Serologic aspects of treating immune thrombocytopenic purpura using intravenous Rh immune globulin

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In patients with immune thrombocytopenic purpura (ITP), IgG autoantibody-coated platelets are phagocytized by mononuclear macrophages, primarily in the spleen. Intravenous Rh immune globulin (IV RhIG) has been used since 1983 to treat D+, nonsplenectomized patients with ITP. The beneficial therapeutic effect of IV RhIG is attributed to competitive inhibition of phagocytosis of IgG-coated platelets by IgG anti-D-coated D+ red blood cells (reticuloendothelial or Fc receptor blockade). Following infusions of IV RhIG in D+ ITP patients, the direct and indirect antiglobulin tests become transiently positive, reflecting passively transferred anti-D and other alloantibodies that were present in the infused IV RhIG. These consistent and predictable serologic findings contrast with the inconsistent and weak anti-D reactivity observed when D– women are treated with relatively small doses of intramuscular RhIG for Rh immunoprophylaxis. The pathophysiology of ITP and the effect of infusing IV RhIG in patients with ITP are illustrated in this review, using computer-generated figures. Immunohematology 2001;17:106–110.

Key Words: immune thrombocytopenic purpura, ITP, RhIG, Rh immune globulin, reticuloendothelial blockade, Fc receptors

When intravenous Rh immune globulin (IV RhIG; WinRho SDF™, NABI, Boca Raton, FL) is issued by a blood bank to treat a patient with immune thrombocytopenic purpura (ITP), it is the only time that a blood product is used with the specific intent of destroying the recipient’s red blood cells (RBCs). This unique circumstance has practical implications for interpreting postinfusion serologic test results and for selecting RBCs for transfusion in IV RhIG-treated patients. The following review is intended to describe the rationale for treating ITP using IV RhIG; to illustrate the effect of IV RhIG, using computer-generated figures; and to provide suggestions for selecting RBCs if such patients require a transfusion.

Pathophysiology of ITP

ITP is an autoimmune disease in which immune dysregulation results in IgG autoantibodies with serologic specificity for epitopes on platelet glycoproteins. Persons with thrombocytopenia may be classified as having primary or secondary thrombocytopenia. Persons with secondary thrombocytopenia may have immune destruction of platelets as a complication of another disease, such as systemic lupus erythematosus, Evans’ syndrome, or an infectious disease (i.e., infectious mononucleosis); or they may have a disease due to decreased production of platelets, such as aplastic anemia. Some patients may have primary thrombocytopenia caused by immune destruction of circulating platelets for which no other illness has been diagnosed. In this situation the exclusion of other diseases is the basis for establishing the diagnosis of primary immune thrombocytopenia, i.e., ITP. Clinically, the most widely used classification categorizes persons with ITP as either children or adults. In this classification, the emphasis is on differences in the expression of ITP in children, who typically present with an acute and transient illness (i.e., less than 6 months’ duration), versus adults, who typically present with an insidious, chronic course of thrombocytopenia (i.e., more than 6 months’ duration). In children with ITP the proportion of affected males and females is approximately equal. In adults, as for all other autoimmune diseases, more females than males are affected.

In ITP, normal-appearing platelets are formed from morphologically normal megakaryocytes, but abnormal IgG autoantibodies attach immunologically to circulating platelets. IgG-coated platelets are detected by Fc...
receptors on mononuclear macrophages, typically in the spleen. The IgG-platelet complex causes membrane of the splenic macrophage to invaginate, and IgG-coated platelets are phagocytized. This mechanism of mononuclear macrophage phagocytosis of IgG-coated platelets is illustrated in Figure 1. To the extent that bone marrow megakaryocytes do not increase production of platelets sufficiently to compensate for the shortened survival of circulating platelets, patients have a decreased platelet count and may develop clinical signs of thrombo-cytopenia. If the decreased platelet count is an isolated abnormality and there are no other factors contributing to a decrease in hemostasis, such as decreased plasma coagulation factor concentrations, the patient is likely to have minimal spontaneous bleeding and relatively few petechia (“dry purpura”). Other patients, whose thrombocytopenia is complicated by the coexistence of other hemostatic deficiencies, such as chronic liver disease and decreased coagulation factor concentrations, abnormal integrity of the capillary system, or drug-related platelet dysfunction, may experience more prominent signs and symptoms of thrombocytopenia, such as bleeding gums, metrorrhagia, and increased susceptibility to bruising (“wet purpura”).

Treatment of ITP

The first successful treatment of ITP was reported in 1913, when Kaznelson reported the successful outcome of splenectomy in a patient with increased bleeding and susceptibility to bruising and a low platelet count. After that report, splenectomy was the treatment of choice for ITP until 1950, when Wintrobe reported increased platelet counts in patients with purpura hemorrhagica after treatment with ACTH or cortisone. For the next three decades, corticosteroids and/or splenectomy were the principal treatments for ITP. For refractory patients, other approaches to treatment were used, such as immunosuppressive drugs, plasmapheresis, and selective immune adsorption of autoantibodies, but these treatments were less likely to be successful and had a secondary role in the management of ITP. In 1981, Imbach et al. reported that intravenous immunoglobulin (IVIG) increased platelet counts in children with ITP. Imbach’s interest in the use of IVIG in ITP began when he noted that two children with low platelet counts who were being treated with IVIG (for other indications) had increased platelet counts after receiving IVIG. Clinical trials soon established the efficacy of IVIG for treating ITP. Splenectomy, corticosteroids, and IVIG became the primary treatments for ITP until 1983, when Salama et al. hypothesized that the beneficial effect of IVIG in ITP may be due to RBC antibodies present in IVIG that cause immune hemolysis of the recipient’s RBCs by splenic sequestration of IgG-coated platelets. Salama et al. postulated that anti-A, anti-B, and other RBC antibodies may have been responsible for hemolysis and decreased hematocrits they had observed in patients treated with IVIG. They stated that, while the main effect of IVIG in ITP may be macrophage Fc receptor blockade by individual IgG molecules, an even more efficient Fc receptor blockade may be induced by IgG-coated RBCs undergoing hemolysis in mononuclear macrophages. They conducted clinical trials using IV RhIG in D+ children and adults with ITP, and the successful outcomes opened a new era for patients with ITP. Subsequent clinical trials using several different IV RhIG products in D+ patients established the efficacy of IV RhIG for the treatment of ITP (Table 1).

Mechanism of Action of IV RhIG in ITP

Figure 1 illustrates how splenic mononuclear macrophage Fc receptors become activated by circulating IgG-coated platelets in untreated ITP, which become phagocytized. Figure 2 illustrates how an infusion of IV RhIG in a D+, nonsplenectomized patient with ITP modifies this situation and increases the circulating platelet count. Shortly after the infusion of IV RhIG, phagocytosis of IgG-coated platelets is competitively inhibited by IgG anti-D-coated D+ circulating RBCs (reticuloendothelial or Fc receptor blockade). Assuming a typical or representative platelet count for
**Table 1.** Selected reports of clinical trials using IV RhIG (anti-D)

<table>
<thead>
<tr>
<th>Year</th>
<th>First author</th>
<th>Principal findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>1984</td>
<td>Salama, A.</td>
<td>Of ten patients treated with IV RhIG (Bioteest, Frankfurt, FRG), eight had an increase in platelet count.</td>
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<tr>
<td>1986</td>
<td>Salama, A.</td>
<td>Of 15 chronic and two acute patients with ITP treated with RhIG (Zentralinstitut fur das Bluttransfusionswesen, Hamburg, FRG; HypRheso-D, Cutter, Emeryville, CA) who were Rh(D)-positive, two cases responded to anti-Rh(A) insignificantly (increment &lt; 20,000/μL) while all others were considered good or excellent.</td>
</tr>
<tr>
<td>1986</td>
<td>Becker, T.</td>
<td>Of 15 D+ children with ITP treated with IV RhIG (Zentralinstitut fur das Bluttransfusionswesen, Hamburg, FRG) all were found to have a rise in platelets.</td>
</tr>
<tr>
<td>1988</td>
<td>Oksenhendler, E.</td>
<td>IV RhIG (Biotransfusion, France) was effective in at least nine out of 14 Rh(D)+ patients with HIV-related ITP.</td>
</tr>
<tr>
<td>1991</td>
<td>Bussel, J.B.</td>
<td>Of 43 nonsplenectomized patients, the mean initial platelet count was 22,000/μL and the mean increase in platelet count was 95,000/μL after treatment with IV RhIG (WinRho, Winnipeg Rh Institute of the University of Manitoba, Winnipeg, Canada).</td>
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<tr>
<td>1992</td>
<td>Gringeri, A.</td>
<td>Of 51 HIV ITP patients, including HIV-related, treated with IV RhIG (Rhesuman, Berna, Italy; Partogamma, Immuno, Italy), 67 percent showed increases in platelet counts.</td>
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<tr>
<td>1992</td>
<td>Andrew, M.</td>
<td>Of 25 patients with ITP, 90 percent responded to IV RhIG (WinRho; Rh Pharmaceuticals, Winnipeg, Manitoba, Canada).</td>
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<tr>
<td>1993</td>
<td>Caglayan, S.</td>
<td>Of 19 children with ITP, 15 responded to IV RhIG (Rleso-Gulap, Seru, Austria) with an increase in average platelet number to 76,000/μL after being administered 100 μg for 5 consecutive days.</td>
</tr>
<tr>
<td>1994</td>
<td>Borgna-Pignatti, C.</td>
<td>Of seven patients with ITP, five had an increase in platelet count (&gt;100,000/μL) after treatment with IM RhIG (Partobulin, Immuno, or Partogamma, Biagini, Pisa, Italy).</td>
</tr>
<tr>
<td>1994</td>
<td>Blanchette, V.</td>
<td>Although IV RhIG (Sandoglobulin, Swiss Red Cross, Basel, Switzerland; WinRho, Winnipeg Rh Institute, Winnipeg, Canada) was easier to administer, the rate of platelet response was significantly slower than that observed in children randomized to receive IV IgG.</td>
</tr>
<tr>
<td>1996</td>
<td>Godeau, B.</td>
<td>Of seven patients with chronic ITP, only one patient showed transient response while all others showed no response to a monoclonal anti-D (MONO-D, Laboratoire Francais du fractionnement et des Biotechnologies, Les Ulis, France).</td>
</tr>
<tr>
<td>1997</td>
<td>Scaradavou, A.</td>
<td>Of 261 nonsplenectomized ITP patients, 72 percent of patients responded to IV RhIG (WinRho or WinRh SD, Cangene, Winnipeg, Manitoba, Canada) with an increased platelet count &gt;20,000/μL.</td>
</tr>
<tr>
<td>2001</td>
<td>Newman, G.C.</td>
<td>Children with acute ITP receiving IV RhIG (WinRho, Cangene Corporation, Winnipeg, Manitoba, Canada) 75 μg/kg/d had overnight platelet increases in seven out of nine cases.</td>
</tr>
<tr>
<td>2001</td>
<td>Bussel, J.B.</td>
<td>There was no overall relationship between response to IV RhIG (WinRho) or IV Ig and response to subsequent splenectomy. However, both a good platelet response in adults to the last IV RhIG and a hemoglobin decrease of ≥2.0 gm/dL appeared to predict response to subsequent splenectomy.</td>
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**Fig. 2.** Postulated mechanism of action of IV RhIG (anti-D) for inducing a transient macrophage Fc receptor blockade to treat ITP in D+ patients. Infused anti-D attaches immunologically to D antigen sites on the patient’s D+ circulating RBCs. Assuming a normal RBC count of approximately 5.0 million/μL and a decreased platelet count of 50,000/μL, IgG-coated RBCs compete for phagocytosis by mononuclear macrophages with a numerical advantage of 500:1. The result is a decrease in circulating RBCs and an increase in circulating platelets. The effect has been described as a “medical splenectomy” and, although the platelets remain coated by IgG, they typically function adequately after surgical splenectomy.

a patient with ITP of approximately 10,000/μL and a normal RBC count of 5,000,000/μL in that patient, the numerical competition of approximately 500 (IgG-coated RBCs) to 1 (IgG-coated platelet) favors phagocytosis of IgG-coated RBCs. Presumably, the physical bulk of phagocytized RBCs leverages the comparatively small dose of IV RhIG (50μg/kg) to result in a comparable degree of Fc receptor blockade as would a much larger dose of IVIG (1gm/kg). This reticuloendothelial or macrophage Fc receptor block, which has been referred to as a “medical splenectomy,” was described earlier by Shulman et al. in their studies of immune destruction of platelets in patients with hereditary spherocytosis. IV RhIG is not indicated for persons who are D- or have had a splenectomy, since clinical trials have shown IV RhIG to be ineffective in these patients.
Serologic Aspects

Most blood bank technologists are acquainted with the weak and transient reactivity of anti-D in the plasma of D− women after an intramuscular injection of conventional RhIG (IM RhIG) for Rh immunoprophylaxis at 28 weeks of pregnancy or after delivery of a D+ infant. Because IM RhIG is injected in muscle and consists of only approximately one-tenth the dose of IV RhIG that is used to treat ITP, circulating anti-D is detectable in the recipient’s plasma for only a few days to a few weeks, if at all. Anti-D titers after injections of IM RhIG are low and do not interfere with Rh(D) type-specific transfusions of serologically compatible D− RBCs to D− women, because there is no serologic incompatibility. In the case of treating D+ patients with ITP with IV RhIG, the circumstance is quite different because (1) a much higher dose of anti-D is given, (2) it is given as an IV bolus, and (3) it is given to a person whose D+ RBCs are serologically incompatible. Thus, an ITP patient treated with IV RhIG not only will develop a positive antibody detection test (indirect antiglobulin test) but also will develop a positive direct antiglobulin test (DAT). Although the composition of alloantibodies in both IM and IV RhIG is similar, and it reflects the expected serologic specificities of alloantibodies in D− alloimmunized women, the considerably larger dose of IV RhIG for ITP causes more alloantibodies to be detectable in recipients’ plasma, such as anti-C, -E, and -G. These additional allo-antibodies may be detected both in the eluate from the DAT-positive samples and by antibody screening by the indirect antiglobulin test. There is relatively little data on the transfusion of D+ RBCs to patients who have been treated for ITP with IV RhIG. However, in our experience1 and in that of others,31 it seems prudent to transfuse D− RBCs, unless there is an urgent need to continue the treatment of ITP to increase the platelet count.1 In that latter situation, it may be argued that by giving D+ RBCs one brings the full dose of IV RhIG to effect competitive inhibition of macrophage Fc receptor function, supporting the primary therapeutic goal of raising the platelet count.

Note: Figures 1 and 2 were produced using Microsoft Word 2000 word processing software. Images were created using Adobe Illustrator software, modified using MGI Photosuite, and inserted in Microsoft Word where needed. Symbols were created using WordArt in Microsoft Word.

References


