CONCISE REPORT

Micropipette Aspiration of Human Blood Platelets: A Defect in Bernard-Soulier's Syndrome

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Previous reports have suggested that platelets from patients with Bernard-Soulier's syndrome (BSS) are not giant cells. Rather, they are normal-sized in suspension, but spread out on glass slides more readily than control cells, yielding the impression of being giant. The present study has used cell sizing techniques, electron microscopy, and micropipette aspiration to evaluate platelets from three patients with BSS. Cell sizing techniques revealed that BSS platelets were considerably larger than normal. The increased size was confirmed in electron microscopic studies of BSS platelets fixed in suspension. However, the BSS platelets did not contain increased amounts of internalized surface membrane considered to be the source of membrane necessary for excessive spreading. A possible explanation for increased spreading of BSS platelets was found in studies of their resistance to deformation in micropipettes. BSS platelets were much less resistant to deformation than normal cells or other abnormal platelets when aspirated under the same negative pressure. Their unusual deformability may explain the tendency of BSS platelets to spread more readily than normal cells on glass slides.

MATERIALS AND METHODS

Blood for the present study was obtained from well characterized normal donors and patients with inherited platelet disorders, after informed consent. The samples were mixed immediately with 3.8% trisodium citrate or citrate-citric acid, pH 6.5 (9.3 mM sodium citrate, 7.0 mM citric acid, and 140 mM dextrose) in a ratio of 9 parts blood to 1 part anticoagulant. Platelet-rich plasma (PRP) was separated from whole blood by centrifugation at 100 g for 20 min at room temperature. Samples of PRP were mixed with an equal volume of the citrate anticoagulant and centrifuged to pellets. Supernatant was discarded and the pellets resuspended in phosphate buffer and incubated with adenosine (5 mM), and theophylline (3 mM) to inhibit activation. Washed platelets were maintained at room temperature until used in specific experiments. Sizing of platelets was carried out in a Coulter Model S+2 and Telefunken electronic particle sizing systems.

Micropipettes with an average internal diameter of 0.7 μm (range 0.6–0.8 μm) were prepared as described previously. In brief, boron silicate capillary pipettes, 1 mM in external diameter, were drawn in a two-stage process. Internal diameters were estimated as reported. The pipettes were filled with phosphate buffer and secured in a micromanipulator. Drops of washed normal and defective platelets were placed on siliconized glass slides mounted on a Reichert microscope equipped with a 100× water-immersion objective and Normarski interference phase-contrast optics. Images were observed directly and displayed on a video monitor and preserved on a video cassette recorder. A calipers was used to measure the lengths of segments aspirated into the pipette during projection on the screen.

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or from videotapes. Lengths were standardized by comparisons with measurements on a 10-μm Zeiss grid projected from the microscope stage onto the videoscreen.

The tip of the pipette was advanced under direct vision, while a constant negative pressure of 10 cm H2O was maintained. Preliminary studies revealed that this pressure uniformly deformed discoid platelets without causing passage through the pipette. The lengths of platelet membrane sleeves aspirated into micropipettes were determined from the videoscreen directly or later from videotapes, as described above. Each data point was based on measurements of 15–20 aspirated platelets. The cells were retained in the pipette for 15–30 sec and then extruded. Observations on the extruded cells continued for 5 min. Means and standard deviations derived from these measurements and the statistical significance were determined using Student's t test.

Electron Microscopy

Samples of platelet-rich plasma from the three patients with BSS evaluated in this study were prepared for study in the electron microscope. The samples were fixed initially in glutaraldehyde, followed by osmic acid, and embedded in Epon 812 according to procedures developed in this laboratory. Thin sections were stained with lead citrate and uranyl acetate and examined in a Philips 301 electron microscope.

Patients

Defective platelets for this study were provided by one patient with thrombasthenia,12 two patients with May-Hegglin anomaly,16,17 two with Epstein's syndrome,18 two patients with gray platelet syndrome,19 and three patients with BSS. The defects in each patient have been characterized in previous morphological, physiologic, and biochemical studies.

RESULTS

Aspiration of Normal Platelets

Samples of platelets from 15 normal donors were evaluated. The average extension length of discoid untreated platelets under a constant negative pressure of 10 cm H2O was 0.7 ± 0.1 μ (Fig. 1). Extension lengths were the same whether generated at the edge or in the center of the disc, suggesting a uniform distribution of the forces influencing membrane resistance to deformation. Upon expulsion from the pipette, platelets regularly regained their discoid form within 1 min.

Aspiration of Abnormal Platelets

Platelets from patients with thrombasthenia, gray platelet syndrome, and Epstein's syndrome responded to micropipette aspiration in the same pipettes as normal platelets (Fig. 1). May-Hegglin syndrome platelets were slightly less resistant than normal, but the difference was not statistically significant. Only BSS platelets were more deformable when aspirated under constant negative pressure of 10 cm H2O, and the difference was striking. All three patients gave similar results. Their platelets were much less resistant to deformation than normal cells when measured by this technique.

Fig. 1. Micropipette elastometry of normal and abnormal platelets (10 cm H2O). Suspensions of washed platelets, prepared as described in the text, were aspirated into glass micropipettes with a mean internal diameter of 0.7 μ. The lengths of aspirated segments on platelets drawn into the micropipette were measured on images projected from videotapes. Each cell was watched for 5 min following extrusion from the pipette to observe the ensuing morphological changes. Fifteen to 20 cells were analyzed to obtain each data point. The vertical bars indicate the mean extension lengths in micrometers, and the linear extensions represent standard deviations of the mean. Untreated control platelets developed extensions of 0.74 ± 0.13 μM and rapidly recovered their discoid form after extrusion from the micropipette. Thrombasthenic (Thromb), gray platelet syndrome (Gray), and Epstein's (Epstein) syndrome platelets were as resistant to deformation in the micropipette as normal cells. May-Hegglin (May-Heg) appeared more deformable than normal, but the difference was not statistically significant. Only Bernard-Soulier's syndrome (BSS) platelets revealed a marked difference from the response of normal cells. Under a negative pressure of 10 cm of H2O, the membrane extensions drawn from BSS cells were 3 times as long as on control platelets.

Platelet Sizing

Normal platelets evaluated in electronic particle systems have a mean platelet volume (MPV) of 8.9 ± 1.5 fl.14 All three BSS patients had large platelets. In 2, the values for MPV were 26.06 and 29.2 fl, respectively. The third patient had an MPV of 18.2 fl.

Ultrastructure

The fine structure of platelets from the three patients with BSS was consistent with the sizing data. Some cells were normal-sized, most were large and some were huge (Fig. 2). Aside from massive size, the morphology of BSS platelets did not differ significantly from normal cells (Fig. 3). Mitochondria, dense bodies, and α-granules were present in the giant platelets. Microtubules were evident in a marginal band, even though giant BSS platelets often appeared to be spherical in form. Elements of the open canalicular systems were apparent in BSS platelets, but did not appear to be overdeveloped.

DISCUSSION

The present investigation has employed the technique of micropipette aspiration to examine the sur-
face characteristics of abnormal platelets. Three patients with the BSS were found to have platelets markedly different from normal cells. Under a constant negative pressure of 10 cm of H$_2$O, sleeve-like segments drawn into micropipettes from BSS cells were nearly three times longer than the extensions aspirated from normal platelets.

The reduced resistance of the surface membranes of BSS platelets to deformation may have been related to their known deficiency in GPIb. If a deficiency in this constituent contributes to increased deformability, then platelets lacking other glycoproteins might also be less resistant than normal. However, a patient with thrombasthenia lacking platelet surface membrane GPIIb and GPIIIa revealed a resistance to deformation identical to normal cells.

Platelets from the three patients with BSS in this investigation were large. It is possible that giant size may predispose to a reduced resistance to deformation. Three other giant platelet syndromes were tested to evaluate the possibility that size is a major factor in mechanical resistance of the surface membrane. Although variations were noted, the deformability of platelets from patients with the gray platelet syndrome, May-Hegglin anomaly, and Epstein’s syndrome did not differ significantly from normal cells on aspiration into micropipettes.

Milton and Frojmovic have suggested that BSS platelets are normal-sized, but unusually deformable. Interaction with glass or other surfaces causes the cells to spread out into thin films, which appear much larger than normal platelets. In order to expand in this manner, BSS platelets would have to synthesize additional membrane or provide it from some other source. Milton and Frojmovic proposed that the open canalicular system was excessively developed in BSS platelets and its extrusion on contact with foreign surfaces, or during shape change, provided the membrane for markedly increased spreading.

The open canalicular system is overdeveloped in certain giant platelet syndromes, such as May-Hegglin anomaly and Epstein’s syndrome. However, giant cells from patients with these disorders do not spread excessively and were not significantly different from normal platelets on aspiration into micropipettes. BSS platelets, on the other hand, do not appear to have excessive amounts of open canalicular system. Platelets from the three patients evaluated in this investigation were studied in the electron microscope. Channels of the open canalicular system were present, but did not appear excessive.

Thus, the circulating platelets of the three patients with BSS evaluated here were very large when fixed in suspension or examined in electronic sizing systems. Because they are giant cells, it is uncertain whether their large size on peripheral blood smears is due to unusual spreading. If they do spread excessively or undergo hypervolumetric shape change, it is unlikely that the membrane for the increased surface area comes from an overdeveloped system of surface connected channels. Rather, the present study provides an alternative explanation. BSS platelets were very deformable and developed surface extrusions nearly
three times longer than normal or other types of defective platelets when aspirated into micropipettes. The decreased resistance to deformation would favor increased spreading and shape change, although it would not provide new membrane for those processes. As the cells are already large in suspension, new sources of membrane would not be necessary. Their unusual pliability alone would permit spreading into thinner films, tending to make them appear unusually large on peripheral blood smears. It is possible that loss of transmembrane association of GPIb with actin-binding protein inside the platelet membrane may be responsible for the unusual deformability of BSS platelets.20

REFERENCES

20. Fox JEB, Baughn AK, Phillips DR: Direct linkage of GPIb to a Mz = 250,000 polypeptide in platelet cytoskeletons. Blood 62(suppl 1):255a, 1983 (abstr)
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