Ten patients with idiopathic thrombocytopenic purpura (ITP) were studied before and following a rise in circulating platelets subsequent to infusions of intravenous gammaglobulin (400 mg/kg/day x 5 days). We quantitated the amount of circulating IgG capable of binding to normal donor platelets in vitro using an RET-monoconal anti-human IgG assay, as well as the amount of IgG associated with the patients' platelets before and following therapy. We found no evidence for a decrease in platelet-specific IgG antibodies in these patients undergoing an acute response to therapy. These data suggest that the short-term efficacy of intravenous gammaglobulin is due to effects other than a substantive reduction in platelet reactive antibodies, such as the alteration of IgG-coated platelet destruction.

**Materials and Methods**

Human subjects. Patients were selected on the basis that they had ITP by previously described criteria,2,6,8 had received high-dose gammaglobulin with a dramatic increase in platelet count within one week of starting therapy, and had plasma samples stored before and following therapy.

Five subjects were childhood ITP patients entered into a randomized study of high-dose gammaglobulin therapy in Italy (group 1, Table 1). Following the diagnosis of active ITP, these subjects were observed for 10 days without treatment before initiating infusion of Endoblin (IMMUNO, Austria) at 400 mg/kg/day for five consecutive days.6a

Five subjects were adult patients treated by the Hematology Service of the University of Alabama at Birmingham for ITP (group 2, Table 1). All had either relapsed or had no response following prior treatments with corticosteroids, vincristine, and/or splenectomy. These 5 patients received infusions of Sandoglobulin® (Sandoz, Basel), 400 mg/kg/day for five consecutive days, without concurrent additional treatment for ITP.

Platelet counts, direct platelet antibody assays, and plasma samples were obtained within 24 hours before the first gammaglobulin infusion ("pre" samples) and on the day following the fifth and last treatment ("post" samples). Plasma samples were stored frozen until further study.

Healthy adult volunteers (blood group O, RhD positive) employed at the University of Alabama at Birmingham were the sources of platelets used in the normal donor platelet antibody-binding assays.
**RESULTS**

Table 1 presents clinical data for the 10 ITP patients treated with high-dose gammaglobulin. All responded to treatment, as demonstrated by an increased circulating platelet count on the day following completion of therapy. Seven of the 9 patients for whom we had complete data also demonstrated a reduction in the amount of platelet-associated IgG (ng/10^9 platelets), as measured by the assay performed on freshly obtained platelets. It should be pointed out that the direct assay for group 1 was performed by an ELISA assay on platelet extracts, which inherently gives higher base line (normal) levels of platelet IgG than the 125I-monoclonal antibody assay on intact platelets used for group 2.

The first two data columns of Table 2 demonstrate that all patients' plasmas had the expected increase in IgG concentration following IV gammaglobulin therapy, with a mean increase of 3.4-fold (from 6.8 to 23.3 mg/mL). The "mock"-treated ITP plasma had a similar increment, from 7.7 to 20 mg/mL IgG.

We next analyzed the amount of IgG in each plasma sample which was capable of binding to normal donor platelets in vitro. Because of the wide range of plasma IgG concentrations, most notably between the "pre" and "post" treatment samples, we used IgG-enriched fractions of the individual plasma samples in order to assay comparable amounts of IgG in each platelet IgG binding determination. Table 2 (data columns 3 and 4) shows the amount of IgG bound per mg of total IgG incubated with a constant number of normal donor platelets. The majority of patient samples demonstrated a decrease in this value "post" treatment; the group as a whole had 32.9 ng of platelet IgG per mg of total IgG "pre" therapy and 15.1 ng of platelet IgG per mg of total IgG "post" therapy. This decrease probably reflects the passive infusion of gammaglobulin that does not contain platelet antibody (unpublished observation) and that dilutes out the specific activity of the patient's platelet antibodies. This is supported by results with the "mock"-treated ITP plasma, which underwent a similar reduction from 39 ng/mg of IgG to 22 ng/mg of IgG following in vitro addition of Sandoglobulin®.
Since the total concentration of IgG per mL of plasma was quantitated "pre" and "post" therapy (columns 1 and 2, Table 2), the concentration of platelet antibody per mL of plasma could be calculated (data columns 5 and 6, Table 2). The patients' plasma samples as a group had a modest increase in circulating platelet antibody, from 254 ng/mL to 426 ng/mL of plasma. There was not a statistically significant difference between the values "pre" and "post" therapy (p = 0.068 by paired t-test analysis). The "mock"-treated ITP plasma sample yielded similar data, with a concentration of 300 ng/mL plasma "pre" therapy and 400 ng/mL "post" therapy (Table 2). These results do not support the hypothesis that therapeutic gammaglobulin produced a reduction in the circulating pool of platelet antibody.

Antibody bound to the platelet surface in vivo represents a second pool of platelet antibodies. As seen in Group 2 patients (Table 1), the amount of IgG antibody associated with the platelet surface was generally reduced following high-dose gammaglobulin therapy. The post-therapy values for platelet-surface IgG for patients BOK, SEH, and MKN were 0.16, 0.16, and 0.12 ng/10^6 platelets, which reflect 640, 640, and 480 IgG molecules/platelet respectively. Patient CNI had a pretherapy value of 2.1 ng/10^6 platelets (8,400 IgG/platelet) and six days later (post-therapy) 0.64 ng/10^6 platelets (2,560 IgG/platelet). However, each of these patients had a dramatically increased number of circulating platelets post-therapy. If one multiplies the amount of platelet-associated antibody per 10^6 platelets by the number of circulating platelets (Table 1), the total amount of platelet-bound antibodies has clearly not been reduced. This calculation is a rough estimate since it includes platelet-specific antibody as well as "nonspecific" IgG on the platelet surface, which amounts to 122 ± 5 molecules/platelet in our studies^6^ and to 79 ± 27 molecules/platelet as reported by George and Saucerman.^1^

**DISCUSSION**

This study was performed in order to re-evaluate the possibility that modulation of platelet IgG autoantibody production is involved in the therapeutic efficacy of high-dose gammaglobulin therapy in ITP. Several authors have suggested that high-dose gammaglobulin is immunomodulatory in vivo, either by alteration of the anti-idiotype network or modulation of suppressor-T lymphocytes, thereby leading to depressed immunoglobulin production, including the production of any platelet-specific antibodies.4,5 Others have suggested that high-dose gammaglobulin may directly interfere with the binding of platelet-specific antibodies to the target antigens.5 In support of these hypotheses, many authors cite the frequently reported observation that as platelet counts increase during gammaglobulin therapy, measured amounts of platelet-associated IgG (eg, ng/10^6 platelets) decrease.

This inverse relationship between platelet count and platelet-associated IgG is not unique to gammaglobulin therapy. The same pattern is generally observed in ITP patients responding to splenectomy, corticosteroids, vincristine, or cyclophosphamide.5 If it is assumed that the rate of platelet autoantibody production remains constant during the response period of rapidly rising platelet counts, then a reduction in the average amount of IgG per platelet could be explained simply by the greater number of platelets available for antibody binding. Additionally, the total body load of platelet-specific antibody could increase (in the absence of an increased rate of antibody production) due to a decreased rate of removal of antibody-platelet complexes in the RES.

We undertook the present study to determine if we could find evidence for suppression of platelet antibody production during the acute response to high-dose gammaglobulin therapy by estimating the amount of platelet-binding IgG, both
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In the plasma and associated with platelets, before and following a therapeutically effective treatment regimen with gammaglobulin. Our data do not suggest that the amount of platelet antibody was reduced by this therapy. We have previously published data to show that preparations of therapeutic gammaglobulin can down modulate human monocyte IgG-Fc receptor binding of IgG-coated platelets in vitro. Therefore, we conclude that in both pediatric and adult ITP patients, the short-term therapeutic effects of high-dose gammaglobulin are more likely to be mediated by inhibition of IgG-Fc receptor function in the RES or by additional factors other than reduction in the amount of whole-body platelet-reactive antibodies.

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