CONCISE REPORT

Anti-RH Immunoglobulin Therapy for Human Immunodeficiency Virus–Related Immune Thrombocytopenic Purpura

By Eric Oksenhendler, Philippe Bierling, Yves Brossard, Claudine Schenmetzler, Pierre-Marie Girard, Maxime Seligmann, and Jean-Pierre Clauvel

The potential hazards of steroids in human immunodeficiency virus (HIV)-infected patients led us to evaluate the effectiveness and safety of anti-D and anti-c Ig in 17 adults with severe HIV-related immune thrombocytopenic purpura (platelet count < 20 × 10^9/L). The 14 Rh+ patients received 12 to 25 μg/kg of anti-D IgG intravenously on two consecutive days. A significant platelet rise above 50 × 10^9/L was obtained in nine patients. Repeated boosters were performed in six cases and were effective in all cases.

immune thrombocytopenic purpura (ITP) is now well recognized as part of the clinical spectrum of the human immunodeficiency virus (HIV)-related disorders.5 The precise immune mechanism involved in peripheral platelet destruction, however, is still under discussion: nonspecific deposition of complement and/or immune complexes and autoantibodies directed against a specific target protein of the platelet membrane have been advocated.

In previous therapeutic trials, the response to prednisone was similar to that obtained in adults with chronic ITP, but the potential immunosuppressive consequences of such therapy have considerably reduced its utilization. High-dose intravenous (IV) polyvalent immunoglobulin can be an effective and safe treatment; however, its widespread application is hampered by high cost, the need for hospitalization, and its usually transient effect.

An alternative mode of therapy, using Rh antibodies (anti-D) injected into Rh-positive patients, has been proposed for adults and children with idiopathic ITP. More recently it has been reported to be effective, in anecdotal cases, of HIV-related thrombocytopenia.3

In this report, we describe the modalities and effects of treatment by Rh antibodies of 17 nonsplenectomized patients with HIV-related severe ITP.

METHODS AND PATIENTS

Laboratory studies. Platelet bound IgG was detected by a direct immunofluorescence test. Serum IgG with antiplatelet activity was evaluated by using an indirect immunofluorescence assay.9 IgG coating of RBCs was assayed by two techniques: (a) the direct antiglobulin test (DAT) was performed by the standard method with commercial anti-IgG and anticomplement sera (Biotransfusion, France). (b) The IgG antibody coating of RBCs was evaluated by a comparative assay: briefly, anti-Rh IgG was adjusted to 1 μg anti-D/mL against the World Health Organization anti-Rh D IgG standard (no. 68/419). A calibration line was established: four samples (0.25, 0.5, 1, and 2 mL) of this solution were incubated (37°C, one hour) with 1 mL of RBCs of the same Rh phenotype as the patient's RBCs. After washings, eluates were prepared, and their anti-D IgG concentration was measured on an autoanalyzer. Eluates from the patient's RBC were simultaneously prepared and studied. The number of IgG molecules on the patient's RBCs was then obtained by reference to the calibration line and by assuming a molecular weight of 160,000 for IgG, a mean corpuscular volume of 100 μm³ for RBCs, and a similar yield of elution for all samples.

The 3 Rh-positive patients had a good response after they were given 20 mL × 2 of plasma containing potent anti-c antibodies. Therapy was well tolerated, and only one patient had significant hemolysis. These data suggest that anti-Rh IgG can be effective and safe in HIV-related thrombocytopenic purpura and that a specific interaction between the RBC antigens and the anti-Rh antibodies is required.

By using these techniques, the DAT had positive results in patients with more than 70 anti-D IgG molecules/RBC.

The anti-c content of the plasma collected from a single immunized donor was evaluated with regard to its agglutination capacity by the same assay.

Patients. Seventeen patients (14 males and three females) entered this pilot trial because of severe thrombocytopenic purpura associated with HIV-positive serology.

Ten patients were IV drug addicts and seven were homosexual men. None of them had Centers for Disease Control–defined acquired immunodeficiency. The mean age was 28.3 years (range 23 to 42). The initial platelet count was below 20 × 10^9/L in all patients, and had a tendency for mild clinical bleeding. A normal or increased megakaryocyte count was observed in an otherwise normal bone marrow aspiration. Eleven patients had received prior therapy including prednisone, high-dose polyvalent IgG, or danazol. None had been splenectomized. Fourteen patients were Rh-positive (D+), whereas the three others were negative (D−). Platelet-bound IgG was detected in 14 of 17 patients, and serum IgG with antiplatelet activity was present in eight of the 15 patients tested (Table 1). The DAT had positive results in one patient.

Treatment schedule. A commercially available preparation of IV anti-D immunoglobulin (Biotransfusion) was used. Anti-D IgG is prepared with pooled plasma from immunized donors or patients by using the Cohn cold ethanol fractionation method followed by an incubation at pH 4 with pepsin traces. Acid-modified anti-D IgG can be used IV,13 which is not the case for most preparations available in the United States.

The 14 Rh-positive patients were treated with two different
regimens: seven patients received a daily dose of 1,000 μg over a 30-minute infusion on two consecutive days. The total dose ranged from 24 to 34 μg/kg. Eight patients were given 25 μg/kg on two consecutive days. Patient 7 received both regimens 4 months apart.

The three Rh-negative patients received on two consecutive days 20 mL of plasma collected from a single immunized donor that contained potent anti-c antibodies. This dosage was equivalent to 24 to 32 μg/kg × 2 of anti-D IgG with regard to the agglutination capacity. All patients were advised of procedures and attendant risks and gave informed consent.

RESULTS

Results with anti-D IgG in Rh+ (D+) patients. Nine of the fourteen patients (64%) had a satisfactory initial response with a rise of their platelet count above 50 × 10⁹/L by days 3 to 12 (Table 2). The platelet count remained above 25 × 10⁹/L for 11 to 37 days. Three further patients achieved a significant platelet increment after a third infusion was performed; however, there was no evidence for a correlation between the dose used and increase in platelet counts.

The DAT results became positive in all cases, with mild biologic signs of increased hemolysis. Thus, haptoglobin levels below 0.5 g/L were a constant finding. The hemoglobin drop ranged from 4 to 22 g/L. RBC-bound IgG levels, evaluated in nine patients after the two infusions, ranged from 75 to 375 molecules/cell. Platelet-bound IgG and serum antiplatelet activity were not modified by therapy.

Among the patients who had an initial response, six received repeated boosters at the initial daily dose (12 to 25 μg/kg) every 2 to 5 weeks. The response was maintained for 3 to 7 months in five patients.

Results with anti-c IgG in Rh- (C-, D-, E-, c+, e+) patients. All three patients receiving the anti-c plasma had good responses, with increases in platelet counts up to 82, 84, and 412 × 10⁹/L, respectively.

Patient 17, who had the best response, experienced a severe hemolysis with a hemoglobin drop from 124 to 74 g/L. When she was given a booster at half of the initial dose on day 25, her platelet increment remained good, although lower (124 × 10⁹/L), and her hemolysis was mild.

DISCUSSION

Previous studies have reported the results of therapy in HIV-related thrombocytopenia: treatment is not justified in patients with mild thrombocytopenia and no clinical bleeding. Prednisone can be effective, but very few patients maintain their response when not receiving steroids. In addition, in view of the potential severe side effects, long-term utilization of glucocorticoids should be avoided.

Splenectomy is successful in about 75% of the patients for whom surgery was warranted because of the persistence of symptomatic profound thrombocytopenia. High-dose IV polyvalent IgG is a potential and safe alternative therapy, although its effects are usually transient.

Our report shows that anti-D IgG were effective in at least nine of 14 Rh-positive patients with HIV-related ITP. This response rate is in the range of that obtained with high-dose polyvalent IgG. Furthermore, it was well tolerated, and mild hemolysis was clinically inapparent. Repeated infusions remained effective in five of six outpatients for 3 to 7 months. Thus, anti-Rh (D) immunoglobulin therapy is an interesting alternative to conventional therapeutic approaches before considering splenectomy.

The precise mechanism responsible for the beneficial effect of this therapy is not yet firmly established. As previously reported, two patients (no. 6, Rh+; and no. 15, Rh−) received consecutively anti-D IgG and anti-c IgG. These data demonstrated that the beneficial effect requires an interaction between the RBC antigens and their specific antibodies because anti-D IgG is effective only in D+.

### Table 1. Main Clinical and Hematological Data in 17 Patients With HIV-Related ITP

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<tr>
<th>Patient</th>
<th>Sex</th>
<th>Age (Yr)</th>
<th>Risk Group</th>
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<th>Rha</th>
<th>Platelet Count (x 10⁹/L)</th>
<th>Platelet-Bound IgG*</th>
<th>Serum Antiplatelet Antibodies†</th>
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Abbreviations: ND, not done; IgG, high-dose immunoglobulin; DNZ, danazol; PDN, prednisone.

*Direct immunofluorescence assay.
†Indirect immunofluorescence assay.
patients whereas anti-c antibodies are effective in c+ patients. These findings strongly argue against the hypothesis of a direct Fc receptor blockade that was suggested by Petri et al.\textsuperscript{17} to explain the immunosuppressive effect of anti-D IgG toward Rh immunization. Our results are in favor of a competitive inhibition of reticuloendothelial system function by sequestration of IgG-coated autologous RBCs and consequently a decrease in IgG-coated platelet destruction.\textsuperscript{18}

The amount of red cell sensitization by anti-D IgG required for therapeutic effect in ITP is still under discussion.\textsuperscript{18} Indeed, within the limited range of doses used (24 to 50 \(\mu\)g/kg), we failed to demonstrate any fair correlation between dose, RBC-bound IgG levels, and platelet increment. These findings corroborate the results of a previous study in which the efficiency of anti-D IgG in ITP was not correlated with the intensity of induced hemolysis.\textsuperscript{20}

Furthermore, in four treated thrombocytopenic patients, RBC survival was only moderately reduced, and the RBC clearance rate tended to normalize after the first days, whereas the platelet increment persisted.\textsuperscript{21} In our experience, despite the persistence of positive results in the DAT and increasing levels of RBC-bound IgG (up to 845 molecules/cell) over long periods of time (1 to 7 months) in patients receiving repeated infusions, only mild hemolysis occurred, whereas the platelet increment remained stable. These findings suggest that hemolysis may not play a direct and major role in the decrease of the Fc-dependent clearing capacity of the monocyte-macrophage system induced by anti-D, IgG-coated RBCs.

Anti-D IgG is an alternative therapeutic approach for HIV-related immune thrombocytopenia: it is an effective, safe, and well-tolerated therapy. Furthermore, the doses used in our patients cost about 15% of the price of 2 g/kg IV polyvalent IgG.

To date, supplies of anti-D IgG in France are sufficient for both routine immunoprophylaxis of Rh immunization and treatment of a few selected patients with thrombocytopenic purpura.

ACKNOWLEDGMENT
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REFERENCES
Anti-RH immunoglobulin therapy for human immunodeficiency virus-related immune thrombocytopenic purpura

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