Editorial

Fifty (or More) Ways to Leave Your Platelets (in a Thrombus)

Lawrence Brass

With a nod to Paul Simon, there may be more ways to activate platelets than there were ways to leave your lover in his song from the 70’s—or at least it seems that way. Gas6 is a vitamin K–dependent, protein-S–related protein found in the α-granules of resting platelets. Once platelet activation has occurred, Gas-6 appears in the fluid phase and on the platelet surface, where it presumably becomes bound after secretion. In this issue of Arteriosclerosis, Thrombosis, and Vascular Biology, Chen et al show that platelets express at least one of the known receptors for Gas-6, the receptor tyrosine kinase, Mer. According to their observations, deletion of the gene encoding Mer in mice does not produce an overt bleeding phenotype or even prolong the bleeding time, but it does inhibit platelet aggregation, diminish the thrombotic response to vascular injury, and provide some protection against disseminated thrombosis. These effects are less profound, but qualitatively similar to those reported several years ago by Angelillo-Scherrer et al in their studies on mice that lack Gas6, and essentially similar to those they have subsequently reported in abstract form using mice that lack either Mer or two related receptors, Axl and Rse (also known as Sky).3

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Gas6 is a 75-kDa protein with several distinct structural domains, starting with an N-terminal domain that includes γ-carboxylated glutamic acid (Gla) residues, a central domain with epidermal growth factor (EGF) repeats and two C-terminal G domains responsible for receptor binding and activation.4,5 Gla residues are glutamic acid residues that have undergone vitamin K–dependent γ-carboxylation, allowing them to support Ca++-dependent binding to phosphatidylserine. Like the Mer knockout, the Gas6 knockout causes decreased secretion and impairs platelet aggregation, particularly at low agonist concentrations. It also provides some degree of protection in three widely-used models of thrombosis in mice: disseminated thrombosis after the intravenous injection of collagen plus epinephrine, vascular occlusion after oxidative injury to the carotid artery, and localized thrombosis caused by venous stasis. Conversely, antibodies to Gas6 inhibit platelet aggregation and improve survival when collagen and epinephrine are injected.2

Thus, Gas6 contributes to platelet activation in vitro and in vivo, and its effects appear to be mediated by one or more members of the Axl/Rse/Mer family of receptor tyrosine kinases. Although interesting in their own right, these observations leave lots of questions unanswered. How does Gas6 affect platelet responses to other agonists? Are its effects mediated solely by the Axl/Sky/Mer family? Which downstream signaling events are involved and are they the same for each of the receptor family members? Does the Gla domain of Gas6 support its binding to the surface of activated platelets? If so, what is the role of surface-bound, as opposed to soluble, Gas6? Are activated platelets the main source of any Gas6 found in the circulation and, if so, what effect does platelet-derived Gas6 have on the biology of other cells that express Axl family members, including endothelial cells and leukocytes?

An equally intriguing question from the clinical standpoint is whether Gas6 function is impaired in patients taking warfarin. Gas6, like coagulation factors II, VII, IX, and X, is a protein that undergoes vitamin K–dependent γ-carboxylation to produce the Gla residues normally found in the Gas6 N-terminal domain. This processing is greatly inhibited or abolished in patients taking warfarin. Impaired Gla formation would presumably interfere with the ability of Gas6 to bind to acidic phospholipids on the surface of activated platelets. Even though Gas6 lacking the Gla domain was previously reported to bind to and activate Rse,6 Nakano et al found that Gla-deficient recombinant Gas6 lost most of its ability to stimulate rat aortic vascular smooth muscle cell (VSMC) proliferation when compared with the properly processed intact protein.7 It also bound less well to cells expressing the receptor. If platelet-derived Gas6 is similarly affected in patients on warfarin, then warfarin might inhibit platelet function, contributing to its antithrombotic effects. Assuming, of course, that the contribution of Gas6 to the aggregation of human platelets in vivo is as great as it seems to be in the mouse knockout studies. To my knowledge, this is still an open issue. Recent evidence suggests that Gas6 contributes to mammalian biology in ways that extend beyond hemostasis. If so, then the potential effects of impaired γ-carboxylation or impaired receptor:ligand interactions will have to be considered in each of them.

So far, these questions have focused on Gas6, but for the non-aficionado the discovery of yet another platelet agonist and yet another knockout that can impair platelet plug formation inevitably leads to the question: why are there so many ways to activate platelets? According to current models, platelet activation is most critical to hemostasis in the arterial circulation, as opposed to the low pressure, stasis-prone venous circulation. Platelets become activated at sites of arterial injury because of the local exposure of collagen plus von Willebrand factor (vWF) within the vessel wall or...
the local generation of thrombin. Collagen exposure is sufficient to form a monolayer of activated platelets, but the formation of a true platelet plug requires a second phase in which additional platelets are recruited and platelet:platelet contacts are formed, initially mediated by the binding of multivalent fibrinogen and vWF molecules to the integrin αIIbβ3 on the platelet surface. This recruitment phase of thrombus formation is accomplished by soluble agonists that are either secreted from platelet dense granules (ADP), synthesized and released as a consequence of platelet activation (TXA2), or generated locally in response to tissue factor exposure (thrombin). Each of these activates a specific class of G protein–coupled receptors on the platelet surface. These receptors share certain characteristics. In general they are rapid response elements that trigger an increase in the cytosolic Ca++ concentration and activate circulating platelets before they move downstream from the point of vascular damage. The critical role of G protein–dependent signaling is reflected by the overt hemorrhagic phenotype and prolonged bleeding time seen in mice that lack the G protein, Giα, the G protein that couples most G protein–coupled receptors to phospholipase C.

How do Gas6 and its receptors fit into this picture? Here there are fewer certainties and more speculation is required. Gas6 doesn’t become available until after α-granule secretion occurs. It is therefore unlikely to play a big role in the initial events of platelet activation. It may, however, help to prolong platelet activation and thereby contribute to thrombus stability. Signals mediated by Gq-coupled receptors in platelets are short-lived, generated quickly but fading away as receptors desensitize, G proteins return to the inactive state, and intracellular Ca++ stores are depleted. Signals generated by Gas6 and other activators of tyrosine kinase-based signaling in platelets start slower, but may last longer. Downstream effectors in these pathways include phosphatidylinositol 3 (PI 3)-kinase and Akt, which can also be activated via members of the Gi family of G proteins. Pharmacological inhibition of PI 3-kinase(s) and loss of PI 3-kinase or Akt expression impair platelet aggregation in ways suggestive of lost stability, producing a phenotype in the aggregometer in which aggregation begins but ends prematurely, especially at low agonist concentrations.

Viewing Gas6 and its receptors in the context of platelet contacts and thrombus stability may, in fact, be helpful to
understanding their contribution. Resting platelets, like other blood cells, do not ordinarily form stable contacts. However, such contacts form once platelets are activated. A variety of observations suggest that the contacts are sites of essential activity, although you might not know this from electron micrographs. These show a fairly bland interface with the gap between platelets estimated to be 10 to 50 nm. The junctional complexes that form between epithelial cells are not evident. However, the short distance between platelets would be expected to permit proteins on one platelet to interact with those on an adjacent platelet. One well-described example is the binding of α5β3 on opposed platelets to fibrinogen or vWF. The integrin serves as both a cell adhesion molecule and a receptor, generating “outside-in” signals through the indirect association of its cytoplasmic domain with intracellular molecules such as PI 3-kinase. Mutation of two tyrosine residues in the β3 cytoplasmic domain produces a pattern of abortive responses reminiscent of those seen with platelets from the Gas6 and Mer knockouts. Extending the parallels between integrin signaling and Gas6/Mer signaling even further, Gas6 has been shown to cause the phosphorylation of the PI 3-kinase substrate, Akt. Other molecules active at the interface between platelets include members of the JAM (Junctional Adhesion Molecule) family and Eph kinase families (EphA4 and EphB1). Like inhibition of Gas6 and integrin signaling, blockade of Eph kinase activation by the ligand, ephrinB1, produces a pattern of reversible platelet aggregation.

Finally, in addition to enabling direct protein interactions, the narrow gap between adjacent platelets in a growing thrombus would be expected to favor the local accumulation of agonists. This would include not only secreted proteins, such as Gas6, but also proteins that are shed from the platelet surface through the action of secreted metalloproteases. Among these are CD40 ligand (CD40L), whose presence in the circulation correlates with significant cardiovascular disease and the semaphorin, CD100, both of which can interact with receptors expressed on platelets. Thus a model emerges in which platelet agonists include not only those that are capable of initiating platelet activation and thrombus formation, but also ones that foster the continued growth and stability of the thrombus. Ultimately, this may be the role of Gas6. As such, Gas6 and other molecules that participate in the later phases of platelet activation may prove to be ideal targets for the development of new antiplatelet therapeutics. After all, what could be better than to interfere with pathological thrombosis while leaving hemostasis intact?

References
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