network Aβ partially blocks the binding of plasminogen to fibrin, thus interfering with fibrin's co-factor ability to co-localize plasminogen and tissue plasminogen activator on the fibrin fiber. The result is reduced plasmin generation. Zamolodchikov and Strickland further show that Aβ also delays the ability of preformed plasmin to degrade the clot, suggesting plasmin binding might also be reduced by Aβ. Hence, these studies suggest 3 independent mechanisms by which Aβ can alter fibrinolysis (see figure). Aβ binding to fibrin(ogen) (1) intercalates into fibrin fibers during formation, promoting the formation of clots with an abnormally dense fiber network; (2) blocks binding of plasminogen to fibrin and therefore fibrin's ability to support plasmin generation; and (3) blocks plasmin-mediated cleavage of fibrin, directly reducing the rate of fibrinolysis. These studies show Aβ can reduce fibrinolysis by associating with the fibrin network either during or after fibrin formation, suggesting that the introduction of Aβ at any point in fibrin's lifespan would have pathologic consequences.

These findings have important implications for AD research. The demonstrated interaction between Aβ and fibrin(ogen) reconciles the leading independent theories that AD results from deposition of Aβ or from other cardiovascular risk factors. Elevated circulating fibrinogen is an established risk factor for both CVD and AD.[7-9] Elevated fibrinogen promotes thrombosis in part via increased fibrin network density and increased resistance to lysis[10] in CVD and likely AD as well. The finding that in AD, increased fibrin stability can also result from interactions between Aβ and fibrin suggests fibrinogen can participate in multiple, independent pathways in the development of AD. Presumably, increased vascular permeability permits fibrin deposition in the cerebrovasculature and Aβ protects fibrin deposits from degradation, culminating in vascular obstruction and fibrin(ogen)-mediated inflammation in the brain. The current findings strongly support continued studies investigating the connection(s) between vascular dysfunction and fibrin(ogen) deposition. Moreover, blocking the binding of Aβ to fibrin by pharmacologically targeting Aβ may enable plasmin(ogen) binding to the network and permit endogenous fibrinolytic mechanisms to clear fibrin deposits. By not targeting the fibrinolytic pathway directly, this "indirect" approach may be expected to have low risk of bleeding.

These findings also have important implications for understanding the contributions of fibrinogen to hemostasis and thrombosis. Other proteins besides Aβ, including fibronectin (reviewed in Wolberg[3]), have been shown to both alter fibrin structure and decrease the rate of fibrinolysis. These two clot properties also co-exist in many thrombotic diseases. Until now, findings of abnormal fibrin network structure were considered sufficient to explain differences in fibrinolysis. However, the current study puts an end to this biologic handwaving because it shows that changes in fibrin network structure may not be sufficient to explain abnormal fibrinolysis in all cases. Could as-yet-unidentified molecules circulating in disease states directly alter binding of fibrinolytic enzymes to fibrin in other thrombotic diseases? Experiments to explicitly measure binding of tissue plasminogen activator and plasmin(ogen) to the fibrin network in cases of reduced fibrinolysis may reveal such novel molecules. As such, this work establishes a new standard for future studies of mechanisms regulating clot structure and stability.

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THROMBOSIS & HEMOSTASIS

Comment on Naik et al, page 3352

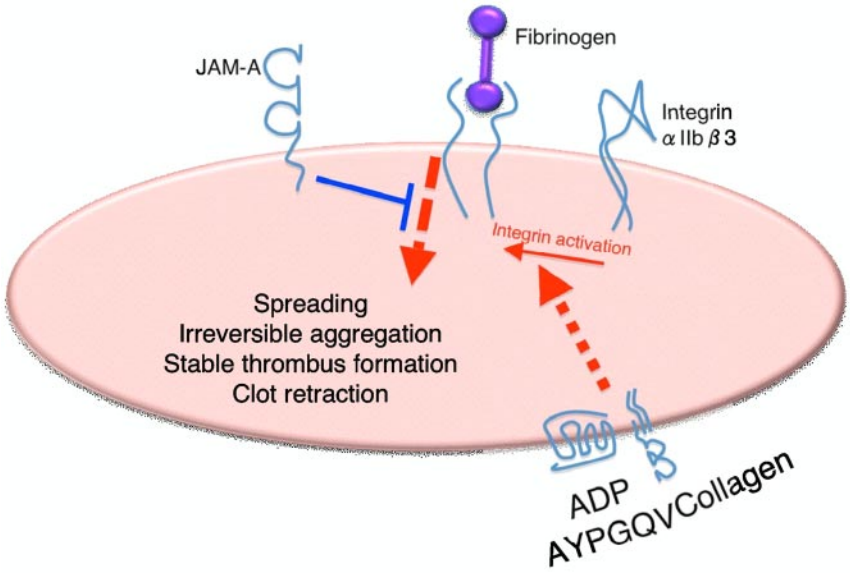
JAMming the signals

Jonathan M. Gibbins UNIVERSITY OF READING

In this issue of *Blood*, Naik and colleagues have identified a new mechanism used by platelets to inhibit the signals that drive their activation through integrin αIIbβ3, which serves to prevent inappropriate or premature thrombus formation.[1]

The activation of platelets at sites of injury or arterial disease is rapid because of the involvement of numerous positive feedback systems. Activated platelets release factors that activate approaching platelets, which similarly secrete activatory factors … and the cycle continues. This chain reaction, which is mediated by platelet agonists of various shapes and sizes, requires effective regulation to limit the extent of response to injury or to prevent accidental activation, which may lead to thrombosis. This is mediated through endogenous inhibitory mechanisms that are able to "turn down the gain" on platelet reactivity or to inhibit the signaling mechanisms used by platelet-stimulating factors such as collagen, thrombin, and ADP.

In recent years the repertoire of such mechanisms has started to become apparent, and these, too, come in various shapes and sizes. The inhibitory effects of prostacyclin (PGI_2) and nitric oxide (NO) that are released by the healthy endothelium are well established, accompanied by the ADP metabolizing



The activation of receptors for collagen, ADP, and AYPGQV (thrombin receptor PAR4 agonist peptide) results in the stimulation of signaling pathways that converge on the activation of the integrin αIIbβ3, its conversion from a low to a high ligand affinity binding state (inside-out integrin signaling). This enables the binding of fibrinogen to the integrin, which stimulates outside-in signaling through the receptor, which is required for platelet spreading, irreversible platelet aggregation, stable thrombus formation, and clot retraction. The absence of JAM-A results in augmented outside-in signaling and associated functional responses. This suggests that JAM-A serves to suppress inappropriate or premature platelet activation through increasing the threshold of activation signals required to trigger platelet function.

activity of CD39 on the healthy endothelial cell surface.[2] Additional soluble endothelium-derived platelet inhibitors such as semaphorin 3A have also been reported.[2] Attention has recently turned to platelet cell adhesion receptors, such as PE CAM-1, G6b, and CEACAM1 that are implicated in limiting the platelet response in scenarios where platelet adhesion occurs, employing inhibitory immune receptor signaling mechanisms of action.[2]

Here, Naik et al add to this repertoire, through the identification of JAM-A as an endogenous inhibitor of platelet activation (see figure).[1] JAM-A is a member of the *Xenopus* (CTX) family of transmembrane cell adhesion molecules that is found within tight junctions between epithelial or endothelial cells, and is also present on the surface of leukocytes and platelets. It is important for the assembly of tight junctions and thereby epithelial barrier function, and is believed to engage in homophilic ligand interactions with JAM-A on opposing cells.[3] Of importance in the study from the Naik laboratory are several lines of evidence to support the involvement of this protein in the regulation of integrin function.[3,4]

Initial experiments to explore the role of JAM-A on platelets suggested that it may have activatory ambitions, although this was found to be because of antibody-mediated cross-linking with the IgG receptor FcγRIIA.[5] Naik et al therefore investigated the function of JAM-A deficient mouse platelets.[1] They found evidence of exaggerated platelet responses in vivo, with decreased bleeding and increased thrombotic responses using a range of experimental approaches. Platelet aggregation to thrombin receptor (PAR4) agonist, ADP, and collagen were enhanced, suggesting a defect in a pathway or feature that is common to each of these. Inside-out signaling to regulate integrin αIIbβ3 affinity and therefore fibrinogen binding was normal, but functions that occur after fibrinogen binding to the integrin, such as spreading and clot retraction, were augmented in platelets lacking JAM-A. This enhanced function was accompanied by increased outside-in signaling through the integrin.

This represents a new mode of platelet inhibitory regulation controlled by JAM-A that is distinct from PECAM-1, G6b, and CEACAM1, which signal through immunoreceptor tyrosine-based inhibitory motifs (ITIMs) to modulate early aspects of activatory platelet signaling,[6-8] and for PECAM-1 to enhance outside-in signaling through integrin αIIbβ3.[9] Similarities are seen, however, with endothelial cell–specific adhesion molecule (ESAM), a CTX family member that also lacks an ITIM. ESAM deficiency results in elevated platelet and thrombotic responses, accompanied by normal inside-out signaling.[10] It is possible therefore, that both CTX family members represent a new cell adhesion–dependent paradigm for platelet regulation. There are, however, inconsistencies in their modes of action because JAM-A deficiency causes enhanced clot retraction and platelet spreading, whereas ESAM deficiency does not.

Further questions remain to be answered to understand where and when platelet JAM-A function might be important. Does JAM-A serve to modulate premature platelet activation, as suggested by Naik and colleagues, or does it come into play on platelet-platelet contact when localized to the points of platelet contact and thereby limit thrombus growth? What does JAM-A bring to the integrin? A phosphatase? To begin to tease these complex questions apart, a priority will be to understand the molecular mechanisms that allow JAM-A to jam integrin αIIbβ3 outside-in signals.

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