Clin Chem Lab Med 2003; 41(3):253–260 © 2003 by Walter de Gruyter · Berlin · New York

Review

Platelet Receptors and Their Role in Diseases

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The absence or deficiency of specific platelet glycoprotein receptors has a well-defined role in causing several rare bleeding disorders such as Bernard-Soulier syndrome or Glanzmann's thrombasthenia. Several new rare disorders caused by defects in other receptors or their signalling pathways have recently been described. Platelet receptors are also often targets for antibodies in pathological conditions. The roles of platelet receptors or their polymorphism variants in diseases such as cardiovascular disorders have started to be intensively investigated over the last 5 years. Many of these findings still remain controversial. Recent evidence points to a fundamental role for platelets and their receptors in the origins of atherosclerosis. Studies on the role of platelet receptors in diseases such as asthma, diabetes and HIV are still at an early stage. Clin Chem Lab Med 2003; 41(3):253–260

Key words: Platelet receptors; Bernard-Soulier syndrome; Glanzmann's thrombasthenia; Idiopathic thrombocytopenia purpura; Receptor polymorphisms.

Abbreviations: BSS, Bernard-Soulier syndrome; GP, glycoprotein; GT, Glanzmann's thrombasthenia; HIT, heparin-induced thrombocytopenia; ITP, idiopathic thrombocytopenia purpura; LRR, leucine-rich repeat; MIDAS, metal ion-dependent adhesion site; PF4, platelet factor 4, VWF, von Willebrand factor.

Introduction

The major physiological function of platelets is haemostasis, prevention of bleeding, and the effect of aspirin has established that they are also involved in its pathological variant, thrombosis. Since then, interest in platelets as a possible target for new anti-thrombotics has increased. The roles of the major receptors in platelet function were to a great extent established by studies on bleeding disorders caused by platelet defects. Bernard-Soulier syndrome (BSS) and Glanzmann's thrombasthenia (GT) provided the basis for studying the function of the glycoprotein (GP) Ib complex and $\alpha_{IIb}\beta_3$, respectively. Milder bleeding disorders have also been related to the absence or deficiency of other platelet receptors such as $\alpha_2\beta_1$, GPVI and $P2Y_{12}$. Many patients with mild bleeding problems caused by platelet defects are seen in haematology departments and still cannot be diagnosed at a molecular level. Molecular diagnosis of the genetic defects in these patients is still a major goal in platelet studies and may be amenable to proteomics approaches.

Cardiovascular diseases are the major cause of death and disability in countries with a western lifestyle and are an ever more serious health problem in developing countries that adopt similar habits. For many years platelets were regarded as innocent bystanders in the development of cardiovascular disease and atherosclerosis. The idea that differences in platelets could affect susceptibility to cardiovascular disorders was not considered. Differences in sequences of coagulation factors clearly influence thrombosis rates, some enhancing and others protective. This observation, together with the discovery of variation in platelet receptor sequences, has caused the role of sequence differences in platelet proteins to be reconsidered, and a large number of papers have appeared over the past few years on various aspects of this problem.

Disorders Caused by Platelet Glycoprotein Receptor Absence or Deficiency

GPIb-V-IX complex

Bernard-Soulier syndrome

Bernard-Soulier syndrome (BSS) is caused by genetic defects in the genes of GPIbα, GPIbβ or GPIX (1–3). Absence or defects in GPV (4) do not cause BSS (5). The absence of GPV on platelets in GPV-null mice leads to a thrombotic disorder rather than a bleeding disorder (6, 7). Patients with BSS have a prolonged bleeding time, large, often giant, platelets and thrombocytopenia (8, 9). The characteristic platelet defect in BSS is lack of response to ristocetin/von Willebrand factor (VWF) or botrocetin/VWF, which is not corrected by normal plasma, while responses to other agonists, except to low doses of thrombin, are normal. BSS is normally an autosomal recessive disorder and homozygous cases are often due to consanguinity. Only one case has been described as autosomal dominant (10). Typical symptoms include nose bleeds, bleeding from the gums and from minor scratches, as well as a tendency to easy bruising. Severe, life threatening bleeding may occur with surgery or major trauma and is normally controlled by platelet or blood transfusions. Women with BSS require special attention for menorrhagia and childbirth; associated problems may be prevented by

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oral contraceptives and appropriate transfusions, respectively. Most patients live relatively normal lives with taking reasonable precautions.

BSS platelets also respond weakly to low doses of thrombin (11) and show abnormal procoagulant responses (12). While they have an increased resting procoagulant state, after stimulation they do not show a normal increase in procoagulant activity. This was partly explained by the recent demonstration of a critical role for GPIbα in platelet responses to thrombin in general (13, 14), and in procoagulant responses in particular (15).

Basic types of BSS vary less than in GT because most molecular defects cause lack of expression of the complex rather than expression of a dysfunctional complex. They can be classed as totally absent – caused by frame shifts, deletions, and major folding defects in any of the three chains α, β, or IX, followed by failure to form a complex or of the complex translocating to the membrane. Frame shifts or deletions in any of the three chains leading to premature termination and therefore lack of the transmembrane domain and production of soluble fragments are major categories. A typical example in GPIbα is W343stop. In GPIX, examples are W126stop and in GPIbβ, a base deletion in A80, resulting in a frame shift and premature termination as well as a W21stop mutation.

Mutations in or near the transmembrane region can lead to frame shifts causing partial expression of functional or dysfunctional molecules. Folding problems can also lead to failure to translocate to the membrane. Such problems are often caused by mutations replacing cysteine residues or introducing abnormal cysteine residues. These types of mutations occur in all three subunits; in GPIbα C209S; in GPIX C73Y and C97Y and in GPIbβ, C8R and Y88C. Mutations within the leucine-rich repeat (LRR) domains may also cause folding problems. Examples in GPIbα are L129P; in GPIX, L40P, N45S, F55S; and in GPIbβ, N63T.

A few mutations in the LRR domains affect folding less radically and a malfunctioning molecule is expressed at variable levels. Those described so far were all mutations of GPIbα: L57F (10), A156V (16), and L179 deletions (17). A few other cases of BSS are known where the mutations lie outside the LRR domains. These include mutations within the disulphide loops at each end of the LRR, causing problems by preventing the loops from folding correctly. In GPIbα, mutations in the C-terminal loops can lead to platelet-type von Willebrand disease (see below). In GPIX, mutations are found in N21G and in GPIbβ in P74R, Y88C, and A108P. These last two mutations lacked disulphide bond formation between GPIbα and GPIbβ in most GPIb molecules.

The only example of a dominant mutation was L57F present in one allele of GPIbα (10). The other allele is normal so this could be explained if a defective, expressed GPIbα interferes with the function of the normal molecule.

Mutations within the promoter region may affect expression levels leading to BSS. A typical example is a mutation in the GATA-1 sequence of one allele of the GPIbβ promoter, which combined with deletion of the other allele in DiGeorge syndrome reduced expression of the complex to produce BSS (18).

A website listing BSS genetic defects (mutations, deletions, *etc.*) is now available (www.bernard-soulier.org).

Platelet-type (or Pseudo) von Willebrand's disease

Platelet-type von Willebrand's disease is a dominant inherited bleeding disorder caused by mutations in GPIbα leading to an increased binding to VWF in resting platelets. This causes platelet activation and aggregation, removal from the circulation by the spleen, and periodic thrombocytopenia. The most active, larger multimers of VWF are removed preferentially, leading to defective haemostasis and hence bleeding risk. Two mutations, G233V and M239V, lie on a loop just below the LRR domain, which has a critical role in binding to the A1 domain of VWF (19). Platelet-type von Willebrand's disease is generally treated very conservatively.

Integrins $\alpha_{IIb}\beta_3$ and $\alpha_v\beta_3$

Glanzmann's thrombasthenia

Glanzmann's thrombasthenia (GT) was first described in 1918 (20) and is both the commonest and the most complex disorder caused by defects in platelet adhesive receptors. This disorder is caused by a defect or absence of one of glycoproteins IIb-IIIa, forming the integrin $\alpha_{IIb}\beta_3$, which were first implicated in 1974–5 (21, 22). This disorder is characterised by poor or absent platelet aggregation to all agonists as well as by fibrinogen deficiency in platelet α-granules in the classic form of this disease. As in BSS the problem can be caused by mutations or deletions preventing expression of one or the other of the subunits by premature termination before the transmembrane region. However, very many variants with different expression levels are caused by mutations affecting folding (9, 23). Both homozygous, as well as compound heterozygous, mutations are known. Because of the number of introns/exons making up the α_{IIb} and β_3 genes, splicing errors are among the known defects, unlike in BSS. X-ray crystallographic structure data for $\alpha_{IIb}\beta_3$ and molecular modelling give some insight into its function (24). Thus, mutations in α_{IIb} that lead to absence of expression, or to defective function while partial expression of the complex is maintained, are often in a seven blade β-propeller structure predicted to be the major ligand binding site of this subunit. Some of these involve calcium-binding domains in the fourth to seventh blades of the propeller. The mutations often change the charge of a residue and include G273D (numbering based on the mature protein) before the first calcium-binding domain and R327H between the second and third calcium-binding domains, G418D before the fourth calcium-binding domain, and a V425D426 deletion at the beginning of the fourth calcium-binding do-

main. A cluster of mutations (P145A, P145L, T176I, and L183P) in the third blade of the propeller are thought to affect ligand binding and cause receptor dysfunction. A further mutation, D224V, in a connecting strand within this structural domain, also led to GT. A Y143H mutation in α_{IIb} was recently described where binding to soluble ligands was absent, whereas cell adhesion and clot retraction were normal (25).

While α_{IIb} subunit expression is restricted to $\alpha_{IIb}\beta_3$, and mutations and deletions of this subunit only affect this integrin, mutations and deletions of the β_3 subunit affect both $\alpha_{IIb}\beta_3$ and $\alpha_v\beta_3$. A large number of mutations and deletions lead to frame shifts and premature termination and hence to failure to express the integrin complex. The β_3 subunit contains a cation-binding domain, classed as a metal ion-dependent adhesion site (MIDAS) structure and a disulphide-rich region recently recognised as a protein disulphide isomerase. Within the MIDAS domain, eight point mutations lead to amino acid replacements causing GT. Two of these, D119Y and D119N, are in the highly conserved DXSXS motif. Three mutations, R214W, R214Q, and R216Q, are near cation coordinating sites and three, D117W, S162L and L262P, are in the MIDAS structural unit. While mutations D119Y and D119N affect function but have little effect on expression, D117W prevents surface expression. Mutations near the cation coordinating sites, R214W, R214Q, and R216Q, increase susceptibility of $\alpha_{IIb}\beta_3$ complexes containing these mutated β_3 subunits to dissociation by calcium chelating agents. The mutations at S162L and L262P reduced surface expression (~30%) as well as causing increased sensitivity of $\alpha_{IIb}\beta_3$ to dissociation by calcium. The S162L mutation results in a complex that does not bind fibrinogen but does support clot retraction, implying that the complex recognises fibrin (26). The recently described L262P mutation caused reduced expression and easily dissociated complexes. Again, cells expressing the mutant complex were able to sustain clot retraction while failing to bind to immobilized fibrinogen (27).

Mutations in cysteine residues within the β_3 subunit include C374Y, C457R with decreased expression, and C542R with absent surface expression.

Mutations in the cytoplasmic domain of β_3 can affect inside-out signalling and activation of the receptor for ligand binding. One in the codon for R724 to a nonsense triplet causes premature termination and deletion of the C-terminal 39 amino acids. Another is the S752P. Platelets from both patients express $\alpha_{IIb}\beta_3$ but do not aggregate in response to platelet agonists. They still adhere to immobilized fibrinogen but do not spread on this surface.

A website listing GT mutations and deletions is now available (http://med.mssm.edu/glanzmanndb)

$P2Y_{12}$ ADP receptor defects

Two families have been identified with a bleeding disorder caused by defects in this receptor (28, 29). The gene for $P2Y_{12}$ has been cloned recently and its functions analysed (30, 31). In one family, the affected members are heterozygous for a dinucleotide deletion in the *$P2Y_{12}$* gene, causing a frame shift and premature termination (31). In one patient, the wild-type allele was not expressed, suggesting an unidentified problem in the promoter region of the gene in this allele. *$P2Y_{12}$* is an important target for several efficient platelet activation inhibitors in clinical use or under development for anti-thrombotic treatment, such as ticlopidine, clopidogrel, and AR-C66096 (31). It has been suggested that the thiol group in the receptor has a role in covalent inhibition by ticlopidine and clopidogrel and R99224. These compounds have different structures and therefore different potential side effects.

Integrin $\alpha_2\beta_1$ deficiencies

In the 1980s, two patients were described with mild bleeding problems related to deficient expression of $\alpha_2\beta_1$ (32, 33). Their platelets responded defectively to collagen but not to other agonists and had low levels of, or absent, $\alpha_2\beta_1$. Further studies of one of these cases showed decreased platelet adhesion to vascular subendothelium as well as poor activation and spreading. These studies, which have been supported by others with α_2 or β_1 knock-out mice (34, 35), suggested that loss of, or inhibition of, $\alpha_2\beta_1$ might not cause major bleeding problems and that inhibition of this receptor might be a possible anti-thrombotic strategy.

Very recently a family was reported whose cells express dysfunctional integrins of the β_1, β_2, and β_3 families. The defect is thought to lie in a cytoplasmic signalling protein common to all three integrin families (36).

GPVI deficiency

The detection of patients lacking or deficient in GPVI was important in identifying this glycoprotein as a critical collagen receptor (37). The first identified patient had platelets with a specific collagen-response defect and had plasma antibodies that recognised GPVI in normal platelets (38). At least three other patients were found, of whom two also completely lacked surface expression of GPVI while the third had 10% of normal amounts.

CD36 deficiency

A small proportion (4–7%) of healthy Japanese and other East Asians lack CD36 (GPIIIb or GPIV in platelets) (39). Recent studies showed that CD36 is also absent in 7–10% of the sub-Saharan populations of Africa (40) and in a very small part (~0.3%) of populations in other parts of the world. Two major adhesive proteins, collagen and thrombospondin, have been suggested as ligands for CD36, but other proteins also bind to this receptor, including plasmodium-infected erythrocytes, and its main function is thought to be as a scavenger for oxidised lipoproteins or a transporter of long-chain fatty acids (41). Other authors found little evidence for a role of CD36 as a collagen receptor. The molecular basis of CD36 deficiency has been identified as a polymor-

phism in codon 90 which, if expressed, would lead to a Ser→Pro shift (42).

Diseases Caused by Antibodies to Platelet Glycoproteins

Autoimmune

The most common diseases caused by platelets are those involving autoimmune responses to components of the platelet surface, mainly glycoproteins, but also glycolipids or proteins associated with platelet lipid surfaces. Antibody binding to these components of the platelet surface leads to platelet activation, aggregation, and depletion by the spleen causing thrombocytopenia. This is called idiopathic thrombocytopenia purpura (ITP). The glycoprotein targets are predominantly GPIIb-IIIa and GPIb-V-IX, implying a role for the amount present, but a very wide range of other platelet glycoproteins have also been implicated in various studies. In rare cases, such antibodies may block the platelet receptor leading to functional disorders such as acquired GT (43) and BSS (44). Side reactions following a viral infection have been suggested as an explanation for why such autoimmune reactions develop.

Drug-related ITP autoantibodies recognising platelet glycoproteins in the presence of a drug

A further category of thrombocytopenia is caused by antibodies to platelet glycoproteins in the presence of a drug essential for both the induction of the antibodies as well as for their effects on the platelets. This can be induced by a number of drugs and is a danger whenever any new drug is introduced. A classic example is quinine or quinidine. Some individuals taking quinine or quinidine for anti-malaria prophylaxis develop thrombocytopenia, which normalises on removal of the drug. On re-exposure to these drugs these individuals rapidly become thrombocytopenic again. The GPIb-V-IX complex on platelets is a major target for these antibodies in the presence of the appropriate drug. The drug is postulated to interact with the platelet glycoprotein producing a neoantigen, recognised as foreign by the immune system. Presumably only some individuals react in this way with a given drug because only they have the possibility to make high affinity antibodies recognising the neoantigen. The range of drugs that can produce such effects is rather wide and it is still unclear whether covalent reactions between the drug and the glycoprotein are necessary or simply a tight, non-covalent reaction. The active species of drugs such as ticlopidine and clopidogrel is formed in the liver, contains a reactive, and reacts covalently with (a) thiol group(s) in the platelet $P2Y_{12}$ ADP receptor.

Heparin-induced thrombocytopenia (HIT)

Heparin-induced thrombocytopenia (HIT) is a special case of a drug-induced, platelet receptor/glycoprotein-related disorder. Although the exact mechanism is still not completely understood, nor why only certain individuals are susceptible, the general basis and how to treat it is now fairly clear (45–47). The new antigen in this case is formed by the association between heparin (or related glycosaminoglycans) and platelet factor 4 (PF4), a small molecule of the chemokine family, synthesised in megakaryocytes, stored in platelet α-granules, and released on platelet activation. Other chemokines from platelets or plasma may also contribute but are not major factors. Although aggregated PF4-heparin-antibody complexes may cause platelet activation by clustering the essential platelet FcγRIIA receptor, it seems more likely that additional platelet receptors containing glycosaminoglycans, such as glypican, can bind to these complexes and their signalling plays a role in the platelet activation.

Diseases Involving Abnormal Functions of Platelet Glycoproteins

The role of platelet receptors in diseases like asthma and diabetes is another area that has been poorly explored so far. Evidence that platelets have receptors for the chemokines RANTES and eotoxin, known to be implicated in asthma, suggests that their involvement needs to be studied more carefully. There is also evidence that platelets are more readily activated or circulate in an activated state in diabetes, contributing to retinopathies and glomerulonephritis. Platelets express low amounts of the FcεRI receptor, which may mediate the effects of IgE/antigen complexes and contribute to the symptoms of asthma.

Diseases Involving Platelet Glycoproteins Acting as Pathogen Receptors

Although this is an area that was neglected for many years, there is increasing evidence that some platelet glycoproteins act as receptors for the binding and internalisation of viruses and, perhaps, other pathogenic micro-organisms. Platelets were ignored in this context because they lack a nucleus and therefore could not contribute directly to viral replication. However, this is not so for megakaryocytes, the platelet precursors, which express the same range of receptors in their final differentiation stage. Growing evidence points to other roles for platelets in providing a refuge and transport for viruses, especially HIV (48), and the possibility that activation of platelets by viruses helps them to circumvent immune responses. Early onset HIV-1 in the absence of AIDS is associated with thrombocytopenia due to increased platelet destruction. All these aspects are at an early stage of investigation and need considerably more research to establish responsible mechanisms. The possible role of platelets in HIV infection *via* their chemokine receptors is an area that is of particular interest.

Polymorphisms in Platelet Glycoproteins Affecting Susceptibility to Cardiovascular Diseases

Amino acid polymorphisms in the principal platelet integrin GPIIb/IIIa ($\alpha_{IIb}\beta_3$) (49) are a major reason for antiplatelet antibodies after transfusion. Myocardial infarction was reported to be commoner in people with the L33 version of the 33L/P polymorphism in GPIIIa (50). GPIIb/IIIa containing this polymorphism binds more avidly to immobilized fibrinogen and shows enhanced outside-in signalling. Platelets with this GPIIb/IIIa polymorphism were reported to form large aggregates more easily (51). However, defective responses to arachadonic acid and thromboxane A2 were reported in the PLA2 polymorphism of β_3 (52).

Homozygosity for Ser843 in GPIIb enhanced the risk of stroke five-fold in high-risk groups of young women with hypertension or diabetes (53), but other studies found no effect on cardiovascular disease or myocardial infarction (54).

The HPA-5 immunogen is a 505 E/K (A/G 1648) polymorphism on α_2 of $\alpha_2\beta_1$ (55, 56). Silent polymorphisms in the coding region of the α_2 gene affect levels of $\alpha_2\beta_1$ expression and the higher level of expression was associated with higher levels of stroke in younger patients. Higher $\alpha_2\beta_1$ expression might lead to easier activation after exposure to collagen following vessel damage or plaque rupture. Several groups (57–59) reported a higher incidence of myocardial infarction or stroke associated with the T807 variant, particularly in patients over 50 years of age. Coronary heart disease and acute myocardial infarction rates were higher in subjects expressing the 807T polymorphism (60). However, other studies found no effect after stenting (61) and no association with malignant arrhythmia (62).

The risk of stroke in young women was two-fold higher in those with the 807T polymorphism (63). An even stronger association was found in a study of a large group but was concentrated among high-risk subgroups. Homozygous carriers of 807TT *vs.* homozygous CC had a 14.1 × risk factor for cardiovascular mortality in high-risk women (≥ 2 risk factors smoking, diabetes, or microalbuminuria) in a large prospective study with 12239 women (64). In this total population, the risk factor of 807TT *vs.* CC was only 1.2 ×.

GPIb-V-IX also contains polymorphisms that might influence cardiovascular disease development. There are three categories of variants. Single amino acid polymorphisms within the LRR domain of GPIbα, such as 145Thr/Met (65), might affect VWF binding to platelets by changing the conformation or flexibility of the LRR domain. The M145 polymorphism showed a trend to higher risk of stroke in young women (63). The second category is variations in the number of copies of a 13 amino acid segment in the O-glycosylated mucin-like domain affecting the distance that the binding sites are held out from the platelet surface (66). The third category involves a polymorphism that was described in the Kozak sequence of the promoter of GPIbα (–5T/C) affecting levels of expression. A strong association was recently reported between a combination of the two polymorphisms, T145M and –5T/C, with the incidence of stroke (67).

Polymorphisms in other receptors have so far been poorly investigated but could also contribute to differences in platelet reactivity. A T102C polymorphism in the serotonin (5-HT) 2A receptor has been associated with non-fatal acute myocardial infarction (68). Some cases of thrombocytopenia were shown to be due to a defect in the thrombopoietin receptor gene (69) and might be expected to reduce susceptibility to cardiovascular disease. The FcγRIIA H/R131 polymorphism (70) might also affect platelet reactivity.

Many of the studies relating differences in the sequence of platelet receptors with increased incidence of cardiovascular disease remain controversial. The effects reported are generally fairly small compared to certain polymorphisms in coagulation factors. They are not negligible but may only show up against a background of overall platelet/coagulation polymorphisms or when several polymorphisms occur in the same individual. An example of a difference that could be detected when both a platelet receptor and a plasma factor were affected is the increased bleeding noted when a polymorphism affecting low levels of expression of $\alpha_2\beta_1$ was combined with mild von Willebrand disease involving decreased amounts of VWF (71). In several recent studies the effect of a platelet polymorphism was quite strong but only if subgroups of subjects with multiple risk factors possibly affecting vascular integrity were considered.

Recent experimental confirmation (72) of the critical role of platelets in the origin of atherosclerosis should increase interest in the effect of variations in the individual glycoprotein receptors on this pathological process.

Acknowledgements

Work performed at the Theodor Kocher Institute was supported by grant #31-063868.00 from the Swiss National Science Foundation.

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Received 10 January 2003, revised 10 February 2003, accepted 14 February 2003

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