

PART I

Concepts in Platelet Physiology, Function, and Measurement



Platelet physiology and the role of the platelet in ischemic heart disease

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Blood platelets – equipped for action

The platelet, a tiny anucleate blood cell 2–4 μm in diameter, has major and diverse roles in health and disease and underlying this is a complex structure that supports a wide range of functional responses [1]. Approximately 1×10^{11} new platelets are released each day into the circulation from bone marrow where they are formed by fragmentation from megakaryocytes [2,3]. Thrombopoietin is the most important cytokine regulating platelet production and some disease states, such as inflammatory conditions, can increase thrombopoietin levels and platelet production [3]. This is of relevance when considering the rate of recovery of haemostatic function (and susceptibility to thrombosis) following exposure to irreversible platelet inhibitors such as aspirin and thienopyridines.

Platelets in the resting state are smooth, discoid cells possessing an open canalicular system and an exterior glycocalyx [1]. Ca^{2+} is sequestered in intracellular stores and released into the cytoplasm upon activation of the platelet by agonists, where it plays a major part in mediating platelet responses to activation [4]. Contained within the platelet cytoplasm are three types of granule, namely α -granules, dense granules and lysosomes [5]. α -Granules are the most abundant granules and contain a large number of proteins, many of which play a role in regulating the balance of thrombosis and fibrinolysis, such as α_2 -antiplasmin and plasminogen activator inhibitor-1 (PAI-1) [6]. The membranes of α -granules also contain proteins that are expressed on the cell surface following platelet activation, including glycoprotein (GP) IIb/IIIa

($\alpha_{IIb}\beta_3$) and P-selectin (CD62P). Dense granules contain concentrated stores of ADP, ATP, pyrophosphate, ionized calcium and 5HT, and release of the contents of dense granules contributes to platelet activation and hemostasis [5,7]. Lysosomes contain numerous acid hydrolases and these are secreted by platelets only in response to strong agonist stimulation. Platelet activation leads to fusion of granules with the open canalicular system and release of granule contents [8].

Platelets possess a cytoskeleton which determines cell shape and consists mainly of microtubules and microfilaments [5]. Reorganization of the platelet cytoskeleton during platelet activation leads to a change in cell shape, with the platelets becoming spherical and extending finger-like projections known as pseudopodia. Activation of platelets also leads to a reorganization of components of the plasma membrane that leads to the assembly of prothrombinase complex on the platelet surface, catalyzing thrombin generation and coagulation [9].

Figure 1.1 provides an overview of mechanisms for platelet activation and associated responses which will be covered in subsequent sections.

The platelet glycoprotein IIb/IIIa complex

The GPIIb/IIIa complex is an adhesion receptor belonging to the integrin gene superfamily and, like other integrins, is a heterodimer composed of an α (α_{IIb}) and a β (β_3) transmembrane subunit [10]. Approximately 40,000 to 80,000 GPIIb/IIIa complexes are present on the surface of each resting platelet and this number can rapidly increase following activation by strong agonists due to exposure of internal receptors normally present within the open canalicular system and α -granule membranes [10]. Activation of platelets induces conformational changes in GPIIb/IIIa so that it can bind fibrinogen, vWF or fibronectin, whereas in the resting state GPIIb/IIIa binds fibrinogen only weakly so as to allow uptake into α -granules [11]. Fibrinogen molecules possess two binding regions for GPIIb/IIIa and act as bivalent ligands, forming cross-bridges between activated platelets and leading to aggregation of activated platelets. In this way, GPIIb/IIIa mediates the so-called “final common pathway” of platelet aggregation, regardless of the stimulatory agent(s) [12]. The cytoplasmic domains of both IIb and IIIa play an important role in this “inside-out” signaling and also mediate “outside-in” signaling, whereby ligand binding to GPIIb/IIIa leads to cytoskeletal reorganization and other post-ligand binding events that amplify platelet activation [13,14]. This explains why therapeutic concentrations of GPIIb/IIIa antagonists inhibit platelet dense granule secretion and platelet procoagulant responses as well as platelet aggregation [15,16], although, through less well understood mechanisms, low concentrations of GPIIb/IIIa antagonists potentiate α -granule release and soluble CD40L release [17,18].

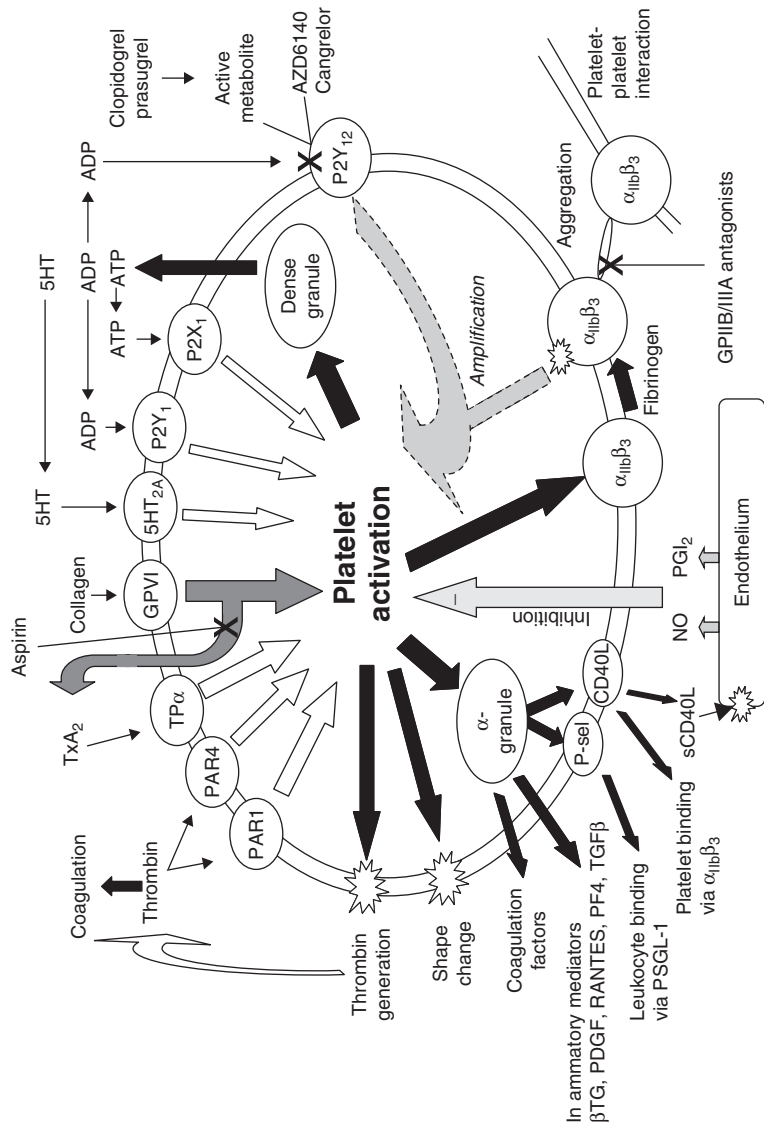


Fig. 1.1 Overview of platelet activation mechanisms and associated functional responses. Numerous agonists including thrombin, TXA₂, collagen, 5HT, ADP and ATP bind to platelet surface receptors to initiate platelet activation and subsequent activation of GPIIb/IIIa (α_{IIb}β₃) mediates platelet aggregation. Collagen binding to GPVI induces TXA₂ formation and release. Dense granules release 5HT, ADP and ATP which amplify platelet activation, with activation of P2Y₁₂ by ADP playing a major role in amplification. α-Granule release promotes thrombus formation and associated inflammatory responses. Microparticle formation and assembly of tenase and prothrombinase on the platelet surface promote thrombin generation. Nitric oxide (NO) and prostacyclin (PGI₂) released by intact endothelium inhibit platelet activation. Adapted from Ref. [27] with permission.

Platelet adhesion and initiation of thrombus formation

The presence on the platelet surface of various receptors that bind to adhesive ligands underlies the ability of platelets to adhere to subendothelial components that are exposed upon injury and breaching of the vascular endothelium, a process that represents the first step in the hemostatic response of platelets [19]. Plasma von Willebrand factor (VWF) can bind to subendothelial components such as collagen, and platelets, via GPIb α in the GPIb-IX-V receptor complex, bind rapidly to immobilized VWF, providing a mechanism for platelet adhesion at high shear rates [19]. $\alpha_2\beta_1$ and GPVI play pivotal roles in the binding of platelets to collagen in the subendothelial matrix, with GPVI playing a dominant role in the subsequent activation of platelets by collagen [19,20]. Initially, weak adhesion is stabilized through integrin activation and binding of VWF to GPIIb/IIIa as well as collagen to $\alpha_2\beta_1$ [20]. Signaling through GPIb-IX-V, GPVI and GPIIb/IIIa leads to powerful activation of platelets and the release of soluble agonists via the following mechanisms: (1) release of dense granule contents containing the soluble agonists ADP, ATP and 5HT; (2) activation of phospholipase A₂ and the formation and release of thromboxane A₂; and (3) platelet procoagulant activity leading to generation of thrombin (Figure 1.1). These soluble agonists bind to platelet receptors that are linked to G proteins and mediate further platelet activation and recruitment of other platelets into platelet aggregates.

Platelet P2 receptors

There are three P2 receptor subtypes on the platelet surface, P2X₁, P2Y₁ and P2Y₁₂ [21]. P2X₁ is a ligand-gated cation channel activated by ATP and plays a role in platelet shape change and collagen-induced platelet activation [21]. P2Y₁ and P2Y₁₂ are G-protein coupled receptors activated by ADP. The P2Y₁ receptor is linked to G_q and initiates ADP-induced platelet activation, this activation being then sustained and amplified via P2Y₁₂, which is coupled to G_i [21–24]. P2Y₁₂ also plays a major role in sustaining and amplifying the responses to numerous agonists since other agonists induce dense granule release and ADP released from dense granules then binds to the P2Y receptors. In addition to sustaining and amplifying platelet aggregation, P2Y₁₂ activation amplifies granule secretion and platelet procoagulant activity [24–26]. This is the basis for the importance of P2Y₁₂ in platelet function and the growing therapeutic success of antagonists that target this receptor [27].

In a similar fashion to ADP's action via the P2Y₁₂ receptor, epinephrine (adrenaline) and norepinephrine (noradrenaline) can also amplify platelet activation via α_{2A} adrenergic receptors but the observation that this requires supraphysiological concentrations of epinephrine and norepinephrine renders the pathophysiological significance of this pathway uncertain [28].

Platelet protease-activated receptors (PARs) and thrombin

PAR1 and PAR4 are expressed on human platelets with PAR1 likely playing the more important role, since it is activated at low thrombin concentrations whereas PAR4 requires higher thrombin concentrations in order to contribute to thrombin-induced platelet responses [29]. Thrombin cleaves the N-terminal exodomain on the PARs leading to a tethered peptide ligand that activates the receptor, a process that can be mimicked by thrombin receptor-activating peptides or TRAPs [29]. Whereas the P2Y₁ receptor is linked only to G_q, PAR1 is linked to both G_q and G12/13 and mediates strong platelet activation as manifest by the extent of granule secretion and procoagulant activity induced by PAR1 activation [29–31]. P2Y₁₂ activation plays a key role in promoting these responses [24,26,32]. GPIIb α may serve as a cofactor at the platelet surface, supporting PAR cleavage by thrombin [29,33]. The presence of PAR1 on other cell types involved in inflammatory responses provides a rationale for targeting this receptor in order to treat thrombotic diseases and associated inflammation [29].

The arachidonic acid pathway and thromboxane A₂

A group of phospholipases, collectively termed phospholipase A₂ (PLA₂), hydrolyse membrane phospholipids, such as phosphatidylcholine and phosphatidylserine, to produce arachidonic acid [34,35]. Arachidonic acid is rapidly converted by cyclooxygenase (COX) to prostaglandin G₂, which is then converted by peroxidase to prostaglandin H₂ (PGH₂) [34,36]. PGH₂ is then rapidly converted by thromboxane synthase to thromboxane A₂ (TXA₂). PGH₂ and TXA₂ are highly labile, potent platelet agonists that can diffuse across the plasma membrane and bind to specific G-protein coupled platelet receptors [30,34,37]. Studies of aspirin (acetylsalicylic acid), which acetylates and irreversibly inhibits COX, demonstrate the role of the arachidonic acid pathway in platelet responses to stimulation by different agonists. Aspirin abolishes platelet macroaggregation induced by arachidonic acid and substantially reduces platelet aggregation induced by low concentrations of collagen [38,39]. It also inhibits the “secondary wave” of macroaggregation induced by ADP, adrenaline and platelet-activating factor in citrated platelet-rich plasma but has little or no effect on platelet aggregation induced by these agonists in media containing physiological levels of divalent cations, indicating a restricted role for the arachidonic acid pathway in platelet activation under physiological conditions [24,40–43]. This explains why effective inhibition of COX by aspirin leaves many aspects of platelet function relatively intact.

Receptor pathways that inhibit platelet activation

The vascular endothelium presents an antithrombotic surface, in part related to the release by intact endothelium of nitric oxide (NO) and prostacyclin

(PGI₂), both of which act on platelet pathways that suppress platelet activation as well as having vasodilatory effects [44]. NO activates platelet guanylyl cyclase, raising platelet cyclic GMP levels and inhibiting agonist-induced rises in cytoplasmic calcium levels [45,46]. Platelet-derived NO also appears to limit thrombus formation and synthetic NO donors may have substantial antithrombotic effects [46,47]. Glutathione peroxidase potentiates inhibition of platelets by NO donors and inherited deficiency of the plasma isoform of this enzyme can lead to childhood ischemic stroke [46].

Endothelial COX-1 and prostaglandin G/H synthase-2 (PGHS-2; known as COX-2) mediate the production of PGI₂, which activates IP receptors on platelets and, via coupling to G_s, mediates an increase in platelet cyclic AMP, which in turn inhibits platelet activation [48,49]. The inhibition of these endothelial COX enzymes by traditional non-steroidal anti-inflammatory drugs (NSAIDs) and newer selective COX-2 inhibitors, and subsequent impairment of endothelial PGI₂ release, underlies the adverse cardiovascular effects of these drugs [49].

Another COX metabolite, PGE₂, has contradictory actions on platelets but, acting at low concentration via EP₃ receptors, may enhance platelet responses by opposing increases in cyclic AMP [50]. It is suggested that release of PGE₂ from inflamed vessel wall, such as atherosclerotic plaque, may counteract the inhibitory effects of PGI₂ and contribute to arterial thrombosis [50,51].

Adenosine has an inhibitory effect on platelet function, acting via A_{2A} receptors and increasing cyclic AMP levels [52]. It is proposed that plasma levels of adenosine increase sufficiently during ischemia or hypoxia to activate these receptors [52]. Adenosine and other A_{2A} receptor agonists have antithrombotic effects in animal models of thrombosis [53]. The antiplatelet drug dipyridamole acts by inhibiting adenosine uptake by blood cells, thereby increasing exposure of platelets to adenosine, as well as by inhibiting platelet cyclic GMP-dependent phosphodiesterase [54].

Platelet procoagulant activity

In their resting state, platelets have asymmetric distribution of aminophospholipids in the surface membrane bilayer with enzymes acting to keep these aminophospholipids (predominantly phosphatidylserine and phosphatidylethanolamine) in the inner layer [55]. Platelet activation is associated with an increase in the cytoplasmic ionized calcium concentration that inhibits the activity of these enzymes and also activates the enzyme scramblase that causes redistribution of the aminophospholipids to the outer layer where they are able to support the assembly of tenase and prothrombinase complexes and subsequent generation of thrombin in plasma. Microparticles are also shed under the action of calpain and these also have procoagulant properties. These processes are triggered by platelet GPVI receptor binding to collagen and play an important role in thrombin generation and arterial thrombogenesis [56].

Thrombin-induced activation of platelets further promotes platelet procoagulant activity, and P2Y₁₂ receptor activation and GPIIb/IIIa receptor outside-in signaling play important roles in amplifying this process, such that antagonists of these receptors inhibit platelet procoagulant responses [16,24,32,57–59].

Inflammatory responses of platelets

α -Granule secretion consequent to platelet activation leads to a number of processes that are considered pro-inflammatory. CD40L and P-selectin are translocated to the platelet surface as a result of fusing of the α -granule membrane with the surface membrane and are then gradually shed into the surrounding medium in their soluble forms. Soluble CD40L (sCD40L) induces cytokine production in vascular cells and may play a role in atherogenesis and restenosis, as well as promoting thrombosis by stabilizing platelet aggregates [60,61]. P-selectin binds to its counter-receptor on leukocytes, PSGL-1, helping to recruit leukocytes and monocyte-derived microparticles into thrombus, and the ensuing cross-talk between platelets, leukocytes and the microparticles promotes thrombogenesis and inflammatory responses [62,63]. Release of the chemokine RANTES by platelets, in conjunction with P-selectin, supports the binding of monocytes to inflamed endothelium and contributes to intimal hyperplasia in murine models [64,65]. β -thromboglobulin, platelet-derived growth factor and platelet factor 4 are other platelet α -granule contents that also contribute to inflammatory responses [6].

Nucleotides released from platelet dense granules may also have pro-inflammatory effects: both ADP and ATP can activate leukocytes whilst ATP acts on P2X receptors on vascular smooth muscle cell (VSMC) and may contribute to VSMC proliferation and migration [66,67]. These nucleotides also act on endothelial cells to promote nitric oxide and prostacyclin release [66].

The role of the platelet in coronary atherothrombosis

Atherothrombosis refers to the process whereby progression of atherosclerosis leads to plaque rupture or erosion, which induces arterial thrombus formation and, in some instances, clinical sequelae such as coronary artery thrombosis causing acute coronary syndromes or sudden cardiac death [68,69]. Coronary arterial plaques that have a thin fibrous cap overlying a lipid-rich core and abundance of inflammatory cells are particularly prone to rupture and are termed “vulnerable” or “high-risk” plaques [68,70]. Exposure of collagen, vWF and fibronectin following endothelial disruption leads to platelet adhesion and activation, as described in the previous section but, furthermore, the exposed lipid-rich core of high-risk plaque is highly thrombogenic, containing abundant tissue factor that initiates the coagulation cascade culminating in thrombin formation, which then leads to platelet activation and fibrin

deposition [68,69]. The central role of thrombin in the thrombosis that ensues following plaque disruption explains why anticoagulants such as heparins, direct thrombin inhibitors and factor Xa inhibitors have beneficial effects in the management of acute coronary syndromes [71–73] in addition to antiplatelet agents that target pathways that are involved in the platelet responses to thrombin and collagen, as described above. As well as exposure of tissue factor in the vessel wall, there is a circulating pool of tissue factor in blood that becomes concentrated at the site of vessel wall injury and contributes to thrombus formation [74]. Platelets, leukocytes and endothelial cells can express tissue factor, which may then be borne on circulating microparticles derived from these cells [75].

The formation of non-occlusive, platelet-rich thrombi over the sites of atherosclerotic plaque erosion or rupture with subsequent release of inflammatory mediators from platelet α -granules and platelet-leukocyte interactions may contribute to the progression of atherosclerotic lesions [76]. Such mural thrombi in coronary arteries may not be immediately associated with any clinical manifestations but instead contribute to the progressive narrowing of the arterial lumen that eventually leads to myocardial ischemia under conditions of increased myocardial oxygen demand and the associated symptom of angina pectoris. Platelets may also contribute to the vascular smooth muscle cell proliferation that leads to restenosis following percutaneous coronary intervention [77].

Conclusion

The rich variety of characteristics of platelets equips them for their role in hemostasis and physiological responses to vascular injury. Except in exceptional circumstances, it is only when the vessel wall becomes diseased that these physiological responses tend to be exaggerated and lead to thrombotic occlusion of the vessel lumen and excessive inflammation of the vessel wall. This explains why the platelet plays such an important role in the various manifestations of ischemic heart disease. Advances in the knowledge of platelet physiology have led to the development of antithrombotic therapies for managing ischemic heart disease and continue to inform the development of novel strategies.

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