Platelet Adhesion and Thrombus Formation on Subendothelium in Platelets Deficient in Glycoproteins IIb-IIIa, Ib, and Storage Granules

By Harvey J. Weiss, Vincent T. Turitto, and Hans R. Baumgartner

Patients whose platelets are deficient in glycoprotein (GP) Ib, IIb-IIIa (thrombasthenia), or granule substances (storage pool deficiency, SPD) were studied to define further the properties of platelets that mediate platelet adhesion and thrombus formation on subendothelium. Both nonanticoagulated and citrated blood were exposed to everted, de-endothelialized rabbit vessel segments under controlled flow conditions and shear rates varying from 650 to 3300 sec\(^{-1}\). Morphometry was used to measure platelet thrombus dimensions and the percentage of the subendothelial surface covered with contact (C) or spreading (S) platelets. Adhesion was defined as C + S. The results in SPD demonstrated (1) reduced thrombus dimensions in \(\delta\)-SPD (pure dense granule deficiency) in proportion to the magnitude of the dense granule defect; (2) an even greater reduction in thrombus dimensions in patients with combined deficiencies of \(\alpha\) and dense granules (\(\alpha\delta\)-SPD); and (3) impaired platelet adhesion at several conditions in \(\alpha\delta\)-SPD and, in \(\delta\)-SPD, a hematocrit-dependent impairment of adhesion in citrated blood at 2,600 sec\(^{-1}\). In thrombasthenia, platelets were present as a monolayer on the subendothelial surface in both nonanticoagulated and citrated blood, indicating an absolute requirement for GPIIb-IIIa in promoting platelet-platelet interaction at all shear rates and perfusion times. Two types of abnormalities in platelet-vessel wall interactions were observed. In nonanticoagulated blood, the percentage of platelets in the C phase was consistently increased at all shear rates, but C + S values were normal. These observations indicate that platelets deficient in GPIIb-IIIa do not spread normally on the subendothelial surface exposed to nonanticoagulated blood. With citrated blood, the C + S value in thrombasthenia was reduced at both 800 and 2,600 sec\(^{-1}\), as in von Willebrand’s disease, and a similar degree of reduction (about 50%) was observed in normal blood treated with a monoclonal antibody to GPIIb-IIIa. The findings, together with theoretical considerations, are consistent with an hypothesis that GPIIb-IIIa mediates the spreading of platelets on subendothelium following the initial attachment through GPIb and that GPIIb-IIIa may be considered an adhesion site on the platelet membrane. Abnormalities of GPIIb-IIIa may, depending on the conditions of study, result in either increased values of C platelets or decreased values of C + S. The results of the study further suggest that a complex interaction of platelet granule factors and membrane GP mediate platelet adhesion and thrombus formation.

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the same conditions, parallel studies were carried out on patients with severe von Willebrand's disease and the Bernard-Soulier syndrome.

In addition, we have also used blood from patients with various types of SPD to examine a possible relationship of platelet α and dense granule defects, and their magnitude, on platelet-platelet interaction (thrombi).

**MATERIALS AND METHODS**

**Perfusion Chamber—Containing Vessel Segments**

The perfusion chamber is an annular device on the core of which is mounted an everted segment of rabbit aorta whose endothelium has been completely removed by a balloon catheter. Chambers of two different sizes, designated original and small, were used in the study. Blood entering the chamber passes through the annular space formed by the subendothelial surface and the outer cylinder wall, thereby permitting platelets to interact with the exposed subendothelial surface. The flow parameters, dimensions, and temperature (37 °C) control for the chamber have been previously described. Vessel segments either 20 mm (nonanticoagulated blood studies) or 14 mm (citrated blood studies) in length were stored in 0.2 mol/L tris (hydroxymethyl) aminomethane (Tris) buffer, pH 7.4, at 4 °C for periods of seven to 28 days prior to use.

**Perfusion Procedure**

Everted vessel segments were exposed at varying shear rates and exposure times to citrated or nonanticoagulated blood. Citrated blood, adjusted for the hematocrit value to a final plasma concentration of 19.7 mmol/L sodium citrate, was circulated through the chamber in a closed system using a peristaltic pump (Holter Extracorporeal Medical Specialties, Inc, King of Prussia, Pa) to maintain the desired blood flow. For studies using nonanticoagulated blood, blood from the antecubital vein was drawn directly into the chamber and was not recirculated. The flow rate was controlled by the Holter pump and was accurately determined after each run by measuring the actual amount of blood perfused. The wall shear rate for each flow rate used was determined as previously described. For flow rates of 10, 20, 40, and 50 mL/min in the small chamber (used for all studies with nonanticoagulated blood), the corresponding wall shear rates are 650, 1,300, 2,600, and 3,300 sec⁻¹. For the original chamber (used in some of the citrate blood studies), flow rates of 40 and 160 mL/min correspond to wall shear rates of 200 and 800 sec⁻¹.

**Morphologic Evaluation**

After perfusion, vessel segments were embedded in an oriented manner, and cross sections 0.8 μm in thickness were stained and evaluated morphometrically by light microscopy. Platelet interaction with the subendothelium was evaluated by two microscopic techniques that were performed on the same cross-sectional area of vessel segment. Platelet interactions were evaluated at a single axial position approximately equidistant from both ends, that is, in studies on nonanticoagulated blood, at a single axial position located 10 mm from the proximal end of the original segment, and in citrated blood studies, at 7 mm from the proximal end. For determining platelet adhesion, the presence of platelets was evaluated at 10-μm intervals, and platelet-subendothelial interaction was evaluated as either C, platelets are attached but not spread on the surface, and S, platelets are spread on the surface and may, in addition, have superimposed aggregates of platelets (thrombi) extending for varying distances into the lumen. Platelet adhesion is defined as C + S, expressed as a percentage of the total number of evaluations (~1,000) per vessel segment. To evaluate more precisely the dimensions of the platelet thrombi, vessel segments are projected onto the recording plate of a manual picture analysis system (MOP/AMOI, Kontron AG., Zürich, Switzerland). The thrombus volume-per-unit surface area is calculated from the total cross-sectional area of all platelet aggregates in a section normalized by the cross-sectional length of the subendothelial surface. Previous studies have shown that the thrombus volume calculated in this manner shows an excellent correlation with the mass of Cr-labeled platelets deposited on the surface. The maximum thrombus height is the average peak-to-base distance for the three tallest thrombi in a section. Further details of the MOP technique have been previously published.

**Subjects**

**Thrombasthenia.** Six patients with platelet abnormalities characteristic of Glanzmann's thrombasthenia were used in various studies. In all patients, the platelets did not aggregate with 5 x 10⁻⁵ mol/L ADP and epinephrine or with collagen (20 μg/mL). Three previously reported patients (M.C., L.W., M.Mo.) and another subject, C.G. (kindly studied by Dr Graham Jamieson, American Red Cross, Bethesda, Md), have decreased amounts of GPIIb and IIIa detected by periodic-acid Schiff (PAS) staining after separation of platelet membrane proteins by polyacrylamide gel electrophoresis. Patients M.Mo. and L.M. were kindly referred for study by Dr Margaret Johnson and have been shown previously to have decreased amounts of GPIIb-IIIa. Other studies on the thrombasthenic patients have shown decreased amounts (C.G., 5%; M.Mo., 15%; and L.M., 10% of normal) of GPIIIa in binding studies using a monoclonal antibody to GPIIIa (kindly performed by Dr Perumal Thiagaragan, Jefferson Medical College, Philadelphia) and markedly reduced or absent amounts of the GPIIb-IIIa complex (M.C., L.W., M.Mo., L.M., and C.G.) detectable by crossed immunoelectrophoresis of Triton (Sigma Chemical Co, St Louis) X-100-solubilized whole platelets against a polycylindrical rabbit anti-human platelet antibody (kindly performed by Dr Ira Sussman, Montefiore Medical Center, New York). The mean plasma factor VIII-related antigen (VIIIIR:Ag) value (±SE) in these patients, determined as previously described, was 1.30 ± 0.15 U/mL (mean normal value, 0.97; range, 0.55 to 1.61).

**Storage pool deficiency.** Nine patients with SPD had either selective deficiencies of platelet-dense granules (d-SPD) and dense granule bound substances or were deficient in both dense and α-granules (αd-SPD). The patients with d-SPD included six with the albinism variant (d-SPD [A], Hermansky-Pudlak syndrome) and three unrelated, nonalbino patients (d-SPD [NA]). Values of platelet ADP, adenosine triphosphate (ATP), and serotonin for most of these patients have been previously reported and, for more recently studied patients, were obtained by previously described methods. Maximal releasable adenine nucleotides (a reflection of the nucleotides specifically stored in dense granules) were obtained by incubating gel-filtered platelets with 5U/mL thrombin at 37 °C and measuring the released ATP and ADP. Average values for patient groups are shown in Table 1 and demonstrate that the most striking dense granule defect (serotonin and secretable adenine nucleotide values) were found in d-SPD(A) (serotonin values 3% of normal and undetectable adenine nucleotide release), whereas the abnormalities were of a lesser magnitude in d-SPD(NA) patients (serotonin values 45% of normal, adenine nucleotide release 32% of normal). Five patients with combined deficiencies of both α and...
Table 1. Patients With Storage Pool Deficiency

| Granule Defect | No. | ATP  | ADP  | Serotonin (x 10) | α-Granules Substances
<table>
<thead>
<tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td>Total</td>
<td>Releasable (ATP + ADP)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>δ-SPD (A)</td>
<td>6</td>
<td>3.8 ± 0.2</td>
<td>0.2 ± 0.1</td>
<td>0</td>
<td>9 ± 4</td>
</tr>
<tr>
<td>(NA)</td>
<td>3</td>
<td>3.9 ± 0.1</td>
<td>0.6 ± 0.1</td>
<td>1.1 ± 0.3</td>
<td>125 ± 35</td>
</tr>
<tr>
<td>αβ-SPD Family C</td>
<td>4</td>
<td>3.7 ± 0.2</td>
<td>0.7 ± 0.2</td>
<td>0.9 ± 0.3</td>
<td>150 ± 24</td>
</tr>
<tr>
<td>Patient J.C.</td>
<td>1</td>
<td>4.3</td>
<td>0.5</td>
<td>0.7</td>
<td>45</td>
</tr>
<tr>
<td>Normal subjects</td>
<td></td>
<td>4.4 ± 0.2</td>
<td>2.3 ± 0.1</td>
<td>3.4</td>
<td>278 ± 10</td>
</tr>
</tbody>
</table>

Abbreviations: A, albinos; NA, non-albinos.

*Data from Weiss et al.

Values are mean ± SE.

Patients with Storage Pool Deficiency

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<th>Granule Defect</th>
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<th>Serotonin (x 10)</th>
<th>α-Granules Substances</th>
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Abbreviations: A, albinos; NA, non-albinos.

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Values are mean ± SE.

dense granules were also studied. Four patients are members of a family (family C) with moderate α-granule deficiency, and one unrelated patient (J.C.) is severely deficient in α-granules and granule-bound substances. The dense-granule deficiencies in patients with αβ-SPD were less severe than in patients with δ-SPD (Table 1). The plasma VIII:Ag values for SPD patients were as follows: δ-SPD (A), 0.94 ± 0.13; δ-SPD (NA), 0.91 ± 0.11; αβ-SPD (family C), 0.66 ± 0.04; and αβ-SPD (J.C.), 0.62.

Other patients. To compare the results obtained in thrombasthenia and SPD with known defects of platelet adhesion, we studied two previously reported patients (T.H. and A.J.) with the Bernard-Soulier syndrome deficient in GPIb, three patients (C.M., D.R., and W.D.) with severe classical von Willebrand’s disease who have markedly decreased plasma levels of the FVIII-complex (VIII:Ag, <0.03 U/mL by Laurell electroimmunoassay; VIII:RCo, <0.03 U/mL; and VIII:C, <0.01 to 0.05 U/mL, assayed as previously described), and one previously reported patient with a marked congenital deficiency of fibrinogen (plasma fibrinogen level, 3 μg/mL). We also studied a patient with another type of platelet membrane defect (Scott syndrome) characterized by an isolated deficiency of platelet procoagulant activity and impaired binding of Factor Xa.

Normal subjects. Control values were obtained by studying normal hospital personnel, ages 25 to 50.

Blood Values

Platelets were counted on blood collected into EDTA by an electronic counting device (model S. Coulter Electronics, Hialeah, Fla). The average values for platelet counts (per μL) were as follows: thrombasthenic patients, 251,000 (187,000 to 340,000); nonalbinos with δ-SPD (NA), 296,000 (264,000 to 353,000); albinos with δ-SPD (A), 292,000 (210,000 to 349,000); family C with αβ-SPD, 197,000 (152,000 to 263,000); and patient, J.C., with αβ-SPD, 295,000. The average count in patients with von Willebrand’s disease was 262,000 (160,000 to 345,000); in the two patients with the Bernard-Soulier syndrome, 85,000 and 103,000; in the patient with fibrinogen deficiency 300,000; and in the patient with the Scott syndrome (Xa binding defect), 338,000. For the normal subjects used in the study, the average value was 262,000 (178,000 to 355,000). All normal subjects with platelet counts greater than 355,000 were excluded from the study.

Monoclonal Antibody to GPIIb-IIIa

This antibody (M148, a generous gift from Professor Roger Hardisty) was produced by the hybridoma technique against human medulloblastoma and has been reported to be directed, in platelets, specifically against GPIIb-IIIa. It induces functional defects in platelet aggregation that are entirely similar to those observed in patients with thrombasthenia. A control monoclonal antibody (UJ13A) known not to bind to platelets was also studied.

Statistics

The significance of differences between mean values was evaluated by Student’s t test, adjusting, where appropriate, for small sample sizes and inhomogeneity of variances, and the correlation coefficient (r) between two variables from the least-square regression line.

RESULTS

Studies Using Nonanticoagulated Blood

In these studies, venous blood was drawn immediately through the annular perfusion chamber for varying periods of time, and the wall shear rate was controlled by adjusting the flow rate. The various parameters of platelet–vessel wall interaction were determined as described in Materials and Methods and included C platelets, S platelets, C + S, thrombus volume per subendothelial surface area, and average maximum thrombus height.

Normal subjects. Analysis of the vessel segments after perfusion of normal blood revealed the presence of platelets attached to the subendothelial surface in varying stages of activation, including a small percentage that had contacted the surface but had not spread, a larger percentage that had spread and degranulated, and platelet thrombi on top of S platelets.

Thrombasthenia. In patients with thrombasthenia, platelets were observed on the subendothelium as a monolayer of both C and S platelets. The absence of thrombus is similar to findings previously reported using citrated blood and indicate a complete absence of platelet-platelet interaction in thrombasthenia even when the studies are performed without the use of anticoagulants.

Values obtained for C + S in thrombasthenia are shown in Fig 1 (top) for different shear rates (650, 1,300, 2,600, and 3,300 sec−1) and exposure times. At no condition was a significant decrease in C + S ever observed (at 1,300 sec−1 and three minutes it was somewhat increased). In contrast, adhesion was decreased at all shear rates studied (650 to
Plates were normal in thrombasthenia, the number of platelets that had contacted the subendothelium without spreading was consistently increased (Fig 1, top), indicating that thrombocytopenic platelets in nonanticoagulated blood do not spread normally on this surface.

Adhesion values (Fig 1, bottom) were somewhat dependent on the shear rate. At 650 sec\(^{-1}\), values in \(\delta\)-SPD were increased (five- and ten-minute perfusion) and in \(\alpha\delta\)-SPD were either normal (five-minute perfusion) or increased (ten-minute perfusion). At 2,600 sec\(^{-1}\), values were normal in \(\delta\)-SPD, but decreased in \(\alpha\delta\)-SPD (Fig 1, bottom, and Table 2).

The most striking impairment observed was in platelet thrombus dimensions (thrombus height and thrombus volume), and the abnormalities were related to the type of SPD. Among patients with \(\delta\)-SPD, thrombus dimensions (thrombus height in particular) were more strikingly decreased in albino patients, \(\delta\)-SPD(A), having severe dense granule defects than in the nonalbino patients with \(\delta\)-SPD(NA) (Table 2). Thrombus dimensions were decreased in general, to an even greater extent in \(\alpha\delta\)-SPD (Table 2). The greatly diminished values of thrombus volume and thrombus height in \(\alpha\delta\)-SPD were particularly evident at 2,600 sec\(^{-1}\) in patient J.C. with the most severe \(\alpha\)-granule defect.

**Storage pool deficiency.** Studies in SPD were performed at shear rates of 650 sec\(^{-1}\) (five- and ten-minute perfusion) and 2,600 sec\(^{-1}\) (two-minute perfusion). Values for \(C + S\), thrombus volume, and thrombus height obtained in subgroups of \(\delta\)-SPD (A and NA) and \(\alpha\delta\)-SPD (family C, and patient J.C.) are shown in Table 2. The pooled \(C + S\) and \(S\) values for the two types of granule defects (\(\delta\) and \(\alpha\delta\)-SPD) are shown in Fig 1 (bottom) for comparison with the other platelet disorders depicted in that Figure.

Studies Using Citrated Blood

Studies using citrated blood were performed at shear rates of 800 and 2,600 sec\(^{-1}\) and a perfusion time of five minutes. As in previous studies\(^3\), the platelet thrombus heights \((14 \pm 2 \text{ and } 14 \pm 4 \mu m)\) and thrombus volumes \((0.81 \pm 0.27 \text{ and } 0.82 \pm 0.30 \mu m^3/\mu m^2)\) in normal subjects were considerably less than the values obtained (Table 2) for nonanticoagulated blood.

### Table 2. Platelet-Subendothelial Interaction in SPD in Nonanticoagulated Blood

<table>
<thead>
<tr>
<th>Shear Rate (sec(^{-1}))</th>
<th>Platelet Adhesion (C + S) (Percent Surface Coverage)</th>
<th>Thrombus Volume ((\mu m^3/\mu m^2))</th>
<th>Maximum Thrombus Height ((\mu m))</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Exposure Time (min)</td>
<td>650</td>
<td>2,600</td>
</tr>
<tr>
<td>Normal subjects (N = 19, 13, 16)*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SPD</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(\delta)-SPD</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A (N = 4, 4, 4)</td>
<td>47 ± 2 59 ± 9 38 ± 4</td>
<td>1.6 ± 0.2 3.5 ± 0.65 1.0 ± 0.45</td>
<td>33 ± 25 46 ± 5 16 ± 3†</td>
</tr>
<tr>
<td>NA (N = 2, 2, 3)</td>
<td>36 ± 4 47 ± 2 29 ± 4</td>
<td>1.5 5.7 1.8 ± 0.5</td>
<td>44 77 41 ± 9‖</td>
</tr>
<tr>
<td>(\alpha\delta)-SPD</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Family C (N = 3, 3, 3)</td>
<td>27 ± 1 41 ± 2 24 ± 15§</td>
<td>0.5 ± 0.1† 2.2 ± 0.6‡ 0.6 ± 0.3‡</td>
<td>21 ± 25 38 ± 25 17 ± 3†</td>
</tr>
<tr>
<td>Patient J.C.</td>
<td>37 75‖ 20</td>
<td>1.2 1.9 0.07‖</td>
<td>39 32‖ 4‖</td>
</tr>
</tbody>
</table>

*No. of subjects for the three flow conditions shown.
†P < .001 relative to normal subjects.
‡P < .01 relative to normal subjects.
§P < .05 relative to normal subjects.
‖P < .05 relative to albino subjects with \(\delta\)-SPD(A).
Greater than 2 SD from the mean value of normal subjects.

Values shown are the mean ± SE for N ≥ 3, average for N = 2, or single value for patient J.C.
**GPIIb-IIIa deficiency.** In contrast to the normal values observed in nonanticoagulated blood, C + S in citrated blood was decreased in thrombasthenia by an average of 50% and 59%, respectively, at both 800 and 2,600 sec$^{-1}$ (Fig 2), but no increase in contact platelets was observed. The magnitude of the adhesion defect was less than in the Bernard-Soulier syndrome (Fig 2) but was similar to that obtained in patients with severe von Willebrand’s disease under the same conditions (Fig 2). In the latter disorder, however, the decreased adhesion (33% and 68% reduction at 800 and 2,600 sec$^{-1}$) was more shear-rate dependent (Fig 2). Addition of a monoclonal antibody against GPIIb-IIIa to normal blood completely eliminated platelet-platelet interaction (absent thrombi) on the subendothelium when evaluated after perfusion for five minutes at a shear rate of 2,600 sec$^{-1}$ and, hence, produced the type of defect observed in thrombasthenia (data not shown). This antibody reduced platelet adhesion at a shear rate of 2,600 sec$^{-1}$ to a level (Fig 3) that was comparable to the values observed in thrombasthenia. In contrast, no inhibition of adhesion was observed at a shear rate of 200 sec$^{-1}$ (Fig 3).

**Storage pool deficiency.** In αβ-SPD, adhesion was decreased significantly ($P < .01$) at 800 sec$^{-1}$ and almost significantly ($P = .07$) at 2,600 sec$^{-1}$ (patient J.C., with the most severe defect, was not, unfortunately, studied under these conditions).

In δ-SPD, adhesion values at 800 sec$^{-1}$ were virtually identical to those obtained in normal subjects (Fig 2). At 2,600 sec$^{-1}$, the value (30.2% ± 7.1%) was 33% less than normal (44.7% ± 4.4%), but this reduction failed to reach statistical significance at the 5% level ($P = .08$). Since considerable variation was obtained among subjects in this group of nine patients with δ-SPD, we examined other parameters that could have influenced adhesion values at 2,600/sec$^{-1}$. Among several parameters that could have influenced adhesion, we found no correlation with the plate-let count or plasma VIII:Ag value. However, a strong correlation ($r = .88, P < .01$) with the hematocrit value was observed, whereas no correlation ($r = .27$) was observed in normal subjects. In addition, as seen in Fig 4, when patients and control subjects were each subdivided into two groups (one with hematocrit values 40 or less and the other with values greater than 40), it could be seen that the decreased adhesion in δ-SPD was limited to those five subjects with the lower hematocrits. In these patients (hematocrit value, 38.4 ± 0.7) the adhesion value was 17.7% ± 3.7%, compared with a value of 44.7% ± 4.6% ($P < .01$) in the subset of control subjects with the comparable hematocrits (39.0 ± 0.6). In contrast, the average adhesion value (Fig 4) in the four patients with higher hematocrit values (43.0 ± 0.4) were not significantly different than in controls with comparable hematocrit values (44.0 ± 0.5).

**Other subjects.** In the patient with severe fibrinogen deficiency, platelet adhesion was either normal (2,600 sec$^{-1}$) or somewhat increased (800 sec$^{-1}$). Adhesion was normal at both shear rates in the patient with Scott syndrome (Xa binding defect) (Fig 2).

**DISCUSSION**

In a previous study, we have described and formally analyzed a model for platelet adhesion on subendothelium in our perfusion system and shown how defects in either initial platelet contact or subsequent platelets spreading could effect the measured values of C, S, and C + S (defined as total adhesion) as a function of time. From the results of that study, we concluded that GPIIb plays a role in platelet adhesion by mediating the initial contact of platelets with the subendothelial surface. A major purpose of the present study was to evaluate a possible role for GPIIb-IIIa in mediating platelet adhesion to subendothelium by studying platelets deficient in these glycoproteins. In addition, we used patients with SPD to evaluate whether granule-bound substances might also mediate adhesion and thrombus formation.

In studies using nonanticoagulated blood, we identified several properties of platelets that influence platelet-platelet interaction (thrombi) on subendothelium. As previously observed with citrated blood,41 platelet thrombi were completely absent in thrombasthenia, indicating that GPIIb-IIIa is required for platelet-platelet interaction. Thrombi were also smaller (but not absent) in patients selectively deficient in dense granules (δ-SPD), and the degree of abnormality was in proportion to the magnitude of the δ-SPD (Table 2). These findings strongly suggest that dense granule–bound substances, in all likelihood ADP, either influence the growth of thrombi on subendothelium or help to maintain thrombus stability in growing thrombi subjected to the...
high-shear stresses (650 to 2,600 sec⁻¹ in the present study) comparable to those encountered physiologically.⁴ The even smaller thrombi (Table 2) observed in patients with combined deficiencies of α and dense granules (αd-SPD) further suggest an additional role for substances, including vWF,³¹ stored in α-granules as well.

Using both nonanticoagulated and citrated blood, we observed abnormalities in the interaction of platelets with the subendothelium that also indicate a possible role for GPIIb-IIIa in mediating platelet adhesion. In nonanticoagulated blood, we found that the number of C platelets in thrombasthenia was consistently higher than in normal blood, even where C + S values were comparable in the two groups (Fig 1), indicating impaired spreading of thrombasthenic platelets on the subendothelial surface. In citrated blood (Fig 2), the 50% to 59% reduction in C + S values were comparable to those observed in von Willebrand's disease (although the abnormality in the latter disorder was some-

what more shear dependent, suggesting different mechanisms for the defect). In addition, when GPIIb-IIIa in normal platelets was blocked by a monoclonal antibody to GPIIb-IIIa, the resulting reduction in platelet adhesion was comparable to that observed in thrombasthenia (Fig 3). The decreased values for C + S that we obtained in the present study in the thrombasthenic patients are at some variance with the conclusions of a previous study using citrated blood and a shear rate of 800 sec⁻¹.⁴¹ However, these earlier studies were conducted at longer perfusion times for which the surface in normal controls approaches surface saturation with platelets, so that potential differences between patients and controls are reduced. The values obtained at the shorter perfusion times of the present experiments are not in the saturation region and, hence, would be more likely to demonstrate differences between patients and controls. In fact, the results are quite compatible with the originally reported patient results for longer perfusion times, which were at the lower limit of normal even in that study.

The results of our study as well as those of a recent report demonstrating a requirement for GPIIb in mediating platelet‐collagen adhesion⁴⁴ indicate that the GPIIb-IIIa complex may be considered an adhesion site on the platelet surface. As previously indicated, the process by which platelets (and other cells¹) adhere to a surface consists of an initial attachment phase (which we denote as C platelets in our morphometric analysis) and a subsequent spreading (measured as S platelets) on the subendothelium. We believe that the results obtained with nonanticoagulated blood (increased values for C, but normal C + S) and citrated blood (decreased C + S values) complement each other in suggesting an abnormality in platelet spreading. In a previous paper, we analyzed, on theoretical grounds, how defects in either platelet C or S will effect the values for C + S as a function of both perfusion time and another critical variable, the rate (K₉) at which platelets detach from the surface.⁴ The model predicted that under conditions in which the rate of detachment was low, the major effect of a platelet spreading defect would be consistently higher values of C at all perfusion times, with relatively unaffected values for C + S. The values we obtained in nonanticoagulated blood are consistent with such a defect. In contrast, an increased detachment rate would produce conditions that are more sensitive for detecting a defect in C + S, but relatively less sensitive for detecting an
increase in platelet C, as observed in our studies on citrated blood. Although we have no direct data to suggest that removal of calcium is more favorable for the detachment of contacted platelets, such a mechanism might explain how a defect in platelet spreading (because of a GPIIb-IIIa deficiency) could result in increased C values in nonanticoagulated blood and decreased C + S values in citrated blood.

Another possible reason why a reduction in C + S in thrombathaenia is observed in citrated but not nonanticoagulated blood is related to potential effects of platelet thrombus size in influencing adhesion. In previous studies, we have shown that the presence of large thrombi tend to reduce platelet adhesion on the surface. Two potential mechanisms may contribute to the reduced adhesion in the presence of thrombus growth, especially in nonanticoagulated blood in which thrombus heights reach 100 to 200 μm, (in citrated blood, thrombus heights rarely exceed 30 μm). One possibility is that platelets near the surface of growing thrombi are depleted in number by being adsorbed by the thrombus mass. The other possibility is that thrombi shelter the surface downstream of the growing thrombus from circulating platelets. Both these mechanisms contribute to the localized depletion of the platelet boundary layer concentration; moreover, the larger the thrombus growth, the greater the depletion. Therefore, the smaller thrombi observed in patients with SPD (Table 2 and Fig 2) could explain the relatively higher C + S values obtained at some conditions in these patients. The aforementioned observations would predict an even greater enhancement of adhesion in thrombathaenia, where thrombi are completely absent, than in SPD. That adhesion was generally normal could, therefore, reflect the combined effects of absent thrombi (tending to increase adhesion) and a true adhesion defect (tending to reduce it). In contrast, thrombi in citrated blood are considerably smaller than in nonanticoagulated blood, thereby reducing the influence of tall thrombi on adhesion and enhancing the possibility of observing decreased C + S values in thrombathaenia.

The mechanism by which GPIIb-IIIa mediates platelet spreading and adhesion on subendothelium remains to be determined. Since adhesion was not decreased (and was, in fact, increased) in a patient with severe fibrinogen deficiency, it is unlikely that the binding of fibrinogen to GPIIb-IIIa plays a role. The recent observations that thrombin and ADP can promote the binding of vWF to platelets through GPIIb-IIIa suggest that this protein, in addition to supporting adhesion through binding to GPIb, may also mediate platelet–vessel wall attachment through binding to GPIIb-IIIa. The decreased adhesion in some patients with δ-SPD (as in fawn-hooded rats with SPD) is also consistent with the recent conclusions of Timmons et al that released platelet ADP could also play a role in this mechanism. However, platelet adhesion in δ-SPD was strongly influenced (at 2,600 sec⁻¹) by the hematocrit value, even within the relatively narrow range of hematocrit values (36 to 44) observed in the study (Fig 4). These findings suggest a possible role for red cells that could bypass the platelet contribution with increasing shear rates, as was previously suggested. This possibility is currently under investigation.

The question whether the aforementioned conclusions are valid must take into account the recent studies that have shown that GPIIb-IIIa–mediated binding of exogenously added vWF does not occur in the presence of physiologic concentrations of fibrinogen. However, this would not necessarily apply to vWF present in platelet α-granules and secreted at the platelet surface following platelet activation. Previous studies, for example, have shown a higher concentration of high–molecular weight multimers of vWF in platelets than in plasma, and it is possible that platelet vWF could have a higher affinity for GPIIb-IIIa than plasma vWF. In addition, whereas the molar ratio of fibrinogen (10⁻⁵ mol/L) to vWF (5 × 10⁻⁹ mol/L for a subunit molecular weight (mol wt) of 220,000 in plasma is about 200, the ratio in platelets is approximately 14 (assuming a fibrinogen concentration of approximately 100 μg/10⁹ platelets, a VIIIIR:Ag concentration of 0.43 U/10⁹ platelets, and an assumption that, as in plasma, 1 unit of VIIIIR:Ag per ml is equal to 5 × 10⁻⁴ mol/L for a subunit mol wt of 220,000). In addition, vWF in subendothelium has also been demonstrated to play a role in mediating platelet adhesion, and it is possible that vWF bound to a surface may more successfully compete with fibrinogen for GPIIb-IIIa on platelets that have initially attached through GPIb4 than does plasma vWF. Finally, α-granule proteins other than vWF (such as fibronectin and thrombospondin) could also play a role in mediating platelet adhesion through their binding to GPIIb-IIIa, and the reduced levels of platelet adhesion that we observed in patients with δ-SPD do not, at present, permit us to choose among several α-granule proteins that could be important. Further studies on the complex interaction of vWF, membrane glycoproteins, and various granule substances could have important implications for understanding hemostasis and thrombosis.

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Platelet adhesion and thrombus formation on subendothelium in platelets deficient in glycoproteins IIb-IIIa, Ib, and storage granules

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