Cell Adhesion Mechanisms in Platelets

David Varga-Szabo, Irina Pleines, Bernhard Nieswandt

Abstract—At sites of vascular injury, platelets come into contact with the subendothelial extracellular matrix which triggers their activation and the formation of a hemostatic plug. This process is crucial for normal hemostasis, but may also lead to pathological thrombus formation causing diseases such as myocardial infarction or stroke. The initial capture of flowing platelets is mediated by the interaction of the glycoprotein (GP) Ib-V-IX complex with von Willebrand factor (vWF) immobilized on exposed collagens. This interaction allows the binding of the collagen receptor GPVI to its ligand and to initiate cellular activation, a process that is reinforced by locally produced thrombin and soluble mediators released from platelets. These events lead to the shift of β1 and β3 integrins on the platelet surface from a low to a high affinity state, thereby enabling them to bind their ligands and to mediate firm adhesion, spreading, coagulant activity, and aggregation. This review summarizes the most important structural and functional properties of these adhesion receptors and briefly discusses their potential as targets for antithrombotic therapy. (Arterioscler Thromb Vasc Biol. 2008;28:403-412)

Key Words: adhesion receptors platelet thrombosis activation

Platelets are small anuclear cell fragments that are produced by megakaryocytes in the bone marrow by the extension of dynamic protrusions into microvessels which appear to be sheared from their transendothelial stems by flowing blood, resulting in the appearance of proplatelets in peripheral blood.1,2 Platelets circulate for approximately 10 days in the blood stream before they are cleared by macrophages in spleen and liver. During their life time, most platelets never undergo firm adhesion in the circulation. Only when the endothelial cell layer of blood vessels is damaged by injuries or pathological alterations, such as found in atherosclerosis, the adhesive potential of platelets becomes evident. Under these conditions, components of the subendothelial extracellular matrix (ECM) are exposed and trigger sudden platelet activation and adhesion. In addition, various soluble stimuli are produced and released from platelets...
which strengthen platelet adhesion and, together with locally produced thrombin, recruit more platelets into the growing thrombus by exposing or activating receptors on the platelet surface which allow platelets to adhere to each other, a process termed aggregation (Figure 1). These events are crucial to prevent posttraumatic blood loss by providing a platelet plug that leads to the closure of smaller defects of the vessel wall and are required for primary hemostasis. On the other hand, uncontrolled thrombus formation in diseased vessels can lead to vascular occlusion and ischemia and infarction of vital organs. One of the major clinical problems in the developed world is arterial thrombosis caused by rupture or erosion of an atherosclerotic plaque leading to platelet adhesion and subsequent thrombus formation in coronary and cerebral arteries causing myocardial infarction and stroke, respectively.

The adhesive properties of platelets have to be tightly regulated to ensure that the cells readily become activated under appropriate conditions to prevent blood loss in cases of vascular injury, while at the same time undesired adhesion, which can lead to thrombotic events, has to be avoided. Platelets, which have evolved relatively late during evolution and which are only found in mammals, possess various adhesion receptors and a sophisticated regulatory machinery to adhere in response to a well-defined set of stimuli. This makes platelets a very instructive model for the cellular mechanisms underlying regulated adhesion. Their central role in ischemic cardio- and cerebrovascular diseases has made the signaling mechanisms and membrane proteins mediating platelet adhesion prime targets for drugs used to treat and prevent arterial thrombosis. A comprehensive discussion of the mechanisms that regulate platelet adhesion and current areas of debate is far beyond the scope of this review. Rather, we focus on the principal receptors that mediate platelet adhesion and aggregation on the damaged vessel wall, the majority of which is exclusively expressed in platelets/megakaryocytes. It is important to note, however, that under pathological conditions platelets can also adhere to the intact activated vascular endothelium by processes involving endothelial as well as platelet P-selectin, its ligand P-selectin glycoprotein ligand (PSGL)-1, as well as GP Ib, and thereby contribute to inflammatory processes by facilitating EC activation and leukocyte recruitment. The mechanisms underlying platelet-EC interaction in inflammation and atherosclerosis have recently been reviewed by Lindemann et al and will not be discussed in detail here.

The first step in the hemostatic cascade is platelet interaction with the exposed ECM which contains a large number of adhesive macromolecules, such as laminin, fibronectin, collagen, and vWF. The mechanisms of platelet adhesion at sites of injury are to a large extent determined by the prevailing rheological conditions. Blood flows with a greater velocity in the center of the vessel than near the wall, thereby generating shear forces between adjacent layers of fluid that become maximal at the wall. The drag, which opposes platelet adhesion and aggregation, increases with the prevailing shear rates. Under conditions of high shear, such as is found in small arteries and arterioles, the initial adhesion of platelets to the ECM is mediated by the interaction between the platelet receptor glycoprotein (GP) Ib and vWF bound to collagen. Although mandatory at high shear, this interaction may not be relevant under conditions of low shear as found in veins and large arteries. The binding of GP Ib to vWF has a fast off-rate and is therefore insufficient to mediate stable adhesion but rather maintains the platelet in close contact with the surface, although it continuously translocates in the direction of blood flow. During this “rolling,” platelets establish contacts with the thrombogenic ECM protein collagen through the immunoglobulin superfamily receptor, GPVI. While GPVI binds collagen with low affinity and thus is unable to mediate adhesion by itself, it triggers intracellular signals that shift platelet integrins to a high-affinity state and induce the release of the secondary mediators adenosine diphosphate (ADP) and thromboxane A2 (TXA2). These agonists together with locally produced thrombin contribute to cellular activation by stimulating receptors that couple to heterotrimeric G proteins (Gα, G12/G13, G). which induce different signaling events and act synergistically to induce full platelet activation (Figure 2). Under conditions, when high concentrations of these soluble agonists are present, this may be sufficient to mediate platelet activation independently of GPVI, as shown experimentally in a model of laser-induced arterial injury in mice, where thrombus formation occurs independently of collagen receptors. The mechanisms of G protein–mediated platelet activation have recently been summarized in an excellent review by Offermanns and will not be discussed here.

Firm adhesion to the ECM is mediated by high-affinity β1 integrins which bind to collagen (α2β1), fibronectin (α5β1), and laminin (α6β1), as well as the major platelet integrin, αIIbβ3, interacting with fibronectin and collagen-bound vWF. In the following, the function of these individual receptors and their ligands will be discussed in more detail.
vWF

A central mediator in the adhesion of platelets is vWF, a multimeric adhesive glycoprotein that contains binding sites for collagen as well as for the 2 major platelet receptors, GPIb and integrin αIIbβ3. vWF is found in the Weibel-Palade bodies of endothelial cells, in the α-granules of platelets, and in the plasma at a concentration of approximately 10 g/mL in humans. The mature subunit of vWF consists of 2050 amino acids and is composed of 4 different repeating domains (A–D). The 3 homologous A domains span residues 497 to 1111 and regulate interaction with different receptors and prothrombotic ligands of the subendothelial matrix. The A1 domain exclusively binds collagen type VI, whereas collagen I and III are bound via the A3 domain. The C1 domain contains the sequence Arg-Gly-Asp (RGD), which represents a binding motif for both of the platelet αIIbβ3-integrins, namely αIIbβ3 and αvβ3. The interaction between vWF and the receptor complex GPIb-V-IX occurs via the A1 domain and is of pivotal importance in the initial adhesion of platelets to the subendothelium under conditions of high shear, such as found in atherosclerotic vessels.

Intracellularly assembled dimers of mature vWF subunits serve as constituents to build large multimers up to 20 MDa in molecular weight. The largest multimers with the greatest thrombogenic potential are stored in platelets and endothelial cells and get secreted on cellular activation or damage. Under normal conditions, soluble vWF does not undergo significant interactions with its platelet receptor, GPIb-V-IX. However, when immobilized on exposed collagen at sites of injury, it becomes a strong adhesive substrate. In vitro studies suggest that conformational changes in the A1 domain of vWF could alter its affinity for interaction partners. Ristocetin, a bacterial-derived antibiotic from Nocardia lurida and botrocetin, a viper venom protein from Bothrops jararaca, induce vWF/GPIb-V-IX interaction under static conditions, whereas under in vivo conditions, the vWF molecule might alter its conformation because of high shear forces and because of the immobilization on a surface. The absence of vWF in all compartments in humans causes severe defects in primary hemostasis and coagulation. Such vWF type 3 patients have strongly reduced factor VIII levels and suffer from spontaneous bleeding. A similar phenotype has also been reported for vWF knockout mice which display massively prolonged bleeding times and defective thrombus formation in vivo, emphasizing the importance of vWF in hemostasis and thrombosis. However, in contrast to GPIb, vWF appears not to be essential for thrombus formation in vivo, as much delayed adhesion still occurs even under arterial flow conditions, indicating that GPIb can initiate adhesion by interacting with other ligands. One of the strongest candidates is thrombospondin-1, which has been shown to interact with GPIb under high shear flow conditions in vitro.

The GPIb-V-IX Complex

The initial contact of platelets with the exposed ECM must occur through the action of a receptor that functions irrespective of cellular activation and thereby facilitates rapid interactions that resist shear forces acting on the cells. This process is mediated by GPIb-V-IX, a highly abundant (25,000 copies per platelet) structurally unique receptor complex exclusively expressed in platelets and megakaryocytes. Four different genes encode the receptor complex, namely the α- and β-subunits of GPIb, GPIX, and GPV, all of which belong to the leucine-rich repeat protein superfamily and are characterized by the presence of 1 or more leucine-rich repeats. GPIbα (135 kDa), which represents the major functional subunit of the receptor complex, is linked by disulphide bonds to 2 GPIbβ subunits (25 kDa). GPIX (22 kDa) and GPV (88 kDa) are noncovalently associated, and...
resulting in an overall receptor stoichiometry of 2:4:2:1.\textsuperscript{13,22} The N-terminal extracellular part of GPIb\textalpha consists of 8 LLRs and contains the binding domains for most interaction partners of the receptor complex, namely vWF, Mac-1, P-selectin, thrombin, high molecular weight kininogen (HMWK), and factor XII (for review see ref. 13). The C-terminal cytoplasmic tail of GPIb\textalpha is composed of 96 amino acids and contains binding sites for putative signaling molecules, such as actin-binding protein and 14-3-3\textgamma.\textsuperscript{23}

In humans, lack or dysfunction of GPIb-V-IX has been associated with the Bernard-Soulier syndrome (BSS), a congenital bleeding disorder characterized by mild thrombocytopenia, giant platelets, and inability of the cells to aggregate in response to ristocetin.\textsuperscript{24} Similarly, mice deficient in GPIb\textalpha or GPIb\textbeta lack the entire receptor complex and reflect human BSS, as they display a severe bleeding phenotype and macrothrombocytopenia. Whereas the macrothrombocytopenia appears to be related to alterations in the cytoplasmic domain of GPIb, the lack of GPV does not cause a BSS-like phenotype and as no mutations within the GPV gene have been described in BSS patients,\textsuperscript{25} and mice deficient in this subunit produce rather normal platelets and show only very minor alterations in hemostasis.\textsuperscript{28,29}

**GPIb\textalpha-Mediated Tethering on vWF**

At the high shear rates found in small arteries and arterioles, the instantaneous onset of the interaction between GPIb\textalpha and the A1 domain of vWF immobilized on collagen or on the surface of activated platelets is crucial for the initial tethering (or capture) of flowing platelets.\textsuperscript{9} This interaction is, however, characterized by a rapid dissociation rate and is insufficient for stable adhesion. This can be illustrated by the rolling of GPIb-IX–expressing cells or platelets on a vWF monolayer at high shear.\textsuperscript{9} Thus, the principal function of GPIb-V-IX is to recruit platelets to the site of injury and to reduce their velocity to enable the interaction of other receptors with the thrombogenic surface. It is not entirely clear how the interaction between GPIb\textalpha and vWF is regulated but this process appears to involve conformational changes in both the receptor and the ligand (recently reviewed in ref.\textsuperscript{30}). Furthermore, phosphorylation of serine 166 of the GPIb\textbeta subunit may negatively regulate vWF binding by the GPIb-V-IX complex.\textsuperscript{31}

Besides its mandatory function as an adhesion receptor, increasing evidence suggests that GPIb-V-IX also acts as a signal transducer that has the capacity to activate integrin \(\alpha IIb \beta 3\) and thereby facilitates firm adhesion of the cells independently of “classical” activating receptors, such as G protein–coupled receptors (GPCRs) or GPVI (see below). It has been proposed that GPIb-V-IX may be involved in immunoreceptor tyrosine activation motif (ITAM) signaling based on the observation that the receptor complex is recruited to lipid rafts,\textsuperscript{32} where it may interact with GPVI\textsuperscript{33} and induce phosphorylation of the Fc receptor (FcR) \(\gamma\)-chain associated ITAM.\textsuperscript{34} This could not, however, be confirmed in other studies.\textsuperscript{35} Others have proposed that GPIb signaling may occur through the association with proteins, such as 14-3-3\textgamma, Src kinases, calmodulin, and PI3-kinase.\textsuperscript{36–38} Recently, adhesion and degranulation promoting adaptor protein (ADAP) was identified as a component of integrin inside-out signaling pathways induced by vWF/GPIb-V-IX and other agonists independent of the FcR\(\gamma\)-chain, indicating complementary integrin \(\alpha IIb \beta 3\) activation by GPVI and GPIb-V-IX.\textsuperscript{39} Despite these observations the vWF/GPIb-V-IX axis is generally considered to be a very weak agonist based on the observation that rolling of platelets on a vWF surface may last for several minutes until \(\alpha IIb \beta 3\)-mediated stable adhesion (via vWF) is seen.\textsuperscript{40}

The specific requirement for GPIb-vWF interaction under conditions of high shear, such as found in diseased arteries, makes it a potentially attractive target for the pharmacological inhibition of pathological thrombus formation in the setting of precipitate diseases such as myocardial infarction or stroke. In line with this, the inhibition of the vWF binding site of GPIb\textalpha by Fab fragments of the inhibitory antibody p0p/B abolished platelet tethering and subsequent thrombus formation at sites of arterial injury in mice.\textsuperscript{41} In addition, it was shown that arterial thrombus formation is abrogated in mice expressing GPIb\textalpha, in which the extracellular domain is replaced by that of the human interleukin (IL)-4 receptor,\textsuperscript{42} confirming the mandatory role of GPIb for adhesion and thrombus formation under arterial flow conditions. Although anti-GPIb\textalpha treatment results in prolonged bleeding times, this does not necessarily translate into an increased bleeding risk. This has only very recently been revealed in a model of ischemic stroke in mice, where anti-GPIb\textalpha Fab fragments (p0p/B) profoundly protected the animals from the development of ischemic brain infarcts but did not increase the incidence of intracranial bleeding compared with control.\textsuperscript{43} An alternative approach to interfere with GPIb-V-IX function could arise from the observation that GPIb\textalpha can be down-regulated from the platelet surface by the membrane-expressed sheddase, a disintegrin and metalloproteinase 17 (ADAM17).\textsuperscript{44} The potential of GPIb-V-IX as an antithrombotic target has recently been reviewed by Vanhoorelbeke et al.\textsuperscript{45}

**Platelet Collagen Receptors**

In contrast to vWF, subendothelial fibrillar collagen is a highly thrombogenic substrate as it directly induces powerful activation of the cells and supports their adhesion, aggregation, and coagulant activity by direct and indirect mechanisms. Of the more than 20 forms of collagen in the human genome, fibrillar types I and III are the major constituents of the ECM of blood vessels and have been the focus of most attention. In addition, the network-forming type IV collagen is the major form in the subendothelial basement membrane. Collagens consist of repeat GXY motifs where G is glycine and X and Y are frequently proline (amino acid code = P) and hydroxyproline (amino acid code = O). Barnes and Farndale demonstrated that the sequence GPO, which makes up approximately 10% of collagens I and III represents the strongest platelet stimulus within the macromolecule\textsuperscript{46} that was later shown to specifically interact with GPVI\textsuperscript{47} (see below). Besides GPIb\textalpha and integrin \(\alpha IIb \beta 3\), which interact with collagen via vWF,\textsuperscript{9} several collagen receptors have been
identified on the platelet surface, most notably the immunoglobulin superfamily member GPVI and integrin α2β1.48,49 The relative contribution of these 2 receptors to platelet-collagen interactions has been intensively debated in the literature. It is now widely accepted that although GPVI serves as an activatory receptor that is essential for platelet activation and aggregation on collagen, integrin α2β1 contributes to firm adhesion and only makes minor contribution to cellular activation.48

**GPVI**

GPVI is the major platelet collagen receptor to mediate cellular activation, which is a prerequisite for efficient adhesion, aggregation, degranulation, and coagulant activity on the matrix protein.48,50 GPVI (62 kDa) is a type I transmembrane receptor expressed exclusively in platelets and megakaryocytes.51 It contains 2 Ig-C2–like extracellular domains formed by disulfide bonds, a mucin-like stalk, a transmembrane region, and a short 51-aa cytoplasmic tail.51 GPVI harbors a positively charged arginine in its transmembrane region which allows it to undergo a noncovalent association with the Fc receptor-γ (FcRγ) chain, the ITAM-bearing signal transducing subunit also complexed with a variety of immune receptors.52,53 The GPVI cytosolic tail contains a proline-rich motif that binds selectively to the SH3 domain of the Src family tyrosine kinases, Fyn, and Lyn. Crosslinking of GPVI by ligand binding brings Fyn and Lyn into contact with the FcRγ-chain, resulting in tyrosine phosphorylation of the ITAM.54,55 The tyrosine kinase Syk then binds to the phosphorylated tyrosine via its SH2 domain and thereby becomes autophosphorylated and activated. Activated Syk, in turn, initiates a signaling cascade that is thought to take place in cholesterol-rich membrane domains known as lipid rafts and involves the formation of a “signalosome” containing the adaptors LAT and SLP-76 as well as effector proteins, most notably phospholipase (PL)Cγ2, phosphoinositide-3 (PI-3) kinase, and small G proteins.56 PLCγ2 subsequently induces the formation of the second messengers 1,2-diacylglycerol (DAG) and inositol 1,4,5-trisphosphate (IP3). DAG activates protein kinase (PK) C, whereas IP3 induces the release of Ca²⁺ from intracellular stores and subsequent Ca²⁺ entry resulting in an increase in cytosolic Ca²⁺ concentrations. A very recent study suggests that store-operated Ca²⁺ entry may play a particular role in this process.57 GPVI-mediated signaling results in strong integrin activation and release of stored mediators, which play a central role for thrombus growth (see below).11,49 GPVI-ITAM signaling can be inhibited by immunoreceptor tyrosine-base inhibition motif (ITIM)-coupled receptors. In platelets, ITIM receptors activate the cytoplasmic tyrosine phosphatases SHP1 and SHP2, thereby inhibiting activation of PLCγ2.58 The major ITIM-containing receptor on the platelet surface is PECAM-1 (CD31) (10,000 copies per cell), which significantly inhibits GPVI signaling, but is also involved in the negative regulation of signals generated by G protein–coupled receptors and the GPIb-V-IX complex.59 PECAM-1 is very highly expressed on endothelial cells (1 million copies per cell) and becomes activated by binding to itself, indicating an important role in the prevention of platelet activation on the endothelial surface. However, the inhibitory function of PECAM-1 has been questioned by recent studies showing that PECAM-1 had only a minor inhibitory effect on platelet activation induced by ITAM signaling and that thrombus formation on collagen was not altered if PECAM-1 was absent.60 Furthermore, Wee et al reported even potentiation of aggregation in PECAM-1–/– platelets in response to CRP,61 suggesting that further experiments will be needed to address these discrepancies.

No patient with a genetic deficiency in GPVI has been reported to date. However, a few GPVI-deficient patients have been described who had anti-GPVI antibodies in their blood which was difficult to explain.62,63 A likely explanation for this observation comes from studies in mice showing that injection of an anti-GPVI antibody (JAQ1) into mice results in a downregulation of the receptor from the platelet surface and a GPVI knockout-like phenotype for a prolonged time period.64 Such GPVI-depleted platelets are not activatable by collagen and do not firmly adhere to the immobilized protein under high or low shear flow conditions and the same is seen with platelets from FcRγ-chain–deficient mice, which also lack GPVI. Although the initial tethering and rolling still occurs through the interaction of GPIb with collagen-bound vWF, the transition to stable adhesion is defective because of the lack of integrin activation.50,63 Direct activation of integrins by Mn²⁺ or the addition of agonists, which activate integrins via inside-out signaling, such as ADP, can restore adhesion of such platelets. Mice genetically engineered to lack GPVI later confirmed the essential role of this receptor for collagen-induced platelet activation and spreading on the matrix protein,65,66 although in one study initial adhesion of GPVI-deficient platelets to collagen was detected.65 Mice lacking the GPVI/FcRγ-chain complex do not exhibit a major bleeding phenotype but they are profoundly protected from experimental arterial thrombosis. At sites of arterial injury markedly reduced platelet attachment and thrombus formation has been observed in FcRγ-chain–deficient,67 GPVI-depleted,41 or GPVI-deficient68 mice. Thus, GPVI is crucial for stable platelet adhesion on collagen surfaces or the ECM, but the receptor is, similar to GPIb-V-IX, unable to mediate this adhesion by itself. Rather, together with GPCRs stimulated by locally produced or released agonists, it mediates cellular activation and the shift of platelet integrins from low to high affinity states, thereby allowing firm adhesion and thrombus growth. Shear-resistant platelet attachment on collagen requires the binding of integrins, notably α2β1 and αIIbβ3, which directly or indirectly (via vWF) bind to the matrix protein, respectively.

**Integrins**

Integrins are widely expressed heterodimeric transmembrane receptors which regulate cell-extracellular matrix (ECM) and cell-cell interactions contributing to a wide variety of physiological processes.69 They are composed of a bigger α- and a smaller β-subunit which are noncovalently linked to each other and are type I transmembrane proteins. Both subunits consist of a large N-terminal extracellular domain which in the heterodimeric state of the integrin form a globular head, a single transmembrane domain and a short cytoplasmic tail.
domain. Integrins are supposed to be expressed in a low affinity state on the surface of resting platelets and will turn into a high affinity state following platelet activation, a process referred to as “inside-out” signaling. Ligand-occupied integrins on the other hand trigger various cellular processes, such as cell spreading, through “outside-in” signaling. Platelets express 3 different β-integrins, namely α2β1 (collagen receptor), α5β1 (fibronectin receptor), and α6β1 (laminin receptor) as well as 2 β3-integrins, αIIbβ3 and αβ3, the latter only being present in very low amounts. Among them, 2 are considered to be of pivotal importance for platelet adhesion and aggregation on the exposed subendothelial matrix: α2β1 and αIIbβ3.

Integrin α2β1
Integrin α2β1 (also termed GPIIb/IIIa, VLA-2, or CD49b/CD29) was the first platelet collagen receptor to be identified and serves mainly as an adhesion receptor. It is present on the platelet surface with a copy number of approximately 2000 to 4000 and can bind to collagen types I, II, III, IV, and XI. The role of integrin α2β1 in hemostasis and thrombosis has been controversial for a long time. Early publications suggested the integrin to be the central platelet collagen receptor that is essential for shear-resistant adhesion to the ECM. However, studies on mice lacking either the α2- or the β1-subunit failed to show a major hemostatic defect and it is now accepted that α2β1 plays a significant but not essential role for the adhesion process as other receptors, most notably integrin αIIbβ3, are also able to mediate shear-resistant platelet attachment to the ECM.

α2β1 is the only platelet surface receptor containing an I- (or A-) domain that is placed between the 2nd and 3rd repeats of the seven-bladed β-propeller of the α2-subunit—most probably present as a protrusion—of the extracellular domain and serves as the primary ligand binding interface of the integrin. There is a metal coordination site integrated into the protruding I-domain. The binding of a Mg+ ion to this site is critical for the recognition of a glutamate residue in the collagen molecule. Mutations affecting the upper surface of this domain caused loss of collagen binding under both static and flow conditions. The I-domain of the α2-subunit contacts the putative metal ion-dependent activation site (MIDAS) (or I-like) domain of the β1-subunit which is neighbored by a plexin-semaphorin-integrin (PSI) domain. The membrane-near extracellular region of β1 is rich in cysteine and disulphide bridges and is suggested to be responsible for conformational changes in the structure of the integrin on inside-out signaling.

Many integrins exist in low affinity states and require cellular activation to undergo a conformational change to efficiently bind their ligands, whereas other integrins have been shown to be constitutively present in a high affinity state. For a long time, platelet α2β1 was thought to belong to the latter group of integrins. An early model of platelet-collagen interactions, the so-called “2-step, 2-site” model incorporated the idea that platelets in a first step firmly bind to collagen via high affinity α2β1 allowing subsequent interaction of a second receptor leading to platelet activation. This second, low affinity receptor was later proposed to be GPVI. However, the idea that α2β1 is active on unstimulated platelets was first challenged by Moroi and coworkers who demonstrated that the affinity of the integrin for soluble collagen increases on cellular stimulation. This suggested that α2β1, like αIIbβ3 (see below), requires an agonist-induced conformational change (via inside-out signaling) to bind to collagen which was further confirmed by the analysis of GPVI-deficient murine platelets. The underlying “inside-out” signaling events are not clear at present but they might be similar to those regulating the affinity of integrin αIIbβ3 with talin binding as the final step (see below). Based on antibody binding studies, in the case of integrin α2β1 a third (so-called intermediate) conformation is suggested to exist, where the physiological ligand collagen can already bind, but antibodies specific for the high-affinity conformation cannot. A recent study that used an antibody that recognizes activation-induced conformational changes in α2β1 suggested that the activation of this integrin is dependent on the previous activation of αIIbβ3. However, the underlying mechanisms have not been identified and it remains to be determined whether the detected conformational changes indeed reflect “activation” of the integrin.

Inside-out activation of α2β1 can be induced through ligation of GPVI as demonstrated by defective adhesion of GPVI-deficient or -inhibited platelets to the matrix protein. Binding of α2β1 to collagen, on the other hand, contributes to cellular activation indirectly by reinforcing GPVI-collagen interactions and directly by a series of intracellular signaling events summarized as “outside-in” signaling. This is very similar to that induced by GPVI, including Src, Syk, SLP-76, and phospholipase (PL) Cy2. Thus, although structurally unrelated, the 2 major collagen receptors share important signaling molecules and act in a cooperative manner, reinforcing each other’s activity. Similar, recent elegant studies suggest that α2β1 and GPIIb have partially overlapping functions and cooperatively mediate primary adhesion on collagen/vWF, reinforced by activation through GPVI. Thus, although minor species-specific differences may exist in the interplay between GPIIb, GPVI, and α2β1 and the relative contribution of each of these receptors very much depends on the experimental conditions, it is now generally accepted that they act synergistically in the process of platelet adhesion, activation, and expression of procoagulant activity on the ECM. However, because the absence of α2β1 has been shown to have no or only minor protective effect in arterial thrombosis, inhibition of the integrin may be a less promising antithrombotic strategy than GPVI or GPIIb inhibition.

Integrin αIIbβ3
Integrin αIIbβ3 (GPIIb/IIIa) is the most abundant glycoprotein on the platelet surface with a number of 60,000 to 80,000 copies per cell and an additional intracellular pool that is exposed on the surface on activation. The affinity of this integrin for its ligands, most notably fibrinogen, fibronectin, and vWF is highly modulatable, and on activation it mediates platelet adhesion, aggregation, and spreading on the exposed extracellular matrix of the injured vessel wall as well as pathological thrombus formation. Lack or dysfunction of αIIbβ3 gives rise to Glanzmann thrombasthenia, a severe
bleeding diathesis associated with impaired adhesion and abolished aggregation of platelets.91 Accordingly, mice lacking the β3integrin resemble the phenotype of Glanzmann thrombasthenia with absent platelet aggregation, reduced clot retraction and greatly reduced fibrinogen uptake into platelets.92 These mice have markedly prolonged tail bleeding times and display spontaneous hemorrhage in all developmental stages. In intravital microscopy studies performed in mesenteric arterioles, β3-null mice do not form any thrombi.93 The integrin heterodimer is formed by noncovalent coupling of the αIIb and the β3 subunit during megakaryopoiesis. The “head” domain of the molecule is formed by the 7-bladed β-propeller and thigh domain in the α-chain and by the I-like domain (or A-domain) and hybrid domains in the β-chain.94–96 and has a size of approximately 8×10 nm as determined by rotary shadowing electron microscopy.97,98 The extracellular part of the α subunit further contains a calF1 and a calF2 domain, whereas the β subunit contains a PSL, a β-tail, and 4 EGF-like domains.94–96 These domains form the L-shaped “stalk” which consists of a “neck” and “body” region. Molecular modeling of the transmembrane region by electron cryomicroscopy and x-ray crystallography suggests that the 2 subunits span the membrane forming an α-helical coiled-coil structure,96 and end in a C-terminal short intracellular tail.

Integrin αIIbβ3 binds several ligands each containing an arginine-glycine-aspartic acid (RGD) sequence, such as fibrinogen, fibrin, vWF, fibronectin, thrombospondin, and vitronectin. Arginine contacts the α-subunit β-propeller and aspartic acid—through a Mg2+—the MIDAS of the β-subunit I-like domain.95 In resting platelets, however, the integrin is in a “low-affinity” or “off” state, which is characterized by a highly bent conformation which keeps the binding site for the RGD sequence hidden. On agonist-induced platelet activation “inside-out” signaling events lead to a conformational switch, the integrin changes into the “high-affinity” or “on” state, and the RGD binding site becomes unmasked. This process called switchblade-like opening can be initiated also passively by manganese ions.99 The “inside-out” activation of αIIbβ3 seems to be regulated through a mechanism that essentially involves the cytoplasmic tails of the 2 subunits. A current model suggests that there is an important—probably charge-regulated—interaction between the cytoplasmic tails of the α- and β-subunits which keeps the integrin in the inactive state.100 Mutagenesis of the membrane-proximal parts of the cytoplasmic tails or deletion of it results in the permanent activation of αIIbβ3 supporting this hypothesis.101,102 It is hypothesized that during “inside-out” signaling the interaction between the subunits is broken up and that the cytoskeletal protein talin-1 is the final effector molecule of this process.100

Talin-1 (270 kDa) is an elongated flexible protein that can bind to the cytoplasmic tail of integrin β3 and can link it to actin through its actin binding site at the C-terminal region.103 The proposed central role of talin in the activation of αIIbβ3 has very recently been confirmed by the analysis of platelets from mice with an induced talin-1 deficiency in platelets. These platelets fail to aggregate in response to all major agonists and display abolished adhesion to fibrinogen and collagen in vitro, the latter suggesting also defective activation of α2β1. In vivo, adhesion and thrombus formation at sites of vascular injury is abrogated (Figure 3) and the animals are unable to arrest bleeding.105 These results provide the first direct evidence that the regulation of β3 and β1 integrins in platelets occurs through similar pathways and may involve the same molecules. Furthermore, they demonstrate that α2β1 indeed requires inside-out activation to bind its ligand, collagen, and the same appears to be true for α5β1 and α6β1 (see below).

Once bound to its ligands, αIIbβ3 induces processes such as spreading and clot retraction and contributes to platelet procoagulant activity through “outside-in” signaling events.70 Recent data suggest that talin is also essential for this process to occur (Figure 3 and ref.105), which stands in contrast to previous reports.106 In addition, the cytoplasmic tails of αIIb and β3 establish further functional connections with a number of signaling molecules. Subunit αIIb interacts with the chloride channel ICln,107 with the calcium- and integrin-binding protein (CIB),108 and with the catalytic subunit of protein phosphatase 1.109 The relevance of these interactions...
is, however, still unclear. More is known about the proteins interacting with the β3 cytoplasmic tail. Filamin, α-actinin, myosin, and skelemin are cytoskeletal proteins and are supposed to be involved in platelet spreading and clot retraction. The Src family kinases—after receptor recruitment—undergo tyrosine autophosphorylation at position 418110 and trigger a signaling cascade that regulates lamellipodia formation.111 Because of the importance of αIIbβ3 integrin in platelet aggregation it has become an attractive pharmacological target for the prevention of ischemic cardiovascular events. Strategies to inhibit its function include antibodies (abciximab), cyclic peptides adapted from a snake venom disintegrin (eptifibatide), and nonpeptide analogues of an RGD peptide (tirofiban and lamifiban) that inhibit ligand binding.112 Although these inhibitors have been beneficial for patients undergoing percutaneous coronary intervention, they do not have widespread clinical use because of their side effect of undesired bleeding.112

Integrins αvβ3, α5β1, α6β1

Beside the 2 major integrins (αIIbβ3 and α2β1) there are further integrins like αvβ3, α5β1 (or VLA-5), and α6β1 (or VLA-6) expressed on the platelet surface. The structure of the α- and β-subunits of these integrins resembles that of αIIb, although small differences exist.113,114 They bind different proteins in the ECM; vitronectin is the preferred ligand of αvβ3 which can, however, also bind fibronectin and osteopontin, α5β1 serves as a receptor for fibronectin, and α6β1 is the major laminin receptor on platelets.115 Although they are all well-established adhesion receptors, their role in platelet attachment at sites of vascular injury has not been assessed in much detail. Recent studies on α2- and β1-deficient mice suggest that integrin α5β1 and α6β1 significantly contribute to the adhesion process in vivo, but they seem to be not essential because of functional redundancy with integrin αIIbβ3.76 Furthermore, laminin-integrin α6β1 interactions have been reported to stimulate platelet spreading through GPVI.116 These observations indicate that these integrins may modulate platelet responses and that their relative importance to the overall processes of adhesion, activation, and spreading is determined by the nature of the exposed ECM, which may significantly differ between individual regions of the body and the type and severity of injury.

Conclusion

In recent years, both in vitro and in vivo studies on the structure and function of platelet adhesion receptors have provided new insights into how platelets interact with the injured vessel wall and thereby initiate primary hemostasis and arterial thrombosis. These developments not only served as a basis for a revised model of platelet adhesion48 but also pointed toward mechanisms that might be of greater relevance for thrombus formation in diseased vessels than for the arrest of bleeding. Interference with these pathways could be the key to the development of powerful yet safe antithrombotics.

Acknowledgments

We acknowledge the present and past members of our laboratory for their work on platelet-vessel wall interactions and their contribution to many of the ideas and concepts that have been discussed in this review.

Sources of Funding

The authors are supported by the Rudolf Virchow Center and the Deutsche Forschungsgemeinschaft (Sonderforschungsbereich 688 and grant Ni556/7-1).

Disclosures

None.

References


Arterioscler Thromb Vasc Biol  March 2008


82. Shattil SJ, Illyes E, Baert J, Pareyn I, van de Walle GR. Integrin α2β1-deficient mice are a model for Glanzmann thrombasthenia showing lam...
Cell Adhesion Mechanisms in Platelets
David Varga-Szabo, Irina Pleines and Bernhard Nieswandt

Arterioscler Thromb Vasc Biol. 2008;28:403-412; originally published online January 3, 2008; doi: 10.1161/ATVBAHA.107.150474

Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2008 American Heart Association, Inc. All rights reserved.
Print ISSN: 1079-5642. Online ISSN: 1524-4636

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://atvb.ahajournals.org/content/28/3/403

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Arteriosclerosis, Thrombosis, and Vascular Biology can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Arteriosclerosis, Thrombosis, and Vascular Biology is online at:
http://atvb.ahajournals.org//subscriptions/