Blinding the Monocytes to Protect the Heart

Israel F. Charo, MD, PhD

Despite the impressive success of statins in lowering cholesterol levels, atherosclerotic heart disease remains the leading cause of death in the Western world. Although a central role for cholesterol, particularly low-density lipoprotein cholesterol, in atherogenesis is undisputed, other contributing factors likely include diabetes mellitus, triglycerides, oxidation of proteins or lipids, and immune responses to self- or bacterial antigens. Whatever the precise agents, an expanding body of evidence strongly implicates inflammation as a final common pathway leading to the formation of unstable atherosclerotic plaques, plaque rupture, and acute myocardial infarction. Consistent with the hypothesis that inflammation plays an important role in plaque instability, a large, prospective, clinical trial found that elevated levels of high-sensitivity C-reactive protein were a strong predictor of future major cardiovascular events, even in patients who met current American Heart Association guidelines for low-density lipoprotein cholesterol levels. Clinical trials are now testing the hypothesis that potent anti-inflammatory drugs, such as methotrexate or interleukin-1b, can reduce the risk of major cardiovascular events in patients who have suffered myocardial infarctions. Ideally, however, one would prefer a drug that more specifically targets vascular inflammation. In this regard, biological insights into the pathogenesis of atherosclerosis have focused attention on circulating blood monocytes.

Landmark studies from Gerrity and Faggiotto et al demonstrated robust recruitment of monocytes to endothelium in swine and nonhuman primates within weeks of the animals being placed on high-fat diets. These monocytes are the precursors of the lipid-laden macrophage foam cells that form the fatty streak, the earliest pathological evidence of atherosclerosis. The mechanism for monocyte recruitment was revealed by the identification of the chemokine monocyte chemoattractant protein-1 (now known as CCL2) at sites of monocyte accumulation in the vessel wall. The observation that monocyte chemoattractant protein-1 is induced by minimally oxidized low-density lipoprotein, and the cloning of the monocyte receptor for monocyte chemoattractant protein-1 (CCR2), genetic deletion of CCR2, or monocyte chemoattractant protein-1, markedly diminished atherosclerotic lesion formation in mice placed on high-fat diets, providing compelling evidence that the monocyte chemoattractant protein-1/CCR2 axis mediated the directed migration of monocytes to areas destined to accumulate foam cells and subsequently develop atherosclerosis. More recent work has demonstrated significant heterogeneity among monocytes and their potentially different roles in atherogenesis. Proinflammatory monocytes, identified by high expression of the surface marker Ly6C, are CCR2+. A second monocyte subset, identified by low expression of Ly6C, is largely CCR2+ and is thought to be involved in the postinflammatory reparative functions of monocyte/macrophages, including mediating the removal of capillary endothelial cells by neutrophils. Although the importance of monocytes in atherogenesis is reasonably well established, at least in rodent models, potential roles of monocytes in the setting of myocardial infarction have not been explored as well.

In this issue of Circulation, Majmudar et al report that siRNA-mediated silencing of CCR2 decreases recruitment of Ly6C+ monocytes to the ischemic myocardium, reduces inflammation, and improves cardiac function in mice after experimental myocardial infarction. Earlier work from this group demonstrated that after ligation of the coronary artery in mice, 2 waves of monocytes are sequentially recruited to the ischemic myocardium. The first wave is dependent on monocyte CCR2 expression and arrives shortly after ligation. The spleen is a major source of these monocytes. The second wave appears 7 days later, is not CCR2 dependent, and may be the precursors of macrophages involved in reparative functions. Inflammation is often viewed as a double-edged sword in which the need for leukocytes to deal with injury/infection is balanced by their propensity to damage tissue if recruited in large numbers. The notion that CCR2 specifically controls the potentially harmful phase of this response is appealing and offers immediate strategies for therapeutic interventions. Complicating this view, however, is the observation that CCR2 is also present on hematopoietic stem cells, the precursors of all monocytes and leukocyte lineages, and directs the recruitment of these stem cells to sites of injury. The net result of deleting CCR2 is thus difficult to predict, as illustrated in the case of liver damage in response to acetaminophen, in which the loss of CCR2 actually leads to more severe injury.

The article by Majmudar et al presents 2 important preclinical advances with the potential to be translated into more optimal management of post–myocardial infarction patients. The first is the introduction of multimodal imaging to simultaneously quantify cardiac inflammation and remodeling. The absence of validated biomarkers or noninvasive imaging techniques has been a significant impediment to the development of therapeutics to reduce cardiovascular inflammation, although the use of fluorine-18 deoxyglucose–positron emission tomography has been explored. In this regard, Tardif et al recently reported results of a phase II...
trial in which patients treated with a 5-lipoxigenase inhibitor had a decrease in noncalcified plaque volume, as determined by coronary artery computed tomography. Majmudar et al have quantified inflammation by measuring the intracellular leukocyte enzyme myeloperoxidase by magnetic resonance imaging. The authors coupled this imaging technique with a positron emission tomography–based method of following factor XIII–dependent cross-linking of extracellular matrix proteins through the development of a fluorine-18–labeled agent. Together, these 2 imaging modalities allowed noninvasive and quantitative measurements of cardiac inflammation and remodeling. Other potential molecular imaging targets for the noninvasive measurement of inflammation include chemokines, integrins, and macrophage activation, as determined by glucose metabolism. Second, this study involved the use of siRNA to specifically neutralize CCR2 and to reduce inflammation. The use of nanoparticles to deliver siRNA to the heart is an elegant and novel demonstration of the utility of this delivery system.

The siRNA approach holds great promise for neutralizing targets that have been difficult to drug with either antibodies or small molecules, but efficient delivery of the siRNA has been a significant challenge. In the case of CCR2, however, other approaches have already advanced to the clinical stage. Gilbert et al reported that neutralization of CCR2 with a monoclonal antibody (MLN1202) reduced high-sensitivity C-reactive protein levels in patients at risk for atherosclerosis. Small-molecule antagonists of CCR2 from several pharmaceutical companies are in human clinical trials for type 2 diabetes mellitus and for diabetic nephropathy. It is unclear whether siRNA will offer unique advantages over these more traditional therapeutics, particularly for neutralizing the activity of a cell surface receptor. Furthermore, the use of siRNA to neutralize CCR2 resulted in a decrease in the number of Ly6C+ monocytes. It is thus difficult in the present article, as well as in the article by Leuschner et al from the same group, to distinguish the effects of specifically neutralizing CCR2 from those related to decreasing the number of circulating Ly6C-hi monocytes. It remains to be seen whether antibody or small-molecule antagonism of CCR2 will uniformly lead to similar decreases in Ly6C-hi monocytes.

There are several uncertainties in extrapolating the results of this study to humans. First, the studies were done in mice maintained on a high-fat diet. Although there are compelling reasons to work in mice, it is also clear that the track record of cardiovascular results in mice translating to humans is mixed, an example being the numerous agents that successfully treated vascular restenosis in murine models but often failed in humans. In addition, the mice were maintained on a very high–fat diet to create vascular lesions that mimick atherosclerotic plaques in humans. Under these conditions, the cholesterol levels are typically much higher than seen in patients and cause a marked monocytosis and increase in the number of circulating Ly6C-hi monocytes. Whether this degree of inflammation exists in post–myocardial infarction patients, whose blood monocyte counts are not this high, is unclear. Second, it will be important to see whether the improvement in cardiac function seen on day 21 after ligation is maintained when evaluated at later time points. Third, as alluded to earlier, although the use of nanoparticle-delivered siRNA is an elegant way to neutralize CCR2, it is likely that either monoclonal antibody or small-molecule antagonists will prove to be easier and more efficient ways to block CCR2. Finally, the safety implications of neutralizing CCR2 are not yet clear. The limited experience thus far in phase I and II human clinical trials is encouraging, but preclinical studies in CCR2-deficient mice have revealed the potential for impaired ability to clear intracellular pathogens. This may become a greater concern if one moves from brief treatments for post–myocardial infarctions patients to long-term treatment of the inflammatory component of atherosclerosis. Similarly, if the siRNA silencing of CCR2 reduces the number of circulating monocytes outside the normal range in patients, this may also raise safety or regulatory issues.

Despite these caveats, the clinical implications of this study are noteworthy. The fact that administering the siRNA 1 day after the creation of the infarct still afforded protection is encouraging. The combination of noninvasive imaging of cardiac inflammation with therapeutics that specially block the trafficking of CCR2+ monocytes to the ischemic myocardium has the potential to add a novel class of drugs to our armamentarium for treating patients with acute myocardial infarctions. Successful treatment of myocardial inflammation in this setting may represent the next quantum leap in the management of patients with atherosclerotic heart disease, especially because current conventional therapies leave the majority of the treatment populations in controlled clinical trials at high residual risk for a cardiovascular event.

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References


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