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INTEGRIN-MEDIATED PLATELET ADHESION

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TABLE OF CONTENTS

1. ABSTRACT

Adhesion of platelets to the damaged subendothelium is a prerequisite reaction for the initiation of hemostasis *in vivo*. Platelet membranes contain high concentrations of integrins and other glycoproteins (GPs) that are involved in the platelet adhesion to the extracellular matrix. In the present review, we focus on two platelet integrins, integrin alphaIIb beta3 (GPIIb/IIIa) and integrin alpha2 beta1 (GPIa/IIa) because these integrins are major components of the platelet membrane proteins and are known to contribute to platelet adhesion to fibrin(ogen) and collagen surfaces, respectively. These integrins bind soluble ligands (fibrinogen or collagen) after platelets are activated but only have low affinity towards these ligands when platelets are in the resting state. We describe the binding properties of these integrins and discuss the mechanism for the activation of these integrins. Platelets can adhere to fibrin(ogen) or collagen immobilized on a surface. When platelets adhere to a collagen- or fibrin-coated surface, they become activated and form aggregates; this is especially prominent under flow conditions. We discuss the contribution of these integrins and non-integrin proteins, GPIb and GPVI, to the platelet adhesion on to the collagen surface, especially under flow conditions, a system that most closely approximates platelet adhesion *in vivo*.

2. INTRODUCTION

In the hemostatic system *in vivo*, the first event after vessel wall damage that occurs is the reaction where platelets adhere to the exposed subendothelium. After these cells adhere, they accumulate at the damaged area, forming a hemostatic plug that stops the leakage of blood from the vessels. This crucial reaction in hemostasis involves a complex series of steps.

Basically, adhesion is the reaction of cells sticking to an insoluble surface, but this does not mean that adhesion is a simple contact of cells and the surface. In the adhesion process, cells also spread over the surface, enabling them to form tighter interactions with the surface. In platelets, many reactions are induced in these cells while they adhere to insoluble surfaces, which sometimes, depending on the type of insoluble surface, induce the activation of platelets and subsequent platelet aggregation. The adhesion process includes at least three reactions: 1) the first interaction (contact, binding), 2) signal transducing and activating reactions inside the cells (e.g., phosphorylation and cytoskeletal reorganization), and 3) spreading or shape change (pseudopod formation, firm adhesion). Subsequently, other reactions, the release reaction, in which the granule contents are released, and aggregation occur. The quick succession or simultaneous occurrence of these events makes it difficult to analyze the molecular mechanism of adhesion, because such investigations would require a separation of these processes so that steps can be analyzed individually. In this chapter, we discuss 1) the interaction of integrins with their ligands, 2) the effects of integrin binding on the signal transduction system in platelets and 3) the contribution of integrins in platelet adhesion, especially under flow conditions.

Platelets contain several integrins including alphaIIb beta3 (GPIIb/IIIa), alpha2 beta1 (GPIa/IIa), alphaV beta3 and alpha5 beta1. The former two integrins were identified as platelet-specific glycoproteins and later found to be members of the integrin family. These two integrins are major components of platelet membrane proteins and their contributions to platelet adhesion have been studied extensively. Thus, we will first describe the properties of these two integrins and the mechanism of interaction of these proteins with ligands individually, and later discuss the contribution of platelet integrins to the adhesion of platelets onto the ligand surface.

3. THE MAJOR INTEGRINS INVOLVED IN ADHESION

3.1 Integrin alphaIIb beta3 (GPIIb/IIIa)

Integrin alphaIIb beta3 was first identified as major membrane glycoproteins of platelets under the name of GPIIb/IIIa , and soon after, its essential physiological roles in platelet aggregation were suggested from the observation that patients with an inherited bleeding disorder, Glanzman's thrombasthenia, had platelets markedly deficient in this glycoprotein (1). Later, integrin alphaIIb beta3 was shown to

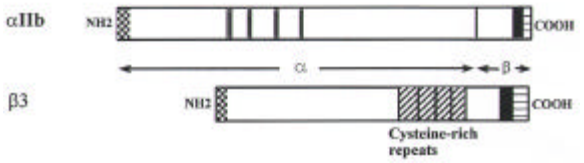

Figure 1. Schematic structure of integrin alphaIIb (GPIIb) and integrin beta3 (GPIIIa). Integrin alphaIIb is composed of two polypeptide chains (alpha and beta). Signal peptide region (checkered rectangles), transmembrane domains (black rectangle) and cytoplasmic tails (horizontally striped rectangles) are shown for both alpha and beta. Integrin alphaIIb contains 4 divalent cation sites (narrow, gray-shaded rectangles), and integrin beta3 contains a cysteine-rich domain (as indicated in the figure, diagonally shaded rectangles).

be a receptor for soluble fibrinogen on activated platelets, and its binding to fibrinogen was necessary for platelet aggregation (2,3). Integrin alphaIIb beta3 is now one of the most extensively studied proteins in the integrin family, and its chemical structure and its function have been reviewed by many authors (4-9).

Integrin alphaIIb beta3 (GPIIb/IIIa) is a heterodimeric complex and is always present as a 1-to-1 complex in platelets. Integrin alphaIIb (GPIIb) is composed of two polypeptide chains (alpha and beta), and integrin beta3 (GPIIIa) is a single chain polypeptide, which is also present in platelets as a complex with integrin alphaV. Their structures and biosynthesis have already been analyzed in detail (10-14). As its characteristic chemical structure, GPIIb has 4 stretches of 12 amino acids homologous to the Ca^{2+}-binding EF hand structure, to which four Ca^{2+} molecules are suggested to bind. Glycoprotein IIIa has a total of 56 cysteine residues, all engaged in disulfide bonds (15), and four cysteine-rich repeats that are a distinguishing feature of the beta-integrin subunit. The structures of integrin alphaIIb and beta3 are summarized in figure 1.

The binding of integrin alphaIIb beta3 to fibrinogen was extensively studied because of its importance in platelet aggregation (16,17). Fibrinogen binds to activated platelets with a dissociation constant of 5-40 x 10^{-8} M, and there are about 40,000 binding sites per platelet. The binding requires the presence of 1-2 mM Ca^{2+} or Mg^{2+}. Integrin alphaIIb beta3 was indicated to recognize a stretch of amino acids at the C-terminal end of the fibrinogen gamma-chain. Integrin alphaIIb beta3 also binds to Arg-Gly-Asp (RGD) peptide, and fibrinogen has two RGD sequences, although these RGD sequences have been reported to be hindered from being able to react with integrin alphaIIb beta3. Thus, fibrinogen is thought to react with integrin alphaIIb beta3 through the C-terminal portion of its gamma-chains. This supports the hypothesis that fibrinogen cross-links platelets to form aggregates, since fibrinogen has two identical C terminals. From data obtained from cross-linking fibrinogen-derived peptides to platelets and observing what peptide sequences of alphaIIb beta3 can inhibit fibrinogen binding to platelets, the fibrinogen binding sites were determined to lie within the following peptide sequences of alphaIIb beta3: amino acid residues 292-314 of GPIIb (18), 109-171 of GPIIIa (19), and 204-231 of GPIIIa (20). Further support for the involvement of amino acids at or near these regions was obtained by analyzing the structure of integrin alphaIIb beta3 from a patient with thrombasthenia (21); the patient's platelets had a defective GPIIIa due to a Arg214 to Trp mutation.

Integrin alphaIIb beta3 has been indicated to have several conformations that expose different epitopes for monoclonal antibodies and bind ligands with different affinities. The resting form of alphaIIb beta3 accounts for most of the integrin on resting platelets, and this form only has very low affinity to bind fibrinogen. When platelets are activated with agonists, integrin alphaIIb beta3 molecules are transformed to the activated form that can bind ligands with high affinity. Furthermore, following the binding of the ligand to the activated integrin, the conformation of alphaIIb beta3 changes to expose a ligand-induced binding site (LIBS) (8,22-24). The dynamic change of the state of integrin alphaIIb beta3 in the process of platelet activation and aggregate formation is an integral part of platelet function.

The activation mechanism of integrin alphaIIb beta3 has been examined by many investigators but is still yet not fully explained. Although agonists would activate platelets through a signaling pathway that includes various classes of signal response elements, the final activation of alphaIIb beta3 results from the interactions of its short cytoplasmic domains with other components at the intracellular side of the plasma membrane. The interaction of the cytoplasmic domain of alphaIIb beta3 would induce a conformational change in the extracellular domain to bind with higher affinity to fibrinogen. Studies performed with recombinant proteins having various mutational changes in the cytoplasmic domains of alphaIIb and/or beta3 indicated the contribution of these cytoplasmic tails to the activation and function of integrin alphaIIb beta3 (25-28). Numerous proteins have been indicated to interact with the cytoplasmic tails of integrin beta, including cytoskeletal proteins (alpha-actinin, paxillin, talin and filamin) (29-32), protein kinases (focal adhesion kinase and integrin-linked kinase-1) (30,33) and potential regulatory proteins (beta3-endonexin, cytohesin-1, integrin cytoplasmic domain-associated protein-1 and calreticulin) (34-37). Beta3-endonexin was identified as a protein that interacts specifically with the cytoplasmic domain of integrin beta3 (38). Beta3-endonexin might be a direct inducer of the activation of integrin alphaIIb beta3 , but its physiological activity in platelet function remains to be analyzed.

The ligand specificity of integrin alphaIIb beta3 is broad. It can bind many adhesive proteins containing the RGD sequence in their structures, including fibrinogen, fibronectin, von Willebrand factor (vWf), collagen and vitronectin. Considering its concentration in plasma, fibrinogen would be a major ligand for alphaIIb beta3 when platelets aggregate physiologically. However, alphaIIb beta3 can also interact with other ligands, as determined by *in vitro* platelet adhesion to surface-immobilized adhesive proteins.

Platelets adhere to a surface of immobilized fibrinogen under both the static condition and the flow condition with low shear rate. This adhesion occurs via integrin alphaIIb beta3 and does not need prior activation. The interaction appears to involve the carboxy terminus of the fibrinogen gamma-chain (39,40). The mechanism of platelet adhesion to fibrinogen will be described later in this review. Integrin alphaIIb beta3 is also involved in platelet adhesion to other matrices. In the adhesion to a vWf-

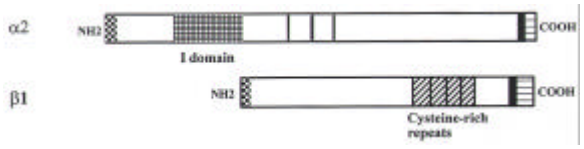

Figure 2. Schematic structure of integrin alpha2 (GPIa) and beta1 (GPIIa). Integrin alpha2 contains an I domain (rectangle with grid pattern) and three divalent cation ion sites (narrow, gray-shaded rectangles). The signal peptide region (checkered rectangles), transmembrane domains (black rectangles), and cytoplasmic tails (horizontally striped rectangles) are shown for both the alpha2 and beta1 chains.

coated surface, alphaIIb beta3 is suggested to bind to vWf after the initial fast interaction of vWf with GPIb and support the firm adhesion of platelets (41-43). In the adhesion to a collagen-coated surface under static and flow conditions, platelets are activated and form aggregates after the initial adhesion, which is dependent on collagen receptors or GPIb of the platelet surface. The inhibition of the function of alphaIIb beta3 by a monoclonal antibody or disintegrin antagonist prevents the formation of these aggregates and severely decreases the extent of adhesion (44,45). The contribution of alphaIIb beta3 to the platelet adhesion to a fibronectin-coated surface under both static and flow conditions was also indicated (46).

3.2 Integrin alpha2 beta1 (GPIa/IIa)

In 1985, Nieuwenhuis et al. described a patient whose platelets showed no reactivity to collagen, and the GPIa in her platelets was found to be decreased to 15-20% of the normal level (47). This was the first indication that GPIa is a physiologically active collagen receptor of platelets. Later, GPIa was indicated to be present as a complex with GPIIa, and the GPIa/IIa complex was found to be identical to the very late activation antigen-2 (VLA-2) on activated T-cells and the class II extracellular matrix receptor (ECMR-II) on fibroblasts (48). The complex has now been identified as a member of the integrin family and designated as integrin alpha2 beta1. Integrin alpha2 beta1 is widely distributed on various cell types, functioning as a collagen receptor (49). However, it appears to function as both a collagen receptor and a laminin receptor on endothelial cells, melanoma cells and epithelial cells. Several reviews have been written emphasizing different aspects of this subject (50-53).

The structures of integrin beta1 and alpha2 were deduced from the corresponding cDNA sequences (54,55). The integrin alpha2-subunit is a single polypeptide chain that contains an I domain insert (residues 140-359) next to its three metal binding domains, a characteristic common to integrin proteins. This I domain is homologous to the collagen binding domain of vWf, and the critical involvement of the I domain for the binding to collagen was indicated (56,57). Recently a three-dimensional structure of the recombinant I domain was determined and a model of the binding of collagen to this protein was presented (58). Integrin beta1 has a similar structure to other integrin beta proteins and contains four cysteine-rich repeats, as shown in figure 2.

The interaction of integrin alpha2 beta1 with collagen requires the presence of Mg^{2+}, whereas the presence of Ca^{2+} ion is inhibitory (59). Although this metal ion dependency of alpha2 beta1 is frequently used to indicate the presence or involvement of this protein, such a metal ion dependency is also exhibited by beta2 integrins and integrin alphavbeta1. Another characteristic of integrin proteins is the process by which their function is activated, which was described for integrin alphaIIb beta3 in the previous section. Several antibodies are known to activate alpha2 beta1, and the epitopes for these antibodies were identified to be small regions of the beta1-subunit, within the sequence composed of residues 207-218 (60). Some antibodies inhibiting alpha2 beta1 also recognize this region. These data suggest that this essential region of integrin beta1 regulates the activity of alpha2 beta1 since the putative binding site for collagen is close to this region (residues 120-182 and 220-231) (60). Other epitope regions of the beta1-subunit were also proposed for other activating antibodies (61,62). These regions are near the transmembrane domain or inside a cysteine-rich domain, and all of these epitopes are located apart from the ligand binding site, so the binding of the latter activating antibodies to these regions would regulate the ligand binding characteristics at a region sterically apart from the ligand binding site by inducing a conformational change of the protein. These data suggest that the activation of alpha2 beta1 would be induced when platelets are activated with agonists. Recently we were able to detect such an activation of alpha2 beta1 in platelets by monitoring the binding of soluble collagen (63). Although there was no binding of soluble collagen to resting platelets, significant binding of soluble collagen was observed after platelets were reacted with the activating antibody TS2/16 or platelet agonists, thrombin, ADP or CRP (collagen-related peptide). The binding was Mg ion dependent and inhibited by an anti-alpha2-beta1 antibody. PGI_2, an inhibitor of platelet function, potently inhibited the activation of platelets to bind soluble collagen induced by each of the tested agonists, with the exception of that induced by TS2/16. These results suggest that integrin alpha2 beta1 is activated when platelets are activated with the agonists and then becomes able to bind soluble collagen with high affinity. Thus it appears that both integrins alphaIIb beta3 and alpha2 beta1 have similar activation mechanisms. Our kinetic analysis of soluble collagen binding indicated that the dissociation constant is $3.5\text{-}9\times10^{-8}$ M and there are 1500-3500 binding sites per platelet, for thrombin- or TS2/16-activated platelets.

At present, 18 types of collagen have been identified. Platelet adhesion to collagen types I to VIII was determined; collagen types I, II, III and IV were shown to react strongly with platelets, but types VI, VII and VIII reacted only weakly (64,65). Antibody to alpha2 beta1 inhibited platelet adhesion to all the collagen types , so integrin alpha2 beta1 would be able to bind with any of these types of collagen (64). Because types I and III collagens are typical fibrous collagens found in bone, tendons, and other connective tissues and easily available, most studies have been done with these types. A specific amino acid sequence in collagen that reacts with alpha2 beta1 was studied , and a short DGEA sequence in the alpha1(I)-CB3 fragment appeared to be a recognition sequence of collagen (66). In contrast to these results, other groups identified the alpha2 beta1 recognition sites in residues 520-528 of the alpha1 (III) collagen triple-helical chain (67,68). Since denatured collagen, gelatin, does not react with platelets at all and does not bind to alpha2 beta1, integrin alpha2 beta1 would interact with the collagen molecule by recognizing its steric structure.

4. MODES OF PLATELET ADHESION TO VARIOUS SURFACES AND THE INVOLVEMENT OF INTEGRINS IN THESE PROCESSES

4.1 Platelet adhesion to the fibrinogen/fibrin surface

Although resting platelets do not bind to soluble fibrinogen, washed platelets can adhere to an immobilized fibrinogen surface under the static condition. The initial attachment, independent of platelet activation, is followed by spreading and irreversible adhesion even in the presence of an activation inhibitor (39). The adhesion is dependent on the interaction of platelet integrin alphaIIb beta3 with the C-terminal portion of fibrinogen immobilized on the surface. Compared to the resting platelets, many more activated platelets adhered to the surface of immobilized fibrinogen (40,69). These results suggested that the activation of platelets, which induces the expression of the high affinity fibrinogen binding sites on the platelet surface, is not necessary for the adhesion on the fibrinogen surface, but rather, activation stimulates the adhesion.

The platelet adhesion to a fibrinogen surface under flow conditions was also independent of platelet activation. Adhesion was observed at lower shear rates, but at a shear rate of 1500/sec or higher, almost no platelets adhered on the fibrinogen surface (41,70-72). Platelets can also adhere to a surface of immobilized fibrin, and the extent of platelet adhesion to the fibrin surface is higher than that to the fibrinogen surface, but still lower than that to the surface of immobilized collagen. Platelets adhere to the fibrinogen surface as a single cells, but the platelets form aggregates on the fibrin surface. The formation of platelet aggregates indicates that platelet activation has occurred during the adhesion of platelets to the immobilized fibrin surface. In fact, the platelet-activating inhibitor PGI_2 inhibited platelet adhesion to the fibrin surface under flow conditions (41). As yet, no mechanism has been proposed to explain how platelets become activated when they adhere to immobilized fibrin. The adhesion to the fibrin or fibrinogen surface was inhibited by an antibody to integrin alphaIIb beta3, but not by an anti-GPIb antibody (41,72), indicating that platelet adhesion to fibrin or fibrinogen depends on integrin alphaIIb beta3, with no contribution from GPIb. In addition, the interaction of platelets with fibrinogen was indicated to be mediated by the C-terminal sequence of the fibrinogen gamma-chain and not by the RGD sequence of the alpha-chain (71). These results show that the platelet adhesion to the fibrinogen or fibrin surface is mediated through the interaction of integrin alphaIIb beta3 and the C-terminus of fibrin(ogen) under the flow condition with low shear rates.

Platelets adhere to fibrinogen even under flow conditions, although we can not observe any binding of soluble fibrinogen to resting platelets. How can we explain this discrepancy? A reasonable explanation can be proposed: This adhesion would be due to the binding reaction between two membrane surfaces that have a high density of ligand and receptor. Although a single binding interaction is weak, many binding interactions between alphaIIb beta3 molecules on the platelet membrane and fibrinogen immobilized on the surface would fix the platelets onto the surface. The theoretical and experimental evidence to support this hypothesis must be obtained in future investigations.

4.2 Platelet adhesion to the collagen surface

The interaction of platelets with collagen is a very complex reaction, whose mechanism has been difficult to elucidate on the molecular level. This is mainly due to the physicochemical properties of the collagen molecule itself. Under physiological conditions, collagen is present in an insoluble form, collagen fibers. Because the multivalent collagen fibers can bind to many proteins, many platelet proteins have been proposed to be collagen receptors. However, at present, only two glycoproteins have met the criteria to make them plausible physiological platelet collagen receptors, integrin alpha2 beta1 (GPIa/IIa) and GPVI (47,73).

Platelet adhesion to collagen-coated wells under static conditions was reported to depend on alpha2 beta1 (59), CD36 (GPIV) (74) and GPVI (44). The physiological contribution of CD36 to platelet adhesion to collagen was brought into question by the finding that individuals lacking this protein showed normal adhesion, with no bleeding abnormalities (75,76). In adhesion assays, platelet adhesion to collagen-coated wells is quantitated by the radioactivity of labeled platelets or by colorimetry. Platelet adhesion was biphasic and not only the adhesion of platelets but also the release reaction of platelets was observed, which means that aggregation was also induced, especially in the second phase of adhesion. Monoclonal antibody against integrin alphaIIb beta3 and disintegrin inhibited the second phase of adhesion. In contrast, monoclonal antibody against integrin alpha2 beta1 and the Fab fragment of an antibody against GPVI inhibited the first phase adhesion. Patient platelets lacking GPVI had an adhesion pattern similar to that for the antibody-inhibited adhesion (44). These findings indicate that alpha2 beta1 and GPVI are involved in the early phase of platelet adhesion on the collagen surface, and platelet activation and aggregation occur subsequently to this initial interaction with collagen.

Since platelets only react with insoluble collagen, a far from ideal ligand for binding studies, it has been difficult to perform experiments to directly measure the interaction between platelets and collagen. However, we have been able to measure the direct binding of soluble collagen to platelets, as was described in the previous section. This Mg^{2+} dependent binding occurs through the interaction of integrin alpha2 beta1 with the soluble collagen molecules. This is in contrast to the binding of microparticles of labeled collagen fibrils to platelets, in which the main portion of the binding was independent of both metal ion and alpha2 beta1 (63). Since the binding of collagen to GP VI is metal ion-independent (73), the binding of insoluble collagen fibrils to platelets would be mainly mediated through GPVI. CRP is a collagen mimetic peptide that can interact with GPVI and induce platelet aggregation. CRP also induced the activation of alpha2beta1. These results suggest that the interaction of insoluble collagen with GP VI induces the activation of alpha2 beta1, and the activated alpha2 beta1 would also be involved in the binding of insoluble collagen. This would be the case in platelet adhesion under static conditions. A few platelets would adhere to the collagen coated-wells in the presence of EDTA. Thus, the adhesion is dependent on the tight binding through the activated alpha2 beta1, which would be activated by the interaction of GPVI with insoluble collagen.

Under physiological conditions, platelets in flowing blood adhere and form aggregates on the exposed subendothelium of blood vessels. As a model that closely approximates this *in vivo* adhesion, platelet adhesion to an immobilized-collagen surface under flow conditions using a flat flow perfusion chamber has been employed. Platelet adhesion

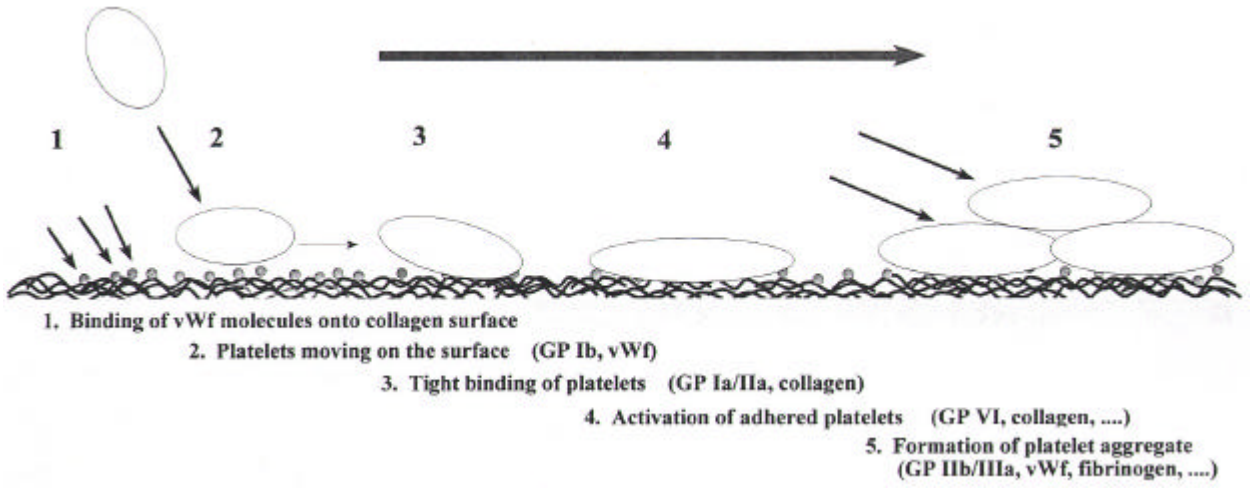

Figure 3. Model of platelet adhesion onto the collagen surface under flow conditions.

was monitored by fluorescent microscopy when platelets were labeled by mepacrine (45,77,78) or by staining after fixing the adhered platelets (79,80). In these experiments, platelets were observed to adhere to the collagen-coated surface and then form aggregates under different shear rates. The platelet adhesion to the collagen-coated surface was dependent on the vWf-GPIb interaction under flow conditions, especially at high shear rate (80-82). vWf binds to collagen with high affinity (83), and antibodies against vWf and GPIb severely inhibited the platelet adhesion to the collagen-coated surface (78,82). Platelet adhesion to the collagen surface was inhibited by a monoclonal antibody against alpha2 beta1 (64). The antibody especially inhibited the firm adhesion of platelets under flow conditions (unpublished observation), and the adhesion of GPIa-deficient platelets to subendothelium was also severely decreased (84). On the other hand, platelet adhesion of GPVI-deficient platelets to the collagen surface was not decreased when it was assayed by Sakariassen's non-fluorescent microscopy method, although measurements by the fluorescent microscopy method indicated that adhesion was decreased. Both methods indicate that the aggregate formation after adhesion was completely absent (45). The absence of platelet aggregates on subendothelium was also observed using GPVI-deficient blood (unpublished result).

From these results, the mechanism of platelet adhesion onto the collagen surface under flow conditions, which would be also applicable to *in vivo* adhesion to subendothelium, can be proposed to occur as follows (figure 3): 1) The first reaction is a binding of circulating vWf molecules to the collagen surface. 2) Platelets in the circulating blood flow interact with the immobilized vWf molecules through GPIb on the platelet surface. This interaction would be very fast but of fairly low avidity, so that platelets would still be moving on the collagen surface but with much reduced speed. 3) While the platelets are moving on the collagen surface with slow velocity, these platelets can react with collagen through integrin alpha2 beta1 and/or GPVI, and thereby, integrin alpha2 beta1 would become activated. The activated alpha2 beta1 molecules bind to collagen with high affinity, and this would stop the platelets from moving on the collagen surface, so they become "arrested". 4) The interaction of platelets with immobilized collagen also would activate platelets through GPVI. 5) The platelet activation induced by GP VI includes the activation of integrin alphaIIb beta3, which stimulates the formation of platelet aggregates under flow conditions. In this model, we currently do not have enough evidence to show that there is a relationship between integrin alpha2 beta1 and GPVI. Although it is possible for GPVI to activate integrin alpha2 beta1, both proteins would contribute to the platelet adhesion rather independently, since GPVI-deficient platelets adhere normally to the collagen surface under flow conditions (45). Thus, steps 3 and 4 would seem to occur quite simultaneously. The ligands of activated alphaIIb beta3 under flow conditions are yet not fully understood. vWf has been proposed to contribute to adhesion by being a ligand for activated integrin alphaIIb beta3 (72,85). The details of the mechanism in each reaction remain to be analyzed in future investigations.

4.3 Platelet adhesion to other surfaces

As described above, vWf is indicated to be involved in platelet adhesion to the collagen surface , especially at high shear stress. The platelet adhesion to a vWf-immobilized surface under flow conditions is characterized by mainly a transient adhesion of platelets on the vWf surface, indicating a binding interaction with a high on-rate and a high off-rate (72,82). The adhesion of platelets to the vWf surface is predominantly dependent on the interaction with GPIb on the platelet surface. We will not describe the mechanism of the interaction of vWf and GPIb because GPIb is not an integrin protein, but the involvement of activated integrin alphaIIb beta3 in the binding with vWf was suggested. The binding to activated integrin alphaIIb beta3 is suggested to induce the firm adhesion of platelets on the vWf surface under shear stress (72,85).

Platelets contain two integrins that can bind to fibronectin, integrin alpha5 beta1 (GPIc/IIa) and integrin alphaIIb beta3 (GPIIb/IIIa). Under static conditions, platelet adhesion to a surface of immobilized fibronectin was observed to depend on GPIc/IIa and not on integrin alphaIIb beta3 (86). Contrary to those results, another group reported that alphaIIb beta3 contributes to the adhesion (87). Under flow conditions, platelet adhesion to the fibronectin surface was maximum at a shear rate of 300/sec; and at shear rates more than 1300/sec, surface coverage by adhering platelets was reduced to less than 4 %. An anti-integrin alphaIIb beta3 antibody and RGD peptide strongly inhibited the platelet adhesion to the fibronectin surface

under both static and flow conditions, but anti-integrin alpha5 beta1 antibody inhibited only 50% of the adhesion under both conditions (46). These results indicated that both integrins contribute to platelet adhesion to the fibronectin surface. Also, the contribution of GPIb and vWf was suggested in the platelet adhesion on fibronectin under flow conditions (88). Thus, the mechanism of platelet adhesion to fibronectin seems to be complex, and the physiological role of this adhesion is also unclear at present.

5. PERSPECTIVES ON THE FUTURE DIRECTION OF RESEARCH ON ADHESION

Platelets have several glycoproteins that react with components of the extracellular matrix and contribute to their adhesion. The structures and functions of these glycoproteins, with the exception of GPVI, have already been analyzed in detail. However, the mechanism of platelet adhesion remains incompletely elucidated. One of the reasons for this is the complexity of adhesion. Adhesion is the interaction of ligands immobilized on a surface and the cell membrane, which expresses many receptors on its surface; and the binding of these two surfaces would induce complex reactions on the cell surfaces themselves as well as inside the cells. Future investigations must concentrate on answering many questions that directly relate to the observed characteristics of the adhesion mechanism. For example, why do resting platelets adhere to a surface of immobilized fibrinogen or vWf , but can not bind to the soluble form of these proteins without first being activated ? We might propose that these ligand proteins might undergo a conformational change as a result of being immobilized to a surface and then become able to bind to the platelet receptors. However, a more plausible explanation would be that the interaction of two surfaces with many binding sites would give tighter binding than the binding of single molecules. After the initial binding of the two surfaces, which might be weak at first, especially at the beginning of contact, many reactions may subsequently occur inside the area where the membrane contacts the extracellular matrix surface; such reactions might include activation of integrin receptors, clustering, or signal transduction. Since platelets spread over the surface and also form pseudopods after adhesion, it is obvious that the adhesion induces many signals to transform platelet shape, although a discussion about these signal transduction systems could not be made in the limited space of this chapter. Briefly stated, the stress induced by the interaction of the platelet membrane and extracellular surface would be spread throughout the entire platelet via signal transduction mechanisms, and thus platelets would be activated to the next level, the aggregation reaction. Therefore, analyzing the adhesion of reconstituted platelet membranes to an immobilized surface under controlled conditions may be a promising technique to analyze adhesion decoupled from accompanying aggregation.

Analyses of platelet adhesion under flow conditions is also very complex, although this method probably approximates the situation *in vivo* most closely. Among the recent results identifying the contribution of platelet membrane proteins and plasma protein in flow adhesion, the interaction of GPIb and vWf was found to be especially important for the adhesion under higher shear stress. Since platelets flow at fast speed, an "apparatus" that can first catch the flowing platelets and tether them to the surface is needed for the adhesion of platelets under flow conditions, in addition to the mechanism of firm adhesion. After platelets are tethered on the surface, integrins work as strong binders that can hold on to platelets even when there is strong shear stress. In this process, integrins need to be activated. Only after the activation do integrins show the strong affinity to ligands. And, here again, the mechanism of integrin activation has yet to be described in detail for any member of the integrin family. So far, extensive studies have been done on integrin alphaIIbbeta3, and although the importance of the cytoplasmic domains of this integrin has been suggested, the mechanism for their participation in adhesion remain unclear. The activation of integrins is one of the mechanisms that restricts thrombus formation to the damaged area in human body and therefore should be analyzed in the future.

The mechanism of platelet adhesion to the collagen-coated surface is similar to the adhesion of neutrophils to endothelial cells. Selectins on neutrophils and on endothelial cells bind to sialylated sugar ligands on other cells and tether cells on the endothelial cell layer. At this stage, neutrophils "roll" on the layer. Then beta2 integrins on the neutrophils are activated and bind firmly to ICAM-1 on the endothelial cells. After the firm adhesion, neutrophils undergo shape changes that allow them to diapedese through interendothelial junctions of the vessel wall (89,90). The rolling of neutrophils in shear flow was simulated, considering many physical and chemical characteristics of these cells (91,92). Simulation of platelet adhesion on vWf with such models would be helpful for understanding the factors affecting the adhesion under flow conditions.

6. REFERENCES

1. Nurden, A. T. and J. P. Caen: An abnormal platelet glycoprotein pattern in three cases of Glanzmann's thrombasthenia. *Br J Haematol* 28, 253-260 (1974)

2. Mustard, J. F., M. A. Packham, R. L. Kinlough-Rathbone, D. W. Perry & E. Regoeczi: Fibrinogen and ADP-induced platelet aggregation. *Blood* 52, 453-466 (1978)

3. Marguerie, G. A., E. F. Plow & T. S. Edgington: Human platelets possess an inducible and saturable receptor for fibrinogen. *J Biol Chem* 254, 5357-5363 (1979)

4. Plow, E. F. and M. H. Ginsberg: Cellular adhesion: GPIIb-IIIa as a prototypic adhesion receptor. *Prog Hemost Thromb* 9, 117-156 (1989)

5. Kieffer, N. and D. R. Phillips: Platelet membrane glycoproteins: Functions in cellular interactions. *Annu Rev Cell Biol* 6, 329-357 (1990)

6. Smyth, S. S., C. C. Joneckis & L. V. Parise: Regulation of vascular integrins. *Blood* 81, 2827-2843 (1993)

7. Calvete, J. J.: Clues for understanding the structure and function of a prototypic human integrin: The platelet glycoprotein IIb/IIIa complex. *Thromb Haemostas* 72, 1-15 (1994)

8. Du, X. and M. H. Ginsberg: Integrin alphaIIb beta3 and

platelet function. *Thromb Haemostas* 78, 96-100 (1997)

9. Abrams, C. and Shattil, S. J.: The platelet integrin, GP IIb-IIIa (alphaIIb beta3). In: Advances in molecular and cell biology. Eds: Bittar, E. E. , Lapetina, E. G., JAI Press Inc., Greenwich, CT, 18, 67-108 (1997)

10. Poncz, M., R. Eisman, R. Heidenreich, S. M. Silver, G. Vilaire, S. Surrey, E. Schwartz & J. S. Bennett: Structure of the platelet membrane glycoprotein IIb. Homology to the alpha subunits of the vitronectin and fibronectin membrane receptors. *J Biol Chem* 262, 8476-8482 (1987)

11. Heidenreich, R., R. Eisman, S. Surrey, K. Delgrosso, J. S. Bennett, E. Schwartz & M. Poncz: Organization of the gene for platelet glycoprotein IIb. *Biochem* 29, 1232-1244 (1990)

12. Fitzgerald, L. A., B. Steiner, S. C. Rall, Jr., S. S. Lo & D. R. Phillips: Protein sequence of endothelial glycoprotein IIIa derived from a cDNA clone. Identity with platelet glycoprotein IIIa and similarity to "integrin". *J Biol Chem* 262, 3936-3939 (1987)

13. Zimrin, A. B., S. Gidwitz, S. Lord, E. Schwartz, J. S. Bennett, G. C. White, II & M. Poncz: The genomic organization of platelet glycoprotein IIIa. *J Biol Chem* 265, 8590-8595 (1990)

14. Duperray, A., A. Troesch, R. Berthier, E. Chagnon, P. Frachet, G. Uzan & G. Marguerie: Biosynthesis and assembly of platelet GPIIb-IIIa in human megakaryocytes: Evidence that assembly between pro-GPIIb and GPIIIa is a prerequisite for expression of the complex on the cell surface. *Blood* 74, 1603-1611 (1989)

15. Calvete, J. J., A. Henschen & J. Gonzalez-Rodriguez: Assignment of disulphide bonds in human platelet GPIIIa. A disulphide pattern for the beta-subunits of the integrin family. *Biochem J* 274, 63-71 (1991)

16. Bennett, J. S. and G. Vilaire: Exposure of platelet fibrinogen receptors by ADP and epinephrine. *J Clin Invest* 64, 1393-1401 (1979)

17. Marguerie, G. A., Ginsberg, M. H., and Plow, E. F.: The platelet fibrinogen receptor. In: Platelets in biology and pathology III. Eds: MacIntyre, D. E., Gordon, J. L., Elsevier, Amsterdam, The Netherlands, 95-125 (1987)

18. Loftus, J. C., E. F. Plow, A. L. Frelinger, III, S. E. D'Souza, D. Dixon, J. Lacy, J. Sorge & M. H. Ginsberg : Molecular cloning and chemical synthesis of a region of platelet glycoprotein IIb involved in adhesive function. *Proc Natl Acad Sci USA* 84, 7114-7118 (1987)

19. D'Souza, S. E., M. H. Ginsberg, S. C.-T. Lam & E. F. Plow: Chemical cross-linking of arginyl-glycyl-aspartic acid peptides to an adhesion receptor on platelets. *J Biol Chem* 263, 3943-3951 (1988)

20. Charo, I. F., L. Nannizzi, D. R. Phillips, M. A. Hsu & R. M. Scarborough: Inhibition of fibrinogen binding to GPIIb-IIIa by a GPIIIa peptide. *J Biol Chem* 266, 1415-1421 (1991)

21. Lanza, F., A. Stierle, D. Fournier, M. Morales, G. Andre, A. T. Nurden & J.-P. Cazenave: A new variant of Glanzmann's thrombasthenia (Strasbourg I). Platelets with functionally defective glycoprotein IIb-IIIa complexes and a glycoprotein IIIa ^{214}Arg-^{214}Trp mutation. *J Clin Invest* 89, 1995-2004 (1992)

22. Frelinger, A. L., III, S. C.-T. Lam, E. F. Plow, M. A. Smith, J. C. Loftus & M. H. Ginsberg: Occupancy of an adhesive glycoprotein receptor modulates expression of an antigenic site involved in cell adhesion. *J Biol Chem* 263, 12397-12402 (1988)

23. Du, X., E. F. Plow , A. L. Frelinger, III, T. E. O'Toole, J. C. Loftus & M. H. Ginsberg: Ligands "activate" integrin $alpha_{IIb}beta_3$ (platelet GPIIb-IIIa). *Cell* 65, 409-416 (1991)

24. Frelinger, A. L., III, X. Du, E. F. Plow & M. H. Ginsberg: Monoclonal antibodies to ligand-occupied conformers of integrin $alpha_{IIb}beta_3$(glycoprotein IIb-IIIa) alter receptor affinity, specificity, and function. *J Biol Chem* 266, 17106-17111 (1991)

25. Shattil, S. J., M. H. Ginsberg & J. S. Brugge: Adhesive signaling in platelets. *Curr Opin Cell Biol* 6, 695-704 (1994)

26. Shattil, S. J. and M. H. Ginsberg: Integrin signaling in vascular biology. *J Clin Invest* 100, 1-5 (1997)

27. Shattil, S. J., J. Gao & H. Kashiwagi: Not just another pretty face: Regulation of platelet function at the cytoplasmic face of integrin alphaIIb beta3. *Thromb Haemostas* 78, 220-225 (1997)

28. O'Toole, T. E., Y. Katagiri, R. J. Faull, K. Peter , R. Tamura, V. Quaranta, J. C. Loftus, S. J. Shattil & M. H. Ginsberg: Integrin cytoplasmic domains mediate inside-out signal transduction. *J Cell Biol* 124, 1047-1059 (1994)

29. Otey, C. A., G. B. Vasquez, K. Burridge & B. W. Erickson: Mapping of the alpha-actinin binding site within the beta1 integrin cytoplasmic domain. *J Biol Chem* 268, 21193-21197 (1993)

30. Schaller, M. D., C. A. Otey, J. D. Hildebrand & J. T. Parsons: Focal adhesion kinase and paxillin bind to peptides mimicking beta integrin cytoplasmic domains. *J Cell Biol* 130, 1181-1187 (1995)

31. Horwitz, A., K. Duggan, C. Buck, M. C. Beckerle & K. Burridge: Interaction of plasma membrane fibronectin receptor with talin- a transmembrane linkage. *Nature* 320, 531-533 (1986)

32. Sharma, C. P., R. M. Ezzell & M. A. Arnaout: Direct interaction of filamin (ABP-280) with the beta 2-integrin subunit CD18. *J Immunol* 154, 3461-3470 (1995)

33. Hannigan, G. E., C. Leung-Hagesteijn, L. Fitz-Gibbon, M. G. Coppolino, G. Radeva, J. Filmus, J. C. Bell & S. Dedhar: Regulation of cell adhesion and anchorage-dependent growth by a new beta 1-integrin-linked protein kinase. *Nature* 379, 91-96

(1996)

34. Shattil, S. J., T. O'Toole, M. Eigenthaler, V. Thon, M. Williams, B. M. Babior & M. H. Ginsberg: Beta3-endonexin, a novel polypeptide that interacts specifically with the cytoplasmic tail of the integrin beta3 subunit. *J Cell Biol* 131, 807-816 (1995)

35. Kolanus, W., W. Nagel, B. Schiller, L. Zeitlmann , S. Godar, H. Stockinger & B. Seed: Alpha L beta 2 integrin/LFA-1 binding to ICAM-1 induced by cytohesin-1, a cytoplasmic regulatory molecule. *Cell* 86, 233-242 (1996)

36. Chang, D. D., C. Wong, H. Smith & J. Liu: ICAP-1, a novel beta 1 integrin cytoplasmic domain-associated protein, binds to a conserved and functionally important NPXY sequence motif of beta 1 integrin. *J Cell Biol* 138, 1149-1157 (1997)

37. Coppolino, M. G., C. Leung-Hagesteijn, S. Dedhar & J. Wilkins: Inducible interaction of integrin alpha2 beta1 with calreticulin. Dependence on the activation state of the integrin. *J Biol Chem* 270, 23132-23138 (1995)

38. Eigenthaler, M., L. Hofferer, S. J. Shattil & M. H. Ginsberg: A conserved sequence motif in the integrin beta3 cytoplasmic domain is required for its specific interaction with beta3-endonexin. *J Biol Chem* 272, 7693-7698 (1997)

39. Savage, B. and Z. M. Ruggeri: Selective recognition of adhesive sites in surface-bound fibrinogen by glycoprotein IIb-IIIa on nonactivated platelets. *J Biol Chem* 266, 11227-11233 (1991)

40. Gartner, T. K., D. L. Amrani, J. M. Derrick, N. E. Kirschbaum, G. R. Matsueda & D. B. Taylor: Characterization of adhesion of "resting" and stimulated platelets to fibrinogen and its fragments. *Thromb Res* 71, 47-60 (1993)

41. Hantgan, R. R., G. Hindriks, R. G. Taylor, J. J. Sixma & P. G. De Groot: Glycoprotein Ib, von Willebrand factor, and glycoprotein IIb:IIIa are all involved in platelet adhesion to fibrin in flowing whole blood. *Blood* 76, 345-353 (1990)

42. Savage, B., S. J. Shattil & Z. M. Ruggeri: Modulation of platelet function through adhesion receptors. A dual role for glycoprotein IIb-IIIa (integrin alphaIIb beta3) mediated by fibrinogen and glycoprotein Ib-von Willebrand factor. *J Biol Chem* 267, 11300-11306 (1992)

43. Ruggeri, Z. M.: Mechanisms initiating platelet thrombus formation. *Thromb Haemostas* 78, 611-616 (1997)

44. Moroi, M., M. Okuma & S. M. Jung: Platelet adhesion to collagen-coated wells: Analysis of this complex process and a comparison with the adhesion to matrigel-coated wells. *Biochim Biophys Acta* 1137, 1-9 (1992)

45. Moroi, M., S. M. Jung, K. Shinmyozu, Y. Tomiyama , A. Ordinas & M. Diaz-Ricart: Analysis of platelet adhesion to a collagen-coated surface under flow conditions: The involvement of glycoprotein VI in the platelet adhesion. *Blood* 88, 2081-2092 (1996)

46. Beumer, S., M. J. W. Ijsseldijk, P. G. De Groot & J. J. Sixma: Platelet adhesion to fibronectin in flow: Dependence on surface concentration and shear rate, role of platelet membrane glycoproteins GP IIb/IIIa and VLA-5, and inhibition by heparin. *Blood* 84, 3724-3733 (1994)

47. Nieuwenhuis, H. K., J. W. N. Akkerman, W. P. M. Houdijk & J. J. Sixma: Human blood platelets showing no response to collagen fail to express surface glycoprotein Ia. *Nature* 318, 470-472 (1985)

48. Kunicki, T. J., D. J. Nugent, S. J. Staats, R. P. Orchekowski, E. A. Wayner & W. G. Carter: The human fibroblast class II extracellular matrix receptor mediates platelet adhesion to collagen and is identical to the platelet glycoprotein Ia-IIa complex. *J Biol Chem* 263, 4516-4519 (1988)

49. Zutter, M. M. and S. A. Santoro: Widespread histologic distribution of the alpha2 beta1 integrin cell-surface collagen receptor. *Am J Pathol* 137, 113-120 (1990)

50. Kehrel, B.: Platelet receptors for collagens. *Platelets* 6, 11-16 (1995)

51. Santoro, S. A., Saelman, E. U. M., and Zutter, M. M. The alpha2 beta 1 integrin: Structure, function, and regulation of a platelet surface collagen receptor. In: Advances in molecular and cell biology. Eds: Bittar, E. E. , Lapetina, E. G., JAI Press Inc., Greenwich, CT, 18, 67-108 (1997)

52. Moroi, M. and S. M. Jung: Platelet receptors for collagen. *Thromb Haemostas* 78, 439-444 (1997)

53. Santoro, S. A. and M. M. Zutter: The alpha2 beta1 integrin: A collagen receptor on platelets and other cells. *Thromb Haemostas* 74, 813-821 (1995)

54. Argraves, W. S., S. Suzuki, H. Arai, K. Thompson, M. D. Pierschbacher & E. Ruoslahti: Amino acid sequence of the human fibronectin receptor. *J Cell Biol* 105, 1183-1190 (1987)

55. Takada, Y. and M. E. Hemler: The primary structure of the VLA-2/collagen receptor alpha2 subunit (platelet GPIa): Homology to other integrins and the presence of a possible collgen-binding domain. *J Cell Biol* 109, 397-407 (1989)

56. Kamata, T., W. Puzon & Y. Takada: Identification of putative ligand binding sites within I domain of integrin alpha2 beta1 (VLA-2, CD49b/CD29). *J Biol Chem* 269, 9659-9663 (1994)

57. Tuckwell, D. S., K. B. M. Reid, M. J. Barnes & M. J. Humphries: The A-domain of integrin alpha2 binds specifically to a range of collagens but is not a general receptor for the collagenous motif. *Eur J Biochem* 241, 732-739 (1996)

58. Emsley, J., S. L. King, J. M. Bergelson & R. C. Liddington: Crystal structure of the I domain from integrin alpha2 beta1. *J*

Biol Chem 272, 28512-28517 (1997)

59. Santoro, S. A.: Identification of a 160,000 dalton platelet membrane protein that mediates the initial divalent cation-dependent adhesion of platelets to collagen. *Cell* 46, 913-920 (1986)

60. Takada, Y. and W. Puzon: Identification of a regulatory region of integrin beta1 subunit using activating and inhibiting antibodies. *J Biol Chem* 268, 17597-17601 (1993)

61. Gibbins, J., J. Asselin, R. Farndale, M. Barnes, C.-K. Law & S. P. Watson: Tyrosine phosphorylation of the Fc receptor gamma-chain in collagen-stimulated platelets. *J Biol Chem* 271, 18095-18099 (1996)

62. Tsuchida, J., S. Ueki, Y. Saito & J. Takagi: Classification of 'activation' antibodies against integrin beta1 chain. *FEBS Lett* 416, 212-216 (1997)

63. Jung, S. M. and M. Moroi: Platelets interact with soluble and insoluble collagens through characteristically different reactions. *J Biol Chem* 273, 14827-14837 (1998)

64. Saelman, E. U. M., H. K. Nieuwenhuis, K. M. Hese, P. G. De Groot, H. F. G. Heijnen, E. H. Sage, S. Williams, L. McKeown, H. R. Gralnick & J. J. Sixma: Platelet adhesion to collagen types I through VIII under conditions of stasis and flow is mediated by GP Ia/IIa (alpha2 beta1-integrin). *Blood* 83, 1244-1250 (1994)

65. Sixma, J. J., G. H. van Zanten, E. U. M. Saelman, M. Verkleij, H. Lankhof, H. K. Nieuwenhuis & P. G. De Groot: Platelet adhesion to collagen. *Thromb Haemostas* 74, 454-459 (1995)

66. Staatz, W. D., K. F. Fok, M. M. Zutter, S. P. Adams, B. A. Rodriguez & S. A. Santoro: Identification of a tetrapeptide recognition sequence for the $alpha_2beta_1$ integrin in collagen. *J Biol Chem* 266, 7363-7367 (1991)

67. Morton, L. F., A. R. Peachey, C. G. Knight, R. W. Farndale & M. J. Barnes: The platelet reactivity of synthetic peptides based on the collagen III fragment alpha1(III)CB4. Evidence for an integrin alpha2 beta1 recognition site involving residues 522-528 of the alpha1(III) collagen chain. *J Biol Chem* 272, 11044-11048 (1997)

68. Glattauer, V., J. A. Werkmeister, A. Kirkpatrick & J. A. M. Ramshaw: Identification of the epitope for a monoclonal antibody that blocks platelet aggregation induced by type III collagen . *Biochem J* 323, 45-49 (1997)

69. Coller, B. S.: Interaction of normal, thrombasthenic, and Bernard-Soulier platelets with immobilized fibrinogen: defective platelet-fibrinogen interaction in thrombasthenia. *Blood* 55, 169-178 (1980)

70. Jirouskova, M., J. E. Dyr, J. Suttnar, K. Holada & B. Trnkova: Platelet adhesion to fibrinogen, fibrin monomer, and fibrin protofibrils in flowing blood- The effect of fibrinogen immobilization and fibrin formation. *Thromb Haemostas* 78, 1125-1131 (1997)

71. Zaidi, T. N., L. V. McIntire, D. H. Farrell & P. Thiagarajan: Adhesion of platelets to surface-bound fibrinogen under flow. *Blood* 88, 2967-2972 (1996)

72. Savage, B., E. Saldivar & Z. M. Ruggeri: Initiation of platelet adhesion by arrest onto fibrinogen or translocation on von Willebrand factor. *Cell* 84, 289-297 (1996)

73. Moroi, M., S. M. Jung, M. Okuma & K. Shinmyozu: A patient with platelets deficient in glycoprotein VI that lack both collagen-induced aggregation and adhesion. *J Clin Invest* 84, 1440-1445 (1989)

74. Tandon, N. N., U. Kralisz & G. A. Jamieson: Identification of glycoprotein IV (CD36) as a primary receptor for platelet-collagen adhesion. *J Biol Chem* 264, 7576-7583 (1989)

75. Yamamoto, N., N. Akamatsu, H. Yamazaki & K. Tanoue: Normal aggregation of GPIV (CD36)-deficient platelets from seven healthy Japanese donors. *Br J Haematol* 81, 86-91 (1992)

76. Saelman, E. U. M., B. Kehrel, K. M. Hese, P. G. De Groot, J. J. Sixma & H. K. Nieuwenhuis: Platelet adhesion to collagen and endothelial cell matrix under flow conditions is not dependent on platelet glycoprotein IV . *Blood* 83, 3240-3244 (1994)

77. Hubbell, J. A. and L. V. McIntire: Visualization and analysis of mural thrombogenesis on collagen, polyurethane and nylon. *Biomaterials* 7, 354-363 (1986)

78. Alevriadou, B. R., J. L. Moake, N. A. Turner, Z. M. Ruggeri, B. J. Folie, M. D. Phillips, A. B. Schreiber, M. E. Hrinda & L. V. McIntire: Real-time analysis of shear-dependent thrombus formation and its blockade by inhibitors of von Willebrand factor binding to platelets. *Blood* 81, 1263-1276 (1993)

79. Sakariassen, K. S., P. A. M. M. Aarts, P. G. De Groot, W. P. M. Houdijk & J. J. Sixma: A perfusion chamber developed to investigate platelet interaction in flowing blood with human vessel wall cells, their extracellular matrix, and purified components. *J Lab Clin Med* 102, 522-535 (1983)

80. Houdijk, W. P. M., K. S. Sakariassen, P. F. E. M. Nievelstein & J. J. Sixma: Role of factor VIII-von Willebrand factor and fibronectin in the interaction of platelets in flowing blood with monomeric and fibrillar human collagen types I and III. *J Clin Invest* 75, 531-540 (1985)

81. Wu, Y.-P., H. H. F. I. Van Breugel, H. Lankhof, R. J. Wise, R. I. Handin, P. G. De Groot & J. J. Sixma: Platelet adhesion to multimeric and dimeric von Willebrand factor and to collagen type III preincubated with von Willebrand factor. *Arterioscler Thromb Vasc Biol* 16, 611-620 (1996)

82. Moroi, M., S. M. Jung, S. Nomura, S. Sekiguchi, A. Ordinas & M. Diaz-Ricart: Analysis of the involvement of the von Willebrand factor-glycoprotein Ib interaction in platelet adhesion

to a collagen-coated surface under flow conditions. *Blood* 90, 4413-4424 (1997)

83. Roth, G. J., K. Titani, L. W. Hoyer & M. J. Hickey: Localization of binding sites within human von Willebrand factor for monomeric type III collagen. *Biochemistry* 25, 8357-8361 (1986)

84. Nieuwenhuis, H. K., K. S. Sakariassen, W. P. M. Houdijk, P. F. E. M. Nievelstein & J. J. Sixma: Deficiency of platelet membrane glycoprotein Ia associated with a decreased platelet adhesion to subendothelium: A defect in platelet spreading. *Blood* 68, 692-695 (1986)

85. Goto, S., D. R. Salomon, Y. Ikeda & Z. M. Ruggeri: Characterization of the unique mechanism mediating the shear-dependent binding of soluble von Willebrand factor to platelets. *J Biol Chem* 270, 23352-23361 (1995)

86. Piotrowicz, R. S., R. P. Orchekowski, D. J. Nugent, K. Y. Yamada & T. J. Kunicki: Glycoprotein Ic-IIa functions as an activation-independent fibronectin receptor on human platelets. *J Cell Biol* 106, 1359-1364 (1988)

87. Gardner, J. M. and R. O. Hynes: Interaction of fibronectin with its receptor on platelets. *Cell* 42, 439-448 (1985)

88. Beumer, S., H. F. G. Heijnen, M. J. W. Ijsseldijk, E. Orlando, P. G. De Groot & J. J. Sixma: Platelet adhesion to fibronectin in flow: The importance of von Willebrand factor and glycoprotein Ib. *Blood* 86, 3452-3460 (1995)

89. Konstantopoulos, K. and L. V. McIntire: Effects of fluid dynamic forces on vascular cell adhesion. *J Clin Invest* 98, 2661-2665 (1996)

90. Lasky, L. A.: Selectins: Interpreters of cell-specific carbohydrate information during inflammation. *Science* 258, 964-969 (1992)

91. Hammer, D. A. and S. M. Apte: Stimulation of cell rolling and adhesion on surfaces in shear flow: General results and analysis of selectin-mediated neutrophil adhesion. *Biophys J* 63, 35-57 (1992)

92. Tozeren, A. and K. Ley: How do selectins mediate leukocyte rolling in venules? *Biophys J* 63, 700-709 (1992)

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