New Links Between Inflammation and Thrombosis

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Abstract—This article is a summary of the Sol Sherry Lecture of the Council on Arteriosclerosis, Thrombosis, and Vascular Biology, which was presented at the Scientific Sessions of the American Heart Association in November 2004. It highlights work from our laboratory, focusing mainly on new aspects of P-selectin and CD40L (CD154) biology and on the interplay of platelets and leukocytes in thrombosis and inflammation. (Arterioscler Thromb Vasc Biol. 2005;25:1-4.)

Key Words:

It was an honor to present a lecture in the memory of Dr. Sol Sherry (1916 to 1993), who is known among hematologists as the “father of thrombolytic therapy.” Sherry is also acclaimed for the large number of trainees whose careers he helped build. Even I am part of this lineage because he was a mentor to Dr Victor Marder, who, in turn, mentored me in my first faculty position in Rochester, NY. It was there that we found that Weibel-Palade bodies, organelles specific to endothelial cells, are secretory granules and that they contain very large von Willebrand factor (vWF) multimers. These multimers are most potent in promoting platelet adhesion during the first step of hemostasis. Several years later, it became apparent that P-selectin is stored in these same granules and mediates adhesion to leukocytes. Thus, we established that the Weibel-Palade body is a prominent link between inflammation and thrombosis. A myriad of stimulatory mediators that result from injury or inflammation, such as thrombin and histamine, cause Weibel-Palade body secretion, leading to surface expression of vWF and P-selectin (Figure). Whereas P-selectin mediates leukocyte rolling, both of these Weibel-Palade body components can support resting platelet rolling under different shear conditions. Activated platelets can also roll on endothelium via their P-selectin but only when the endothelium is inflamed and expresses a yet unidentified P-selectin ligand. Inflamed endothelium also strongly supports leukocyte rolling because it expresses new ligands induced by inflammation such as E-selectin and vascular cell adhesion molecule-1 that, like P-selectin, mediate leukocyte rolling. Because of the similarities between the behavior of leukocytes and platelets and the molecular mechanisms recruiting both cell types to the vessel wall, we asked: Are platelets involved in inflammation? Are leukocytes involved in hemostasis? As is discussed below, the answer to both questions is yes.

Role of Platelets in Chronic and Acute Inflammation

The goal of inflammation is to rapidly recruit leukocytes to the site of injury or infection. P-selectin on endothelium plays an important role in neutrophil and monocyte recruitment. Therefore, it was no big surprise when Arthur Beaudet’s and our groups found that P-selectin is important for the migration of monocytes to atherosclerotic lesions in mouse models of atherosclerosis. But P-selectin is also expressed at high density on activated platelets after it is released from α-granules and could help in leukocyte recruitment to atherosclerotic lesions. Therefore, Peter Burger asked whether it is platelet or endothelial P-selectin that is most important for atherosclerosis. The question was answered by performing bone marrow transplants generating animals with only endothelial or platelet P-selectin on the apolipoprotein E–deficient (apoE−/−) background that promotes atherosclerosis. The chimeric animals were allowed to age for 7 months, and then their aortic sinus lesions were analyzed. Only animals with endothelial P-selectin developed lesions of significant size, demonstrating that endothelial P-selectin is crucial for atherosclerotic lesion growth. Interestingly, a comparison of animals expressing endothelial P-selectin showed 30% larger lesions in mice with platelet P-selectin than in mice lacking the platelet P-selectin. Thus, platelets and their P-selectin contribute to atherosclerotic lesion development. The degree of lesion maturation (presence of smooth muscle cells and calcification) was dependent on endothelial as well as platelet P-selectin. Therefore, it appears that signaling induced by P-selectin may stimulate monocytes/macrophages to produce more chemoattractants or growth factors, leading to larger numbers of smooth muscle cells in the lesions. Russell Ross had already proposed a role for platelets in atherosclerosis in the 1980s, but it was only in 2002 to 2003 that 3 groups demonstrated this experimentally through very different approaches. In addition to our work described above, Massberg et al showed that platelet...
depletion or inhibition of platelet adhesion delayed fatty streak (early atherosclerotic lesion) formation in mice,17 and Ley’s group observed that repeated infusion over several weeks of activated platelets into apoE−/− mice promoted lesion formation.18 Here again, platelet P-selectin was required for the atherogenic effect.

Because platelet P-selectin is expressed only on activated platelets, for it to have an effect in the bone marrow transplant study described above,14 some platelets also had to become activated in circulation. In our group, Vandana Dole is now asking whether the presence of large numbers of activated platelets, such as would occur at the time of surgery or in pathologic situations such as unstable angina, could systemically affect endothelial function and perhaps induce inflammation. She has found that numbers of rolling leukocytes were increased several-fold in wild-type mice 2 hours after infusion of wild-type–activated platelets. The effect was transient because leukocyte rolling returned to baseline levels 7 hours after infusion. Resting platelets or activated platelets lacking P-selectin did not activate endothelium (V. Dole, unpublished observations). Thus, it appears that the presence of activated platelets in circulation rapidly and transiently induces systemic leukocyte rolling, giving the animal a head start on inflammation. Whether this effect is directly onto endothelium or by an intermediate generated from platelet/leukocyte interaction via P-selectin remains to be clarified. What is evident now is that P-selectin expressed on endothelium or on activated platelets is a major player in acute and chronic inflammation.

Procoagulant Activity of P-Selectin

Because P-selectin is stored in the same granules as the platelet adhesion molecule vWF, could it also have a role in hemostasis? An early observation by Palabrica et al indicated that it might be the case. She observed that in the presence of an antibody to P-selectin, less fibrin was deposited on platelets adherent to thrombogenic grafts implanted in ba-

How Does P-Selectin Induce Procoagulant Activity and Promote Fibrin Deposition?

P-selectin appears to have 2 distinct roles in the process. First, through signaling by P-selectin glycoprotein ligand-1 (PSGL-1), it induces the formation of MPs from leukocytes, most
likely monocytes. The plasma levels of these MPs depend on levels of P-selectin expression. Thus, significant injury or chronic inflammation might increase the P-selectin/PSGL-1–dependent MP production. In mice, MP count increases with age, but this is not the case in PSGL-1−/− mice, indicating that the increase in MP count perhaps results from more inflammation (P-selectin expression) with age. Paul Ridker has shown that elevated soluble P-selectin (sP-selectin) in plasma increases the risk of future cardiovascular events such as myocardial infarction and stroke. High levels of sP-selectin appear undesirable and may reflect poor health. Preliminary results from our laboratory indicate that the ΔCT mice, with several-fold elevated sP-selectin and consequently presenting unusually high procoagulant activity, have defects in their blood–brain barrier (J. Kisucka, unpublished results), showing how widely disregulated P-selectin expression can affect vascular function.

A second role of P-selectin in augmenting coagulation is in the recruitment of leukocyte-derived MPs to thrombi (Figure). P-selectin expressed on activated platelets captures the MPs via PSGL-1. This concentrates the procoagulant activity at the thrombus site, just where it is needed. The importance of this blood-borne TF may depend on the quality of the injury. In situations in which lots of TF is exposed to blood, the blood-borne TF may be less important than in puncture wounds. MP-containing TF may also stabilize fibrin long after it was deposited and when the wound is fully covered by platelets. This is likely why inhibitors of P-selectin/PSGL-1 may act as thrombolytic agents dipping the coagulation balance toward thrombolysis. Another situation in which blood-borne TF may play an important role is thrombosis at sites of stasis. Ischemia causes upregulation of P-selectin on endothelium, and this may promote capture of MPs and explain formation of larger thrombi in deep vein thrombosis models in the ACT mice than in wild-type.

Could the procoagulant power of P-selectin be captured in a situation in which the intrinsic coagulation pathway is defective? Apparently, yes. To strengthen the extrinsic pathway of coagulation and thus the total capacity to generate thrombin, we treated mice deficient in Factor VIII with P-selectin/PSGL-1 with P-selectin/PSGL-1 to produce more MPs. In this way, in the mouse model of hemophilia A, we could normalize plasma-clotting parameters and tail-bleeding time. Our results point to a new strategy for treatment of hemophilia patients, in particular, those with alloantibodies.

**Role of CD40 Ligand (CD40L) in Thrombosis**

Another new molecular link between inflammation and thrombosis is the cytokine CD40L (also called CD154 or gp39). CD40L is a trimeric transmembrane protein that is a member of the tumor necrosis factor (TNF) family. It is not only expressed on the cells of the immune system but also on activated platelets. Similar to P-selectin, CD40L is stored in platelets and translocates to the plasma membrane on activation. CD40L is an important molecule regulating immune responses by interacting with its receptor CD40, a member of the TNF receptor family. This interaction induces antibody isotype switching, and absence of CD40L in humans results in hyper IgM syndrome. CD40L binding to CD40 on endothelium induces inflammation, and this biological activity is also preserved in platelet CD40L. The interaction of platelet CD40L with CD40, which is also found on platelets, induces CD40L shedding by proteolytic cleavage, and the soluble protein (sCD40L) no longer has the inflammatory activity of the transmembrane receptor. The proinflammatory activity of CD40L is also reflected in its prominent role in atherosclerosis. Because CD40L is expressed on activated platelets, we asked whether it plays a role in thrombosis and whether this role is dependent on CD40. Our initial observations showed that thrombi forming in injured arterioles in CD40L-deficient mice were highly unstable and that, in contrast, CD40-deficient mice had normal thrombus formation. David Phillips noted that CD40L has a KGD sequence, a sequence known to bind to the major platelet integrin αIIbβ3. His laboratory provided us with recombinant forms of sCD40L. Whereas the wild-type version of the protein could, on infusion, restore stability of CD40L-deficient thrombi, the protein mutated in the KGD sequence could not. Binding studies confirmed that indeed, sCD40L binds to the platelet integrin via the KGD sequence and showed that CD40L triggers “outside-in” signaling in the platelet by inducing αIIbβ3 cytoplasmic domain phosphorylation.

In conclusion, there are many links between the processes of thrombosis and inflammation (Figure). In nature, hemostatic and inflammatory responses often occur together as, for example, a reaction to an animal bite that produces bleeding and infection. Therefore, it is not surprising that some of the first response mechanisms, such as secretion of Weibel–Palade bodies, are shared in thrombosis and inflammation. In addition, it is apparent that molecules involved in these defense mechanisms, such as P-selectin and CD40L, may have independent functions in both pathways. The physiological processes of thrombosis and inflammation should not be viewed in isolation because they greatly influence each other, and the more we continue to scrutinize them, the more interconnections we are likely to find.

**Acknowledgments**

The work from our laboratory was funded by grants R37HL41002, R01 HL53756, and P01 HL56949 from the National Heart, Lung, and Blood Institute of the National Institutes of Health. I would like to thank Jaime Lazarte and Lesley Cowan for help with preparation of this manuscript.

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