

Platelet Receptors

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Abstract Well-understood functions for “traditional” platelet receptors are described, but “newer” receptors are equally discussed. Receptors are described biochemically (structure, ligand(s), protein partners, and function) and whenever possible, their clinical importance (mutations, polymorphisms, syndrome) are highlighted.

Keywords Platelet receptors • Adhesion • Aggregation • Amplification • Stabilization • Thrombosis • Bleeding

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1 Introduction

At sites of vascular injury, platelet receptor–ligand interactions are essential for the recruitment of circulating platelets to exposed subendothelial extracellular matrix components, as well as for the subsequent platelet activation and their aggregation. It is not surprising that those receptor–ligand interactions (Fig. 1), which control the initial capture of flowing platelets have attracted a lot of attention. Especially in smaller arteries and arterioles, where shear forces in blood are rather high, interactions between the platelet receptor glycoprotein membrane complex Ib (GPIb) and von Willebrand factor (VWF) are crucial. Synthesized by endothelial cells, VWF multimers are deposited in the subendothelial matrix; VWF is also recruited from the circulation onto collagen fibers, exposed to the blood in the ruptured vasculature (Savage et al. 1998). VWF tethers with GPIb, i.e. its fast off-rate does not allow stable platelet adhesion to the injured vessel wall. Or, platelet GPIb targets platelets to the vessel wall, where they roll over (sub)endothelium in the direction of the blood flow. Firm platelet adhesion implicates additional receptor–ligand couples (Table 1). Of prime importance are specific sequences on exposed collagen fibrils, recognized by the platelet collagen receptor GPVI, a process triggering potent platelet activation and complementing the minor activation brought about by the GPIb–VWF couple. These events switch on intracellular

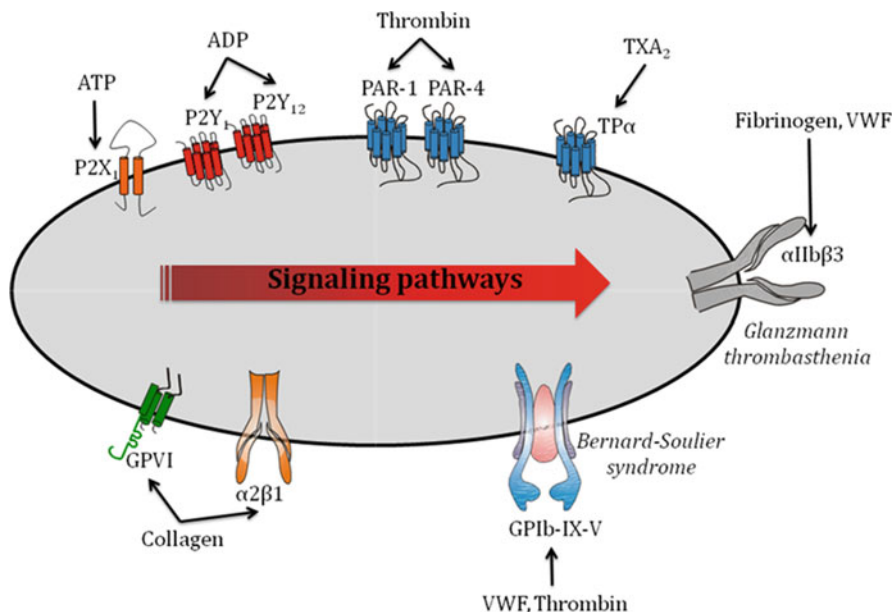


Fig. 1 Major platelet receptor–ligand interactions. Overview of well-known receptors on platelets and of their mode of activation driving to $\alpha\text{IIb}\beta 3$ activation. Mutations in GPIb-IX-V and $\alpha\text{IIb}\beta 3$ genes rise to Bernard–Soulier syndrome and Glanzmann thrombasthenia, respectively

Table 1 Platelet receptors in recruitment, adhesion, and aggregation

Receptors	Family	Ligands	Comments
Initiation of platelet recruitment			
GP1b-IX-V complex	Leucine-rich repeat family	VWF, thrombin, FXI, FXII, P-selectin, HK, Mac-1, TSP-1	Bernard–Soulier syndrome
Platelet adhesion and aggregation			
GPVI	Ig superfamily	Collagen, laminin	
$\alpha_2\beta_1$	Integrins	Collagen	
$\alpha_5\beta_1$		Fibronectin	
$\alpha_6\beta_1$		Laminin	
$\alpha_v\beta_3$		Vitronectin, fibrinogen, VWF, osteopontin	
$\alpha_{IIb}\beta_3$		Fibrinogen, fibrin, VWF, TSP-1, fibronectin, vitronectin	Glanzmann thrombasthenia
CD148	Tyrosine phosphatase receptor	unknown	Regulation of GPVI
CLEC-2	C-type lectin receptor	Podoplanin (platelets? CLEC-2?)	

signaling, amongst others leading to a conformational shift of membrane β -integrins on the platelet surface from a low to a high affinity state. This causes respectively firm platelet adhesion to collagen via $\alpha_2\beta_1$, in turn triggering platelet spreading, but also platelet aggregation via platelet $\alpha_{IIb}\beta_3$ interactions with fibrinogen, these interactions being essential in central blood vessels exposed to moderate shear stress (Varga-Szabo et al. 2008). To maintain hemostasis, this scheme requires additional fine-tuning via many more receptors, ranging from receptors recognized by soluble mediators, themselves released and produced by platelets, to receptors for thrombin, generated upon coagulation activation and receptor–co-receptor stabilizing pathways, all synergizing to control platelet activation and to arrest blood loss (Table 2).

Physiological platelet activation implies that platelets within the forming plug are sufficiently close to allow formation of direct and indirect bridges between adjacent platelets, allowing paracrine action by platelet-released molecules, favoring the transfer of information as in a neurological or immunological synapse. Together with fibrin formation on the activated platelets, this tight contact also restricts the diffusion of plasma factors, preventing premature fibrinolysis by plasmin over the growing thrombus. The late aggregate-stabilizing events prevent early disaggregation and/or embolization of the fresh platelet aggregate (Rivera et al. 2009) (Table 3).

Platelet receptors also mediate platelet interactions with activated/inflamed vascular endothelium and participate in pro-inflammatory leukocyte interactions with the vessel wall (Pitchford et al. 2003), but this is beyond the scope of this chapter. The currently available list of receptors with a specific role in platelet activation is probably incomplete. Novel proteomic and genomic strategies have hypothesized new candidate receptors, even when only based on the presence in

Table 2 Platelet receptors in the amplification phase

Receptors	Family	Ligands	Comments
P2Y ₁	G protein-coupled receptors	ADP	
P2Y ₁₂		ADP	
PAR1		Thrombin	High affinity
PAR4		Thrombin	Low affinity
TP α		Thromboxane	
PGE ₂ receptor (EP3)		PGE ₂	
PAF receptors		1- <i>O</i> -alkyl-2-acetyl- <i>sn</i> -glycero-3-phosphocholine	PAF: platelet-activating factor
Lysophosphatidic acid receptor		Lysophosphatidic acid	
Chemokine receptors		Chemokines	
V1a vasopressin receptor		Vasopressin	
A2a adenosine receptor		Adenosine	
β 2 adrenergic receptor		Epinephrine	
Serotonin receptor	Ion channel	Serotonin (5-hydroxytryptamin)	
Dopamine receptor		Dopamine	
P2X ₁		ATP	
c-mpl		TPO	
Leptin receptor	Tyrosine kinase receptor	Leptin	
Insulin receptor		Insulin	
PDGF receptor		PDGF	

such factors of a transmembrane and an extracellular domain. Consequently, some of these candidate receptors are orphans at present, without known ligand, even when murine knock-out models or/and genetic data (SNPs and mutations) suggest their involvement in (patho)physiological hemostasis (Fig. 2).

2 GPIb-IX-V in Initiation of Platelet Recruitment

Following arterial vascular injury, subendothelial collagen fibrils are disrupted and exposed to the circulation, alongside other subendothelial factors, including VWF, laminin, proteoglycans, fibronectin, vitronectin, etc. Whereas subendothelial VWF is believed to be bound to collagen VI (Hoylaerts et al. 1997), exposed collagen I and III fibers further recruit circulating VWF, providing a network of highly multimeric VWF strands, capable of tethering the platelet receptor glycoprotein Ib α (GPIb α), thus initiating platelet rolling on damaged (sub)endothelium (Wu et al. 2000).

Table 3 Platelet receptors in the stabilization phase and in the negative regulation of platelet activation

Receptors	Family	Ligands	Comments
Stabilization			
Ephr	Tyrosine kinase receptor	Ephrin	
Axl/Tyro3/Mer		Gas-6	
P-selectin	C-type lectin receptor family	PSGL-1, GPIb, TF	Soluble P-selectin: biomarker
TSSC6	Tetraspanins	?	
CD151		?	
CD36	Class B scavenger receptor	TSP1, oxLDL, VLDL, oxPL, collagen type V	Many functions
TLT-1	Ig superfamily	Fibrinogen?	TLT-1 soluble form correlated with DIC
PEAR1	Multiple EGF-like domain protein	?	Phosphorylated after platelet contact
Negative regulation			
VPAC1	G protein-coupled receptors	PACAP	
PECAM-1	Ig superfamily	PECAM-1, collagen, glycosaminoglycans	
G6b-B		?	
PGI ₂ receptor (IP)	G protein-coupled receptors	PGI ₂	Prostacyclin released from endothelial cells
PGD ₂ receptor		PGD ₂	
PGE ₂ receptor (EP4)		PGE ₂	

2.1 Biochemical Description

Four distinct transmembrane proteins, GPIb α (135 kDa), GPIb β (26 kDa), GPIX (20 kDa), and GPV (82 kDa), assemble at the surface of bone marrow megakaryocytes to constitute a functional GPIb receptor. The four subunits are encoded by genes mapping to chromosomes 17p12 (*GPIBA*), 22q11.2 (*GPIBB*), 3q29 (*GP5*), and 3q21 (*GP9*). These genes belong to the leucine-rich family of proteins and are almost exclusively expressed in platelets, with the exception of faint expression in certain endothelial cells (Wu et al. 1997). One complex of GPIb is formed by two chains of GPIb α , 2 GPIb β , 2 GPIX et 1 GPV (2 :2 :2 :1). Assembly of the GPIb-IX-V complex depends critically on a molecular chaperone in the endoplasmic reticulum: gp96 (grp94, HSP90b1) (Staron et al. 2011). GPIb α , GPIb β , and IX are present at 25,000 copies per platelet, GPV at 12,500 copies. GPIb α , GPIb β , and GPIX are closely associated and are all required for efficient bioavailability of GPIb. Deficiency of a single subunit dramatically decreases the surface expression of the whole complex. GPV is more loosely associated and its absence does neither prevent the expression of GPIb, nor its interaction with VWF. It plays a role during thrombin binding to GPIb α (see below).

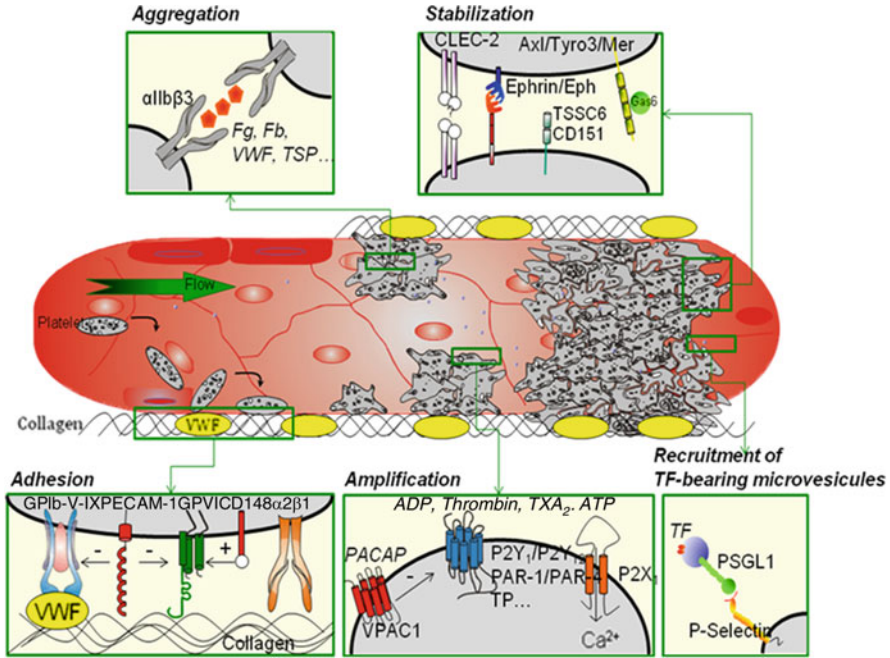


Fig. 2 Overview of platelet receptors involved in platelet activation. Vascular injury induces the exposure of the subendothelial extracellular matrix and leads to the deceleration of circulating platelets, enabling sustained contacts of platelet receptors with components of the ECM and leading to platelet activation. This platelet adhesion at the site of vessel damage is the first step in thrombus formation. It involves interactions of von Willebrand factor (VWF) with GPIb-IX-V and of collagen with GPVI and integrin $\alpha 2\beta 1$. Platelet activation is associated with a change in platelet shape, granule secretion, thromboxane A_2 (TXA_2) synthesis, and intracellular signaling events leading to integrin $\alpha IIb\beta 3$ activation. Its activation leads to a change in its conformation, enabling it to bind to ligands, such as fibrinogen and VWF. This promotes platelet–platelet interactions (platelet aggregation) and thrombus formation. Together with fibrin formation on the activated platelets, this tight contact prevents early disaggregation and/or embolization of the fresh platelet aggregate via various receptors and ligands

GPIb α contains 8 Leucine-Rich Repeat domains, one negatively charged region, containing 3 sulfated tyrosines, an O and N-glycosylated region, and a transmembrane and cytoplasmic domain. Platelet activation induces transient clearance of GPIb from the platelet surface, followed by a slow reappearance to normal levels, between 30 and 60 min. Translocation appears to be associated with the formation of GPIb clusters.

2.2 GPIb, an Omnivalent Receptor

The extracellular domain comprises the binding site for VWF, P-selectin, and Mac-1. In addition to its dynamic role in platelet recruitment onto VWF, the GPIb membrane complex functions as a receptor for coagulation factor XII

(Bradford et al. 2000), XI (Baglia et al. 2002), thrombin, the latter interaction studied in large detail (Adam et al. 2003; De Marco et al. 1994) and HK (High-molecular-weight kininogen) (Lanza 2006). Hence, GPIb is a receptor linking primary and secondary hemostasis. Thrombin binding to GPIb α appears to favor presentation of thrombin to its proper receptor, the Protease-Activated-Receptor 1 (see below). GPV may act as a negative modulator of thrombin-induced platelet activation, since cleavage of GPV by thrombin unmasks GPIb-IX and facilitates binding of thrombin to GPIb α (Ramakrishnan et al. 2001). GPIb interacts with vascular P-selectin, pointing to its function in inflammatory platelet pathways (Romo et al. 1999).

2.3 Bernard–Soulier Syndrome

Defects in three GPIb encoding genes give rise to a serious bleeding diathesis, accompanied by morphological platelet anomalies (giant platelets), collectively defined as the Bernard–Soulier Syndrome (BSS). BSS is a rare hereditary thrombocytopathy, first described in 1948 by Bernard and Soulier in a young male patient who had severe mucocutaneous bleeding, prolonged bleeding time with normal platelet count, and abnormally large platelets. Ultrastructural studies of affected platelets show a dilated open canalicular system, prominent dense tubular system, and vacuolization (Lanza 2006).

The BSS usually presents in newborn and during infancy, or early childhood as epistaxis, and/or gingival bleeding. Later bleeding complications will be associated with menstruation, trauma, surgery, or gastric ulcers. The BSS is rare with a reported prevalence of 1 in 1,000,000. It is an autosomal recessive disorder, linked with consanguinity (Lanza 2006). Patients have a prolonged bleeding time, thrombocytopenia, and larger platelets than the normal individual due to defective thrombopoiesis in GPIb defective megakaryocytes (Poujol et al. 2002).

Few mutations were reported that cause a gain of function in the GPIb α chain, leading to the so-called platelet-type GPIB (Pincus et al. 1991), showing a phenotype similar to that of certain subtypes of von Willebrand disease (Franchini et al. 2008).

3 Platelet Adhesion and Aggregation

3.1 Collagen Receptors

Collagens are the most abundant proteins in the subendothelial extracellular matrix (20–40% of total proteins in the aorta). In addition to providing mechanical strength to the blood vessel wall, collagen is essential in platelet adherence and platelet plug formation. Nine types of collagen reside in the vasculature, but only fibrillar collagen

type I, III, V, and VI and nonfibrillar collagen type IV and VIII are thrombogenic. Although platelets have various receptors for collagen, such as GPVI, $\alpha 2\beta 1$, p65, p47, TIIICBP, GPIV, the integrin $\alpha 2\beta 1$ and GPVI are considered major receptors for binding to collagen and activation of platelets (Nuytens et al. 2011).

3.1.1 GPVI-FcR γ

Biochemical Description

Glycoprotein VI (GPVI) is a 63-kDa transmembrane protein consisting of two immunoglobulin (Ig)-like domains in the extracellular region connected to a highly glycosylated linker, a transmembrane domain, and a cytoplasmic tail. GPVI is a member of the Ig-like receptors within the leukocyte Ig-like receptor complex (LRC) on human chromosome 19q13.4. GPVI is expressed exclusively in platelets and megakaryocytes (around 3,700 copies per platelet), where it associates with the transmembrane adapter protein FcR γ . Surface expression of GPVI is dependent on FcR γ stabilization, through a salt bridge between the transmembrane GPVI domain residue Arg²⁷² and FcR γ (Asp residues). The FcR γ is a covalent-linked homodimer with each chain containing one copy of an ITAM (Immunoreceptor Tyrosine-based Activation Motif) defined by the presence of two YxxL sequences separated by seven amino acids (Clemetson and Clemetson 2001). Phosphorylation of the ITAM motif by two Src kinases (Fyn and Lyn) associated with GPVI initiates platelet signaling, leading to potent platelet activation. GPVI is expressed on platelets as a mixture of monomers and dimers, with a stoichiometry of one GPVI to each FcR γ -chain covalent dimer. The affinity of collagen for monomeric GPVI is too low to mediate activation to physiological concentrations of collagen, but dimeric GPVI forms a unique conformation with higher affinity for collagen (Miura et al. 2002). F(ab)₂ fragments of antibodies to this structure induce platelet activation, while Fab fragments block activation by collagen. These results suggest a minimal signaling model in which activation is achieved through cross-linking of two GPVI dimers.

Moderate Bleeding in Patients

The role of GPVI deficiency for bleeding complications has remained uncertain for a long time. Early reports on modest bleeding complications could not be interpreted due to incomplete GPVI deficiencies reported (Arthur et al. 2007). Even, the study of various knock-out models did not clearly elucidate the role of GPVI in platelet function, with various mouse models showing different degrees of functional deficiency, depending on the model chosen (Konstantinides et al. 2006; Mangin et al. 2006; Massberg et al. 2003). The paradigm, that GPVI is a major collagen receptor on platelets, despite a moderate bleeding diathesis in men and mice, has recently been resolved by two different groups. They described

independent patients with a proven genetic GPVI defect. Collagen-induced platelet activation was absent, whereas GPVI quantification by flow cytometry evidenced incomplete antigen absence. With complete functional GPVI deficiency, only a mild bleeding phenotype was reported in both cases (Dumont et al. 2009; Hermans et al. 2009), suggesting that additional platelet–collagen interactions play an equally important role in platelet physiology, the next candidate being $\alpha 2\beta 1$.

3.1.2 $\alpha 2\beta 1$

Biochemical Description

The integrin $\alpha 2\beta 1$ (or VLA2, CD49b/CD29, GPIa/IIa) is a collagen receptor composed of a 150-kDa $\alpha 2$ chain and a 130-kDa $\beta 1$ chain. $\alpha 2\beta 1$ is expressed at $1,730 \pm 500$ copies per platelet, with an expression profile ranging from 900 to 4,000 copies per platelets. $\alpha 2$ is the only platelet subunit to contain an I domain, a 200-residue inserted sequence. The crystal structure of this domain indicates that it comprises seven helices surrounding a core of five parallel β -strands, a short antiparallel β -strand, and a C-terminal helix (Clemetson and Clemetson 2001). The I-domain metal ion coordinating residues D151, T221, and D254 are all important for collagen binding to the integrin. The I-domain prefers Mg^{2+}/Mn^{2+} in this site. Several recognition sequences by $\alpha 2\beta 1$ have been identified in collagen I and III: GFOGER, GLOGER, GASGER, GROGER, and GLOGEN, with different hierarchy and affinity in their recognition profile (Siljander et al. 2004). This interaction is dependent on Mg^{2+} and the GER sequence. $\alpha 2\beta 1$ recognizes these sequences in the resting state, but platelet activation by classical agonists via intracellular signal transduction pathways will activate $\alpha 2\beta 1$ via a structural rearrangement of the $\alpha 2\beta 1$ domains (Cosemans et al. 2008), causing them to upregulate their affinity for their preferred ligand sequences, stereochemically positioned at regular positions on bundled collagen fibrils (Siljander et al. 2004).

Variable Membrane Expression and Function

Even when no strict deficiencies in $\alpha 2\beta 1$ have been reported, hereditary variation in $\alpha 2\beta 1$ receptor density is well known, at least in part due to several $\alpha 2\beta 1$ allelic polymorphisms, such as the 873G/A and 807C/T polymorphisms (Moshfegh et al. 1999). The C807T and G873A polymorphisms are risk factors for thrombotic pathologies such as myocardial infarction. In some patients with inherited thrombocytopenia, a reduced $\alpha 2\beta 1$ membrane density is coupled to prolonged bleeding times and severely reduced in vitro platelet adhesion to ligands for $\alpha 2\beta 1$ (Santoro 1999). Yet, platelet aggregation tests in vitro manifest subnormal to normal platelet responses to collagen.

3.1.3 CD148 in Optimal Platelet Response to Collagen

General Description

The CD148 gene maps to chromosome 11p11.2, encoding a protein consisting of a large, glycosylated extracellular region containing eight fibronectin type III repeats, a single transmembrane domain, and a single cytoplasmic protein tyrosine phosphatase (PTP) domain. CD148 is the only receptor-like protein tyrosine phosphatase identified in platelets. CD148 is expressed in many cell types, including epithelial and endothelial cells, fibroblasts, and most hematopoietic cells. CD148 null mice exhibited a bleeding tendency and defective arterial thrombosis. CD148 plays a critical role in regulating GPVI/FcR γ chain expression. Indeed, CD148-deficient mouse platelets show reduced GPVI expression. Basal *Src family kinases* (SFK) activity was found to be markedly reduced in CD148-deficient platelets, resulting in global hyporesponsiveness to agonists that signal through SFKs, including collagen and fibrinogen. CD148 maintains a pool of active SFKs in platelets by directly dephosphorylating the C-terminal inhibitory tyrosines of SFKs that are essential for platelet activation (Ellison et al. 2010; Senis et al. 2009).

CD148 in Disease

In view of the moderate bleeding complications in GPVI deficiency, it seems not surprising that CD148-related platelet dysfunction has not been reported. However, dysregulation of CD148 has been noted in other tissues: comparative analysis of tissue samples from normal gut and from patients with active Crohn's disease showed that leucocytes expressing CD148 are significantly upregulated in inflamed tissues. Furthermore, a marked loss of CD148 immunoreactivity was apparent in some carcinomas (Autschbach et al. 1999). Further work will need to define whether CD148 deficiencies affect GPVI-dependent platelet signaling reactions.

3.1.4 C-Type Lectin-Like Receptor 2: CLEC-2

Biochemical Description

The CLEC-2 gene maps to chromosome 12 and codes for a type II membrane protein C-type lectin receptor family with an extracellular carbohydrate-like recognition domain (CRD-like) that lacks the conserved amino acids for binding to sugars. It has a cytoplasmic tail of 31 amino acids that contains a single conserved YxxL sequence (known as a hem-immunoreceptor tyrosine-based activation motif, hem-ITAM). CLEC-2 is highly expressed on megakaryocytes and platelets, and at low level on peripheral blood mouse neutrophils. CLEC-2 was discovered via the snake venom rhodocytin, also known as aggrexin and isolated from the venom of the Malayan

pit viper, *Calloselasma rhodostoma*. It was initially thought to mediate platelet activation through $\alpha 2\beta 1$ and GPIb α based on the ability of high concentrations of antibodies to block activation. However, rhodocytin did not bind recombinant $\alpha 2\beta 1$ and activates platelets deficient in integrin $\alpha 2\beta 1$, GPIb α , and GPVI. Thus, rhodocytin appears to activate platelets through a novel receptor. Also, an antibody to CLEC-2 induces potent activation of human platelets, establishing CLEC-2 as a novel platelet activation receptor (Suzuki-Inoue et al. 2006). Furthermore, by using podoplanin—a ligand of CLEC-2 expressed on the surface of cells such as kidney podocytes, lung type I alveolar cells, and lymphatic endothelial cells but absent from vascular endothelial cells and platelets and an anti-CLEC-2 antibody, it was shown that Syk mediates phosphorylation of CLEC-2, with Src family kinases playing a critical role further downstream.

Recently, three studies claim, respectively deny a role for CLEC-2 in platelet activation at arteriolar shear rates. However, the underlying function is still elusive, as platelets do not express podoplanin, the only known endogenous ligand of CLEC-2. It is possible that CLEC-2 is involved in thrombus stabilization through homophilic interactions (Hughes et al. 2010; May et al. 2009; Suzuki-Inoue et al. 2010). Whether it plays a role in platelet plug formation, in contact with extravascular tissue, remains to be demonstrated.

3.2 Integrins

Integrins play an essential role in the cell metabolism of every cell, including platelets, the only difference being that the integrin ligands of platelets are partly extracellular matrix bound and insoluble, and partly soluble and present in the circulation. Correspondingly, heterodimeric receptors of the $\beta 1$ and $\beta 3$ integrin families mediate platelet adhesion and aggregation. In resting platelets, integrins are expressed in a low-affinity state but they shift to a high-affinity state and efficiently bind their ligands in response to cellular activation (Kasirer-Friede et al. 2007). The integrin receptor for collagen on platelets, $\alpha 2\beta 1$ has been discussed in the previous paragraph, but platelets have several integrins at their disposal.

3.2.1 $\alpha 5\beta 1$

General Description

$\alpha 5\beta 1$ is the principal platelet receptor for matrix fibronectin through its RGD sequence. $\alpha 5\beta 1$ supports resting platelet adhesion to fibronectin in static conditions. However, this interaction is unable to promote platelet tyrosine phosphorylation, calcium oscillation, or lamellipodia formation. The $\alpha 5\beta 1$ –fibronectin interaction is shear stress sensitive and quickly loses avidity at increasing shear stress. Thus, the

role of $\alpha 5 \beta 1$ may be limited to initiating the interaction of resting platelets with matrix fibronectin especially in injuries in the larger blood vessels, where shear forces are low, promoting the subsequent engagement of other integrins and other receptors to amplify platelet responses.

3.2.2 $\alpha 6 \beta 1$

General Description

$\alpha 6 \beta 1$ is the principal laminin receptor in platelets, not requiring platelet activation in order to bind laminin. Cations Mn^{2+} , Co^{2+} , and Mg^{2+} support adhesion while Ca^{2+} , Zn^{2+} , and Cu^{2+} do not. Binding of platelets to laminin through $\alpha 6 \beta 1$ does not induce platelet aggregation but platelets adherent to laminin trigger signaling pathways, inducing filopodia formation, with PI3K and cdc42 activities being higher than in platelets activated through $\alpha IIb \beta 3$ (see below). Laminin has been known for many years to support adhesion of platelets through integrin $\alpha 6 \beta 1$, but its ability to activate GPVI was only recently discovered by demonstration of spreading of mouse platelets on a laminin surface and loss of activation in the absence of GPVI. The interaction between laminin and GPVI is dependent on the initial interaction with integrin $\alpha 6 \beta 1$, in contrast to collagen which initiates platelet activation through GPVI. This difference between the two matrix proteins may reflect the tenfold lower affinity of laminin for GPVI or the presence of a subpopulation of constitutively active $\alpha 6 \beta 1$. While the weak nature of the activation of GPVI by laminin argues against a significant role in the prevention of major bleeds, it is ideally suited to facilitate vessel repair after minor damage without the risk of forming occlusive thrombi (Ozaki et al. 2009).

3.2.3 $\alpha IIb \beta 3$

Biochemical Description

$\alpha IIb \beta 3$ is the most abundant surface-expressed integrin in platelets (40,000–80,000 copies per platelet), with an additional pool that can be recruited from internal membranes upon agonist-induced platelet activation. It is the major receptor on the platelet, also functionally (see below). The mature αIIb and $\beta 3$ subunits are 148-kDa and 95-kDa proteins, respectively. $\alpha IIb \beta 3$ binds several RGD containing ligands, including fibrinogen, fibrin, VWF, vitronectin, fibronectin, and thrombospondin. While ligand recognition typically occurs through an RGD tract, in the case of fibrinogen (the major platelet $\alpha IIb \beta 3$ ligand), investigations of the $\alpha IIb \beta 3$ -mediated cell adhesion to immobilized fibrinogen have evidenced that the fibrinogen synergy gamma (400–411) sequence (dodecapeptide) by itself promotes cell attachment by initiating $\alpha IIb \beta 3$ clustering and recruitment of intracellular proteins, while the RGD

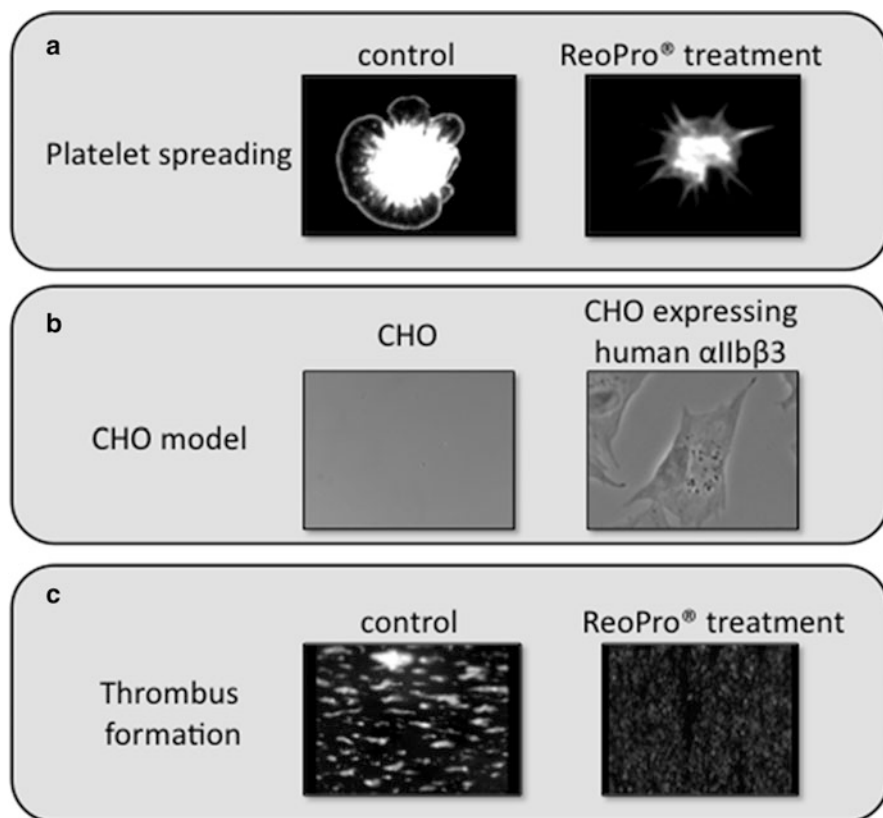


Fig. 3 Central role of $\alpha\text{IIb}\beta 3$ in platelet activation. (a) Human platelets plated over a fibrinogen matrix during 25 min and upon additional incubation with (a, left) or without ReoPro[®] for 10 min, (a, right) (monoclonal antibody that blocks $\alpha\text{IIb}\beta 3$). Treatment with ReoPro[®] impairs platelet spreading and induces the detachment of platelets from the fibrinogen matrix. (Platelets were stained with phalloidin–rhodamine for F-actin). (b) Chinese Hamster Ovary cells (CHO) without $\alpha\text{IIb}\beta 3$ or transfected with the human integrin, spread over a fibrinogen matrix. Only cells expressing integrin show cell adhesion and spreading. (c) Whole blood was perfused over a collagen matrix at a shear rate of $1,500\text{ s}^{-1}$ during 4 min and thrombus formation monitored with fluorochrome (rhodamine). The treatment of blood with ReoPro[®] impairs platelet aggregation and thrombus formation, responsible for formation of a monolayer of platelets owing to GPIb and collagen receptors, interacting with collagen-bound VWF and specific collagen sequences, respectively

motif subsequently acts as a molecular switch on the $\beta 3$ subunit to induce a conformational change necessary for full cell spreading (Salsmann et al. 2006).

This dominant integrin on the platelet surface mediates platelet aggregation through binding of plasma fibrinogen and serves as the principal receptor for platelet adhesion in vivo. The shift of integrin $\alpha\text{IIb}\beta 3$ from a low- to a high-affinity state is considered the “final common pathway” of platelet activation. This shift is an absolute requirement for platelet $\alpha\text{IIb}\beta 3$ to interact with fibrinogen (Fig. 3), itself a ligand with two receptor interaction sites, i.e., enabling interaction with separate platelets, constituting the basis of platelet aggregation.

Pathophysiology

The function of $\alpha\text{IIb}\beta 3$ was first highlighted in the so-called Glanzmann thrombasthenia (GT) syndrome, a rare autosomal recessive bleeding syndrome affecting the megakaryocyte lineage and characterized by lack of platelet aggregation due to quantitative or qualitative defects in $\alpha\text{IIb}\beta 3$. The sites of bleeding in GT are clearly defined: purpura, epistaxis, gingival hemorrhage, and menorrhagia are nearly constant features; gastrointestinal bleeding and hematuria are less common but can cause serious complications. GT platelets attach to subendothelium after injury, but platelet spreading on the exposed surface is defective. In addition to defective thrombus formation and clot retraction, other $\alpha\text{IIb}\beta 3$ dependent processes are often absent. Although GT is a very rare disorder, occurring in $\approx 1:1,000,000$ individuals worldwide, research efforts have led to the characterization of more than 170 distinct genetic abnormalities within $\alpha\text{IIb}\beta 3$, resulting in quantitative and qualitative deficiencies of the receptor on the platelet surface. All mutations for αIIb and $\beta 3$ are classified in the following database at <http://sinaicentral.mssm.edu/intranet/research/glanzmann/menu>. Nonsense mutations and splice site mutations with frameshift are common. Certain mutations predominate in ethnic groups such as in Israel and in the French gypsy population. Recently, the estimated age of the French gypsy mutation founder was 300–400 years (Coller and Shattil 2008; Fiore et al. 2011; Kasirer-Friede et al. 2007; Nurden 2006; Nurden and Nurden 2008).

The current treatment options for GT are transfusion with normal human platelets or via therapeutic agents, limiting fibrinolysis (Amicar, tranexamic acid) or generating a procoagulant tendency (NovoSeven[®]). Unfortunately, frequently antibodies develop to transfused platelets, destroying them. In addition, the beneficial effect of expensive hemostatic agents is relatively short lived. Recently hematopoietic stem cell gene transfer was explored as a strategy to improve platelet function within a canine model for GT. Approximately 5,000 $\alpha\text{IIb}\beta 3$ receptors formed on 10% of platelets. These modest levels allowed platelets to adhere to fibrinogen, form aggregates, and mediate retraction of a fibrin clot. These results fuel the hope that gene therapy could become a practical approach for treating inherited platelet defects, one day (Fang et al. 2011).

3.2.4 $\alpha\text{v}\beta 3$

General Description

In general, the $\beta 3$ gene is widespread, with $\alpha\text{v}\beta 3$ being expressed in many cell types, including endothelial cells, osteoblasts, smooth muscle cells, and leukocytes, throughout the vascular bed. In platelets it is only present in a few hundred copies per platelet. There are notable differences between $\alpha\text{v}\beta 3$ and $\alpha\text{IIb}\beta 3$ in platelets. $\alpha\text{v}\beta 3$ can bind several RGD-containing ligands, including osteopontin and adenovirus penton base, but vitronectin is the preferred ligand for $\alpha\text{v}\beta 3$. High-affinity $\alpha\text{v}\beta 3$ can be induced by agonists such as ADP and by direct integrin modulators

such as DTT and MnCl_2 . Activated $\alpha_v\beta_3$ on platelets can bind osteopontin, present in atherosclerotic plaques and in the wall of injured but not normal arteries (Kasirer-Friede et al. 2007; Nurden 2006).

3.3 *Receptors in Amplification Phase*

Soluble platelet agonists play a critical role in platelet activation and thrombus formation. TXA_2 is synthesized by activated platelets ADP, ATP are released from damaged red blood cells and platelet dense granules, all serving to amplify ongoing platelet activation and recruit circulating platelets in developing platelet aggregates. These agonists activate platelets via G-protein-coupled receptors (GPCRs), a family of 7-transmembrane domain receptors that transmit signals through heterotrimeric G proteins and via the ion channel P2X_1 . Early traces of thrombin generated during the initiation phase of coagulation by microvesicular tissue factor deposited on blebbing platelets (Varga-Szabo et al. 2008) will give rise to amplifying thrombin-induced platelet activation via protease-activated receptors (PARs), long before coagulation activation has come to completion. Thus, ADP will control platelet activation via two platelet receptors, i.e., P2Y_1 (Gq coupled) and P2Y_{12} (Gi coupled), whereas ATP will induce rapid activation via Ca^{2+} influx through P2X_1 . Thrombin bound onto GPIb will rapidly activate PAR1 in human platelets, whereas on murine platelets, thrombin can likewise be presented to PAR4 by PAR3. PAR4 requires higher thrombin concentrations and is not the prime thrombin receptor on human platelets.

Prostanoids are a family of bioactive lipid mediators that are formed by cyclooxygenase from arachidonic acid liberated from the cell membrane. They are involved in numerous physiological activities, including platelet aggregation, vasorelaxation and vasoconstriction, local inflammatory response, and leucocyte–endothelial cell adhesion. Amplification of platelet activation by TXA_2 synthesis and binding to the TXA_2 /prostaglandin H2 receptor (TP) receptor are well known, because they constitute the aspirin-sensitive arm of platelet activation. By contrast, prostacyclin (PGI_2) and PGD_2 are known to inhibit platelet aggregation, whereas PGE_2 potentiates or inhibits platelet response in a dose-dependent manner.

3.3.1 P2Y_1

Biochemical Description

The P2Y_1 receptor is a 42-kDa protein that contains 373 amino acid residues and is widely distributed in many tissues including heart, blood vessels, smooth muscle cells, neural tissue, testis, prostate, ovary, and platelets. About 150 P2Y_1 receptor binding sites are expressed per platelet. Although present at the platelet surface, P2Y_1 is also abundantly represented in membranes of α -granules and elements of

the open canalicular system. The P2Y₁ receptor is absolutely required for ADP-induced platelet aggregation. Its pharmacological inhibition or genetic deficiency results in complete absence of platelet aggregation and shape change in response to ADP. ADP is a more potent agonist than ATP and their 2-methylthio derivatives are more potent than the parent compounds. UTP, UDP, CTP, and GTP are inactive. ATP is, in fact, a partial agonist at the P2Y₁ receptor and so, at low levels of receptor expression it acts as an antagonist. Overall, P2Y₁ accounts for about 20–30 % of the total ADP binding sites on the platelet surface (Gachet 2008).

Functional Variability

A common genetic variant at the P2Y₁ locus (dimorphism, 1622AG) is associated with platelet reactivity to ADP. This genotype effect partly explains the interindividual variation in platelet response to ADP, which may have clinical implications with regard to thrombotic risk.

3.3.2 P2Y₁₂

Biochemical Description

P2Y₁₂, which maps to chromosome 3q21-q25, is present in platelets, endothelial cells, glial cells, and smooth muscle cells. It contains 342 amino acid residues, including 4 extracellular Cys residues at positions 17, 97, 175, and 270: Cys 97 and Cys 175, which are linked by a disulphide bridge, are important for receptor expression; 2 potential N-linked glycosylation sites at the extracellular amino-terminus may modulate its activity.

P2Y₁₂ receptors exist predominantly as homo-oligomers situated in lipid rafts. On treatment with the active metabolite of clopidogrel (which covalently inhibits P2Y₁₂), the homo-oligomers are disrupted into nonfunctional dimers and monomers that are sequestered outside the lipid rafts.

ADP and some of its analogs, such as 2-methylthio-ADP and (*N*)-methanocarba-2-methylthio-ADP, stimulate P2Y₁₂, whereas adenosine triphosphate and its triphosphate analogs act as antagonists.

P2Y₁₂, the G_i-coupled platelet receptor for adenosine diphosphate (ADP), plays a central role in platelet function.

Patient Bleeding

Patients with congenital P2Y₁₂ defects display a mild to moderate bleeding diathesis, characterized by mucocutaneous bleedings and excessive postsurgical and posttraumatic blood loss. Defects of P2Y₁₂ should be suspected when ADP,

even at high concentrations ($\geq 10 \mu\text{M}$), is unable to induce full, irreversible platelet aggregation (Gachet 2008).

3.3.3 P2X₁

Description

Platelet dense granules also release ATP upon activation, reactive with an ion channel present on platelets, i.e. P2X₁. P2X₁ is a widely distributed ligand-gated ion channel, highly expressed in human megakaryocytes and platelets. ATP is the physiological agonist and ADP is an antagonist. Rapid desensitization of the P2X₁ receptor during platelet preparation made this receptor go unnoticed in vitro, for a long time. The P2X₁ gene maps to chromosome 17p13.2, encoding 399 amino acids, organized in two transmembrane domains (TM1 and TM2) separated by a large extracellular domain containing ten cysteine residues. Three molecules of ATP bind the extracellular domain of P2X₁, triggering conformational changes, resulting in the opening of a cationic pore, allowing rapid changes in the membrane permeability for monovalent and divalent cations, including Ca²⁺, Na⁺, and K⁺. Activation of P2X₁ receptors triggers transient shape change, with platelets converted from a discoid to spherical shape. P2X₁ receptor activation furthermore amplifies platelet responses to low concentrations of other platelet agonists, with rapid kinetics. Yet, P2X₁ contributes equally to low and high levels of thromboxane A₂ receptor activation. More significantly, P2X₁ receptor activation has been shown to be essential for enhanced platelet adhesion and thrombus formation under high shear rates (Oury et al. 2004). During various studies, P2X₁ has distinguished itself as a potential new drug target for antithrombotic therapy, especially for those conditions where mild long-term risk correction is required. P2X₁, indeed, seems a “safe” target, its inhibition causing mild effects on platelet function (Hu and Hoylaerts 2010).

3.3.4 Thromboxane Receptor

Biochemical Description

TXA₂ is generated from its precursor arachidonic acid through the cyclooxygenase pathway (Patrino et al. 2005). The TP receptor or TXA₂/prostaglandin (PG) H₂ receptor (57 kDa) is a membrane-bound seven transmembrane receptor, G-protein coupled and distributed widely in the cardiovascular system. Human TP receptors exist in two isoforms, TP α and TP β , which differ in their C-terminal intracytoplasmic region. Both receptors are encoded by the same gene, but result from alternative splicing. Even though mRNA for both TP α and TP β is found in platelets, only TP α is expressed in platelets, with TP β being expressed in endothelial cells. TP α -mediated signal transduction occurs via several G proteins,

including Gq and G_{12/13}. TP receptors are also expressed in other cell types, relevant to atherothrombosis, such as smooth muscle cells, macrophages, and monocytes (Habib et al. 1999; Hirata et al. 1996).

Clinical Importance

In several patients, platelet dysfunction has been attributed to an abnormal platelet TXA₂ receptor. One mutation (Arg⁶⁰-Leu) in the first cytoplasmic loop of TP α was found in patients with a mild bleeding disorder, characterized in aggregation tests by altered responses to TXA₂, TXA₂ analogs, and other agonists (but not thrombin).

3.3.5 Prostaglandin E2 Receptors

Description

During inflammation, the synthesis of prostanoids in endothelial cells and smooth muscle cells is highly increased. The biosynthesis of PGE₂ is enhanced by inflammatory mediators in vascular smooth muscle cells and macrophages.

PGE₂ shows a biphasic, concentration-dependent effect on platelet aggregation. Although high concentrations inhibit platelet aggregation, lower concentrations enhance it. PGE₂ activates four G protein-coupled receptors EP1, EP2, EP3, and EP4. Each of these receptors has a distinct pharmacological signature and intracellular signal transduction. Stimulation of EP3 receptors results in elevation of free intracellular calcium levels, whereas stimulation of EP2 and EP4 receptors usually increases intracellular cAMP levels through activation of G α s protein, resulting in a decrease of intracellular calcium levels. Human platelets contain mRNA for EP1 receptor, all of the EP3 splice variants, and EP4. However mRNA for EP2 receptors is lacking in platelets (Paul et al. 1998).

The proaggregatory effect of PGE₂ has been ascribed to the activation of the EP3 receptor, leading to inhibition of the increase in cAMP, increased mobilization of calcium, and elevated P-selectin expression in platelets. Mice lacking this receptor show an increased bleeding tendency and a decreased susceptibility to thromboembolism (Ma et al. 2001). It has also been demonstrated that PGE₂ produced by atherosclerotic plaques in mice can facilitate arterial thrombosis, acting via EP3 (Gross et al. 2007).

Defects in the EP3 receptor gene are not yet discovered in humans.

Recently, a selective EP4 agonist (ONO AE1-329) has been shown to inhibit platelet aggregation, calcium mobilization, and upregulation of P-selectin. Furthermore EP4 activation enhances the inhibitory effect of aspirin. Conversely, two chemically distinct EP4 antagonists, GW627368x and ONO AE3-208, abrogated the inhibitory effect of ONO AE1-329 on platelet aggregation (Philipose et al. 2010). EP4 receptors might play an important role in the control of hemostasis by mediating the inhibitory effect of PGE₂, thereby balancing out the proaggregatory

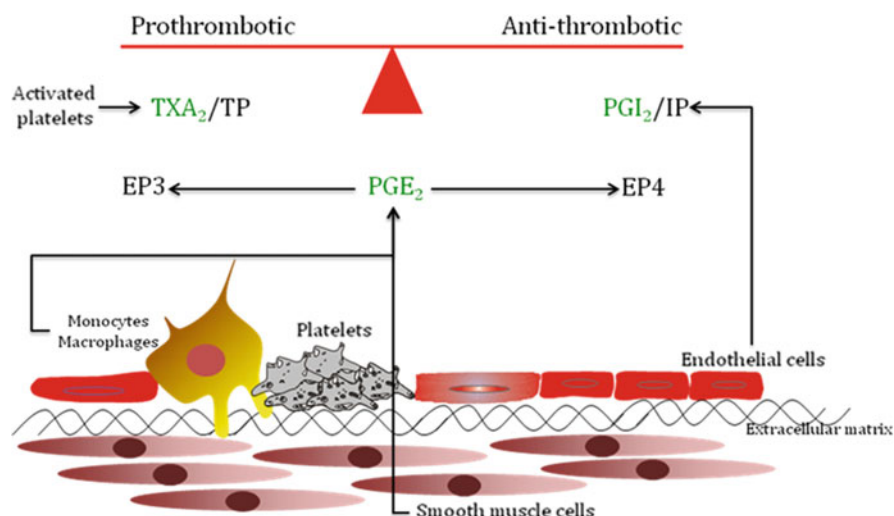


Fig. 4 The balance between thrombotic and antithrombotic effects of prostanoids. In response to vascular injury, PGI_2 produced by endothelial cells opposes the enhanced prothrombotic effect of TXA_2 produced by platelets. Smooth muscle cells, monocytes, and macrophages (accumulate in atherosclerotic plaques) release prostanoids such as PGE_2 during inflammation. PGE_2 shows a biphasic, dose-dependent effect on platelet aggregation

effect of EP_3 receptors (Fig. 4). EP_4 agonists might constitute a novel class of antithrombotic agents and be clinically useful in cases where aspirin or ADP antagonists are not warranted or are insufficient (Philipose et al. 2010).

3.3.6 Prostacyclin (PGI_2) Receptor

Prostaglandin I_2 (PGI_2) or prostacyclin is a derivative of arachidonic acid, released by vascular endothelial cells, serving as a potent vasodilator, inhibitor of platelet aggregation, and moderator of vascular smooth muscle cell proliferation–migration–differentiation (antiatherosclerotic) through a specific membrane-bound receptor, the prostacyclin receptor (IP receptor) (Fig. 4). The receptor belongs to the prostanoid family of G protein-coupled receptors and is widely distributed throughout the body with pre-dominant cardiovascular expression on platelets and smooth muscle cells. The receptor has a molecular weight of 37–41 kDa (depending upon different states of glycosylation) and has been categorized as a Class A rhodopsin-like GPCR. Glycosylation of the extracellular domain is necessary for ligand binding, receptor activation, and membrane localization. In the cytoplasmic domain, a number of serine residues (S328 and S374) are thought to be phosphorylated by GPCR kinases or second-messenger-activated kinases (PKC and PKA) and play potential roles in either agonist-induced phosphorylation and/or kinase-mediated receptor desensitization (Stitham et al. 2007). Prostaglandins have two structural features, a

cyclopentane ring and side chains that are recognized by their receptor to stabilize ligand binding. The binding pocket of the receptor can accommodate the cyclopentane rings of PGI₂, PGE₁, and PGE₂. The IP is most commonly associated with coupling to the G α s subunit of the heterotrimeric G-protein, which upon receptor activation stimulates membrane-bound adenylyl cyclase to catalyze the formation of the second messenger, cAMP. Animal studies using prostacyclin receptor knock-out mice have revealed increased propensities towards thrombosis, intimal hyperplasia, atherosclerosis, restenosis, as well as reperfusion injury.

Modification of IP and TP Function by Heterodimerization

IP and TP associate to form homo- and heterodimers. Interestingly, heterodimerization with the IP facilitated coupling of the TP α to cAMP generation (an IP-like cellular response) (Wilson et al. 2004) and rendered the TP sensitive to regulation by IP agonists (Wilson et al. 2007). Therefore, when IP and TP are present in the same cell, a common occurrence in cardiovascular tissues, cAMP can be generated via the PGI₂-IP/IP and TxA₂-IP/TP pathways. This signaling shift likely contributes to the limit placed by the IP on the deleterious cardiovascular effects of TP activation. One variant, IP^{R212C}, which occurs at low frequency (0.8 % in white and Asian cohorts), is associated with accelerated cardiovascular diseases. IP^{R212C} exerts a dominant action on the wild-type IP and TP α through dimerization. This likely contributes to accelerated cardiovascular disease in individuals carrying the variant allele (Ibrahim et al. 2010).

Clinical Importance

Two polymorphisms, which result in the amino acid mutations Val25Met and Arg21His were reported. The latter polymorphism exhibited a significant decrease in signaling upon activation of the IP receptor (Stitham et al. 2002). Other dysfunctional human prostacyclin receptor variants were described, some having defects in binding, activation, and/or protein stability/folding. Mutations (M113T, L104R, and R279C) in three highly conserved positions demonstrated severe misfolding resulting in impaired ligand binding and activation of cell surface receptors. In a cohort study, major coronary artery obstruction was significantly increased in the dysfunctional mutation group in comparison with the silent mutations (Stitham et al. 2011). Circulating platelet–leukocyte mixed conjugates and platelet microparticles are potential markers of inflammation in atherothrombotic disease. Epoprostenol (a synthetic salt of PGI₂ clinically used in pulmonary hypertension and transplantation as a potent inhibitor of platelet aggregation) inhibits human platelet–leukocyte conjugate and platelet microparticle formation in whole blood. Epoprostenol might reduce the inflammatory cell contribution to pulmonary hypertension and thrombosis (Tamburrelli et al. 2011).

3.3.7 Thrombin Receptors

Biochemical Description

As explained earlier, platelet activation by thrombin partially depends on GPIb-IX-V, but is primarily assured by two protease-activated receptors (PAR), i.e., PAR-1 and PAR-4. PARs consist of four members (PAR1-4), PAR-3 and PAR-4 being the mouse platelet receptors for thrombin. GPIb contains a high affinity binding site for thrombin within residues 268–287 at the N-terminal globular domain and can bind two separate thrombin molecules by interacting with both exosite I and II of thrombin. Binding of thrombin (immobilized, proteolytically inactive) to GPIb induces platelet adhesion and spreading and secretion; platelets from patients with the BSS which lack GPIb display impaired thrombin responsiveness (Adam et al. 2003). Or, GPIb is an enhancer of the thrombin response. PAR-1 and PAR-4 are activated by a unique irreversible proteolytic cleavage within the first extracellular loop exposing an N-terminus that serves as a tethered ligand. Short synthetic peptidomimetics of the N-terminus sequences, corresponding to the new N-terminus, upon cleavage by thrombin (SFLLR for PAR-1 and GYPGQV for PAR-4) can activate these receptors directly, reproducing most of the action of thrombin on platelets. This dual-receptor signaling model for thrombin implies that PAR-1 is the primary mediator, activating platelets at low concentration vs. PAR-4 as a back-up receptor, activated at higher thrombin concentrations. Qualitative differences in the dynamics of PAR-1 and PAR-4 activation might be relevant for sustained optimal platelet responses to thrombin. Thus, the PAR-4 mediated Ca^{2+} mobilization is slower and more prolonged than that of PAR-1 and it is switched off more slowly.

No patient has been identified with congenital deficiencies of PAR receptors. Pharmacological PAR-1 inhibitors are in development for the prevention of platelet-dependent thrombosis (Oestreich 2009).

3.4 Receptors in Stabilization Phase

Stabilization of platelet aggregates is a dynamic process requiring contact-dependent signaling and outside-in signaling through integrins, particularly $\alpha\text{IIb}\beta 3$. Signals emanate from $\alpha\text{IIb}\beta 3$ once ligand binding has occurred, thus triggering essential events for thrombus stabilization, such as cytoskeletal reorganization, enlarging platelet aggregates, development of a procoagulant surface, and clot retraction. These processes increase the local concentration of soluble platelet agonists inside the aggregate consisting of degranulating platelets. In addition to integrins, other actors perpetuate the platelet plug (Rivera et al. 2009).

3.4.1 Eph Kinase Receptors

Biochemical Description

Eph kinases are receptor with ligands, the ephrins, expressed on the surface of cells. Eph kinases are receptor tyrosine kinases with an extracellular ligand binding domain and an intracellular tyrosine kinase domain. The Eph B subfamily is distinguished from the Eph A subfamily by an insertion within the extracellular domain that helps to define the ligand preferences for the receptor. Interactions between Eph kinases and ephrins on adjacent cells play a central role in neuronal patterning and vasculogenesis. Human platelets express EphA4, EphB1, and ephrinB1. The former is constitutively associated with $\alpha\text{IIb}\beta_3$ in both resting and activated platelets. Clustering of either EphA4 or ephrinB1 causes platelet adhesion to immobilized fibrinogen, but blockade of Eph/ephrin interactions hampers clot retraction by impairing β_3 phosphorylation. This results in inhibition of platelet aggregation at low agonist concentrations and results in smaller thrombi on collagen-coated surfaces under arterial flow conditions, causing premature disaggregation. These effects are partially due to the ability of ephrin B1 to activate Rap1, a Ras family member that supports integrin activation in platelets (Prevost et al. 2003, 2005).

3.4.2 Gas-6 and Its Receptors

Biochemical Description

Gas-6 (growth arrest-specific gene 6) is a vitamin K-dependent protein implicated in cell growth, adhesion, and migration, through its interactions with Tyro 3, Axl, and Mer tyrosine kinase receptors (TAM family). The TAM receptors are three related protein receptor tyrosine kinases that were first cloned in 1991 as orphan receptors, widely expressed in the vertebrate nervous system. The three receptors consist of two N-terminal immunoglobulin domains, followed by two fibronectin-III-like domains attached via a single-pass α -helical transmembrane domain to an intracellular tyrosine kinase domain. In common with other receptor tyrosine kinases, the functional receptors form both hetero- and homodimers. The structure of receptor–ligand complexes causes two N-terminal immunoglobulin domains to mediate binding to the ligand.

Mouse Gas-6 is found in plasma and in platelets' granules from which it is secreted upon activation. In contrast, in man Gas-6 is predominantly present in plasma. Mice deficient in Gas-6 or in one of its receptors show abnormal platelet responses to agonists and are protected against thrombosis, suggesting a major role of this axis in thrombus formation and vascular wall homeostasis. Recently, the role of Gas-6 in human platelet function has been clarified. Gas-6 reinforces $\alpha\text{IIb}\beta_3$ outside-in signaling by activation of PI-3 K and Akt, and promotes β_3 phosphorylation and therefore clot retraction (Angelillo-Scherrer et al. 2005). These effects

constitute in fact, an enhancement and perpetuation of the thrombus-stabilizing role of ADP. The inhibition of Gas-6 signaling has been proposed as an attractive target for novel antithrombotic drugs (Cosemans et al. 2006, 2010).

3.4.3 Tetraspanin Superfamily

Tetraspanins, also called tetraspans or the transmembrane 4 superfamily (TM4SF), have four transmembrane domains, intracellular N and C-termini, and two extracellular domains (EC1, EC2), one short and one longer, typically 100 amino acid residue long loop. The key features are four or more cysteine residues in the EC2 domain, with two in a highly conserved “CCG” motif. Generally, tetraspanins are often thought to act as scaffolding proteins, anchoring multiple proteins to one area of the cell membrane.

CD151

Biochemical Description

The tetraspanin superfamily member CD151 (previously termed PETA-3/SFA-1) is broadly expressed in vascular, hematopoietic, and immune compartments, and is especially abundant in epithelia, endothelia, cardiac muscle, smooth muscle, megakaryocytes, platelets, and the immune system. CD151 is functionally linked to hemidesmosome formation, cancer metastasis, neurite outgrowth, vascular morphogenesis, cell migration, integrin trafficking, wound healing, immune responsiveness, and hemostasis. Recently, it has been found that CD151 appears to regulate fibrinogen-binding proteins, including integrin α IIb β 3. The absence of CD151 leads to smaller, unstable thrombi being formed in vivo (Orlowski et al. 2009).

TSSC6

General Description

Tumor-suppressing subchromosomal transferable fragment cDNA 6 (TSSC6), also previously known as Pan hematopoietic expression (Phemx), is a tetraspanin superfamily member. Its C-terminal cytoplasmic domain is relatively large (33 and 99 amino acids in mouse and human, respectively) compared to other tetraspanin superfamily members. TSSC6 is specifically expressed in hematopoietic organs and tissues where it may play a role in hematopoietic cell function. Absence of platelet TSSC6 affects the secondary stability of arterial thrombi in vivo upon vascular injury, by regulating integrin α IIb β 3 “outside-in” signaling events (Goschnick et al. 2006).

3.4.4 CD36

Biochemical Description

CD36, also known as scavenger receptor, GPIIb, GPIV, GP88, FAT, SCARB3, or PASIV, is expressed on the surface of most cells, such as platelets, monocytes, endothelial cells, smooth muscle cells, and cardiomyocytes. The human gene is located on chromosome 7. The CD36 protein consists of a single peptide chain of 474 amino acids and has a molecular weight of 80–90 kDa. On platelets 10,000–25,000 molecules are present. CD36 has a “hairpin-like” configuration, containing two transmembrane domains, one near the N-terminus and the other near the C-terminus, separated by a large, glycosylated extracellular loop. Platelet ligands of CD36 identified include TSP1, oxidized phospholipids (oxPL), oxidized low-density lipoprotein (oxLDL), and long chain fatty acids. CD36 was first described as a collagen type I and III receptor on platelets. However, platelets from CD36-deficient patients respond normally to collagen stimulation. Even when CD36 binding to the nonfibrillar type V collagen is documented, today it is clear that CD36 is not a primary collagen receptor (Nergiz-Unal et al. 2011). Yet, platelet activation is modulated by CD36 interactions with various ligands. A new role for TSP-1 in promoting platelet aggregation through modulation of an inhibitory signaling pathway has been demonstrated. Through CD36, TSP-1 prevents cAMP/protein kinase A (PKA) signaling. Indeed, TSP-1 triggers CD36-dependent signals that reduce platelet sensitivity to PGE₁, diminishing its ability to inhibit platelet aggregation and arrest under conditions of flow (Roberts et al. 2010). On the other hand, oxLDL formed in the setting of hyperlipidemia and atherosclerosis can activate platelets in a CD36-dependent manner.

Clinical Relevance

The level of platelet CD36 surface expression is highly variable among individuals from a general population. This variability affects the functional response of platelets to oxLDL and is associated with inheritance of specific genotypic polymorphisms at the *CD36* locus in both Caucasians and African Americans (Ghosh et al. 2011).

3.4.5 TLT-1

General Description

Triggering Receptors Expressed on Myeloid cells (TREM)s are involved in the activation of various cell types of the innate immune system, including monocytes, macrophages, microglia, and neutrophils. The family is characterized by a single V-set immunoglobulin (Ig) domain, a short cytoplasmic tail, and a charged residue

in the transmembrane domain. TREM-like transcript-1 (TLT-1), a type 1 single Ig domain orphan receptor specific to platelet and megakaryocyte alpha-granules, relocates to the platelet surface upon platelet stimulation and its longer cytoplasmic tail carries a canonical ITIM (Immunoreceptor Tyrosine-based Inhibition Motif) capable of becoming phosphorylated and of binding Src homology-containing protein tyrosine phosphatase-1 (SHP-1), identifying TLT-1 as the only putative inhibitory member of the TREM cluster. The ability of anti-TLT-1 scFv to block aggregation of washed platelets suggested that TLT-1 facilitates thrombosis by interacting with a ligand or ligands on or in activated platelets. TLT-1 may act in concert with α IIB β 3 to facilitate fibrinogen/platelet interactions and/or higher order platelet aggregation (Giomarelli et al. 2007; Washington et al. 2009).

Patients

Septic patients, in contrast to healthy individuals, have substantial levels of soluble TLT-1 (sTLT-1) in their plasma that correlated with the presence of disseminated intravascular coagulation (DIC). The sTLT-1 directly promotes platelet aggregation *in vitro* at clinically relevant concentrations, which suggests that TLT-1 may be a novel, platelet-specific, secondary activation factor (see Sect. 4).

3.4.6 PEAR1

General Description

PEAR1 (Platelet Endothelial Aggregation Receptor-1) (also known as MEGF12 or JEDI) is a transmembrane protein of the multiple EGF-like domain protein family of 150 kDa. PEAR1 is mainly expressed in platelets, endothelial cells, and also in satellite glial cell precursors, where it is necessary for apoptotic neuron clearance in the embryonic dorsal root ganglia via an engulfment activity. PEAR1 is composed of an extracellular EMI domain (Emilin domain), 15 extracellular epidermal growth factor-like repeats (EGF-like repeats), and multiple cytoplasmic tyrosines and prolines. The intracellular domain structure contains 5 proline-rich domains and an NPXY motive, which may serve as a phosphotyrosine binding site. During platelet aggregation, PEAR1 is phosphorylated at Tyr-925 and Ser-953/1029, upon its oligomerization, in an α IIB β 3-dependent manner. PEAR1 phosphorylation is also observed, independently of α IIB β 3 during physical platelet approximation via centrifugation. Thus PEAR1 was hypothesized to be a platelet–platelet contact receptor (Nanda et al. 2005).

Functional Relevance

A possible function for PEAR1 was substantiated by several studies that have linked polymorphisms in PEAR1 with increased or decreased platelet responses to various

agonists. A PEAR1 promoter-region variant (rs2768759) was associated with increased aggregation in PRP, most strongly in response to epinephrine, in both pre- and postaspirin treatment conditions (Herrera-Galeano et al. 2008). Increased expression of PEAR1 might be an important cause of hyperactivity (Herrera-Galeano et al. 2008) and genetic variation within PEAR1, particularly rs41299597, seems to lead to an increased membrane expression of PEAR1 in activated platelets and elevated responsiveness to GPVI ligands (Jones et al. 2009a). A genome-wide meta-analyses linked the minor allele of the PEAR1 SNP (rs12566888) to a drop in aggregation response towards ADP and epinephrine in the European and African-ancestry sample (Johnson et al. 2010).

3.4.7 P-Selectin/PSGL-1 Couple

General Description

P-selectin is a cell adhesion molecule of the selectin family of 140 kDa. The primary ligand for P-selectin is PSGL-1 (P-selectin glycoprotein ligand-1), constitutively found on all leukocytes. The transient interactions between P-selectin and PSGL-1 allow leukocytes and activated platelets to roll along the venular endothelium. The formation of a fibrin network upon activation of the coagulation cascade is a critical event contributing to thrombus stability. Recent studies with a laser injury-induced thrombosis model in mice expressing a low level of tissue factor (TF) have shown that this fibrin formation depends on the monocyte-derived TF carried by microvesicles, with minimal contribution of vessel wall TF. These microvesicles are captured onto the thrombus through the interaction between P-selectin expressed on the surface of activated platelets and PSGL-1 present on microvesicles, hence delivering TF to the growing thrombus. Mice deficient in either PSGL-1 or P-selectin display thrombi with little TF and reduced thrombin generation, resulting in hampered thrombus size (Abdullah et al. 2009; Morel et al. 2008; Ramacciotti et al. 2009).

Cardiovascular Patients

Soluble P-selectin is present in the blood and circulates in man at 100 ng/ml. Elevated plasma sP-selectin is a major predictive factor of cardiovascular events related to platelet turnover and its activation and function. Increased expression of P-selectin is observed in coronary artery disease, acute myocardial infarction, stroke, and peripheral artery diseases.

3.5 *Receptors in Negative Regulation of Platelet Activation*

Arterial thrombus formation is a dynamic process, in which a state of surface passivation is preferable to limit thrombus growth. The essential roles of nitric

oxide (NO) and prostacyclin (PGI₂) in the negative regulation of platelets to prevent uncontrolled thrombosis have been well established. However, it is now recognized that various receptors with and without ITIM domain inhibit thrombus formation actively.

3.5.1 ITIM-Containing Receptors

ITIMs are defined by a consensus sequence (L/I/V/S)-X-Y-X-X-(L/V) and are commonly found in pairs separated by 15–30 amino acid residues. ITIM-containing receptors were originally identified by their ability to inhibit signaling by ITAM receptors. For example, PECAM-1 causes mild inhibition of platelet activation by the ITAM and ITAM-like receptors, GPVI and CLEC-2, and by the G protein-coupled receptor agonist, thrombin. The latter action is similar to that of G6b-B (see below), which upon cross-linking by a specific antibody inhibits activation of platelets by the GPVI-specific agonist, collagen-related peptide, and the G protein-coupled receptor agonist ADP.

3.5.2 PECAM-1

General Description

Platelet activation can be inhibited through the adhesion molecule PECAM-1 (or CD31). Like GPVI, PECAM-1 is a member of the Ig superfamily, with 6 extracellular Ig domains, transmembrane domain, and cytoplasmic tail. The cytoplasmic domain of PECAM-1 contains an ITIM, which becomes phosphorylated upon stimulation by homophilic interactions and/or clustering, facilitating the recruitment of tyrosine, serine/threonine, or possibly lipid phosphatases, and the consequent inhibition of kinase-dependent signaling. PECAM-1 plays a role in attenuating thrombus formation involving GPVI, GPIb, and thrombin-mediated platelet activation (Jones et al. [2009b](#)).

3.5.3 G6b-B

Description

The list of novel plasma membrane proteins identified via proteomics includes the immunoglobulin superfamily member G6b of 241 amino acids (26-kDa), which undergoes extensive alternate splicing. G6b-B undergoes tyrosine phosphorylation and association with the SH2 domain-containing phosphatase, SHP-1, in stimulated platelets suggesting that it may play a novel role in limiting platelet activation. G6b-B is the only one of these variants to contain both a transmembrane region and two ITIM that support binding to the two SH2 domain-containing protein tyrosine

phosphatases, SHP1 and SHP2. The second ITIM in G6b-B is located around 20 amino acids downstream of the first ITIM and has a slightly different sequence mentioned earlier (TXYXXV) (Mori et al. 2008).

3.5.4 VPAC1/PACAP

Description and Patients

The pituitary adenylate cyclase-activating polypeptide (PACAP) is a neuropeptide of the vasoactive intestinal peptide/secretin/glucagon superfamily. Studies in two related patients with a partial trisomy 18p revealed three copies of the PACAP gene and elevated PACAP concentrations in plasma. Patients suffer from severe mental retardation and have a bleeding tendency with mild thrombocytopenia. The PACAP receptor (vasoactive intestinal peptide/pituitary adenylate cyclase-activating peptide receptor 1 VPAC1) in platelets is coupled to adenylyl cyclase activation. Increased basal cAMP levels in patients' platelets have been found, providing a basis for the reduced platelet aggregation in these patients. Megakaryocyte-specific transgenic overexpression of PACAP in mice correspondingly increased PACAP release from platelets, reduced platelet activation, and prolonged the tail bleeding time. The therapeutic potential to manage arterial thrombosis or bleeding by administration of PACAP mimetics or inhibitors is being considered (Freson et al. 2004).

4 Proteolysis/Shedding of Platelet Receptors

Metalloproteinase-mediated ectodomain shedding of platelet receptors has emerged as a new mechanism for modulating platelet function. By regulating surface expression of GPVI and GPIIb α , shedding not only irreversibly downregulates GPVI/GPIIb α functions, but generates proteolytic fragments that might be unique biomarkers or modulators in plasma. Altered expression levels of GPIIb α /GPVI are associated with both thrombotic propensity and platelet aging, suggesting an additional role in platelet clearance. Recently, the molecular GPVI shedding has been described to be dependent on FXa or/and other proteinases. Indeed, coagulation-induced GPVI shedding via FXa downregulates GPVI under procoagulant conditions. FXa inhibitors have an unexpected role in preventing GPVI downregulation. Furthermore, various platelet proteinases (at least ADAM10 and 17) regulate GPVI shedding (Bender et al. 2010).

Furthermore, the TLT-1 extracellular domain is shed from platelets during activation. Therefore, the possibility that sTLT-1 might be released under pathological conditions, resulting in changes in platelet function during those disease states has been advanced. Data from two independent cohorts confirmed the presence of increased levels of sTLT-1 in sepsis patients. The finding that patients who died

during sepsis had increasing levels of sTLT-1, whereas those who survived showed a decline in sTLT-1 during this same period, suggesting that monitoring of sTLT-1 levels could be an important prognostic indicator. Similarly, the correlation between sTLT-1 and D-dimers indicates association of sTLT-1 with the clinical manifestations of disseminated intravascular coagulation (DIC). These data substantiated the involvement of TLT-1 in the response to sepsis and indicate that sTLT-1 may provide a significant clinical tool for the diagnosis of disseminated intravascular coagulation associated with sepsis (Washington et al. 2009). Other proteins can be shed, like P-selectin, ICAM-1, and PECAM-1, the latter shed from human platelets during high shear stress. However, the concentrations released in plasma are insufficient to affect platelet activation; they may serve to monitor platelet activation and vascular injury in coronary artery disease. Indeed, a rapid increase of PECAM-1 has been reported after myocardial infarction (Soeki et al. 2003).

Recently, a clinical study showed that soluble P-selectin can establish the diagnosis of deep vein thrombosis (DVT) with a cutoff point of 90 ng/mL, when combined with a Wells score ≥ 2 , with a positive predictive value of 100%. Also, soluble P-selectin can exclude the diagnosis of DVT with cutoff points below 60 ng/mL, when combined with a Wells score < 2 , with a negative predictive value of 96%. Based on their data, 32% of patients could potentially be diagnosed with DVT without the need of imaging exams (Ramacciotti et al. 2011). One of the conclusions of this study is that such biomarkers (like P-selectin) could establish or exclude the diagnosis for thrombosis in case where ultrasound imaging is not available. Also, in patients with the antiphospholipid syndrome, sP-selectin measurements had a good prognostic value in thrombosis prediction (Devreese et al. 2010).

5 Conclusion

Different types of receptors control platelet function at various levels, as highlighted earlier. Translation of receptor-delivered signals occurs via a series of intracellular signaling pathways, discussed in larger detail in other chapters. There is a wealth of platelet-specific and platelet-prone receptors (see “Key Messages”); yet we only have limited pharmacological tools to control platelet function in secondary thrombosis prevention or in bleeding (see “Knowledge Gaps”). The new functional insight in the so-called secondary platelet receptors should accelerate the development of additional pharmaceutical drugs to control platelet function.

Knowledge Gaps

- Some receptors are orphans i.e., without know ligand.
- Interplay between platelet receptors activation in hemostasis and its consequences in inflammation, immune system, wound healing, angiogenesis, antibacterial activity, etc.
- Limited clinical phenotypes after identification of the large number of functional polymorphisms and mutations in various receptors.
- Limited molecular understanding of the role in hemostasis and thrombosis of newly identified potential membrane receptors via genome-wide linkage association studies.
- Limited pharmacological translation of basal knowledge into new drugs.

Key Messages

- Receptors can be classified into categories, depending on their role in adhesion, aggregation, amplification, and stabilization.
- Hemostasis is only achieved when the majority of receptors act in concert. There is limited redundancy in receptor function.
- Polymorphisms in receptors or their expression add to large variability in platelet responses to various agonists.
- Newly discovered receptors may guide development of new antithrombotics.

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