Possible Thrombosthenin Defect in Glanzmann’s Thrombasthenia

By Francois Booyse, Donna Kisieleski, Ruth Seeler, and Max Rafelson, Jr.

Microquantitation of thrombosthenin by single radial immunodiffusion in conjunction with a specific immuno-histochemical antibody staining technique has indicated an immunologic defect or absence of the surface-localized thrombosthenin (S-thrombosthenin) in platelets from Glanzmann’s thrombasthenic patients. In addition, ADP- and ATP-induced changes of the surface of normal platelets could not be demonstrated with Glanzmann’s thrombasthenic platelets.

GLANZMANN’S THROMBASTHENIA is a rare but well-studied bleeding disorder in which the abnormality is the apparent unresponsiveness of the platelet membrane to external stimuli. These patients have a normal platelet count, prolonged bleeding time, defective or absent clot retraction, low platelet fibrinogen, and absence of platelet aggregation in the presence of ADP or thrombin. Clot retraction is partially restored by the addition of magnesium. However, the primary reason for poor clot retraction appears to be the inability of these platelets to aggregate normally and not to a defect in clot retraction per se.

No satisfactory explanation for these in vitro abnormalities in thrombasthenia has been advanced. The platelet content of the actomyosinlike contractile protein, thrombosthenin, has been measured in patients with thrombasthenia and reported to be normal. However, it is unlikely that the semiquantitative technique employed could detect the small differences in thrombosthenin content described in this report. The purpose of this report is to describe a possible abnormality in the thrombosthenin localized on the surface of platelets (S-thrombosthenin or surface thrombosthenin) from two patients with Glanzmann’s thrombasthenia using the following two newly developed techniques: microquantitation of thrombosthenin by single radial immunodiffusion, and specific immunohistochemical staining of surface-localized platelet antigens using a soluble unlabeled antibody-enzyme complex.

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MATERIALS AND METHODS

Both thrombasthenic patients showed hemorrhagic symptoms that began with bruising and epistaxis. They also suffered innumerable nasal hemorrhages in addition to ecchymosis and prolonged bleeding from minor lacerations. The partial thromboplastin times, prothrombin times, and insolubility of the clots in urea were normal. Platelet counts ranged between 200,000-555,000 platelets/cu mm. Bleeding times were always greater than 10 min, and clot retraction absent. Platelet adhesion was 0%, and no aggregation occurred with collagen, epinephrine, and $2 \times 10^{-6} M$ and $2 \times 10^{-4} M$ ADP. Both these patients, therefore, fulfill the classical description of Glanzmann's thrombasthenias.

Isolated platelets and platelet-rich plasma (PRP) were prepared from 5-10 ml of freshly drawn blood by differential centrifugation as previously described. Standard ACD (formula A-USP) was used as anticoagulant.

Immunohistochemical staining was carried out on freshly isolated platelets, fixed briefly (10 min) with 4 volumes of 4% warm (37°C) gluteraldehyde, containing 0.1 M cacodylate buffer, pH 6.7. Details of the antibody staining technique have been described elsewhere.

Platelets were treated with horseradish peroxidase by a modification of the method described by Behnke. Instead of adding the horseradish peroxidase directly to PRP, the enzyme was added to fixed platelets. PRP (2 ml) was routinely fixed for 30 min with 4 volumes of 4% warm (37°C) gluteraldehyde. The fixed platelets were pelleted at 1100 g for 10 min, washed twice with saline, and incubated with 3 ml of saline containing 3 mg of horseradish peroxidase for 1 hr at room temperature. ADP- and ATP-induced surface changes were studied by adding these agents in $1 \times 10^{-6} M$ and $0.5 \times 10^{-6} M$ final concentrations, respectively, to warm PRP and by stopping the activation process after 2-4 sec by the addition of 4 volumes of 4% warm (37°C) gluteraldehyde. These activated platelets were then washed with saline and treated with horseradish peroxidase.

Thrombosthenin was quantitated by the technique of single radial immunodiffusion using absorbed monospecific rabbit antihuman thrombosthenin serum.

RESULTS

The thrombosthenin content of normal platelets obtained by single radial immunodiffusion is $15.2 \pm 1.62\%$ (2 SD) of the total platelet protein. Analysis of two patients with Glanzmann's thrombasthenia gave thrombosthenin values between 12.9% and 13.2% of the total platelet protein (Table 1). This 2–2.3% decrease corresponds well with the normal content of S-thrombosthenin of 1–2% of the total platelet protein reported previously.

Immunohistochemical staining of fixed, intact normal platelets with rabbit antihuman thrombosthenin serum showed the presence of surface-localized or S-thrombosthenin on the outer surface of these cells (Fig. 1a,b). However, specific antibody staining of Glanzmann's thrombasthenic platelets did not

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<th>Table 1. Microquantitation of Thrombosthenin in Normal (12 Individuals) and Glanzmann's (2 Patients) Platelets</th>
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<td>Thrombosthenin Content* (%)</td>
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*Expressed as percentage of total platelet protein.†Mean ±2 SD.
show any surface reaction, suggesting the absence or immunologic defect of S-thrombosthenin in these platelets (Fig. 2).

Normal platelets undergo very extensive and rapid (within 2–4 sec) surface changes when treated with aggregating agents such as thrombin, ADP, or actomyosin dissociating agents such as ATP and salyrgan.5,9 These extended surface structures can then be studied and visualized by their increased and extensive nonspecific interaction with horseradish peroxidase as shown in Fig. 3a,b. When thrombasthenic platelets are treated with ADP and ATP, followed by horseradish peroxidase treatment, no such surface changes are produced (Fig. 3c,d). It, therefore, appears that the surface of thrombasthenic platelets is completely unresponsive to ADP and ATP activation under the conditions described here.

DISCUSSION

Thrombosthenin comprises about 15% of the total platelet protein. By means of the specific immunohistochemical staining technique and controlled KCl extractions, we have shown that this protein is localized both on the platelet surface and in the cytoplasm.5,6 Approximately 6–10% of the total thrombosthenin, representing about 1–2% of the total platelet protein, is localized on the platelet surface and has been designated surface or S-thrombosthenin. The remaining 90–95% of the protein, representing about 13% of the total platelet protein, is located within the platelet and has been designated cytoplasmic or C-thrombosthenin.6 In addition, we have proposed a molecular model for platelet aggregation directly involving the formation of interplatelet S-thrombosthenin bridges between aggregating platelets.10 Such interplatelet bridges do, in fact, form and have been shown to contain immunohistochemically identifiable thrombosthenin.5

The surface changes induced by various agents, such as thrombin, ADP, and ATP, have been attributed to changes in the S-thrombosthenin. Immuno-
Fig. 2. Antibody-stained Glanzmann's thrombasthenic platelets. (A) Control, using normal rabbit serum; (B) sample, using antithrombosthenin serum. × 19,000.

Fig. 3. Surface changes induced with ADP and ATP, stained with specific antibody. (A) Normal platelets, ADP-activated; (B) normal platelets, ATP-activated; (C) Glanzmann's platelets, ADP-activated; (D) Glanzmann's platelets, ATP-activated. × 26,000.
histochemical staining has indicated that the surface extensions formed, prior to interplatelet bridge formation, does contain thrombosthenin. The absence of antibody staining in conjunction with the decreased amount of total thrombosthenin and lack of surface activation with ADP and ATP would, therefore, seem to suggest that thrombasthenic platelets are either lacking in S-thrombosthenin or have this protein altered in such a manner that it does not react with specific antithrombosthenin serum. In the latter case, the altered S-thrombosthenin must also be unresponsive to thrombin, ADP, and ATP. Although we prefer the former explanation, this point must be resolved by further experimentation. In either case, a specific defect in S-thrombosthenin provides a reasonable explanation for at least part of the surface defect described for thrombasthenic platelets and also provides additional evidence in favor of a contractile protein model for platelet aggregation.

REFERENCES

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