages of plaque formation [55]. It has been suggested that the “resident” intimal CD11c⁺ cells significantly increase in number in advanced atherosclerosis based on the number of CD11c⁺MCII⁺ cells present [54]. Although expansion of cells matching this phenotype is evident, it is difficult to discriminate between the resident *versus* the recruited immune cell populations based on these markers alone since recruited cells can adopt these phenotypes [54,56]. Additionally, there is remarkable immune cell heterogeneity in atherosclerotic aorta. Recent scRNAseq studies identified up to 11 distinct leukocyte populations in the atherosclerotic aorta when compared to control aortas, with diverse macrophage and dendritic cell populations [58*,59**]. Therefore,

lineage tracing experiments during the different stages of atherosclerosis are needed to enable the distinction between resident and *de novo* recruited cells.

CONCLUSIONS

In the last five years, our understanding of the spectrum of endothelial – monocyte / macrophage has significantly expanded along with the realization that macrophages do take long-term residence in organs, including the vascular wall. Their roles are broad and, not surprisingly, dependent on environmental conditions.

Research focus would benefit from combinatorial studies that bring lineage tracing and functional analysis in the context of both physiological and pathological conditions. Major obstacles in clarifying specific functions relate to the potential for functional compensation. Thus, studies that aim at understanding cellular redundancy by selectively eliminating lineages at specific times and sites would aid in clarifying to what degree can monocytes substitute for developmentally-derived long-term lineages. As the functional interdependencies between endothelium and mononuclear lineages become untangled, the information will expand our ability to systematically evaluate how cell-cell interactions impacts tissue adaptability and resilience to stressors.

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** of outstanding interest

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KEY POINTS

- The vascular wall is home to heterogeneous populations of monocytes and macrophages, all with distinct phenotypes, functions, and origins.
- The endothelium helps regulates monocyte conversion and macrophage maturation.
- Resident, perivascular macrophages support blood vessel integrity.
- Arterial regeneration requires monocytes and macrophages.

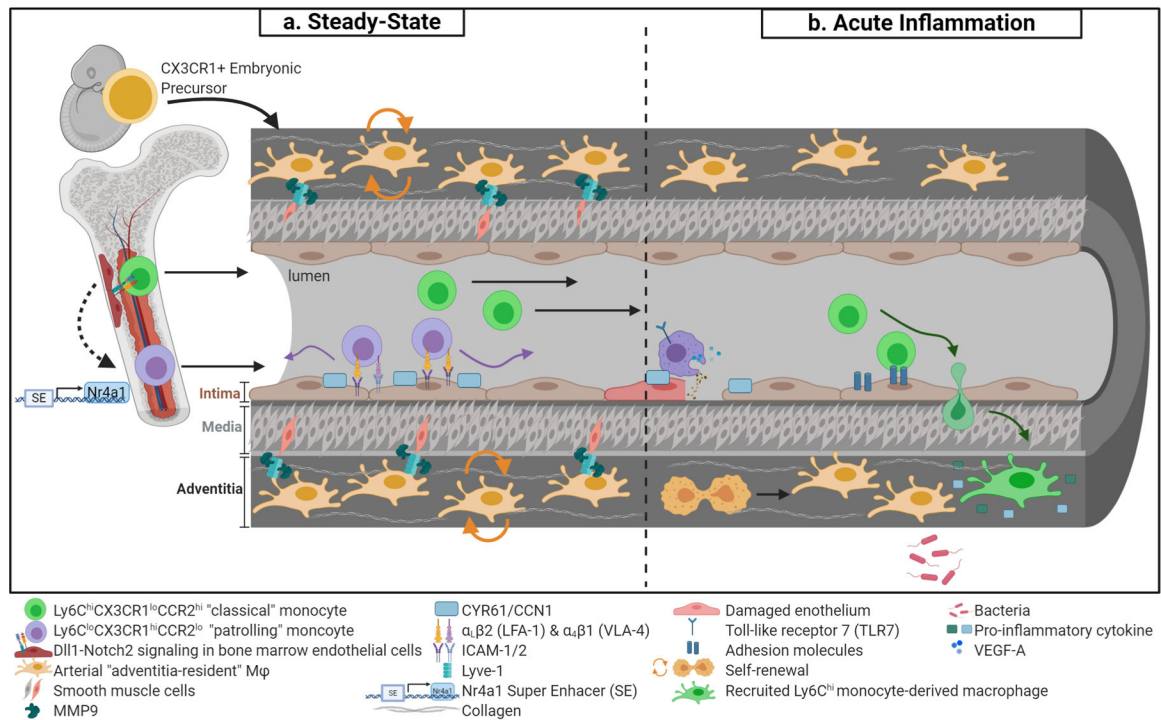


Figure 1. Recruitment and dynamics of vascular and perivascular monocyte/macrophages.

(a) Arterial "adventitia-resident" macrophages are derived from CX3CR1+ embryonic precursors and seed the perivascularity prior to birth (orange cells). Throughout adulthood, adventitia-resident macrophages are maintained by self-renewal. Adventitia-resident macrophages regulate collagen content via matrix metalloproteinase-p (Mmp9) proteolysis, which is dependent on Lyve-1 and smooth muscle cell binding. At steady-state, Ly6C^{hi} classical monocytes (in green) are the precursors to Ly6C^{lo} patrolling monocytes (in purple). Monocyte conversion is dependent on Dll1/Notch2 signaling in distinct vascular beds. Additionally, Nr4a1 super enhancer regulates patrolling monocyte development and is essential for monocyte survival. In the circulation, patrolling monocytes crawling requires α_Lβ2 integrin (or LFA-1) and other adhesion molecules highlighted. Furthermore, endothelial cystein-rich angiogenic inducer 61 (Cyr61 or Ccn1) promotes efficient surveillance. (b) Patrolling monocytes remove cellular debris from the endothelial surface at sites of necrosis in a TLR-7-dependent manner. At sites of injured endothelium, patrolling monocytes may facilitate endothelial proliferation through secretion of Vegfa. Moreover, activated endothelium upregulates adhesion molecules that enable monocyte capture, rolling, and extravasation into tissue and migrate to inflammatory stimuli. Recruited monocytes differentiate into macrophages as part of a pro-inflammatory response. Adventitia-resident macrophages are severely depleted during bacterial infection but are able to regain steady-state levels through self-renewal.

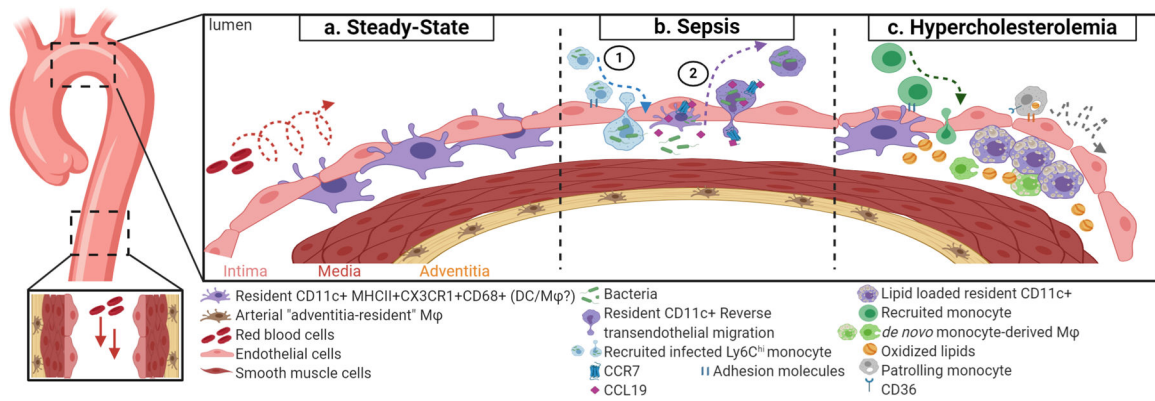


Figure 2. Intimal resident CD11c+ myeloid cells in arteries during health and disease.

(a) In regions that experience disturb blood flow, like the lesser curvature of the aortic arch, resident CD11c+ myeloid cells (in purple) accumulate in the subintimal space of healthy aortae. Resident CD11c+ myeloid cells rarely accumulate in descending aortae, which experiences uniform laminar blood flow. (b) 1. Circulating monocytes (in blue) become infected with bacteria, gain access to the subintima space, and disseminate the infection. 2. Upon infection, resident CD11c+ myeloid cells upregulate Ccl19 and Ccr7, which is necessary for these cells clear the infection via reverse transendothelial migration, and enter the circulation. (c) At early stages of hypercholesterolemia, resident CD11c+ myeloid cells uptake oxidized lipids, contributing to the initial lesion bed. Additionally, CD36-mediate uptake of oxidized lipid by patrolling monocytes increases crawling.