Targeting a Monocyte Subset to Reduce Inflammation
Siamon Gordon

Therapeutic siRNA Silencing in Inflammatory Monocytes in Mice
Leuschner et al

Recently in Nature Biotechnology, Leuschner et al described an elegant method of limiting the recruitment of a proinflammatory subset of monocytes in mouse models of inflammation. Although providing prospects for therapeutic application, especially in cardiovascular diseases, it raises questions that make it necessary to proceed with caution.

In this technical tour-de-force, Leuschner et al exploited the findings that murine macrophages that consist of two defined subsets differing in Ly6 antigen expression also differ in their expression of a major chemokine receptor, CCR2, responsible for the recruitment of circulating Ly6C hi CCR2+ monocytes to peripheral sites of inflammation. They utilized lipid nanoparticles containing siRNA against CCR2 to deplete the recruitment of this subset and to attenuate inflammation in several models of vascular injury (myocardial infarction, atherosclerotic plaques), islet cell transplantation in diabetic mice, and tumor-associated macrophage recruitment. These striking results raise the possibility of limiting the deleterious effects of selected monocyte subsets while preserving housekeeping and clearance functions of resident tissue macrophages, which are essential for host homeostasis and survival. By optimizing the nanoparticle delivery system and the siRNA used for silencing, combined with sophisticated imaging in defined mouse models of disease, they achieved striking improvements over currently available anti-CCR2 antibodies or small molecule inhibitors.

Figure summarizes the experimental strategy, which is based on previous knowledge of monocyte subpopulations and the importance of CCR2+ monocyte recruitment to sites of a range of metabolic and inflammatory stimuli. Inhibition of the inflammatory monocyte subset was demonstrated by gene ablation or reduction of CCR2 expression and antibody blockade of the actions of MCP-1, its ligand, in vitro and in vivo. The nanoparticles, which had been optimized for biocompatible delivery, localized rapidly to spleen, liver, and bone marrow after intravenous delivery; the encapsulated siRNA, selected for nuclease stability and reduced immuno-stimulation, effectively decreased CCR2 message and protein content, and reduced the levels of circulating Ly6C hi monocytes and their migratory activity. They demonstrated partial selectivity for Ly6 hi monocytes compared with Ly6 lo monocytes and other phagocytes, polymorphonuclear leukocytes and myeloid dendritic cells, and, to a greater extent, for lymphocytes. The inflammatory disease models included acute ischemia reperfusion injury after coronary artery ligation, chronic inflammatory atherosclerosis plaque development in apolipoprotein E–deficient mice, survival of pancreatic allografts in streptozotocin-induced diabetes, and two transplantable tumors, a lymphoma and colorectal carcinoma. These experiments illustrate the role of CCR2+ monocyte depletion in limiting acute and chronic inflammatory lesions delivered in advance of lesion development or therapeutically after pathology had been established. The authors exploited and extended their previous discovery that murine splenic red pulp provides a major and important reservoir of monocytes for mobilization to injured organs.

The goal of selective therapeutic modulation of monocyte/macrophage phenotype has become the “holy grail” of much contemporary research, not only in cardiovascular disease, where they are the major purveyors of inflammatory pathology, but also in obesity and malignancy, which they promote. Circulating monocytes in mice and humans occur as two well-characterized subsets, with differential expression of chemokine receptors (CX3CR1) as well as CCR2, and with differential expression of a range of other genes encoding...
receptors such as the FcR and CD16, as demonstrated by microarray analysis. Their life history and entry into tissues varies with differential recruitment in inflammation compared with the resident tissue macrophage populations present in the steady state. The patrolling subpopulation of monocytes remains intravascular, utilizing the adhesion molecule LFA-1 to perform poorly understood trophic functions for the endothelium. Recruitment of monocytes by microbial ligands can result in the acquisition of dendritic cell–associated properties such as expression of a surface lectin related to DC-SIGN. These properties are responsible for homing to T-cell areas and have the ability to capture and present antigens to lymphoid cells. Interconversion of macrophages and dendritic cells within tissues also has been described.

There is a clear distinction in the origin and distribution of inflammatory recruited monocytes and resident more sessile macrophage populations, which are constitutionally present in tissues in the absence of overt inflammation. The recently recruited monocytes can differentiate rapidly into macrophages that display enhanced turnover and shorter survival times than resident macrophages. They adapt to the unique local microenvironments in different organs and alter their expression of adhesion and chemokine receptors that determine their localization; they can die by apoptosis or necrosis, or they can emigrate readily from sites of inflammation involving molecules, such as netrin, by poorly understood mechanisms.

Recent studies have highlighted the complexity of resident macrophage populations in different tissues. Epidermal Langerhans cells and microglia arise from yolk sac and may turn over locally, albeit at a low level, compared with bone marrow–derived mononuclear phagocytes. The abundant resident macrophage populations in tissues such as liver (Kupffer cells), gut, and lung (alveolar macrophages), as well as in lymphoid organs, are also phenotypically variable and distinct from inflammatory monocyte-derived cells. Once outside the vascular compartment, macrophages are modulated by the local connective tissue matrix, as well as cytokines and possible microbial products, and display considerable phenotypic heterogeneity. Their variability extends far beyond the simplistic classifications of M1 (proinflammatory, interferon γ-induced) and M2 (antiinflammatory, TH2 cytokine–induced) phenotypes associated with bacterial stimuli, cell-mediated immune activation and allergy, or parasitic infection.

What, then, are the implications of extensive monocyte/macrophage heterogeneity for selective targeting, and to what extent does the present study fall short of achieving the holy grail of “surgical” ablation of undesired functions? It will be considerably more difficult to access extravascular macrophages, although local delivery, eg, by the subcutaneous or intratracheal route, can be envisaged. This aspect could be a particular advantage in manipulating the cardiovascular system for therapeutic purposes. More problematic is the dynamic complexity, plasticity, and phenotypic variability of tissue macrophages, especially in relation to optimal recruitment and regulation of inflammatory cells to prevent persistent inflammation and to achieve controlled repair. If mono-cytic CCR2-dependent recruitment is hindered, then can other chemokine receptors or myeloid cells, including LY6Clo monocytes or polymorphonuclear leukocytes, compensate with attendant risks of tissue injury? Is antecedent inflammation not required for subsequent repair? This is suggested by the authors in a study of the role of extramedullary monocytopoiesis in infarct healing and accelerated evolution of heart failure after reduced inflammatory cell recruitment. Could the risk of atherosclerotic plaque vulnerability decrease but entail a greater risk of aneurysm? Above all, will the risk of infection be enhanced, along with inability of the host to mount a rapid and effective innate and acquired immune response? Inhibition of monocyte recruitment by antiCD11b/CD18 antibody in an autoimmune model of diabetes resulted in reduced lymphoid cell recruitment, which could be beneficial or detrimental to the host by recruiting regulatory or activatory subpopulations of T lymphocytes. Similarly, monocyte-derived dendritic cells can be tolerogenic or activatory and host-protective or pathogenic. In summary, the two-edged sword of immune-mediated host defense against injury is delicately balanced and may be tilted to pathological repair by monocyte depletion.

Finally, humans and mice do express many differences as well as common features. The importance of a major splenic reservoir in humans requires further investigation. The authors suggest that the approach followed in regard to CCR2 can be extended to other myeloid and lymphoid populations. Nanoparticles provide a more concentrated delivery system, and targeting a particular monocyte subset could well be more efficient by removing a whole cell, rather than targeting a single molecule. Although promising, and perhaps more effective than present small molecule inhibitors and antibodies for therapy, these can be expected to improve in potency and specificity. However, cardiovascular disease may provide a particularly favorable field for targeting inflammation by the nanoparticle/siRNA strategy so elegantly developed here.

Acknowledgments

The author is grateful to Frederic Geissmann and Simon Yona for useful discussions.

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_Circ Res._ 2012;110:1546-1548
doi: 10.1161/RES.0b013e31825ec26d

_Circulation Research_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/110/12/1546

An erratum has been published regarding this article. Please see the attached page for:
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The error has been noted and corrected in the online version of the article, which is available at http://circres.ahajournals.org/content/110/12/1546.full.
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DOI: 10.1161/RES.0b013e3182640d29