Neutrophils in Atherosclerosis

Alarmin Evidence of a Hit and Run?

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Inflammation and activation of immune cells are key mechanisms in the development of atherosclerosis. Elegant experimental studies, typically performed in compound mutant mice susceptible to diet-induced atherosclerosis, indicate important roles for monocytes, T lymphocytes, and mast cells in lesion formation (Figure). Moreover, inflammatory pathways promote thrombosis, a late complication of atherosclerosis responsible for myocardial infarction and ischemic stroke. However, a direct contribution of neutrophils as the first line of immune offense (ie, acute inflammation) in atherosclerosis is controversial and uncertain. In this issue of Circulation Research, Doring and colleagues report that the neutrophil secondary granule protein cathelicidin (CRAMP in mice, LL37 in humans) directly promotes atherosclerosis by enhancement of the recruitment of inflammatory monocytes.

Early histological studies of human atherosclerotic plaque identified a rich infiltrate of immune cells including monocyte/macrophages and T lymphocytes, but failed to identify significant neutrophilic accumulation. The paradigm of atherosclerosis as a chronic inflammatory condition gained further traction as chemokines responsible for monocyte and lymphocyte trafficking were found to regulate atherosclerosis. Yet, tantalizing indirect evidence implicating neutrophils in the initiation, progression, and complications of atherosclerosis has also emerged. Epidemiological studies have correlated peripheral blood leukocyte counts, comprised mainly of neutrophils, with coronary artery disease. In the apoE-deficient (apoE−/−) mouse model, hyperlipidemia induces neutrophilia and the degree of neutrophilia is positively correlated with the extent of early atherosclerotic lesion formation. In turn, neutropenic mice display reduced plaque sizes at early but not late stages of atherosclerotic lesion formation. Finally, activated neutrophils have been identified at sites of plaque rupture and superficial endothelial cell erosion in patients with acute coronary syndromes. Nonetheless, whether neutrophils truly contribute to early atherosclerotic lesion formation and the precise mechanisms of neutrophil-driven atherosclerosis remain unclear.

Doring and colleagues report that neutrophil-derived cathelicidin, a member of the alarmin family, promotes atherosclerosis by enhancement of the recruitment of inflammatory monocytes. This group of structurally heterogeneous, endogenous molecules are rapidly released on damage and exert immune cell recruiting and activating properties. Here, Doring et al report that doubly deficient CRAMP−/−/ApoE−/− mice exhibit reduced atherosclerotic lesion sizes with lower macrophage numbers compared to singly deficient apoE−/− mice alone. In atherosclerotic aortas, CRAMP was detected specifically in neutrophils but not in monocytes or macrophages. By use of intravital microscopy, CRAMP was found to be deposited by activated neutrophils on inflamed endothelium of large arteries. Mechanistic experiments supported the conclusion that, in this location, cathelicidins promoted adhesion of inflammatory monocytes in a formylpeptide receptor-dependent manner.

This study challenges several important concepts regarding the key stimuli and mechanisim(s) for inflammatory cell recruitment in atherosclerosis. Seminal observations from Massberg and coworkers indicate that platelets are the critical proximate sensor of vascular injury. Platelets are capable of adhering directly to the intact endothelial monolayer even in the absence of endothelial disruption. During this adhesive process, platelets are activated to release proinflammatory cytokines (interleukin [IL]-1β, chemoattractants [RANTES, NAP-2], and CD40 ligand [CD40L]). This interaction of platelets with endothelial cells triggers the expression of adhesion molecules to promote the adhesion of leukocytes to the endothelium. Importantly, platelets adhere to the vascular endothelium of the carotid artery in ApoE−/− mice before the development of manifest atherosclerotic lesions, and prolonged blockade of platelet adhesion in ApoE−/− mice profoundly reduced leukocyte accumulation in the arterial intima and attenuated atherosclerotic lesion formation. In this paradigm, neutrophil secretion of CRAMPs might be expected to be initiated as a consequence of platelet activation, but upstream of monocyte trafficking. Thus, the proposed role of neutrophils in early atherosclerosis will need to be further scrutinized in the context of these other proximate steps in the pathogenesis of atherosclerosis.

These data also add an additional layer of complexity to the mechanisms of leukocyte trafficking in atherosclerosis. Traditionally, the recruitment of circulating leukocytes into inflamed tissue requires multiple adhesive and signaling events to occur in a coordinated fashion. The first step is selectin-dependent attachment and rolling, followed by firm adhesion and chemotaxis. Firm adhesion is accomplished in...
an integrin-dependent fashion via members of the $\beta_2$-integrin family. Mac-1 (CD11b/CD18, $\alpha_M \beta_2$) is the most abundant $\beta_2$-integrin on neutrophils and monocytes, and broadly regulates inflammation in response to diverse forms of vascular injury in vivo via its interaction with platelet GPIb.$^{12}$ Previously, the dominant chemotactic receptors for monocytes have been the chemokine receptors CCR2, CCR5, and CX3CR1.$^{13}$ The work of Doring and colleagues suggests that monocyte recruitment may also be highly dependent on the formyl-peptide receptor via alarmins in a nonredundant fashion.

Despite the notion of atherosclerosis as a form of chronic inflammation, the work of Doring and coworkers$^{2}$ suggests that neutrophils play a role in the initiation of murine atherosclerosis. That they can only be transiently identified in plaque is consistent with previous reports of their absence histologically. What then are the factors that lead to the inclusion of neutrophils early and their exclusion in more advanced lesions? And how do these concepts apply to human atherosclerosis and the important clinical problem of transition from stable to vulnerable plaque?

Understanding the broader functions of CRAMP/LL37 derived from distinct cellular compartments in vascular homeostasis and disease will be required to determine the clinical significance of these findings. First, is selective inhibition of neutrophil cathelicidin achievable in vivo? Second, although cathelicidins are largely restricted to neutrophils, previous studies have reported the presence of cathelicidins in macrophages and endothelial cells in human plaque specimens,$^{14}$ therefore what is the consequence of inhibiting endothelial-derived cathelicidin? Third, what is the most appropriate timing of cathelicidin inhibition? Studies from neutropenic mice indicated reduced plaque sizes at early, but not late stages of atherosclerotic lesion formation. The studies by Doring and coworkers$^{2}$ were limited to assessment of lesion area following 4 weeks of high-fat diet. Later time points (eg, 20–24 weeks) will be helpful in determining whether atherosclerotic lesion formation is truly attenuated without late catch-up. Furthermore, the importance of neutrophils in early lesion formation suggests that inhibitors will need to be administered early in the disease process, focusing primarily on asymptomatic individuals with risk factors alone or subclinical disease. Fourth, what are the mechanisms for the antiatherosclerotic effects of targeting cathelicidin beyond inflammatory cell recruitment? CRAMP and other alarmins have been implicated in other cellular functions critical to atherogenesis, including foam cell formation and ROS production. Perhaps, alarmins also contribute to the thrombotic complications of atherosclerosis by altering protease expression and fibrous cap integrity and tissue factor expression. Fifth, are there possibly deleterious consequences of inhibiting cathelicidin function with respect to vessel repair and regeneration after vascular injury? The contribution of neutrophils and neutrophilic granule proteins to arterial healing after injury has been examined previously. Indeed, neutrophil-borne cathelicidin appears to promote
reendothelization and thereby limit neointima formation after stent implantation. Follow-on studies translated these findings to an animal model using a neutrophil-instructing, biofunctionalized, miniaturized Nitinol stent coated with LL-37. This stent reduced in-stent restenosis in a mouse model of atherosclerosis, suggesting that LL-37 may promote vascular healing after interventional therapy.

Despite remarkable advances in understanding the immunologic pathophysiology of atherosclerotic lesion formation in mice, we still lack definitive evidence of causation in human atherosclerosis. Dr Peter Libby, one of the pioneers of the inflammation and atherosclerosis hypothesis, stated presciently that “experimental atherosclerosis in animals furnishes an important research tool, but extrapolation to humans requires care.” Although the nonlipid effects of statins are tantalizing, we have not yet identified a disease modifying, anti-inflammatory therapy akin to other chronic inflammatory diseases such as rheumatoid arthritis, psoriasis, and multiple sclerosis.

The contribution by Döring et al2 in this issue of Circulation Research highlights the emergence of additional considerations related to murine atherosclerosis that may guide the study of human disease. The involvement of the neutrophils appears to be transient—“hit and run”—contributing to vascular injury in a time- and stimulus-dependent manner. The implication that the natural history of murine atherosclerosis is multiphasic with regard to specific cellular components suggests that targeted therapies will have to be tailored to disease activity (ie, subclinical disease versus advanced disease versus acute coronary syndromes). Thus, understanding how to safely and efficiently translate these experimental observations might lead to new clinical applications, perhaps targeting neutrophil cathelicidin.

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References
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