Commentary

A new ''kid'' on the platelet thrombin receptor ''block'': Glycoprotein Ib–IX–V

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If you cut yourself, your hemostatic system will recruit circulating platelets and plasma fibrinogen/fibrin to staunch the loss of blood. These two arms of hemostasis combine to produce the hemostatic plug, comprised of aggregated platelets and deposited fibrin. ''Plug'' formation is regulated in terms of time, place, and extent so as to minimize bleeding from injured arteries/veins, while avoiding any obstruction of nearby uninjured vessels. Such finely tuned regulation entails crosstalk between the platelet and the fibrin pathways, and nature has chosen the plasma protease, thrombin, acting on platelet thrombin receptor(s) as the vital link between the two systems. This linkage is the topic of studies by Ramakrishnan and colleagues (1) in this issue of PNAS, who have applied ''knockout-mouse'' technology to identify a heretofore unappreciated platelet thrombin receptor, glycoprotein (GP) Ib–IX–V (1).

Thrombin is the major product of the plasma coagulation "cascade" of sequential "zymogen-to-protease" steps. As the culmination of a series of proteolytic cleavages, thrombin converts fibrinogen to fibrin, which deposits at sites of bleeding or thrombosis as the fibrinous portion of a hemostatic plug or a thrombotic mass. However, thrombin plays an alternate role in platelet function as a powerful agonist for platelet activation and aggregation. For decades, despite much effort and clear evidence that thrombin *both* bound to and stimulated the platelet, no thrombin receptor could be found. Then, a decade ago, Coughlin and colleagues (2, 3) identified a unique seven-transmembrane domain, G protein-coupled receptor for thrombin, the first member (PAR-1) of the current family of four protease activated receptors. As the PAR name implies, these receptors undergo proteolytic cleavage by thrombin, release a peptide, and unmask a new ligand within their own NH_2-terminal region (the "tethered ligand"), capable of self-activating the somewhat shortened receptor. The platelets of different species possess different PAR complements (PAR1,4 in human), and the paradigm of thrombin binding to a PAR, cleaving/exposing a ligand within the receptor, and inducing signal transduction has been firmly established as a central feature of platelet physiology. Furthermore, the importance of each of these events (thrombin generation, fibrin deposition, and platelet aggregation) in human physiology and pathophysiology is well documented by the widespread use and efficacy of specific inhibitors of each (warfarin, heparin, plasminogen activators, and antiplatelet agents; ref. 4). However, one aspect of thrombin's action on platelets remained unexplained: its interactions with the GP Ib–IX–V surface receptor (5).

Platelets possess two major surface receptors, GP Ib–IX–V and GP IIb–IIIa that differ in their family of origin (leucine-rich vs. integrin), function (adhesion vs. aggregation), and ligand [von Willebrand factor (vWf) vs. fibrinogen], respectively. As generally described, the GP Ib–IX–V receptor bears little resemblance to a thrombin receptor. Comprised of four distinct peptide chains, (GPs Ibα, 140 kDa; Ibβ, 22 kDa; IX, 20 kDa; V, 85 kDa) that interrelate through direct protein–protein interactions, common possession of leucine-rich repeats, and shared absence in a congenital deficiency state (Bernard–Soulier syndrome), the GP Ib–IX–V receptor displays affinity for its vWf ligand only under high shear conditions generated by rapidly flowing blood. Consequently, this unique receptor–ligand pair provides the critical contact point that arrests and anchors platelets at injured areas in the arterial circulation and, after adhesion, activates the GP IIb–IIIa integrin system for subsequent aggregation. However, none of the features appeared to involve thrombin or thrombin receptors, except for (*i*) the fact that thrombin receptor-like domains are encoded by the Ibα and V genes (6), and (*ii*) the intriguing but heretofore puzzling observations that the GP Ibα chain binds thrombin whereas GP V is cleaved by the enzyme (7, 8). The juxtaposition of thrombin interactions with a shear-dependent receptor generated much speculation, such as GP Ib–IX–V as a "way station" for thrombin delivery to the PAR receptors, but an explanation awaited close analysis of GP V knockout mice (9, 10).

At first glance, GP V seems to be a minor player in the GP Ib–IX–V system. GP Ib–IX alone, lacking V, is capable of nearly complete adhesive function and surface expression, and GPV is present in only half of the Ib–IX complexes (11). The platelets of GP V −/− mice, possessing only the Ib–IX form of the receptor, display nearly normal function, except for their modest, and perhaps entirely unexpected, *increase* in reactivity to thrombin (9). This initial insight, GP V as an *inhibitor* of the platelet response to thrombin, led to the current progress (1). Key reagents were protease-active thrombin (capable of activating both PARs and Ib–IX) as contrasted to protease-inactive thrombin (activating only Ib–IX/incapable of activating PARs). Ramakrishnan *et al*. (1) show that removal of GP V, by gene targeting or thrombin pretreatment, permits platelet activation by protease-inactive thrombin, a previously invisible response because of the presence of GP V. The investigators go on to demonstrate an *in vivo* role of GP Ib–IX in intact animals again through the use of protease-inactive thrombin. A main conclusion is that GP Ib–IX–V acts as a thrombin receptor only after it jettisons its GP V chain/inhibitor, either through thrombin cleavage or knockout. Thus, GP V has attained its recent prominence in platelet physiology through its absence.

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What is the importance of this observation? First, it adds a new and poten-

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tially critical avenue for thrombin activation of platelets and will immediately stimulate work on the similarities, differences, and relative contributions of GP Ib–IX–V and PARs as signaling receptors. GP V, once cleaved by thrombin, does not appear to contribute a tethered ligand, and no evidence suggests that Ib–IX is coupled to G proteins. However, dimerization of Ib–IX complexes in response to thrombin binding is a likely avenue for study, previewed by figure 6 in the paper by Ramakrishnan *et al*. (1), as is the connection between activated Ib–IX and ADP secretion. Second, one would like to know how thrombin binding is affected by GP V removal, how GP Ib shear dependence relates to thrombin affinity, and how the redistribution of surface Ib–IX that follows thrombin activation might influence signaling. Third, clinical investigators may search for inhibitors or modifiers of this new, and perhaps central, platelet agonist pathway in an effort to find more effective antiplatelet agents.

As always, a new ''kid on a block'' will stimulate many ''locals'' to reconsider and reconstitute their concepts concerning platelet responses to thrombin.

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