

von Willebrand Factor Interaction with the Glycoprotein IIb/IIIa Complex Its Role in Platelet Function as Demonstrated in Patients with Congenital Afibrinogenemia

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Abstract

We have studied three afibrinogenemic patients, who had only trace amounts of plasma and platelet fibrinogen as measured by radioimmunoassay, and demonstrate here that the residual aggregation observed in their platelet-rich plasma is dependent upon von Willebrand factor (vWF) binding to the platelet membrane glycoprotein (GP)IIb/IIIa complex. The abnormality of aggregation was more pronounced when ADP, rather than thrombin, collagen, or the combination of ADP plus adrenaline was used to stimulate platelets. With all stimuli, nevertheless, the platelet response was completely inhibited by a monoclonal antibody (LJP5) that is known to block vWF, but not fibrinogen binding to GPIIb/IIIa. Addition of purified vWF to the afibrinogenemic plasma resulted in marked increase in the rate and extent of aggregation, particularly when platelets were stimulated with ADP. This response was also completely blocked by LJP5. Addition of fibrinogen, however, restored normal aggregation even in the presence of LJP5, a finding consistent with the knowledge that antibody LJP5 has no effect on platelet aggregation mediated by fibrinogen binding to GPIIb/IIIa. Two patients gave their informed consent to receiving infusion of 1-desamino-8-D-arginine vasopressin (DDAVP), a vasopressin analogue known to raise the vWF levels in plasma by two- to fourfold. The bleeding time, measured before and 45 min after infusion, shortened from >24 min to 12 min and 50 s in one patient and from 16 min to 9 min and 30 s in the other. Concurrently, the rate and extent of ADP-induced platelet aggregation improved after DDAVP infusion. The pattern, however, reversed to baseline levels within 4 h. The concentration of plasma vWF increased after DDAVP infusion, but that of fibrinogen remained at trace levels. We conclude that vWF interaction with GPIIb/IIIa mediates platelet-platelet interaction and may play a role in primary hemostasis.

Introduction

Fibrinogen and von Willebrand factor (vWF)¹ are both essential for normal platelet function. Prolongation of the bleeding time

is typically observed in patients with congenital deficiency of either protein, the corresponding disease states being known as afibrinogenemia and von Willebrand disease (1–4). Specific receptors exist on the platelet membrane for both proteins. Considerable clinical and experimental evidence indicates that vWF interaction with the platelet membrane glycoprotein (GP) Ib functions in promoting the initial attachment of platelets to the damaged vessel wall (adhesion) (5, 6), whereas fibrinogen interaction with the GPIIb/IIIa complex is necessary for subsequent formation of the platelet plug through platelet-platelet contact (aggregation) (7, 8).

In addition to binding to GPIb, vWF also interacts with GPIIb/IIIa (9, 10). Fibrinogen and vWF compete for binding to GPIIb/IIIa, however, and, at physiological plasma concentrations of the two proteins, fibrinogen binding far exceeds that of vWF (11–13). This latter observation has put in question the physiological relevance of vWF binding to GPIIb/IIIa. On the other hand, it has long been known that platelet aggregation is not completely abrogated in patients with afibrinogenemia (14–16) as it is in typical patients with Glanzmann thrombasthenia, a condition in which the GPIIb/IIIa binding site on platelets is lacking (17, 18). Moreover, the bleeding time is prolonged in afibrinogenemic individuals but not to the extent usually seen in severe von Willebrand disease or Glanzmann thrombasthenia.

In the studies reported here we provide evidence that vWF interaction with GPIIb/IIIa is responsible for the residual degree of aggregation seen in afibrinogenemia. Moreover, we demonstrate that increasing the plasma levels of vWF may ameliorate the bleeding abnormality of these patients.

Methods

Patients. The three afibrinogenemic patients who participated in this study (B.P., V.M., and M.M.) belong to three different kindreds and have been previously described in detail (19–21). The plasma fibrinogen level, measured by radioimmunoassay (courtesy of Dr. Edward F. Plow, Research Institute of Scripps Clinic), was <5 µg/ml in B.P. and V.M., and 8.4 µg/ml in M.M. (range of normal values = 2.5–4 × 10³ µg/ml). The intraplatelet fibrinogen concentration was 3, 3.3, and 5.7 µg/10⁹ platelets in B.P., V.M., and M.M., respectively (range of normal values = 360–550 µg/10⁹ platelets). All patients, as well as normal controls, gave their informed consent before donating blood, in accordance with the Declaration of Helsinki. They had received no drugs for at least 2 wk. The two patients (V.M. and M.M.) who received infusion of 1-desamino-8-D-arginine vasopressin (DDAVP) were fully informed of the investigational purpose of the treatment. These experiments were performed in Pordenone (Italy) in accordance with the guidelines of the local Human Subjects Experimentation Committee. DDAVP was infused at the dose of 0.4 µg/kg of body weight, according to a previously published protocol (22). Bleeding time (Simplate II, General Diagnostics, Morris Plains, NJ) and platelet aggregation were tested before, and then 45 min and 4 h after the infusion. For aggregation studies, the count in the platelet-rich plasma was adjusted to similar values in all samples and the experiments were performed within 30 min from blood collection.

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1. *Abbreviations used in this paper:* DDAVP, 1-desamino-8-D-arginine vasopressin; GP, platelet membrane glycoprotein; vWF, von Willebrand factor.

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vWF antigen concentration in plasma was measured by electroimmunoassay (23).

Sample collection and aggregation studies. Blood was collected using polypropylene syringes and 19-gauge needles and immediately transferred into polypropylene tubes containing $\frac{1}{10}$ final volume of 3.1% (0.105 M) trisodium citrate. Platelet-rich plasma was prepared by collecting the supernatant after each of two or three successive centrifugation steps performed at 1,200 *g* for 60 s at room temperature (22°–25°C). All aggregation studies were performed in siliconized glass cuvettes, at 37°C, with constant stirring at 1,200 rpm and with a platelet count between 2.5 and 3×10^8 /ml. The stimuli used included ADP (Sigma Chemical Co., St. Louis, MO), adrenaline (Parke-Davis and Co., Detroit, MI), collagen (equine collagen fibrils; Hormon-Chemie, Munich, Federal Republic of Germany), and human alpha thrombin (the generous gift of Dr. John W. Fenton, II, New York State Department of Health, Albany, NY). Aggregation was recorded as increase in light transmittance through the stirred platelet suspension.

Monoclonal antibody. The anti-GPIIb/IIIa monoclonal antibody LJP5 has been recently described in detail (24). It blocks vWF binding to stimulated platelets without affecting fibrinogen binding. Monovalent Fab fragment of this antibody was prepared as described (24). It was stored in 0.02 M Tris · HCl–0.10 M NaCl buffer, pH 7.35.

Purification of fibrinogen and vWF. Fibrinogen was purified from blood collected in acid-citrate-dextrose anticoagulant, and containing 0.1 M (final concentration) epsilon-aminocaproic acid, using the glycine precipitation method of Kazal et al. (25). A final gel filtration step through an 80 × 5-cm 4B-CL Sepharose column (Pharmacia Fine Chemicals, Uppsala, Sweden) was performed to remove traces of contaminating vWF. In the purified fibrinogen preparation, 95% of the protein was clotted by addition of alpha thrombin. vWF was purified following methods previously described in detail (26). In addition, purified vWF was treated with a rabbit antihuman fibrinogen IgG, coupled to Sepharose 4B-CL beads, to remove trace contamination of fibrinogen. The preparation of purified vWF had >100 U of ristocetin cofactor activity and vWF antigen per milligram of protein and had a multimeric structure like that of plasma vWF (10). Other characteristics of these purified proteins, as obtained in our laboratories, were recently reported (10, 24).

Binding studies. vWF was radiolabeled with ^{125}I as described (10). The binding of ^{125}I -vWF to thrombin-stimulated washed platelets was performed as previously reported in detail (10). The method for preparation of washed platelet suspensions using the albumin density gradient technique has also been reported (24, 27).

Results

Platelet-rich plasma from the three afibrinogenemic patients showed minimal aggregation when stimulated with ADP at 1.65 μM , but more evident aggregation when the ADP concentration was raised to 6.25 μM (Fig. 1). Addition of purified vWF to the plasma resulted in increased aggregation at either concentration of ADP (Fig. 1). At the higher concentration of ADP, the rate and extent of platelet aggregation observed after the addition of 75 $\mu\text{g}/\text{ml}$ of vWF were similar to those seen after addition of 700 $\mu\text{g}/\text{ml}$ of fibrinogen, but at the lower ADP concentration the effect of fibrinogen was much more pronounced than that of vWF (Fig. 1). The ADP-induced aggregation of afibrinogenemic platelet-rich plasma, whether with or without addition of purified vWF, was markedly inhibited by the anti-GPIIb/IIIa monoclonal antibody LJP5 (Fig. 2). Accordingly, this antibody inhibited ^{125}I -vWF binding to normal or afibrinogenemic platelets stimulated by ADP plus adrenaline (Table I). In previous studies we have shown that antibody LJP5 has no effect on the binding of fibrinogen to GPIIb/IIIa (see reference 24 for full details). Moreover, antibody LJP5 has no effect on the aggregation of normal platelet-rich plasma (Fig. 3). In accordance with the latter finding, afibrinogenemic platelet-rich plasma to

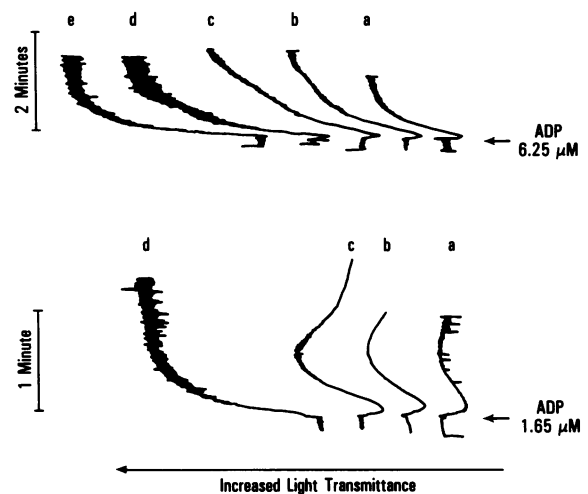


Figure 1. Aggregation of afibrinogenemic platelet-rich plasma stirred at 37°C. Traces a–d in the lower panel were observed after addition of ADP at a concentration of 1.65 μM (added at arrow); traces a–e in the upper panel with ADP at 6.25 μM . (Lower panel) Platelet-rich plasma from patient B.P. (platelet count adjusted to 2.5×10^8 /ml) was tested as such (a) or after addition of purified vWF (b, 33 $\mu\text{g}/\text{ml}$; c, 82 $\mu\text{g}/\text{ml}$) or purified fibrinogen (d, 780 $\mu\text{g}/\text{ml}$). (Upper panel) Platelet-rich plasma from patient V.M. (platelet count adjusted to 3.0×10^8 /ml) was tested as such (a) or after addition of purified vWF (b, 9.8 $\mu\text{g}/\text{ml}$; c, 18.5 $\mu\text{g}/\text{ml}$; d, 75 $\mu\text{g}/\text{ml}$) or purified fibrinogen (e, 700 $\mu\text{g}/\text{ml}$). Note the different time scale due to the use of two different recorders.

which fibrinogen had been added aggregated normally, even in the presence of antibody LJP5 (Fig. 4).

Afibrinogenemic platelet-rich plasma showed aggregation when stimulated with collagen or alpha thrombin. With the latter stimulus, both the rate and extent of aggregation were similar to normal, even without addition of exogenous fibrinogen or vWF (Fig. 5). Nevertheless, the aggregation of afibrinogenemic platelet-rich plasma induced by collagen or thrombin was markedly inhibited in the presence of antibody LJP5, demonstrating, therefore, that it was mediated by vWF interaction with GPIIb/IIIa (Fig. 5).

The plasma levels of vWF increased in the two afibrinogenemic patients treated with DDAVP, but the concentration of fibrinogen remained extremely low (Table II). The bleeding time, measured 45 min after the infusion of DDAVP, was shorter than it was before treatment (Table II). Moreover, the extent of ADP-induced platelet aggregation, tested as the most abnormal aggregation parameter in the patients, increased 45 min after the DDAVP infusion, but returned towards baseline levels after 4 h (Fig. 6). These findings demonstrated that increasing the plasma levels of vWF *in vivo* had similar effects as the addition of purified vWF to the afibrinogenemic platelet-rich plasma *in vitro*.

Discussion

Fibrinogen is considered an essential cofactor for platelet aggregation, and its role in this process has been correlated to the occupancy of a specific receptor on the platelet membrane GPIIb/IIIa complex (8, 28, 29). In patients whose platelets lack this receptor, as is the case in Glanzmann thrombasthenia, platelet function is severely impaired (17, 18), but in patients

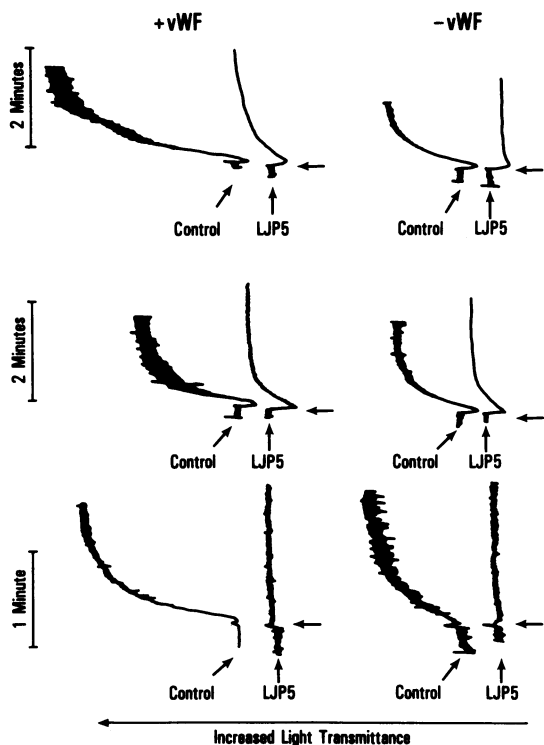


Figure 2. Effect of antibody LJP5 (monovalent Fab fragment) on aggregation of afibrinogenemic platelet-rich plasma. Each experiment was performed without (curves on the right) or with (curves on the left) addition of purified vWF. Control curves contained 0.02 M Tris · HCl–0.1 M NaCl buffer, pH 7.35, instead of antibody. The latter was incubated for 2 min at 37°C before addition of ADP (indicated by horizontal arrows). The platelet count was 3×10^8 /ml in all cases. (Lower panel) Patient B.P.: vWF added, 33 μ g/ml; LJP5, 50 μ g/ml; ADP, 6.25 μ M. (Middle panel) Patient M.M.: vWF added, 39 μ g/ml; LJP5, 66 μ g/ml; ADP, 8 μ M. (Upper panel) Patient V.M.: vWF added, 38 μ g/ml; LJP5, 70 μ g/ml (curve on the right) or 140 μ g/ml (curve on the left); ADP, 6.25 μ M.

with afibrinogenemia the abnormality is less dramatic (14–16, 19–21). The skin bleeding time is prolonged in afibrinogenemia, but usually not to the extent that it is in Glanzmann thrombasthenia. Moreover, platelet aggregation, tested in the aggregometer, is always completely abrogated in thrombasthenic patients who are severely deficient in GPIIb/IIIa, whereas some degree of aggregation is always observed in afibrinogenemia, even when the levels of plasma and platelet fibrinogen are essentially unmeasurable. In fact, as also shown in the present study, the degree

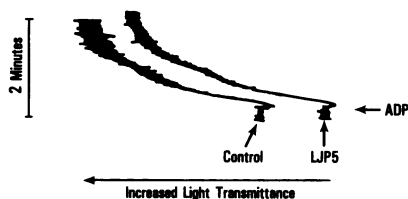


Figure 3. Lack of effect of antibody LJP5 (monovalent Fab fragment) on aggregation of normal platelet-rich plasma (platelet count, 2.5×10^8 /ml). LJP5 concentration was 140 μ g/ml; ADP (added at arrow) was 6.25 μ M. The control curve contained 0.02 M Tris · HCl–0.1 M NaCl buffer, pH 7.35, instead of antibody.

Table I. Effect of Antibody LJP5 on the Binding of 125 I-labeled vWF to Normal or Afibrinogenemic Platelets Stimulated with ADP Plus Adrenaline

	125 I-vWF added μ g/ml	125 I-vWF bound (μ g/ 10^9 platelets)	
		Control	LJP5
Patient M.M.	3.2	2.74	0.24
	21.6	10.31	3.75
Normal	3.2	3.01	0.21
	21.6	11.45	2.14

The binding was measured by mixing washed platelets (1×10^8 cells/ml) with 125 I-vWF (at the indicated concentrations) in the presence of ADP and adrenaline (20 μ M each). LJP5 Fab (160 μ g/ml), or 0.02 M Tris · HCl–0.1 M NaCl buffer, pH 7.35, was also added (the latter indicated as control). The mixtures were incubated for 30 min at 22°–25°C before separating bound from free 125 I-vWF by centrifugation through 20% sucrose (see reference 10 for technical details). All indicated concentrations are final. The results shown were obtained after subtracting from the total amount of 125 I-vWF bound the amount that was bound in similar mixtures prepared with the only omission of ADP and adrenaline.

of aggregation of afibrinogenemic platelet-rich plasma is only slightly less than normal when potent stimuli, like thrombin, are used. The initial response of platelets to relatively high concentrations of ristocetin is normal in both afibrinogenemia (21) and Glanzmann thrombasthenia (30), since a different platelet receptor, namely GPIb, and a different adhesive glycoprotein, namely vWF, are involved in this response (10). At lower ristocetin concentrations, however, aggregation may be abnormal in both conditions (21, 30). This finding may reflect the participation of fibrinogen in platelet aggregation mediated by vWF binding to GPIb, as suggested by recent studies (31, 32).

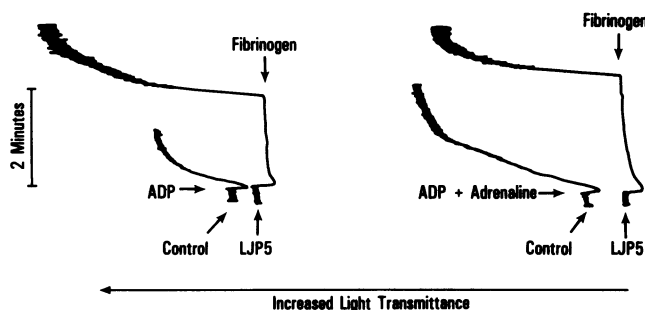


Figure 4. Aggregation of afibrinogenemic platelet-rich plasma. The two curves on the right show the aggregation induced by the combination of ADP and adrenaline (control curve, 6.25 μ M each) and the blocking effect exerted by antibody LJP5 (140 μ g/ml) in the presence of the same agonists. Addition of purified fibrinogen (800 μ g/ml) to the mixture containing LJP5 resulted in prompt platelet aggregation. The two curves on the left show a similar experiment performed using ADP alone, 6.25 μ M, as platelet agonist. LJP5 concentration was 70 μ g/ml. Platelet-rich plasma (platelet count, 3×10^8 /ml) was from patient V.M. in both cases.

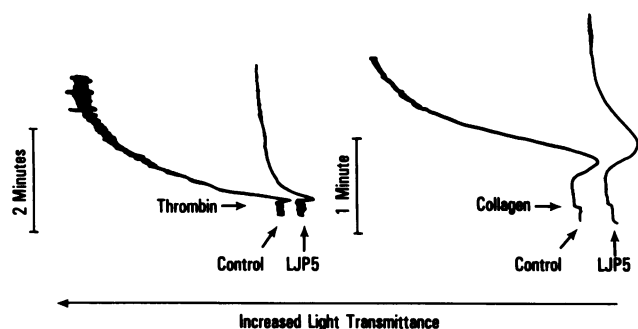


Figure 5. Aggregation of afibrinogenemic platelet-rich plasma. The two curves on the *right* (patient B.P.) demonstrate the aggregating effect of collagen (1 µg/ml) in the absence (control) or in the presence of antibody LJP5 (50 µg/ml). The two curves on the *left* (patient V.M.) demonstrate the aggregating effect of human alpha thrombin (2 NIH U/ml) in the absence (control) or in the presence of antibody LJP5 (200 µg/ml). Platelet count was 3×10^8 /ml in both cases.

The reasons for the residual platelet function observed in afibrinogenemia have not been elucidated until now (33). Previously published observations have suggested that purified vWF may, in the absence of added fibrinogen, mediate the aggregation of washed platelets (34), but these studies have not addressed the nature of the membrane receptor involved and have not ruled out the possible participation of platelet-derived fibrinogen in the process. Recent work, however, has demonstrated that other adhesive glycoproteins, namely vWF and fibronectin, may, as well as fibrinogen, occupy the GPIIb/IIIa receptor (9, 10, 35). We have investigated the possible role of vWF in the aggregation of afibrinogenemic platelets by using a recently described monoclonal antibody, identified as LJP5, which is directed against the platelet membrane GPIIb/IIIa complex. This antibody selectively blocks the binding of vWF to GPIIb/IIIa but has no effect on fibrinogen binding nor on the aggregation of normal platelet-rich plasma (24). Moreover, in yet unpublished studies (performed by Drs. Edward F. Plow and Mark H. Ginsberg, the Research Institute of Scripps Clinic), it has been established that

Table II. Effect of Infusion of DDAVP in Two Afibrinogenemic Patients

Patient	Bleeding time		Plasma vWF		Plasma fibrinogen	
	B	A	B	A	B	A
	min	min	U/dl	U/dl	µg/ml	µg/ml
V.M.	>24	12.50	190	386	<5	<5
M.M.	16	9.30	73	349	9	14.5
Normal range	2.31–6.01		49–145		2,043–4,145	

Plasma vWF indicates the concentration of vWF antigen, measured by electroimmunoassay and expressed in arbitrary units per deciliter, where 100 U/dl is the content of average normal plasma. B indicates the results obtained immediately before the infusion of DDAVP; A indicates the results obtained 45 min after the infusion. The normal range of values is that observed in untreated resting individuals. Note that patient M.M. is the only one of the three patients studied who consistently showed measurable, although very low, amounts of plasma fibrinogen (see Methods).

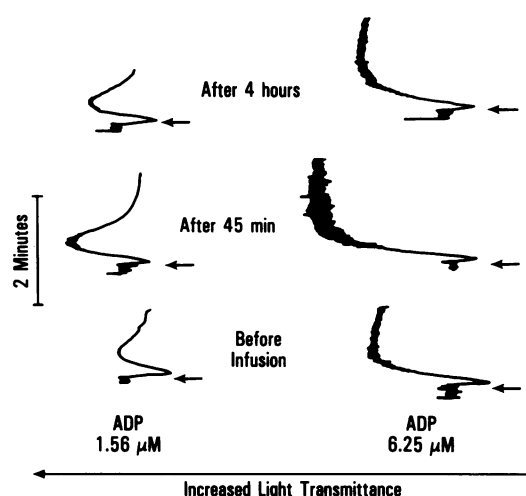


Figure 6. Aggregation of afibrinogenemic platelet-rich plasma before or after infusion of DDAVP. Patient M.M. (platelet count, 3×10^8 /ml). Curves on the *right* obtained with ADP (added at arrow) at a final concentration of 6.25 µM; curves on the *left* with ADP at 1.56 µM. The extent of aggregation, as well as the size of platelet aggregates (demonstrated by the extent of light scattering), were increased after DDAVP infusion.

LJP5 does not interfere with the binding of fibronectin to GPIIb/IIIa. We report here that antibody LJP5 has a marked inhibitory effect on platelet aggregation in afibrinogenemia, thus demonstrating that, in the absence of fibrinogen, platelet-platelet interaction is mediated by vWF binding to GPIIb/IIIa.

The aggregation of afibrinogenemic platelet-rich plasma in response to agonists like thrombin, collagen, or the combination of ADP plus adrenaline is less abnormal than in response to ADP alone. Such finding may be related to the fact that more vWF is likely to be released from the platelet α -granules in response to thrombin, or other strong stimuli, than it is in response to ADP. The released platelet vWF may then contribute to mediating aggregation. In any case, the inhibitory effect of antibody LJP5 demonstrates that the aggregation of afibrinogenemic platelet-rich plasma in response to all the agonists tested is mediated by the interaction of vWF with GPIIb/IIIa. Only minute amounts of fibrinogen are present in the platelets of patients like those described here (see Results). Although this fibrinogen, as well as vWF, is released from platelets, its contribution to aggregation is likely to be irrelevant, presumably as a consequence of its extremely low concentration. This concept is supported by the observed inhibitory effect exerted by antibody LJP5, which has no effect on platelet aggregation mediated by the binding of fibrinogen to the GPIIb/IIIa receptor (see reference 24 and Figs. 3 and 4).

Recently, it has been reported that fibronectin may also be involved in mediating platelet aggregation, as demonstrated by the inhibitory effect of an anti-fibronectin monoclonal antibody on the aggregation of gel-filtered platelets induced by thrombin and the ionophore A-23187 (36). Our results suggest that, in afibrinogenemic platelet-rich plasma, aggregation is mediated mainly by vWF, as shown by the minimal residual aggregation observed in the presence of antibody LJP5, which does not block fibronectin binding to GPIIb/IIIa. It is interesting to note, nevertheless, that at least three adhesive glycoproteins (namely fi-

brinogen, vWF, and fibronectin) may be involved in mediating platelet-platelet interactions.

Larger amounts of vWF than normally present in the circulation must be added to afibrinogenemic platelet-rich plasma in order to improve the aggregation in response to ADP. This finding can be explained by the observation, previously reported in several distinct studies (10, 12, 13, 34, 37, 38), that the amount of vWF bound to GPIIb/IIIa in response to ADP is considerably less than that bound in response to thrombin. It appears, however, that the abnormality of primary hemostasis observed in afibrinogenemic patients is reflected better by the abnormal pattern of ADP-induced aggregation rather than the nearly normal response to thrombin.

The aggregation induced by ADP in afibrinogenemic platelet-rich plasma was improved when vWF levels in the circulation were increased by the infusion of DDAVP. It is of interest that, after administration of DDAVP into two patients, a transitory shortening of the bleeding time was observed in parallel with an amelioration of the aggregation pattern. The bleeding time, as well as ADP-induced platelet aggregation *in vitro*, were not completely normalized, however. This suggests that the interaction of vWF with GPIIb/IIIa may promote platelet-platelet interaction, but cannot substitute completely for the function of fibrinogen in primary hemostasis, even at the high vWF plasma levels attained after treatment with DDAVP. Moreover, it should be considered that other, yet undefined mechanisms, in addition to the binding of vWF to the GPIIb/IIIa receptor, may explain the effect of DDAVP on primary hemostasis in the patients here described. Nevertheless, our studies suggest that DDAVP may be an additional, useful tool in the therapy or prevention of bleeding in afibrinogenemia, particularly in view of its ease of use and lack of side effects (39). It is important to point out that the administration of DDAVP has no effect on the plasma levels of fibrinogen. Therefore, it may only help improve formation of the platelet plug by increasing the plasma levels of vWF and cannot improve the clotting defect of afibrinogenemic patients.

In conclusion, our studies demonstrate that the binding of vWF to GPIIb/IIIa promotes platelet aggregate formation in the absence of fibrinogen. This function is distinct from that seen after binding of vWF to GPIb, as it involves different platelet agonists and distinct membrane receptors. In spite of the possible role in mediating events of primary hemostasis in afibrinogenemic patients, however, the question remains of whether vWF binding to GPIIb/IIIa may also have a physiologic role in normal. Although minimal binding of vWF is expected to occur at the relative concentrations of vWF and fibrinogen that are present in plasma, the situation may be different at sites of vascular injury, where release of vWF from platelets and altered endothelial cells, as well as its interaction with subendothelial structures, may create conditions favoring vWF binding to GPIIb/IIIa. In this regard, our work may provide the molecular basis to explain previously published observations, showing that (a) interaction of platelets with platelets adhering to glass beads requires vWF and is enhanced in the absence of fibrinogen (40); and (b) thrombus formation, in addition to platelet adhesion, is abnormal in patients with von Willebrand disease who lack vWF (41). Thus, vWF might contribute, by means of its binding to GPIIb/IIIa and with the essential participation of fibrinogen, to create a stable platelet aggregate in the process of normal hemostasis.

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References

1. Rabe, F., and E. Salomon. 1920. Ueber Faserstoffmangel im Blute bei einem Falle von Haemophilie. *Deutsches Arch. fuer Klin. Med.* 132: 240-244.
2. Caen, J., and S. Inceman. 1963. Considerations sur l'allongement du temps de saignement dans l'afibrinogénémie congénitale. *Nouv. Rev. Fr. Hematol.* 3:614-615.
3. von Willebrand, E. A. 1931. Ueber hereditaere Pseudohaemophilie. *Acta. Med. Scand.* 76:521-550.
4. Ruggeri, Z. M., and T. S. Zimmerman. 1985. Platelets and von Willebrand disease. In *Seminars in Hematology*. P. K. Schick and H. Stormorken, editors. Grune & Stratton, Inc., New York. 203-218.
5. Weiss, H. J., T. B. Tschopp, H. R. Baumgartner, I. I. Sussman, M. M. Johnson, and J. J. Egan. 1974. Decreased adhesion of giant (Bernard-Soulier) platelets to subendothelium. Further implications on the role of the von Willebrand factor in hemostasis. *Am. J. Med.* 57:920-925.
6. Nurden, A. T., and J. P. Caen. 1975. Specific roles for surface glycoproteins in platelet function. *Nature (Lond.)* 255:720-722.
7. Marguerie, G. A., E. F. Plow, and T. S. Edgington. 1979. Human platelets possess an inducible and saturable receptor specific for fibrinogen. *J. Biol. Chem.* 254:5357-5363.
8. Collier, B. S., E. I. Peerschke, L. E. Scudder, and C. A. Sullivan. 1983. A murine monoclonal antibody that completely blocks the binding of fibrinogen to platelets produces a thrombasthenic-like state in normal platelets and binds to glycoproteins IIb and/or IIIa. *J. Clin. Invest.* 72: 325-338.
9. Ruggeri, Z. M., R. Bader, and L. De Marco. 1982. Glanzmann thrombasthenia: deficient binding of von Willebrand factor to thrombin-stimulated platelets. *Proc. Natl. Acad. Sci. USA.* 79:6038-6041.
10. Ruggeri, Z. M., L. De Marco, L. Gatti, R. Bader, and R. R. Montgomery. 1983. Platelets have more than one binding site for von Willebrand factor. *J. Clin. Invest.* 72:1-12.
11. Schullek, J., J. Jordan, and R. R. Montgomery. 1984. Interaction of von Willebrand Factor with human platelets in the plasma milieu. *J. Clin. Invest.* 73:421-428.
12. Gralnick, H. R., S. B. Williams, and B. S. Collier. 1984. Fibrinogen competes with von Willebrand factor for binding to the glycoprotein IIb/IIIa complex when platelets are stimulated with thrombin. *Blood.* 64:797-800.
13. Pietu, G., G. Cherel, G. Marguerie, and D. Meyer. 1984. Inhibition of von Willebrand factor-platelet interaction by fibrinogen. *Nature (Lond.)* 308:648-649.
14. Gugler, E., and E. F. Luescher. 1965. Platelet function in congenital afibrinogenemia. *Thromb. Diath. Haemorrh.* 14:361-373.
15. Inceman, S., J. Caen, and J. Bernard. 1966. Aggregation, adhesion, and viscous metamorphosis of platelets in congenital fibrinogen deficiencies. *J. Lab. Clin. Med.* 68:21-32.
16. Weiss, H. J., and J. Rogers. 1971. Fibrinogen and platelets in the primary arrest of bleeding. *N. Engl. J. Med.* 285:369-374.
17. Caen, J. P., P. A. Castaldi, J. C. Leclerc, S. Inceman, M. J. Larriue, M. Probst, and J. Bernard. 1966. Congenital bleeding disorders with long

bleeding time and normal platelet count. I. Glanzmann's thrombasthenia (report of fifteen patients). *Am. J. Med.* 41:4-26.

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

19. Girolami, A., G. Zacchello, and R. D'Elia. 1971. Congenital afibrinogenemia. A case report with some considerations on the hereditary transmission of this disorder. *Thromb. Diath. Haemorrh.* 25:460-468.

20. Girolami, A., R. Venturelli, and G. Bareggi. 1972. A report of a case of congenital afibrinogenemia. *Blut.* XXIV:23-31.

21. Girolami, A., L. De Marco, L. Virgolini, R. Peruffo, and F. Fabris. 1975. Platelet adhesiveness and aggregation in congenital afibrinogenemia. An investigation of three patients with posttransfusion, cross-correction studies between two of them. *Blut.* 30:87-100.

22. Ruggeri, Z. M., P. M. Mannucci, A. B. Federici, R. Lombardi, and T. S. Zimmerman. 1982. Multimeric composition of Factor VIII/von Willebrand factor following administration of DDAVP: implications for pathophysiology and therapy of von Willebrand's disease subtypes. *Blood.* 59:1272-1278.

23. Ruggeri, Z. M., P. M. Mannucci, S. L. Jeffcoate, and G. I. C. Ingram. 1976. Immunoradiometric assay of Factor VIII related antigen, with observations in 32 patients with von Willebrand's disease. *Br. J. Haematol.* 33:221-232.

24. Trapani Lombardo, V., E. Hodson, J. Roberts, T. J. Kunicki, T. S. Zimmerman, and Z. M. Ruggeri. 1985. Independent modulation of von Willebrand factor and fibrinogen binding to the platelet membrane glycoprotein IIb/IIIa complex as demonstrated by monoclonal antibody. *J. Clin. Invest.* 76:1950-1958.

25. Kazal, L. A., S. Amsel, O. P. Miller, and L. M. Tocantins. 1963. The preparation and some properties of fibrinogen precipitated from human plasma by glycine. *Proc. Soc. Exp. Biol. Med.* 113:989-994.

26. De Marco, L., and S. S. Shapiro. 1981. Properties of human asialo-Factor VIII. A ristocetin-independent platelet-aggregating agent. *J. Clin. Invest.* 68:321-328.

27. Walsh, P. N., D. C. B. Mills, and J. G. White. 1977. Metabolism and function of human platelets washed by albumin density gradient separation. *Br. J. Haematol.* 36:281-296.

28. Lee, H., A. T. Nurden, A. Thomaidis, and J. P. Caen. 1981. Relationship between fibrinogen binding and the platelet glycoprotein deficiencies in Glanzmann's thrombasthenia Type I and Type II. *Br. J. Haematol.* 48:47-57.

29. Bennett, J. S., J. A. Hoxie, S. F. Leitman, G. Vilaire, and D. B. Cines. 1983. Inhibition of fibrinogen binding to stimulated human platelets by a monoclonal antibody. *Proc. Natl. Acad. Sci. USA.* 80:2417-2421.

30. Chediak, J., M. C. Telfer, B. Vander Laan, B. Maxey, and I.

Cohen. 1979. Cycles of agglutination-disagglutination induced by ristocetin in thrombasthenic platelets. *Br. J. Haematol.* 43:113-126.

31. De Marco, L., A. Girolami, S. Russell, and Z. M. Ruggeri. 1985. Interaction of asialo von Willebrand factor with glycoprotein Ib induces fibrinogen binding to the glycoprotein IIb/IIIa complex and mediates platelet aggregation. *J. Clin. Invest.* 75:1198-1203.

32. De Marco, L., A. Girolami, T. S. Zimmerman, and Z. M. Ruggeri. 1985. Interaction of purified IIB von Willebrand factor with the platelet membrane glycoprotein Ib induces fibrinogen binding to the glycoprotein IIb/IIIa complex and initiates aggregation. *Proc. Natl. Acad. Sci. USA.* 82:7424-7428.

33. Soria, J., C. Soria, J. Y. Borg, M. Mirshahi, H. Pigué, P. Tron, C. Fessard, and J. P. Caen. 1985. Platelet aggregation occurs in congenital afibrinogenemia despite the absence of fibrinogen or its fragments in plasma and platelets, as demonstrated by immunoenzymology. *Br. J. Haematol.* 60:503-514.

34. Fujimoto, T., and J. Hawiger. 1982. Adenosine diphosphate induces binding of von Willebrand factor to human platelets. *Nature (Lond.)* 297:154-157.

35. Ginsberg, M. H., J. Forsyth, A. Lightsey, J. Chediak, and E. F. Plow. 1983. Reduced surface expression and binding of fibronectin by thrombin-stimulated thrombasthenic platelets. *J. Clin. Invest.* 71:619-624.

36. Dixit, V. M., D. M. Haverstick, K. O'Rourke, S. W. Hennessy, T. J. Broekelmann, J. A. McDonald, G. A. Grant, S. A. Santoro, and W. A. Frazier. 1985. Inhibition of platelet aggregation by a monoclonal antibody against human fibronectin. *Proc. Natl. Acad. Sci. USA.* 82:3844-3848.

37. Fujimoto, T., S. Ohara, and J. Hawiger. 1982. Thrombin-induced exposure and prostacyclin inhibition of the receptor for factor VIII/von Willebrand factor on human platelets. *J. Clin. Invest.* 69:1212-1222.

38. Di Minno, G., S. Shapiro, P. M. Catalano, L. De Marco, and S. Murphy. 1983. The role of ADP secretion and thromboxane synthesis in factor VIII binding to platelets. *Blood.* 62:186-190.

39. Mannucci, P. M., Z. M. Ruggeri, F. I. Pareti, and A. Capitanio. 1977. 1-Deamino-8-D-Arginine vasopressin: a new pharmacological approach to the management of haemophilia and von Willebrand's disease. *Lancet.* i:869-872.

40. McPherson, J., and M. B. Zucker. 1975. Platelet retention in glass bead columns: adhesion to glass and subsequent platelet-platelet interactions. *Blood.* 47:55-67.

41. Turitto, V. T., H. J. Weiss, and H. R. Baumgartner. 1984. Platelet interaction with rabbit subendothelium in von Willebrand's disease. Altered thrombus formation distinct from defective platelet adhesion. *J. Clin. Invest.* 74:1730-1741.