CD40-CD40L and Platelet Function
Beyond Hemostasis

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Traditionally, platelets have been thought of as anuclear, subcellular fragments derived from megakaryocytes circulating in blood as small discs and mitigating hemorrhage. This hemostatic process requires platelet activation, a complex chain of events involving rapid structural changes that activate adhesion receptors, remodel the cytoskeleton, and lead to the eventual synthesis and secretion of various platelet-derived factors. The result of this cascade is the formation of platelet-dependent thrombosis that primarily attenuates bleeding but, in pathophysiological settings, contributes to occlusive vascular events. These clinical realities have led to the bias that platelets are primarily involved in thrombosis and hemostasis. This bias has been reflected in the predominant use of platelet aggregometry as the standard method for quantifying platelet activation ex vivo. This technique, however, only identifies platelet-platelet binding (homotypic aggregates) and, in clinical settings, has promoted a relatively simplistic vision of platelet function. The focus on platelet-dependent thrombosis has made the platelet aggregate a common therapeutic target in syndromes involving vascular occlusion. However, merely preventing the process of platelet-platelet binding may not always translate into clinical efficacy as seen in the recent disappointing results associated with the use of the oral IIb/IIIa inhibitors.1

Recently, studies have suggested that platelets also participate in inflammatory reactions. Platelets are known to produce inflammatory mediators including platelet-derived growth factor, platelet factor 4, and transforming growth factor-β. Platelets are also known to bind, via P-selectin (CD62P) expressed on the surface of activated platelets to the leukocyte receptor, P-selectin glycoprotein ligand-1 (PSGL-1). The relevance of this binding is supported by a study demonstrating that the infusion of recombinant soluble human form of P-selectin glycoprotein ligand-1 in an animal model of vascular injury reduced myocardial reperfusion injury and preserved vascular endothelial function.2 The clinical implications of heterotypic aggregates are shown by studies demonstrating that after acute myocardial infarction, circulating monocyte-platelet aggregates are both increased and are a more sensitive marker of in vivo platelet activation than platelet surface P-selectin.3 Plaque rupture promotes activation of the inflammatory responses, and the consistent finding of heterotypic aggregates highlights the close interaction between inflammation and thrombosis in vascular disease.

Contributing to our understanding of the role of platelets in inflammation are CD40-CD40 ligand (CD40L) interactions. CD40 is a membrane glycoprotein belonging to the tumor necrosis family receptor superfamily and its ligand CD40L (CD154) is a glycoprotein from the tumor necrosis factor family. CD40-CD40L interactions are central in immune responses and inflammation. Ligation of CD40 on various vascular cells contributes to the pathogenesis of atherosclerotic, thrombotic, and inflammatory processes.4,5 On endothelial cells or monocytes, the engagement of CD40 leads to the synthesis of adhesion molecules, chemokines, and tissue factor and causes the activation of matrix metalloproteinases. CD40L has also been detected in platelets6 where, after stimulation, it is translocated to the platelet surface (Figure). The surface-expressed CD40 ligand is then cleaved from the platelet over a period of minutes to hours subsequently generating a soluble fragment (soluble CD40 ligand or sCD40L). It is estimated that >95% of the circulating sCD40L is derived from platelets. Found to be increased on platelets in fresh thrombus,4 sCD40L has been shown to be elevated in cardiovascular disease7,8 and associated with increased cardiovascular risk in apparently healthy women.9

Although sCD40L has been characterized as a marker of thrombotic diseases, much less is known about its direct role in platelet function. It has been suggested that CD40L is an α₃β₃, ligand, a platelet agonist, and contributes to stability of arterial thrombi.6 In this issue of Circulation Research, Inwald and colleagues10 add to the limited information concerning the effects of sCD40L on platelet function and demonstrate that CD40 ligation may be a mechanism for platelet activation. After incubation of platelets with the trimeric form of sCD40L (sCD40LT), they showed enhanced CD62P expression as well as dense and α-granule release. Interestingly, the presence of a glycoprotein IIb/IIIa blocking agent did not alter sCD40LT-induced CD62P expression. In addition, β-thromboglobulin and [³⁵S]-5-HT were shown to be present after ligation reflecting α- and dense granule release, respectively. An intriguing part of this study is the confirmatory data using subjects with X-linked hyper-IgM syndrome and known absence of CD40L expression. The ability to utilize these subjects is particularly interesting in platelet-dependent studies as genetic manipulation in cell culture is not possible.
At issue is the form and concentration of sCD40L used in the studies. In the experiments, the concentration of sCD40LT used was 1 µg/mL although platelet activation was present at concentrations as low as 100 ng/mL. Although these concentrations are similar to those reported in the literature for CD40L activation of other cell types, it is still greater than the levels reported in patients (50 ng/mL). Although this discrepancy may question the clinical relevance of the findings of Inwald et al., it is possible that local concentrations in active areas of thrombotic or inflammatory disease are higher. This would allow a dose-dependent mechanism for targeting the effects of sCD40L. Further kinetic studies and assessment of in vivo concentrations are needed to answer the question of relevance in relation to the levels used in these studies. In addition, the trimeric form of sCD40L was used in the studies and it has been shown that the specific activity of sCD40L may depend on its form as a trimer or monomer. Although the membrane-bound CD40L may be trimeric, there is debate concerning the soluble form. Further studies, specifically in platelets, would be required to clarify this question.

In the studies by Inwald et al., platelet activation via sCD40LT occurred in the presence of a α₃β₃ inhibitor. This appears to be in contrast to previous work that demonstrates that sCD40L is an α₃β₃ ligand. However, a more intriguing interpretation is that this is not only a novel means of platelet activation but that the platelet may be activated in distinct ways in different settings. In the present study by Inwald et al., incubation with sCD40LT resulted in significantly less CD62P expression compared with platelet activation with thrombin receptor agonist peptide 1-6 (SFLLRN). In addition, the increase in Pac-1 is minimal with sCD40L compared with SFLLRN. Differences in dense granule release were also detected with thrombin receptor stimulation leading to >5 times [¹¹C]-5-HT levels compared with sCD40LT incubation. Similar results were not shown for β-thromboglobulin and further analysis of α-granules as well as the effects of other agonists would be of interest. However, it may be possible that in areas of relevant concentrations, the presence of sCD40L leads to selective activation and granule release but excludes the formation of homotypic aggregates. This possibility is strengthened by the finding that sCD40LT enhanced platelet-neutrophil formation occurs in the setting of minimal α₃β₃ activation and that platelets from subjects deficient in CD40L aggregated normally. However, in the setting of high shear, α₃β₃ may be engaged leading to thrombus formation. Thus, these differences could allow for a partial separation of the thrombotic and inflammatory roles of platelets. The mechanism for the sCD40L-induced cascade of platelet activation is not known. In the present study, the experiments suggesting that there was no change in calcium flux after sCD40L stimulation are too limited to draw conclusions or to infer that protein kinase C signaling is responsible. However, the possibility that ligation of CD40, as well as binding to α₃β₃, can lead to outside-in signaling in the absence of inside-out signaling is intriguing. However, not all means of inside-out stimulation in platelets are calcium-dependent. To conclude that CD40-CD40L interactions are resulting in outside-in signaling or G protein–independent activation requires further examination.

Although many questions remain, these findings help to clarify if sCD40L is released to further drive platelet-dependent thrombus formation or inflammatory reactions in the vasculature. The partial and selective activation that appears after engagement of CD40 suggests that the platelet is involved in both functions and that it may have the capacity to be differentially regulated. Continuing to define the role of platelets in inflammation and the extent to which its thrombotic functions are complementary will be challenging. The anuclear platelet is being rediscovered as an intriguing link between thrombosis and inflammation, and future research should provide great insight into platelet-dependent mechanisms leading to the regulation of vascular disease.
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References

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