

Published in final edited form as:

Trends Immunol. 2011 October ; 32(10): 470–477. doi:10.1016/j.it.2011.05.001.

Monocyte trafficking in acute and chronic inflammation

Molly A. Ingersoll¹, Andrew M. Platt¹, Stephane Potteaux^{1,2}, and Gwendalyn J. Randolph¹

¹Department Gene and Cell Medicine and the Immunology Institute, 1425 Madison Avenue, Mount Sinai School of Medicine, New York 10029, USA.

Abstract

Environmental signals at the site of inflammation mediate rapid monocyte mobilization and dictate differentiation programs whereby these cells give rise to macrophages or dendritic cells. Monocytes participate in tissue healing, clearance of pathogens and dead cells, and initiation of adaptive immunity. However, recruited monocytes can also contribute to the pathogenesis of infection and chronic inflammatory disease, such as atherosclerosis. Here, we explore monocyte trafficking in the context of acute inflammation, relying predominantly on data from microbial infection models. These mechanisms will be compared to monocyte trafficking during chronic inflammation in experimental models of atherosclerosis. Recent developments suggest that monocyte trafficking shares common themes in diverse inflammatory diseases; however, important differences exist between monocyte migratory pathways in acute and chronic inflammation.

Monocyte Phenotypic Heterogeneity

Monocytes are heterogeneous circulating blood cells poised to rapidly extravasate into inflamed tissues. In the bone marrow (BM), a common Lin⁻cKit^{hi}CD115⁺CX3CR1⁺Fit3⁺ progenitor cell, termed macrophage and DC precursor (MDP), gives rise to monocytes and numerous subsets of macrophages and DCs [1]. Two major subsets of blood monocytes have been described in mice, humans, and other species [2–4]. The two murine subsets, classical (which express Ly6C, recognized by the anti-Gr1 antibody) and nonclassical monocytes (Ly6C^{lo}), are distinguished by differential expression of chemokine receptors, particularly CCR1, CCR2, and CX3CR1 (fractalkine receptor) [3–6]. Classical monocytes are known to exit the BM in a CCR2-dependent fashion [7] and CCR2 ligands CCL2 (MCP-1) and CCL7 (MCP-3) help maintain homeostatic levels of monocytes in the circulation [8]. Mechanisms controlling the egress of non-classical monocytes from the BM remain elusive and, indeed, it remains to be directly demonstrated that they arise in the BM. In addition to the BM, the spleen is home to both subsets of mature monocytes which appear phenotypically similar to the two subsets found in blood (Text Box 1) [9].

Analogously, CD115⁺ human monocytes can be separated into two major subsets, typically based on their differential expression of CD14 and CD16 [6, 10]. The CD14⁺⁺CD16⁻ subset, equivalent to mouse classical monocytes, is also CCR2⁺, whereas the CD14⁺CD16⁺ (also called CD14^{dim}CD16⁺) subset lacks CCR2 and constitutes the non-classical population

© 2011 Elsevier Ltd. All rights reserved.

Corresponding author: Ingersoll, M.A. (molly.ingersoll@mssm.edu).

²Present address: PARCC - INSERM U970, 56 rue Leblanc, 75015 Paris, France

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

[6]. Nonclassical CD16⁺ monocytes can be further divided by their high or low levels of CD14 expression [11]. Recent work reveals a difference between the minor CD14⁺CD16⁺ and CD14^{dim}CD16⁺ monocyte populations in their capacity to become activated and secrete key inflammatory cytokines in response to different stimuli [12]. CD14⁺CD16⁻ and CD14⁺CD16⁺ respond to TLR2 and TLR4 ligands, whereas CD14^{dim}CD16⁺ monocytes respond to viral stimuli through TLR7 and TLR8. In addition, these data are in agreement with previous work [6] suggesting that CD14^{dim}CD16⁺ monocytes are the counterparts of nonclassical monocytes in mice. Differential gene expression profiles, including many genes involved in cell trafficking, between the monocyte subsets are well conserved between mice and humans, suggesting mouse models of trafficking may reveal clues to human monocyte behavior in disease [6]. However, when applying observations made in mouse experimental models to human disease, it is important to recognize a few key differences, such as subset ratio in the blood, that exist between humans and mice. Here, we discuss recent advances in the mechanisms controlling monocyte trafficking during acute and chronic inflammation and address controversial issues such as the role of CCR2 in directing monocytes to inflamed tissues.

Monocyte Functional Heterogeneity

As might be predicted from their differential gene expression patterns, monocyte subsets also differ functionally. Nonclassical monocytes demonstrate a patrolling behavior along blood vessel walls [13] and accumulate in non-inflamed peripheral tissues such as spleen, lung, and liver when adoptively transferred [3]. While lineage tracer studies suggest that it is unlikely that nonclassical monocytes contribute to dendritic cell (DC) populations in steady state peripheral organs, it is possible they contribute to resident macrophage populations [14]. Consensus on the homing patterns of both monocyte subsets has not been reached, but there is overwhelming evidence that recruitment of classical monocytes dominates early in inflammatory responses. For example, classical monocytes infiltrate inflamed tissues, such as in sterile peritonitis models, more robustly than or to the exclusion of their nonclassical counterparts in mice and rats, and are specifically increased in the circulation during systemic or chronic infection [3, 4, 15, 16]. Only classical monocytes migrate to the skin of mice receiving an intracutaneous injection of latex microspheres [17] and in a model of skeletal muscle injury, only classical monocytes migrate to injured tissue [18]. However, after engulfing dying cells, they differentiated into cells resembling nonclassical monocytes, which mediated tissue repair mechanisms [18]. By contrast, after myocardial infarction, both monocyte subsets appear to home to the same tissue at different stages of inflammation (Figure 1) [19]. Specifically, whereas the classical subset of monocytes first seeds the infarcted heart and exhibits inflammatory functions, the nonclassical subset is recruited at a later stage and promotes tissue healing by expressing high amounts of vascular endothelial growth factor [19]. In this report, the two subsets are under the control of distinct trafficking mechanisms, with the classical subset being recruited via CCR2 and nonclassical monocytes utilizing a CX3CR1-dependent pathway [19]. Dependence on CX3CR1 may be due to survival rather than recruitment, as this chemokine receptor is important for nonclassical monocyte homeostasis [20, 21]. To add a level of complexity, nonclassical monocytes were reportedly recruited earlier than classical monocytes, or even neutrophils, to the peritoneum following intraperitoneal injection of *Listeria monocytogenes* [13]. Given these disparate outcomes in different models, the relative contributions of classical monocyte recruitment and subsequent differentiation versus sequential monocyte subset recruitment to injured tissues merit further study.

The exact relationship between these subsets and stage at which they diverge during development is unclear. Although phenotypically distinct monocyte subsets exist in the BM and blood, most studies suggest that these subsets represent the same cells at different

maturation stages [4, 22, 23]. Indeed, in the absence of inflammation, grafted classical monocytes can home back to the BM, differentiate into nonclassical monocytes, and return to the bloodstream in mice [23]. Monocyte subset conversion, after adoptive transfer of classical monocytes, also occurs in rats in the absence of further proliferation [15]. Further understanding of the pathways that govern monocyte subset conversion is critical to advance efforts to manipulate subset frequency to attenuate inflammation and/or enhance tissue repair.

Monocyte trafficking during acute inflammation: *Listeria* infection as a prototypical monocyte migration model

Monocyte migration to sites of inflammation typically occurs after neutrophil infiltration and can be sustained for days (Figure 1). Initial experiments exploring monocyte subset behavior in the context of inflammation used sterile inflammatory triggers, such as thioglycollate or lipopolysaccharide (LPS), even if the inciting agent was microbial in origin [3, 4, 15]. More recently, studies have focused on the role of classical monocytes in inflammatory models of bacterial, viral and parasitic infections and the role this subset serves in the host response.

Monocyte behavior in the context of infection is probably best characterized in Gram-positive opportunistic pathogen *Listeria monocytogenes* models [24]. *Listeria* infection provokes a robust monocytosis, an observation that led to the original name *Bacterium monocytogenes* for an unknown bacterium in 1926 [25]. More recent work demonstrated that *Listeria* infection promotes monopoiesis in the BM that is dependent on TLR signaling and is sustained by ongoing infection [26]. Intravenously administered *Listeria* invade splenocytes and hepatocytes and escape into the cytosol [25], which is required for the expression of the CCR2 ligands, CCL2 (MCP-1) and CCL7 (MCP-3), in target organs [27, 28]. These chemokines induce CCR2⁺ classical monocyte infiltration that controls infection directly and mediates initiation of adaptive immunity [24, 29]. Deletions in CCL2 or CCL7 result in reduced, but not ablated, monocyte recruitment, which correlates with an increase in bacterial burden [28]. This outcome is an intermediate phenotype, as compared to infection of *Ccr2*^{-/-} mice, where monocyte migration to infected spleen and liver is severely abated, and both bacterial burden and mortality are increased [24, 29, 30]. CCR2 signaling is thought to be the primary mechanism for monocyte migration to *Listeria*-infected tissues and mice deficient in CCR5 do not display increased susceptibility to infection [31]. *Ccr2*^{-/-} mice exhibit a specific accumulation of monocytes in the BM under both homeostatic and inflammatory conditions, suggesting that CCR2 is required for mediating monocyte egress from the BM during infection [7]. Using CCL2 reporter mice and conditional knockouts, mesenchymal stem cells and CXCL12-abundant reticular cells have been identified as the major producers of CCL2 in the BM, regulating monocyte egress in response to TLR ligands [32]. The failure to observe a role for CCR2 in tissue-specific homing in this work [7] and others indicates that CCR2 either does not play a role at this step or has a redundant role with other chemoattractants. See Text Box 2 for further discussion.

Beyond *Listeria*: The role of chemokine receptors in additional infection models

Listeria models demonstrate the critical role of CCR2 in mediating robust trafficking of classical monocytes, primarily by controlling BM egress. Using similar tools, such as *Ccr2*, *Ccl2*, or *Ccl7*^{-/-} mice, CCR2-dependent migration of classical monocytes appears to be a critical component of the host response to a wide variety of microbial infections (Figure 1), although not always to the benefit of the host.

Aerosol *Mycobacterium tuberculosis* infection induces chemokine expression, including CCL2 in lung tissue [33]. Moreover, *Ccr2*^{-/-} mice have impaired monocyte and T cell migration to the lung and increased susceptibility to high-dose infection [33]. Similarly, absence of CCL2 contributes to reduced monocyte and IFN- γ -producing T cell infiltration in the *M. tuberculosis*-infected lung [34]. *Ccr2*^{-/-} chimeric mice reconstituted with *Ccr2*^{+/+} monocytes and *Ccr2*^{-/-} T cells and infected with *M. tuberculosis* addressed whether decreased T cell recruitment is a direct result of the absence of CCR2 or an indirect result of reduced monocyte infiltration [35]. In these mice, T cells were recruited to infected lungs in comparable numbers independently of CCR2, suggesting that *M. tuberculosis* susceptibility in *Ccr2*^{-/-} mice depends predominantly on monocyte infiltration [35]. Interestingly, treatment with poly-IC, a synthetic analog of dsRNA that acts as a TLR3 agonist, of mice infected with *M. tuberculosis* renders the mice more susceptible to infection, through the robust recruitment of classical monocytes to the lung, which support bacterial growth in this model [36]. This suggests that there is a fine balance in the magnitude of monocyte recruitment that controls infection in response to *Mycobacterium* infection.

While CCR2 deficiency is beneficial during Dengue virus infection, prolonging host survival and minimizing tissue damage [37], it is detrimental in a model of West Nile virus infection of the brain [38]. Similar to *M. tuberculosis* infection, CCL2 neutralization is beneficial for the host after West Nile virus infection [39], whereas CCR2 deficiency negatively affects host survival [38], suggesting that moderating monocyte infiltration minimizes tissue damage while still controlling viral replication.

Gram-positive uropathogens, such as *Staphylococcus saprophyticus* that preferentially infect the kidney, induce only modest monocyte recruitment, despite kidney-specific expression of monocytic chemokines CCL2, CCL3, and CCL5 [40]. By contrast, CCR2-mediated, CCL2-expendable, monocyte recruitment to the kidney in a model of acute ischemic-reperfusion injury is robust and harmful to the host [41]. Both of these models induce multiple monocytic chemokines but exhibit surprisingly different monocyte trafficking patterns, suggesting further investigation is needed to determine the role of monocytes in defense against pathogens and tissue injury in the kidney. Monocyte recruitment during uropathogenic *E. coli* (UPEC) infection may be controlled by several chemokines expressed robustly in the bladder, including CCL2, CCL3, CCL4, and CCL5 [42] but the contribution of each of these ligands is unknown. Monocyte infiltration correlates with significantly reduced UPEC, suggesting that monocytes likely play a critical role in bacterial eradication [42]. In support of this idea, recent analysis reveals that TLR4 polymorphisms and expression levels on human monocytes correlate with susceptibility to acute and chronic urinary tract infection in adult patients [43]. Seemingly contradictory findings demonstrate that UPEC infection of *Ccr2*^{-/-} mice reveals no dependency on monocyte infiltration for reduction in bacterial burden [44, 45]. However, the apparently self-limiting strain used in these studies [44, 45] is rapidly cleared from the wildtype host despite inoculum fifty times higher than those used in similar infection models where UPEC robustly colonize bladder tissue [42], thus complicating data interpretation.

Fate of Recruited Monocytes: TipDCs and immunity

Classical monocytes recruited to *Listeria*-infected spleen undergo differentiation into so-called TNF and iNOS-producing DCs or TipDCs [29]. Subsequent to this study, cells resembling TipDCs have been identified in bacterial infection models of *Brucella melitensis* [46], UPEC [44], and *Salmonella* [47]. Development of TipDCs have also been observed in viral infections such as influenza [48], and parasitic infection models, including *Trypanosoma brucei* [49], *Leishmania major* [50], and *Toxoplasma gondii* infection [51] (Figure 1).

In *Salmonella* infection, recruited classical monocytes differentiate further once they have reached the infected gut, and about 20–30% express TNF α or iNOS [47]. However, these cells are, in fact, poor antigen-presenting cells, despite expressing CD11c, MHC II and co-stimulatory molecules [47]. Further, while these cells are critical in controlling bacterial burden, *Salmonella* has been shown to block monocyte-derived DC accumulation in lymph nodes when injected into the skin [47, 52, 53], due at least in part to differences in the extracellular matrix of the host [54].

In a model of oral inoculation of *Toxoplasma gondii*, classical monocytes are recruited to the villi of the small intestine in a CCR2-dependent manner [51]. Mice succumb to infection more rapidly in the absence of either CCR2 or CCL2 [55]. Recruited monocytes differentiate into cells resembling TipDCs, with the ability to produce TNF- α and iNOS, which serves to eliminate local parasite burden [51]. The authors of this study stress that the TNF α and iNOS-producing cells identified in the gut of *T. gondii*-infected mice do not express CD11c [51, 56], differentiating them from TipDCs identified during *Listeria* infection [29]. However, it is possible that they are the same cells, as TipDCs are described as being CD11c^{int} [29], which may be difficult to discern by immunofluorescent detection methods such as those used in the *T. gondii* study [51]. Of note, these cells may be the same cells identified in a peritoneal parasite infection model as recruited Gr1⁺ CD68⁺ macrophages [57].

During infection, most studies have focused on initial innate immune responses, demonstrating that TipDCs play a critical role in controlling pathogen burden, but do not demonstrate, for instance, that their absence leads to impaired adaptive immunity. Indeed, TipDCs are not necessary for CD4⁺ or CD8⁺ T priming and activation in *Listeria* infection [29]. One exception to this is a recent study describing the accumulation in lymph nodes of two DC subsets that arise during *Leishmania major* skin infection [58]. One subset migrates to lymph nodes through lymphatic vessels from infected skin while the second appears to directly enter lymph nodes from the circulation [58]. Monocyte-derived DCs from the skin exhibit superior ability to activate T cells in this system [58]. As cells identified as TipDCs are the predominant cell infected in a *L. major* model [50], it is reasonable to conclude that here, these *L. major*-associated TipDCs behave as DCs, contributing to the initiation of adaptive immune responses. This is only one example and further investigation into the role of TipDCs in eradicating pathogens or in antigen presentation are needed.

One important question is how monocytes that enter the lymph node through the HEV, such as observed in *L. major* infection, gain access to antigen for presentation after they arrive and locally differentiate into DCs in the ensuing hours and days [58–61]. Inflamed lymph nodes (LNs) contain a population of CD11c^{lo}CD11b⁺Gr1⁺ cells that phenotypically resemble monocytes, and appear to seed the LN through the HEV in a CCR2-dependent manner [15, 60, 62]. The recruitment of these cells may be a consequence of inflammatory signals that enter the LN from the lymph. In particular, CCL2 made in inflamed organs is rapidly transported to the HEV through the conduit system in the LN to recruit monocytes along HEVs [62]. It has been shown that these recruited monocytes undergo rapid differentiation into DC-like cells [58, 59], but they may preserve some aspects of their former monocyte phenotype for some period of time [15]. Do these monocytes have sufficient access to antigen that drains through conduits [63]? Alternatively, could antigen be passed on from DCs that emigrated to lymph nodes via the afferent lymphatic vessel from sites of inflammation [64]? It is important to address these possibilities in order to better understand the role of monocyte arrival to lymph nodes through the HEV in immune responses.

Monocyte trafficking during chronic inflammation: studies in atherosclerosis

Nearly all infection models that have studied monocyte trafficking have done so in an acute phase of infection. To gain a better picture of monocyte trafficking during chronic inflammation, one can turn to studies of the chronic inflammatory disease atherosclerosis. Atherosclerosis begins very early in life as deposition of lipid and infiltration of monocytes creates fatty streaks in the intima of arteries. Advanced atheromatous plaques are characterized by accumulation of monocyte-derived foam cells and modified lipoproteins, with lipid and dying foam cells contributing to the formation of a molten necrotic core [65]. Even before the disease begins, CD11c⁺ cells accumulate in areas of the artery that will, upon elevated cholesterol in the plasma, go on to form plaques [66]. In these nascent plaques, as in all stages of the disease, both monocyte subsets are recruited, where they rapidly accumulate cholesterol esters to become foam cells, using a combination of chemokine receptors that include CCR2, CCR5, and CX3CR1 [67].

Contribution of monocytosis and monocyte subsets to disease progression

Similarly to *Listeria* infection [25], monocytosis develops in apoE-deficient hypercholesterolemic mouse models and contributes significantly to the pool of monocytes available in circulation [16, 69]. Monocytosis appears to be initiated and maintained by the accumulation and retention of cholesterol in plasma membrane signaling rafts of hematopoietic cells, rendering these cells more sensitive to hematopoietic cytokines that drive monocyte precursor proliferation [70]. The number of circulating monocytes positively correlates with plaque size in mice and is thought to be an independent risk factor for cardiovascular disease in humans [71]. In mice, where monocytosis is dominated by the classical subset, this subset enters plaques that form in the aortic arch of *ApoE*^{-/-} mice at a greater frequency than the nonclassical subset [16, 69]. However, recruitment of nonclassical monocytes approaches the rate of classical monocyte entry into aortic sinus plaques (Figure 2) [72]. Thus, in contrast to acute infection or inflammation models, both monocyte subsets significantly contribute to plaque and the ratio at which they do so differs in different anatomic locations. It is uncertain if both subsets participate in disease progression from the onset or if the contribution of nonclassical monocytes increases later. In another model of chronic inflammation, chronic liver injury, only CCR2⁺ classical monocytes are recruited and appear to drive liver fibrogenesis [68]. Findings from atherosclerosis models, as well as this liver study, indicate that CCR2-dependent classical monocyte infiltration shapes disease progression during chronic inflammation.

Turning down the dial for recruitment of both monocyte subsets promotes regression

Recruited or locally differentiated nonclassical monocytes are thought to initiate repair of injured tissue [18, 19]. Thus, during reversal of atherosclerosis, one might hypothesize that the sustained recruitment of nonclassical monocytes would be desired. However, in a model of regression, in which apoE-deficient mice were treated with apoE-encoding vectors to reverse disease, both subsets of monocytes are halted from entering plaques [72]. Indeed, this cessation in recruitment accounted for the reduction in plaque macrophage burden (Figure 2) [72]. It remains possible, however, that certain aspects of “healing” during disease regression do require macrophages of a certain phenotype, but ongoing nonclassical monocyte recruitment may not be needed for remodeling and repair.

Concluding remarks

Monocyte recruitment to sites of infection, and in atherosclerosis, includes the recruitment of classical monocytes by chemokine signaling. A weakness in the current body of literature is that too few scenarios of chronic infection have been studied. Further investigation into monocyte behavior in chronic infection, such as in *M. tuberculosis*, may reveal a more prominent role for nonclassical monocytes, for example. Perhaps the function of nonclassical monocytes is in tissue repair, but few studies demonstrate that nonclassical monocytes are recruited to damaged tissue. Those that do are predominately in the setting of chronic atherosclerosis where there is no healing. Future studies are needed to address open questions and to bring the biology of monocyte subsets into sharper focus.

Text Box 1: Focus on the splenic monocyte reservoir

The spleen contains a reservoir of more than a million monocytes, with the two subsets represented in equal proportion, analogous to the blood [9]. In a model of ischemic heart injury, the classical monocyte population is recruited to the heart in an angiotensin II-dependent manner [9, 73]. Splenectomy or inhibitors of angiotensin converting enzyme reduce the number of monocytes arriving at the injured heart, which subsequently promotes healing of the damaged tissue by reducing the level of pro-inflammatory cytokines at the site of injury [9, 73]. Of note, while this study demonstrates a substantial decrease in spleen-associated monocytes that correlates with increased classical monocyte cell numbers at the injured heart, it does not directly demonstrate if only classical monocytes or if both subsets can leave the spleen. Further, although many infection models illustrate that monocytes respond to infected tissues through robust infiltration, the role of splenic reservoir monocytes has not been addressed in the context of infection. Do splenic monocytes mobilize to infected tissues? In addition to angiotensin II, what other factors mobilize splenic monocytes? Do they contribute to pathogen clearance or host tissue damage? One clue to the role of splenic monocytes arises from *Listeria* infection. While CCR2-dependent monocyte recruitment from the BM via the blood to the spleen is thought to be critical for controlling *Listeria* infection, there are no differences in bacterial burden between wildtype and *Ccr2*^{-/-} animals until day 3 in the spleen [27] as compared to day 1 in the liver [30]. These results suggest that local splenic monocytes may be able to control bacterial burden in the spleen for a short period of time. Experiments depleting or otherwise altering the splenic reservoir would shed light on these questions. Moreover, the potential role splenic monocytes play in infection of organs other than the spleen is of particular interest.

Text Box 2: Escape from the bone marrow – the only role for CCR2?

Classical monocyte recruitment relies heavily on CCR2 and its ligands, CCL2 and CCL7. While its importance in BM egress is undisputed [7, 32], the role of CCR2 in mediating monocyte homing to tissues is unclear. Using CCR2 reporter mice and mixed (*Ccr2*^{+/+} or *Ccr2* monocytes) adoptive transfer into infected mice, it was shown that post-BM egress, CCR2 dispensable for migration to *Listeria*-infected hepatic foci [30]. Here, monocyte trafficking to the liver was mediated by the upregulation of ICAM and CD44 expression and blocked by anti-CD11b, CD44 or ICAM antibodies [30]. Similarly, transferred *Ccr2*^{+/+} or *Ccr2*^{-/-} monocytes trafficked comparably to the gut in a toxoplasmosis model [51], supporting the conclusion that other signals drive monocyte recruitment to infected tissues. By contrast, CCR2-dependent migration appears to be required for monocyte recruitment from blood to the gut mucosa during colitis [74]. Further, *Ccr2*^{-/-} monocyte trafficking to the brain is reduced in West Nile virus infection when *Ccr2*^{+/+} and *Ccr2*^{-/-} monocytes are transferred into *Ccr2*^{-/-} mice at day 7 post-infection [38]. While this model shows a defect in CCR2-mediated recruitment of circulating monocytes, analysis

of the ratio between *Ccr2*^{+/+} and *Ccr2*^{-/-} monocytes in the blood and in the BM revealed that the *Ccr2*^{-/-} donor monocytes accumulate in the BM as early as 3 hours post transfer [38]. Indeed, a proportion of classical monocytes transplanted into non-irradiated mice return to the BM within 1 day [23]. Therefore, while transferring monocytes into inflamed hosts superior to using *Ccr2*^{-/-} mice or *Ccr2*^{-/-} BM chimeric mice to investigate the requirement for CCR2 beyond BM egress, all approaches are confounded by the return of transferred cells the BM. Following transfer of a 1:1 mix of *Ccr2*^{+/+} and *Ccr2*^{-/-} monocytes to the circulation, cells will return to the BM. However, only the *Ccr2*^{+/+} monocytes can egress and thus the transferred ratio will be skewed in the blood, as found in the West Nile infection model [38]. The differential recruitment of *Ccr2*^{+/+} and *Ccr2*^{-/-} bone marrow cells to the colitic gut may be CCR2- dependent but the observation may actually reflect a skewed ratio of transferred monocytes in the blood. Indeed, that any *Ccr2*^{-/-} monocytes are found in inflamed tissues suggests that CCR2 is not necessary for trafficking once monocytes are in circulation. However, given that many peripheral sites of inflammation express CCL2, it seems unlikely that CCL2 would not recruit monocytes to these sites. Supporting this conclusion, over-expression of CCL2 in pancreatic islets specifically recruits mononuclear cells to the islets while expression of the murine gammaherpesvirus M3 protein, which binds murine chemokine receptors, blocks this migration [75]. Lung-specific over-expression of CCL2 increases the number of mononuclear phagocytes and alveolar macrophages in the lung and confers additional protection against *Mycobacterium bovis* bacille Calmette-Guérin infection [76]. It is likely then, that CCR2 signaling plays a role in mediating recruitment of monocytes from the blood to the tissue, but that redundant pathways, including expression of non-chemokine chemoattractants, have masked this role.

Acknowledgments

We acknowledge the researchers who have contributed to this field but whose work is not cited or was cited through review articles because of space limitations. The laboratory's work is supported by NIH grants AI061741, AI049653, and HL096539 to GJR, an American Heart Association (AHA) Established Investigator Award 0740052 to GJR and a Ruth L. Kirschstein National Research Service Award F32HL096291 to M.A.I.

References

1. Fogg DK, et al. A clonogenic bone marrow progenitor specific for macrophages and dendritic cells. *Science*. 2006; 311(5757):83–87. [PubMed: 16322423]
2. Ziegler-Heitbrock L. The CD14+ CD16+ blood monocytes: their role in infection and inflammation. *J Leukoc Biol*. 2007; 81(3):584–592. [PubMed: 17135573]
3. Geissmann F, Jung S, Littman DR. Blood monocytes consist of two principal subsets with distinct migratory properties. *Immunity*. 2003; 19(1):71–82. [PubMed: 12871640]
4. Sunderkotter C, et al. Subpopulations of mouse blood monocytes differ in maturation stage and inflammatory response. *J Immunol*. 2004; 172(7):4410–4417. [PubMed: 15034056]
5. Gordon S, Taylor PR. Monocyte and macrophage heterogeneity. *Nat Rev Immunol*. 2005; 5(12):953–964. [PubMed: 16322748]
6. Ingersoll MA, et al. Comparison of gene expression profiles between human and mouse monocyte subsets. *Blood*. 2010; 115(3):e10–e19. [PubMed: 19965649]
7. Serbina NV, Pamer EG. Monocyte emigration from bone marrow during bacterial infection requires signals mediated by chemokine receptor CCR2. *Nat Immunol*. 2006; 7(3):311–317. [PubMed: 16462739]
8. Tsou CL, et al. Critical roles for CCR2 and MCP-3 in monocyte mobilization from bone marrow and recruitment to inflammatory sites. *J Clin Invest*. 2007; 117(4):902–909. [PubMed: 17364026]
9. Swirski FK, et al. Identification of splenic reservoir monocytes and their deployment to inflammatory sites. *Science*. 2009; 325(5940):612–616. [PubMed: 19644120]

10. Passlick B, Flieger D, Ziegler-Heitbrock HW. Identification and characterization of a novel monocyte subpopulation in human peripheral blood. *Blood*. 1989; 74(7):2527–2534. [PubMed: 2478233]
11. Ancuta P, et al. Fractalkine preferentially mediates arrest and migration of CD16+ monocytes. *J Exp Med*. 2003; 197(12):1701–1707. [PubMed: 12810688]
12. Cros J, et al. Human CD14dim monocytes patrol and sense nucleic acids and viruses via TLR7 and TLR8 receptors. *Immunity*. 2010; 33(3):375–386. [PubMed: 20832340]
13. Auffray C, et al. Monitoring of blood vessels and tissues by a population of monocytes with patrolling behavior. *Science*. 2007; 317(5838):666–670. [PubMed: 17673663]
14. Jakubzick C, et al. Lymph-migrating, tissue-derived dendritic cells are minor constituents within steady-state lymph nodes. *J Exp Med*. 2008; 205(12):2839–2850. [PubMed: 18981237]
15. Yrlid U, Jenkins CD, MacPherson GG. Relationships between distinct blood monocyte subsets and migrating intestinal lymph dendritic cells in vivo under steady-state conditions. *J Immunol*. 2006; 176(7):4155–4162. [PubMed: 16547252]
16. Tacke F, et al. Monocyte subsets differentially employ CCR2, CCR5, and CX3CR1 to accumulate within atherosclerotic plaques. *J Clin Invest*. 2007; 117(1):185–194. [PubMed: 17200718]
17. Qu C, et al. Role of CCR8 and other chemokine pathways in the migration of monocyte-derived dendritic cells to lymph nodes. *J Exp Med*. 2004; 200(10):1231–1241. [PubMed: 15534368]
18. Arnold L, et al. Inflammatory monocytes recruited after skeletal muscle injury switch into antiinflammatory macrophages to support myogenesis. *J Exp Med*. 2007; 204(5):1057–1069. [PubMed: 17485518]
19. Nahrendorf M, et al. The healing myocardium sequentially mobilizes two monocyte subsets with divergent and complementary functions. *J Exp Med*. 2007; 204(12):3037–3047. [PubMed: 18025128]
20. Jakubzick C, et al. Blood monocyte subsets differentially give rise to CD103+ and CD103– pulmonary dendritic cell populations. *J Immunol*. 2008; 180(5):3019–3027. [PubMed: 18292524]
21. Landsman L, et al. CX3CR1 is required for monocyte homeostasis and atherogenesis by promoting cell survival. *Blood*. 2009; 113(4):963–972. [PubMed: 18971423]
22. Tacke F, et al. Immature monocytes acquire antigens from other cells in the bone marrow and present them to T cells after maturing in the periphery. *J Exp Med*. 2006; 203(3):583–597. [PubMed: 16492803]
23. Varol C, et al. Monocytes give rise to mucosal, but not splenic, conventional dendritic cells. *J Exp Med*. 2007; 204(1):171–180. [PubMed: 17190836]
24. Serbina NV, et al. Monocyte-mediated defense against microbial pathogens. *Annu Rev Immunol*. 2008; 26:421–452. [PubMed: 18303997]
25. Stavru F, Archambaud C, Cossart P. Cell biology and immunology of *Listeria monocytogenes* infections: novel insights. *Immunol Rev*. 2011; 240(1):160–184. [PubMed: 21349093]
26. Serbina NV, et al. Selective expansion of the monocytic lineage directed by bacterial infection. *J Immunol*. 2009; 183(3):1900–1910. [PubMed: 19596996]
27. Serbina NV, et al. Sequential MyD88-independent and -dependent activation of innate immune responses to intracellular bacterial infection. *Immunity*. 2003; 19(6):891–901. [PubMed: 14670305]
28. Jia T, et al. Additive roles for MCP-1 and MCP-3 in CCR2-mediated recruitment of inflammatory monocytes during *Listeria monocytogenes* infection. *J Immunol*. 2008; 180(10):6846–6853. [PubMed: 18453605]
29. Serbina NV, et al. TNF/iNOS-producing dendritic cells mediate innate immune defense against bacterial infection. *Immunity*. 2003; 19(1):59–70. [PubMed: 12871639]
30. Shi C, et al. Monocyte trafficking to hepatic sites of bacterial infection is chemokine independent and directed by focal intercellular adhesion molecule-1 expression. *J Immunol*. 2010; 184(11): 6266–6274. [PubMed: 20435926]
31. Zhong MX, et al. Chemokine receptor 5 is dispensable for innate and adaptive immune responses to *Listeria monocytogenes* infection. *Infect Immun*. 2004; 72(2):1057–1064. [PubMed: 14742553]

32. Shi C, et al. Bone Marrow Mesenchymal Stem and Progenitor Cells Induce Monocyte Emigration in Response to Circulating Toll-like Receptor Ligands. *Immunity*. 2011 **In Press, Corrected Proof**.
33. Scott HM, Flynn JL. Mycobacterium tuberculosis in chemokine receptor 2-deficient mice: influence of dose on disease progression. *Infect Immun*. 2002; 70(11):5946–5954. [PubMed: 12379669]
34. Kipnis A, et al. Role of chemokine ligand 2 in the protective response to early murine pulmonary tuberculosis. *Immunology*. 2003; 109(4):547–551. [PubMed: 12871221]
35. Peters W, et al. CCR2-dependent trafficking of F4/80dim macrophages and CD11cdim/intermediate dendritic cells is crucial for T cell recruitment to lungs infected with Mycobacterium tuberculosis. *J Immunol*. 2004; 172(12):7647–7653. [PubMed: 15187146]
36. Antonelli LR, et al. Intranasal Poly-IC treatment exacerbates tuberculosis in mice through the pulmonary recruitment of a pathogen-permissive monocyte/macrophage population. *J Clin Invest*. 2010; 120(5):1674–1682. [PubMed: 20389020]
37. Guabiraba R, et al. Role of the chemokine receptors CCR1, CCR2 and CCR4 in the pathogenesis of experimental dengue infection in mice. *PLoS One*. 2010; 5(12):e15680. [PubMed: 21206747]
38. Lim JK, et al. Chemokine receptor Ccr2 is critical for monocyte accumulation and survival in West Nile virus encephalitis. *J Immunol*. 2011; 186(1):471–478. [PubMed: 21131425]
39. Getts DR, et al. Ly6c+ "inflammatory monocytes" are microglial precursors recruited in a pathogenic manner in West Nile virus encephalitis. *J Exp Med*. 2008; 205(10):2319–2337. [PubMed: 18779347]
40. Kline KA, et al. Characterization of a novel murine model of Staphylococcus saprophyticus urinary tract infection reveals roles for Ssp and SdrI in virulence. *Infect Immun*. 2010; 78(5):1943–1951. [PubMed: 20176795]
41. Li L, et al. The chemokine receptors CCR2 and CX3CR1 mediate monocyte/macrophage trafficking in kidney ischemia-reperfusion injury. *Kidney Int*. 2008; 74(12):1526–1537. [PubMed: 18843253]
42. Ingersoll MA, et al. G-CSF induction early in uropathogenic Escherichia coli infection of the urinary tract modulates host immunity. *Cell Microbiol*. 2008; 10(12):2568–2578. [PubMed: 18754853]
43. Yin X, et al. Association of Toll-like receptor 4 gene polymorphism and expression with urinary tract infection types in adults. *PLoS One*. 2010; 5(12):e14223. [PubMed: 21151974]
44. Engel D, et al. Tumor necrosis factor alpha- and inducible nitric oxide synthase510 producing dendritic cells are rapidly recruited to the bladder in urinary tract infection but are dispensable for bacterial clearance. *Infect Immun*. 2006; 74(11):6100–6107. [PubMed: 16966414]
45. Engel DR, et al. CCR2 mediates homeostatic and inflammatory release of Gr1(high) monocytes from the bone marrow, but is dispensable for bladder infiltration in bacterial urinary tract infection. *J Immunol*. 2008; 181(8):5579–5586. [PubMed: 18832716]
46. Copin R, et al. MyD88-dependent activation of B220-CD11b+LY-6C+ dendritic cells during Brucella melitensis infection. *J Immunol*. 2007; 178(8):5182–5191. [PubMed: 17404301]
47. Rydstrom A, Wick MJ. Monocyte recruitment, activation, and function in the gut-associated lymphoid tissue during oral Salmonella infection. *J Immunol*. 2007; 178(9):5789–5801. [PubMed: 17442963]
48. Aldridge JR Jr, et al. TNF/iNOS-producing dendritic cells are the necessary evil of lethal influenza virus infection. *Proc Natl Acad Sci U S A*. 2009; 106(13):5306–5311. [PubMed: 19279209]
49. Bosschaerts T, et al. Tip-DC development during parasitic infection is regulated by IL-10 and requires CCL2/CCR2, IFN-gamma and MyD88 signaling. *PLoS Pathog*. 2010; 6(8)
50. De Trez C, et al. iNOS-producing inflammatory dendritic cells constitute the major infected cell type during the chronic Leishmania major infection phase of C57BL/6 resistant mice. *PLoS Pathog*. 2009; 5(6):e1000494. [PubMed: 19557162]
51. Dunay IR, et al. Gr1(+) inflammatory monocytes are required for mucosal resistance to the pathogen Toxoplasma gondii. *Immunity*. 2008; 29(2):306–317. [PubMed: 18691912]
52. Rotta G, et al. Lipopolysaccharide or whole bacteria block the conversion of inflammatory monocytes into dendritic cells in vivo. *J Exp Med*. 2003; 198(8):1253–1263. [PubMed: 14568983]

53. Rydstrom A, Wick MJ. Salmonella inhibits monocyte differentiation into CD11c hi MHC-II hi cells in a MyD88-dependent fashion. *J Leukoc Biol.* 2010; 87(5):823–832. [PubMed: 20124491]
54. Rotta G, et al. Contrasting roles of SPARC-related granuloma in bacterial containment and in the induction of anti-Salmonella typhimurium immunity. *J Exp Med.* 2008; 205(3):657–667. [PubMed: 18316416]
55. Robben PM, et al. Recruitment of Gr-1+ monocytes is essential for control of acute toxoplasmosis. *J Exp Med.* 2005; 201(11):1761–1769. [PubMed: 15928200]
56. Dunay IR, Sibley LD. Monocytes mediate mucosal immunity to *Toxoplasma gondii*. *Curr Opin Immunol.* 2010; 22(4):461–466. [PubMed: 20537517]
57. Mordue DG, Sibley LD. A novel population of Gr-1+ activated macrophages induced during acute toxoplasmosis. *J Leukoc Biol.* 2003; 74(6):1015–1025. [PubMed: 12972511]
58. Leon B, Lopez-Bravo M, Ardavin C. Monocyte-derived dendritic cells formed at the infection site control the induction of protective T helper 1 responses against *Leishmania*. *Immunity.* 2007; 26(4):519–531. [PubMed: 17412618]
59. Cheong C, et al. Microbial stimulation fully differentiates monocytes to DC-SIGN/CD209(+) dendritic cells for immune T cell areas. *Cell.* 2010; 143(3):416–429. [PubMed: 21029863]
60. Nakano H, et al. Blood-derived inflammatory dendritic cells in lymph nodes stimulate acute T helper type 1 immune responses. *Nat Immunol.* 2009; 10(4):394–402. [PubMed: 19252492]
61. Nagaoka K, et al. Expression of C-type lectin, SIGNR3, on subsets of dendritic cells, macrophages, and monocytes. *J Leukoc Biol.* 2010; 88(5):913–924. [PubMed: 20724666]
62. Palframan RT, et al. Inflammatory chemokine transport and presentation in HEV: a remote control mechanism for monocyte recruitment to lymph nodes in inflamed tissues. *J Exp Med.* 2001; 194(9):1361–1373. [PubMed: 11696600]
63. Sixt M, et al. The conduit system transports soluble antigens from the afferent lymph to resident dendritic cells in the T cell area of the lymph node. *Immunity.* 2005; 22(1):19–29. [PubMed: 15664156]
64. Allan RS, et al. Migratory dendritic cells transfer antigen to a lymph node-resident dendritic cell population for efficient CTL priming. *Immunity.* 2006; 25(1):153–162. [PubMed: 16860764]
65. Tabas I. Consequences and therapeutic implications of macrophage apoptosis in atherosclerosis: the importance of lesion stage and phagocytic efficiency. *Arterioscler Thromb Vasc Biol.* 2005; 25(11):2255–2264. [PubMed: 16141399]
66. Paulson KE, et al. Resident intimal dendritic cells accumulate lipid and contribute to the initiation of atherosclerosis. *Circ Res.* 2010; 106(2):383–390. [PubMed: 19893012]
67. Gautier EL, Jakubzick C, Randolph GJ. Regulation of the migration and survival of monocyte subsets by chemokine receptors and its relevance to atherosclerosis. *Arterioscler Thromb Vasc Biol.* 2009; 29(10):1412–1418. [PubMed: 19759373]
68. Karlmark KR, et al. Hepatic recruitment of the inflammatory Gr1+ monocyte subset upon liver injury promotes hepatic fibrosis. *Hepatology.* 2009; 50(1):261–274. [PubMed: 19554540]
69. Swirski FK, et al. Ly-6Chi monocytes dominate hypercholesterolemia-associated monocytes and give rise to macrophages in atheromata. *J Clin Invest.* 2007; 117(1):195–205. [PubMed: 17200719]
70. Yvan-Charvet L, et al. ATP-binding cassette transporters and HDL suppress hematopoietic stem cell proliferation. *Science.* 2010; 328(5986):1689–1693. [PubMed: 20488992]
71. Combadiere C, et al. Combined inhibition of CCL2, CX3CR1, and CCR5 abrogates Ly6C(hi) and Ly6C(lo) monocytes and almost abolishes atherosclerosis in hypercholesterolemic mice. *Circulation.* 2008; 117(13):1649–1657. [PubMed: 18347211]
72. Potteaux S, et al. Suppressed monocyte recruitment drives macrophage removal from atherosclerotic plaques of Apoe^{-/-} mice during disease regression. *The Journal of Clinical Investigation.* 2011 **in press**.
73. Leuschner F, et al. Angiotensin-converting enzyme inhibition prevents the release of monocytes from their splenic reservoir in mice with myocardial infarction. *Circ Res.* 2010; 107(11):1364–1373. [PubMed: 20930148]
74. Platt AM, et al. An independent subset of TLR expressing CCR2-dependent macrophages promotes colonic inflammation. *J Immunol.* 2010; 184(12):6843–6854. [PubMed: 20483766]

75. Martin AP, et al. The chemokine decoy receptor M3 blocks CC chemokine ligand 2 and CXC chemokine ligand 13 function in vivo. *J Immunol.* 2006; 177(10):7296–7302. [PubMed: 17082648]
76. Schreiber O, et al. Mice that overexpress CC chemokine ligand 2 in their lungs show increased protective immunity to infection with *Mycobacterium bovis* bacille Calmette-Guerin. *J Infect Dis.* 2008; 198(7):1044–1054. [PubMed: 18694332]

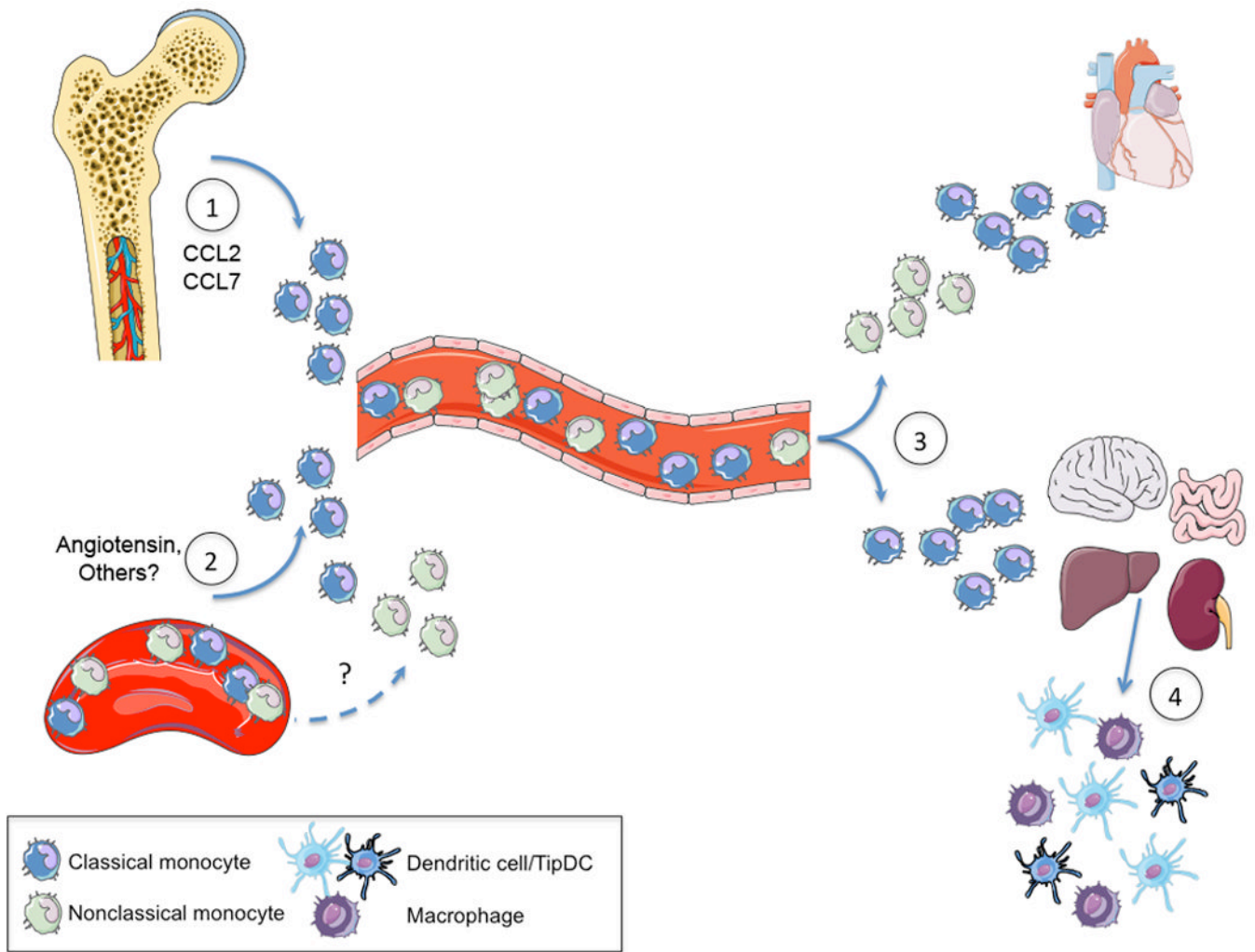


Figure 1. Monocyte trafficking in acute injury or infection

(1) Signals including CCR2-ligand (CCL2 and CCL7) binding induce classical monocyte egress from bone marrow during inflammation or infection [32]. (2) Angiotensin II induces monocyte egress from the splenic reservoir during myocardial infarction [9]. While an increase in total monocytes is observed in the blood concomitant with a decrease in splenic monocytes [9], it is not clear if nonclassical monocytes are released from the spleen during myocardial infarction. (3) Classical monocytes arrive first at the injured heart, followed by non-classical monocytes [19]. Only classical monocytes are recruited to injured or infected sites such as brain, gut, liver and kidney. (4) Upon recruitment, monocytes may differentiate into macrophages, dendritic cells, or TipDCs.

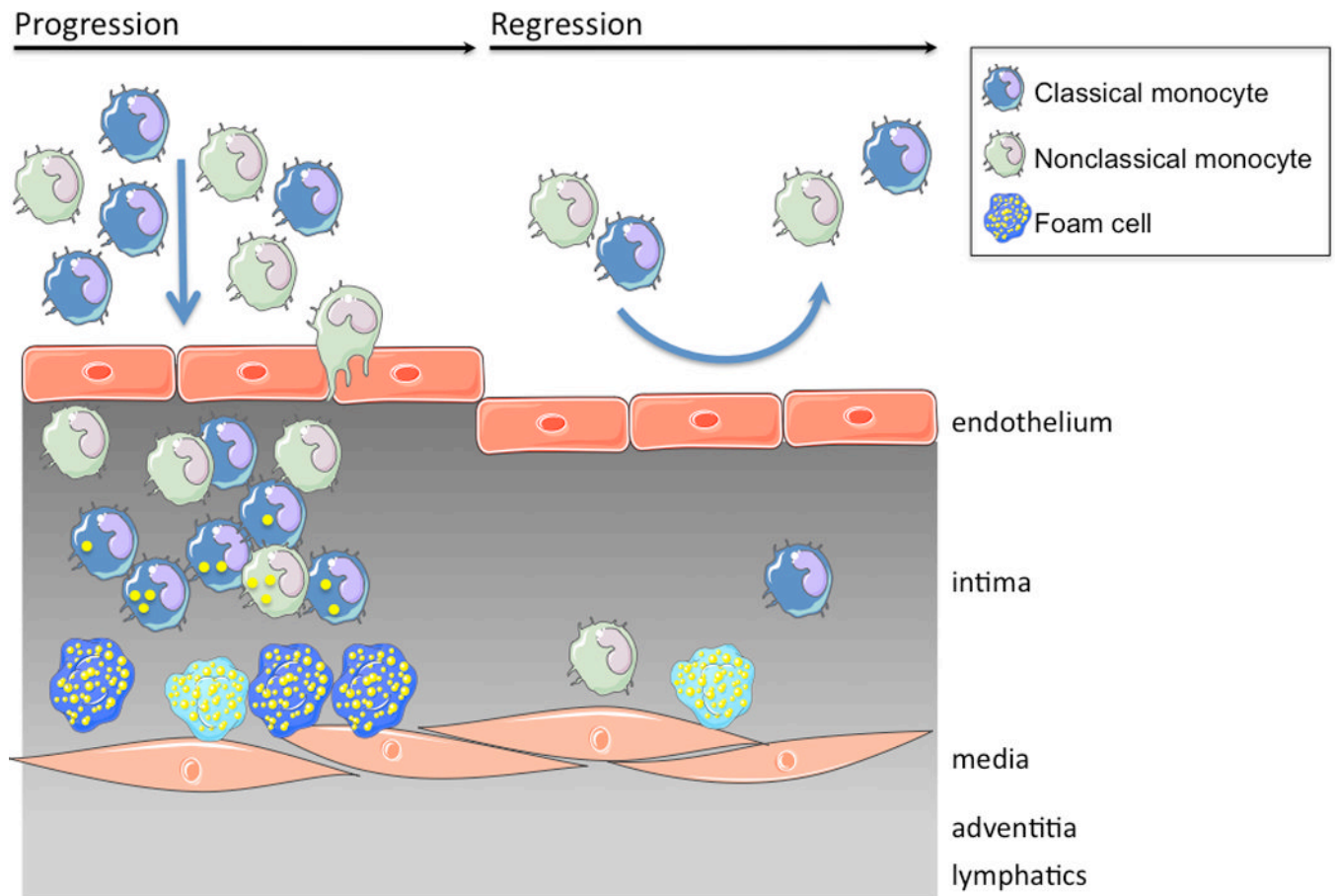


Figure 2. Monocyte recruitment during in atherosclerosis

During progressive disease, both classical and nonclassical monocytes are recruited to sites of activated endothelium through CCR2, CCR5, and CX3CR1 signaling pathways [67]. Monocytes accumulate in the intima, take up lipid (yellow dots), and develop into lipid-laden foam cells. During regressive disease, recruitment of both subsets is reduced [72]. In contrast to the acute injured heart model, where recruited nonclassical monocytes contribute to tissue healing [19], in atherosclerosis, healing is correlated with a reduction in total monocyte recruitment.