Platelets and Chemokines in Atherosclerosis
Partners in Crime

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Abstract—It becomes increasingly evident that blood platelets do not only exert important functions in hemostasis and thrombus formation but are also involved in atherosclerotic vascular disease. A major portion of the underlying mechanisms is related to an intricate functional interaction of platelets with chemokines, which have also been implicated in atherogenesis and neointima formation: (1) Platelets can induce the secretion of chemokines in different cells of the vascular wall; (2) In combination with primary agonists, certain chemokines can potentiate platelet aggregation and adhesion; (3) Activated platelets can release and deposit chemokines and precursors on vascular cell surfaces, which trigger atherogenic recruitment of vascular cells or modulate crucial processes such as angiogenesis and lipoprotein metabolism; (4) Surface-adherent platelets can bind and present vascular cell-derived chemokines to trigger arrest of circulating mononuclear cells. The close linkage between platelets and chemokines as culprits in the pathogenesis of vascular diseases may provide a valuable target for selective interventions. (Circ Res. 2005;96:612-616.)

Key Words: atherosclerosis ■ neointima ■ plaque ■ monocyte ■ adhesion

Blood platelets play critical roles in hemostasis, providing rapid protection against bleeding and catalyzing the formation of stable blood clots via the coagulation cascade. Recently, activated platelets have not only been implicated in thrombosis but also in inflammatory reactions, immune responses, and in distinct aspects of atherosclerosis.1,2 Importantly, an intermittent injection of activated platelets has been shown to exacerbate the formation of native atherosclerotic lesions, a process involving platelet surface receptors that facilitate mononuclear cell recruitment.3 Among the array of chemokines found to be expressed in atherosclerotic lesions,4 the deposition of the most abundant platelet chemokine, platelet factor 4 (PF4; CXCL4), has been correlated with lesion severity and symptomatic atherosclerosis, suggesting that persistent platelet activation may contribute to the evolution of vascular lesions and supporting the rationale for chronic antiplatelet therapy in patients at risk for atherosclerosis.5 The spectrum of molecular mechanisms by which platelets and chemokines are functionally interlinked to promote atherosclerosis and neointimal hyperplasia is the focus of this concise review.

Platelets Induce Chemokine Secretion in Endothelial and Smooth Muscle Cells
Several studies have demonstrated the capability of activated platelets to induce an inflammatory reaction in cells of the vascular wall. The expression of CD40 ligand on activated platelets has been shown to rapidly occur after in vitro stimulation and during thrombus formation in vivo.5 Similar to other proinflammatory cytokines, platelet CD40 ligand induces endothelial activation and secretion of chemokines, such as monocyte chemoattractant protein-1 (MCP-1; CCL2) and interleukin-8 (IL-8; CXCL8), thereby generating crucial signals for distinct steps of leukocyte recruitment and extravasation at sites of vascular injury or initiating an inflammatory response of previously intact endothelium.6 Consequently, the disruption of CD40 ligand-induced signaling by antibody inhibition or genetic deletion has been shown to reduce the formation and progression of atherosclerotic lesions in mice.7,8 The induction of endothelial MCP-1 secretion by stimulated platelets has also been attributed to an activation of nuclear factor κB-dependent transcriptional mechanisms, possibly involving platelet-derived IL-1β.9 By similar mechanisms, platelet adhesion to subendothelial smooth muscle cells (SMCs) has been shown to increase MCP-1 secretion by SMCs, as well as their migratory properties, which appears to be particularly relevant in the context of arterial injury and restenosis.10 Thus, the direct activation of vascular cells by stimulated platelets resulting in the secretion of chemokines is an important component contributing to the initiation of vascular inflammation in atherosclerotic vascular disease (Figure, A).
Chemokines Potentiate Activation of Platelet Functions by Primary Agonists

The primary agonists adenosine diphosphate, thrombin, and collagen can directly activate platelet functions such as aggregation and adhesion. Recent research has shed light on a novel role of chemokines in stimulating or potentiating platelet activation when acting in combination with low levels of primary agonists (Figure, B). Although previous work on platelet α-granule proteins has suggested that PF4 can modulate aggregation and secretion induced by low agonist levels, its receptor on platelets remains elusive. More recently, stromal cell–derived factor-1 (SDF-1; CXCL12), which can be detected in atherosclerotic plaques, has been shown to induce platelet activation and aggregation via CXCR4 expressed on platelets, implying a potential role in atherogenesis. The macrophage-derived chemokines thymus- and activation-regulated chemokine (TARC; CCL17) and MDC (CCL22) present in atherosclerotic lesions have also been demonstrated to induce platelet activation via their receptor CCR4, which has been identified in platelets. Notably, however, the ability of chemokines to stimulate platelet functions has been reported to depend on the presence of low levels of agonists, such as thrombin, or plasma components. Likewise, the membrane-bound chemokine fractalkine overexpressed in atherosclerosis and after vascular injury has been shown to enhance platelet activation, degranulation, and adhesion to collagen, involving adenosine phosphates. This may promote thrombogenesis in vascular disease. As the most eminent chemokine protagonists participating in platelet activation, fractalkine and SDF-1 have been found to be upregulated in neointimal SMCs, which become luminally exposed after arterial denudation injury. The proinflammatory phenotype of these SMCs may not only support inflammatory recruitment of mononuclear cells but may also contribute to the activation of circulating platelets in the context of vascular injury (Figure, B). Moreover, because increased serum levels of SDF-1α have been associated with stable coronary disease, SDF-1α is more likely to be involved in neointimal hyperplasia or thrombus formation after injury than in thrombogenic complications of native atherosclerosis.

Platelets Release and Deposit Chemokines, Triggering Monocyte Arrest on Endothelium

The activation of platelets discussed above leads to the release of multiple chemokines, including CCL3, regulated on activation, T-cell expressed and secreted (RANTES; CCL5), CCL7, CCL17, CXCL1, CXCL5, or CXCL8, as well as precursors for CXCL7, such as β-thromboglobulin, from the α-granules. The secretion of platelet-derived chemokines can be stimulated by classical primary agonists, such as thrombin, by CD40 ligand-bearing T cells via CD40 expressed on platelets or by oxidized low-density lipoprotein (LDL), as shown for the release of CXC chemokines in patients with coronary artery disease. After its release by stimulated platelets, the CC chemokine RANTES can be immobilized on the surface of activated microvascular or aortic endothelial cells. The interactions of activated platelets with monocytes and atherosclerotic arteries can result in the delivery of the platelet-derived chemokines RANTES and PF4 to the monocyte surface and early atherosclerotic endothelium. Moreover, RANTES can be de-
tected on the luminal lining of neointimal lesions.20,21 The deposition of RANTES appears to be particularly effective under flow conditions supporting platelet rolling and is instrumental in triggering the subsequent arrest of monocytes or T cells on inflamed and early atherosclerotic endothelium in carotid arteries.18,20,21 Indeed, the delivery of RANTES and the effects on monocyte recruitment are mediated by mechanisms dependent on platelet P-selectin, as shown by using P-selectin–deficient platelets or mice repopulated with P-selectin–deficient bone marrow.3,21 Accordingly, injection of activated wild-type but not P-selectin–deficient platelets increased the size of atherosclerotic lesions in apolipoprotein E–deficient mice.3 A crucial involvement of RANTES in the development of native atherosclerotic lesions and in neointima formation after wire-induced arterial injury has been confirmed by treatment of atherosclerosis-prone mice with the peptide antagonist Met-RANTES.22 Thus, it has become evident that the deposition of RANTES is a principle mechanism by which activated platelets can support and sustain atherogenic monocyte recruitment (Figure, C).

The process of P-selectin–dependent RANTES delivery can also be emulated by platelet-derived microparticles. Platelet microparticles are formed in a process of microvesiculation during platelet activation, and elevated levels have been associated with inflammatory processes during vascular pathogenesis and increased cardiovascular risk.23 In diabetic patients, levels of platelet-derived microparticles, P-selectin, and RANTES are concomitantly increased.34 Platelet-derived microparticles have been shown to confer chemokine receptors, namely CXCR4, to mononuclear cells (eg, hematopoietic stem cells), thereby facilitating their recruitment.28 Recently, platelet microparticles have been implicated as transfer modules mediating the immobilization of RANTES on activated early atherosclerotic endothelium to trigger atherogenic monocyte arrest (Weber et al, unpublished data, 2004). Because the exposure to platelet microparticles has been shown to induce the expression of endothelial adhesion molecules in a process requiring arachidonic acid metabolism, platelet microparticles may similarly be involved in the upregulation of endothelial chemokine expression.26 Conversely, platelet chemokines and their receptors (eg, CX3CR1) may play a crucial role in the generation and composition of platelet microparticles.

**Interactions With PF4 Modulate Atherogenic Functions of Lipoproteins and Chemokines**

PF4 is the most abundant CXC chemokine released from platelet α-granules. With regard to the accumulation of LDL-derived cholesterol in macrophages as a pathogenetic feature of atherosclerosis potentially modulated by platelets, PF4 has been shown to interfere with binding and degradation of LDL through its receptor, a process that could promote the formation of oxidized LDL.27 Moreover, PF4 can directly bind to oxidized LDL, increases its binding to vascular cells and macrophages depending on the presence of proteoglycans such as chondroitin sulfate, and enhances the esterification of oxidized LDL in macrophages.28 Given that PF4 and oxidized LDL colocalize in macrophage-derived foam cells of atherosclerotic lesions, this mechanism likely promotes vascular lipid accumulation in vivo. Although displaying only weak chemotactic properties, PF4 has been implicated in promoting the adhesion of leukocytes and progenitor cells and strongly inhibits hematopoiesis and angiogenesis.29,30 Recently, PF4 has been found to bind hematopoietically active IL-8 and to block IL-8–mediated signaling in progenitor cells, providing a novel mechanism for modulation of hematopoiesis by PF4.31 Similar interactions may also be relevant for the PF4-mediated inhibition of endothelial chemotaxis and angiogenesis induced by IL-8 or basic fibroblast growth factor.29,30 Recently, a nonallelic variant of PF4 released by platelets has been identified, which was even more potent in inhibiting endothelial chemotaxis and angiogenesis.32 Because blocking the closest murine IL-8 ortholog KC (CXCL1) can delay endothelial recovery and enhance neointima hyperplasia after arterial injury in atherosclerosis-prone mice,33 the effects of PF4 may also lead to an exacerbation of lesion formation. Notably, a heterophilic interaction has also been revealed for PF4 and RANTES (Figure, C), which promoted the stimulation of monocyte arrest on activated endothelium, whereas PF4 conceivably inhibited IL-8–triggered arrest.34 Similarly, PF4 and RANTES immobilized on endothelial cells can also act in concert to induce monocyte arrest (Weber et al, unpublished data, 2004). Whereas the amplification of monocyte arrest required structural motifs important in RANTES oligomerization and the presence of chondroitin sulfate,34 the functional involvement of an alternatively spliced form of CXCR3 as a recently described receptor for PF4 expressed in endothelial cells remains to be determined in detail.35 Conversely, the interaction with RANTES may affect or amplify the effects of PF4 in promoting the survival of emigrated monocytes and their differentiation into macrophages.36

**Platelets Present Vascular Chemokines to Mononuclear Cells After Arterial Injury**

Murine models of atherosclerosis have unraveled a central role of MCP-1 and its receptor CCR2 in native and neointimal lesion formation.37–39 In hyperlipidemic mice or after arterial injury, an upregulation of MCP-1 expression has been detected in medial SMCs, but increased MCP-1 levels were also found in serum and in platelets adherent to the denuded artery. In vitro studies revealed that recombinant or SMC-derived MCP-1 binds to the surface of adherent platelets and triggers monocyte arrest in flow.38 Surface-adherent platelets do not contain MCP-1 but show staining for MCP-1 after endothelial denudation, whereas peak serum levels of MCP-1 precede platelet coverage and peak MCP-1 expression in the injured wall, implying that locally secreted MCP-1 is retained and presented by platelets at the site of injury.39 This is in accordance with a study reporting low-affinity binding of MCP-1 to platelets despite a lack of functional CCR2 and extends evidence that chemokines can be concentrated on the surface of activated platelets, possibly via binding to proteoglycans.40–42 The blockade of MCP-1 did not interfere with monocyte arrest in arteries with native atherosclerosis but profoundly inhibited monocyte accumulation in denuded arteries after injury. This suggests a distinctive contribution of MCP-1 to monocyte arrest after endothelial denudation.
depending on the presence of adherent platelets for local binding and concentration of MCP-1. A causal relationship between MCP-1–dependent monocyte arrest on platelets and neointimal hyperplasia is inferred by reduced plaque area and macrophage content in hyperlipidemic mice with genetic deletion of CCR2. Recent evidence also infers an involvement of SDF-1α in neointimal hyperplasia and recruitment of circulating progenitor cells, giving rise to SMCs after arterial injury. Early after denudation of apolipoprotein E–deficient carotid arteries, the expression of SDF-1α was not only detectable in medial SMCs but also in conjunction with luminal platelets bound to the injured surface. Blocking SDF-1α inhibited the recruitment of peripheral blood progenitor cells in freshly injured carotid arteries, and their arrest on platelets adherent to extracellular matrix in vitro was potentiated by exposure to SDF-1α via its receptor CXCR4 (Weber et al, unpublished data, 2004). Beyond the secretion and deposition of platelet-derived chemokines, the binding and presentation of chemokines by surface-adherent platelets represents a novel mechanism by which platelets can support chemokine-triggered recruitment of monocytes or progenitor cells in the context of arterial injury (Figure, D). As opposed to the inhibition of IL-8–induced endothelial migration and angiogenesis by platelet-derived PF4, the presentation of MCP-1 or SDF-1α by adherent platelets may assist in the CCR2-dependent mononuclear cell recruitment crucial for atherosclerosis or in the CXCR4-dependent recruitment of endothelial progenitor cells involved in neovascularization. On the other hand, similar to IL-8 identified in human coronary plaques, these chemokines may promote progression of atherosclerosis by mediating plaque neovascularization.

Functional Interactions Between Platelets and Chemokines as Targets for Intervention

An intricate functional relationship between platelets and chemokines emerges from the multiple interactions discussed above and illustrated in the synopsis of the Figure. The mechanisms provide a framework for synergistic functions and allow insights into the deleterious basis for proatherogenic, proinflammatory, or thrombogenic effects exerted by two prime suspects in the pathogenesis of vascular disease. The notion that platelets and chemokines can act as mutual and bidirectional effectors implies the existence of feedback loops amplifying and aggravating their role for instance in atherogenic and neointimal recruitment of mononuclear cells. Chemokines can stimulate and potentiate platelet activation and adhesion, resulting in turn in the release of platelet-derived chemokines and possibly more efficient retention and presentation of vascular chemokines by platelets adhering to collagen of the extracellular matrix. Conversely, platelets may induce the secretion of chemokines for monocyte recruitment from endothelial cells or SMCs and, at the same time, may be activated by membrane-bound chemokines on luminally exposed SMCs. Chemokines secreted from platelet α-granules and deposited on the luminal surface of early atherosclerotic endothelium are instrumental in triggering monocyte recruitment, and at the same time, monocyte arrest and the function of emigrated macrophages in lipoprotein binding and metabolism is modulated by PF4 released from activated platelets. Differential effects of platelet-derived PF4 in inhibiting IL-8–induced endothelial cell migration, while enhancing RANTES-induced monocyte arrest, suggest the option of selectively interfering with effects resulting from the heterophilic interactions of specific chemokine family members. For instance, blocking the “heteromerization” of RANTES and PF4 by specific antibodies or antagonists may disrupt a deleterious mechanism amplifying monocyte recruitment without affecting functions of these molecules in physiological immune surveillance or hemostasis (ie, the role of RANTES in inflammation or of PF4 in activating protein C). Similarly, findings that specific proteoglycans (eg, chondroitin sulfate) are required for the effects of platelet chemokines may open avenues for a selective intervention (eg, by neutralization of or with glycosaminoglycans). This may for the first time enable options for targeting specific functions of platelets and chemokines as culprits in the pathogenesis of atherosclerotic vascular disease without undesirable systemic side effects. Although most of the data supporting the hypothesis presented herein may be derived from in vitro studies, investigations addressing the relevance of these mechanisms in vivo are clearly warranted and under way. For instance, although mice repopulated with bone marrow deficient in CXCR4 show reduced neointima formation after arterial injury, insinuating a role of CXCR4 on progenitor cells or platelets, studies dissecting the involvement of CXCR4 or CX3CR1 in platelet activation in vivo (eg, by platelet-specific genetic deletion) remain elusive. Moreover, evidence for a contribution of platelets to chemokine delivery or endothelial activation can be gathered in mice repopulated with bone marrow deficient in platelet degranulation (eg, because of genetic deletion of the small G-protein Gq) or in platelet signal proteins (eg, CD40 ligand). Such studies should ultimately clarify the relevance of individual chemokine-dependent mechanisms engaged or sustained by platelets in vivo.

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References

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