Neutrophils, Hypercholesterolemia, and Atherogenesis

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A critical role for hypercholesterolemia in the pathogenesis of atherosclerosis is firmly established. However, we still have much to learn about how hypercholesterolemia leads to the development of atherosclerotic heart disease and the spectrum of cellular participants involved. Atherosclerosis is viewed as a chronic inflammatory process, with our focus of attention dwelling on the role of cells recognized within the artery wall, including endothelial cells, monocyte-derived cells, and T lymphocytes. Hypercholesterolemia-induced activation of each of these cells has been mechanistically linked to multiple early processes in atherosclerosis, including upregulation of key adhesion proteins, chemokines, and cytokines that cumulatively orchestrate inflammatory cell trafficking into the vessel wall. A critical role for neutrophils, however, in the early atherosclerotic process has been conspicuously absent from the mainstream line of thought, no doubt because neutrophils are rarely observed in early atherosclerotic plaque compared with other inflammatory cell types.

Interestingly, a growing body of evidence points toward participation of neutrophils in later stages of atherosclerotic heart disease and its acute complications. Examination of both coronary artery segments obtained at autopsy and atherectomy specimens from subjects with unstable angina confirms that neutrophil infiltration is common within culprit lesions in subjects who experience an acute coronary event. Similarly, examination of human carotid atherosclerotic plaques has revealed that high neutrophil numbers are strongly associated with histopathological features of rupture-prone lesions, suggesting a role for neutrophils in plaque destabilization. Evidence for the involvement of neutrophils in plaque vulnerability has also come from both biochemical and immunohistochemical analyses of culprit plaques within human carotid endarterectomy specimens. Multiple neutrophil-specific proteases with links to matrix protein degradation such as elastase, neutrophil gelatinase-associated lipocalin, matrix metalloproteinase-9, CD66b, and proteinase 3 both colocalize with intraluminal sites of hemorrhage and are positively correlated with the presence of additional neutrophil proteins such as α1-antitrypsin/elastase complexes, myeloperoxidase, and α-defensins. Evidence of neutrophil activation, as monitored by a reduction in leukocyte myeloperoxidase content across the coronary vasculature (a so-called transcoronary inflammatory gradient), has been directly observed in patients with unstable angina. Additionally, myeloperoxidase release, presumably via neutrophil activation, has also been reported as an early event in acute myocardial infarction, apparently preceding myocardial injury. Elevated systemic levels of myeloperoxidase, the most abundant protein in neutrophils, are associated with enhanced incident risk for major adverse cardiac events among subjects who present with chest pain or acute coronary syndrome. Myeloperoxidase and other neutrophil granule proteins are present within human atherosclerotic lesions. Thus, evolving evidence suggests neutrophil involvement in atherosclerotic plaque progression and acute plaque destabilization/vulnerability.

Is there a role then for neutrophils in very early stages of atherosclerosis mediated via hypercholesterolemia? Interestingly, studies from nearly 3 decades ago in nonhuman primates suggested so. The time course of cellular recruitment into fatty streaks induced by a high-cholesterol diet was examined through detailed histopathological examination of the early cellular components of aortic fatty streaks in cholesterol-fed African green monkeys. Surprisingly, although the anticipated cellular participants were observed within fatty streaks, after a high-cholesterol diet, the majority of lesions examined showed intimal neutrophils. It has taken several decades, but further support for a role for neutrophils in atherogenesis has recently been reported. Zernecke et al induced neutrophil depletion via antibody administration and observed marked decreased atherosclerotic lesion size in Apoe−/− mice. The chemokine receptor CXCR4 and its ligand CXCL12 (stromal-derived factor 1) play a critical role in regulating both bone marrow neutrophil emigration and resorption of senescent neutrophils back to the bone marrow. In further studies, Zernecke et al induced elevations in neutrophil levels by modulation of the CXCR4/CXCL12 axes and observed significant increases in both atherosclerotic lesion and necrotic core area size. In additional recent studies in Apoe−/− mice, fluorescently tagged neutrophils and monocytes were used in combination with flow cytometry, confocal microscopy, and intravital microscopy. Surprisingly, neutrophilic granulocytes were shown to serve as a major cellular component of atherosclerotic lesions in Apoe−/− mice, particularly within shoulder regions where they may even outnumber monocyte/macrophages. Moreover, the majority of leukocytes interacting with endothelium on lesion shoulders are neutrophils, suggesting a significant recruitment of these cells to plaque.

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In this issue of Circulation, Drechsler and colleagues\(^\text{20}\) add substantially to this evolving story and provide convincing data to support a role for hypercholesterolemia-induced neutrophilia as a critical enabling process in early stages of atherosclerosis. Apoe\(^{-/-}\) mice fed a high-fat diet demonstrated neutrophilia, with circulating neutrophil levels correlating with early atherosclerotic lesions. The mechanisms through which a high-fat diet elevated peripheral neutrophil numbers were shown to be multifactorial, including stimulation of granulopoiesis via tumor necrosis factor– and interleukin-17–mediated generation of granulocyte colony-stimulating factor, enhanced bone marrow mobilization (presumably via higher levels of CXCL1), and reduced peripheral clearance of senescent neutrophils (presumably via reduced CXCL12). Fluorescence-activated cell sorter analysis of digested aortas from mice fed a high-fat diet for different periods of time showed that neutrophils were prominent cellular infiltrates within the first month, with rapid reductions in numbers with longer durations of diet. Importantly, both intravital microscopy studies of large arteries (carotid) in monocyte-depleted Lysm\(^{green/gfp}\) Apoe\(^{-/-}\) mice, in which only neutrophils are fluorescent, and immunohistochemical analyses (with neutrophil-specific marker Lys6G) of aortic roots of Apoe\(^{-/-}\) mice on a high-fat diet for 1 month confirmed early transmural infiltration of neutrophils. Through the use of multiple individual genetic knockouts, roles for CCR1, CCR2, CCR5, and CXCR2 were shown to be critical for early neutrophilic artery infiltration. Differential presentation of platelet-derived CCL5, the ligand for CCR1 and CCR5, was shown to be the underlying cause of the neutrophil recruitment specifically to the larger (carotid) artery through multiple approaches, including the use of selective platelet depletion, treatment with an antagonist to P-selectin, or treatment with an inhibitor to platelet glycoprotein IIb/IIIa. Finally, the link between aortic neutrophil infiltration and early atherosclerosis was demonstrated by selectively depleting neutrophils in Apoe\(^{-/-}\) mice at differing time points. Significant reductions (\(\approx 50\%\)) in aortic root lesions were observed only at early (eg, 1 month) time points.

The studies by Drechsler et al do not reveal the underlying mechanism through which hypercholesterolemia-induced neutrophil recruitment promotes early atherosclerotic changes. However, they do point toward new potential avenues for therapeutic targeting. The role of CCR1 and CCR5 for neutrophilic recruitment selectively to arterial versus venous sites represents one potential option. Numerous neutrophil proteins now serve as candidates for both further investigation and therapeutic targeting. Neutrophil granule proteins have been shown to play a role in recruitment of inflammatory monocytes,\(^{21}\) and granule proteins like myeloperoxidase show numerous mechanistic links with atherosclerotic heart disease at multiple stages in the evolution of the atherosclerotic process.\(^{22}\) Whether or not interfering with neutrophil involvement in atherosclerotic heart disease development and its complications is a successful therapeutic approach in humans remains to be determined.

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**References**


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