

Chapter 2

Platelet Physiology

Matthew D. Linden

Abstract

Platelets are cell fragments which circulate in blood. They are of pivotal importance in blood clot formation, affecting thrombosis and haemostasis. By rapidly altering the activation and expression of surface receptors, platelets are able to quickly undergo structural and phenotypic changes in response to stimulation, such as collagen exposure on injured vascular endothelium. This response to stimulation allows platelets to become adhesive, aggregate to form a thrombus, and release a variety of mediators affecting coagulation, inflammation, and chemotaxis at the site of injury. Therefore, in addition to their critical role in thrombosis and haemostasis, platelets also play a role in immunity, inflammation, wound healing, haematologic malignancies, and metabolic disorders. The role of platelets in disease, particularly in atherothrombosis, is increasingly the focus of current research and antiplatelet therapy plays a significant role in the prevention and treatment of atherothrombotic and inflammatory diseases.

Key words Platelet physiology, Platelet structure, Granules, Platelet activation, Platelet receptors

1 Introduction

Platelets are small cells of large importance in medicine. They are involved in many pathophysiological processes, such as thrombosis, haemorrhage, inflammation, and cancer. The involvement of platelets in disease may be a direct primary disorder of platelet number and function, or an indirect result of the critical role of platelets in thrombosis, such as with coronary artery disease, stroke, peripheral vascular disease, and diabetes.

2 Platelet Structure

Platelets are subcellular fragments released from megakaryocytes that circulate in blood as small, granular, anuclear discs. These 3.0 by 0.5 μm discoid cells circulate in laminar blood flow near the apical surface of the endothelium for about 7 days or until they become activated, whereupon they undergo rapid metamorphic

changes to spread and adhere to damaged endothelial surfaces, release granules and aggregate with other platelets (1).

Platelets have a dynamic glycocalyx covered with glycoprotein receptors necessary to facilitate platelet adhesion, aggregation, and signal activation, principally the mobile receptors glycoprotein (GP) Ib–IX–V complex and integrin $\alpha_{\text{IIb}}\beta_3$ (the GPIIb–IIIa complex) (2). Anionic phospholipid, phosphatidylserine from the platelet membrane facilitates and acts as the site of formation of the prothrombinase complex of coagulation, and contains tissue factors—exposed on activation and decrypted with the release of microparticles—which also contributes to coagulation.

Below the plasma membrane of platelets lies a complex microtubule and microfilament cytoskeleton capable of dynamically changing platelet cell shape and surface area with activation (2). They contain many granules and bodies with haemostatic mediators, adhesion molecules, signaling molecules, calcium, ATP, ADP, and serotonin which can be released to the site of clot formation or expressed on the platelet surface in response to platelet activation (3).

2.1 The Platelet Cytoskeleton

The cytoskeleton of platelets is a spectrin mesh reinforced by a microtubule core and a rigid network of cross-linked actin filaments which maintains the discoid shape and maintains cell integrity against high shear forces as blood flow forces platelets against endothelium, and allows for transformation of the platelet shape with activation (1).

The spectrin membrane of platelets is a two-dimensional assembly of spectrin strands interconnected to each other in a mesh by actin filaments (4). Each molecular end of the spectrin molecule has an actin-binding site. Actin is the most abundant molecule in platelets; much of it is cross-linked into a rigid cytoplasmic network by homodimeric filamin (FLNa and FLNb) and α -actinin (5). These proteins act as “scaffolding molecules” which bind, via the carboxyl terminus, and localize molecules such as GTPase, sal A, sac, rho, cdc42, Trio and Toll, kinases, phosphatases, and transmembrane proteins, particularly the glycoprotein Ib $_{\alpha}$ subunit of the von Willebrand factor (vWF) receptor, adjacent to the plasma membrane (6).

A single coiled microtubule core, which sits in the cytoplasm, just beneath the plasma membrane, along the thin edge of the platelet, is responsible for the characteristic discoid shape of resting platelets (7).

When activated, the platelet cytoskeleton undergoes significant alteration to facilitate shape change, attachment to and spreading of platelets over the damaged endothelium. Activation also initiates other platelet responses such as secretion of granules (see Subheading 2.2) which moves adhesion receptors to the cell surface and releases agonists and mediators to the circulation, activates biochemical pathways—such as stimulating the synthesis and

release of thromboxane, and causes conformational changes in platelet surface receptors—such as activation of the $\alpha_{\text{IIb}}\beta_3$ integrin to allow fibrinogen and vWF binding and thus platelet aggregation (8).

Platelet shape change is a complex, actin-dependent process that involves reorganization of the platelet cytoskeleton and assembly of new actin filaments. This process follows a reproducible sequence as the platelet activates and spreads, beginning with spherizing as a result of a transient rise in cytosolic calcium concentration in response to activation of phospholipase C secondary to platelet receptor binding (9). Phospholipase C hydrolyzes membrane-bound polyphosphoinositide P_2 (PIP_2) into inositol triphosphate (IP_3) (9). IP_3 then binds to dense granules, releasing the calcium stored in them (9). Furthermore, active transport of extracellular calcium across the plasma membrane by calcium channels occurs (10). The increased intracellular calcium causes a conformational change in gelsolin, allowing it to bind and F-actin and cleave filaments, resulting in the loss of normal discoid platelet shape and spherizing of the cell (10).

New actin filaments are assembled from unpolymerized pools of actin in the cytoplasm and attach to the barbed end of the filaments fragmented by gelsolin (11). These filaments extend, causing lamellar spreading of the platelet, before the actin is capped by CapZ, preventing further filament assembly (11, 12). This extension unfolds invaginations of the plasma membrane and surface-connected open canalicular system to increase surface area of the platelet. During activation, underlying actin filaments also become tethered to integrin $\alpha_{\text{IIb}}\beta_3$. Through calcium- and rho kinase-stimulated phosphorylation of platelet myosin, contractile force is applied to the actin, $\alpha_{\text{IIb}}\beta_3$, and the fibrinogen bound to it, causing clot retraction (13). The action of myosin phosphorylation is also involved in the secretion of granules and the modulation of platelet surface receptors, such as the down-regulation of glycoprotein Ib (GPIb)–IX–V complex from the surface after activation (1).

2.2 Platelet Granules and Secretion

Platelets contain α -granules, dense granules, and lysosomes (3). Secretion of granule contents releases haemostatic mediators at the site of vascular injury, or causes expression of surface molecules which facilitate cellular adhesion. α -granules are the most numerous of the platelet organelles, and are 200–500 nm in diameter with a highly organized interior substructure divided into different zones. They contain a variety of adhesion molecules, chemokines, coagulation and fibrinolysis proteins, growth factors, immunologic molecules, and other proteins (Table 1) (3, 14). Dense granules contain ionic calcium, magnesium, phosphate, and pyrophosphate as well as ATP, GTP, ADP, and GDP nucleotides and the transmitter serotonin (15). Platelet lysosomes, though few in number,

Table 1
Components of alpha granules

Adhesion molecules	Fibronectin, fibrinogen, integrin $\alpha_{IIb}\beta_3$, integrin $\alpha_v\beta$ P-selectin, von Willebrand factor
Chemokines	β -Thromboglobulin, growth-regulated oncogene α , interleukin 8, macrophage inflammatory protein 1 α , monocyte chemotactic protein 3, neutrophil-activating protein, platelet factor 4, RANTES
Coagulation proteins	Factor V, factor VIII, high-molecular-weight kininogen, multimerin
Fibrinolysis proteins	α_2 -Macroglobulin, plasminogen, plasminogen activator inhibitor 1
Growth factors	Basic fibroblast growth factor, epidermal growth factor, hepatocyte growth factor, insulin-like growth factor 1, platelet-derived growth factor, transforming growth factor β , vascular endothelial growth factor
Immunologic molecules	BIH globulin, c1 inhibitor, factor D, IgG
Other proteins	α_1 antitrypsin, albumin, osteonectin

contain acid hydrolases, cathepsins, and lysosomal membrane proteins similar to lysosomes in other cells (3).

Exocytosis of platelet granules and secretion of granular contents occur after cells are activated by specific ligands that interact with platelet membrane receptors through G-protein couple signaling. Diglycerol, which results from the cleavage of membrane phosphatidylinositol 4,5 bisphosphate (PIP₂) to IP₃, activates protein kinase C (PKC) which acts in synergy with calcium ions released from dense granules by IP₃ to amplify the secretion of granules (see Subheading 4). Changes to the cytoskeleton associated with platelet activation facilitate the development, targeting, and exocytosis of secretory granules (16, 17). Platelet shape change can occur without secretion, particularly when signaling via the G_i but not G_q pathways takes place, but secretion cannot take place if the cytoskeletal rearrangement is inhibited (18).

2.3 Microparticles

Activated platelets release two types of membrane vesicles: (1) platelet-derived microparticles (PMPs) budded from the plasma membrane, and (2) exosomes, which are smaller than PMP and released from α -granules during secretion (19).

Signal transduction resulting from agonists such as thrombin or collagen-binding receptors on the surface of platelets, and the resulting elevation of intracellular calcium, results in the activation of several enzymes, such as calpain and PKC, which facilitate PMP production by degrading structural proteins including actin-binding protein, talin, and the heavy chain of myosin (20, 21). Concurrently, the platelet cell membrane loses its organized asymmetrical distribution and negatively charged aminophospholipids phosphatidylserine (PS) and phosphatidylethanolamine (PE) become expressed

on the surface, facilitating interaction with the coagulation system and the tenase complex (see Subheading 5). PS and PE are therefore also expressed on the PMPs which bud off from the platelet, and thus PMPs are procoagulant particles (22, 23). Furthermore, platelets and PMPs share glycoprotein receptors such as GPIb, PECAM-1, and integrin $\alpha_{IIb}\beta_3$, and subpopulations may express P-selectin from platelet granules, suggesting that PMPs can participate in cellular interactions, adhesion, and aggregation (19).

In addition to being formed as a result of agonist-induced platelet activation, PMPs may form as a result of complement activation/damage to platelets, and platelet aging and destruction, and may be released directly from megakaryocytes in platelet genesis (19).

3 Platelet Receptors

Platelets express a variety of different receptors on their surface, most having a direct role in haemostasis by activating the platelet in response to agonists, as adhesive receptors interacting with the damaged cell wall, or aggregating with other platelets and other cells to contribute to thrombus formation (24). By changing the expression and activation status of these surface molecules, platelets are able to markedly change their phenotype to carry out physiological functions or deal with pathological events. Table 2 summarizes the known platelet receptors, their structural family, function, and importance. A number of the more major receptors are discussed in detail.

3.1 GPIb–IX–V

GPIb is a heterodimeric transmembrane protein consisting of a disulfide-linked 140 kDa alpha chain and 22 kDa beta chain (25, 26). It exists on the platelet surface in complex non-covalent association with GPIX and platelet GPV to form the receptor for vWF and mediates platelet adhesion to the arterial circulation (27). vWF, which is present in blood, must undergo conformational alterations through binding to collagen prior to being recognized and bound by the GPIb alpha chain of the receptor complex on platelets. Furthermore, this process is much more efficient when high shear stress exerts conformational changes on both the vWF and the GPIb–IX–V complex (27).

Interaction between the GPIb–IX–V complex on the surface of platelets and vWF bound to collagen, while insufficient to firmly adhere platelets to the matrix, allows them to roll along the matrix and facilitates firm adhesion via other surface proteins. However, binding of vWF to the receptor complex causes transmembrane signaling through phosphorylation of the intracellular chain of GPIb beta, resulting in activation of $\alpha_{IIb}\beta_3$ to its ligand-receptive

state (see Subheading 3.2), and acts synergistically with signals generated from the binding of collagen to GPVI and $\alpha_2\beta_1$ (Table 2) (28, 29).

In addition to binding collagen–vWF and causing platelet activation, GPIb–IX–V also acts as a receptor for Mac-1 on leukocytes (30). Leukocytes and activated platelets bind primarily through interaction of P-selectin on the activated platelet to constitutively express P-selectin glycoprotein ligand 1 (PSGL-1) on the leukocyte (31). This proximity and heterotypic aggregation of cells allow the N-terminal region globular region of GPIb alpha to recognize the alpha chain of activated Mac-1 (25, 32). This process is thought to mediate firm aggregation of the platelet and

Table 2
Platelet membrane receptors

Class	Family	Receptor	Function
Integrins	β_3	$\alpha_{IIb}\beta_3$	The glycoprotein IIb–IIIa complex, also CD61/CD41. A unique platelet receptor that is essential for fibrinogen binding and platelet aggregation
		$\alpha_v\beta_3$	Vitronectin receptor of low importance and low expression on platelets
	β_1	$\alpha_2\beta_1$	The glycoprotein Ia–IIa complex, also CD49b. A major collagen adhesion receptor on platelets. In combination with GPIb–IX–V complex, allows firm adhesion and platelet activation in response to collagen stimulation
		$\alpha_5\beta_1$	Fibronectin receptor with a supplementary role in platelet adhesion at injury sites
		$\alpha_6\beta_1$	Laminin receptor with a supplementary role in platelet adhesion at injury sites
	β_2	$\alpha_L\beta_2$	May play a role in regulation or caspase activation. Expressed in platelet granules and only found on the surface of platelets that have undergone activation and secretion
Leucine-rich repeat (LRR)	Toll-like receptors	GPIb–IX–V	Pivotal platelet receptor in initiating and propagating haemostasis and thrombosis with a number of ligands including collagen, von Willebrand Factor (vWF), thrombospondin, P-selectin, and leukocyte integrin Mac-1
		TLR1	Lipopolysaccharide receptors which increase platelet adhesion to fibrinogen and increase platelet activation to agonists such as thrombin. Present on about 40% of human platelets
		TLR2	
		TLR4	
		TLR6	
		TLR9	

(continued)

Table 2
(continued)

Class	Family	Receptor	Function
Seven-transmembrane receptors	Thrombin receptors	PAR1	After cleavage by thrombin acts as a ligand and receptor signaling platelet activation
		PAR4	
		P2Y ₁	Mediates transient platelet shape change and aggregation by ADP binding via G _q signaling leading to calcium release and activation of phospholipase C
	ADP receptors	P2Y ₁₂	Inhibits adenylyl cyclase by ADP binding via G _{ai2} signaling and amplified mobilization of cytoplasmic calcium by P2Y ₁
		Thromboxane receptor A ₂	Activates platelets via phospholipase A2 and phospholipase C through G _q , G _{i2} , and G _{12/13} . Important for autocrine amplification of platelet activation following binding of other receptors
		PGI ₂ receptor	Binds endothelial prostacyclin to inhibit platelet activation via G _s signaling and adenylyl cyclase
		PGD ₂ receptor	Similar in mechanism but distinct from PGI ₂ receptor
		PGE ₂ receptor	Potentiates platelet response to ADP at low concentrations, but inhibits aggregation at high concentrations
	Lipid receptors	Platelet-activating factor receptor	Activates platelet via G _q and G _i protein signaling
		Lysophosphatidic acid receptor	Causes shape change, degranulation, and aggregation
	Chemokine receptors	CXCR4	May be involved in megakaryocytosis and platelet-leukocyte interactions
		CCR4	
		CCR3	
		CCR1	
	Vasopressin receptor	V _{1a} receptor	Causes rapid but reversible platelet activation via G _{q11} signaling
	Adenosine receptor	A _{2a} receptor	Inhibits vasopressin or PAF-induced platelet activation via G _s signaling
	Epinephrine receptor	β2-Adrenergic receptor	Augments platelet activation caused by other agonists
	Serotonin receptor	5-HT _{2A} receptor	Causes autocrine platelet activation and degranulation via G-protein and calcium signaling
	Dopamine receptor	D3	Involved in dopamine uptake and may inhibit platelet function
		D5	
Immunoglobulin superfamily	GPVI	GPVI	Major collagen receptor

leukocyte, as well as transmigration of the leukocyte through the mural thrombus to sites of vascular injury, thus providing a potentially important role for this receptor in inflammation.

GPIb–IX–V also plays a role in tethering of platelets to endothelium via binding of the receptor to P-selectin expressed by endothelial cells (33), and has two binding sites for thrombin, important in the response of platelets to low concentrations (<0.1 U/mL) of thrombin (32, 34), while the PAR family of receptors are more important where higher concentrations are present (see Subheading 3.3). The receptor complex also contributes to the soluble coagulation system through interactions with high-molecular-weight (HMW) kininogen, as well as coagulation factors XII and XI (34, 35).

Despite the advances in understanding of the role of GPIb–IX–V in vWF tethering, maintenance of coagulation, inflammation, and signaling, much remains to be learned about the functions of this unique and complicated receptor complex (25).

3.2 $\alpha_{IIb}\beta_3$

Integrin $\alpha_{IIb}\beta_3$, also known as the GPIIb–IIIa complex, is the most abundant glycoprotein on platelet membranes, and plays an important role in platelet aggregation and signaling as well as interaction with the blood coagulation system and other cell types—such as endothelial cells. $\alpha_{IIb}\beta_3$ binds several adhesive proteins, including fibrinogen (coagulation factor I), prothrombin (coagulation factor II), vWF, fibronectin, neural cell adhesion molecule L1, and vitronectin (36). Much of its binding activity is due to KQAGDV and RGD recognition sequences and binding sites (37).

$\alpha_{IIb}\beta_3$ maintains a low affinity for ligand binding on resting circulating platelets. However conformational changes in $\alpha_{IIb}\beta_3$ as a result of inside-out signaling secondary to ligand binding of agonist receptors (such as ADP, thrombin), as well as clustering of the receptor following release of $\alpha_{IIb}\beta_3$ from the cytoskeleton, lead to a large increase in receptor affinity for its various ligands and increased adhesive capacity (8). Once activated, $\alpha_{IIb}\beta_3$ results in both platelet adhesion to the site of vascular injury and aggregation of platelets causing thrombus propagation. Binding of activated $\alpha_{IIb}\beta_3$ to immobilized fibrinogen and vWF at the site of vascular injury results in spreading of the platelet, firm adhesion of the platelet to the vascular wall, and outside-in signaling leading to amplification of platelet activity, while binding of activated $\alpha_{IIb}\beta_3$ to soluble fibrinogen and vWF promotes cell-to-cell adhesion and platelet aggregation as well as outside-in signaling (36). Therefore $\alpha_{IIb}\beta_3$ is both critical to the adhesive and aggregatory properties of activated platelets as well as essential in the amplification of activation signaling from agonist receptors.

3.3 *Thrombin Receptors*

Thrombin plays a critical role in the regulation of haemostasis and thrombosis primarily through its proteolytic function within the

coagulation cascade. In addition to this action, thrombin also exerts profound effects on a diverse range of cells, including platelets, through cleavage of a number of G-protein-coupled, seven-transmembrane domain, protease-activated receptors (PARs) (38).

PAR1 is the predominant receptor for thrombin-mediated platelet activation and secretion in humans (39, 40). Thrombin interacts with a hirudin-like DKEYPF binding domain on the N-terminus of PAR1, facilitating cleavage at an LDPR/SFLLR sessile bond and generating a new amino terminus, leading to self-activation via a tethered ligand mechanism, resulting in G-protein signaling (40–42). Synthetic peptides containing at least the first five amino acids of the tethered ligand (SFLLR), known as thrombin receptor-activating peptides (TRAP), are able to effect receptor activation without the need for receptor proteolysis, and is commonly employed in laboratory analysis of PAR1 activation without triggering the coagulation system (39, 43). PAR1 is the primary low-dose thrombin receptor on human platelets.

PAR3 is minimally expressed in human platelets. Like PAR1, PAR3 is thought to utilize a hirudin-like domain for thrombin interaction and cleavage at a LPIKTFRGAP sequence generating a tethered ligand (44). However, synthetic peptides of the tethered ligand do not cause PAR3 activation, suggesting that a conformational change in the receptor caused by cleavage is required before the tethered ligand is recognized. PAR3 is the primary low-dose thrombin receptor in mice, but plays little role in humans due to its minimal expression on human platelets.

PAR4, while less abundant than PAR1, is readily expressed on human platelets, although it is much less sensitive to cleavage by thrombin than PAR1 or PAR3 as it lacks the hirudin-like binding sequence of these molecules (45). Instead, thrombin cleaves PAR4 at the Arg-Gly bond in the PAPRGYPGQV sequence, resulting in an exposure of a tethered ligand that binds the PAR4 receptor and activates G-protein signaling. Like PAR1, synthetic peptidomimetics of the tethered ligand can elicit a thrombin-like response from PAR4 without the need for cleavage (45). PAR4 is the principal high-dose thrombin receptor on both mouse and human platelets.

Upon PAR activation, a series of distinct signaling pathways mediated by $G\alpha_s$, $G\alpha_i$, $G\alpha_q$, and $G\alpha_{12/13}$ proteins are initiated (see Subheading 4). These signaling events involve phosphoinositide hydrolysis, protein phosphorylation, increased cytosolic calcium, and suppression of cyclic AMP and ultimately converge in cytoskeletal actin reorganization and integrin activation (10, 46). Shape change resulting in actin reorganization results in the internalization or blebbing off (microparticles) of cleaved thrombin receptors, while cell spreading exposes PAR receptors from the canalicular system to the platelet surface, increasing the potential for further platelet activation by thrombin (47).

The platelet GPIb–IX complex is also a receptor for thrombin (see Subheading 3.1). While the functional relevance of thrombin–GPIb–IX binding on platelet activation is unclear, functional GPIb–IX–V complex is required for optimal thrombin responsiveness in humans, although not necessarily functioning directly in the signal transduction mechanism, and may simply be a result of localization of thrombin to the platelet surface through GPIb–IX–V binding.

3.4 P2Y Nucleotide Receptors

Nucleotide receptors are classified into P1 (A_1 through A_4 adenosine receptors) and P2 (ATP and ADP receptors) (24). The P2 receptors are further categorized as ion-channel linked (P2X) or G-protein linked (P2Y). Two P2Y receptors play a major role in platelet aggregation; these are the G_q -coupled receptor P2Y₁ and the G_i -coupled receptor P2Y₁₂ (48, 49). Both receptors are bound to and activated by adenosine diphosphate (ADP), while adenosine triphosphate (ATP) acts as an antagonist for both.

P2Y₁ signals through phospholipase C and is responsible for the mobilization of intracellular calcium ions, thereby mediating shape change and aggregation (50). P2Y₁₂ inhibits adenylate cyclase, thus inhibiting cyclic adenosine monophosphate (cAMP) production, leading to increased platelet activation via dephosphorylation of vasodilator-stimulated phosphoprotein (VASP), which in turn leads to activation of the $\alpha_{IIb}\beta_3$ integrin and decreased inhibition of calcium mobilization and granule release (50).

4 Platelet Signaling

The link between the myriad of surface receptors and their effects on platelet function is network of signaling molecules and regulators including heterotrimeric G-proteins that associate surface receptors and intracellular effectors, Ras proteins that act as GTP-binding switches, and phospholipases that signal via hydrolysis of phosphoinositides and formation of prostanoids, lipid kinases, protein tyrosine kinases, and serine/threonine kinases that regulate enzyme activity (24). In general, this involves activation of phospholipase C and PI 3-kinase-dependent pathways and suppression of cAMP and adenylyl cyclase, which normally act to prevent platelet activation.

In vivo platelet activation is usually initiated by collagen and vWF or, in the case of pathology, thrombin. In collagen-induced platelet activation, collagen and vWF bind to several molecules on the platelet membrane (see Subheading 2) including GPIb–IX–V complex, GPVI, and integrin $\alpha_{IIb}\beta_3$ and $\alpha_2\beta_1$. Collagen causes clustering of GPVI and its constitutively associated Fc receptor γ -chain (51). This leads to phosphorylation of the γ -chain by Src kinases

which associate with Syk and phosphorylation and activation of phospholipase $C\gamma_2$ ($PLC\gamma_2$). This results in the production of IP_3 —which opens Ca^{2+} channels and thus increases intracellular calcium, and diacylglycerol (DAG) which activates PKC, which phosphorylates serine/threonine to activate $\alpha_{IIb}\beta_3$ to expose fibrinogen-binding sites for aggregation, shape change, and activation. Thus the activation of platelets is a slow process, requiring polymerized collagen and clustering of GPVI. Coincident binding of vWF to $\alpha_{IIb}\beta_3$ to GPIb slows the platelet and localizes it to the endothelium long enough for this process to occur (24).

In contrast, thrombin induces platelet activation via a G-protein-dependent mechanism which results in faster and more intense activation of $PLC\beta$. Thrombin binds to and cleaves the N-terminus of the PAR 1 and 4 in humans. This cleavage exposes a new N-terminus which acts as a tethered ligand (39), signaling via $G_{q\alpha}$, G_{12} , and G_i with downstream activation to a host of intracellular effectors (38, 52). $G_{q\alpha}$ activates $PLC\beta$, resulting in the generation of IP_3 from PIP_2 and release of intracytosolic calcium, and activated PKC from DAG (53, 54). Thus $G_{q\alpha}$ is responsible for integrin activation and fibrinogen binding and increased cytosolic calcium. G_{12} is coupled to guanine nucleotide exchange factors (GEF) which activate Rac and Rho signaling pathways to uncap actin filaments and reorganize the cytoskeleton to produce shape change, degranulation, and spreading (55). G_i inhibits adenylyl cyclase, resulting in diminished cAMP and thus promoting platelet activation (38).

Following initial activation of the platelet, additional circulating platelets are activated and recruited to the thrombus by the local accumulation of molecules that are secreted by the platelets in the primary thrombus, such as ADP or thromboxane A_2 (TxA_2) (Fig. 1). The signaling pathways involved in this extension phase of platelet thrombus formation are predominantly through high-affinity G-protein-coupled receptors similar to thrombin, often with multiple G-proteins activated by the same receptor, and thus rapid amplification of activation can take place. ADP is released by degranulation, while thromboxane is generated by cyclooxygenase from arachidonic acid in response to platelet activation. Both ADP and TxA_2 release result in further platelet activation and recruitment of nearby resting platelets into the growing thrombus in a self-propagating cycle. Binding of the thromboxane prostanoid (TP) receptor leads to shape change via G_q and Rho signaling, and activation of PKC via $PLC\beta$ and G_{12} , similar to $P2Y_1$. This stimulated further release of TxA_2 and amplification of the platelet thrombus formation.

Once $\alpha_{IIb}\beta_3$ has been activated by PLC, it binds fibrinogen and platelet aggregation can occur. The close cell-to-cell contact between platelets in aggregation gives rise to another phase of

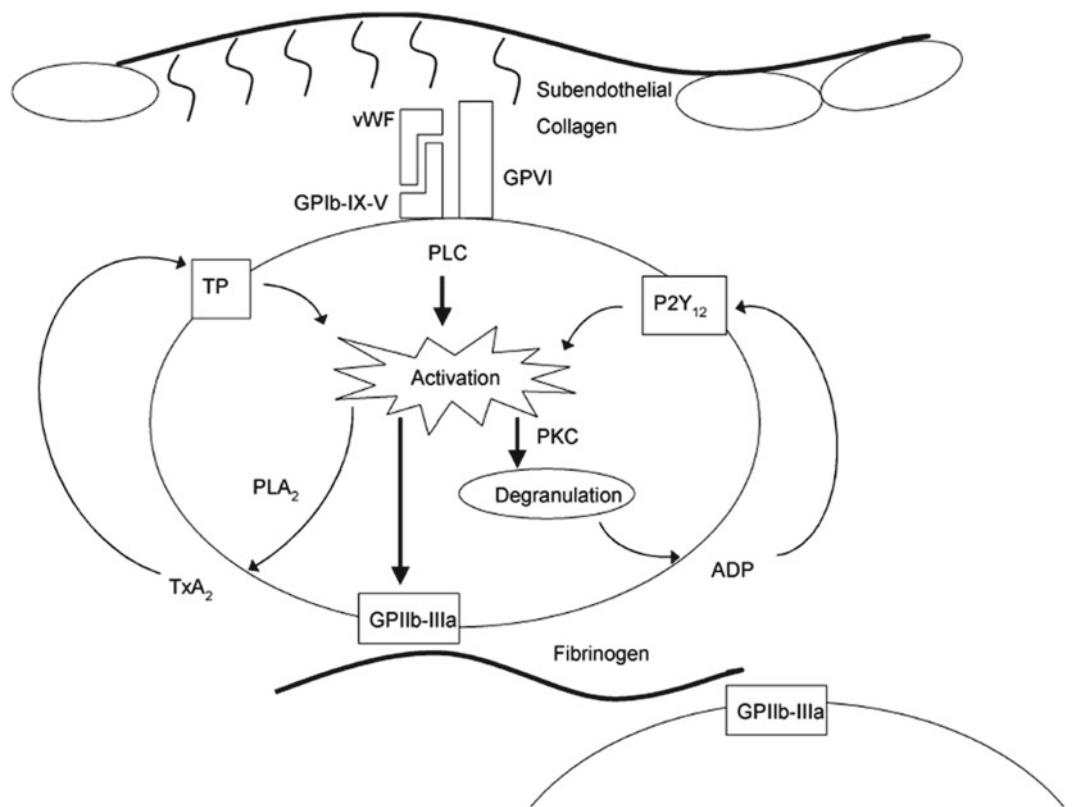


Fig. 1 Platelet activation is normally initiated by exposure of collagen and vWF. This leads to release of ADP through degranulation and synthesis of thromboxane A₂. Both of these bind constitutively expressed receptors on the platelet surface, and represent a self-perpetuating signal amplification loop, and a means of recruiting nearby resting platelets. An activation-dependent conformational change in GPIIb-IIIa allows fibrinogen binding and therefore platelet aggregation

Table 3
G-protein-coupled receptors expressed on human platelets

G-protein	Receptors	Function
G _i	A _{2A} -adrenergic, CXCR4, P2Y ₁₂ , PAR1	Decrease cAMP by inhibiting adenylyl cyclase Increase IP ₃ /DAG via PLC Increase 3-PPIs
G _q	PAF, PAR1, PAR4, P2Y ₁ , TPα, TPβ, vasopressin 1	Increase IP ₃ /DAG via PLC
G ₁₂	PAR1, PAR4, P2Y ₁ , TPα, TPβ	Actin assembly and reorganization
G _s	Prostaglandin I receptor	Increases cAMP by adenylyl cyclase

signaling that results in outside-in signaling through integrins and binding of ephrins to Eph kinases (56). Table 3 lists the G-proteins expressed on human platelets, their associated receptors, and function.

5 Platelet Interactions

Platelet physiology cannot be considered in isolation from the other components of blood and the vascular system which interact to contribute to haemostasis and thrombosis. Platelets bind to injured or activated endothelium, while leukocytes bind to activated platelets and the heterotypic thrombus serves as a major pro-coagulant site (57) and plays an important role in inflammation. These multicellular interactions are mediated by adhesive molecules such as P-selectin from platelet granules, PSGL-1 on leukocytes and endothelium, and GPIb (31).

5.1 Platelet–Leukocyte Interactions

Platelets are a central cellular interface of thrombotic and inflammatory processes (58) and modulate this interface by binding to leukocytes and altering their function (57, 59). Correspondingly, platelets play a pivotal role in recruitment of monocytes and neutrophils to sites of vascular injury, and thus to the atherosclerotic plaque (60). Platelet activation, formation of leukocyte–platelet aggregates (monocyte–platelet aggregates and neutrophil–platelet aggregates), and platelet secretion of inflammatory modulators that affect leukocyte function, such as CD40 ligand (CD40L), are associated with the development of atherosclerosis (61–63), stable coronary artery disease (CAD) (64), unstable angina (65), myocardial infarction (MI) (66), and events following percutaneous coronary intervention (PCI) (66), with a greater magnitude in patients experiencing late clinical events (67).

P-selectin (CD62P) is a component of the α -granule membrane and is not normally expressed on the surface of platelets (59, 68–70). Upon platelet activation α -granule-soluble contents are released and P-selectin is exposed on the platelet surface (59, 69, 70). In vitro, the activation-dependent increase in platelet surface P-selectin is not reversible over time (71, 72). However, in vivo, circulating degranulated platelets rapidly lose their surface P-selectin, yet continue to circulate and function (73).

Surface expression of P-selectin on platelets mediates the initial adhesion of activated platelets to monocytes and neutrophils via PSGL-1, which is constitutively expressed on the surface of these leukocytes (59, 74–76).

Following initial tethering to activated platelets via P-selectin/PSGL-1 interaction, leukocyte activation occurs through signaling via PSGL-1 and platelet-secreted chemokines and lipid mediators (77–82). This response in turn causes activation and upregulation of the Mac-1 integrin ($\alpha_M\beta_2$, CD11b/CD18) on the monocyte surface, allowing firm adhesion to platelets via bridging fibrinogen bound to the activated glycoprotein IIb–IIIa integrin ($\alpha_{IIb}\beta_2$, CD61/CD41) (57, 80), and via direct interaction with GPIb α (30) on the platelet surface. Other adhesion molecules such as

LFA-1 on the monocytes interacting with ICAM-2 on platelets may play a role in stabilizing heterotypic aggregates (83). However, P-selectin/PSGL-1 interaction is first required before these stable secondary adhesions may take place. The exact physiologic significance of leukocyte–platelet aggregation is unknown, but it may represent targeting of both cell types to appropriate inflammatory or haemostatic sites (84).

5.2 Platelet– Endothelial Cell Interaction

Resting platelets under shear roll on activated endothelial cells via interaction of endothelial P-selectin with platelet surface GPIb α or PSGL-1 (33, 85) and vWF interaction with GPIb α (86). This rolling accumulates platelets at the site of injury, and allows for other interactions to take place (85). Following platelet activation, a more stable association between endothelial cells and platelets occurs, mediated by GPIIb–IIIa-bound fibrinogen on platelets binding to ICAM-1 and $\alpha_v\beta_3$ on endothelial cells (87).

5.3 Platelet– Coagulation System Interaction

Platelet adherence and aggregation at the site of vascular injury not only serves to form a platelet-rich haemostatic plug or thrombus but also serves as a site of activation and assembly of the coagulation system, to direct its haemostatic potential to the site of injury and prevent systematic widespread intravascular clot formation. Activated platelets regulate propagation of the coagulation system by releasing granule components that trigger and propagate the coagulation cascade, express specific high-affinity receptors for coagulation components which protect them from inactivation, act as the site for assembly of the tenase and prothrombinase complex formation, and amplify the initial stimulus leading to a rapid and localized thrombin burst (88).

6 Summary

Platelets are specialized, anuclear cells not only of pivotal importance in thrombosis and haemostasis but which also play a role in innate immunity, inflammation, wound healing, haematologic malignancies, and metabolic disorders. Platelets have considerable cross talk with other cells, including other platelets, leukocytes, and endothelial cells. They also interact with the coagulation cascade and the humoral immune system. This is accomplished by a complex array of surface receptors, adhesion proteins, integrins, and glycoproteins coupled to multiple signaling pathways which orchestrate initiation, extension, and propagation of platelet activation both with redundancy and the ability to modulate function. The role of platelets in disease, particularly in atherothrombosis, is increasingly the focus of current research and antiplatelet therapy plays a significant role in the prevention and treatment of atherothrombotic and inflammatory diseases.

References

- Hartwig JH (2002) Platelet structure. In: Michelson AD (ed) *Platelets*. Academic, San Diego, CA, pp 37–52
- Boyles J, Fox JE, Phillips DR, Stenberg PE (1985) Organization of the cytoskeleton in resting, discoid platelets: preservation of actin filaments by a modified fixation that prevents osmium damage. *J Cell Biol* 101:1463–1472
- Reed GL (2002) Platelet secretion. In: Michelson AD (ed) *Platelets*. Academic, San Diego, CA, pp 181–195
- Hartwig JH, DeSisto M (1991) The cytoskeleton of the resting human blood platelet: structure of the membrane skeleton and its attachment to actin filaments. *J Cell Biol* 112:407–425
- Tablin F, Reeber MJ, Nachmias VT (1988) Platelets contain a 210 K microtubule-associated protein related to a similar protein in HeLa cells. *J Cell Sci* 90(Pt 2):317–324
- Kovacsovics TJ, Hartwig JH (1996) Thrombin-induced GPIb-IX centralization on the platelet surface requires actin assembly and myosin II activation. *Blood* 87:618–629
- Schwer HD, Lecine P, Tiwari S, Italiano JE Jr, Hartwig JH, Shivdasani RA (2001) A lineage-restricted and divergent beta-tubulin isoform is essential for the biogenesis, structure and function of blood platelets. *Curr Biol* 11:579–586
- Shattil SJ, Hoxie JA, Cunningham M, Brass LF (1985) Changes in the platelet membrane glycoprotein IIb/IIIa complex during platelet activation. *J Biol Chem* 260:11107–11114
- Berridge MJ (1984) Inositol trisphosphate and diacylglycerol as second messengers. *Biochem J* 220:345–360
- Brass LF (1999) More pieces of the platelet activation puzzle slide into place. *J Clin Invest* 104:1663–1665
- Hartwig JH (1992) Mechanisms of actin rearrangements mediating platelet activation. *J Cell Biol* 118:1421–1442
- Carlier MF, Didry D, Erk I, Lepault J, Van Troys ML, Vandekerckhove J, Perelroizen I, Yin H, Doi Y, Pantaloni D (1996) Tbeta 4 is not a simple G-actin sequestering protein and interacts with F-actin at high concentration. *J Biol Chem* 271:9231–9239
- Knezevic I, Leisner TM, Lam SC (1996) Direct binding of the platelet integrin alphaIIb beta3 (GPIIb-IIIa) to talin. Evidence that interaction is mediated through the cytoplasmic domains of both alphaIIb and beta3. *J Biol Chem* 271:16416–16421
- Niewiarowski S, Holt JC, Cook JJ (1994) Biochemistry and physiology of secreted platelet proteins. In: Coleman RW, Hirsh J, Marder VJ, Salzman EW (eds) *Haemostasis and thrombosis: basic principles and clinical practice*. Lippincott, Philadelphia, PA, pp 546–556
- Fukami MH (1997) Dense granule factors. In: von Bruchhausen F, Walter U (eds) *Platelets and their factors*. Springer, New York, pp 419–432
- Knight DE, Hallam TJ, Scrutton MC (1982) Agonist selectivity and second messenger concentration in Ca²⁺-mediated secretion. *Nature* 296:256–257
- Chang JD, Ware JA (1997) Ca²⁺ and Protein Kinase C in Platelets. In: Bittar EE & Lapretina EG (eds) *Advances in Molecular and Cell Biology Vol 18: The Platelet*. JAI Press, Greenwich, pp 275–310
- Shirakawa R, Yoshioka A, Horiuchi H, Nishioka H, Tabuchi A, Kita T (2000) Small GTPase Rab4 regulates Ca²⁺-induced alpha-granule secretion in platelets. *J Biol Chem* 275:33844–33849
- Nieuwland R, Sturk A (2002) Platelet-derived microparticles. In: Michelson AD (ed) *Platelets*. Academic, San Diego, CA, pp 255–265
- Wiedmer T, Shattil SJ, Cunningham M, Sims PJ (1990) Role of calcium and calpain in complement-induced vesiculation of the platelet plasma membrane and in the exposure of the platelet factor Va receptor. *Biochemistry* 29:623–632
- Wiedmer T, Sims PJ (1991) Participation of protein kinases in complement C5b-9-induced shedding of platelet plasma membrane vesicles. *Blood* 78:2880–2886
- Sims PJ, Faioni EM, Wiedmer T, Shattil SJ (1988) Complement proteins C5b-9 cause release of membrane vesicles from the platelet surface that are enriched in the membrane receptor for coagulation factor Va and express prothrombinase activity. *J Biol Chem* 263:18205–18212
- Sims PJ, Wiedmer T, Esmon CT, Weiss HJ, Shattil SJ (1989) Assembly of the platelet prothrombinase complex is linked to vesiculation of the platelet plasma membrane. Studies in Scott syndrome: an isolated defect in platelet procoagulant activity. *J Biol Chem* 264:17049–17057
- Clemetson KJ (2002) Platelet receptors. In: Michelson AD (ed) *Platelets*. Academic, San Diego, CA, pp 65–84
- Lopez JA, Berndt MC (2002) The GPIb-IX-V complex. In: Michelson AD (ed) *Platelets*. Academic, San Diego, CA, pp 85–104
- Lopez JA (1994) The platelet glycoprotein Ib-IX complex. *Blood Coagul Fibrinolysis* 5:97–119

27. Fredrickson BJ, Dong JF, McIntire LV, Lopez JA (1998) Shear-dependent rolling on von Willebrand factor of mammalian cells expressing the platelet glycoprotein Ib-IX-V complex. *Blood* 92:3684–3693
28. Ramakrishnan V, DeGuzman F, Bao M, Hall SW, Leung LL, Phillips DR (2001) A thrombin receptor function for platelet glycoprotein Ib-IX unmasked by cleavage of glycoprotein V. *Proc Natl Acad Sci U S A* 98:1823–1828
29. Kroll MH, Hellums JD, McIntire LV, Schafer AI, Moake JL (1996) Platelets and shear stress. *Blood* 88:1525–1541
30. Simon DI, Chen Z, Xu H, Li CQ, Dong J, McIntire LV, Ballantyne CM, Zhang L, Furman MI, Berndt MC, Lopez JA (2000) Platelet glycoprotein Ib α is a counterreceptor for the leukocyte integrin Mac-1 (CD11b/CD18). *J Exp Med* 192:193–204
31. McEver RP (2006) P-Selectin/PSGL-1 and other interactions between platelets, leukocytes and endothelium. In: Michelson AD (ed) *Platelets*. Academic, San Diego, CA, pp 231–250
32. Kansas GS (1996) Selectins and the ligands: current concepts and controversies. *Blood* 88:3259–3287
33. Romo GM, Dong JF, Schade AJ, Gardiner EE, Kansas GS, Li CQ, McIntire LV, Berndt MC, Lopez JA (1999) The glycoprotein Ib-IX-V complex is a platelet counterreceptor for P-selectin. *J Exp Med* 190:803–814
34. Berndt MC, Phillips DR (1981) Interaction of thrombin with platelets: purification of the thrombin substrate. *Ann N Y Acad Sci* 370:87–95
35. Baglia FA, Badellino KO, Li CQ, Lopez JA, Walsh PN (2002) Factor XI binding to the platelet glycoprotein Ib-IX-V complex promotes factor XI activation by thrombin. *J Biol Chem* 277:1662–1668
36. Hato T, Ginsberg MH, Shattil SJ (2002) Integrin α IIb β 3. In: Michelson AD (ed) *Platelets*. Academic, San Diego, CA, pp 105–116
37. Cierniewski CS, Byzova T, Papierak M, Haas TA, Niewiarowska J, Zhang L, Cieslak M, Plow EF (1999) Peptide ligands can bind to distinct sites in integrin α IIb β 3 and elicit different functional responses. *J Biol Chem* 274:16923–16932
38. Tolentino AR, Bahou WF (2002) Thrombin receptors. In: Michelson AD (ed) *Platelets*. Academic, San Diego, CA, pp 117–138
39. Vu TK, Hung DT, Wheaton VI, Coughlin SR (1991) Molecular cloning of a functional thrombin receptor reveals a novel proteolytic mechanism of receptor activation. *Cell* 64:1057–1068
40. Rasmussen UB, Vouret-Craviari V, Jallat S, Schlesinger Y, Pages G, Pavirani A, Lecocq JP, Pouyssegur J, Van Obberghen-Schilling E (1991) cDNA cloning and expression of a hamster α -thrombin receptor coupled to Ca^{2+} mobilization. *FEBS Lett* 288:123–128
41. Bizios R, Lai L, Fenton JW II, Malik AB (1986) Thrombin-induced chemotaxis and aggregation of neutrophils. *J Cell Physiol* 128:485–490
42. Liu LW, Vu TK, Esmon CT, Coughlin SR (1991) The region of the thrombin receptor resembling hirudin binds to thrombin and alters enzyme specificity. *J Biol Chem* 266:16977–16980
43. Vu TK, Wheaton VI, Hung DT, Charo I, Coughlin SR (1991) Domains specifying thrombin-receptor interaction. *Nature* 353:674–677
44. Ishihara H, Connolly AJ, Zeng D, Kahn ML, Zheng YW, Timmons C, Tram T, Coughlin SR (1997) Protease-activated receptor 3 is a second thrombin receptor in humans. *Nature* 386:502–506
45. Xu W-F, Anderson H, Whitmore TE, Presnell SR, Yee DP, Ching AC, Gilbert T, Davie EW, Foster DC (1998) Cloning and characterization of the human protease-activated receptor 4. *Proc Natl Acad Sci U S A* 95:6642–6646
46. Brass LF, Manning DR, Cichowski K, Abrams CS (1997) Signaling through G proteins in platelets: to the integrins and beyond. *Thromb Haemost* 78:581–589
47. Brass LF (1997) Thrombin receptor antagonists: a work in progress. *Coron Artery Dis* 8:49–58
48. Ayyanathan K, Webbs TE, Sandhu AK, Athwal RS, Barnard EA, Kunapuli SP (1996) Cloning and chromosomal localization of the human P2Y₁ purinoceptor. *Biochem Biophys Res Commun* 218:783–788
49. Hollopeter G, Jantzen H-M, Vincent D, Li G, England L, Ramakrishnan V, Yang R-B, Nurden P, Nurden A, Julius D, Conley PB (2001) Identification of the platelet ADP receptor targeted by antithrombotic drugs. *Nature* 409:202–207
50. Jin J, Kunapuli SP (1998) Coactivation of two different G protein-coupled receptors is essential for ADP-induced platelet aggregation. *Proc Natl Acad Sci U S A* 95:8070–8074
51. Kehrel B, Wierwille S, Clemetson KJ, Anders O, Steiner M, Knight CG, Farndale RW, Okuma M, Barnes MJ (1998) Glycoprotein VI is a major collagen receptor for platelet activation: it recognizes the platelet-activating quaternary structure of collagen, whereas CD36, glycoprotein IIb/IIIa, and von Willebrand factor do not. *Blood* 91:491–499

52. Woulfe D, Yang J, Prevost N, O'Brien P, Brass LF (2002) Signal transduction during the initiation, extension, and perpetuation of platelet plug formation. In: Michelson AD (ed) *Platelets*. Academic, San Diego, CA, pp 197–213
53. Hung DT, Wong YH, Vu TK, Coughlin SR (1992) The cloned platelet thrombin receptor couples to at least two distinct effectors to stimulate phosphoinositide hydrolysis and inhibit adenylate cyclase. *J Biol Chem* 267: 20831–20834
54. Hung DT, Vu TH, Nelken NA, Coughlin SR (1992) Thrombin-induced events in non-platelet cells are mediated by the unique proteolytic mechanism established for the cloned platelet thrombin receptor. *J Cell Biol* 116: 827–832
55. Klages B, Brandt U, Simon MI, Schultz G, Offermanns S (1999) Activation of G12/G13 results in shape change and Rho/Rho-kinase-mediated myosin light chain phosphorylation in mouse platelets. *J Cell Biol* 144:745–754
56. Shattil SJ (1999) Signaling through platelet integrin α IIb β 3: inside-out, outside-in, and sideways. *Thromb Haemost* 82: 318–325
57. Barnard MR, Linden MD, Frelinger AL III, Li Y, Fox ML, Furman MI, Michelson AD (2005) Effects of platelet binding on whole blood flow cytometry assays of monocyte and neutrophil procoagulant activity. *J Thromb Haemost* 3:2563–2570
58. Libby P, Simon DI (2001) Inflammation and thrombosis: the clot thickens. *Circulation* 103:1718–1720
59. McEver RP (2002) P-selectin/PSGL-1 and other interactions between platelets, leukocytes, and endothelium. In: Michelson AD (ed) *Platelets*. Academic, San Diego, CA, pp 139–155
60. Palabrica T, Lobb R, Furie BC, Aronovitz M, Benjamin C, Hsu YM, Sajer SA, Furie B (1992) Leukocyte accumulation promoting fibrin deposition is mediated in vivo by P-selectin on adherent platelets. *Nature* 359:848–851
61. Huo Y, Schober A, Forlow SB, Smith DF, Hyman MC, Jung S, Littman DR, Weber C, Ley K (2003) Circulating activated platelets exacerbate atherosclerosis in mice deficient in apolipoprotein E. *Nat Med* 9:61–67
62. Schonbeck U, Libby P (2001) CD40 signaling and plaque instability. *Circ Res* 89: 1092–1103
63. Schonbeck U, Libby P (2001) The CD40/CD154 receptor/ligand dyad. *Cell Mol Life Sci* 58:4–43
64. Furman MI, Benoit SE, Barnard MR, Valeri CR, Borbone ML, Becker RC, Hechtman HB, Michelson AD (1998) Increased platelet reactivity and circulating monocyte-platelet aggregates in patients with stable coronary artery disease. *J Am Coll Cardiol* 31:352–358
65. Ott I, Neumann FJ, Gawaz M, Schmitt M, Schomig A (1996) Increased neutrophil-platelet adhesion in patients with unstable angina. *Circulation* 94:1239–1246
66. Michelson AD, Barnard MR, Krueger LA, Valeri CR, Furman MI (2001) Circulating monocyte-platelet aggregates are a more sensitive marker of in vivo platelet activation than platelet surface P-selectin: studies in baboons, human coronary intervention, and human acute myocardial infarction. *Circulation* 104:1533–1537
67. Mickelson JK, Lakkis NM, Villarreal-Levy G, Hughes BJ, Smith CW (1996) Leukocyte activation with platelet adhesion after coronary angioplasty: a mechanism for recurrent disease? *J Am Coll Cardiol* 28:345–353
68. Michelson AD, Linden MD, Barnard MR, Furman MI, Frelinger AL (2006) Flow Cytometry. In: Michelson AD (ed) *Platelets*. Academic Press, San Diego, CA, pp 545–565
69. Hsu-Lin SC, Berman CL, Furie BC, August D, Furie B (1984) A platelet membrane protein expressed during platelet activation and secretion: studies using a monoclonal antibody specific for thrombin-activated platelets. *J Biol Chem* 259:9121–9126
70. Stenberg PE, McEver RP, Shuman MA, Jacques YV, Bainton DF (1985) A platelet α -granule membrane protein (GMP-140) is expressed on the platelet membrane after activation. *J Cell Biol* 101:880–886
71. Michelson AD, Benoit SE, Kroll MH, Li J, Rohrer MJ, Kestin AS, Barnard MR (1994) The activation-induced decrease in the platelet surface expression of the glycoprotein Ib-IX complex is reversible. *Blood* 83:3562–3573
72. Ruf A, Patscheke H (1995) Flow cytometric detection of activated platelets: comparison of determining shape change, fibrinogen binding, and P-selectin expression. *Semin Thromb Hemost* 21:146–151
73. Michelson AD, Barnard MR, Hechtman HB, MacGregor H, Connolly RJ, Loscalzo J, Valeri CR (1996) *In vivo* tracking of platelets: circulating degranulated platelets rapidly lose surface P-selectin but continue to circulate and function. *Proc Natl Acad Sci U S A* 93: 11877–11882
74. Larsen E, Celi A, Gilbert GE, Furie BC, Erban JK, Bonfanti R, Wagner DD, Furie B (1989)

- PADGEM protein: a receptor that mediates the interaction of activated platelets with neutrophils and monocytes. *Cell* 59:305–312
75. Hamburger SA, McEver RP (1990) GMP-140 mediates adhesion of stimulated platelets to neutrophils. *Blood* 75:550–554
 76. Linden MD, Furman MI (2005) Monocyte-platelet aggregates in patients with ischemic heart disease. In: Morrow DA, Cannon C (eds) *Cardiovascular biomarkers: pathophysiology and disease management*. Humana, Totowa, NJ, pp 487–493
 77. Hidari KI, Weyrich AS, Zimmerman GA, McEver RP (1997) Engagement of P-selectin glycoprotein ligand-1 enhances tyrosine phosphorylation and activates mitogen-activated protein kinases in human neutrophils. *J Biol Chem* 272:28750–28756
 78. Blanks JE, Moll T, Eytner R, Vestweber D (1998) Stimulation of P-selectin glycoprotein ligand-1 on mouse neutrophils activates beta 2-integrin mediated cell attachment to ICAM-1. *Eur J Immunol* 28:433–443
 79. Evangelista V, Manarini S, Sideri R, Rotondo S, Martelli N, Piccoli A, Totani L, Piccardoni P, Vestweber D, de Gaetano G, Cerletti C (1999) Platelet/polymorphonuclear leukocyte interaction: P-selectin triggers protein-tyrosine phosphorylation-dependent CD11b/CD18 adhesion: role of PSGL-1 as a signaling molecule. *Blood* 93:876–885
 80. Weber C, Springer TA (1997) Neutrophil accumulation on activated, surface-adherent platelets in flow is mediated by interaction of Mac-1 with fibrinogen bound to alphaIIb beta3 and stimulated by platelet-activating factor. *J Clin Invest* 100:2085–2093
 81. Weyrich AS, Elstad MR, McEver RP, McIntyre TM, Moore KL, Morrissey JH, Prescott SM, Zimmerman GA (1996) Activated platelets signal chemokine synthesis by human monocytes. *J Clin Invest* 97:1525–1534
 82. Ostrovsky L, King AJ, Bond S, Mitchell D, Lorant DE, Zimmerman GA, Larsen R, Niu XF, Kubes P (1998) A juxtacrine mechanism for neutrophil adhesion on platelets involves platelet-activating factor and a selectin-dependent activation process. *Blood* 91:3028–3036
 83. Diacovo TG, deFougerolles AR, Bainton DF, Springer TA (1994) A functional integrin ligand on the surface of platelets: intercellular adhesion molecule-2. *J Clin Invest* 94:1243–1251
 84. Rinder HM, Bonan JL, Rinder CS, Ault KA, Smith BR (1991) Dynamics of leukocyte-platelet adhesion in whole blood. *Blood* 78:1730–1737
 85. Frenette PS, Denis CV, Weiss L, Jurk K, Subbarao S, Kehrel B, Hartwig JH, Vestweber D, Wagner DD (2000) P-Selectin glycoprotein ligand 1 (PSGL-1) is expressed on platelets and can mediate platelet-endothelial interactions in vivo. *J Exp Med* 191:1413–1422
 86. Andre P, Denis CV, Ware J, Saffaripour S, Hynes RO, Ruggeri ZM, Wagner DD (2000) Platelets adhere to and translocate on von Willebrand factor presented by endothelium in stimulated veins. *Blood* 96:3322–3328
 87. Bombeli T, Schwartz BR, Harlan JM (1998) Adhesion of activated platelets to endothelial cells: evidence for a GPIIb/IIIa-dependent bridging mechanism and novel roles for endothelial intercellular adhesion molecule 1 (ICAM-1), alpha v beta3 integrin, and GPIb alpha. *J Exp Med* 187:329–339
 88. Bouchard BA, Butenas S, Mann KG, Tracy PB (2002) Interactions between platelets and the coagulation system. In: Michelson AD (ed) *Platelets*. Academic, San Diego, CA, pp 229–253

Haemostasis

Methods and Protocols

Monagle, P. (Ed.)

2013, XII, 431 p., Hardcover

ISBN: 978-1-62703-338-1

A product of Humana Press