**Novel Therapeutic Targets at the Platelet Vascular Interface**

Lawrence F. Brass, Li Zhu, Timothy J. Stalker

**Abstract**—Platelet activation in vivo can be part of the hemostatic response to injury or a pathological response to disease. In either setting, platelets adhere to the vessel wall and to each other, forming a closely packed mass interspersed with fibrin. Recent studies have identified new molecules on the platelet surface and within platelets that support and regulate thrombus growth and stability, ensuring that platelet accumulation after injury is sufficient to stop bleeding, but not so exuberant that vascular occlusion occurs. An understanding of how this balance is achieved helps to illuminate the events of platelet activation and, at the same time, provides potential targets for new classes of antiplatelet agents. *(Arterioscler Thromb Vasc Biol. 2008;28:s43–s50)*

**Key Words:** platelets ▪ cell adhesion molecules ▪ integrins ▪ semaphorins ▪ ephrins ▪ Eph kinases ▪ vascular biology

This article is part of a multi-part CME-certified activity titled Translational Therapeutics at the Platelet Vascular Interface. In order to achieve all of the activity’s learning objectives, please read all of the components of the activity listed in the Table of Contents and follow the “Instructions for Participation and Obtaining CME Credit” outlined prior to the Introduction.

The broad outlines of platelet activation are familiar. Platelets evolved as an efficient means to respond to injuries that produce holes in a high pressure, closed circulatory system and, to a great extent, platelet attributes reflect the demands placed on them. To be maximally useful and minimally harmful, circulating platelets must be able to sustain repeated collisions with the normal vessel wall without premature activation, recognize the distinguishing features of a damaged wall, cease forward movement on recognition of damage, adhere to collagen despite continued blood flow, and acquire the ability to stick to each other, forming a stable plug of the correct size. Viewed in these terms, pathological thrombus formation occurs when diseases or drugs subvert the mechanisms that are designed to prevent unwarranted platelet activation, producing a local accumulation of platelets where none was needed or wanted.

In the setting of vascular injury, platelets are activated by collagen and thrombin. Polymerized collagen fibrils that are present within the vessel wall as a complex with von Willebrand factor (vWF) become exposed to the circulation when the overlying endothelial cell monolayer is breached. Platelets tumbling on the periphery of the moving blood stream are captured when glycoprotein (GP) Ib on the platelet surface binds to the VWF A1 domain, establishing contacts that slow the forward motion of the platelet long enough for platelet activation to occur via signaling events downstream of receptors for collagen (GPs VI and Ib), thrombin (PAR1 and PAR4), and adenosine diphosphate (ADP) (P2Y1 and P2Y12).

In general terms, the formation of a stable platelet plug can be thought of as occurring in 3 stages: initiation, extension, and perpetuation. Initiation by collagen or thrombin produces a platelet monolayer that supports further thrombin generation and the subsequent adhesion of activated platelets to each other. Extension occurs when additional platelets are recruited and activated, sticking to each other and accumulating on top of the initial monolayer. Thrombin, ADP, and thromboxane A2 (TXA2) play an important role in recruitment, activating platelets via G protein–coupled receptors. Signaling downstream of these receptors activates $\alpha_{\text{IIb}\beta_3}$, allowing the integrin to serve as a cell adhesion molecule and making cohesive interactions between platelets possible. Perpetuation refers to the later events of platelet plug formation that help to stabilize the platelet plug and prevent premature disaggregation. Examples include outside-in signaling through integrins and other cell adhesion molecules and signaling through receptors whose ligands are located on the surface of adjacent platelets. The net result is a hemostatic plug or thrombus composed of activated platelets embedded within a cross-linked fibrin mesh, a structure sturdy enough to withstand the shear forces generated by flowing blood in arterial circulation.

For the most part, this description has withstood the test of time, evolving as new molecules are shown to be participants...
in or regulators of hemostatic plug formation. However, a number of essential questions remain unanswered. For example, how is the magnitude of the thrombotic response calibrated so that neither too few nor too many platelets accumulate in response to injury? How is an inadequate response avoided so that rebleeding does not occur? Conversely, what limits the response so that vascular occlusion and subsequent tissue ischemia are avoided? What happens after $\alpha_{IIb}\beta_3$ is activated and platelets adhere to each other? Are interactions required between platelets beyond those mediated by $\alpha_{IIb}\beta_3$?

This review addresses some of these issues, emphasizing recent discoveries of novel contact-dependent and contact-facilitated interactions that affect thrombus growth and stability and, as a result, help to optimize the growth of the platelet plug (Figure 1).

**The Platelet Synapse**

In contrast to many other types of cells, platelets are not normally in stable contact with each other, but develop such contacts once platelet activation has begun. Electron micrographs of aggregated platelets show the close proximity of the plasma membranes of adjacent platelets. Estimates for the width of the gap between adjacent platelets range from as little as zero to as much as 50 nm. In theory, the short distance between platelets makes it possible for molecules on the surface of one platelet to bind to molecules on an adjacent platelet and for information to be passed, much like a neurological or immunologic synapse. This could be a direct interaction, as when one cell adhesion molecule binds to another in trans, or an indirect interaction, such as occurs when multivalent adhesive proteins link activated $\alpha_{IIb}\beta_3$ on adjacent platelets. In either case, these interactions can theoretically provide both an adhesive force and a secondary source of intracellular signaling. Junctional molecules have been detected in platelets, as have some of the molecules associated with junction formation in other types of cells, but it has not been established that contacts between platelets mimic the tight and adherens junctions found, for example, between endothelial and epithelial cells. Nonetheless, there is increasing evidence that biologically meaningful interactions do occur between adjacent platelets and that some platelet surface molecules accumulate at sites of contact (Figure 2).

In concept, close contacts between platelets not only allow platelet-platelet interactions to occur, but can also limit the diffusion of plasma molecules into the gaps between platelets and prevent the escape of platelet activators from the gaps. This might, for example, limit the access of plasmin to embedded fibrin, thereby helping to prevent premature dissolution of the hemostatic plug. It might also foster the accumulation of platelet activators within a protected environment in which higher concentrations can be reached and maintained. Seen in this context, clot retraction, a long-described phenomenon whose role has remained elusive, can be viewed as a mechanism for narrowing the gaps between platelets and increasing the local concentration of soluble ligands within the gaps. Clot retraction is dependent on the interaction between actin/myosin complexes and the cytoplasmic domain of $\alpha_{IIb}\beta_3$.

Any list of molecules that participate in contact-dependent and contact-facilitated interactions between platelets is likely to be incomplete, but a number of participants and potential participants have been identified. Those that will be discussed here include integrins and other cell adhesion molecules, direct interactions between receptors and surface-attached ligands, and bioactive molecules that are either secreted or proteolytically shed from the surface of activated platelets (Figure 2). Some of these molecules also play a role in the interaction of platelets with other types of cells, but the focus of this review will be on platelet-platelet interactions.

**Integrins**

There are approximately 80,000 copies of $\alpha_{IIb}\beta_3$ on the surface of platelets and, when bound to fibrinogen, fibrin, or vWF, the activated integrin provides the dominant cohesive strength that holds platelet aggregates together. $\alpha_{IIb}\beta_3$ distributes across the surface of resting platelets, but tends to cluster at sites between activated platelets (Figure 2). The term “inside-out signaling” has been applied to the events that lead to integrin activation. “Outside-in signaling” refers to the events that occur downstream of activated integrins once ligand binding has occurred.

![Figure 1. Optimizing the response to injury. Platelet and fibrin accumulation after vascular injury is intended to limit further blood loss without compounding the original injury by occluding blood flow. In this context, the optimal hemostatic response is one that is large enough to be stable, but not so large that it occludes the vascular lumen, producing downstream ischemia and further tissue damage. Recent studies show that optimization is an active process, involving proteins on the platelet surface as well as regulatory molecules within the platelet.](image-url)
large part on the formation of protein complexes that link to the integrin cytoplasmic domain. Some of the protein-protein interactions that involve the cytoplasmic domains of αIIbβ3 help regulate integrin activation; others participate in outside-in signaling and clot retraction. Talin binding is thought to be one of the critical final events in integrin activation. Some interactions require the phosphorylation of tyrosine residues Y773 and Y785 (Y747 and Y759 in mice) in the αIIbβ3 cytoplasmic domain by tyrosine kinases in the Src family. Substitution of phenylalanine for these tyrosines produces mice whose platelets tend to disaggregate and that show impaired clot retraction and a tendency to rebleed from tail bleeding-time sites. Fibrinogen binding to the extracellular domain of activated αIIbβ3 stimulates a rapid increase in the activity of Src family members and Syk. Studies of platelets from mice lacking these kinases suggest that these events are required for the initiation of outside-in signaling and for full platelet spreading, irreversible aggregation, and clot retraction. Loss of clot retraction is also a hallmark of αIIbβ3-deficient platelets in patients with Glanzmann thrombasthenia, a bleeding disorder in which activated platelets fail to stick to each other.

**PECAM-1**

Integrins are not the only molecules found at the interface between platelets. Some have been known about for some time but have newly assigned functions in platelets that appear to counter original ideas about their roles. A better way to view them at this point is as platelet surface molecules that can enter into homophilic or heterophilic interactions in trans that modulate the growth and stability of platelet plugs. The net effect can be positive or negative. One example is platelet endothelial cell adhesion molecule-1 (PECAM-1; CD31). PECAM-1 is a type-1 transmembrane protein with 6 extracellular immunoglobulin (Ig) domains. The most membrane-distal Ig domain is able to support homotypic interactions in trans. The C terminus contains phosphorylatable tyrosine residues capable of binding the tyrosine phosphatase, SHP-2. Loss of PECAM-1 expression causes increased responsiveness to collagen in vitro and increased

---

**Figure 2.** The platelet synapse. Activated platelets come into close contact, producing a network of cell junctions where surface molecules on adjoining platelets can interact and gaps where locally secreted and shed molecules can accumulate. The left part of the figure shows human platelets that have been activated with the PAR1 agonist, SFLLRN, and then allowed to spread on a fibrinogen-coated surface before being stained with an antibody to the β subunit of the integrin αIIbβ3. Antibody staining is enriched at sites of platelet-platelet contact and relatively diminished, but not absent, elsewhere on the platelet surface. The right part of the figure is a conceptual view of the platelet synapse and illustrates many of the molecules and cell-cell interactions that are discussed in the text.
thrombus in vivo. The data are consistent with a model in which PECAM-1 brings SHP-2 near its substrates, including the GP VI signaling complex.\textsuperscript{11–13} This suggests that PECAM-1 provides a braking effect on collagen signaling and thereby helps to prevent either unwarranted platelet activation or overly exuberant growth of platelet thrombi that might otherwise occlude the vessel lumen and cause ischemia.

**CTX Family Members**

The repertoire of molecules that can potentially engage between platelets also includes 4 members of the CTX family (junctional adhesion molecule [JAM]-A, JAM-C, endothelial cell–selective adhesion molecule [ESAM], and CD226), each of which has an extracellular domain with 2 Ig-like domains, a single transmembrane region, and a cytoplasmic tail that terminates in a PDZ domain binding site. PDZ domains are structural features found in numerous cytosolic proteins that support protein–protein interactions. The length of the cytoplasmic domain and the probable specificity of the PDZ ligand domain varies among the different members of the CTX family. JAM-A localizes to tight junctions of endothelial and epithelial cells and is also found on monocytes, neutrophils, and lymphocytes. JAM-C has been found on endothelial cells, lymphatic vessels, dendritic cells, and natural killer cells. JAM-A contributes to cell-cell adhesion by forming trans interactions involving the N-terminal Ig domain. However, JAMs also support heterotypic interactions, including binding to integrins via their membrane proximal Ig domain.\textsuperscript{14,15} JAM-A was originally described as the antigen for a platelet-activating antibody.\textsuperscript{16} JAM-A has also been shown to induce platelet adhesion and spreading via interactions with immobilized JAM-A in vitro, suggesting that it might play a role in homotypic platelet-platelet and platelet-endothelial interactions. It also appears to mediate heterophilic interactions between platelets and leukocytes by binding to \(\alpha_{\beta_2}\).\textsuperscript{14,17} Similarly, JAM-C was found to bind \(\alpha_\text{M}\beta_2\) integrin on leukocytes, suggesting a role for JAM-C in platelet-leukocyte interactions.\textsuperscript{15}

Two other CTX family members expressed on platelets are ESAM and CD226.\textsuperscript{18–20} The ESAM cytoplasmic domain is longer than the corresponding domain of JAM-A or JAM-C, and contains proline-rich regions as well as a C-terminal PDZ ligand domain. ESAM was first identified as a cell adhesion molecule in endothelial cells, where it has been shown to bind via homotypic interactions, colocalize with tight junction proteins, and bind to the PDZ domain–containing adaptor protein MAGI-1.\textsuperscript{21} ESAM expression on the platelet surface increases when platelets are activated. Our recent studies show that ESAM localizes to the junctions between activated platelets, and loss of ESAM expression in ESAM knockout mice increases platelet aggregation in response to multiple agonists and promotes thrombus growth in vivo after laser-induced vascular injury.\textsuperscript{22} This suggests that ESAM, like PECAM-1, serves a restraining role on thrombus growth and stability. Preliminary studies suggest that this may also be true for JAM-A.\textsuperscript{23} CD226 has been shown to participate in the binding of activated platelets to endothelial cells in vitro.\textsuperscript{24} Endothelial cells express nectin-2, so it is possible that it serves as a ligand for platelet CD226 as well. It is unknown at this time whether platelets express nectins, or whether CD226 also mediates platelet-platelet adhesion. It is unknown whether CD226 localizes to platelet junctions when activated platelets come into contact with each other. A recent brief report on CD226\textsuperscript{1−}\textsuperscript{2} mice describes a mild impairment in megakaryocyte differentiation.\textsuperscript{25} There are no reports yet on platelet function in these mice.

**CD2 Family Members**

Signaling lymphocytic activation molecule (SLAM; CD150) and CD84 are members of the CD2 family of homophilic adhesion molecules that have been studied extensively in lymphocytes, but have now been shown to be expressed in platelets as well.\textsuperscript{26–28} The members of the family are type-I membrane GPs in the Ig superfamily. Notable differences among them are in the cytoplasmic domain, which supports binding interactions with a variety of adaptor/partner proteins. SLAM and CD84 are expressed on the surface of resting as well as activated platelets and become tyrosine phosphorylated during platelet activation, but only if aggregation is allowed to occur. Immobilized CD84 causes platelet activation in vitro. Mice that lack SLAM have a defect in platelet aggregation in response to collagen or a PAR4-activating peptide, but a normal response to ADP and a normal bleeding time.\textsuperscript{29} In a mesenteric vascular injury model, female SLAM\textsuperscript{−−} mice showed a marked decrease in platelet accumulation. Male mice were normal.\textsuperscript{26} The presence of SLAM, CD84, and 2 of their known adaptor proteins (SAP and EAT-2) in platelets provides a novel mechanism by which close contacts between platelets can help to support thrombus stability.

**Receptor-Ligand Interactions at the Junctions Between Platelets**

Direct contacts between platelets can promote signaling by more than one mechanism. In addition to signaling events that occur downstream from cell adhesion molecules, there are cell surface receptors that interact in trans with cell surface ligands (Figure 2). One example is the family of Eph receptor tyrosine kinases and their ligands, known as ephrins. Ephrins are cell surface proteins with either a glycosphatidylinositol (GPI) anchor (the ephrin A family) or a transmembrane domain (the ephrin B family). The cytoplasmic domains of the ephrin B family members include phosphorylatable tyrosine residues and a PDZ target domain. Contact between an ephrin-expressing cell and an Eph-expressing cell causes signaling in both cells, a phenomenon known as bidirectional signaling. Eph kinases and ephrins are best known for their role in neuronal organization and brain development, and as markers distinguishing arteries from veins during vasculogenesis. We have found that human platelets express EphA4, EphB1, and ephrinB1\textsuperscript{29} and, more recently, ephrinA1 (Wu and Brass, unpublished observation, 2007). Clustering of ephrinB1 promotes platelet aggregation. Blockade of Eph/ephrin interactions inhibits platelet aggregation at low agonist concentrations and limits the growth of platelet thrombi on collagen-coated surfaces under arterial flow conditions. It also impairs \(\beta_3\) phosphorylation, which inhibits clot retrac-
tion by impairing the interaction of myosin with the β3 cytoplasmic domain. EphA4 colocalizes with αmβ2 at sites of contact between aggregated platelets. EphB1, ephrinB1, and ephrinA1 may do so as well, although this has not been established. Collectively, these observations suggest a model in which the onset of aggregation brings platelets into close proximity and allows ephrinB1 to bind to EphA4 and EphB1. 

Sema4D and Its Receptors

A second example of a cell surface ligand/receptor interaction made possible by close contacts between platelets is the binding of the ligand sema4D to its receptors, CD72 and plexin-B1. Semaphorins are a large family of structurally related proteins distinguished in part by whether they are secreted, held to the cell surface by a GPI anchor, or have an integral transmembrane domain. Like Eph kinases and ephrins, semaphorins are best known for their role in the central nervous system, but individual family members have been found elsewhere. Semaphorins have in common a 500 amino acid residue extracellular “sema” domain that forms a 7-bladed propeller structure. Sema4D (or CD100) is a 150-kDa type I membrane GP that forms a disulfide-linked homodimer. It was originally studied in the context of lymphocytes where it is expressed on T cells and, to a lesser extent, B cells. Sema4D(−/−) mice are viable, but show defective B-cell development, impaired T-cell activation, and blunted immune responses. Our recent studies show that platelets express sema4D and that platelets from sema4D(−/−) mice have impaired responses to collagen with a rightward shift in the dose/response curve for platelet aggregation and Akt activation. Studies performed in vivo show that the time to occlusion is prolonged when the carotid artery is exposed to FeCl3, and that Rose Bengal injury of cremaster muscle arterioles causes stable occlusion within 4 minutes in approximately half of matched wild-type mice, but none of the sema4D(−/−) mice. We have also observed a reduction in infarct size in sema4D(−/−) mice after temporary occlusion of the left anterior descending coronary artery.

Our current working model is that the effects of sema4D in platelets are mediated at least in part by CD72 and plexin-B1, both of which are present on human platelets. Plexin-B1 is a high affinity sema4D receptor previously shown to be expressed on endothelial cells. CD72 is a 45-kDa immunoreceptor tyrosine-based inhibitory motif (ITIM) domain-containing type II transmembrane protein that has been shown to affect intracellular signaling in B cells through its ability to associate with the tyrosine phosphatase SHP-1 and the adaptor protein Grb2. Plexin-B1 has a type-I PDZ ligand domain at its C terminus, enabling it to interact with cytosolic proteins that have 1 or more PDZ domains. In endothelial cells, plexin-B1 is coupled to Rho- and Akt-dependent signaling pathways and there is some evidence that this is the case in platelets as well. There is also evidence that plexin-B1 can form signaling complexes with the receptor tyrosine kinase, Met. The plexin-B1 knockout mouse was recently reported. The mice appear to develop normally. Studies on their platelets are pending. Although much more remains to be learned, the evidence available so far suggests that sema4D plays a previously unsuspected role in supporting thrombus growth and stability. After platelet activation is well on its way, sema4D is gradually shed from the platelet surface, a point that will be discussed in the next section.

Is the Gap Empty?

The gaps between aggregated platelets potentially provide a safe harbor in which platelet-derived molecules can accumulate. In addition to the proteins that are secreted from platelet α-granules, activated platelets release agonists such as ADP and TxA2 and presumably continue to do so even after thrombus formation has begun. Platelets also shed surface molecules, including GP Ibα, GP V, GP VI, P-selectin, CD40 ligand (CD40L) (see below), and sema4D. This is a regulated process, not a random event. Cleavage of each of these proteins can be prevented with inhibitors of metalloproteases and in the case of GP Ibα, GP V, and sema4D, a role for a particular metalloprotease, ADAM17, has been established through studies on platelets from mice that lack it. The advantage that the platelet derives from shedding surface proteins can be surmised, but is not always entirely clear. Downregulation of responsiveness to collagen has been proposed as the benefit derived from shedding GP VI. Loss of the GP Ibα exodomain, which serves as the primary vWF receptor on platelets, would also impair platelet interactions with collagen, but because cleavage occurs after platelet activation, the impact on thrombus prevention is not readily apparent. It is possible that the reduction in net surface charge that would accompany loss of the negatively charged GP Ibα exodomain allows platelets to pack more closely together in a thrombus. It may also expose interaction sites on the remaining extracellular portion of GP Ibα that are occluded in the intact molecule, but this remains entirely speculative. In contrast to glycoproteins Ib and VI, where cleavage of the exodomain may predominantly serve to impair function, cleavage of CD40L and sema4D gives rise to bioactive fragments that can stimulate platelets as well as other nearby cells.

CD40 Ligand

The CD40 ligand (CD40L; CD154) is a 33-kDa transmembrane protein that is present on the surface of activated platelets, but not resting platelets. Its appearance on the platelet surface is followed by the gradual release of an 18-kDa exodomain fragment of CD40L. Both the surface-bound and the soluble form of CD40L (sCD40L) are trimers. Platelets also express CD40, the 48-kDa transmembrane protein that acts as a receptor for CD40L. Unlike CD40L, CD40 is detectable on the surface of resting as well as activated platelets. CD40L is a member of the tumor necrosis factor (TNF) family and platelet-derived CD40L or activated CD40L-expressing platelets can elicit responses from endothelial cells and monocytes that appear to be proatherogenic. The extracellular portion of CD40L includes a binding domain for CD40 and a KGD (RGD in
mice) integrin-recognition sequence. The binding of sCD40L to activated platelets is blocked by mutations in the KGD sequence and by antibodies to \( \alpha_{IIb} \beta_{3} \). Binding occurs to platelets that lack CD40, but not to platelets that lack \( \beta_{3} \). Consistent with a role in thrombus formation, CD40(L\(^{-/-}\)) mice show delayed occlusion after vascular injury and decreased thrombus stability.\(^{53}\) Loss of CD40 had no apparent effect.\(^{53}\) Notably, platelet aggregation is normal in CD40L\(^{-/-}\) platelets, although growth of platelet aggregates on collagen-coated surfaces under shear stress is affected.\(^{53,58}\) Taken together, these results suggest that the effects of CD40L on platelets are mediated by \( \alpha_{IIb} \beta_{3} \), not CD40, and that platelet behavior in the relatively low shear conditions present in an aggregometer cuvette may not be fully reflective of the events that are relevant to thrombus stability in vivo.

**Sema4D Shedding**

A second example of a biologically active molecule that is shed from the surface of activated platelets is sema4D. Sema4D has already been mentioned as the platelet surface ligand for CD72 and plexin-B1 on nearby platelets. Time course studies show that there is progressive cleavage and shedding of the sema4D extracellular domain once platelet activation has occurred and irrespective of the agonist used to activate the platelets.\(^{54}\) The rate at which sema4D is shed from activated platelets, like the rate of CD40L shedding, lags well behind the rate of platelet aggregation, requiring 30 to 60 minutes to reach completion. The exodomain can be retrieved from the fluid phase intact and continues to be a disulfide-linked dimer, leaving behind a fragment that includes the cytoplasmic domain, the transmembrane domain, and a small piece of the extracellular domain. Shedding is sensitive to metalloprotease inhibitors and fails to occur in mouse platelets that lack ADAM17 or in human platelets incubated with an ADAM17-selective inhibitor.\(^{34}\) This suggests that at least some of the shed sema4D exodomain should be protected in the gaps between platelets, whereas the rest is washed downstream where it can interact with plexin-B1 on endothelial cells (Figure 3).\(^{36,37,40,59}\)

An example of a ligand that is secreted from platelets rather than being shed from the platelet surface is growth-arrest specific gene 6 (Gas6). In rodent platelets, Gas6 is found in \( \alpha \)-granules.\(^{60-62}\) Secreted Gas6 is a ligand for the receptor tyrosine kinases Tyro3, Axl, and Mer, all of which are expressed on platelets. Because Tyro3 family members have been shown to stimulate PI 3-kinase and PLC\(\gamma\), a reasonable hypothesis is that secreted Gas6 can bind to its receptors on the platelet surface and cause signaling. Platelets from Gas6\(^{-/-}\) mice were found to have an aberrant response to agonists in which aggregation terminates prematurely.\(^{61}\) Furthermore, although the tail-bleeding times of the Gas6\(^{-/-}\) mice were normal, the mice were resistant to thrombosis,\(^{61}\) as are mice lacking any 1 of the 3 Gas6 receptors. Platelets from mice lacking Gas6 receptors also failed to aggregate normally in response to agonists.\(^{63-65}\) Biochemical studies showed that Gas6 signaling promotes \( \beta_{3} \) phosphorylation and, therefore, clot retraction.\(^{64}\) Collectively, these results are consistent with a role for secreted Gas6 in promoting integrin outside-in signaling and helping to perpetuate and stabilize platelet plug formation. Most granule secretion occurs after the onset of aggregation, and Gas6 secreted into the confined spaces between platelets might achieve high local concentrations and cause receptor activation.

**Conclusion**

There is now ample evidence that the signaling events that support platelet activation continue after integrin activation, granule secretion, and platelet aggregation have begun. These events rely in part on interactions *in trans* between platelet surface molecules and on the accumulation of bioactive molecules in the gap between adjacent platelets. Some can reasonably be expected to promote the growth and stability of the hemostatic plug, support clot retraction, and help to maintain the plug in place until wound healing is complete or at least well under way. Others may have the opposite effect, helping to place an upper limit on platelet accumulation and thrombus size. These events can take advantage of the close proximity between platelets once aggregation begins and may
even extend to the contacts that develop between platelets, endothelial cells, and leukocytes as the hemostatic plug evolves.

Sources of Funding
This work was supported by grants from the NIH Heart, Lung, and Blood Institute.

Disclosures
None.

References


