Platelet biology

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INTRODUCTION

The platelet is a circulating anucleate disc-shaped cell, responsible for initiation of the hemostatic mechanisms that repair injury to the vascular endothelium.

The biology underlying these platelet functions will be discussed here. Discussions of platelet function testing as well as congenital and acquired disorders of platelet function and an overview of hemostasis are presented separately. (See "Platelet function testing" and "Congenital and acquired disorders of platelet function" and "Overview of hemostasis".)

The biology of the platelet precursor cells (megakaryocytes) and platelet formation are discussed elsewhere. (See "Megakaryocyte biology and the production of platelets".)

Platelet functions other than those enumerated above (eg, the role of platelets in inflammatory and immune responses, as well as specific disorders characterized by local or systemic platelet activation) have been reviewed elsewhere [1].

NORMAL VALUES

The normal range for platelet counts in the adult is discussed separately. (See "Diagnostic approach to the adult with unexplained thrombocytopenia", section on 'Definitions and areas of concern' and "Approach to the patient with thrombocytosis", section on 'Terminology'.)

OVERVIEW OF PLATELET FUNCTION

Role in hemostasis — Platelets are responsible for the first phase of hemostasis, referred to as primary hemostasis [2]. There are four major steps in this process (figure 1):

- Adhesion to the site of injury
- Activation and secretion
- Aggregation
- Interaction with coagulation factors

Circulating platelets do not normally encounter the connective tissue matrix that lies beneath vascular endothelial cells. Once a break within the integrity of this vascular lining occurs, platelets are exposed to, and interact with, collagen fibrils. Platelet interactions with collagen not only provide a surface for platelet adhesion, but also serve as a strong stimulus for platelet activation. This results in signaling pathways that induce platelets to change their shape, spreading along the collagen fibrils and to secrete thromboxane A2 and ADP into the circulation. The released thromboxane A2 and ADP stimulate neighboring platelets, causing them to become activated and in turn to secrete additional thromboxane A2 and ADP.

Activated platelets not only secrete thromboxane A2 and ADP, they also directly bind to the circulating coagulation protein fibrinogen, via the abundant platelet integrin glycoprotein (GP)IIb/IIIa (also known as alphaIIb/beta3) [3,4]. Fibrinogen (and fibrin) can simultaneously bind two GPIIb/IIIa receptors and can therefore function as a link between two platelets (figure 1). This platelet-fibrinogen-platelet connection initiates the process of platelet aggregation [2]. Since each platelet has 40,000 to 80,000 copies of GPIIb/IIIa on its surface, very large clumps (or aggregates) of platelets can assemble at the site of platelet activation [5,6]. A cross-linked fibrin clot ultimately stabilizes the growing platelet aggregate. (See "Disorders of fibrinogen", section on 'Biology'.)

In addition to collagen, ADP, and thromboxane A2, other agonists can activate platelets at sites of vascular injury. Tissue factor, which is expressed on all non-vascular cells, is exposed to circulating blood upon disruption of the protective endothelial layer of the vasculature. Tissue factor can interact with factor VIIa to promote local coagulation, and ultimately the generation of thrombin, the most potent of the platelet agonists. Platelets facilitate this process by providing procoagulant phospholipids that accelerate thrombin generation (figure 2). Consequently, platelet activation and fibrin deposition are intimately linked, maximizing the growth and strength of the hemostatic plug. (See "Overview of hemostasis", section on 'Clotting cascade and propagation of the clot'.)

Platelets have also been shown to contribute to a variety of processes not related to hemostasis. These additional biologic roles for platelets include the dissemination of cancer metastasis, liver regeneration, inflammatory arthritis, and immunity against pathogens.

Many, but not all, of these functions for platelets are mediated by the secretion of their granules, which contain large amounts of cytokines and growth factors. Because platelets contain so many growth factors within their granules, some investigators have attempted to accelerate wound healing by injecting platelets suspended within plasma (Platelet-Rich Plasma). Although there are widely reported anecdotes and testimonies about the merits of Platelet-Rich Plasma therapy, well-conducted, randomized trials of administering Platelet-Rich Plasma have shown this approach to be of no benefit. The evidence is discussed in separate topic reviews. (See "Overview of the management of overuse (persistent) tendinopathy", section on 'Autologous blood and platelet-rich plasma injection' and "Investigational approaches to the management of osteoarthritis", section on 'Platelet-rich plasma' and "Plantar fasciitis", section on 'Unproven treatments' and "Hamstring muscle and tendon injuries", section on 'PRP and other biologic injections' and "Elbow tendinopathy (tennis and golf elbow)", section on 'Platelet-rich plasma and other biologic injections'.)

Drugs with antiplatelet actions — A number of drugs are used specifically for their ability to interfere with platelet function, especially in cardiology and vascular medicine. (See "Congenital and acquired disorders of platelet function", section on 'Therapeutic antiplatelet agents' and "Acute ST-elevation myocardial infarction: Antiplatelet therapy", section on 'Rationale'.)

The following mechanisms are discussed in the relevant sections of this review:

- Interference with the thrombin receptor (see 'PAR1 (thrombin receptor)' below)
- Interference with ADP binding (eg, clopidogrel) (see 'P2Y1 and P2Y12 (ADP receptors)' below)
- Interference with prostaglandin synthesis (eg, aspirin) (see 'Eicosanoids and arachidonate' below)
- Interference with cyclic AMP (eg, dipyridamole) (see 'Cyclic AMP and cyclic GMP' below)
- Inhibition of the GPIIb/IIIa receptor (eg, abciximab) (see 'GPIIb/IIIa activation' below)

Use of these agents is discussed in the following separate reviews:

- Cardiovascular disease prevention (see "Overview of the prevention of cardiovascular disease events in those with established disease (secondary prevention) or at very high risk" and "Aspirin in the primary prevention of cardiovascular disease and cancer")
- Acute coronary syndrome (non-ST elevation) (see "Acute non-ST-elevation acute coronary syndromes: Early antiplatelet therapy")
- Acute coronary syndrome (ST elevation) (see "Acute ST-elevation myocardial infarction: Antiplatelet therapy")

- Coronary stenting (see "Long-term antiplatelet therapy after coronary artery stenting in stable patients")
- Stroke prevention (see "Antithrombotic therapy for the secondary prevention of ischemic stroke")

Other drugs used in cardiovascular medicine and/or as antithrombotic agents (eg, nitrates, heparin, thrombolytic agents), may also have a direct or indirect effect on platelet function. These agents are discussed separately. (See "Congenital and acquired disorders of platelet function", section on 'Therapeutic antiplatelet agents'.)

Certain classes of drugs, including some of those listed above, may also have toxic effects on the bone marrow, the developing megakaryocyte, and/or the mature platelet through a number of different mechanisms, including, but not limited to, antibody formation (eg, abciximab, clopidogrel, heparin). These are discussed separately. (See "Drug-induced immune thrombocytopenia", section on 'Mechanisms of DITP'.)

PLATELET AGONISTS AND THEIR RECEPTORS

Most platelet agonists, including thrombin, ADP, epinephrine, and thromboxane A2, stimulate cell surface receptors that span the platelet membrane. The cytoplasmic surface of these receptors interacts with heterotrimeric G-proteins. These G proteins consist of a GTP-binding alpha subunit and beta-gamma heterodimers (figure 3) [7]. When GDP is bound to the alpha subunit, the G-proteins are inactive. Agonist-stimulated receptors promote the replacement of GDP with GTP, switching on the G protein. G protein-coupled receptors typically have a limited duration of activity, after which they are turned off by phosphorylation and, in some cases, cleared from the cell surface by endocytosis.

The subclass of their alpha subunit characterizes the function of different G-protein heterotrimers, and consequently, the role of the receptors that associate with them. Receptors that associate with G-proteins containing a G alpha-q subunit stimulate phosphoinositide hydrolysis by phospholipase Cbeta (PLCbeta) [8]. Receptors coupled with G alpha-subclass "s" stimulate cyclic AMP (cAMP) formation, while those associated with G alpha-subclass "i" inhibit the production of cAMP [9]. G alpha-subclass "i"-coupled receptors also stimulate phosphatidylinositol 3-kinase (PI3Kgamma) and phospholipase Cbeta. Finally, G alpha-subclass "12/13"-coupled receptors stimulate signaling pathways leading to platelet shape-change and phosphoinositide hydrolysis [10].

Platelet agonists are sometimes classified as strong or weak, presumably reflecting differences in the sets of intracellular signals they evoke. Strong agonists (eg, thrombin, collagen) potently stimulate phosphoinositide hydrolysis and are relatively unaffected by inhibitors of cyclooxygenase (COX; eg, aspirin). The weaker agonists (eg, ADP, epinephrine)

have little or no ability to induce phosphoinositide hydrolysis and are more dependent on an intermediate effect of inducing thromboxane A2 formation to mediate their effects.

PAR1 (thrombin receptor) — Thrombin is arguably the most potent activator of platelets. When added at picomolar (ie, 10^{-12} Molar) concentrations to platelets in vitro, it causes phosphoinositide hydrolysis, thromboxane A2 formation, protein phosphorylation, and an increase in the cytosolic free Ca^{++} concentration. These pathways lead to platelet shape change, aggregation, and granule secretion [8]. Thrombin receptors are activated by cleavage at a specific site, exposing a new N-terminus (the "tethered ligand") that interacts with residues in the second extracellular loop of the receptor [11]. This interaction results in the activation of the G-proteins on the cytoplasmic surface of the receptor, and consequently additional signaling events.

The primary thrombin receptor in human platelets is known as the Protease-Activated Receptor-1, or PAR-1, to distinguish it from three closely related receptors, PAR-2, PAR-3, and PAR-4. There are approximately 2000 copies of the G alpha-subclass q-, or G alpha-subclass 12/13-, coupled PAR-1 on the surface of human platelets [12]. Since platelets synthesize very little protein, they have no real ability to replace cleaved receptors with new ones. Therefore, human platelets usually respond to thrombin only once.

PAR-1 antagonists — Vorapaxar and atopaxar are orally active selective PAR-1 antagonists that block thrombin-mediated platelet activation without interfering with thrombin-mediated cleavage of fibrinogen [13]. Atopaxar was being evaluated in clinical trials and has not been approved by the US Food and Drug Administration (FDA). Vorapaxar was approved by the FDA in 2014. A number of trials have demonstrated their efficacy in preventing thrombosis in patients undergoing percutaneous coronary intervention as well as in patients with atherothrombotic disease, although at the cost of increased bleeding.

GPIa/IIa and GPVI (collagen receptors) — Subendothelial collagen has been long recognized as an important initiator of platelet responses, serving both a substrate for platelet adhesion and as a potent platelet agonist. The interaction between platelets and collagen is complex. The collagen responses are accompanied by phosphoinositide hydrolysis, thromboxane A2 formation, protein phosphorylation, and an increase in cytosolic Ca^{++} [14]. As with thrombin, cyclooxygenase (COX) inhibitors retard, but do not eliminate, platelet responses to collagen, suggesting that thromboxane A2 formation is not essential for this response.

Platelets possess two different types of collagen receptors [14]:

- Glycoprotein Ia/IIa
- Glycoprotein VI

These are discussed in more detail below.

Glycoprotein Ia/IIa — The first collagen receptor discovered was alpha2beta1 (also known as VLA-2, platelet GPIa/IIa). It is a member of the integrin family of adhesion receptors and primarily serves as an anchor for platelets to attach to collagen exposed after disruptions in the vascular endothelial layer (figure 1) [15]. A patient with an acquired deficiency of this integrin (GPIa/IIa) exhibited a mild bleeding diathesis [16].

Glycoprotein VI — The identification of a patient with a mild bleeding diathesis who had normal amounts of GPIa/IIa, but markedly reduced amounts of platelet glycoprotein VI (GPVI), suggested that GPVI also served as an important collagen receptor [17]. Experiments using receptor-blocking antibodies, heterologously expressed receptors, and genetically altered mice have elucidated the relative roles of each of these proteins [15,18]. GPVI serves as the primary collagen agonist receptor and is responsible for collagen-induced platelet aggregation and secretion. It also can serve as a collagen-adhesion receptor, at least under low shear conditions.

One patient with a mild bleeding disorder, a moderately reduced platelet count, and an autoantibody that bound to GPVI-positive platelets has been described [19]. This patient's platelets failed to become activated in response to collagen and inefficiently adhered to immobilized collagen under conditions of arterial shear. Other patients with a more severe bleeding disorder, often associated with immune dysfunction or autoimmune disease have been described [20].

P2Y1 and P2Y12 (ADP receptors) — Adenosine diphosphate (ADP) is stored in platelet dense granules and released upon platelet activation. ADP interacts with two G-protein-coupled platelet receptors, $P2Y_1$ and $P2Y_{12}$ [21,22]. These receptors display activity to a range of pyrimidine and purine agonists [23]. When ADP stimulates the platelet G alpha-subclass q-coupled $P2Y_1$ receptor, it causes phosphoinositide hydrolysis, thromboxane A2 formation, protein phosphorylation, and an increase in cytosolic Ca^{++}. ADP also stimulates the G alpha-subclass i-coupled $P2Y_{12}$ receptor that inhibits cAMP formation. Studies using platelets derived from knockout mice, or several different ADP receptor antagonists, have demonstrated that simultaneous stimulation of both $P2Y_1$ and $P2Y_{12}$ is required for the full ADP-response in platelets [21].

Abnormalities of the platelet P2Y12 receptor — Congenital deficiency of the $P2Y_{12}$ receptor is an autosomal recessive disorder associated with excessive bleeding, prolonged bleeding time, reversible platelet aggregation in response to weak agonists, impaired aggregation in response to low concentrations of collagen or thrombin, and, most typically, failure to achieve full and irreversible platelet aggregation to ADP, even at very high

concentrations [24]. A variety of different mutations in the $P2Y_{12}$ receptor have been described, although they are all extremely rare [23].

The platelet $P2Y_{12}$ receptor can be inhibited by several drugs, which include the following:

- Clopidogrel
- Ticlopidine
- Prasugrel
- Ticagrelor
- Cangrelor

These are discussed in more detail in the following sections.

Clopidogrel and ticlopidine — Several patients with bleeding defects have been described whose platelets show greatly diminished responsiveness to ADP [25]. This phenotype is not dissimilar from that produced by the thienopyridine antiplatelet agents ticlopidine and clopidogrel. Both are prodrugs that depend on metabolism by cytochrome P450 isoenzymes for their anti-platelet effects. Both ticlopidine and clopidogrel have been shown to inhibit platelet aggregation ex vivo and to prolong the bleeding time in humans. The degree of prolongation of the bleeding time is equivalent to or greater than that of aspirin, and the effect of thienopyridines and aspirin appears additive. (See "Acute non-ST-elevation acute coronary syndromes: Early antiplatelet therapy", section on 'P2Y12 use'.)

Effects of ticlopidine and clopidogrel on platelet aggregation and the bleeding time may be seen within 24 to 48 hours of the first dose, but are not maximal for four to six days. Moreover, the effects may last for 4 to 10 days after the drugs have been discontinued. This may be explained by their extended half-life after multiple dosing or by irreversible effects on platelets.

Although the target of these two drugs was initially elusive, it was ultimately proven that their metabolites block the platelet $P2Y_{12}$ receptor. Ex vivo studies indicate that ticlopidine and clopidogrel inhibit ADP-mediated signaling, and thereby indirectly impair fibrinogen binding to its platelet receptor, GPIIb/IIIa, and inhibit platelet aggregation in response to many agonists, particularly ADP. The effect on ADP-induced platelet aggregation seems to account for the observed decrease in responses to low concentrations of other agonists, since ADP released from dense granules plays a role in those responses. Consistent with this hypothesis, aggregation in response to high concentrations of thrombin or collagen are normal.

Some patients appear to be "resistant" to clopidogrel. This is sometimes attributable to polymorphisms within the $P2Y_{12}$ gene, or more commonly, the cytochrome P450 system may be responsible for reduced effectiveness of clopidogrel in patients with cardiovascular

disease, with a resultant increased risk for vascular events [26]. Alternatively, the metabolism of clopidogrel can be altered by concurrent medications including warfarin or proton pump inhibitors, although the clinical significance of these interactions is unclear. This subject is described in detail separately. (See "Clopidogrel resistance and clopidogrel treatment failure".)

Prasugrel — Prasugrel is a third-generation orally active thienopyridine that also must be converted to an active metabolite by the cytochrome P450 (CYP) system. However, its activation is not adversely affected by the common CYP polymorphisms that can inhibit the effectiveness of clopidogrel [27-29]. The use of prasugrel to reduce the risk of thrombotic cardiovascular events in patients with acute coronary syndrome being managed with coronary artery stents is discussed separately. (See "Acute non-ST-elevation acute coronary syndromes: Early antiplatelet therapy".)

Ticagrelor — Ticagrelor is not a thienopyridine, but is an orally active $P2Y_{12}$ receptor blocker. It does not require metabolic conversion to an active form, so it has the advantage of a more rapid onset of action [22]. Ticagrelor binds directly to the $P2Y_{12}$ receptor and can more completely inhibit the sustained platelet aggregation response to ADP than clopidogrel. The use of ticagrelor for reducing thrombotic cardiovascular events in patients with acute coronary syndromes is discussed separately. (See "Acute ST-elevation myocardial infarction: Antiplatelet therapy", section on 'Patients receiving primary PCI' and "Acute non-ST-elevation acute coronary syndromes: Early antiplatelet therapy", section on 'Cangrelor'.)

Cangrelor — Cangrelor is another non-thienopyridine adenosine triphosphate (ATP) analogue that blocks the $P2Y_{12}$ receptor. Potential advantages of this intravenously administered agent are its rapid onset of action and rapid return of platelet function after cessation. (See "Antithrombotic therapy for elective percutaneous coronary intervention: Clinical studies", section on 'Cangrelor'.)

Adrenergic receptors — Platelet responses to epinephrine are mediated by G alpha-subclass i (or its highly related G alpha-subclass z)-coupled alpha(2)-adrenergic receptors [30]. Although high doses of epinephrine induce platelet activation in vitro, platelets do not aggregate when stimulated with physiologic doses of epinephrine alone. In this respect, epinephrine is unique among platelet agonists. It is thought that stress-induced epinephrine in vivo increases the ability of platelets to respond to low doses of other platelet agonists.

Epinephrine is a potent inhibitor of cyclic AMP (cAMP) formation, but this alone is not sufficient to trigger platelet activation. High doses of epinephrine cause phospholipase C activation ex vivo. However, this can be suppressed by aspirin, suggesting that it is dependent upon thromboxane A2 formation.

Thromboxane receptor — Thromboxane A2 is produced from arachidonate in platelets by the aspirin-sensitive cyclooxygenase (COX) pathway (figure 4). Once formed, thromboxane A2 can passively diffuse across the plasma membrane and activate other platelets via a G alpha-subclass q-coupled receptor known as the thromboxane receptor (also called thromboxane A2 receptor or prostanoid TP receptor). Thromboxane A2, like ADP, amplifies the initial signal for platelet activation, thereby helping to stimulate additional platelets. This process is effective locally, and is limited by the brief half-life of thromboxane A2. This helps to confine the spread of platelet activation to the original area of injury.

Of note, platelets only express COX-1. Therefore, aspirin and other non-selective COX inhibitors (table 1) block platelet activation; in contrast, selective COX-2 inhibitors do not do so. This contributes to the increased cardiovascular risks associated with COX-2 inhibitors. (See 'Aspirin' below and "Overview of COX-2 selective NSAIDs", section on 'Lack of platelet inhibition and use during anticoagulation'.)

A number of stable endoperoxide/thromboxane analogs have been synthesized, including U46619. When added to platelets ex vivo, U46619 causes platelet shape change, aggregation, secretion, phosphoinositide hydrolysis, protein phosphorylation, and an increase in cytosolic Ca^{++}, while having little effect on cAMP formation [31]. Similar responses are seen when platelets are incubated with exogenous arachidonic acid. Since the effects of arachidonate can be inhibited with aspirin, they are thought to be largely due to thromboxane A2 formation.

SECOND MESSENGERS AND BEYOND

After platelet activation by an agonist, intracellular signaling is needed for cytoskeletal reorganization, fibrinogen receptor exposure, and granule secretion. Two pathways that are central to platelet activation are the phosphoinositide hydrolysis pathway and the eicosanoid synthesis pathway.

- The phosphoinositide hydrolysis pathway begins when phospholipase C cleaves membrane phosphatidylinositol 4,5-bisphosphate (PIP2) to form inositol 1,4,5-trisphosphate (IP3) and diacylglycerol, both of which serve as second messengers (figure 5).

- The eicosanoid pathway begins when phospholipase A2 releases arachidonate from membrane phospholipids to form thromboxane A2. As mentioned above, most of the agonists that activate platelets do so via G protein coupled receptors, and it is the activation of G-proteins that is the first step in the intracellular signaling pathway leading to platelet second messenger formation.

Inositol signaling: phospholipase C and PI 3-kinases — One of the earliest responses of platelets to most agonists is the activation of phospholipase C (PLC) (figure 3). Platelets contain beta and gamma forms of this enzyme. The beta forms are activated by G proteins, while the gamma forms (predominantly PLC gamma2) are regulated by tyrosine phosphorylation [32,33]. PLC beta is thought to be primarily responsible for the rapid burst of phosphoinositide hydrolysis that occurs during platelet activation by agonists such as thrombin and thromboxane A2.

In general, PLC(beta1) and PLC(beta3) respond best to G alpha, particularly members of the G alpha-subclass q family, while PLC(beta2) responds best to G beta-gamma. Based upon studies with pertussis toxin and genetically modified mice, PAR-1 in platelets is thought to be coupled to PLCbeta by G alpha isotypes derived from Gq- or G12/13-coupled receptors, and to a lesser extent by G beta-gamma derived from G-subclass i [8,34]. Thromboxane A2 receptors are coupled to PLCbeta by G alpha derived from Gq- or G12/13-coupled receptors [8]. Once activated, phospholipase C hydrolyzes PI-4,5-P(2) into DAG and IP(3) (figure 5) [35,36]. Once generated, DAG then activates PKC, which in turn phosphorylates pleckstrin (a protein critical for granule secretion) [37]. IP(3) binds to receptors in the dense tubular system and releases sequestered Ca^{++} into the cytosol [38,39]. Additional discussion of these events is presented below.

Phosphatidylinositol 3-kinases (PI3K) are a group of enzymes that phosphorylate the D-3 position of the inositol ring of phosphatidylinositol to produce phosphatidylinositol 3-phosphate (PI 3-P), phosphatidylinositol 3,4-bisphosphates (PI 3,4-P2), and phosphatidylinositol 3,4,5-triphosphates (PI 3,4,5-P2 or PIP3) (figure 5) [40]. Several isoforms of PI3K that phosphorylate PI, PI 4-P, and PIP2 have been described in humans, and they are classified according to their catalytic subunit: p110alpha, p110beta, p110gamma, and p110delta.

Using pharmacologic inhibitors, and platelets obtained from genetically modified mice, investigators have begun to understand the role of different PI3K isoforms in platelets and their downstream effectors [41]:

- Mice lacking the p110gamma catalytic subunit of PI3K have a defect in ADP-mediated platelet aggregation [42].
- Mice lacking the p110alpha or p110beta subunit of PI3K have a defect in signaling events initiated by the platelet collagen receptor, GPVI, whereas there is no defect in platelet activation following stimulation by other platelet agonists such as ADP or thrombin [43].

Together, the literature suggests that PI3K is involved in both the initial activation of GPIIb/IIIa and the subsequent stabilization of the GPIIb/IIIa/fibrinogen interaction that

leads to irreversible platelet aggregation [44].

Cytosolic calcium ions — Calcium ions (Ca^{++}) serve as intracellular second messengers, and like protein kinases, affect enzyme activity and protein- protein interactions. Based upon studies with intracellular probes such as Fura-2, the cytosolic free Ca^{++} concentration in resting platelets is approximately 0.1 microM. Strong agonists, such as thrombin or collagen cause an increase to ≥1.0 microM. Weaker agonists, particularly epinephrine, may have little or no effect on cytosolic Ca^{++}.

When platelets are activated, the cytosolic Ca^{++} concentration increases because of a combination of Ca^{++} release from the dense tubular system and Ca^{++} influx across the plasma membrane [45]. The trigger for Ca^{++} release from the dense tubular system is 1,4,5-IP3, and the mediators of Ca^{++} influx from the extracellular fluid are store-operated calcium channels (SOCC). The rise in cytosolic Ca^{++} contributes to platelet activation by stimulating enzymes that are not optimally active at low Ca^{++} concentrations. Examples of these include cPLA(2), phospholipase C, phosphorylase kinase, gelsolin, calpain, and myosin light chain kinase.

Protein kinase C — Protein kinase C (PKC) isozymes are a family of serine/threonine kinases that play an essential role in the signal transduction mechanisms after activation of receptors [46]. PKC has been identified as the cellular receptor for the lipid second messenger diacylglycerol (DAG), and it is therefore a key enzyme in the signaling events that follow activation of receptors coupled to phospholipase C (PLC). PKC isozymes phosphorylate multiple cellular proteins on serine and threonine residues. Although some discrepancies between different studies on PKC isozyme expression exist, platelets probably express PKC alpha, beta, delta, epsilon, eta, theta, and perhaps zeta and lambda [47].

PKC isozymes control a variety of functions, including aggregation, release of granular contents, mobilization of intracellular calcium, and regulation of cell shape. PKC isozymes also play an important role in megakaryocyte differentiation. A prominent PKC substrate is pleckstrin. Mice lacking pleckstrin have a complete loss of PKC-mediated granule secretion [37].

Eicosanoids and arachidonate — The passive release of thromboxane A2 from platelets is another mechanism to amplify platelet activation. Eicosanoids are formed from the arachidonate released from membrane phospholipids by phospholipase A2 during platelet activation [48]. Platelet phospholipase A2 is stimulated by the rise in the cytosolic Ca^{++} that accompanies platelet activation. Once released from membrane phospholipids, arachidonate can be metabolized to thromboxane A2 by cyclooxygenase-1 (COX-1) [48].

Once formed, thromboxane A2 can diffuse across the plasma membrane and activate other platelets through their thromboxane A2 receptors. This leads to platelet shape change,

aggregation, secretion, phosphoinositide hydrolysis, protein phosphorylation and an increase in cytosolic Ca^{++}, while having little effect on cAMP formation.

Aspirin — Aspirin acetylates COX-1, causing it to be irreversibly inactivated (figure 6). Since platelets lack the ability to synthesize significant amounts of protein, inactivation of COX-1 by aspirin blocks thromboxane A2 synthesis for the life of the platelet (ie, approximately seven days). Platelets that do not synthesize thromboxane A2 normally have impaired stimulation by ADP, epinephrine, arachidonic acid, and low doses of collagen and thrombin, but normal responses to the major platelet agonists, collagen and thrombin. Hence, aspirin is the most commonly used drug for antithrombotic therapy. (See "Aspirin in the primary prevention of cardiovascular disease and cancer".)

Platelet prostaglandin synthesis in an adult is nearly completely inhibited by a single 325 mg oral dose of aspirin or by as little as 30 mg taken daily for 7 to 10 days. Aspirin is one of the few drugs that prolongs the bleeding time in humans and appears to do so by blocking platelet aggregation rather than adhesion. (See "Platelet function testing", section on 'The in vivo bleeding time'.)

In individuals with normally functioning platelets, the effect of aspirin on the bleeding time is slight (generally no more than 1.2 to 2.0 times the pre-aspirin bleeding time). The bleeding time may remain prolonged for four days after aspirin has been discontinued; platelet aggregation tests may remain abnormal for up to a week, until the affected platelets are replaced by unaffected ones.

Other NSAIDs — In contrast to aspirin, other nonsteroidal antiinflammatory drugs (NSAIDs) inhibit COX enzymes reversibly [49]. Even though these drugs may cause a transient prolongation of the bleeding time, the platelet defects induced by NSAIDs are usually not clinically significant. In fact, it has been shown that ibuprofen can be administered safely to patients with hemophilia A [50,51]. Agents that are selective for COX-2 have no effect on platelet function. (See "Nonselective NSAIDs: Overview of adverse effects", section on 'Antiplatelet effects' and "Overview of COX-2 selective NSAIDs", section on 'Lack of platelet inhibition and use during anticoagulation'.)

Other protein kinases — In addition to serine/threonine kinases such as PKC, platelets contain a large number of tyrosine kinases, some of which become active during platelet activation. Human platelets contain tyrosine kinases that are receptors for extracellular ligands, as well as large number of nonreceptor (cytoplasmic) tyrosine kinases, including Btk, Tec, Src, Fyn, Lyn, Hck, and Syk. Some of these play critical roles in collagen-induced platelet activation.

Inhibitors of phosphotyrosine phosphatases, such as vanadate, promote platelet activation. In general, tyrosine phosphorylation can serve two roles. It can have a regulatory effect on

the phosphorylated protein, perhaps by causing a conformational change, or it can provide a binding site for modular domains located in other proteins, such as SH2 domains. Although studies of knockout mice have identified the role of some of these proteins in signaling pathways downstream of the GPVI collagen receptor, the function of most tyrosine kinases in platelets is still incompletely understood [52].

Cyclic AMP and cyclic GMP — Agents that raise the cyclic AMP (cAMP) concentration in platelets inhibit platelet activation, but the mechanism by which this occurs is unclear. In general, the effects of cAMP are thought to be mediated by cAMP-dependent protein kinase, also known as protein kinase A [53]. Platelet substrates for this enzyme include the 24 kDa beta chain of glycoprotein Ib, actin-binding protein and myosin light chain, VASP and Rap1B. Raising cAMP levels causes a number of specific changes in platelet function, including impaired phosphoinositide hydrolysis, a smaller increase in the cytosolic free Ca^{++} concentration in response to agonists, and an accelerated uptake of Ca^{++} into the dense tubular system.

PGI2 released from activated endothelial cells elevates platelet cAMP levels by stimulating receptors on the platelet surface that are coupled to adenylyl cyclase via G proteins. This results in an inhibition of platelet activation. Consequently, mice lacking the PGI2 receptor show an increased risk of thrombosis [54]. Most platelet agonists suppress cAMP formation by inhibiting adenylyl cyclase via one or more of the G-subclass i family members that are expressed in platelets.

Dipyridamole — The drug dipyridamole (Persantine) exerts antiplatelet effects by inhibiting cAMP phosphodiesterase, thus raising cAMP levels within platelets [53]. However, its efficacy as an antiplatelet agent has been debated. (See "Congenital and acquired disorders of platelet function", section on 'Dipyridamole'.)

Sildenafil — Sildenafil, an agent used for erectile dysfunction in men, is a phosphodiesterase-5 inhibitor, and potentiates the ability of nitric oxide (NO) to inhibit platelet aggregation in vitro by preventing platelet cGMP catabolism [55-57]. (See "Sexual activity in patients with cardiovascular disease", section on 'Sildenafil' and "Treatment of male sexual dysfunction", section on 'Phosphodiesterase-5 inhibitors'.)

Cilostazol — Cilostazol, a specific inhibitor of cAMP phosphodiesterase in platelets and vascular smooth-muscle cells, is a potent antiplatelet agent and vasodilator. (See "Antithrombotic therapy for elective percutaneous coronary intervention: Clinical studies", section on 'Cilostazol' and "Management of claudication due to peripheral artery disease", section on 'Cilostazol'.)

PLATELET ACTIVATION

Shape change — One of the more dramatic events during platelet activation is the metamorphosis that occurs when platelets adhere and spread on exposed collagen fibrils or become activated in the circulation by soluble factors such as thrombin or ADP [58]. In either case, platelets lose their distinct discoid shape and acquire an irregular morphology with multiple filopodial projections. This transformation is associated with, and largely due to, cytoskeletal rearrangements within the platelet.

Platelet cytoskeletal proteins are arranged in three major structures: a cytoplasmic actin network, a rim of membrane-associated cytoskeleton, and a marginal band consisting of a microtubule coil. Together, these lend support to the platelet plasma membrane and give shape to both resting and activated platelets.

The cytoplasmic actin network is composed of actin filaments and associated proteins. Actin is a 42 kDa protein which accounts for as much as 20 percent of total platelet protein [59]. In resting platelets, 40 to 50 percent of the actin is present as filamentous F-actin, and the remainder is present as globular monomeric G-actin. The shift to increase the proportion of F-actin to 70 to 80 percent during platelet activation involves a coordinated sequence of events in which the actin filaments present in resting platelets are severed and the resultant smaller fragments used as the nidus for new, longer actin filaments.

This process is thought to be regulated in part by the increase in levels of a phosphatidylinositol (PI-4,5-P2) that accompanies platelet activation [60]. At the same time, myosin is phosphorylated by myosin light chain kinase and becomes associated with F-actin, forming filaments that are anchored to the platelet plasma membrane by attachment, via actin-binding protein, to the GPIb/IX complex.

The cytoskeletal rim is composed of actin, filamin, P235 (talin), vinculin, spectrin, alpha-actinin and several membrane glycoproteins. Filamin is an elongated 280 kDa protein that is present in platelets and functions as an actin binding protein. In resting platelets, filamin is part of a semi-rigid array that helps to maintain the platelet's discoid shape and limits the lateral movement of GPIb. This role is analogous to that performed by spectrin in erythrocytes.

When platelets are activated, actin filaments form and attach to actin-binding protein. Later, the rising cytosolic Ca^{++} concentration activates calpain that cleaves actin-binding protein, severing the link to GPIb.

The third major structural element in platelets is the marginal band [61]. This microtubule coil is a single tightly-wound polymer of tubulin that encircles the platelet perimeter and helps to maintain its discoid shape. During platelet activation, the microtubule coil contracts. Initially it was thought contraction of the marginal band was required for stable adhesion of platelets under arterial shear pressures. However, newer evidence has

challenged the nature of this marginal band and its function during both resting and activated platelet states [61,62].

GPIIb/IIIa activation — The process of transforming GPIIb/IIIa (alphaIIb-beta3) on the platelet surface into a competent receptor for fibrinogen was one of the most elusive aspects of platelet signaling. It is the final common pathway in platelet responses to most agonists, making it a frequent target for drug development. Circulating platelets do not normally bind fibrinogen or stick to each other unless they have been activated. The reasons for this are multiple, but are ultimately due to the inability of fibrinogen or fibrin to bind to the resting conformation of GPIIb/IIIa.

Platelet activation alters the conformation, or competency, of GPIIb/IIIa, allowing fibrinogen binding. The process whereby intracellular events alter GPIIb/IIIa on the cell surface is referred to as "inside-out" signaling (figure 1) [3,63]. The process requires the binding of talin and kindling-3 to the cytoplasmic tail of GPIIIa. The binding of these two proteins to the cytoplasmic side of the receptor opens the extracellular side and thereby allows it to bind fibrinogen. Normally, this should occur only at sites of vascular injury. Additionally, a series of intracellular signaling events are initiated and propagated, including tyrosine and serine/threonine kinase and phosphatase activation, as a consequence of fibrinogen binding and platelet aggregation (so called "outside-in" signaling) [64].

Working against this tendency to platelet activation are a number of internal and external controls that dampen the intracellular signals that would otherwise allow inappropriate platelet activation, thereby contributing to such complications as myocardial infarction or stroke. These controls include tight regulation of the cytosolic Ca^{++} concentration, intracellular phosphatases that limit signaling through kinase-dependent pathways, extracellular ADPases that hydrolyze released ADP, and the inhibitory effects of PGI2 and nitric oxide (NO) released from endothelial cells. Collectively, these provide a threshold that helps to prevent platelet activation at inappropriate times and places.

GPIIb/IIIa inhibitors — GPIIb/IIIa antibodies and receptor antagonists (eg, abciximab, tirofiban, eptifibatide) inhibit the final common pathway of platelet aggregation, the crossbridging of platelets secondary to fibrinogen binding to the activated GPIIb/IIIa receptor (figure 1).

These agents may also prevent initial adhesion of platelets to the vessel wall. Their use in coronary heart disease is discussed separately. (See "Early trials of platelet glycoprotein IIb/IIIa receptor inhibitors in coronary heart disease", section on 'The platelet glycoprotein IIb/IIIa receptor' and "Acute non-ST-elevation acute coronary syndromes: Early antiplatelet therapy", section on 'Glycoprotein IIb/IIIa inhibitors'.)

Secretion (granule exocytosis) — Another important function of platelets is the release of a variety of substances that stimulate or inhibit platelets or other blood and vascular cells, a process that can be modified in the presence of aspirin [65]. These can covalently modify the thrombus to affect its mechanical properties, as well as regulate coagulation, contribute to cell adhesive events, and modulate the growth of cells of the vessel wall. Platelets contain three types of granules:

- Dense granules – Platelet agonists (eg, ADP, ATP, serotonin) which serve to amplify platelet activation
- Alpha granules – Proteins which enhance the adhesive process (eg, fibrinogen, fibronectin, vitronectin, von Willebrand factor)
- Lysosomal granules – Glycosidases and proteases which have an unclear function in platelet biology [66].

Building upon lessons learned about the role of the SNARE complex (**s**oluble **N**-ethylmaleimide-sensitive factor **a**ttachment protein **re**ceptors) in neuronal cell exocytosis, there has been a substantial increase in our understanding of platelet secretion. Platelets do have the three basic components of the SNARE machinery: t-SNAREs (target receptors), v-SNAREs (vesicle-associated membrane receptors), and soluble components (including NSF and NSF-attachment proteins) [66]. The SNARE machinery regulates the association and subsequent fusion of vesicles with membranes. This process involves a small GTP-binding protein called Arf6 [67]. Studies have shown that patients with gray platelet syndrome have a defect in a scaffolding protein called NBEAL2 [68]. The available evidence suggests that NBEAL2 is critical for shuttling proteins synthesized within the endoplasmic reticulum to the alpha granule. Absence of this protein leads to absence of alpha granules within platelets [69]. (See "Congenital and acquired disorders of platelet function", section on 'Storage pool disorders'.)

SUMMARY

- Platelets are responsible for initiating the hemostatic mechanisms that repair injury to the vascular endothelium. This is accomplished via the following major platelet functions (figure 1). (See 'Overview of platelet function' above.)
    - Platelet interactions with collagen and other agonists (see 'Platelet agonists and their receptors' above)
    - Intracellular signaling (see 'Second messengers and beyond' above)
    - Platelet activation and shape change (see 'Platelet activation' above and 'Shape change' above)
    - Secretion of granule contents (see 'Secretion (granule exocytosis)' above)

- A number of drugs (ie, antiplatelet agents) are used specifically for their ability to interfere with various aspects of platelet function, and have greatest uses in cardiology and vascular medicine. (See 'Drugs with antiplatelet actions' above.)

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