Glycoprotein Ilb-llla and Glycoprotein IV Expression on Bernard-Soulier Syndrome Platelets

To the Editor:

We have read with interest the article by Kunishima et al in which they report a new molecular variant of Bernard-Soulier syndrome (BSS). Kunishima et al report a patient with a mean platelet volume of 14.7 μm³. Later in the text, they describe binding levels of monoclonal antibody (MoAb) anti-glycoprotein (GP) Ilb-Illla comparable to the control, as measured by flow cytometric techniques. One expects that a larger mean platelet volume should result in an increased surface area on platelets and thus in increased expression of glycoproteins normally present on platelet surface. We have access to a BSS patient with an increased mean platelet volume (15.9 μm³) in whom the binding of MoAb anti-GP Ilb-Illla (CD41a) was markedly increased with respect to normal controls. Moreover, a parallel increase in the binding of anti-GP IV (CD36) on platelet membrane was also found. Platelet membrane mean fluorescence intensity (in arbitrary units) was for anti-CD41a 195 ± 52 in normal controls and 440 in the BSS patient, whereas for anti-CD36 it was 156 ± 45 (mean ± SD, n = 5) in normal controls and 450 in the BSS patient (Fig 1). Our data indicate a consistent increase in the presence of both GP Ilb-Illla and GP IV on abnormally large platelets. These findings are perfectly compatible with normal density of those glycoproteins if we take into consideration the larger surface area of BSS.

Our study was performed in whole blood collected in citrate phosphate dextrose (citrate final concentration, 119 nmol/L) with paraformaldehyde (final concentration, 0.3%). Immunolabeling of platelets with conjugated MoAbs (Immunotech, Marseille, France) was performed in whole blood using dual-color analysis. Fluorescence bound to platelets was analyzed in a FACScan (Becton Dickinson, Mountain View, CA). We wonder whether these discrepancies could be caused by the different techniques used. Kunishima et al used an indirect staining method in which they first had to obtain platelet-rich plasma. It is possible that the bigger platelets could be spun down with red blood cells and that only those with a smaller size remained in the supernatant. In our opinion, direct fluorescence methods applied to BSS patients' whole anticoagulated blood circumvent most of the inconveniences caused by selective sedimentation of the largest platelets, a problem that might have affected the experiments previously referred to.

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REFERENCES


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**Fig 1.** The binding of MoAbs anti-GP Ilb-llla (CD41a) and anti-GP IV (CD36) to the platelet surface of a normal control platelets (—); representative of 5 experiments performed) and a BSS patient (—). Platelets in whole blood were doubly labeled with fluorescein isothiocyanate (FITC)- and phycoerythrin-conjugated MoAbs. Each histogram depicts data obtained from 5,000 individual platelets.