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Review

Platelet adhesion receptors and (patho)physiological thrombus formation

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Summary. In thrombus formation associated with hemostasis or thrombotic disease, blood platelets first undergo a rapid transition from a circulating state to an adherent state, followed by activation and aggregation. Under flow conditions in the bloodstream, this process potentially involves platelet-platelet, platelet-endothelium, platelet-subendothelial matrix, and platelet-leukocyte interactions. Specific adhesion receptors on platelets mediate these interactions, by engaging counter-receptors on other cells, or non-cellular ligands in the plasma or matrix. The glycoprotein (GP) Ib-IX-V complex on platelets initiates adhesion at high shear stress by binding the adhesive ligand, von Willebrand Factor (vWF). GP Ib-IX-V may also mediate platelet-endothelium or platelet-leukocyte adhesion, by recognition of P-selectin or Mac-1, respectively. Other membrane glycoproteins, such as the collagen receptor GP VI, may trigger platelet activation at low shear rates. Engagement of GP Ib-IX-V or GP VI leads ultimately to platelet aggregation mediated by the integrin, αIIbß3 (GP IIb-IIIa). This review will focus on recent advances in understanding structure-activity relationships of GP Ib-IX-V, its role in initiating thrombus formation, and its emerging relationships with other vascular cell adhesion receptors.

Key words: Glycoprotein Ib-IX-V, Platelets, Thrombosis, von Willebrand Factor

Introduction

The classical view of thrombus formation in the hemostatic response to vessel wall injury is that circulating platelets recognize exposed subendothelial matrix, adhere to the site, become activated, and recruit additional platelets to produce an aggregate or thrombus. Similarly, in thrombotic disease, platelet aggregation may be induced by sclerotic plaque rupture to expose the underlying matrix, or by pathological turbulent shear stress in blocked vessels (Kroll et al., 1996; Andrews et al., 1997; Savage et al., 1998). At high shear stress in the vasculature, both platelet adhesion and shear-induced platelet aggregation are initiated by the platelet membrane glycoprotein (GP) Ib-IX-V complex binding to the adhesive glycoprotein, von Willebrand Factor (vWF) present in plasma or the subendothelial matrix. More recently, it has been recognized that platelets may roll on the surface of the matrix or endothelium prior to arrest (Frenette et al., 1995, 1998; Savage et al., 1996; Denis et al., 1998; Cranmer et al., 1999; Andre et al., 2000; Kulkarni et al., 2000). Interestingly, the process of platelet rolling on the vessel wall followed by tight adhesion resembles the inflammatory response, where circulating leukocytes first roll, then become tightly adherent on activated endothelium, prior to extravasation (Berndt et al., 2001; Siegelman, 2001). Adhered, activated platelets attached to vessel wall matrix also support rolling of leukocytes under flow (Katayama et al., 2000; Simon et al., 2000). Many of the adhesion receptors that mediate leukocyte adhesion are also involved in platelet-matrix, platelet-endothelium or platelet-leukocyte adhesion.

The network of interactions between vascular cell adhesion receptors is shown in Fig. 1. Receptors that are reportedly expressed on platelets are highlighted. As evident from this diagram, interactions commonly occur between receptors from particular protein families. For instance, integrins may recognize one or more members of the immunoglobulin (Ig) superfamily, and vice-versa (Fig. 1). In a similar vein, selectins favor an association with sulfated, sialomucin-like receptors. Notably, many of the receptors in Fig. 1 have the capacity to bind to more than one counter-receptor. This confers the ability of these receptors to mediate adhesion between different types of vascular cells. For example, GP Ib-IX-V of the leucine-rich repeat protein family enables platelets to interact with endothelial cells via P-selectin (Romo et al., 1999), and with neutrophils or monocytes via the αMß2 integrin, Mac-1 (Simon et al., 2000), as well as to interact with subendothelial matrix by binding vWF.

This review will primarily focus on GP Ib-IX-V,

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which plays a central role in platelet adhesive interactions at high shear stress (Fig. 1), and additionally regulates platelet activation by an interaction with α-thrombin.

Leucine-rich repeat receptor: the GP Ib-IX-V complex

GP Ib-IX-V is a complex of four membrane-spanning polypeptides, GP Ibα, GP Ibß, GP IX and GP V (Fig. 2) (López, 1994). All of these subunits are members of the leucine-rich protein family, and contain one (GP Ibß and GP IX), seven (GP Ibα) or fifteen (GP V) conserved ~24-residue leucine-rich repeats in their extracellular domains. The repeats in each subunit of GP Ib-IX-V are flanked at their N- and C-termini by conserved disulfide-looped sequences. These repeats, and often their flanking sequences, are distributed in a wide range of proteins from diverse species (López, 1994). GP Ibα contains a highly O-glycosylated mucin-like domain that separates its globular N-terminal region from the platelet membrane. This N-terminal region of 282 amino acid residues consists of an N-terminal flank sequence (His1-Ile35), seven leucine rich repeats (Leu36-Ala200), a C-terminal flank sequence (Phe201-Gly268), and an anionic sequence (Asp269-Glu282) which contains three sulfated tyrosine residues (Tyr276, Tyr278 and Tyr279). This 282-residue sequence of GP Ibα is the major ligand-binding region of the GP Ib-IX-V complex, with binding sites for vWF, P-selectin, Mac-1, thrombin, factor XII and high molecular weight kininogen (refer below). Another notable feature of the GP Ib-IX-V structure is a thrombin cleavage site on GP V (Fig. 2), which results in a soluble extracellular fragment of GP V being released following thrombin treatment of platelets (Berndt and Phillips, 1981).

The cytoplasmic domain of the GP Ib-IX-V complex is made up of the cytoplasmic tails of GP Ibα (~100 residues), GP Ibß (~34 residues), GP V (~16 residues) and GP IX (~5 residues). The cytoplasmic sequence of GP Ibα contains a binding site for the cytoskeletal protein, actin-binding protein, within Thr536-Phe568 (Andrews and Fox, 1992; Cunningham et al., 1996). Signaling proteins such as 14-3-3ζ (Du et al., 1994) and the p85 subunit of phosphatidyl inositol (PI) 3-kinase (Munday et al., 2000) also interact with the cytoplasmic domain of the complex. 14-3-3ζ binds to the C-terminal sequence of GP Ibα [Ser606-Gly-His-Ser(P)-Leu] (Du et al., 1996; Andrews et al., 1998). Ser-609 has recently been shown to be stably phosphorylated in resting platelets, but the responsible kinase has not been identified (Bodnar et al., 1999). There is also a 14-3-3-binding sequence, Arg-Leu-Ser-Leu-(Ser/Thr)-Asp-Pro, within the cytoplasmic domain of GP Ibß, encompassing the protein kinase A (PKA) phosphorylation site at Ser166 (Du et al., 1996; Andrews et al., 1998; Calverley et al., 1998). Phosphorylation of Ser166 in GP Ibß

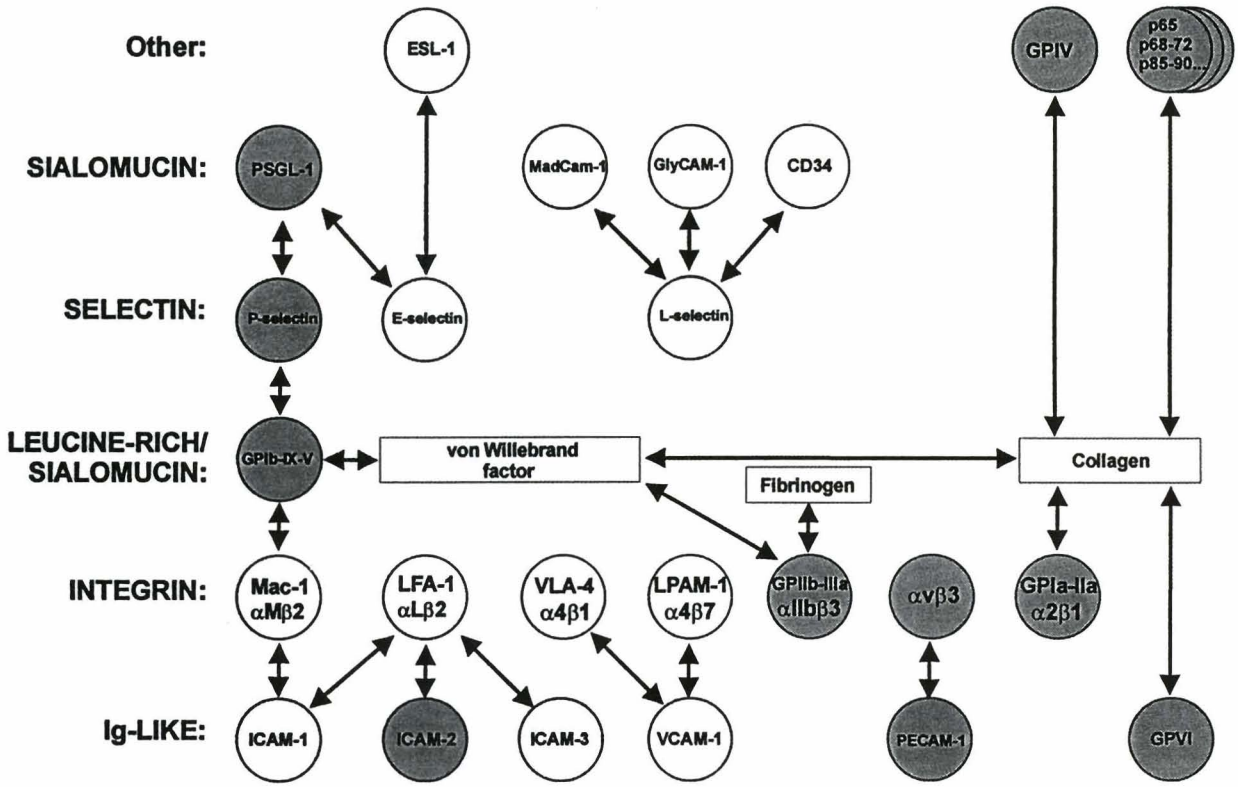


Fig. 1. Networking of adhesion receptors (circled) on vascular cells. Horizontal rows correspond to protein families as indicated in the column on the left. Shaded circles represent receptors that are reportedly expressed on platelets.

enhances 14-3-3ζ association (Calverley et al., 1998; Feng et al., 2000), and also inhibits actin polymerization in response to platelet activation (Fox and Berndt, 1989). The potential functional significance of these interactions between the cytoplasmic domain of GP Ib-IX-V and actin-binding protein, 14-3-3ζ and PI 3-kinase is discussed below.

Interaction of GP Ib-IX-V and vWF

In contrast to normal blood, thrombus formation is dramatically impaired in blood from patients with Bernard-Soulier syndrome (deficient or dysfunctional platelet GP Ib-IX-V) or type 3 von Willebrand's disease (lack of plasma and platelet vWF) (Sadler et al., 1995; López et al., 1998; Tsuji et al., 1999). In the absence of functional GP Ib-IX-V or vWF, platelet-vessel wall interaction is defective at high shear (>1,210 s^{-1}), but normal at low shear (<340 s^{-1}) (Tsuji et al., 1999). Studies with Bernard-Soulier syndrome or type 3 von Willebrand's disease platelets suggest the interaction between GP Ib-IX-V and vWF is critical for thrombus formation at high shear, whereas other receptors mediate platelet adhesion and aggregation at low shear.

vWF is a multimeric, disulfide-linked glycoprotein composed of subunits of 2,050 residues (Ruggeri, 1999). vWF has a modular structure, consisting of domains D'-D3-A1-A2-A3-D4-B1-B2-B3-C1-C2. The A1 domain, encompassing the intramolecular Cys509-Cys695 disulfide bond, contains the binding site for GP Ibα (Andrews et al., 1997). This recognition site is not in an active state in the native molecule, preventing binding of plasma vWF to platelets in the normal circulation, but undergoes conformational activation when associated with subendothelial matrix. The vWF A1 domain may also be activated *in vitro* by non-physiological modulators, such as the bacterial glycopeptide, ristocetin, from *Nocardia lurida* (Berndt et al., 1992), or the viper venom proteins, termed botrocetins, from *Bothrops jararaca* (De Luca et al., 1995b; Fujimura et al., 1996; Andrews and Berndt, 2000b). These structurally different reagents induce conformational activation of the A1 domain by binding to distinct sites. Ristocetin mainly recognizes a negatively charged, proline-rich sequence, Glu700-Asp709, flanking the Cys509-Cys695 disulfide bond (Berndt et al., 1992; De Luca et al., 2000), whereas botrocetins bind to more positively-charged site(s) within the disulfide loop (Andrews et al., 1997; Andrews and Berndt, 2000b). The anti-vWF monoclonal antibody 6G1 also activates the vWF A1 domain, like ristocetin, by an interaction with the proline-rich sequence, Glu700-Asp709 (De Luca et al., 2000). Another anti-vWF A1 domain antibody, MoAb724, also induces GP Ib-dependent platelet aggregation, but appears to mimic botrocetin (Depraetere et al., 1998). In contrast, a viper venom protein, bitiscetin, from *Bitis arietans* activated the A1 domain of vWF to bind GP Ib-IX-V by binding to the vWF A3 domain, a region that also interacts with collagen type III (Obert et al., 1999).

vWF may also be activated by a number of congenital gain-of-function mutations within the vWF gene (Type 2b von Willebrand's disease). These result in mainly single amino acid substitutions within the A1 domain (Andrews et al., 1997). Many of these are clustered at the C-terminal end of a postulated GP Ibα-binding sequence, Asp514-Glu542 (Berndt et al., 1992). A discontinuous sequence(s) of vWF may also be involved in GP Ib-IX-V binding, since mutation of Lys599Ala in a recombinant vWF fragment inhibited the

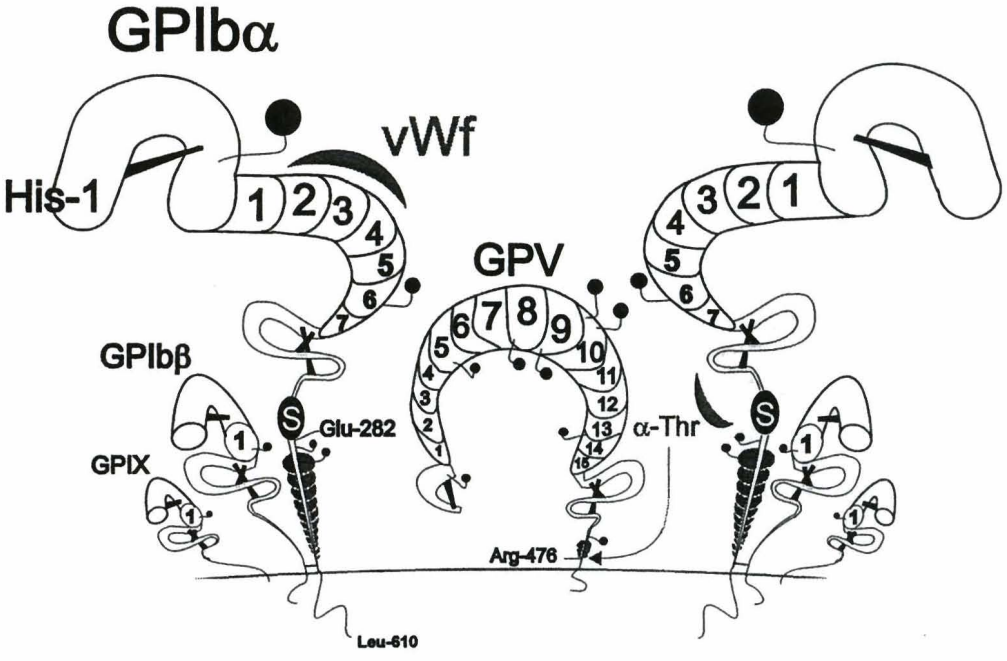


Fig. 2. Schematic of the GP Ib-IX-V complex, consisting of GP Ibα, GP Ibß, GP IX and GP V in the ratio 2:2:2:1. The N-terminal, ligand-binding domain of GP Ibα (His1-Glu282) consists of seven leucine-rich repeats, the disulfide-linked N-terminal and C-terminal flank sequences, and the sulfated tyrosine containing sequence (Asp269-Glu282). Critical regions of GP Ibα for binding vWF (leucine-rich repeats 2-4) and α-thrombin (sulfated region) are indicated. Potential N-linked glycosylation sites (Asn-X-Ser/Thr) are also indicated (shaded circles).

ristocetin-dependent interaction (Matsushita et al., 2000). High physiological (>650 s^{-1}) or pathological (>1,200 s^{-1}) shear rates may also activate vWF, although shear-dependent vWF binding to GP Ib-IX-V may also involve activation at the level of the receptor (López et al., 1998).

Elements implicated in recognition of vWF by GP Ib-IX-V occur within all four structural regions of the N-terminal 282 residues of GP Ibα: the N-terminal flank (His1-Ile35), the seven leucine-rich repeats (Leu36-Ala200), the C-terminal flank (Phe201-Gly268), and the sulfated tyrosine sequence (Asp269-Glu282). This presumably reflects conformational requirements of the receptor for optimal vWF binding. Supporting a functional role for the leucine-rich repeats is a form of Bernard-Soulier syndrome where GP Ibα is expressed in a dysfunctional form that does not bind vWF (López et al., 1998). This form of GP Ibα contains a single amino acid substitution (Leu57/Phe) within the first leucine-rich repeat (Miller et al., 1992). Similarly, GP Ibα associated with the Bolzano variant of Bernard-Soulier syndrome contains an Ala156/Val mutation within the sixth leucine-rich repeat, and this mutation causes dysfunctional vWF binding, but normal thrombin binding (Ware et al., 1993). Further, we have recently produced a series of canine-human and human-canine chimeras of recombinant GP Ibα, corresponding to structural domain boundaries. Binding of chimera-expressing cells to soluble vWF in the presence of ristocetin, or to immobilized vWF under flow in the absence of modulators, suggested that leucine-rich repeats 2-4 of GP Ibα were a critical requirement for vWF binding (Shen et al., 2000). These chimera-expressing cell lines were also used to map epitopes for a panel of anti-GP Ibα monoclonal antibodies that bound to human, but not canine, GP Ibα (Shen et al., 2000). Antibodies that strongly inhibited ristocetin-induced binding (AK2, Hip1, 6D1) mapped to sites within the first four leucine-rich repeats. Another inhibitory antibody, AP1, mapped to the C-terminal flank, whilst partially blocking antibodies, MB45 and AN51, mapped to the N-terminal flank. Other antibodies that preferably blocked botrocetin-induced vWF binding, VM16d or SZ2, recognized the C-terminal flank or sulfated tyrosine sequence, respectively (Ward et al., 1996; Shen et al., 2000). Earlier studies identified synthetic peptides based on leucine-rich repeat sequences, Thr81–Leu95 or Leu136–Leu150, as well as downstream sequences, Asp235–Lys262, Ser251–Tyr279, Gly271–Glu285, as potentially mediating ristocetin- or botrocetin-dependent vWF binding (Katagiri et al., 1990; Vicente et al., 1990).

There is emerging evidence suggesting the disulfide-looped C-terminal domain flanking the leucine-rich repeats is involved in regulating vWF binding. The C-terminal flank domain (Phe201-Gly268) is composed of two disulfide loops by virtue of disulfide bonds between Cys209-Cys248 and Cys211-Cys264 (López, 1994). Congenital gain-of-function mutations within the GP Ibα gene ("platelet-type" or "pseudo" von Willebrand's disease) result in single amino acid substitutions, Gly233/Val or Met239/Val, within the first of the two disulfide-loops (Miller et al., 1991; Russell and Roth, 1993; Miller, 1996). Mutation of Gly233 or Met239 to valine in recombinant GP Ibα, in addition to the artificial mutations Asp235/Val or Lys237/Val, also result in a gain-of-function phenotype (Marchese et al., 1999; Dong et al., 2000). In contrast, an Ala238/Val mutation resulted in partial loss of function (Dong et al., 2000).

Finally, the sulfated tyrosine sequence of GP Ibα, Asp269-Glu282, also contributes to vWF binding under certain conditions. Studies with recombinant GP Ibα expressed in mammalian cells provided evidence that blocking sulfation of Tyr276, Tyr278 and Tyr279 diminished ristocetin-dependent vWF binding, and more strongly inhibited botrocetin-dependent binding (Dong et al., 1994; Marchese et al., 1995). An unsulfated, cathepsin G-generated fragment of native GP Ibα (His1-Leu275) was an order of magnitude less effective than a sulfated mocarhagin-derived fragment (His1-Glu282), a trypsin fragment (His1-Arg293) or the full length soluble receptor at inhibiting botrocetin-dependent vWF binding (Ward et al., 1996). Further, mutagenesis of recombinant GP Ibα showed that anionic residues between Asp252–Asp277 were involved in botrocetin-dependent vWF binding, and contributed to a lesser extent to ristocetin-dependent binding, while residues between Glu281–Asp287 showed a much greater affect on botrocetin-dependent binding (Murata et al., 1991). While these sites are potentially important in regulating platelet adhesion to vWF, analysis of the modulator-independent interaction of GP Ibα with vWF under flow suggests this interaction more closely parallels ristocetin-dependent vWF binding (Dong et al., 2001). Both interactions predominantly involve the more N-terminal sites described above Some antibodies, however, against either GP Ibα or vWF only inhibit the shear-dependent interaction (Cauwenberghs et al., 2000; Dong et al., 2001).

Mac-1 binding to GP Ib-IX-V

The leukocyte integrin, Mac-1 (αMß2) is a recently described ligand for GP Ib-IX-V (Simon et al., 2000). Inhibition studies with monoclonal antibodies and receptor fragments showed that the interaction involved the I domain of Mac-1 (homologous to the vWF A1 domain), and the N-terminal region of GP Ibα containing the leucine-rich repeats and flanking sequences (His1-Glu282). One of the inhibitory anti-GP Ibα antibodies, VM16d, mapped into the C-terminal flank of GP Ibα, and this antibody also blocks vWF binding induced by botrocetin and thrombin-dependent platelet aggregation (Mazurov et al., 1991; Shen et al., 2000). Wild type, but not Mac-1 deficient mouse neutrophils, bound to purified GP Ibα or adherent platelets. These findings imply that GP Ib-IX-V may mediate binding of platelets to leukocytes *via* Mac-1.

This interaction could thereby promote inflammation at thrombotic or atherosclerotic sites (Simon et al., 2000). In particular, this binding interaction may be most relevant to transmigration of macrophages through mural thrombus (Simon et al., 2000), a process required for vessel remodeling post-angioplasty.

P-selectin, a further GP Ib-IX-V ligand

P-selectin is an adhesion receptor of the selectin family, and is associated with the α-granules of platelets and the Weibel-Palade bodies of endothelial cells. It is expressed on the cell surface following activation (Kansas, 1996). vWF is also found in both of these storage organelles. P-selectin is a transmembrane glycoprotein, consisting of an N-terminal Ca^{2+}-dependent lectin-like domain, an epidermal growth factor-like domain, and nine complement regulatory protein repeats in the extracellular region. On activated endothelial cells, P-selectin mediates initial contact and rolling of circulating neutrophils by a specific interaction with P-selectin glycoprotein ligand-1 (PSGL-1). Elements from within both the lectin-like and epidermal growth factor-like domains are involved in PSGL-1 recognition (Kansas et al., 1994; Mehta et al., 1997). A recent report also places PSGL-1 on platelets (Frenette et al., 2000). Although its precise role on platelets has not been fully defined (Frenette et al., 2000), it could be involved, along with GP Ib-IX-V, in supporting platelet rolling on activated endothelium.

PSGL-1 and GP Ibα show a notable degree of structural similarity (Andrews et al., 1997, 1999; Berndt et al., 2001). Both GP Ibα and PSGL-1 are sialomucins that contain sulfated tyrosine sequences within their N-terminal domains. Like PSGL-1, GP Ibα also specifically binds P-selectin (Romo et al., 1999). P-selectin-expressing cells adhered to immobilized GP Ibα, whilst GP Ibα-expressing cells adhered and rolled on either purified P-selectin or activated endothelial cells. Unlike P-selectin binding to PSGL-1, binding to GP Ibα was independent of both Ca^{2+}, and receptor fucosylation. However, binding to both PSGL-1 and GP Ibα appeared to involve, at least in part, their sulfated tyrosine motifs (De Luca et al., 1995a; Pouyani and Seed, 1995; Romo et al., 1999). The physiological consequences of GP Ibα binding to P-selectin are unclear, however the interaction potentially contributes to platelet adhesion to activated endothelium. Supporting a role in thrombus formation, recent studies using intravital microscopy in mice where the P-selectin-GP Ib-IX-V interaction was blocked showed attenuated rolling of platelets, as well as leukocytes, on the vessel wall (Katayama et al., 2000).

Interaction of GP Ib-IX-V with α-thrombin, factor XII and kininogen

The evidence that GP Ib-IX-V interacts with α-thrombin, and facilitates thrombin-dependent platelet activation, has recently been reviewed (Berndt et al., 2001). The N-terminal region of GP Ibα contains a high affinity binding site for thrombin (López, 1994; Greco et al., 1996), and deficiency or blockade of GP Ib-IX-V impairs thrombin-dependent platelet aggregation (Yamamoto et al., 1985; Ward et al., 1996; López et al., 1998; De Candia et al., 1999). The sulfated tyrosine sequence of GP Ibα (Asp269-Glu282) constitutes a major thrombin recognition sequence (De Marco et al., 1994; Gralnick et al., 1994; Marchese et al., 1995; Ward et al., 1996; Mazzucato et al., 1998; De Cristofaro et al., 2000). A second site within the leucine-rich repeats (Phe216-Thr240) may also participate in thrombin binding (Katagiri et al., 1990; McKeown et al., 1996). The demonstration of a direct relationship between thrombin binding to the sulfated region of GP Ibα and cleavage of the seven-transmembrane thrombin receptor, PAR-1, suggested GP Ibα may function as a cofactor for thrombin-dependent PAR-1 activation (De Candia et al., 2001). Thrombin also cleaves GP V near the membrane (Arg476) to release an extracellular soluble fragment (Berndt and Phillips, 1981). Recent studies using platelets from GP V-null mice suggest the GP Ib-IX-V complex is itself a thrombin receptor (Ramakrishnan et al., 2001). It was shown that proteolytically-inactive thrombin activates GP V null platelets, as well as wild-type mouse and human platelets, after cleavage of GP V by thrombin. Catalytically inactive thrombin also induced thrombosis in GP V null mice but not wild-type mice. Thrombin binding to GP Ibα following cleavage of GP V initiated signaling responses and ADP secretion (Ramakrishnan et al., 2001). These findings imply a role for GP V as a negative regulator of thrombin-dependent platelet activation. Interestingly, expression of GP V, along with GP Ibα, is required for optimal binding of thrombin to L cells transfected with GP Ibα and/or GP V (Dong et al., 1997).

Thrombin binding to GP Ib-IX-V also accelerates coagulation at the activated platelet surface (Dormann et al., 2000). Thrombin binding to GP Ibα enhances phospholipid exposure on the platelet surface, and this interaction contributes to increased thrombin generation. In contrast, it has been reported that GP Ibα binding to thrombin causes conformational changes in thrombin that allosterically reduce cleavage of fibrinogen (or peptide substrates) (Li et al., 2000). The physiological consequences of thrombin binding to GP Ib-IX-V, therefore, is likely to depend on thrombin concentration, the extent of GP V cleavage, and the prevalence of inhibitors of the thrombin-GP Ibα interaction. For example, two newly described GP Ib-IX-V ligands, factor XII and high molecular weight kininogen, regulate thrombin binding to GP Ibα, and inhibit thrombin-dependent platelet aggregation (Bradford et al., 1997, 2000; Joseph et al., 1999). Interestingly, the region of factor XIIa necessary for binding GP Ibα (Bradford et al., 2000), has sequence similarity to fibronectin type II modules, as well as epidermal growth factor-like domains also found in P-selectin (refer above).

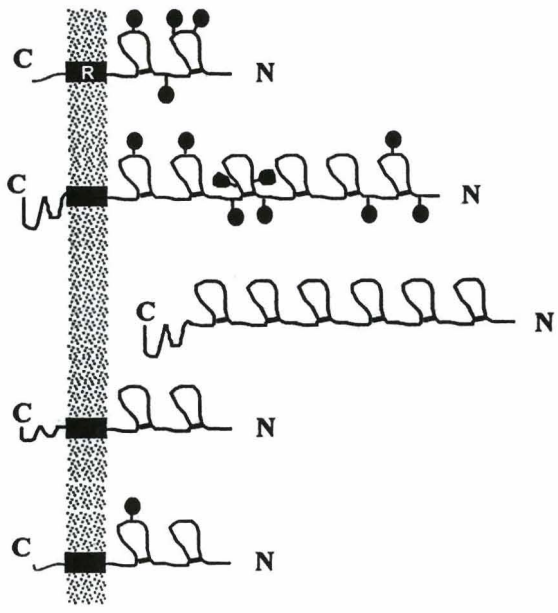
Platelet immunoglobulin-like receptors: PECAM-1, ICAM-2 and GP VI

The catalogue of receptors of the immunoglobulin (Ig) superfamily on platelets (Fig. 3) has recently expanded with the cloning of the collagen receptor, GP VI (Clemetson et al., 1999; Ezumi et al., 2000; Jandrot-Perrus et al., 2000). GP VI contains two extracellular Ig-like domains, a sialomucin core, a transmembrane domain and a short cytoplasmic tail. An arginine residue within the transmembrane domain, and elements within the cytoplasmic tail, mediate the association of GP VI with the Fc receptor gamma chain (FcRγ) (Zheng et al., 2001). FcRγ is commonly associated with T-cell receptors, contains an ITAM (immunoreceptor tyrosine-based activation motif) in its cytoplasmic domain, and has been shown to be critical in GP VI-dependent platelet activation (Watson, 1999; Zheng et al., 2001). Additional collagen receptors, including α2ß1, may also mediate platelet responses to collagen (Chiang, 1999; Nakamura et al., 1999; Watson, 1999; Kamiguti et al., 2000).

Other adhesion receptors of the Ig family found on platelets include ICAM-2, which interacts with the leukocyte integrin LFA-1 (αLß2), and PECAM-1 that interacts with the integrin αvß3 (Fig. 1) (Newman et al., 1990; Diacovo et al., 1994). PECAM-1, found on all vascular cell types, also forms homotypic interactions with PECAM-1 on other cells, and regulates endothelial cell adhesion at cellular junctions (Andrews and Berndt, 2000a). PECAM-1, which shows increased expression following activation (Metzelaar et al., 1991; Cramer et al., 1994), potentially mediates platelet-leukocyte adhesion. PECAM-1 also appears to play a role in recruitment of protein-tyrosine phosphatases in activated platelets, *via* a cytoplasmic ITIM (immunoreceptor tyrosine-based inhibitory motif) domain (Hua et al., 1998). PECAM-1 may also attenuate platelet responses to stimulation by collagen (Patil et al., 2001). ICAM-2 contributes to neutrophil adhesion to activated platelets under flow, along with neutrophil Mac-1 and LFA-1 (Kuijper et al., 1998). Fibrinogen associated with platelet αIIbß3 also plays a role in this process (Weber and Springer, 1997; Kuijper et al., 1998). Finally, another newly identified Ig family receptor on platelets, F11R, contains two extracellular Ig domains, is phosphorylated in thrombin- or collagen-stimulated platelets, and has a potential though undefined role in platelet function (Sobocka et al., 2000).

GP Ib-IX-V-dependent signaling: association with Fc receptors and/or GP VI

Mechanisms of signal transduction following engagement of GP Ib-IX-V by vWF remain a source of conjecture. For example, is signaling that leads to activation of αIIbß3 following platelet adhesion to vWF initiated by direct signal transduction through GP Ib-IX-V, cross-linking of one or more GP Ib-IX-V complexes, or by the involvement of associated receptors?



Protein	Distribution	Binding Partner(s)
GP VI	Platelets	Collagen
PECAM-1	Platelets Endothelial cells Leukocytes	PECAM-1 αvβ3
sPECAM-1		
ICAM-2	Platelets Endothelial cells Leukocytes	LFA-1 (αLβ2)
F11R	Platelets Endothelial cells Lymphocytes	

Fig. 3. Adhesion receptors of the Ig-like superfamily found on platelets. The stippled bar represents the plasma membrane, with the extracellular domain on the right hand side. Solid circles represent potential N-linked glycosylation sites.

Supporting a mechanism involving receptor cross-linking, platelet activation results from engagement of GP Ib-IX-V by native vWF in the presence of ristocetin or botrocetin, by asialo-vWF in the absence of modulators, by soluble vWF under shear, by immobilized vWF under flow, or by recombinant vWF A1 domain multimerized by expression on the surface of COS-7 cells (Kroll et al., 1996; Schulte am Esch et al., 1997; Gu et al., 1999; Berndt et al., 2001). In contrast, a monomeric fragment of vWF encompassing the A1 domain, Glu480-Gly718, inhibits binding of native or asialo-vWF, but does not induce platelet activation (Andrews et al., 1989). A number of presumably monovalent, ~25-kDa GP Ibα-binding viper venom proteins of the C-type lectin family similarly block vWF binding, but do not activate platelets. However, cross-linking of the GP Ibα-binding protein, echicetin, by IgMk has been reported to activate platelets (Navdaev et al., 2001), raising the possibility that cross-linking of GP Ibα on platelets can initiate signals leading to αIIbß3-dependent aggregation. Higher molecular weight forms of GP Ibα-binding snake proteins, such as 50-kDa alboaggregin-A, are also potent platelet agonists (Andrews et al., 1996; Kowalska et al., 1998; Falati et al., 1999), supporting a receptor cross-linking mechanism. Consistent with this supposition, a vWF A1 domain fragment induced phosphorylation of Syk in platelets, and its association with Src, but only native multimeric vWF induced activation of αIIbß3 (Satoh et al., 2000). Other recent reports suggest alboaggregin-A may activate platelets by stimulating GP VI (Asazuma et al., 2001; Dormann et al., 2001). In this case, GP Ib-IX-V may act as a cofactor for alboaggregin-A interacting with GP VI, in a comparable manner to GP Ibα's role in promoting thrombin-dependent cleavage of PAR-1. Masking GP Ibα by antibodies, venom proteins or proteolysis impairs alboaggregin-A-dependent platelet aggregation (Andrews et al., 1996; Asazuma et al., 2001; Dormann et al., 2001), in a similar manner to the effect of GP Ibα blockade on thrombin-dependent PAR-1 cleavage (De Candia et al., 2001). Both alboaggregin-A (Dormann et al., 2001) and thrombin (Ramakrishnan et al., 2001) have also been reported to signal through GP Ibα. It is interesting to speculate that vWF binding to GP Ibα, like alboaggregin-A and thrombin, might similarly lead to stimulation of additional signaling receptors.

Evidence in the literature supports the linkage of GP Ib-IX-V with ITAM-containing Fc receptors (Falati et al., 1999; Torti et al., 1999). Firstly, GP Ibα and FcgRIIα have been shown by fluorescence energy transfer analysis to be proximal on the platelet surface (Sullam et al., 1998). Aggregation of platelets by FcγRIIa stimuli is blocked by anti-GP Ibα antibodies, while vWF-dependent signaling is at least partially inhibited by the anti-FcγRIIa antibody, IV.3 (Sullam et al., 1998; Torti et al., 1999). A direct interaction between FcγRIIa and GP Ibα has also been shown by yeast two-hybrid analysis (Sun et al., 1999). In this case, an Arg542-Arg544 sequence of GP Ibα was suggested to be an interactive site from the affect of mutagenesis of these residues on formation of the complex. In addition to GP Ib-IX-V, FcγRIIa may also be proximal to αIIbß3 (Berndt et al., 1993).

Secondly, FcRγ was shown to be co-associated with GP Ibα in platelets after their stimulation by alboaggregin-A (Falati et al., 1999), a reagent that potentially binds GP VI in addition to GPIbα (Asazuma et al., 2001; Dormann et al., 2001). It is interesting that these findings infer an association of GP Ibα with FcRγ and/or a topographical relationship with GP VI. Finally, an anti-GP Iba monoclonal antibody, SZ2, that maps to the sulfated sequence of GP Ibα (Ward et al., 1996; Shen et al., 2000), blocks platelet aggregation induced by both vWF and collagen (Ruan et al., 1987). This would further support the possible topographical association of GP Ib-IX-V with either GP VI or other collagen receptors such as α2ß1.

Interaction of GP Ib-IX-V with signaling molecules, 14-3-3ζ and PI 3-kinase

Signaling molecules associated with GP Ib-IX-V have been identified that could be early factors in the pathway linking binding of vWF to activation of αIIbß3. Firstly, 14-3-3ζ associated with the cytoplasmic domain of GP Ib-IX-V (refer above), may be involved in this pathway. In CHO cells co-expressing GP Ib-IX and αIIbß3, vWF binding to GP Ib-IX upregulates αIIbß3 function, and these cells adhere and spread on vWF in an αIIbß3-dependent manner (Gu et al., 1999; Yap et al., 2000; Zaffran et al., 2000). However, deletion of the 14-3-3ζ binding site at the C-terminus of GP Ibα inhibited GP Ib-IX-induced activation of αIIbß3, and prevented cell spreading on vWF (Gu et al., 1999). Shear-dependent platelet aggregation initiated by vWF binding to GP Ibα results in dissociation of 14-3-3ζ from GP Ib-IX (Feng et al., 2000). Spreading of CHO GP Ib-IX/αIIbß3 cells on vWF was also blocked by the PI 3-kinase inhibitor, wortmannin (Gu et al., 1999). This result complemented other recent findings that PI 3-kinase forms a complex with GP Ib-IX-V and 14-3-3ζ in platelets (Munday et al., 2000). In the latter study, the association of GP Ib-IX-V with PI-3 kinase and 14-3-3ζ may facilitate translocation of these signaling proteins to the activated cytoskeleton. Binding of 14-3-3ζ to GP Ibß is also likely to be functionally important in the platelet response to vWF, since spreading of CHO cells co-transfected with GP Ib-IX and αIIbß3 on vWF is blocked by treating the cells with prostaglandin E1, that increases cAMP levels and induces PKA-dependent phosphorylation of GP Ibß (Bodnar et al., 1999). 14-3-3ζ binding to GP Ibß, therefore, potentially regulates GP Ib-IX-V-dependent signaling, as well as cytoskeletal rearrangement (Fox and Berndt, 1989).

GP Ib-IX-V and the cytoskeleton

Cytoskeletal rearrangements in platelets are associated with shape change, spreading, secretion and/or aggregation (Fox and Meyer, 1998). The GP Ib-

IX-V complex is linked to cytoskeletal actin filaments *via* a sequence within the cytoplasmic tail of GP Ibα, Thr536-Phe568, that binds actin-binding protein (Andrews and Fox, 1992; Cunningham et al., 1996). It has been shown recently that vWF binding to normal platelets, to Glanzmann's thrombasthenic platelets lacking αIIbß3, or to CHO cells expressing GP Ib-IX, induces actin polymerization and re-organization of the cytoskeleton (Yuan et al., 1999). In recombinant GP Ibα on CHO cells, deletion of the binding site for actin-binding protein prevented stable adhesion at high shear, whereas adhesion at low shear was normal (Cranmer et al., 1999). In contrast, inhibiting actin polymerization (using cytochalasin D or latrunculin B) in platelets, Glanzmann's thrombasthenic platelets, or GP Ib-IX-expressing CHO cells markedly enhanced vWF binding (Mistry et al., 2000). Similarly, the shear rate threshold for vWF-dependent aggregation was reduced from 3,000 s^{-1} to 500 s^{-1}. Similarly, Englund et al. showed deleting the actin-binding protein binding region from GP Iba enhanced vWF binding under static or flow conditions (Englund et al., 2001). These results would imply that attachment of GP Ib-IX-V to the cytoskeleton in resting platelets is a negative regulator of vWF binding. Interestingly, in CHO cells expressing recombinant GP Ibα, deletion of the cytoplasmic sequence involved in actin-binding protein attachment abrogated the functional gain associated with cytochalasin D treatment (Mistry et al., 2000).

Final comments

The multi-functional platelet adhesion receptor, GP Ib-IX-V, plays a central role in platelet responsiveness to injury or pathological shear stress. GP Ib-IX-V binds vWF in the vessel wall matrix or plasma, P-selectin on platelets or endothelial cells, and Mac-1 on neutrophils or monocytes, as well as other ligands such as α-thrombin, factor XII and high molecular weight kininogen. Future studies should define the precise role for each of these interactions in the initiation and progression of thrombosis. In addition, the binding sites on GP Ib-IX-V specific for these different ligands remain to be determined. Finally, the way in which recognition of these various GP Ib-IX-V-binding ligands is regulated, under normal *versus* pathological conditions, also remains to be resolved. A detailed understanding of these elements of GP Ib-IX-V function should ultimately enable therapeutic blockade of thrombosis, with minimal impairment of hemostasis.

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