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CXCR4 Blockade Recruits Neutrophils Into the Plaque
Julie Sainz, Masataka Sata

The importance of inflammation in the initiation and development of atherosclerosis has been extensively studied over the last decades.1-2 In particular, much effort has been devoted to elucidating the role of chemokines and their receptors, which play a central part in the atherogenic immune response. Indeed, interfering with the chemokine–chemokine receptor system emerged as both an exciting tool to dissect the complex inflammatory response and a promising therapeutic target.3 Although participation of other inflammatory cells such as macrophages and T cells is well documented, less attention has been paid to the involvement of neutrophils. Neutrophil infiltrates are seldom observed within human atherosclerotic plaques in comparison with other inflammatory cells. However, neutrophils have been detected at the sites of plaque erosion or rupture on atherectomy specimens from the patients with unstable angina and on autopsy samples from the patients with acute myocardial infarction.4 Moreover, the increase in proinflammatory molecules occurring with coronary artery diseases correlates with the peripheral neutrophil count,5 which, hence, may be useful as a predictor of complex coronary stenosis or myocardial infarction. A histological analysis of human carotid endarterectomy specimens suggested that intraplaque hemorrhage could convey neutrophils into the atherosclerotic lesion, spreading into the necrotic core, thus participating in its protease enrichment.6 Although these clinical studies have suggested the potential contribution of neutrophils to plaque progression and vulnerability, few animal studies have been performed to determine the role of neutrophils in the pathogenesis of atherosclerosis. The molecular mechanism by which neutrophils accumulate at the advanced plaques remains largely unknown.

In the current issue of Circulation Research, Zernecke et al provide compelling evidence that neutrophils participate in atherosclerotic lesion development.7 Neutrophils are a critical component of the innate immune response against a wide array of infectious agents.8 Furthermore, neutrophils may be a major contributor to tissue damage in inflammatory diseases, such as rheumatoid arthritis and adult respiratory distress syndrome.8 Neutrophils are normally produced exclusively in the bone marrow, and their release into the systemic circulation is tightly regulated to maintain homeostatic levels in blood. Although the mechanisms regulating neutrophil homeostasis and trafficking are poorly understood, recent evidence suggests that the chemokine receptor CXCR4 and its ligand CXCL12 (stromal-derived factor 1, SDF-1) play a critical role in the retention of granulocytic precursors within the bone marrow microenvironment.8-10 Zernecke et al hypothesized that blockade of CXCR4/CXCL12 signaling could enable neutrophil recruitment to the atherosclerotic lesions. To investigate the role of the CXCR4/CXCL12 axis in the pathogenesis of atherosclerosis, the authors performed (1) long-term administration of the CXCR4 antagonist AMD3465 in apolipoprotein E-null (ApoE−/−) mice, (2) deletion of CXCR4 expression in the bone marrow of ApoE−/− and LDL receptor–null (LDLR−/−) mice, (3) systemic infusion of neutrophils coupled to fluorescently labeled latex beads into ApoE−/− mice, and (4) systemic administration of an anti–polymorphonuclear leukocyte antibody into ApoE−/− mice.

Treatment of ApoE−/− mice with constant delivery of the CXCR4 antagonist AMD3465 led to an increase in the relative number of neutrophils in the bone marrow, accompanied by an increased production of lysozyme, especially in mature neutrophils. AMD3465 also increased neutrophils in the peripheral blood with an appearance of more immature band population. The authors report that AMD3465 treatment did not modify CXCR4 expression, phagocytosis, reactive oxygen species production, calcium flux regulation, or adhesion properties of circulating neutrophils. However, CXCR4 blockade not only increased neutrophil migration from the bone marrow but also decreased the returning of senescent neutrophils back to the bone marrow after they circulated.7,10 Thus, the global rate of both fresh and senescent circulating neutrophils was increased. Subsequently, AMD3465 increased the number of neutrophils in aortic root plaques and adventitia. The relative content of neutrophils among all inflammatory plaque cells increased, whereas that of macrophage or T cells was unchanged. The infiltrated neutrophils were functionally active, as attested by their production of myeloperoxidase, gelatinase-associated lipocalin (NGAL), matrix metalloproteinase-9, and elastase. Exaggerated infiltration of neutrophils was associated with upregulation of interferon-γ, the CXCR2 ligand CXCL1, and tissue factor. Furthermore, AMD3465 increased the proportion of apoptotic neutrophils, mainly located on the luminal side of the atherosclerotic plaque, as well as that of macrophages. As a whole, these phenomena led to a significant increase of atherosclerotic lesion and necrotic core area size.

The opinions expressed in this editorial are not necessarily those of the editors or of the American Heart Association.

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(Circ Res. 2008;102:154-156.)

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Circulation Research is available at http://circres.ahajournals.org
DOI: 10.1161/CIRCRESAHA.107.170241
Zernecke et al confirmed the contribution of neutrophils to plaque formation by showing that anti-polymorphonuclear leukocyte antibody administration decreased neutrophil content and lesion size in ApoE<sup>−/−</sup> mice. Furthermore, following infusion of normal neutrophils coupled to fluorescently labeled latex beads, positive cells were found in the aortic root and arch of the mice. Interestingly, depletion of CXCR2 expression on the infused neutrophils inhibited their accumulation inside the plaque, emphasizing the importance of this chemokine receptor for neutrophil infiltration ability. Consistent with this notion, previous studies have reported that the balance between CXCR4 and CXCR2 expression tightly regulates the phenotype, function, and trafficking of neutrophils. Taken together, these findings indicate that blockade of the CXCR4/CXCL12 axis results in expansion of neutrophils in peripheral blood and that circulating neutrophils are recruited into the atherosclerotic plaque through CXCR2/CXCL1 interaction, at least in part (Figure).

Although Zernecke et al convincingly demonstrate the protective role of CXCR4/CXCL12 in the pathogenesis of atherosclerosis, the underlying mechanisms responsible for neutrophil participation in plaque formation may be more complex. CXCR4 has other ligands than CXCL12, such as the macrophage inhibitory factor. The authors have reported previously that inhibition of macrophage inhibitory factor could decrease atherosclerotic plaque area, whereas CXCL12 blockade could not. Conversely, CXCL12 may bind to other receptors than CXCR4, such as CXCR7. Moreover, the CXCR4/CXCL12 axis has been implicated in recruitment and homing of bone marrow–derived circulating vascular progenitor cells that contribute to vascular repair, angiogenesis, and neointima formation. These findings emphasize the complexity in the interconnections of the chemokine pathways involved in vascular remodeling and underline the importance of elucidating the redundancy of alternative chemokine ligand/receptor combinations.

Recent studies suggest that trafficking of cells through the adventitia may play a role in atherogenesis. In the current report, AMD3465 increased the number of neutrophils in the adventitia at the aortic root. Similarly, Van Leeuwen et al have observed an accumulation of neutrophils not only in atherosclerotic lesions but also in the adventitia in LDLR<sup>−/−</sup> mice. The precise role of neutrophils in the adventitia near atherosclerotic lesions remains a tantalizing mystery. Moreover, the molecular signaling that recruits neutrophils as well as other inflammatory cells into the adventitia remains to be clarified.

In summary, the present work by Zernecke et al clearly demonstrates a previously unappreciated role of neutrophils in the pathogenesis of atherosclerosis. The CXCR4/CXCL12 axis plays a protective role in regulating neutrophil infiltration into the atherosclerotic plaques. Further understanding of the molecular mechanism by which neutrophils are recruited into the advanced plaques will lead to development of new therapeutic and diagnostic strategy for the patients with atherosclerotic diseases, particularly for those with acute coronary syndrome.

**Sources of Funding**

Supported, in part, by grants from the Ministry of Education, Culture, Sports, Science, and Technology; the Ministry of Health, Labour and Welfare; and the Takeda Science Foundation.

**Disclosures**

None.

**References**


**Key Words:** neutrophil ▪ atherosclerosis ▪ CXCR4 ▪ chemokines ▪ CXCL12
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Circ Res. 2008;102:154-156
doi: 10.1161/CIRCRESAHA.107.170241
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/102/2/154

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