Congenital Disorders of Platelet Signal Transduction

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After injury to the blood vessel, platelets adhere to the exposed subendothelium by a process (adhesion) that involves the interaction of a plasma protein, von Willebrand factor (vWF), and a specific protein on the platelet surface, glycoprotein Ib (GPIb; the Figure). Adhesion is followed by recruitment of additional platelets that form clumps, a process called aggregation (cohesion). This involves binding of fibrinogen to specific platelet surface receptors—a complex comprising glycoproteins IIb-IIIa (GPIIb-IIIa). Activated platelets release the contents of their granules (secretion or release reaction), such as ADP and serotonin from dense granules, which subsequently cause recruitment of additional platelets. In addition, platelets play a major role in coagulation mechanisms; several key enzymatic reactions occur on the platelet membrane–lipoprotein surface. A number of physiological agonists interact with specific receptors on the platelet surface to induce responses, including a change in platelet shape from discoid to spherical, aggregation, secretion, and liberation of arachidonic acid. The Ca2+ concentration; InsP3 functions as a messenger to mobilize intracellular messenger molecules, including Ca2+ ions, products of phosphoinositide (PI) hydrolysis by phospholipase C (PLC; diacylglycerol [DG] and inositol 1,4,5-triphosphate [InsP3]), TxA2, and cyclic nucleotides (cAMP; the Figure). These subsequently induce or modulate the various platelet responses of Ca2+ mobilization, protein phosphorylation, aggregation, secretion, and liberation of arachidonic acid. The interaction between the agonist receptors and the key intracellular effector enzymes (eg, PLA2, PLC, adenyl cyclase) is mediated by a group of GTP-binding proteins that are modulated by GTP. As in most secretory cells, platelet activation results in a rise in cytoplasmic ionized calcium concentration; InsP3 functions as a messenger to mobilize Ca2+ from intracellular stores. DG activates protein kinase C (PKC), and this results in the phosphorylation of the 47-kDa protein pleckstrin. PKC activation is considered to play a major role in platelet secretion and in the activation of GPIIb-IIIa. Numerous other mechanisms, such as phosphorylation of proteins by nonreceptor tyrosine kinases, also play a role in signal transduction. A detailed description of the platelet activation mechanisms is beyond the scope of this review. Inherited or acquired defects in the above platelet mechanisms may lead to an impaired platelet role in hemostasis.

Congenital Disorders of Platelet Function

Disorders of platelet function are characterized by highly variable mucocutaneous bleeding manifestations and excessive hemorrhage after surgical procedures or trauma. A majority of patients, but not all, have a prolonged bleeding time. Platelet aggregation and secretion studies provide evidence for the defect but are not always predictive of the severity of clinical manifestations. The platelet dysfunction in these patients arises by diverse mechanisms.1-3 The Table 1 provides a classification based on the platelet functions or responses that are abnormal (the Figure). In patients with defects in platelet–vessel wall interactions, adhesion of platelets to the subendothelium is abnormal. The 2 disorders in this group are von Willebrand disease (vWD), due to a deficiency or abnormality in plasma vWF,4 and the Bernard-Soulier syndrome, in which platelets are deficient in GPIb (and GPV and IX), and the binding of vWF to platelets is abnormal.5 Disorders characterized by abnormal platelet-platelet interactions (aggregation) arise because of a severe deficiency of plasma fibrinogen (congenital afibrinogenemia) or because of a quantitative or qualitative abnormality of the platelet membrane GPIIb-IIIa complex (Glanzmann thrombasthenia).6 Patients with defects in platelet secretion and signal transduction are a heterogeneous group, lumped together for convenience of classification rather than on the basis of an understanding of the specific underlying abnormality. The major common characteristic in these patients, as currently perceived, is an inability to release intracellular (dense) granule contents on activation of platelet-rich plasma with agonists such as ADP, epinephrine, and collagen. In aggregation studies, the second wave of aggregation is blunted or absent. A small proportion of these patients have a deficiency of dense granule stores (storage pool deficiency). In some of the other patients, the impaired secretion results from aberrations in the signal transduction events that govern end responses such as secretion and aggregation. This review will focus on these patients, who are encountered more often than are those with thrombasthenia or the Bernard Soulier syndrome. Last are the patients who have an abnormality in interactions of platelets with proteins of the coagulation

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system; the best described is the Scott syndrome. In addition to the aforementioned groups, there are patients who have abnormal platelet function associated with systemic disorders such as Down syndrome and the May-Hegglin anomaly, in which the specific aberrant platelet mechanisms are still unclear.

Disorders of Platelet Secretion and Signal Transduction

As a unifying theme, patients lumped in this heterogeneous group generally manifest impaired secretion of granule contents and an absence of the second wave of aggregation on stimulation of platelet-rich plasma with ADP and epinephrine; responses to collagen, thromboxane analogue (U46619), arachidonic acid, and platelet-activating factor (PAF) may also be impaired. Platelet function is abnormal in these patients either when the granule contents are diminished (storage pool deficiency [SPD]) or when there is an aberration in the activation mechanisms governing aggregation and secretion (the Table).

Deficiency of Granule Stores

The term storage pool deficiency (SPD) refers to patients with deficiencies in platelet content of dense granules (δ-SPD), α-granules (α-SPD), or both types of granules (δ-α-SPD). The Quebec platelet disorder is an autosomal dominant disorder associated with abnormal proteolysis of α-granule proteins, deficiency of platelet α-granule multimerin (a factor V-binding protein), and markedly impaired aggregation with epinephrine as a striking feature.

Defects in Platelet Signal Transduction (Primary Secretion Defects)

Signal transduction mechanisms encompass processes that are initiated by the interaction of agonists with specific platelet receptors and include responses such as G-protein activation and activation of effectors such as PLC and PLA2. If the key components in signal transduction are the surface receptors, the G proteins, and the effectors, then evidence now exists for specific platelet abnormalities at each of these levels.

Defects in Platelet-Agonist Interaction: Receptor Defects

These patients have impaired responses because of an abnormality in the platelet surface receptor for a specific agonist. Such receptor defects have been documented for epinephrine, collagen, ADP, and TxA2. Hirata et al17 have described an Arg 60 to Leu mutation of the human TxA2 receptor in a dominantly inherited bleeding disorder. Patients...
described by Cattaneo et al.14,16 and Nurden et al.15 have a defect in the interaction of ADP with one of its receptors. Because ADP and TXA2 play a synergistic role in platelet responses to several agonists, patients with these receptor defects manifest abnormal responses to multiple agonists. A few patients have been described in whom platelet responses to collagen only are blunted and are associated with deficiencies in membrane glycoproteins, including GPⅠa and GPⅠb. GPⅠb-deficient platelets have been reported to have impaired collagen activation of tyrosine kinase Syk but not c-Src.21

**Defects in G-Protein Activation**

G proteins are a heterogeneous group of proteins that link surface receptors and intracellular effector enzymes and constitute an important potential aberrant locus leading to platelet dysfunction. Convincing evidence for such a defect has been provided by Gabbeta et al.22 in a patient with a mild bleeding disorder, abnormal aggregation and secretion responses to a number of agonists, and diminished GTPase activity in response to G-protein α-subunit function on activation. This patient had a selective decrease in the platelet membrane Gαs subunit but normal levels of Gαi, Gαo, Gαz, and Gε. She has been reported to have impaired Ca2+ mobilization23 and diminished release of free arachidonic acid from phospholipids on platelet activation.24 Essentially identical abnormal platelet findings have been reported in the Gαo-deficient knockout mouse.25 Impaired G-protein activation has also been reported in patients with the TXA2 receptor defect.18,19

**Defects in Phospholipase C Activation, Calcium Mobilization, Pleckstrin Phosphorylation, and Tyrosine Phosphorylation**

Several patients have been identified who have a relatively mild bleeding diathesis and impaired dense granule secretion, although their platelets have normal granule stores and, in general, synthesize substantial amounts of TXA2.26,27 These patients have abnormal aggregation and secretion particularly in response to weaker agonists (ADP, epinephrine, and PAF); the response to relatively stronger agonists such as arachidonate and high concentrations of collagen may be normal. Such patients are far more common than those with SP or defects in TXA2 synthesis. Lages and Weiss26 have described 8 such patients who had decreased initial rates and extents of aggregation in response to ADP, epinephrine, and U44169. Defects in early platelet-activation events were postulated in these patients. They subsequently demonstrated in one of these patients a defect in phosphatidylinositol hydrolysis and phosphatic acid formation,28 and pleckstrin phosphorylation.29

An early response to platelet stimulation is the rise in cytoplasmic Ca2+ concentration. Therefore, attention has been focused on this process to explain the impaired aggregation and secretion. In several patients, defects in calcium mobilization have been proposed on the basis of impaired platelet responses to the calcium ionophore A23187; however, this evidence is indirect at best. Direct evidence has been provided that some of these patients have impaired Ca2+ mobilization on platelet activation.23,30 Detailed studies in 2 patients with impaired aggregation and secretion revealed that the resting cytoplasmic Ca2+ concentration was normal but the peak Ca2+ concentrations after activation with ADP, collagen, PAF, or thrombin were diminished,30 with abnormalities in both the release of Ca2+ from intracellular stores and the influx of extracellular Ca2+.23 Further studies showed a defect in platelet formation of InsP3, the key intracellular mediator of Ca2+ release and DG and in pleckstrin phosphorylation,31 indicating a defect in PLC activation. Human platelets contain at least 7 PLC isozymes in the quantitative order PLC-γ2 > PLC-β2 > PLC-β3 > PLC-β1 > PLC-γ1 > PLC-δ1 > PLC-β4.32 Studies in 1 of these patients revealed a selective deficiency in PLC-β2 with normal levels of other PLC isoforms.32 These studies provide strong evidence that PLC-β2, a G protein–linked PLC isozyme, plays a major physiological role in platelet responses to activation. In line with these studies, knockout mice deficient in PLC-β2 have impaired Ca2+ mobilization in neutrophils.33

Several other studies provide evidence for defects in signaling mechanisms, phosphatidylinositol metabolism, and protein phosphorylation in patients with abnormal platelet aggregation and secretion.28,29,34,35 Holmsen et al.34 described a patient with abnormal platelet aggregation and dense granule secretion who had impaired release of free arachidonic acid and phosphoinositide hydrolysis on thrombin activation. However, no studies were performed on Ca2+ mobilization or Ins1,4,5P3 production, and the platelets had reduced GPⅠb and IIa as well. Another patient has been described with impaired platelet responses and diminished phosphoinositide metabolism in whom the altered stimulus-response coupling has been attributed to abnormal membrane phospholipid composition.36 Fuse et al.10 have reported a patient with a mild bleeding disorder whose platelets had impaired aggregation, secretion, InsP3 formation, and Ca2+ mobilization in response to a TXA2 mimetic (STA2) associated with normal TXA2 formation. Interestingly, GTPase activity on activation with STA2 was also impaired, leading to the conclusion that the platelets had an abnormality in coupling between the TXA2 receptor and PLC. In the patient described by Mitsui,35 the abnormal platelet aggregation was associated with decreased TXA2-induced InsP3 formation but with normal TXA2 receptors and GTPase activity on stimulation with TXA2 analogue U46619, suggesting an abnormality in PLC activity downstream from the receptor. In an analysis of 5 patients with absent TXA2-induced aggregation, Fuse et al.30 found evidence for a receptor defect in 3 patients; in the other 2, the primary abnormality appeared distal to the receptor. Together the above studies provide evidence for abnormalities in signal transduction pathways in patients with diminished platelet aggregation and secretion responses.

Yang et al.37 have summarized detailed studies on signaling mechanisms in 8 patients with abnormal aggregation and secretion in response to several different surface receptor-mediated agonists despite the presence of normal dense granule contents. Both PKC-induced pleckstrin phosphorylation and cytoplasmic Ca2+ mobilization play a major role in aggregation/secrection on activation. Receptor-mediated Ca2+
mobilization and/or pleckstrin phosphorylation was abnormal in 7 of the patients. It was postulated that combined platelet activation with a cell-permeable direct PKC activator, 1,2-di-octanoyl-sn-glycerol, and ionophore A23187, which possibly bypasses 2 major intracellular mediators (InsP3 and DG), may induce normal dense granule secretion in patients with impaired receptor-mediated secretion. Platelet activation with a combination of ADP and either 1,2-di-octanoyl-sn-glycerol or A23187 improved secretion in 4 patients. However, a combination of 1,2-di-octanoyl-sn-glycerol and A23187 induced normal secretion in platelet-rich plasma in all patients, suggesting that the ultimate process of exocytosis or secretion per se is intact and that impaired secretion in these patients results from abnormalities in early signal transduction events.

There is growing evidence that protein phosphorylation by tyrosine kinases (members of the Src-kinase family, the focal adhesion kinase [FAK] family, pp72syk, and the Janus [JAK] kinase family) plays an important role in platelet signal transduction.37 In thrombasthenia38,39 and the Scott syndrome,40 tyrosine phosphorylation of several proteins is impaired on platelet activation. In these disorders, this defect is a result of the primary abnormality in the GPIIb-IIIa complex and in phospholipid scrambling, respectively.37,39,40

**Signal Transduction Defects and Activation of GPIIb-IIIa Complex**

Activation of GPIIb-IIIa and platelet fibrinogen binding, a prerequisite for aggregation, is a signal transduction–dependent process and has been linked to PKC activation. Therefore, it is likely that abnormalities in signaling mechanisms may impair the activation of GPIIb-IIIa on platelets. Evidence that this is indeed the case is provided by the decreased activation of otherwise normal platelet GPIIb-IIIa complexes in a patient with marked abnormal platelet aggregation and impaired pleckstrin phosphorylation.41 The number and ligand-binding capacity of the GPIIb-IIIa complex were intact. A similar abnormality in GPIIb-IIIa activation has been observed in platelets with the Gq deficiency,22 attesting to the role of Gq in GPIIb-IIIa activation. Moreover, the defect in GPIIb-IIIa activation provides a cogent explanation for abnormalities in initial aggregation responses noted by Lages and Weiss26 in a number of their patients. Diminished activation of GPIIb-IIIa secondary to upstream signal transduction defects may be a more common mechanism than defects in the GPIIb-IIIa complex per se in patients with blunted aggregation.25

**Abnormalities in Arachidonic Acid Pathways and Thromboxane Production**

A major platelet response to activation is liberation of arachidonic acid from phospholipids and its subsequent oxygenation to TxA2. TxA2 production plays a synergistic role in the response to several agonists. Patients have been described with impaired liberation of arachidonic acid from membrane phospholipids during platelet stimulation.22,24,34 Several patients have been described with platelet dysfunction associated with congenital deficiencies of cyclooxygenase and thromboxane synthase.1

**Defects in Cytoskeletal Assembly**

The Wiskott-Aldrich syndrome (WAS) is an X-linked inherited disorder affecting T lymphocytes and platelets and characterized by thrombocytopenia, immunodeficiency, and eczema.42 Several platelet abnormalities, including dense granule deficiency, have been reported in WAS. WAS arises from mutations in the gene coding for a novel protein of 502 amino acids that binds to several other signaling proteins, including Cdc42 (a GTPase) and p47nck (a Src homology 3 domain containing adapter protein).42,43 This protein constitutes a link between the cytoskeleton and signaling pathways and is a key regulator of cytoskeletal assembly.43

**Relative Frequencies and Therapy of Various Platelet Abnormalities**

Thrombasthenia and the Bernard-Soulier syndrome are rare disorders. Although there are no published data, patients currently classified in the heterogenous category of defects in platelet secretion and signal transduction probably constitute the most frequently encountered inherited platelet function abnormalities, excluding vWD. In our experience, the SPD is present in <10% to 15% of patients with congenital platelet defects. Abnormalities in thromboxane production occur in ~20% of patients. A large proportion of the remaining patients with abnormal aggregation and secretion demonstrate adequate dense granule stores and produce substantial amounts of TxA2. In some of these patients, there is evidence for defects in the signaling mechanisms. In this heterogeneous group, the underlying mechanisms still need to be established. Platelet transfusions have been the major therapeutic modality to manage bleeding in patients with intrinsic platelet defects, and this approach needs to be individualized. A viable alternative is intravenous administration of desmopressin or 1-desamino-8-D-arginine vasopressin (DDAVP), which shortens the bleeding time in a number of patients, particularly those with normal dense granule stores.44,45 This response is dependent on the underlying mechanism leading to the platelet dysfunction.44,45

**Conclusions**

There has been a tremendous advance in our understanding of the role of platelets in hemostasis, and part of this has resulted from characterization of the experiments of nature, such as patients with platelet function disorders. In the vast majority of patients with inherited platelet dysfunction, the underlying mechanisms remain unknown. A better delineation of the involved platelet mechanisms is required and will translate into development of novel therapeutic strategies for a diverse group of disorders—hemorrhagic, thrombotic, atherosclerotic, and proliferative—in which platelets are involved.

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