Monocytes: Protagonists of Infarct Inflammation and Repair After Myocardial Infarction

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Myocardial infarction (MI) is the most frequent cause of heart failure, which is an incapacitating disease with high prevalence and broad socioeconomic impact. In 2008 in the United States, 5.7 million people suffered from heart failure, and more than 287,000 people died.1 Timely revascularization of ischemic myocardium reduces acute infarct mortality, and current standard therapy with β blockers and angiotensin-converting enzyme (ACE) inhibitors curbs development of post-MI heart failure. For example, ACE inhibitor treatment reduced mortality from 25% to 20% in the Survival and Ventricular Enlargement (SAVE) trial.2 Although this is a major advance, long-term mortality remains high. The combination of reduced acute infarct mortality due to efficient acute care and insufficient options to treat infarct survivors chronically has contributed to an increased heart failure prevalence (Figure 1).3

The need to understand and treat heart failure better has motivated clinicians and basic scientists to explore new therapeutic strategies to repair the failing heart, for instance, with stem cells.4,5 Augmentation of intrinsic wound healing that occurs during the first 1 to 2 weeks after MI is a prospective approach with the potential to prevent heart failure. During this period, the infarct is highly active biologically.6–8 Delicate granulation tissue undergoes rapid turnover of cells and of structural components such as the extracellular matrix. Preexisting collagen is digested and new matrix is laid down. During these extensive changes of tissue architecture, the vulnerable wound is exposed to the mechanical stress of cycling intraventricular pressure and myocardial contraction, and the heart can undergo profound and deleterious changes in ventricular geometry and function. In the short term (days, weeks), poor healing can lead to infarct expansion and left ventricular dilatation and in some cases to infarct rupture and death. In the long term (months, years), filling pressure, wall stress, and left ventricular volume can increase and propagate adverse remodeling, leading to heart failure and a poor prognosis.7,9,10 Conversely, “sufficient” healing preserves left ventricular geometry and prevents heart failure. In this review, we propose that the quality of infarct healing shortly after injury determines the fate of the patient for years to come.

Clinicians are well aware of the adverse impact of diabetes mellitus, atherosclerosis, and immunosuppression on tissue repair in leg ulcers, diabetic retinopathy, or surgical wounds. However, the wound in the ischemic heart, possibly because it is concealed beneath the skin, has only recently drawn attention as a potential therapeutic target. Consequently, the impact of comorbidities on healing processes in the heart is poorly understood. This review focuses on the role of monocytes, immune cells that dominate healing of the injured myocardium within the first 2 weeks after MI. We discuss recruitment and function of monocyte subsets and their dual role as key inflammatory components of atherosclerotic disease and as central regulators in infarct healing.

Monocyte Subset Phenotypes and Their Specific Functions

The central function of the immune system is response to injury and infection. The innate immune system, which is typically the first to respond, consists of cells of the myeloid lineage that include neutrophils and monocytes. During injury, these cells accumulate quickly to eliminate dead or dying tissue. The response is massive but “blunt,” as it lacks the specificity emblematic of adaptive immunity. Studies continue to demonstrate, however, that innate immunity is nuanced and regulated by a plethora of signals; although responses can be “blunt” from the perspective of antigen recognition, they are remarkably specific spatiotemporally and quantitatively. One line of investigation that is particularly demonstrative of this has focused on monocytes and their subsets.

Monocytes belong to the mononuclear phagocyte system, a categorization that encompasses multiple cell types of shared ontogeny at various stages of differentiation with essential roles in development, inflammation, and host defense.11–12 Mononuclear phagocytes were discovered by Elie Metchnikoff a century ago. Monocytes are produced in the bone marrow from macrophage and dendritic cell progenitors13,14 and, on maturation, enter the circulation in a process that depends on the chemokine receptor CCR2.15,16 Monocytes circulate freely17 or patrol18 blood vessels for several days19 but differentiate irreversibly to either macrophages or dendritic cells (DC) on tissue infiltration (the spleen is a notable exception; see Splenic Reservoir Monocytes and Infarct Healing). In recent years, many investigators have focused on delineating the relationships between monocytes and DC.
Convincing data now indicate that in the steady state, a dedicated common dendritic cell precursor gives rise to pre-dendritic cells, classical DC, and another kind of type I interferon-producing DC, called a plasmacytoid DC, without monocyte intermediates.20–24 These cells are important to the homeostasis of the organism. They reside in lymphoid tissues such as the lymph nodes or spleen and play important roles in orchestrating adaptive immunity. However, during inflammation (eg, after MI), monocytes can give rise to inflammatory DC or macrophages that accumulate in large numbers in target sites.25–30

The ability of monocytes to differentiate to various cell phenotypes suggests remarkable “plasticity” in response to the environment. The prevailing belief is that circulating monocytes are relatively uncommitted and that their eventual phenotype depends entirely on the tissue environment. In 1989, Passlick and colleagues31 reported that human monocytes can be divided into 2 subsets according to expression of CD16 and CD14. The dominant subset represents 85% of the monocyte pool and expresses CD14 at high levels and is low or negative for CD16 (CD16−), whereas the minor subset is low for CD14 but high for CD16 (CD16+).32,33 CD16+ monocytes produce tumor necrosis factor α (TNF-α) in vitro34 and increase in the circulation in certain inflammatory conditions.35–37 Accumulating evidence suggests, however, that CD16+ monocytes are inflammatory; for example, they express high levels of CCR2, a receptor for an inflammatory chemokine monocyte chemotactic protein 1 (MCP-1) and can release myeloperoxidase.11,38 It is possible that both subsets resemble human CD16− monocytes, initially termed resident because of the capacity to accumulate regardless of inflammation11,40 and later shown to exhibit “patrolling” behavior,18 may be important in the resolution of inflammation.30

**Monocyte/Macrophage Response After MI**

Neutrophils accumulate in the infarcted myocardium in the first hours after onset of ischemia and peak after one day in a process that depends on the chemokines interleukin (IL) 8 (CXCL8) and CXCL1 and the adhesion molecules L- and P-selectin and intercellular adhesion molecule 1 (reviewed in 6–8,47). Thereafter, monocytes and their lineage descendant macrophages dominate the cellular infiltrate. The presence, time course, and importance of these phagocytes have been investigated in rodent and large animal models.30,48–52 The studies confirm that monocytes/macrophages dominate the cellular infiltrate for the first 2 weeks after MI and participate in infarct wound healing. Germline deletion of MCP-153 or its receptor CCR254 point to a central role of this chemokine/chemokine receptor pair in the recruitment of monocytes to the infarct. In addition, some adhesion molecules, such as vascular cell adhesion molecule 1, are upregulated in the infarct55 and may contribute to monocyte recruitment through binding the integrin very late antigen-4 on their cell surface.

In ischemia-reperfusion injury, any inflammation is likely harmful. Neutrophils and monocytes/macrophages release proteolytic enzymes and reactive oxygen species and exacerbate the injury by harming myocytes that survived the ischemic period. Preclinical studies have shown that anti-inflammatory treatment can be beneficial because it decreases the infarct size-to-area-at-risk ratio after ischemia-reperfusion injury (recently reviewed in 56–58). However, none of these strategies have been translated into the clinic, and it is unknown whether reparative monocytes play a role in this type of injury.

In unreperfused MI, experimental data report both negative54,59,60 and positive30,61–64 correlations between monocyte/macrophage numbers and healing/left ventricular remodeling. To reconcile these seemingly conflicting results, we argue that adequate wound healing after death of a large number of myocytes requires a monocyte/macrophage response that balances inflammatory and reparative functions. Indeed, in contrast to ischemia-reperfusion injury, unreperfused MI requires that a large portion of the necrotic myocardium is replaced with scar tissue, a process that requires monocytes. However, either broad suppression of inflammation or unbridled inflammatory activity may stall the reparative functions mediated by these cells.
Monocytes/macrophages have similar functions in skin wounds and myocardial infarcts: The cells (1) release inflammatory mediators, such as inducible nitrous oxide synthase, reactive oxygen species, interferon γ, TNF-α, IL-1, IL-6, and macrophage inflammatory protein 1 α; (2) phagocytose apoptotic and necrotic myocytes and neutrophils and other debris; (3) release proteases such as matrix metalloproteinases (2, 9, and 13), urokinase-type plasminogen activator, and cathepsins, which digest the preexisting collagen network and facilitate cell movement; (4) promote angiogenesis through vascular endothelial growth factor and fibroblast growth factor secretion; (5) transport reparative enzymes and prosurvival factors such as transglutaminases; and (6) stimulate collagen synthesis and deposition by myofibroblasts through release of transforming growth factor β and fibroblast growth factor.

The sum of these functions positions monocytes and their tissue descendants as key regulators of infarct healing. However, the antagonistic nature of these functions presents a conundrum: How can a cell be destructive and reparative at the same time? The existence of monocyte and macrophage subsets provides a possible solution to this tension. A study in a murine model of coronary ligation found that the monocyte response in the myocardium is temporally biphasic. Proinflammatory Ly-6C<sup>high</sup> monocytes dominate on days 1 to 4 (phase 1) and promote digestion of infarcted tissue and removal of necrotic debris, whereas reparative Ly-6C<sup>low</sup> monocytes dominate during the resolution of inflammation (phase 2) and propagate repair (Figure 2). Monocyte subsets express different chemokine receptors and thus respond differentially to chemokines released from the cardiac wound. MCP-1 (also known as CCL2) is released during phase 1 and recruits Ly-6C<sup>high</sup> (CCR2<sup>+</sup> CX3CR1<sup>low</sup>) monocytes preferentially. In the absence of CX3CR1, Ly-6C<sup>low</sup> monocytes do not accumulate during phase 2. Concomitantly, fractalkine expression is decreased in phase 1 but increases in phase 2, suggesting that it recruits Ly-6C<sup>low</sup> (CCR2<sup>−</sup> CX3CR1<sup>high</sup>) monocytes during phase 2. Once recruited, monocyte subsets mediate distinct biological activities: Ly-6C<sup>high</sup> monocytes express TNF-α, IL-1β, myeloperoxidase, matrix metalloproteinases, cathepsins, and plasminogen activator urokinase and are therefore potently inflammatory, whereas Ly-6C<sup>low</sup> monocytes express IL-10, transforming growth factor β and the proangiogenic factor vascular endothelial growth factor and are therefore reparative. The biological properties of monocyte subsets and their sequential recruitment to infarcts...
correlate well with the time course of tissue healing: The early inflammatory and digestive phase 1 is followed by active resolution of inflammation and tissue repair in phase 2. A well-coordinated biphasic monocyte response is necessary for proper healing. Abrogation of phase 1 impairs the removal of dead cardiac myocytes and debris, whereas abrogation of phase 2 decreases the generation of microvessels and the deposition of collagen.

Patients with acute MI show a similar biphasic monocyte response.67 A longitudinal study of a cohort of 36 patients over 2 weeks after MI identified that circulating inflammatory CD16+ monocytes expanded first (peak on day 2.6), followed by CD16+ monocytes (peak on day 4.8). These findings are in line with studies in mice, which showed that the equivalent Ly-6Chigh and Ly-6C(low) monocytes peak at similar times in the infarct (on day 3 and 5 to 7, respectively).90 Similar prognostic value of bone monocyte levels has been described in patients with stroke.68 Because the clinical studies evaluated monocytes in blood only, additional investigations are needed to determine how the monocyte responses in blood and tissue are related.

The paradigm shift from a monophasic to biphasic monocyte response after MI offers new therapeutic strategies. For instance, it could be beneficial to modulate the timing of recruitment or the ratio of subsets to emphasize tissue repair. The distinct recruitment mechanisms of monocyte subsets (MCP-1–dependent for Ly-6Chigh cells, but fractalkine-dependent for Ly-6C(low) cells) offer reasonable targets to control monocyctic phases and the number of inflammatory monocytes in the infarct.

Atherosclerosis Induces Chronic Monocytosis

Myocardial infarction triggers an acute inflammatory response, whereas atherosclerosis is considered a chronic inflammatory disease. Despite their frequent concurrence, the interconnection between the chronic and acute inflammatory conditions is mostly neglected. Next we review briefly the role of monocytes/macrophages in atherosclerosis and then discuss the impact of atherosclerosis on infarct healing.

Multiple studies indicate that atherosclerosis is a multifactorial disease that mobilizes metabolic and inflammatory pathways.59,70 It has been known for a long time that atherosclerotic lesions in humans and mice contain macrophages. Lesional macrophages ingest oxidized lipoproteins and, on prolonged residence in atheroma, acquire morphological characteristics of foam cells. Recent reports in mouse models of atherosclerosis have also revealed the presence of dendritic cells, either in the steady state aorta71 or within tertiary lymphoid structures that develop adjacent to the adventitia.72 Direct involvement of monocytes in initiation and progression of atherosclerosis was demonstrated in mice with decreased macrophage colony-stimulating factor receptor expression.73 These studies indicate that monocytes and their tissue descendants are active participants in disease progression rather than passive responders of ongoing inflammation. The conclusions are consistent with the view, championed by Libby,69 Ross,74 and others, that atherosclerosis is an inflammatory disease.

Figure 3. The repertoire of circulating inflammatory monocytes (inset shows a sorted Ly-6C(high) monocyte expands over time in mice with hyperlipidemia. Adapted from Swirski et al.84

Appreciation of monocytes and macrophages in atherosclerosis raised many questions about their trafficking and function. Multiple studies have now demonstrated that chemokines and their cognate receptors drive monocyte infiltration to the growing atheroma.75–79 The best-described chemokine/receptor pairing is MCP-1/CCR2, but fractalkine/CX3CR1 and macrophage inflammatory protein 1 α/CCR5 are also important. Fate-mapping experiments designed to explore the dynamics of monocyte recruitment have revealed their continuous accumulation in the growing lesion.80,81 Macrophages in the progressive lesion produce proteases, cathepsins, myeloperoxidase, and other inflammatory mediators70 that typically associate with so-called unstable plaques, a designation relevant to human, if less so to mouse, atherosclerosis.82,83

The discovery of monocyte and macrophage heterogeneity necessitated the evaluation of subsets in the context of atherosclerosis. Studies have shown that hypercholesterolemic mice gradually accumulate Ly-6C(high) (CCR2(high)) monocytes in the circulation and the growing lesions (Figure 3).84,85 Although Ly-6C(low) monocytes increase less severely, they ingest oxidized LDL and likely differentiate to dendritic cells on tissue infiltration.86,87 On accumulation, Ly-6C(high) monocytes differentiate to macrophages and contribute to inflammation.84,88–90 However, Ly-6C(high) monocytes can also differentiate to dendritic cells during inflammation, whereas Ly-6C(low) monocytes are known to differentiate to macrophages (for a recent review discussing the ontogeny of monocytes, macrophages, and dendritic cells, refer to Geissmann et al14). The designation of macrophages as either M1 or M2, the former denoting cells that are activated through so-called classical triggers such as lipopolysaccharide or interferon γ and the latter referring to alternative activation through IL-4 or IL-13, has led to the idea that Ly-6C(high) monocytes preferentially become M1 macrophages, whereas Ly-6C(low) monocyte can become M2 macrophages; further study is required to determine the strength of this relationship.91 It is possible that the eventual subsets’ phenotype combines environmentally dependent (arguing for plasticity of monocytes) and environmentally independent (arguing for determinism of monocytes) signals. Future studies will need to show more precisely the differential participation of subsets in atherosclerosis and whether the cells can be manipulated to influence the course of disease. Monocyte heterogeneity, then, links atherosclerosis and its complications, especially because monocytosis that occurs in athero-
sclerosis generates a pool of inflammatory cells that are capable of infiltrating the injured myocardium.

**Impact of Atherosclerosis-Related Blood Monocytosis on Infarct Healing**

The vast majority of myocardial infarcts are caused by occlusion of a coronary artery after the rupture of an inflamed atherosclerotic plaque. Yet until recently, most data on myocardial infarction and heart failure have been generated in animals that lack the heightened inflammatory state of atherosclerosis and associated chronic monocytosis. A recent study of MI in hypercholesterolemic apoE<sup>−/−</sup> mice with preexisting atherosclerosis and systemic monocytosis may recapitulate the clinical situation more faithfully. The study found that hypercholesterolemic mice recruit more Ly-6C<sup>high</sup> monocytes in infarcts and that these monocytes persist longer (prolonged phase 1). The compromised monocyte response associates with impaired infarct healing and accelerated left ventricular remodeling. Serial cardiac magnetic resonance imaging showed that the hypercholesterolemic mice exhibit enhanced left ventricular dilatation and an increased propensity to develop heart failure. The study also showed that blood monocytosis by itself (ie, in the absence of atherosclerosis or hypercholesterolemia, but induced by lipopolysaccharide injections), is sufficient to recapitulate the prolonged and heightened Ly-6C<sup>high</sup> monocyte–associated inflammation in the infarct, the acceleration of left ventricular dilatation, and the development of heart failure. Thus these animal studies indicate that high blood monocyte counts and increased recruitment of the cells into the infarct adversely affect healing and promote left ventricular dilatation (Figure 4).

The experimental data mentioned above are in line with clinical studies that investigated monocyte blood levels at the time of infarction and chronic left ventricular remodeling. Specifically, Tsujioka et al<sup>67</sup> correlated the blood level of the inflammatory CD16<sup>+</sup> monocyte subset during acute MI with magnetic resonance imaging–derived ejection fraction 6 months later and found that patients with increased blood levels of inflammatory monocytes at the time of MI were more prone to develop heart failure. Taken together, these observations indicate that CD16<sup>−</sup> monocytes represent prospective therapeutic targets after MI.

**Figure 4.** Atherosclerosis is associated with an increased number of inflammatory monocytes, cells that are also centrally involved in the wound healing response after MI. The cartoon illustrates how increased recruitment of Ly-6C<sup>high</sup> monocytes impairs healing and favors development of heart failure in apoE<sup>−/−</sup> mice and is based on findings by Panizzi et al. The magnetic resonance images show a long- and short-axis view of a mouse with a large anterolateral infarct 21 days after coronary ligation. LV indicates left ventricular; EF, ejection fraction.

**Figure 5.** A, The spleen stores large numbers of monocytes clustered in the subcapsular red pulp. Intravital microscopy of monocytes obtained in a mouse that expresses GFP under the CX3CR1 promoter. B, After MI, monocytes increase their motility, enter the vasculature and depart from the spleen. Time-lapse intravital microscopy of the spleen 24 hours after coronary artery ligation. C, Within a day after MI, the spleen releases half of its monocyte reservoir. Hematoxylin and eosin stain of a mouse spleen. GFP indicates green fluorescent protein. Adapted from Swirski et al.<sup>64</sup>
Splenic Reservoir Monocytes and Infarct Healing

It has long been thought that circulating blood monocytes differentiate irreversibly into dendritic cells or macrophages on tissue entry. Recent findings, however, indicate that bona fide undifferentiated monocytes can be stored in large amounts in the splenic red pulp. In case of an emergency, these monocytes are quickly released into blood and thus represent an important resource that the body uses to regulate inflammation. In the steady state, splenic monocytes are found in large numbers in the cords of the subcapsular red pulp; they organize in clusters of 20 to 50 cells around the entire organ (Figure 5A). The cells are distinct from previously described iron-recycling red-pulp macrophages and from marginal zone macrophages and dendritic cells. Splenic and blood monocytes exhibit the same morphology, do not differ in their gene-expression profile, are comparably phagocytic, and can differentiate into macrophages or dendritic cells in vitro. However, splenic monocytes are virtually immotile and vastly outnumber their equivalents in circulation.

In response to ischemic myocardial injury, splenic monocytes enter the bloodstream and relocate to the infarct (Figure 5B and 5C). The studies, performed in mouse and rat models, indicate that the spleen can contribute 40% to 75% monocytes to the ischemic myocardium. The deployment of splenic monocytes involves angiotensin II (Ang II), the levels of which increase after MI. The angiotensin type 1 receptor, expressed by splenic monocytes, dimerizes on interaction with the hormone. This event induces a variety of effector programs within the cell, including cytoskeletal rearrangement and chemotaxis-induced migration. Time-lapse in vivo studies have revealed that the splenic monocytes that respond to Ang II increase their motility, encounter neighboring venous sinuses or collecting veins, enter the bloodstream (Figure 5B), and become available for redistribution in circulation.

A 1977 study following 740 American servicemen who had been splenectomized because of trauma sustained during World War II revealed an increased mortality from ischemic heart disease when compared with a similar size sample of veterans who had not been splenectomized. These results suggest that reservoir splenic monocytes also play a significant role in humans. Decisive testing of this hypothesis requires further investigation.

Efficient healing after MI depends on a coordinated mobilization of monocytes to the ischemic myocardium. Therefore, it will be important to explore the molecular mechanisms that orchestrate the release of splenic monocytes. The findings could lead to new therapeutic options that promote or prevent the mobilization and activation of monocytes (or their subsets) and therefore inflammation. Patients with monocytosis-associated inflammatory disorders (eg, atherosclerosis) and who develop MI are likely to mount an exaggerated inflammatory monocyte response in the infarct. Thus decreasing the availability of monocytes immediately after MI could be favorable to these patients. Because monocyte deployment from the spleen at least in part depends on Ang II, it should be tested whether targeting of the hormone (eg, with ACE inhibitors) or its receptors (eg, with angiotensin type 1 receptor antagonists) controls the biodistribution of monocytes. ACE inhibitors are already part of the standard therapy for heart failure, and patients usually receive these drugs within days after ischemia, but their specific impact on monocyte trafficking is unknown.

Monocyte Recruitment and Healing Outcome: A Parabolic Relationship?

Healing necessitates a balanced inflammatory response. On the one hand, monocytes and macrophages are needed to remove necrotic tissue, trigger angiogenesis, and initiate collagen synthesis by myofibroblasts; on the other hand, these cells can secrete proteases and oxygen radicals in abundance and can therefore compromise tissue integrity. The cells’ prolonged presence or exaggerated number hampers swift resolution of inflammation and prevents formation of a durable extracellular matrix. Through extrapolation of experimental and clinical data, we propose that the outcome after nonreperfused MI is related to the number of monocytes that accumulate within the first 2 weeks (Figure 6). The relationship is parabolic: If the infarct recruits insufficient numbers of monocytes, wound healing is delayed because debris is neither cleared nor replaced with granulation tissue and collagen matrix (blue stars in Figure 6). If inflammatory monocytes persist too long, the reparative functions of Ly-6Cslow monocytes, myofibroblasts, and endothelial cells are impaired (red stars in Figure 6). The scenario represented by red stars in Figure 6 is relevant to patients with atherosclerosis who suffer from a high inflammatory burden. However, as data depicted by blue stars suggest, indiscriminate depletion of monocytes will also be detrimental. A therapeutic goal to prevent heart failure, then, is to shift the monocyte response to a hypothetical vertex that denotes “optimal” healing (green star in Figure 6). Thus the inflammation in the healing heart should be (1) monitored with monocyte imaging to identify patients in need of therapeutic intervention, and (2) targeted by tailored therapy to modulate the recruitment of monocyte subsets. Additionally, future studies should explore how already-established thera-
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None.

References


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