Activated Platelets Jam Up the Plaque

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Platelets are, in essence, cellular fragments produced in the bone marrow that circulate in the bloodstream as sensors of vascular damage. Although the specialized role of platelets in protecting the vasculature by forming clots that prevent bleeding (hemostasis) has been recognized for over a century, modern biomedical research has revealed that platelets also contribute to protection against pathogens and participate in inflammation. In the absence of injury, platelets circulate as small discoid-shaped particles that contain multiple receptors on their surface and granules in their interior that are in turn filled with bioactive molecules. It is only when danger or injury is detected that the content of these granules is released, receptors become activated, and platelets become active constituents of thrombotic or inflammatory responses. The delicate balance between preserving vascular integrity while avoiding detrimental thrombo-inflammatory reactions demands robust mechanisms to control platelet activation, and failure of these mechanisms can lead to pathology. Indeed, platelets are known instigators of vascular injury during deep vein thrombosis, various forms of pulmonary distress, or sepsis.1

Notwithstanding the obvious association of platelets with acute thrombo-inflammatory reactions, they have also been associated with atherosclerosis, a chronic inflammatory disease of large arteries. Atherosclerosis results from the formation of atheromatous plaques characterized by the accumulation of inflammatory leukocytes.2 These plaques build up for long periods of time in a process believed to require the continuous stimulation of the endothelial cells that coat the interior of the blood vessels. In the early stages of disease, vascular activation allows recruitment of monocytes that eventually become lipid-laden macrophages, the cells believed to perpetuate inflammation.3 In advanced stages of atherosclerosis, plaque rupture and formation of large thrombi contribute to life-threatening ischemic events. With the progressive understanding of the middle and later stages of atherosclerosis, however, a remaining task in the field is to identify the early cellular and molecular triggers of arterial inflammation and plaque growth.

In this issue of Circulation Research, Karshovska et al provide firm evidence that platelet activation significantly contributes to the development of atherosclerosis. The study builds on previous work showing that elevated platelet counts, platelet deposition on arterial beds, or repeated transfer of activated platelets into athero-susceptible Apoe−/− mice (mice deficient in apolipoprotein E fed a high-fat diet) aggravated atherosclerotic lesions.4,5 A fundamental contribution of the present study, however, is the use of a genetic model of platelet hyperactivation that better mimics a clinical scenario in which patients are continuously exposed to thrombotic and inflammatory mediators produced by these cells. To generate such a model, the authors used Apoe−/− mice in which the functional adhesion molecule A (JAM-A) receptor was specifically eliminated from platelets. Work from the Naik laboratory had shown that JAM-A, a member of the immunoglobulin superfamily, provides a cell-intrinsic brake to platelet activation in response to various agonists. In accordance with this finding, the authors show that JAM-A-deficient platelets formed enlarged thrombi when exposed to fibrillar collagen but showed no additional evidence of exacerbated responses, indicating that the cause of platelet overactivation originates from the environment (ie, adhesive substrates) and is transduced through adhesion receptors, namely the integrin αIβ3. They further complement previous studies6 by showing that phosphorylated JAM-A in resting platelets associates with this integrin and allows incorporation to the complex of CSK, a kinase that inhibits c-Src, and thus prevents signals derived from the environment (referred to as outside-in signaling; Figure) from causing exaggerated platelet activation. Interestingly, the authors provide evidence that the phosphatase PTPN1 recruited to this complex on binding to fibrinogen is responsible for the dephosphorylation of JAM-A and enhanced platelet activation.

Having dissected the molecular components that mediate outside-in activation, the authors then used the platelet-specific JAM-A mutant mice to probe the consequence of platelet hyper-reactivity in the development of atherosclerosis. These experiments were highly informative because they revealed a temporal and anatomic predilection for platelet-enhanced atherosclerosis: hyper-reactive platelets significantly enlarged early lesions, especially in regions with increased susceptibility to plaque formation (ie, aortic roots). Interestingly, not only were plaques bigger in these mice but they also displayed a more advanced phenotype. These findings are in agreement with the protective roles of JAM-A in other models of vascular inflammation,7 and suggest that platelets both contribute to the expansion of preexisting lesions and can trigger the formation of new ones in more resistant arterial regions. These observations linking platelet activation with atherosclerosis are also interesting because they reconcile the well-known activating properties of turbulent flow on platelets8 with the increased susceptibility of arterial regions that display high turbulence to developing atheromatous lesions.
While searching for the mechanisms by which hyper-reactive platelets enhanced plaque development, Karshovska et al. discovered that the number of intraplaque macrophages (macrophages and T lymphocytes) was elevated, as were the levels of proinflammatory cytokines and chemokine receptors. These elevations were again specific to the early stages of disease. Several studies have shown that platelets and myeloid leukocytes display reciprocal cooperation for recruitment into the inflamed vasculature; for example, neutrophils recruited to TNF-α-stimulated venules efficiently capture circulating platelets, and this has been recently demonstrated to also occur in nascent atherosclerotic lesions. In turn, activated platelets can secrete and deposit chemokines on the surface of leukocytes and endothelial cells, thereby enhancing adhesion of the leukocytes on atherosclerotic surfaces. In agreement with these previous studies, Karshovska et al. show that JAM-A-deficient platelets release more CXCL4 and CCL5—two potent inflammatory chemokines—into the plasma, thereby creating a proatherogenic milieu that facilitates their own deposition and further recruitment of monocytes and neutrophils onto atherosclerotic regions of arteries. Importantly, many of these effects could be blocked by pretreatment with an αIIBβ3 antagonist, indicating that they likely originate from outside-in-mediated activation of platelets. Because CXCL4 and CCL5 have been shown to form heterophilic complexes that synergize to promote monocyte recruitment and the formation of neutrophil extracellular traps (DNA-based structures produced by neutrophils that can be found in atherosclerotic areas), these findings provide a reasonable mechanistic scenario to explain the initial inflammatory reactions that occur in the context of atherosclerosis. These findings are also consistent with the growing appreciation that neutrophils, like platelets, are active players in early atherogenesis, and with the notion that monocytes that infiltrate during the first stages of disease are the most relevant source of intraplaque macrophages.

The convincing demonstration that activated platelets promote and accelerate atherosclerosis opens exciting avenues to dissect the mechanisms that underlie this arterial disease. For example, platelets may drive the accumulation, and provoke secondary activation, of myeloid leukocytes by engaging specific leukocyte domains or through cooperation with other cellular processes; if true, these pathways could be targeted to prevent chronic endothelial damage and plaque growth. In addition, the study suggests that chronic activation of platelets could be blunted by targeting the PTPN1 phosphatase associated with the JAM-A/β3 integrin complex. These and other provocative possibilities could provide for the overwhelming demand for effective antiatherogenic strategies in the clinic.

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**Disclosures**
None.

**References**

**Figure.** Junctional adhesion molecule A (JAM-A) deficiency in platelets exacerbates outside-in signaling through elevated c-Src activity (inset), causing platelet hyperreactivity, adhesion, and degranulation, with increased secretion of CXCL4 and CCL5 into the plasma, ultimately causing leukocyte recruitment and acceleration of early-phase atherosclerosis. CSK indicates c-src tyrosine kinase; PTPN1, protein tyrosine phosphatase non-receptor type 1; and SMCs, smooth muscle cells.
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