Acquired Thrombasthenia due to Inhibitory Effect of Glycoprotein IIbIIIa Autoantibodies

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ABSTRACT: **Background:** A 75 year old patient presenting with mucocutaneous bleeding was diagnosed with acquired thrombasthenia. The diagnosis was based on lack of platelet aggregation with adenosine diphosphate (ADP), arachidonic acid and collagen, and normal aggregation induced by ristocetin.
Objective: To study the mechanism of platelet function inhibition in a patient with acquired thrombasthenia.
Methods: Aggregation assays of platelets from the patient and healthy controls were performed. In addition, anti-glycoprotein (GP) IIbIIIa antibodies binding to normal platelets in the presence or absence of the patient's serum was studied by flow cytometry.
Results: Aggregation of normal platelets in the presence of patient's plasma was inhibited four- and 2.5-fold in the presence of ADP and arachidonic acid respectively, while collagen-induced aggregation was completely abolished. Ristocetin-induced aggregation was normal. The patient's serum inhibited binding of commercial anti-glycoprotein IIbIIIa antibodies to normal platelets twofold by flow cytometry. Treatment with anti-CD20 monoclonal antibody (rituximab) normalized the patient's platelet aggregation.
Conclusions: These results suggest that the patient developed inhibitory anti-GPIIbIIIa autoantibodies that caused acquired thrombasthenia.

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KEY WORDS: anti-GPIIbIIIa antibodies, acquired thrombasthenia, rituximab, platelet aggregation

The glycoprotein IIbIIIa integrin is a platelet receptor that binds the arginyl-glycyl-aspartic acid sequence of fibrinogen, von Willebrand factor, fibronectin, and vitronectin. GPIIbIIIa-fibrinogen binding enables platelet aggregation. The crucial role of GPIIbIIIa in hemostasis is demonstrated in Glanzmann thrombasthenia, a life-long bleeding disorder caused by hereditary GPIIbIIIa deficiency. Acquired thrombasthenia may be induced by autoantibodies specific to platelet GPIIbIIIa or isoantibodies due to repeated transfusion of normal platelets. It is characterized by variable degrees of mucocutaneous bleeding, at times life-threatening [1]. Routine coagulation tests as well as platelet volume and morphology are normal. Platelets aggregate in response to ristocetin but will fail to do so with other agonists, such as adenosine diphosphate, thrombin, collagen and epinephrine [1]. Acquired thrombasthenia due to autoantibodies has been associated with immune thrombocytopenic purpura [2,3], tacrolimus therapy for renal transplantation [4], acute lymphoblastic leukemia [5], non-Hodgkin's lymphoma, hairy cell leukemia, multiple myloma, Castelman's disease, and myelodysplastic syndrome [6]. The presence of autoantibodies to GPIIbIIIa was documented in several cases by enzyme-linked immunosorbent assay [3], immunoelectrophoresis [7], Western blotting [8], and rarely, flow cytometry [9].

Treatment consists of corticosteroids, chemotherapy, plasma exchange [10], protein A cepharose immunoadsorption [11], high dose intravenous immunoglobulin [12], recombinant factor VIIa [13] and rituximab [6].

GPIIbIIIa = glycoprotein IIbIIIa

PATIENT DESCRIPTION

A patient with systemic lupus erythematosus and antiphospholipid antibody syndrome developed acquired thrombasthenia syndrome. The diagnosis was established by the lack of platelet aggregation with collagen, ADP, epinephrine and arachidonic acid, while maintaining normal aggregation with ristocetin. The aim of the present work was to study the mechanism of inhibition of platelet function in this patient.

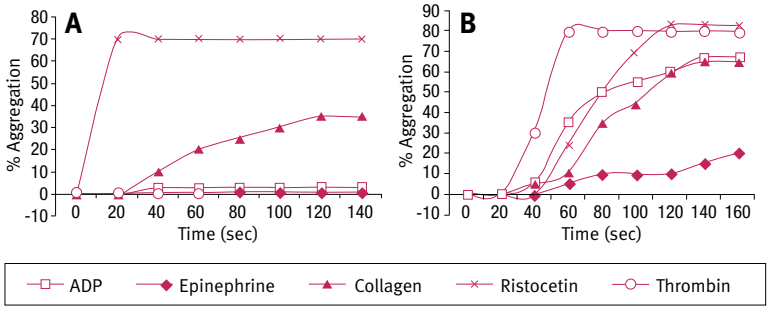
MATERIALS AND METHODS

PATIENT HISTORY

A 75 year old woman with SLE and antiphospholipid antibody syndrome was diagnosed at age 55 years. She developed ITP at age 60. Splenectomy resulted in remission with nor-

ADP = adenosine diphosphate
SLE = systemic lupus erythematosus
ITP = immune thrombocytopenic purpura

Figure 1. A patient's platelet aggregation with various agonists: **[A]** at diagnosis and **[B]** after rituximab treatment. Aggregations of PRP were performed as described in the Methods section. Agonist concentrations were as follows: ADP 4 µM, epinephrine 4 µM, collagen 4 mg/ml, thrombin 1 u/ml, arachidonic acid 0.25 mg/ml, ristocetin 1.5 mg/ ml



malization of platelet count. Fifteen years later, the patient presented with variable degrees of mucocutaneous bleeding and an episode of gastrointestinal hemorrhage. Platelet counts were within normal range (184 x 103 to 284 x 103/µl), and platelet morphology was normal. Antiphospholipid antibody test was positive. Laboratory evaluation revealed prolonged bleeding time of 15 minutes (normal 0.5–8 min) and normal clot retraction. The diagnosis of acquired thrombasthenia was made on the basis of a lack of platelet aggregation in response to ADP, epinephrine, arachidonic acid, and thrombin, and normal ristocetin-induced aggregation [Figure 1A]. The patient was resistant to standard therapy with steroids, cyclophosphamide, azathioprine and IVIG. Anti-CD20 monoclonal antibody (rituximab) 375 mg/m²/week was administered for 4 weeks, resulting in normalization of platelet aggregation [Figure 1B] and cessation of bleeding manifestations.

Eight months later there was a recurrent mucocutaneous bleeding accompanied by a lack of platelet aggregation similar to that observed before the first course of rituximab. The patient received a second course of rituximab. Similar to the effect of the first course of treatment, the bleeding symptoms resolved and platelet aggregation tests normalized. Six years after the second course of rituximab the patient is in complete remission.

MATERIALS

The following materials were used: collagen (Collagen Reagent Horn Nycomed, Germany), ADP, epinephrine, arachidonic acid and ristocetin (Helena Laboratories, Beaumont, TX, USA), thrombin (Omrix Biopharmaceuticals, Tel Aviv, Israel), fluorescein isothiocyanate-conjugated monoclonal anti-GPIIbIIIa antibody (Chemicon, Temecula, CA, USA), and FITC-conjugated non-immune immunoglobulin G (Serotec, Oxford, UK).

IVIG = intravenous immunoglobulin
FITC = fluorescein isothiocyanate

METHODS

The study was approved by the Ethics Committee of the hospital. Informed consent was obtained from the patient and the controls.

- **Platelet preparation.** The patient and controls were asked not to ingest any medications for at least 10 days prior to venipuncture. The patient was allowed to receive 10 mg prednisone and 50 mg azathiaprine. Blood from the patient and healthy volunteers was drawn into a 3.2% sodium citrate tube. Platelet-rich plasma was prepared by centrifugation of whole blood at 180 x g for 15 min. Platelet-poor plasma was prepared by centrifugation of PRP at 2000 x g for 10 min. Serum was prepared by allowing whole blood collected in a test tube to clot by leaving it undisturbed at room temperature for 15–30 minutes. The clot was removed by centrifuging at 1000–2000 x g for 10 minutes in a refrigerated centrifuge.

- **Platelet aggregation.** All experiments were performed using the AggRAM® four-channel aggregation analyzer (Helena Laboratories, Beaumont, TX, USA) and PAP4 aggregometer (Bio/Data Corporation, Horsham, PA, USA). PRP, 200 µl, from the patient or normal volunteers, or a mix of patient PPP and normal PRP (1:1) was aggregated at 37°C with 20 µl of agonist, at the following final concentrations: ADP 1, 2, 4 µM; epinephrine 1, 2, 4 µM; collagen 1, 2, 4 mg/ml; thrombin 0.5,1 u/ml; arachidonic acid 0.25 mg/ml; ristocetin 1.5 mg/ml. Each agonist was tested at least 4 times, and maximal aggregation was determined after 6 min.

- **Flow cytometry.** For the flow cytometry analysis (Beckman Coulter Epics XL-MCL, High Wycombe, UK), normal PRP was pre-incubated with either normal or patient serum diluted 1:50 for 30 min at 22°C, followed by incubation with FITC-conjugated monoclonal anti-GPIIbIIIa antibody for 15 min at 22°C. FITC-conjugated non-immune IgG was used as the negative control, and normal PRP as the positive control.

RESULTS

Platelet aggregation tests were performed at diagnosis and following rituximab treatment. At diagnosis, no aggregation of the patient's PRP was observed in the presence of ADP, epinephrine and thrombin, while collagen-induced aggregation was inhibited to 30% [Figure 1A]. In contrast, normal aggregation with ristocetin was observed [Figure 1A]. Repeated

PRP = platelet-rich plasma
PPP = platelet-poor plasma
IgG = immunoglobulin G

Figure 2. Aggregation of normal PRP in the presence of the patient's PPP (1:1, open symbols) compared to aggregation of normal PRP (closed symbols). The concentrations were as follows: ADP 4 µM, collagen (Coll) 4 mg/ml, arachidonic acid (Arac) 0.25 mg/ml

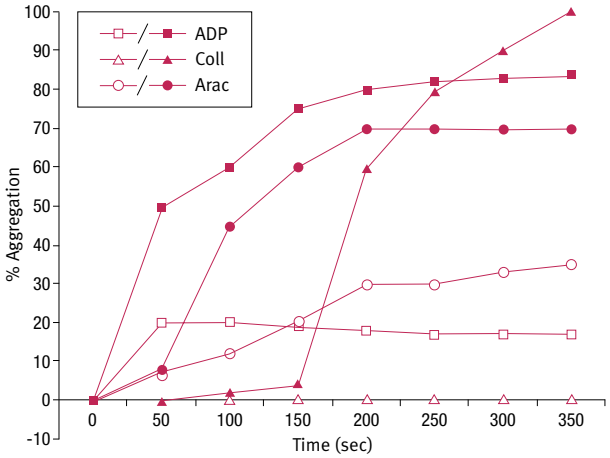
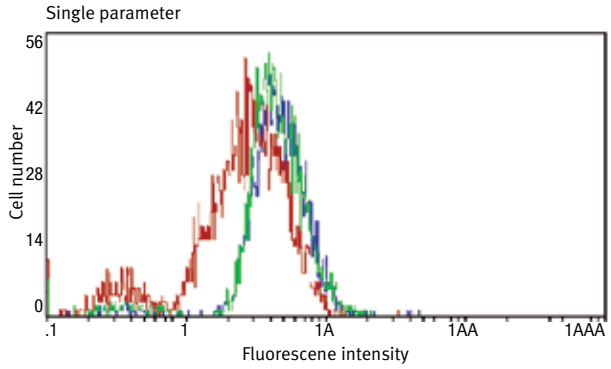


Figure 3. Flow cytometry analysis of the effect of the patient's serum on binding of commercial GPIIbIIIa antibody to normal platelets. The effect of the patient's and normal serum is represented by fluorescence intensity: red = patient's serum, blue = normal serum, green = no serum



aggregation testing after treatment with rituximab showed normalization of platelet aggregation with all the agonists except epinephrine [Figure 1B].

To test the hypothesis that the patient's plasma may inhibit aggregation of normal platelets we performed aggregation of normal PRP in the presence of the patient's PPP. Addition of the patient's PPP to normal PRP (1:1) inhibited ADP- and arachidonic acid-induced aggregation four- and 2.5-fold, while collagen-induced aggregation was completely inhibited [Figure 2]. No effect of the patient's PPP on ristocetin-induced platelet aggregation was observed.

Since platelet aggregation is dependent on intact GPIIbIIIIa, we evaluated whether the inhibitory GPIIbIIIIa activity was present in the patient's serum.

We tested the effect of the patient's serum on binding of commercial GPIIbIIIa antibodies to normal platelets by flow cytometry. The patient's serum inhibited binding of commercial anti-GPIIbIIIa antibodies to normal platelets twofold [Figure 3, red line]. Normal serum had no effect on the binding of anti-GPIIbIIIa antibody to platelets [Figure 3, blue line].

These results suggest that the patient has autoantibodies with anti-GPIIbIIIa activity, causing acquired thrombasthenia.

DISCUSSION

This report provides evidence for the inhibitory effect of the patient's plasma on platelet function, resulting in acquired thrombasthenia. Since this inhibitory effect involves GPIIbIIIa-mediated activities of platelets (aggregation and GPIIbIIIa binding), it is tempting to suggest that the inhibitory effect was mediated by anti-GPIIbIIIa antibody in the patient's serum.

Previous studies have reported the presence of autoantibodies against GPIIbIIIa in acquired thrombasthenia, in line with the absence of platelet aggregation and manifestations of mucocutaneous bleeding [11,14]. However, the mechanism underlying the impact of autoantibody-mediated acquired thrombasthenia platelet dysfunction remains unclear. In some reports, autoantibody binding to platelet GPIIbIIIa resulted in functional defects, whereas in others it had no effect [15].

Platelet dysfunction should be suspected in patients with ITP or SLE with mucocutaneous bleeding and platelet counts of ≥ 50 x 103 /µl. In two series, 13 of 19 patients with ITP and 22 of 35 patients with SLE demonstrated impaired platelet aggregation to ADP, epinephrine, or collagen [16].

It is possible that the antiphospholipid antibody present in our patient's plasma exerted anti-GPIIbIIIa activity, leading to acquired thrombasthenia. However, the possibility that another autoantibody developed, which interfered with the function of GPIIbIIIa, cannot be excluded.

Anti-CD20 (rituximab) is a human-mouse chimeric monoclonal antibody that targets the B cell CD20 antigen, causing a specific B cell depletion [17]. Rituximab had been used successfully in the treatment of antibody-mediated hemostatic disorders [6,18] and for acquired immune cytopenias [3]. This treatment was given to the patient based on the beneficial effect of anti-CD20 on other immune mediated disorders. Indeed, rituximab treatment resulted in a longstanding remission without any bleeding manifestations and normal platelet aggregation.

Thus, the beneficial response to rituximab achieved in our patient suggests that it may be a potential therapeutic option for intractable immune mediated anti-GPIIbIIIa acquired thrombasthenia.

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