Editorial

Platelet-Derived CD40L
The Switch-Hitting Player of Cardiovascular Disease

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Studies focusing on cellular and molecular mechanisms that regulate atherosclerosis have fed scientific journals for decades, nearly as long as it takes an atherosclerotic plaque to grow, rupture, and eventually induce vascular occlusive events.

See p 981

Three closely linked lines of research have now merged. In the 1980s, concepts crystallized on the role of lipids (eg, oxidized LDL, elevated cholesterol) in the genesis of atherosclerotic plaque. In the 1990s, use of antplatelet agents proved that platelet aggregation caused thrombotic ischemic events resulting from the rupture of plaques in advanced lesions and from the vascular injuries inflicted by percutaneous interventions (PCI). Now, atherosclerosis is recognized as an inflammation-mediated disease involving multiple interactions between leukocytes, cells of the vessel wall, and platelets. Indeed, recent studies of predictors of cardiovascular disease focused on cellular and molecular mechanisms that regulate atherosclerosis have fed scientific journals for decades, nearly as long as it takes an atherosclerotic plaque to grow, rupture, and eventually induce vascular occlusive events.

CD40L

CD40L is a trimeric, transmembrane protein of the tumor necrosis factor family that was originally identified on cells of the immune system (activated CD4+ cells, mast cells, basophils, eosinophils, and natural killer cells). The role of CD40L in the immune response involves binding to its receptor on B cells, CD40, to induce B-cell proliferation, generate memory B cells, block B-cell apoptosis, and mediate antibody class switching. However, it was subsequently shown that CD40L and CD40 are also present on several cells of the vasculature, including endothelial cells, smooth muscle cells, monocytes, and macrophages. Additionally, the pioneering work of Henn and collaborators\(^3,4\) showed that CD40L and CD40 also exist in platelets. CD40L is cryptic in unstimulated platelets but is rapidly presented to the platelet surface after platelet stimulation. The surface-expressed CD40L is subsequently cleaved over a period of minutes to hours, generating a soluble fragment termed sCD40L that remains trimeric (Figure 2). Studies on the cellular distribution of CD40L indicate that >95% of the circulating CD40L exists in platelets. This suggests that platelet stimulatory events must be considered in the biological and pathological context of CD40L function.

CD40L and sCD40L are known to have structural domains that allow these proteins to have multiple functions. First, the tumor necrosis factor homology domain allows for binding to its receptor, CD40. Second, the lysine-arginine-glutamic acid (KGD) motif, which remains part of the sCD40L cleavage product, allows for its binding to glycoprotein (GP) IIb/IIIa.\(^5\) Third, the trimeric structure of CD40L and of the soluble cleavage product allows for the induction of signaling reactions when bound to receptors. The functional activities of platelet CD40L are reflective of these multiple domains. When expressed on the surface of platelets and exposed to CD40-bearing vascular cells, platelet-associated CD40L is capable of initiating various inflammatory responses, including expression of inflammatory adhesion receptors (eg, E-selectin, vascular cell adhesion molecule-1 [VCAM-1], intercellular adhesion molecule-1), expression of tissue factor, and release of chemokines (eg, monocyte chemotactic protein-1 [MCP-1], interleukin-6, and interleukin-8).\(^3,6\) Although the repertoire of functional activities for the released product remain to be described, it is known that sCD40L is also proinflammatory,\(^2\) although other studies failed to observe these activities.\(^4\) Studies of mice harboring a CD40L gene deletion have shown that the KGD motif on this protein is also functional. CD40L-/- mice have a thrombosis defect, but infusion of recombinant sCD40L normalizes this deficiency, demonstrating the prothrombotic activity of this protein. This activity of sCD40L can in part be attributed to the KGD peptide sequence found near the carboxyterminus of the protein, which enables it to bind directly to GP IIb/IIIa.\(^5\) Thus, sCD40L has the potential to mediate several events within the vasculature.

Inflammatory Mediators of Atherosclerosis: The Key Role of CD40L

Initial studies utilizing mouse models of atherosclerosis (eg, LDL receptor (LDLR)-/- and ApoE-/- mouse strains) emphasized the critical role of leukocytes. Indeed, deficiencies in molecules involved either in leukocyte rolling (endothelial selectins), leukocyte recruitment (MCP-1), or leukocyte ar-
rest (VCAM-1) reduced the size of atherosclerotic plaques, mainly through a reduction of lipid deposition, smooth muscle cell proliferation, and migration. Additional studies showed that infiltrated B and T lymphocytes were also involved. These cells, in addition to macrophages and vascular cells, release various cytokines and growth factors to promote the migration and proliferation of smooth muscle cells and induce the expression of proinflammatory cytokines from vascular cells and matrix metalloproteinases from resident cells in the atheroma. (2) Thrombosis. sCD40L stabilizes platelet-rich thrombi. (3) Restenosis. sCD40L inhibits the reendothelialization of the injured vessel, potentially leading to the activation and proliferation of smooth muscle cells.

Figure 1. Three functions of sCD40L released from platelets during thrombosis. sCD40L is released from platelet-rich thrombi and contributes to various steps in atherosclerotic lesion progression: (1) Inflammation. sCD40L induces the production and release of proinflammatory cytokines from vascular cells and matrix metalloproteinases from resident cells in the atheroma. (2) Thrombosis. sCD40L stabilizes platelet-rich thrombi. (3) Restenosis. sCD40L inhibits the reendothelialization of the injured vessel, potentially leading to the activation and proliferation of smooth muscle cells.

Restoration of plaque stability, most likely because of the release of matrix metalloproteinases.

The Platelet–CD40L Axis

The involvement of platelets and other elements of the hemostatic/thrombotic system in atherosclerosis is part of a concept first postulated by the late Dr Russell Ross. It suggests that chronic activation of the vessel wall contributes to the recruitment of platelets, which in turn allows further endothelium damage. The theory of platelet recruitment on a physically intact but functionally dysregulated endothelium seems even more relevant, because activated endothelial cells support platelet rolling, their translocation, and occasionally, their adherence. Pioneering studies of Dr E.J. Bowie and coworkers also established that the lack of von Willebrand Factor (the principal ligand mediating platelet aggregation under high shear rates) affected atherosclerotic lesion progression, which further validated this hypothesis. Because platelets in their α-granules possess a large range of proinflammatory molecules, such as transforming growth factor-β, platelet factor-4, RANTES, and P-selectin, a direct link between platelets, inflammation, and atherosclerosis was far from being speculative.

Platelets, however, are also the primary source of circulating CD40L, begging the question about its role in the progression of atherosclerotic disease, including the formation of thrombotic occlusions. Recent studies demonstrate that platelet CD40L becomes mobilized in acute coronary thrombotic indications. For example, increased levels of sCD40L are a consequence of various procedures known to have thrombotic and inflammatory components, including PCI and cardiac surgery requiring cardiopulmonary bypass. Increased levels of sCD40L are also found in patients with acute coronary syndromes and peripheral arterial occlusive disease. Indeed, elevated plasma levels of sCD40L are a risk factor for future cardiovascular events in apparently healthy women. An indication of the inflammatory activity of sCD40L comes from transfusion medicine. Storage of platelet concentrates for clinical transfusion is known to release up to 50% of the platelet CD40L. Transfusion
of concentrates into patients results in CD40L-dependent febrile responses.\textsuperscript{15}

The production of sCD40L from platelets and its thrombotic activity appear to be intimately linked to the platelet integrin GP Ib/IIa. GP Ib/IIa is known to be involved in sCD40L production because GP Ib/IIa antagonists attenuate the release of sCD40L from activated platelets in vitro.\textsuperscript{16} These antagonists block release from stimulated platelets even in the absence of aggregation, demonstrating a direct role for GP Ib/IIa in the cleavage mechanism. Secondly, direct binding of sCD40L to GP Ib/IIa indicates that the ability of sCD40L to promote and stabilize platelet thrombosis under high shear rates is a result of direct interactions between these 2 proteins.\textsuperscript{5}

**Restenosis**

Given the close link between inflammation and restenosis, it is perhaps not surprising that a linkage exists between CD40L and the response to vascular injury. How could CD40L be involved in restenosis? Additionally, is the CD40L involved in these activities derived from platelets? PCI is known to disrupt the endothelium, resulting in the exposure of thrombogenic surfaces that support the adhesion, activation, and aggregation of platelets. The platelet-rich thrombi may be a source of localized high concentrations of proinflammatory CD40L, both on the surface of platelets and in the immediate environment as they shed sCD40L. The article by Urbich et al\textsuperscript{1} in this issue of Circulation provides a mechanism by which the sCD40L generated by thrombosis could promote restenosis. These authors demonstrate that CD40L expressed on the surface of activated platelets and T cells, and the sCD40L released from platelets, inhibit growth factor–induced human umbilical vein endothelial cell migration while not affecting cell proliferation and cell death. Their studies also show that CD40L–induced inhibition of migration is achieved by generation of free radicals and inhibition of NO production. From these observations, they speculate that the interaction of the intact or sCD40L with CD40 could inhibit reendothelialization of an injured vessel, thereby enhancing the restenotic process. The assumption that inhibition of endothelial cell mobility will translate into restenosis in vivo is an exciting and promising hypothesis.\textsuperscript{17}

**Conclusions and Questions**

The mechanisms responsible for initiating atherosclerotic lesions are undoubtedly diverse. However, the emerging data on CD40L suggest the evolution of a new paradigm for the role of platelets in inflammation and atherosclerotic lesion progression. The triad of functional activity of CD40L in atherosclerotic models, high content in platelets, and mobilization during platelet thrombosis provides a readily testable hypothesis and places platelet-derived CD40L squarely in the forefront as an important, mitigating factor in this disease.

Still, several questions arise. Does the sCD40L systemically generated by activated platelets in circulation or locally by acute thrombosis impact subsequent thrombosis, lesion progression, or restenosis? Will the ability of GP Ib/IIa antagonists to block sCD40L release in vitro translate into the inhibition of sCD40L release in acute coronary thrombotic indications like acute coronary syndromes or as result of PCI? Is the activity of antiplatelet agents limited to blocking occlusion and subsequent ischemia, or do these agents have effects that translate into the inhibition of atherosclerotic lesion progression? Is this the mechanism by which the short-term inhibition of thrombosis with GP Ib/IIa antagonists (eg, <20 hours) in the setting of PCI translates into a prolonged inhibition of the accrual of events (eg, up to a year or longer), as was observed in the Evaluation of c7E3 for the Prevention of Ischemic Complications (EPIC) and Enhanced Suppression of Platelet Receptor GP-Ib/IIa using Integrilin Therapy (ESPRIT) studies.\textsuperscript{18,19}

sCD40L is now known to be elevated in the plasmas of individuals with a broad spectrum of inflammatory conditions, such as rheumatoid arthritis,\textsuperscript{20} sickle cell anemia,\textsuperscript{21} and systemic lupus erythematosus.\textsuperscript{22} Do these conditions involve CD40L in immunity? Or, because most of the circulating CD40L exists in platelets, do these pathologies involve platelet-derived CD40L? The answer to these questions may indicate that platelets and platelet-derived products may be involved in a broader spectrum of human pathology than is presently realized.

**References**


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