Monocytes in Myocardial Infarction

Partha Dutta, Matthias Nahrendorf

Abstract—Myocardial infarction (MI) is the leading cause of death in developed countries. Though timely revascularization of the ischemic myocardium and current standard therapy reduce acute mortality after MI, long-term morbidity and mortality remain high. During the first 1 to 2 weeks after MI, tissues in the infarcted myocardium undergo rapid turnover, including digestion of extracellular matrix and fibrosis. Post-MI repair is crucial to survival. Monocytes recruited to the infarcted myocardium remove debris and facilitate the repair process. However, exaggerated inflammation may also impede healing, as demonstrated by the association between elevated white blood cell count and in-hospital mortality after MI. Monocytes produced in the bone marrow and spleen enter the blood after MI and are recruited to the injured myocardium in 2 phases. The first phase is dominated by Ly-6chigh monocytes and the second phase by Ly-6clow monocytes. Yet the number of Ly6Clow macrophages in later healing stages. Understanding the signals regulating monocytosis after MI will help design new therapies to facilitate cardiac healing and limit heart failure. (Arterioscler Thromb Vasc Biol. 2015;35:00-00. DOI: 10.1161/ATVBAHA.114.304652.)

Key Words: hematopoiesis ■ macrophages ■ monocytes ■ myocardial infarction

Monocytes

The innate immune system initiates defense against microorganisms quickly and efficiently, and monocytes are innate immunity’s major players. Monocytes comprise 10% and 4% of human and mouse blood leukocytes, respectively. The main subset of CD115+ monocytes in mice express high levels of Ly-6c, CCR2, and CD62L and low level of CX3CR1.1 Ly-6chigh monocytes are recruited to inflamed sites and produce high levels of proinflammatory cytokines, such as tumor necrosis factor-α and interleukin-1β. Hence, Ly-6chigh monocytes are named inflammatory monocytes. The second subset of mouse monocytes expresses a high level of CXCR4, and a low level of Ly-6c. They reside in blood vessels in steady state and may play important roles in scavenging oxidized lipids, dead cells, and pathogens.2 Both monocyte subsets circulate in the blood and survey steady-state tissue by transporting self-antigens to lymph nodes with minimal differentiation to macrophages, but they can differentiate into macrophages and dendritic cells at sites of inflammation.3 At least 3 subsets of monocytes exist in humans. CD14++CD16+ monocytes resemble mouse Ly-6chigh monocytes, CD14+CD16+ monocytes have proinflammatory roles, and CD14+CD16+ monocytes exhibit patrolling behavior similar to mouse Ly-6chigh monocytes.

Macrophage Origins

During embryonic development, various organs are seeded by macrophages derived from yolk sac or liver progenitors. Most of these macrophages can self-maintain by homeostatic proliferation.4,5 Such self-maintenance was first investigated in microglia, which respond to various injuries, including CNS damage, and can self-renew without blood monocyte contribution.6 Yona et al7 used constitutive and conditional CX3CR1 reporter mice to demonstrate that tissue-resident macrophages, including Kupffer cells and lung, splenic, and peritoneal macrophages, are established before birth and can replenish themselves in adulthood by local proliferation. These data were consistent with the findings by Schulz et al,8 who described that the origin of yolk sac–derived tissue resident macrophages is independent of Myb, a transcription factor required for hematopoietic stem cells (HSC) and monocyte development. Taken together, these studies, in addition to others, indicate that many tissue-resident macrophages are not derived from monocytes in steady state, at least in young mice. Two notable exceptions are dermal and intestinal macrophages. The dermis is populated by various myeloid cells, including macrophages and dendritic cells. Dermal macrophages are highly phagocytic but do not efficiently activate T cells, whereas dermal dendritic cells have strong T-cell stimulatory capacity. In a recent study, Tamoutounour et al9 found that, after 8 weeks of parabiosis, ≈20% of dermal macrophages were parabiont-derived, indicating their monocytic origin. Intestinal macrophage maintenance also depends on blood monocytes.10,11 A recent study12 showed that although yolk sac and fetal macrophages seed the lamina propria, they begin to wane right after birth and are replaced by blood monocytes. This process depends on CCR2 and commensal...
Nonstandard Abbreviations and Acronyms

HSC  hematopoietic stem cells
M-CSF  macrophage colony-stimulating factor
MI  myocardial infarction

but gut microbiota; mice maintained in germ-free conditions have fewer colon macrophages than those in regular housing.

Like other organs, the heart contains macrophages in steady state. A recently published study characterized cardiac macrophage subsets and investigated their origins. The authors reported 4 cardiac macrophage subsets expressing varying levels of Ly-6c and major histocompatibility complex class II. Cardiac macrophages are derived from yolk sac and fetal monocyte progenitors and are replenished by local proliferation in steady state. Yet in injury, such as myocardial infarction (MI), cardiac macrophages are replaced by blood monocytes. Contrary to the notion that cardiac macrophages self-maintain by proliferation, a recent study demonstrated that embryonic-derived cardiac macrophages are continuously replenished by blood monocytes. 

Monocyte Production in Steady State

Monocytes develop from bone marrow HSC after going through several progenitor stages, including common myeloid progenitor, granulocyte/macrophage progenitor, and macrophage/dendritic cells progenitor. Blood monocyte development depends on macrophage colony-stimulating factor (M-CSF). M-CSF–deficient op/op mice have drastic reductions in blood monocyte numbers and atherosclerotic plaque burden if crossbred with LDLR−/− mice. M-CSF is also involved in tissue-resident macrophage proliferation. Tagliani et al found that macrophage proliferation in the uterus during pregnancy was driven by M-CSF. Moreover, the proliferating macrophages produced higher levels of mcp-1, a CCR2 ligand in the myometrium, leading to extravasation of Ly-6chigh monocytes. Another example of M-CSF–dependent macrophage proliferation is that the cytokine induces Gata6−/− macrophages. 

Several transcription factors, such as PU.1, determine HSC differentiation into common myeloid progenitor rather than common lymphoid progenitors. PU.1 binds to GATA-1 and inhibits commitment toward a megakaryocyte–erythroid progenitor, which facilitates myeloid differentiation. Moreover, PU.1 represses mast cell development. Other transcription factors, such as CCAAT/enhancer-binding protein (Cebpa), early growth response gene and interferon consensus sequence-binding protein, also determine myeloid versus lymphoid lineage fate. Surprisingly, Cebpa expression in B and T lymphocytes can transdifferentiate them into macrophages.

Ikaros, a transcription factor that encodes a family of hematopoietic-specific zinc finger proteins, is a central regulator of lymphocyte differentiation.

Transcription factors involved in Ly-6chigh versus Ly-6clow monocyte generation are not well understood, with the exception of the orphan nuclear hormone receptor Nr4a1 (also known as Nur77), which is involved in Ly-6clow monocyte production and survival. However, some reports indicate that the transcription factor is dispensable for Ly-6clow macrophage production.

Monocyte Production After MI

MI activates adrenergic signaling that alerts bone marrow niche cells, which reduce production of HSC retention factors (Figure 1). Consequently, HSC egress from the bone marrow and seed in the spleen. This triggers extramedullary hematopoiesis and monocyte production. Within 24 hours after MI, the spleen’s monocyte reservoir is released. Splenectomy experiments indicated that the organ may contribute as much as half of the monocyte population recruited to the infarct. Within 4 days after MI, the splenic monocyte reservoir refills by proliferation and differentiation of HSC and progenitors. In the spleen, HSC proliferation is stem cell factor–dependent. Neutralizing stem cell factor reduces HSC proliferation and monocyte production. Splenic monocyte production from hematopoietic progenitors also depends on interleukin-10, interleukin-3, and GM-CSF. Currently,
mechanics of splenic HSC maintenance are mostly unknown. We recently found that macrophages are important players in splenic HSC retention, as depleting splenic macrophages with M-CSF receptor knockdown or diphtheria toxin in CD169-DTR mice mobilized splenic HSC and reduced monocyte production.41 Interestingly, splenic macrophages retain HSC via vascular cell adhesion protein 1.

Monocyte Release From Hematopoietic Organs in Steady State and After MI
Monocyte release from the bone marrow follows the circadian rhythm, peaking at ZT4 and reaching nadir at ZT 16.37 Blood monocytes’ diurnal rhythm is linked to fluctuation of several clock genes, such as Bmal1, Nrl1, and Dbp. Diurnal variation of monocyte egress from the bone marrow and increased release during inflammation is driven by changing Mcp-1 levels in the blood. Listeria monocytogenes infection triggers higher blood monocyte levels and results in higher mortality because of massive cytokine storm. Consistent with this, toll-like receptor 9 expression is also highly improved the adaptive immune response.38 Like monocyte fluctuation, mortality and morbidity after MI also follow circadian rhythms. Disrupted diurnal levels aggrivated myocardial remodeling and function after MI.39 During the first 5 days after MI, a critical time for scar formation, there are high macrophage levels in the infarct. Homozygous clock mutant mice exhibited similarly aggravated ventricular remodeling after MI, which accorded with the idea that blood monocyte levels, regulated by circadian rhythm, may determine myocardial repair post-MI. This theory was also supported by a clinical study40 reporting that infarct size peaked at 1:00 AM in patients with ST-segment-elevation MI.

Monocytes leave the bone marrow during diseases, such as infections, atherosclerosis, and MI. Monocyte release after lipopolysaccharides challenge was accompanied by elevated Mcp-1 production by mesenchymal stem cells and Cxcl12-abundant reticular cells lining bone marrow sinusoids. Conditional deletion of Mcp-1 from these cells significantly reduced monocyte egress after lipopolysaccharides challenge. In addition to mesenchymal stem cells, bone marrow endotelial cells may produce Mcp-1 after MI. Similar to monocyte release after lipopolysaccharides challenge, these cells can also produce Mcp-1, resulting in their mobilization into the blood. However, this hypothesis remains to be investigated. As mentioned above, the spleen functions as monocyte reservoir. After MI, monocyte departure from the splenic red pulp depends on angiotensin II-angiotensin 1 receptor signaling.33 Angiotensin II infusion in mice reproduced MI-induced motility of splenic monocytes and their release into the blood.41

Monocyte Recruitment to the Myocardium After MI
After MI, circulating monocytes produced in the bone marrow and spleen are recruited to the infarct in 2 phases,42 with the first phase dominated by Ly-6chigh monocytes. Recruitment of Ly-6chigh monocytes is CCR2-dependent. Ccl2 and Ccl7, both ligands for CCR2, are expressed at high levels in infarcted myocardium.43,44 B cells in ischemic myocardium are likely the source of Ccl7 after MI. Depleting B cells resulted in improved ventricular function accompanied by reduced monocyte recruitment.44 In the second phase of post-MI monocyte response, Ly-6chlow monocyte recruitment depends on Cxcr,45 However, compared with the early recruitment of inflammatory monocytes, far fewer Ly6chlow monocytes are recruited to the infarct, and Ly6chhigh monocytes can give rise to Ly6chlow macrophages in later healing stages.46 Other mononuclear chemoattractants, such as Ccl3 and Ccl4, are also highly expressed in infarcts,46 but their role in myocardial injury remains unstudied. In addition, ELR-containing Cxc chemokines, which are strong neutrophil chemoattractants, are present in the infarct at high levels.47

Monocyte/Macrophage Functions After MI
The 2 sequential monocyte/macrophage phases are both important for healing after acute MI. Ly-6chhigh monocytes give rise to early inflammatory macrophages, and both clear damaged tissue by phagocytosis and secreting proteolytic enzymes. In the second phase, Ly-6chlow macrophages facilitate wound healing and regeneration by promoting myofibroblast accumulation, collagen deposition, and angiogenesis. Infiltrated monocytes may also interact with extracellular matrix in the damaged myocardium, leading to fibronectin release.48 Fibronectin stabilizes the infarct and reduces infarct rupture. Once in the infarct, monocytes differentiate into macrophages in the presence of M-CSF. Macrophages promote angiogenesis, fibroblast proliferation, and extracellular matrix deposition. Myofibroblasts, which are modified fibroblasts and α-smooth muscle actin-positive, are the major sources of collagen in the infarct. Myofibroblast differentiation is transforming growth factor-β-dependent. Macrophages also play a role in organ regeneration. Though MI in adult mammals leads to scarring and diminished ventricular function, neonatal mouse hearts can regenerate after MI without scarring,49 but depleting cardiac macrophages impedes this repair process. A recent study50 showed that embryonic-derived cardiac macrophages promote angiogenesis and healing after myocardial damage. Consistent with this, salamander limb regeneration also depends on macrophages.51 Although inflammation is required for cellular debris removal and new tissue formation after ischemic injury, exaggerated inflammation may impede the healing process, as shown in ApoE−/− mice with coronary ligation.52 Accordingly, blood monocyte count after MI positively correlates with left ventricular end-diastolic volume and negatively correlates with ejection fraction in patients.53 Mcp-1-deficient mice have markedly less monocyte recruitment to the infarct and, consequently, significantly fewer macrophages therein.43 Even though Mcp-1-deficient and wild-type mice had similar infarct sizes, the Mcp-1-deficient mice had improved ventricular function, thereby indicating monocytes’ importance in myocardial healing after MI.

Conclusions
Monocytosis ensues during MI and is vital to eradicating pathogens in systemic or local infection. However, because
exaggerated monocytosis impairs healing, as discussed above, curbing monocyte recruitment to the infarct may improve ventricular function.\(^5\) In addition, MI-induced monocytosis exacerbates other cardiovascular complications. In a clinical study by Han et al., patients with ST-segment-elevation MI had accelerated nonculprit coronary artery lesion atherosclerosis. Consistent with the finding in mice,\(^3\) patients with acute MI had higher splenic metabolic activity as determined by whole body fluorine-18 fluorodeoxyglucose positron emission tomography–computed tomography.\(^5\)

Although the positron emission tomographic imaging agent reports glucose uptake and is not cell-type specific, the data may indicate higher hematopoietic progenitor proliferation in the spleen after MI. By blocking the β-adrenergoreceptor, we decreased HSC egress from the bone marrow and thereby reduced monocytosis after MI in ApoE\(^{-/-}\) mice.\(^3\) In addition, splenic HSC proliferation depends on stem cell factor, and stem cell factor neutralization reduced extramedullary monocytosis after MI. Monocyte release from hematopoietic organs depends on angiotensin-II receptor signaling and CCR2 and could be a target for drug development. After this line of inquiry, we found that CCR2 knockdown decreased inflammatory monocyte recruitment to the infarct, thereby facilitating the healing process. Like monocyte production and migration, macrophage polarization may be harnessed to reduce complications of myocardial ischemia, especially heart failure.\(^9\)

Sources of Funding

This work was supported by National Institutes of Health (K99HL121076, R01HL095657, R01HL114477, R01HL117829, and R01NS084863).

Disclosures

None.

References


Monocytes in Myocardial Infarction
Partha Dutta and Matthias Nahrendorf

Arterioscler Thromb Vasc Biol. published online March 19, 2015;
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/early/2015/03/19/ATVBAHA.114.304652

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/