

BRIEF COMMUNICATION**Molecular Defect in Platelets From Patients With Bernard-Soulier Syndrome**

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An IgG antibody isolated from the serum of a patient with the Bernard-Soulier syndrome induced platelet agglutination in the platelet-rich plasma of 50 normal subjects regardless of their ABO, K0^a, K0^b, HLA, or PI^{A1} types. This antibody was non-reactive with platelets from three other Bernard-Soulier syndrome patients. Indirect immunoprecipitation tests using this serum (or purified IgG) and soluble membrane antigens labeled with ¹²⁵I that had been extracted from normal platelets by the nonionic detergent Nonidet P-40 gave a single radioactive peak at 150,000 MW in sodium dodecyl sulfate-polyacrylamide gel electrophoresis. These findings strongly suggest that the antigenic determinant reacting with this antibody is absent from platelets of Bernard-Soulier syndrome patients and that the deficient molecule is of 150,000 MW. The role of this molecule in subendothelial adhesion and macromolecular-mediated aggregation is discussed.

BERNARD-SOULIER SYNDROME is an inherited bleeding disorder¹ characterized by giant platelets which are unable to aggregate with ristocetin as well as with bovine factor VIII, while aggregation induced by adenosine diphosphate (ADP) is apparently normal.^{2,3} The long bleeding time in this syndrome is associated with a decreased adhesion of giant platelets to vascular subendothelium.⁴

The occurrence of specific antibodies against a normal platelet antigenic determinant might be expected in sera from patients with molecular platelet membrane defects receiving platelet transfusions. Such antibodies could be used to characterize the defect.⁵ We have had the opportunity of studying the serum of a Bernard-Soulier syndrome patient which reacted with all normal platelets tested but did not react with platelets of other Bernard-Soulier syndrome patients. This report deals with the characterization of a platelet protein which is deficient in this syndrome.

MATERIALS AND METHODS*Patient*

The patient had the features of Bernard-Soulier syndrome and the clinical findings were reported in the first description of the disease.¹ Since this report, the patient had received several platelet transfusions for bleeding episodes, and, at the time of study, 5×10^{11} platelets were infused. The serum samples were obtained 8 days after this transfusion.

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Agglutination Tests

Freshly drawn blood was anticoagulated with disodium EDTA at a final concentration of 0.5% in plastic tubes and centrifuged (450 g for 10 min at 4°C) in order to obtain platelet-rich plasma (PRP). Fifty μ l of PRP (1.5×10^7 platelets) were mixed on a glass slide with 100 μ l of serum (or serum dilution) to be tested which had previously been heated (56°C for 30 min) and absorbed (vol/vol) on BaSO₄ (Prolabo, Paris, France). The slide was incubated for 30 min at 22°C on a rotating Kline agitator (100/min). Agglutination was read microscopically (magnification $\times 150$).⁶ Platelet agglutination tests were also performed on citrated PRP in the presence of the antibody after 1 min of incubation at 37°C using a Born MKII microaggregometer (Department of Pharmacology, Royal College of Surgeons, London, England).

Complement Fixation Tests

The microtechnique used was performed as previously described.⁷

Indirect Immunoprecipitation of Soluble Antigens

¹²⁵I was used to label platelet surface proteins using the lactoperoxidase method as described by Marchalonis.⁸ ¹²⁵I (2 mCi; CEA, France) was mixed with a platelet suspension (23×10^9 platelets in 11 ml of phosphate-buffered saline, pH 7.2). The following reagents were then added to the suspension: 5 μ l of KI (0.1 M), and 250 μ g of lactoperoxidase (Sigma Chemical Co., St. Louis, Mo.). The suspension was kept at room temperature for 30 min with occasional shaking. Fifty μ l of 10 mM H₂O₂ was added to the suspension at 0, 7, 14, and 21 min. The labeled platelets were then washed six times. Platelets were obtained from a non-HLA-A1 donor (HLA-A2, B8/A2, BW18) since the patient's serum contained a weak anti-HLA-A1 antibody detected by complement-dependent lymphocytotoxicity tests.⁹

Soluble antigens were extracted by incubating 23×10^9 platelets with the nonionic detergent Nonidet P-40 (Shell Chemical Co., New York) at a final concentration of 0.05% (vol/vol) at 22°C for 30 min in a Tris-HCl buffer, 0.05 M, pH 7.4, containing 0.15 M NaCl.¹⁰ After centrifugation for 90 min at 165,000 g, 5 ml of supernatant containing 60% of the platelet radioactivity was used as soluble extract. Then 50- μ l aliquots of soluble extract were incubated with 2 μ l of the patient's serum or IgG (10 and 6 mg of IgG per ml, respectively) for 1 hr at 22°C and precipitated with excess rabbit anti-human IgG (for 1 hr at 37°C and 24 hr at 4°C; a single line was seen on immunoelectrophoresis with rabbit immune serum against normal human serum). The precipitate was washed three times. Preliminary indirect immunoprecipitation curves with the patient's serum were performed in order to determine the ratio of anti-IgG antibodies to the serum and the ratio of serum-labeled antigens which gave the maximum precipitate and ensured total IgG precipitation. No residual antiplatelet activity was found in the supernatants.

Molecular Weight Determination

The precipitate was dissolved at 100°C for 5 min in 1% sodium dodecyl sulfate, urea 5 M (Merck, Darmstadt, West Germany), and 2-mercaptoethanol 0.15 M final concentrations (Serva, Heidelberg, West Germany) and submitted to electrophoresis in 7.5% polyacrylamide gel in the presence of 0.1% sodium dodecyl sulfate in the electrode buffer of pH 8.3.¹¹ Bovine serum albumin (69,000 MW; Sigma), human IgG (light chains 25,000 MW, heavy chains 55,000 MW; Miles Chemical Co., Elkhart, Ind.), lactoperoxidase (84,000 MW; Sigma), and chicken lysozyme (14,600 MW; Serva) were used as molecular weight markers. The gel was cut into 1-mm slices and the radioactivity was counted in a gamma ray counter (Intertechnique, France). The controls included normal serum, anti-HLA-A1 alloimmune serum and saline in place of the patient's serum. Six μ l of soluble proteins without antibody were submitted to electrophoresis in 7.5% polyacrylamide gel under the same conditions as the immune precipitates. The radioactivity of proteins from soluble extracts precipitable with trichloroacetic acid 12.5% (vol/vol; Prolabo) for 30 min at 22°C was measured.

RESULTS

The agglutination test with the patient's serum or IgG and platelets from 50 healthy unrelated individuals was invariably positive to a dilution of 1:500,

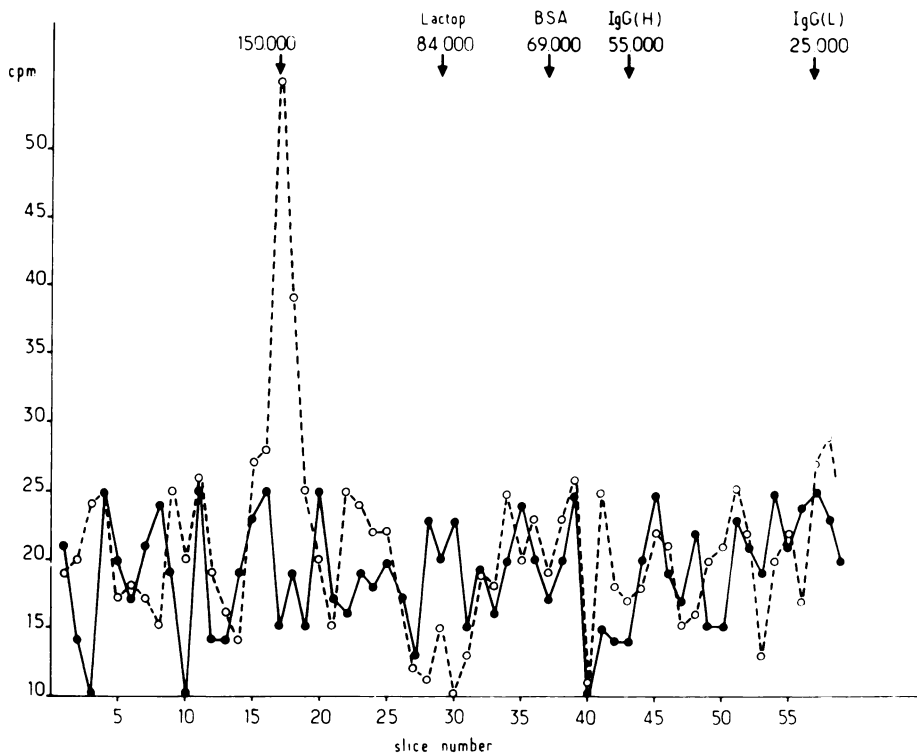


Fig. 1. Electrophoresis in sodium dodecyl sulfate-polyacrylamide gel of precipitate formed by sequential addition of 2 μ l of the patient's serum to 50 μ l platelet soluble extracts, incubation for 1 hr at 22°C, and then addition of an excess of rabbit anti-human IgG and further incubation (○). The radioactivity peak corresponds to a protein of 150,000 \pm 10,000 MW. Same experiment was performed with normal human serum instead of the patient's serum (●). Molecular weight markers incorporated into the gel were human IgG (L, light chains; H, heavy chains) bovine serum albumin (BSA) and lactoperoxidase (Lactop).

whatever the HLA, K0^a, K0^b, PI^{A1}, and ABO type. The results were consistently negative with platelets of three other Bernard-Soulier patients as well as with the patient's own platelets. The serum did not react in complement fixation tests with normal platelets.

The molecular weight of the molecule bearing the antigenic determinant reacting with the patient's serum was determined using indirect immunoprecipitation of labeled soluble antigens. After electrophoresis on sodium dodecyl sulfate-polyacrylamide gel, a single radioactive peak was observed which accounted for 0.5% of the total precipitable proteins by trichloroacetic acid. The molecular weight of the corresponding molecule was 150,000 \pm 10,000 (Fig. 1). Three independent experiments with the patient's serum and IgG gave the same results. No peak was detected with the immunoprecipitate of rabbit anti-human IgG, with soluble proteins, or with immunoprecipitates made from normal human serum or anti-HLA-A1 alloimmune serum instead of the patient's serum (Fig. 1). Results of polyacrylamide gel electrophoresis with soluble extracts showed three major peaks (150,000, 120,000, and 100,000 MW) and two other peaks corresponding to proteins of low molecular weight (50,000 and 28,000 MW) (Fig. 2).

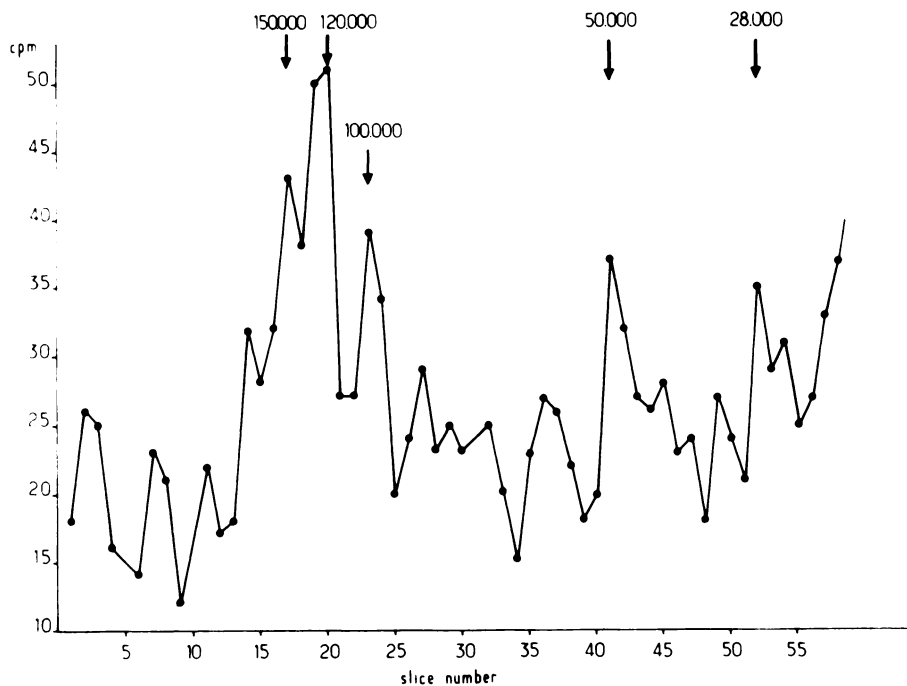


Fig. 2. Electrophoresis in sodium dodecyl sulfate-polyacrylamide gel of platelet soluble extract (6 μ l).

DISCUSSION

The antiplatelet antibody found in a polytransfused Bernard-Soulier syndrome patient was clearly different from the usual agglutinating platelet antibodies which are generally directed against KO^a , KO^b , $P1^{A1}$ antigens. The antibodies found in the patient's serum recognized an antigenic determinant which is present on normal platelets but clearly is absent from those of other Bernard-Soulier syndrome patients.

Nurden and Caen¹² have suggested that one of the three major glycoproteins in normal platelet extracts is missing from platelets of Bernard-Soulier syndrome patients. They estimated the molecular weight of this protein at 155,000. The molecular weight of the molecule bearing the antigenic determinant under study has been estimated by electrophoresis in sodium dodecyl sulfate-polyacrylamide gel to be 150,000. It is reasonable to suppose that the patient's serum detected this glycoprotein deficient in the Bernard-Soulier syndrome. These results with a human antibody are in agreement with those which have been obtained with a rabbit antibody.¹³

Such antibodies raised in patients and directed against deficient molecules were previously used to characterize a molecular deficiency in thrombasthenia.⁵ The deficient molecule in thrombasthenia had a molecular weight of 120,000. Therefore, we conclude that these two platelet diseases (thrombasthenia and Bernard-Soulier syndrome) have two distinct molecular deficiencies involving proteins of molecular weight 120,000 and 150,000, respectively.

The antibody from the patient's serum used at subagglutinating dilution *in vitro* induced a specific Bernard-Soulier-like defect in normal platelets, as evidenced by the fact that aggregation with ristocetin and bovine factor VIII was inhibited, while ADP-mediated aggregation was unaffected.¹⁴ Furthermore, it has been reported that adhesion of normal platelets to rabbit aorta subendothelium is decreased in the presence of this antibody.¹⁴ These experiments suggest that the antigen recognized by the antibody is necessary for the aggregation-inducing agents bovine factor VIII and ristocetin and for adhesion to subendothelium.

Conversely, the serum previously described as recognizing the molecular deficiency in thrombasthenia inhibited ADP aggregation but not ristocetin aggregation.⁵ Thus, these two sera clearly define two molecular deficiencies in thrombasthenia and Bernard-Soulier syndrome corresponding to two different functions.

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