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COMMENTARY

PATHOPHYSIOLOGICAL SIGNIFICANCE OF INTEGRIN EXPRESSION BY VASCULAR ENDOTHELIAL CELLS

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Endothelium is a monolayer of cells in close opposition, lining all blood vessels, the endocardium and lymph vessels. As a uniquely positioned blood-tissue barrier, the endothelial lining interacts with cellular and soluble components of the blood as well as with the underlying subendothelial tissue. In this strategic location the behavior of endothelial cells can be a critical determinant in pathophysiological processes such as inflammation, thrombosis, atherosclerosis and metastatic spread of neoplastic cells. In this respect, adhesion of endothelial cells to extracellular matrix proteins is one of the processes that is important for the maintenance of the integrity of the vessel wall, for repair of vascular injury, and for initiating and controlling the formation of new vessels.

Recently a number of molecules have been identified, exposed at the outer surface of the plasma membrane of endothelial cells, that serve an important role in mediating the anchorage of these cells to extracellular matrix proteins. These molecules have in common that they serve as a recognition site for divalent or multivalent proteins, such as fibronectin, collagen and fibrinogen, containing the tripeptide arginine-glycine-aspartic acid (RGD). Immunochemical studies, protein structure and amino acid sequence data derived from cDNA clones revealed that these functionally related molecules, in fact, comprise a family of widely distributed cell-surface receptors, now generally named "integrins" [1], involved in cell–cell and cell–substrate interactions [1–4]. A major contribution to our knowledge of the structure and function of these endothelial cell surface receptors stemmed from the observation that vascular endothelial cells synthesize a plasma membrane protein that is immunochemically and biochemically closely related to the platelet glycoprotein (GP) IIb/IIIa complex [5, 6]. Platelet GP IIb/IIIa, an abundant membrane protein involved in platelet–platelet interaction and platelet adhesion and spreading to the subendothelium, has been the subject of numerous studies, and its mode of action has been described in great detail [7]. This knowledge, then, provided a firm basis for further studies on the biology of related receptor molecules expressed by vascular endothelial cells and also led to the identification of several other distinct members of the integrin family shared by platelets and endothelial cells [8].

Although the structure and function of integrins produced by a variety of cell types have now been described in detail and their similarities in terms of structure and mode of action have been appreciated, insight into the pathophysiology of disorders associated with molecular defects or structural abnormalities of integrins is limited. For instance, structural defects in certain platelet integrins may lead to severe platelet dysfunction and, consequently, a severe bleeding diathesis [9]. In view of the structural and functional similarities between integrins expressed by different cell types, it is conceivable that these defects are not only restricted to platelets but may also involve the endothelium. Similarly, platelet integrins may carry alloantigens that under certain conditions may give rise to an immunogenic response and, as a result, antibody-mediated thrombocytopenia or platelet dysfunction [10]. If integrin-associated alloantigens are also expressed by endothelial cells, in particular when these integrins are exposed at the apical surface of the endothelium, platelet-associated alloimmune disorders could be associated with endothelial alloimmune disorders. In this commentary, we wish to provide a picture of our current knowledge of the polar expression and heterogeneity of endothelial cell adhesion receptors with special reference to structural variations and functional abnormalities.

Structural and functional properties of endothelial cell integrins

Integrins comprise a family of structurally homologous surface receptors, consisting of non-covalently associated α- and β-subunits, that serve a role in controlling the anchorage of cells to their extracellular matrices. The integrins identified so far are putative transmembrane glycoprotein complexes, connected via the carboxy-terminal end of either the α-chain or the β-chain (or both) with the cytoskeleton. On the basis of the homology of the β-chains, three subfamilies of surface receptors have been identified. All members within each integrin subfamily are constructed of identical β-subunits but distinct, although homologous, α-chains [1–4, 7]. Two of the subfamilies have been localized on endo-

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Table 1. Related integrins on platelets and endothelial cells

	Endothelial cells		Platelets		Ligands
Subfamily	Member	Subunits	Member	Subunits	
VLA	VLA-2	$\alpha 2/\beta 1$	GPIa/IIa	$\alpha 2/\beta 1$	Collagens
	VLA-5	$\alpha 5/\beta 1$	GPIc*/IIa	$\alpha 5/\beta 1$	Fibronectin
	VLA-6	$\alpha 6/\beta 1$	GP1c/IIa	$\alpha 6/\beta 1$	Laminin
Cytoadhesin	Vitronectin receptor	$\alpha V/\beta 3$	GPIIb/IIIa Vitronectin receptor	$\alpha IIb/\beta 3$ $\alpha V/\beta 3$	Fibrinogen Fibronectin Vitronectin von Willebrand factor

* The $\alpha 5$- and $\alpha 6$-subunits on platelets differ slightly in electrophoretic mobility.

thelial cells and blood platelets: the VLA subfamily and the cytoadhesins (Table 1). Thus far, four members of these subfamilies have been identified on endothelial cells. Three of them belong to the VLA subfamily. The other integrin, the vitronectin receptor, is a member of the cytoadhesin subfamily. These integrins are also expressed by a variety of other cell types, including fibroblasts, epithelial cells, and also blood platelets [1–4, 7, 11]. Several members of the VLA subfamily, but not the cytoadhesins, are expressed by leukocytes as well.

Several moelcules that may serve as a ligand for platelet GP IIb/IIIa have been identified, including von Willebrand factor, fibrinogen and fibronectin [7], all glycoproteins that are constructed of two or more identical or very similar constituent units containing divalent or multivalent receptor binding sites. The kinetics and biochemistry of the ligand–receptor interaction have been studied in detail. Also, the structurally closely related endothelial vitronectin receptor may bind these molecules, as suggested by the observations that endothelial cells readily adhere and spread on substrates coated with von Willebrand factor, fibrinogen or vitronectin [12]. These interactions may be quenched with antibodies to the vitronectin receptor, suggesting that indeed this receptor serves an adhesive function mediated by these ligands. From these studies it seems clear that endothelial cells tend to organize themselves as a sheet of polarized cells that express the vitronectin receptor at the basolateral side, and, in this location may mediate and promote adhesion, spreading and migration of the endothelium.

A point of interest is whether the vitronectin receptor is also exposed at the lumenal side of the endothelium and, as such, serves as a recognition site for circulating blood components or endothelial proteins exocytosed to the lumenal surface of the cell, such as von Willebrand factor or fibronectin. Thus far, there is only limited evidence that the vitronectin receptor is also exposed at the lumenal side of the endothelium. Fibrinogen specifically binds to apparently intact, confluent monolayers of cultured endothelial cells [13]. However, it is difficult to distinguish between cell-mediated binding and binding of fibrinogen to the extracellular matrix. Similarly, immunofluorescent studies using non-permeabilized endothelial cells suggests that, in addition to basolateral binding sites, fibrinogen also binds to the apical cell surface [14]. Recent studies on the identification of the fibrinogen receptor on endothelial cells indicate that the vitronectin receptor is involved in the fibrinogen binding [15]. Antibodies to platelet GP IIb/IIIa and RGD containing peptides inhibit fibrinogen binding to endothelial cells, suggesting that indeed the vitronectin receptor is involved in fibrinogen–endothelial cell interaction. Taking into account that the plasma fibrinogen concentration (about 1 μM) is about ten times higher than the estimated binding constant (approximately 0.1 μM), it is tempting to speculate that the vascular lining is covered with a monomolecular film of fibrinogen which may effectively contribute to hemostatic plug formation upon vascular damage.

It has also been reported that the von Willebrand factor, another ligand of both the vitronectin receptor and platelet GP IIb/IIIa, accumulates at the cell surface when endothelial cells are stimulated to release this hemostatic protein from their intracellular storage vesicles, the Weibel–Palade bodies [16]. Previously, we have shown that the von Willebrand factor, secreted after stimulation with phorbol myristate acetate, predominantly accumulates at the apical side of monolayers of endothelial cells [17]. It is tempting to speculate that this pool of cellular von Willebrand factor may accumulate at the lumenal cell surface through the vitronectin receptor and, as such, acts as an effective hemostatic agent upon vascular damage. Further studies should reveal whether this hypothesis is correct.

It should be noted that studies on the binding of ligands, such as fibrinogen, to the vitronectin receptor have been performed with apparently resting endothelial cells. Thus far, it is assumed that, unlike platelet GP IIb/IIIa, the function of the vitronectin receptor is not regulated by stimulation of the cell. Similar to platelets, the endothelial cell is a secretory cell and rapidly responds to agonists such as thrombin [18, 19] which together with von Willebrand factor secretion may induce perturbation of the plasma membrane [20]. It is possible, therefore, that activation of endothelial cells also alters the affinity of the vitronectin receptor, and in this way provides a regulatory mechanism of cell–ligand interaction.

Another point of interest is the role of vitronectin (S-protein) as a ligand for the vitronectin receptor. Previously it has been shown that, similar to fibrinogen and von Willebrand factor, vitronectin, an abundant plasma protein, also promotes adhesion and spreading of endothelial cells to surfaces coated with

this ligand [12]. Therefore, vitronectin also may serve an important role in controlling the anchorage and repair of the endothelium upon vascular damage. As the plasma concentration of vitronectin is relatively high (about 4 μM), it might be expected that, similar to fibrinogen, vitronectin may also be present in complexed form at the lumenal surface of the endothelium. If so, this could be of particular physiological significance. It has been shown recently that vitronectin may serve as a carrier protein for the endothelial-type plasminogen activator inhibitor (PAI-1) [21, 22]. PAI-1 is a serine protease inhibitor, produced by endothelial cells and other cell types, that rapidly decays after release from the cell [23]. When bound to vitronectin, however, PAI-1 is much more stable [21]. Also, vitronectin associated with the extracellular matrix is able to bind and stabilize PAI-1 [22]. As such, vitronectin, and indirectly its membrane receptor, may confer antifibrinolytic activity to the endothelial lining and the subendothelial matrix. Indeed, it has been shown that cultured endothelial cells contain a storage pool of active PAI-1, associated with the plasma membrane of these cells, which is readily accessible to exogenous plasminogen activators such as t-PA or urokinase [24]. It seems possible, therefore, that the vitronectin receptor serves more biological functions than acting as a protein that mediates cell–matrix interactions.

Microheterogeneity of platelet and endothelial cell integrins

At the surface of the platelet membrane genetically determined structures are expressed that may give rise to alloimmunization of otherwise genetically normal individuals, for instance by blood transfusion or pregnancy [10]. These allotypic determinants or alloantigens include antigens such as blood group- and HLA-antigens, that are shared with other blood cells and tissue cells. In addition, platelets express alloantigens which were thought to be platelet specific, i.e. are not expressed by other cell types. Several different antigen systems have been identified that belong to the latter class of platelet-specific alloantigens (see Table 2). All the systems so far described are biallelic with a high and a low frequency allele [25].

Advances in the ability to characterize platelet membrane constituents have led to identification of the relationship between certain of these platelet-specific alloantigens and platelet membrane glycoproteins. Of major importance to these studies was the demonstration that platelets from patients with Glanzmann's thrombasthenia, which are deficient in GP IIb and IIIa, were found to be lacking the PL^{A1} or Zw^a antigen [26, 27]. Data indicating that the PL^{A1} or Zw^a antigen resides on GP IIIa represented the initial assignment of a platelet-specific antigen to a discrete membrane glycoprotein. This approach, using platelets with distinct abnormalities of membrane constituents and techniques that permit adequate separation of membrane proteins, has led to the identification of the proteins that carry the antigens.

The majority of the platelet-specific alloantigens identified thus far appeared to be located on platelet integrins, including the GP IIb/IIIa and the GP Ia/IIa (VLA-2) complex (Table 2). The demonstration that certain alloantigens are carried by integrins and the discovery of the structural homology of the platelet integrins with endothelial cell adhesion receptors led to the observation that endothelial cells also express platelet alloantigens [30, 31]. Immunoprecipitation and immunoblotting experiments revealed that only the alloantigens that are carried by the β-chain of the platelet integrin (e.g. GP IIIa) are also carried by the endothelial counterpart (e.g. the β-chain of the vitronectin receptor). Similarly, other cell types, such as smooth muscle cells and fibroblasts, express alloantigens associated with the β-chain of their cytoadhesin [32]. As the respective β-chains of these receptor molecules are structurally closely related, if not identical, this is not an unexpected finding, although it took almost 30 years after the discovery of platelet-specific alloantigens [33, 34] before it was appreciated that the previously accepted view of the platelet specificity was no longer tenable. Alloantigens, known to reside on platelet GP IIb (e.g. Bak^a or Lek^a), were not found on endothelial cells [31], probably because of substantial structural differences between platelet GP IIb and the α-chain of the vitronectin receptor.

Table 2. Platelet specific alloantigens

Antigen	Antigen location		Frequency (%)
	Platelets	Endothelial cells	
Zw^a or PL^{A1}	GP IIa	β3*	97.0
Zw^b or PL^{A2}			27.0
Bak^a or Lek^a	GP IIb	ND†	91.0
Yuk^a or Pen^a	GP IIIa	β3	1.7
Yuk^b or Pen^b			99.9
Br^a	GP Ia or IIa	α2 or β1	20.0
Br^b			99.0
PL^{E1}	GP Ib‡		99.9
PL^{E2}			5.0

* Also detected on fibroblasts and smooth muscle cells.
† Not detectable.
‡ Endothelial cells produce a membrane protein immunologically closely related to platelet GP Ib [28], a surface receptor that mediates the adhesion of platelets to the vessel wall but shows no structural homology with the integrins [29].

Taken together, it is clear now that cytoadhesins expressed by various cell types, including endothelial cells, fibroblasts and smooth muscle cells, share distinct genetic markers of the α-chain of the cytoadhesins such as the Zw and Yuk system. Preliminary studies conducted in our laboratory indicate that an alloantigen carried by the platelet GPIa/IIa (or VLA-2) complex, the so-called Br-antigen, is expressed by endothelial cells as well. Thus, not only the cytoadhesins but also other subfamilies of the integrins are polymorphic in nature. Similarly, an antigenic polymorphism of the α-chain of the LFA subfamily has been demonstrated [35].

The structural variability of integrins is also of

clinical significance. Zw^a or PL^{A1} is of special importance clinically because of its high antigenicity and its role in causing, in most instances, platelet disorders caused by alloimmunization, such as neonatal alloimmune thrombocytopenia (NAIT) and post-transfusion purpura (PTP). NAIT results from alloimmunization of the mother against an antigen on the platelets of her fetus. Alloantibodies pass the placenta and destroy the fetal platelets and/or impair their function. In most cases, NAIT is caused by Zw^a (or PL^{A1}) incompatibility [10, 25]. In Caucasians 2.4% of the women are Zw^a negative and their chance of having an incompatible fetus is about 1% [25]. However, probably because of its apparent association with some antigens in the HLA-system, notably HLA-DR3 [36], the incidence of NAIT is lower than expected (1 per 2000 births). In Japan, where Zw^a incompatibility is rare (most if not all Japaneses are Zw^a positive), Yuk^b incompatibility is an important cause of NAIT. Similarly, PTP, a rare but also a potentially severe and life-threatening reaction to transfusion with blood or platelets, is, in almost all of the proven cases, caused by Zw^a-alloimmunization. Usually the patient is Zw^a-negative and strong antibodies to Zw^a, probably resulting from an anamnestic response due to exposure to the same sensitizing antigen, sometimes years prior to the inciting transfusion, may be detected in the serum. Similar to NAIT, this alloimmune disorder seems to be associated with HLA-DR3.

From the clinical and serological data reported so far, it seems clear that at least the Zw^a-antigen is of pathogenetic importance in alloimmune disorders. As endothelial cells, fibroblasts, smooth muscle and probably other cell types as well express Zw^a [30–32], it is conceivable that not only platelets but also these cells are involved in these disorders. In particular, endothelial cells, which are directly exposed to the blood, may be involved. Taking into account that the vitronectin receptor, the membrane receptor that carries the Zw-antigens, is also exposed at the lumenal side of the endothelium [14], this is not unlikely. Antibodies to Zw^a may interfere with endothelial cell function, as has been demonstrated with platelets [37], or may cause endothelial damage (vasculitis), similar to anti-endothelial cell antibodies in autoimmune diseases such as systemic lupus erythematosus (SLE) which may cause cell lysis [38]. Also, if platelet and endothelial alloantigens do not properly match, alloimmunization may contribute to allograft rejection. Thus far, there is no evidence in support of this view. However, it is not unlikely, that because of unfamiliarity with the apparently widespread polymorphism of the integrins, clinicians may have overlooked the pathophysiological significance of alloimmune disorders apparently restricted to only one cell type (i.e. platelets).

Also, in auto-immune thrombocytopenia a significant number of antibodies is directed against antigenic determinants on the GP IIb/IIIa complex, although these determinants apparently differ from Zw antigens [39]. If these antibodies also cross-react with the vitronectin receptor on endothelial cells, they may affect endothelial cell function as well and may contribute to the bleeding diathesis in patients with autoimmune thrombocytopenia.

Molecular basis of integrin abnormalities

From several studies it seems clear that the α- and β-subunits of the integrins are encoded by separate genes and are post-translationally processed and assembled to mature complexes before these molecules are exocytosed and inserted into the plasma membrane [40, 41]. The knowledge that the α- and β-polypeptides are biosynthesized as separate molecules which only after association into complexes are transported to the cell surface, may provide a clue to our understanding of molecular defects underlying deficiencies of the expression of these adhesin receptor molecules. Thus far, the deficiencies of one entire subfamily of the integrins and two individual members belonging to distinct subfamilies have been reported. The well-known and thus far only documented subfamily deficiency is the leukocyte adhesion deficiency (LAD), a human genetic disease with serious defects in many leukocyte functions [42]. In this disorder, the common β-chain of the three heterodimeric receptors belonging to the leucocyte adhesin receptor subfamily is missing, leading to the absence of all three receptor molecules on leukocyte surfaces. From these observations stemmed the generally accepted view, supported by substantial experimental evidence, that the surface expression of the integrin is governed, at least in part, by a correct intracellular assembly of the constituent units of the receptor. This hypothesis explains why in deficiency of the common β-chain all three receptor molecules on leukocyte surfaces are absent.

Despite structural homologies between the β-chains of the various integrin subfamilies, no cell types other than lymphocytes, polymorphonuclear cells and monocytes/macrophages are deficient in integrin expression in LAD. This observation suggests that the genetic defect in this disorder is most likely due to a small change in the nucleotide sequence of the coding region of unique peptide domains on the β2-chain or a defect restricted to the regulatory region of the gene encoding the β2-subunit, and not that of the β-chain of other integrins. The latter view is supported by the observation that the cellular distribution of the leukocyte adhesion receptor subfamily is restricted to leucocytes only.

Also, in Glanzmann's thrombasthenia, an autosomal recessive bleeding disorder most commonly characterized by a markedly reduced expression of the GP IIb/IIIa complex [9], deficiency of this cytoadhesin may be due to defective synthesis of either GP IIb or GP IIIa or both. As GP IIb and IIIa are encoded by genes which, although separate, are in close proximity (chromosome 17) [43, 44], it is difficult to judge whether one of the two subunits of the receptor could be held responsible for the defect or if a defect in regulatory elements controlling the synthesis of both subunits underlies the deficiency. Recent studies on the analysis of the genomic DNA obtained from several related and unrelated patients with Glanzmann's thrombasthenia suggested that the structural genes coding for GP IIb and GP IIIa did not contain major deletions or insertions [45]. Rather, these studies suggested that the deficiency is due to a defect in regulatory regions of the genes

encoding either GP IIb or GP IIIa or both. Indeed, in most cases of Glanzmann's thrombasthenia, reduced but detectable amounts of apparently normal GP IIb/IIIa are detected.

Studies conducted in our laboratory suggest that in Glanzmann's thrombasthenia the defect is primarily due to defective synthesis of the α-subunit (GP IIb) of the surface receptor responsible for adequate platelet function. Endothelial cells isolated from the umbilical cord vein of a newborn with Glanzmann's thrombasthenia and severely reduced amounts of GP IIb/IIIa on his platelets, synthesized and expressed normal amounts of the α- and β-subunits of the vitronectin receptor [46]. Similarly, smooth muscle cells isolated from the umbilical cord arteries of this patient were not defective in synthesis and expression of this receptor [32]. In view of the close homology of the β-subunits of the cytoadhesins, it seems most likely then that in this patient the defect is caused by defective synthesis of the α-subunit (GP IIb) of the GP IIb/IIIa complex and not of the β-subunit. Taking into account that a number of adherent cell types, including endothelial cells, smooth muscle cells and fibroblasts express adhesion receptor molecules which are under the same genetic control and are structurally closely related to the GP IIb/IIIa complex, it is conceivable that defective synthesis of the common β-subunit would lead to defective expression of this integrin subclass on all of these cell types, a condition which is probably incompatible with life. The accumulated data so far suggest that Glanzmann's thrombasthenia is caused by an isolated defect of the synthesis of GP IIb (α-subunit); related molecules are normally produced by other cell types. In Fig. 1, the biosynthesis and assembly of cytoadhesins are schematically depicted. This model provides a possible rationale of the isolated deficiency of GP IIb/IIIa in Glanzmann's thrombasthenia. More detailed studies on the DNA nucleotide sequence of the coding region could reveal whether this view is correct. Recently, variants of Glanzmann's thrombasthenia have been reported in which GP IIb/IIIa is normally present but appears to be functionally defective [48]. Apparently, structurally defective subunits, either GP IIb or GP IIIa (or both), may associate but do not function properly (e.g. defective ligand binding). Whether these defects are also apparent at the endothelial cell level is not known. Again, studies at the cDNA or mRNA level could contribute to our understanding of abnormalities both at the cellular and molecular levels.

Recently, a patient with a bleeding diathesis was described [49], characterized by defective collagen-induced platelet aggregation and adhesion to surfaces coated with collagen type III, and markedly reduced amounts of GP Ia, the α-subunit of the VLA-2 complex (Table 1). VLA-2 serves as a receptor for various types of collagens [50] and explains why the platelets of the patient deficient in the α-subunit are unresponsive to collagen. It is not clear from this report whether GP IIa (the β-subunit of VLA-2) is markedly reduced as well, as would be expected from the view that proper intracellular assembly of the receptor is a prerequisite for cell-surface expression of both subunits [51]. It is not known whether this disorder is restricted to platelets or whether other cell types which produce VLA-2, including endothelial cells, are affected as well. We have found recently that cultured endothelial cells express a VLA-receptor that is very similar, if not identical, to the VLA-2 complex of platelets [52]. On the basis of these findings one might expect that the surface expression of VLA-2 by endothelial cells is also defective in this patient.

Concluding remarks

A large body of information on the structure and function of endothelial cell integrins stemmed from studies on platelet integrins. Platelets offer an abundant source of at least five members of the integrin family that are structurally and functionally similar to integrins expressed by endothelial cells. The

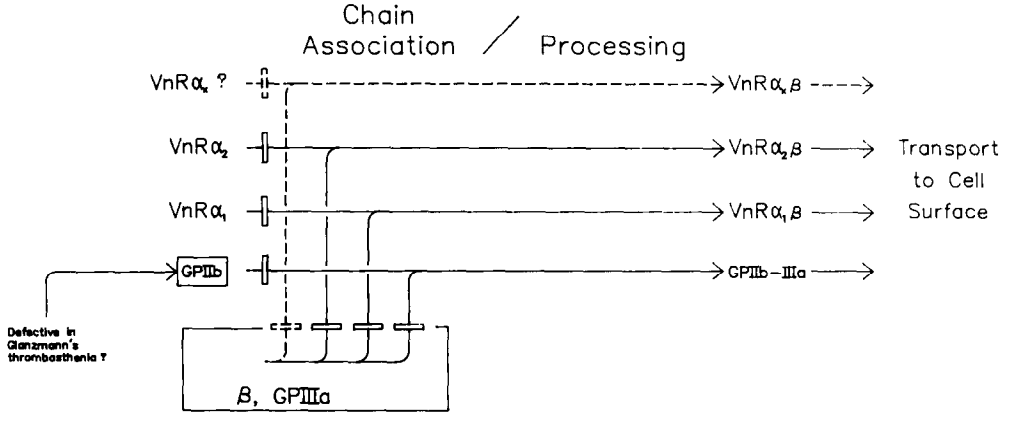

Fig. 1. Biosynthesis of the cytoadhesin family. In normal cells (e.g. endothelial cells, megakaryocytes), the precursors for each subunit are synthesized separately, become non-covalently associated in α/β complexes (GP IIb/IIIa, vitronectin receptor) and are transported to the plasma membrane. So far two structurally different α-subunits have been identified (GP IIb and VnRα or αV). This model, adapted from a model that describes the molecular defects of leucocyte adhesion deficiency [47], also provides for the synthesis of other as yet unidentified α-subunits. Deficiency on the biosynthesis of an α-chain (e.g. GP IIb) leads to an isolated deficiency of the corresponding α/β complex (e.g. GP IIb/IIIa).

nature and biological regulation of the functional state of a number of these platelet receptors have been studied intensively, and it is anticipated that platelet studies will further contribute to our understanding of how endothelial cell integrins act at the molecular level. Because of structural and functional similarities between platelet and endothelial integrins, genetically determined- or antibody-mediated dysfunction of platelet integrins may be of broader clinical significance than was previously anticipated.

REFERENCES

1. Hynes RD, A family of cell surface receptors. *Cell* **48**: 549–554, 1987.
2. Ruoslahti E and Pierschbacher MD, New perspectives in cell adhesion: RGD and integrins. *Science* **238**: 491–497, 1987.
3. Hemler ME, Adhesive protein receptors on hematopoietic cells. *Immunol Today* **9**: 109–113, 1988.
4. Ginsberg MH, Loftus JC and Plow EF, Cytoadhesins, integrins, and platelets. *Thromb Haemost* **59**: 1–12, 1988.
5. Fitzgerald LA, Charo IF and Phillips DR, Human endothelial cells synthesize membrane glycoproteins similar to platelet membrane GP IIb and GP IIIa. *J Biol Chem* **260**: 10893–10896, 1985.
6. Leeksma OC, Zandbergen-Spaargaren J, Giltay JC and van Mourik JA, Cultured human endothelial cells synthesize a plasma membrane protein complex immunologically related to the platelet glycoprotein IIb/IIIa complex. *Blood* **67**: 1176–1180, 1986.
7. Phillips DR, Charo IF, Parise LV and Fitzgerald LA, The platelet membrane glycoprotein IIb–IIIa complex. *Blood* **71**: 831–843, 1988.
8. Giltay JC and van Mourik JA, Structure and function of endothelial cell integrins. *Haemostasis* **18**: 376–389, 1988.
9. Clemetson KJ and Lüscher EF, Membrane glycoprotein abnormalities in pathological platelets. *Biochim Biophys Acta* **947**: 53–73, 1988.
10. Shulman NR and Jordan JV Jr, Platelet immunology. In: *Hemostasis and Thrombosis, Basic Principles and Clinical Practice* (Eds. Colman RW, Hirsh J, Marder VJ and Salzman EW), 2nd Edn, pp. 452–529. JB Lippincott, Philadelphia, 1987.
11. Lam SC-T, Plow EF, D'Souza SE, Cheresh DA, Frelinger AL III and Ginsberg MH, Isolation and characterization of a platelet membrane protein related to the vitronectin receptor. *J Biol Chem* **264**: 3742–3749, 1989.
12. Charo IF, Bekeart LS and Phillips DR, Platelet glycoprotein IIb–IIa-like proteins mediate endothelial cell attachment to adhesive proteins and the extracellular matrix. *J Biol Chem* **262**: 9935–9938, 1987.
13. Dejana E, Languino LR, Polentarutti N, Balconi G, Ryckewaert JJ, Larrieu MJ, Donati MB, Mantovani A and Marguerie G, Interaction between fibrinogen and cultured endothelial cells: induction of migration and specific binding. *J Clin Invest* **75**: 11–18, 1985.
14. Dejana E, Languino LR, Colella S, Corbascio GC, Plow E, Ginsberg M and Marchisio PC, The localization of a platelet GP IIb–IIIa-related protein in endothelial cell adhesion structures. *Blood* **71**: 566–572, 1988.
15. Languino LR, Colella S, Zanetti A, Andrieux A, Ryckewaert JJ, Charon MH, Marchisio PC, Plow EF, Ginsberg MH, Marguerie G and Dejana E, Fibrinogen-endothelial cell interaction *in vitro*: a pathway mediated by an Arg-Gly-Asp recognition specificity. *Blood* **73**: 734–742, 1989.
16. Sporn LA, Marder VJ and Wagner DD, Inducible secretion of large, biologically potent von Willebrand factor multimers. *Cell* **46**: 185–190, 1986.
17. van Buul-Wortelboer MF, Brinkman HJM, Reinders JH, van Aken WG and van Mourik JA, Polar secretion of von Willebrand factor by endothelial cells. *Biochim Biophys Acta* **1011**: 129–133, 1989.
18. Levine JD, Harlan JM, Harker LA, Joseph ML and Counts MB, Thrombin mediated release of factor VIII antigen from human umbilical vein endothelial cells in culture. *Blood* **60**: 531–534, 1982.
19. Loesberg G, Gonsalves MD, Zandbergen J, Willems C, van Aken WG, Stel HV, van Mourik JA and de Groot PG, The effect of calcium on the secretion of factor VIII-related antigen by cultured human endothelial cells. *Biochim Biophys Acta* **763**: 160–168, 1983.
20. de Groot PG, Gonsalves MD, Loesberg C, van Buul-Wortelboer MF, van Aken WG and van Mourik JA, Thrombin-induced release of von Willebrand factor from endothelial cells is mediated by phospholipid methylation. Prostacyclin synthesis is independent of phospholipid methylation. *J Biol Chem* **259**: 13329–13334, 1984.
21. Declerck PJ, De Mol M, Alessi M-C, Baudner S, Pâques E-P, Preissner KT, Müller-Berghaus G and Collen D, Purification and characterization of a plasminogen activator inhibitor 1 binding protein from human plasma, identification as a multimeric form of S protein (vitronectin). *J Biol Chem* **263**: 15454–15461, 1988.
22. Mimuro J and Loskutoff DJ, Purification of a protein from bovine plasma that binds to type 1 plasminogen activator inhibitor and prevents its interaction with extracellular matrix. Evidence that the protein is vitronectin. *J Biol Chem* **264**: 936–939, 1989.
23. Schleef RR and Loskutoff DJ, Fibrinolytic system of vascular endothelial cells. Role of plasminogen activator inhibitors. *Haemostasis* **18**: 328–341, 1988.
24. Sakata Y, Okada M, Noro A and Matsuda M, Interaction of tissue-type plasminogen activator and plasminogen activator inhibitor 1 on the surface of endothelial cells. *J Biol Chem* **263**: 1960–1969, 1988.
25. von dem Borne AEGKr and Ouwehand WH, Immunology of platelet disorders. In: *Clinical Haematology*, Ballière Tindall, London, (Ed. Caen JP), pp. 749–781.
26. Kunicki TJ and Aster RH, Deletion of the platelet-specific alloantigen PL^{A1} from platelets in Glanzmann's thrombasthenia. *J Clin Invest* **61**: 1225–1231, 1978.
27. van Leeuwen EF, von dem Borne AEGKr, von Riesz LE, Nijenhuis LE and Engelfriet CP, Absence of platelet-specific alloantigens in Glanzmann's thrombasthenia. *Blood* **57**: 49–54, 1981.
28. Sprandio JD, Shapiro SS, Thiagarajan P and McCord S, Cultured human umbilical vein endothelial cells contain a membrane glycoprotein immunologically related to platelet glycoprotein Ib. *Blood* **71**: 234–237, 1988.
29. Lopez JA, Chung DW, Fujikawa K, Hagen FS, Papayannopoulou T and Roth JG, Cloning of the α-chain of human platelet glycoprotein Ib: A transmembrane protein with homology to leucine-rich α_2-glycoprotein. *Proc Natl Acad Sci USA* **84**: 5615–5619, 1987.
30. Leeksma OC, Giltay JC, Zandbergen-Spaargaren J, Modderman PW, van Mourik JA and von dem Borne AEGKr, The platelet alloantigen Zw^a or PI^{A1} is expressed by cultured endothelial cells. *Br J Haematol* **66**: 369–373, 1987.
31. Giltay JC, Leeksma OC, von dem Borne AEGKr and van Mourik JA, Alloantigenic composition of the endothelial vitronectin receptor. *Blood* **72**: 230–233, 1988.
32. Giltay JC, Brinkman HJM, von dem Borne AEGKr and van Mourik JA, Expression of the alloantigen Zw^a on human vascular smooth muscle cells and foreskin

fibroblasts. A study on normal individuals and patient with Glanzmann's thrombasthenia. *Blood* **74**: 965–970, 1989.
33. van Loghem JJ, Dorfmeyer H and van der Hart H, Serological and genetical studies on a platelet antigen (Zw). *Vox Sang* **4**: 161–169, 1959.
34. Shulman NR, Aster RH, Leitner A and Hiller MC, Immunoreactions involving platelets. V. Post-transfusion purpura due to a complement-fixing antibody against a genetically controlled platelet antigen. A proposed mechanism for thrombocytopenia and its relevance in "autoimmunity." *J Clin Invest* **40**: 1597–1620, 1961.
35. Pischel KD, Martin SD, Springer TA, Woods VL and Bluestein HG, Polymorphism of lymphocyte function-associated antigen-1 demonstrated by a Lupus patient's alloantiserum. *J Clin Invest* **79**: 1607–1614, 1987.
36. de Waal LP, van Dalen CM, Engelfriet CP and von dem Borne AEGKr, Alloimmunization against the platelet-specific Zw^a Antigen, resulting in neonatal alloimmune thrombocytopenia or posttransfusion purpura, is associated with the supertypic DRW52 antigen including DR3 and DRW6. *Hum Immunol* **17**: 45–53, 1986.
37. van Leeuwen EF, Leeksma OC, van Mourik JA, Engelfriet CP and von dem Borne AEGKr, Effect of the binding of anti-Zw^a antibodies on platelet function. *Vox Sang* **47**: 280–289, 1984.
38. Cines DB, Lyss AP, Reeber M, Bina M and DeHoratius RJ, Presence of complement-fixing anti-endothelial cell antibodies in systemic lupus erythematosus. *J Clin Invest* **73**: 611–625, 1984.
39. van Leeuwen EF, van der Ven JThM, Engelfriet CP and von dem Borne AEGKr, Specificity of autoantibodies in autoimmune thrombocytopenia. *Blood* **59**: 23–26, 1982.
40. Bray PF, Rosa JP, Lingappa VR, Kan YW, McEver RP and Shulman MA, Biogenesis of the platelet receptor for fibrinogen: evidence for separate precursors for glycoproteins IIb and IIIa. *Proc Natl Acad Sci USA* **83**: 1480–1484, 1986.
41. Duperray A, Berthier R, Chagnon E, Ryckewaert JJ, Ginsberg M, Plow E and Marguerie G, Biosynthesis and processing of platelet GP IIb–IIIa in human megakaryocytes. *J Cell Biol* **104**: 1665–1673, 1987.
42. Anderson DC and Springer TA, Leucocyte adhesion deficiency: an inherited defect in the Mac-1, LFA-1, and p150,95 glycoproteins. *Annu Rev Med* **38**: 175–194, 1987.
43. Bray PF, Rosa J-P, Johnston GI, Shiu DT, Cook RG, Lau C, Kan YW, McEver RP and Shuman MH, Platelet glycoprotein IIb: chromosomal localization and tissue expression. *J Clin Invest* **80**: 1812–1817, 1987.
44. Rosa J-P, Bray PF, Gayet O, Johnston GI, Cook RG, Jackson KW, Shuman MA and McEver RP, Cloning of glycoprotein IIIa cDNA from human erythroleukemia cells and localization of the gene to chromosome 17. *Blood* **72**: 593–600, 1988.
45. Russell ME, Seligsohn U, Coller BS, Ginsberg MH, Skoglund P and Quertermous T, Structural integrity of the glycoprotein IIb and IIIa genes in Glanzmann thrombasthenia patients from Israel. *Blood* **72**: 1833–1836, 1988.
46. Giltay JC, Leeksma OC, Breederveld C and van Mourik JA, Normal synthesis and expression of endothelial IIb/IIIa in Glanzmann's thrombasthenia. *Blood* **69**: 809–812, 1987.
47. Springer TA, Thompson WS, Miller LJ, Schmalstieg FC and Anderson DC, Inherited deficiency of the Mac-1, LFA-1, p150,95 glycoprotein family and its molecular basis. *J Exp Med* **160**: 1901–1918, 1984.
48. George JN and Nurden AT, Inherited disorders of the platelet membrane: Glanzmann's thrombasthenia and Bernard–Soulier syndrome. In: *Hemostasis and Thrombosis. Basic Principles and Clinical Practice* (Eds. Colman RW, Hirsh J, Marder VJ and Salzman EW), 2nd Ed, pp. 726–740. JB Lippincott, Philadelphia, 1987.
49. Nieuwenhuis HK, Akkerman JWN, Houdijk WPM and Sixma JJ, Human blood platelets showing no response to collagen fail to express surface glycoprotein Ia. *Nature* **318**: 470–472, 1985.
50. Kunicki TJ, Nugent DJ, Staats SJ, Orchekowski RP, Wayner EA and Carter WG, 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.
51. Heino J, Ignotz RA, Hemler ME, Crouse C and Massagué J, Regulation of cell adhesion receptors by transforming growth factor-β. *J Biol Chem* **264**: 380–388, 1989.
52. Giltay JC, Brinkman HJM, Modderman, PW von dem Borne AEGKr and van Mourik JA, Human vascular endothelial cells express a membrane protein complex immunochemically indistinguishable from the platelet VLA-2 (glycoprotein Ia–IIa) complex. *Blood* **73**: 1235–1241, 1989.