Vox Sang. *45:* 40–47 (1983)

0042–9007/83/0451–0040 $2.75/0

Severe Delayed Hemolytic Transfusion Reaction Complicating an ABO-Incompatible Bone Marrow Transplantation

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Abstract. A 26-year-old, blood group O bone marrow transplant recipient experienced a severe, delayed hemolytic transfusion reaction 6 days following transplantation of marrow from his HLA-mixed lymphocyte culture – identical, blood group AB sister. The patient's pretransplant serum contained both anti-A (IgG titer = 1:128; IgM = 1:32) and anti-B (IgG = 1:16; IgM = 1:64) which was reduced by a two-plasma volume plasma exchange followed by transfusion of four units of incompatible, donor type red cells. The patient experienced no immediate adverse reaction. On the 6th posttransplant day, he became acutely dyspneic. His hematocrit dropped to 18%; the direct antiglobulin test was positive for IgG and complement; anti-A and anti-B were eluted from his red cells. His peripheral blood smear demonstrated extensive agglutination resembling a mixed field reaction. This case demonstrates that significant morbidity may be associated with major ABO-incompatible bone marrow transplantation, that the transfusion of incompatible red cells should be undertaken with extreme caution, and that efforts should be continued to develop methods of pretransplant in vitro red cell removal from the infused bone marrow.

In bone marrow transplantation, engraftment and graft versus host disease are independent of ABO compatibility [1–7]. However, it is important to remove recipient isohemagglutinins directed against donor red blood cells to prevent the occurrence of a hemolytic transfusion reaction from red blood cells contained in the bone marrow. This has been accomplished by large volume plasma exchange, at times followed by the infusion of incompatible donor-type red blood cells to absorb remaining antibody [1, 3–7]; by immunoadsorption of isohemagglutinins over a column containing immobilized antigen [8]; or by removing the majority of the incompatible red blood cells from the bone marrow inoculum [2, 5, 9–12]. When these methods are utilized to remove recipient antibody, it is not unusual to find a positive direct antiglobulin test and occasionally low titers of circulating antibody in the early posttransplant period [3, 4]. However, biochemical evidence of hemolysis is very unusual; and there are no published

reports of delayed hemolytic transfusion reactions. We report a blood group O bone marrow transplant recipient who exhibited a severe delayed hemolytic transfusion reaction 6 days posttransplant from a blood group AB donor.

Case Report

The patient was a 26-year-old white male who presented with acute lymphocytic leukemia in July, 1977, 3 years prior to transplantation. Initial remission was achieved with vincristine, prednisone, intrathecal methotrexate, and 2,400 rad prophylactic cranial irradiation. He suffered a bone marrow and testicular relapse in November, 1979, and a second marrow relapse in April, 1980. After achieving his third marrow remission, he was admitted for bone marrow transplantation.

The pretransplant cytoreduction and immunosuppression regimen consisted of cyclophosphamide, 60 mg/kg/day i.v. × 2 days (days –7 and –6); 3 rest days (days –5, –4, –3), and total body irradiation, 750 rad administered on day –2 through anterior and posterior ports in a single dose at 26 rad/min using a 4-MeV linear accelerator [13]. Because of ABO incompatibility between donor and recipient, an 11-liter plasma exchange (two times the patient's estimated plasma volume) was performed over 5.5 h on the day prior to transplant using an Aminco cell separator with a plasma flow rate of 33 cm^3/min. The plasma removed was replaced with 6 liters of 5% human albumin and 5 liters of group AB fresh frozen plasma. During the 10 h following plasma exchange, four units (approximately 840 cm^3) of incompatible group AB (donor type) red blood cells were transfused to absorb residual anti-A and/or anti-B antibody prior to the marrow infusion. He experienced no adverse reaction to the transfusion of these cells in spite of in vitro incompatibility (+1 macroscopic reactions in saline, at room temperature, at 37°C, and in the antiglobulin test).

Bone marrow was obtained from the donor and transfused as previously described [14]. The volume of transfused marrow was 2,290 cm^3 containing 3.28×10^8 nucleated cells/kg recipient body weight and approximately 785 cm^3 of red blood cells. The patient experienced no adverse reaction to the marrow infusion.

Posttransplant, graft versus host disease prophylaxis included methotrexate, 15 mg/m^2 i.v. on day 1; 10 mg/m^2/dose i.v. on days 3, 6, 11, and weekly thereafter; antithymocyte globulin, 15 mg/kg/dose × 7 doses, given on alternative days beginning on day +8, and hydrocortisone, 100 mg/m^2/day on days 7–20. Pneumocystis prophylaxis with trimethoprim/sulfamethoxazole was begun on day +1. Blood component support posttransplant consisted of group O (recipient type) red blood cells and granulocyte concentrates. Platelets transfused prophylactically to maintain peripheral blood platelet count $\geq$20,000/mm^3 were group AB (donor type) whenever available; occasionally it was necessary to transfuse group A, B, or O platelets.

Posttransplant, the patient was clinically well for the first 5 days. During this period, his only transfusions were three group AB units of single donor platelets collected by apheresis. On the 6th posttransplant day he became acutely dyspneic. His hematocrit of 18% had fallen from 30% during the preceding 36 h. The diagnosis of a severe delayed hemolytic transfusion reaction was made on the basis of: rapid drop in hemoglobin in the absence of bleeding; indirect hyperbilirubinemia, extensive agglutination on peripheral blood smear, positive direct antiglobulin test (IgG and C′ detected), and the presence of anti-A and anti-B eluted from the patient's red cells. He improved rapidly following therapy with fluids, corticosteroids, and the transfusion of several units of group O red blood cells.

Bone marrow biopsy on day 14 demonstrated early evidence of engraftment. Further complications included sepsis, subcutaneous abcess, oral mucositis with herpes simplex, cytomegalovirus isolated from urine, and interstitial pneumonitis. Autopsy on day 25 revealed hemorrhagic esophagitis and cytomegalovirus pneumonitis.

Materials and Methods

Erythrocyte typing was performed with commercially prepared reagents according to the manufacturer's instructions. Mixed field estimates were made microscopically by a single observer (J.S.) by estimating the number of free and agglutinated cells in several fields and expressing the number of agglutinated cells as a percentage of the total. Antibody elutions were performed by the heat method [15]. Direct antiglobulin testing was performed using routine methods and commercially available sera. Monospecific antiglobulin se-

rums (anti-IgG and anti-C3) were obtained from Ortho Diagnostics, Raritan, N.J. Anti-A and anti-B IgM antibody titers were determined by incubation of saline-suspended red cells at room temperature for 30 min. Anti-A and anti-B IgG antibody titers were determined by incubating dithiothreitol-treated serum with albumin-suspended red cells at 37 °C for 30 min followed by addition of antiglobulin [16]. Immunofluorescence staining for IgG, IgM, and C3 was performed on acetone-fixed, air-dried peripheral blood smears as previously described [17]. Typing for HLA-A and B locus antigens was performed using antisera with known specificity for 14 A locus and 22 B locus antigens. The bidirectional mixed lymphocyte culture was performed utilizing previously described techniques [18].

Results

The bone marrow donor and recipient were identical for HLA-A and B loci; both were HLA-A1, B8/Aw31, B40. The patient's cell response to stimulation by donor cells in the mixed lymphocyte culture assay and the reciprocal mixed lymphocyte culture were in the negative range. The patient was blood group O; the donor was group AB.

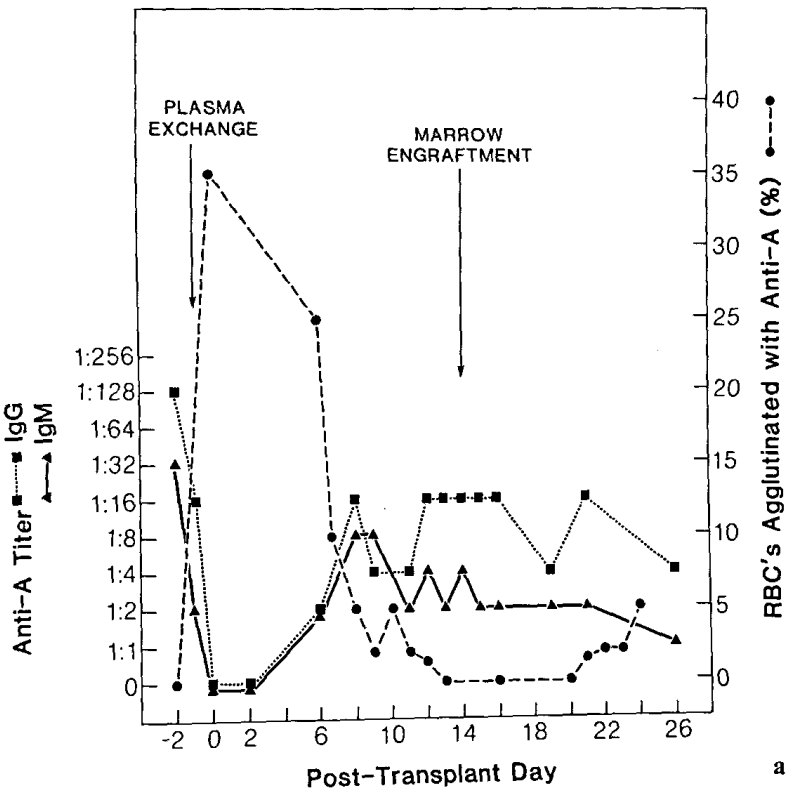
In table I are shown the decreases in anti-A and anti-B as a result of the plasma exchange and the pretransplant transfusion of group AB (donor type) red cells which were incompatible in crossmatch (macroscopic reactions observed in saline at room temperature, at 37 °C, and in the antiglobulin test). No anti-A or anti-B was detectable in the serum at the time of the marrow infusion, which included approximately 785 cm^3 of red cells. Anti-A and anti-B reappeared in the patient's serum following the day 6 hemolytic episode and transfusion of four units of group O red cells, and remained detectable throughout the remainder of the hospital course (fig. 1).

The percentage of circulating red cells which agglutinated with anti-A and anti-B, zero prior to plasma exchange, reached a peak of 35 and 30%, respectively, posttransplant, dropped sharply following the hemolytic episode, became undetectable at 2 weeks posttransplant, and reappeared in small numbers after day +19 (fig. 1).

The direct antiglobulin test which was negative prior to transplantation was positive on day 6 posttransplant using broad spectrum Coombs serum, monospecific IgG, and complement (C3). Anti-A and anti-

Table I. Effects of plasma exchange and infusion of incompatible (donor type) red blood cells on the titers of various antibodies

Antibody	Titer		
	preexchange	postexchange pre-RBC infusion	post-RBC infusion premarrow infusion
Anti-A			
IgM	1:32	1:2	0
IgG	1:128	1:16	0
Anti-B			
IgM	1:64	1:2	0
IgG	1:8	1:1	0



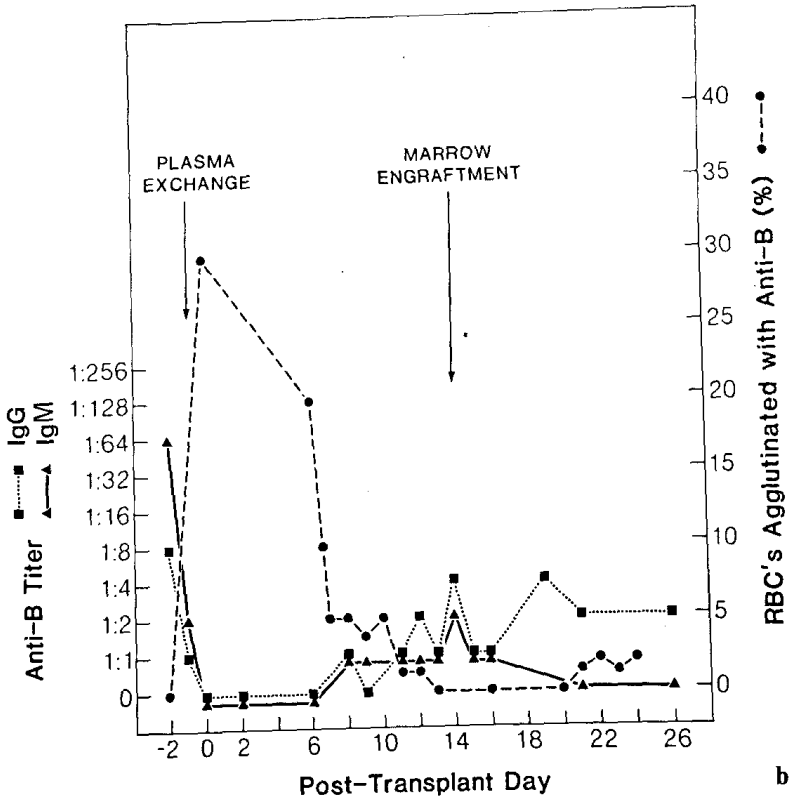


Fig. 1. Blood group O bone marrow transplant recipient. Graphs illustrating anti-A (**a**; IgG and IgM) and anti-B (**b**; IgG and IgM) titers over the course of the patient's hospitalization in relationship to the percentage of circulating red cells which agglutinated with anti-A and anti-B, respectively. Shown is the acute decrease in the percentage of circulating cells which agglutinated in the presence of anti-A and anti-B, and the rise in anti-A and anti-B coincident with the hemolytic transfusion reaction on posttransplant day 6.

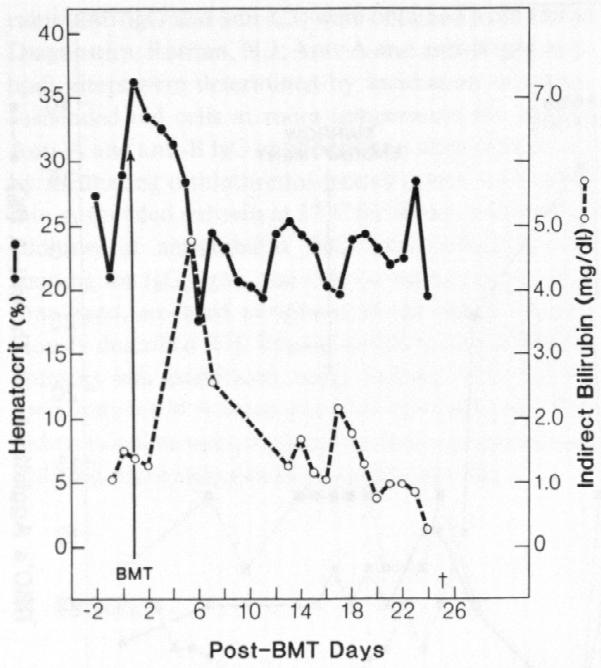


Fig. 2. Relationship between hematocrit and serum level of indirect bilirubin throughout the transplant course, demonstrating the simultaneous occurrence of the hematocrit nadir and indirect bilirubin peak. BMT = Bone marrow transplant.

B were eluted from the patient's circulating cells. Figure 2 illustrates the changes in serum bilirubin and hematocrit throughout the patient's course. Prior to transplant, the total and indirect bilirubin were within the normal range. Following the pretransplant infusion of incompatible red cells, the total bilirubin rose to 1.7 mg/dl (normal <1.2); the indirect reacting fraction rose to 1.5 mg/dl (normal < 0.9). In association with the hemolytic episode, total bilirubin peak was 4.8 mg/dl. Subsequently, values returned to normal range.

Figure 3 illustrates the evolution of the peripheral blood smear. Red blood cell morphology was normal prior to transplant. In chronological order, the following abnormalities were noted: appearance of bite cells; spherocytes, singly, then as clusters of cells; frank aggregates, and prominent, fine, intererythrocytic bridges. Figure 4 illustrates immunofluorescence on a peripheral

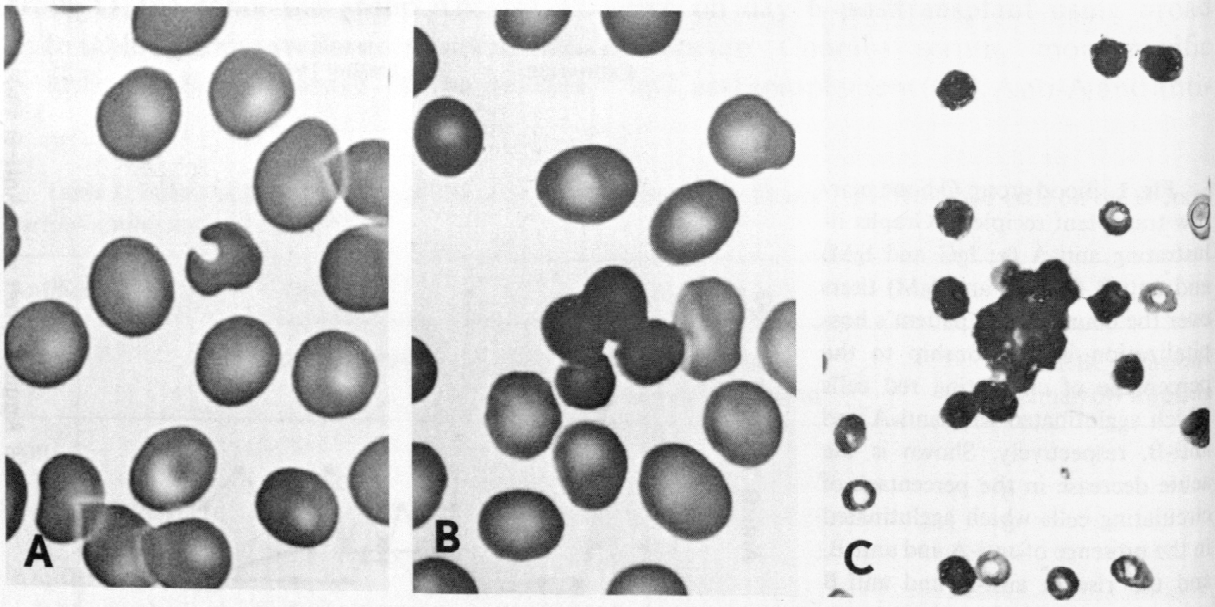


Fig. 3. Peripheral blood smears demonstrating: (**A**) Bite cell, 2nd posttransplant day. × 1,000. (**B**) Spherocyte cluster, 4th posttransplant day. ×1,000. (**C**) Red blood cell aggregate, 6th posttransplant day. ×400.

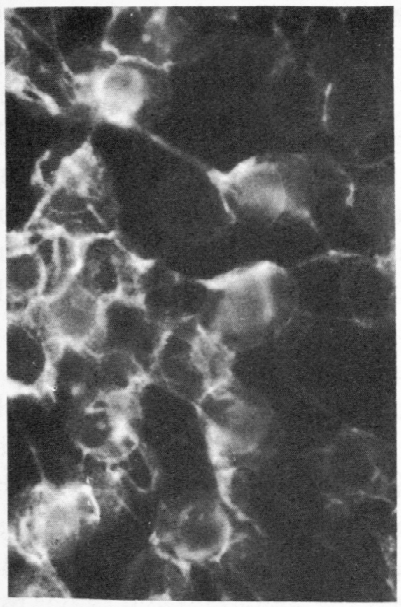


Fig. 4. Peripheral blood smear demonstrating red blood cell aggregates, stained with fluorescein-labeled anti-IgG, 9th posttransplant day. ×1,000.

blood smear from the 9th posttransplant day, demonstrating prominent staining of the red blood cells and the intercellular bridges with IgG. Complement (C3) stained weakly; IgM was essentially negative.

Discussion

Successful bone marrow transplantation despite ABO incompatibility between donor and recipient has frequently been reported without an increased risk of graft rejection or graft versus host disease [1–7]. However, invariably one or more of several possible pretransplant procedures has been utilized to minimize the morbidity resulting from hemolytic transfusion reactions. Reported methods of patient preparation for major ABO-incompatible transplant where the recipient has ABO antibodies directed against donor red cells include: whole blood or plasma exchange [1, 3–7, 12], immunoadsorption of antibodies during plasmapheresis onto a column containing artificially synthesized blood group antigens [8], transfusion of incompatible red cells, and in vitro pretransfusion marrow processing to remove red blood cells [2, 5, 9–12].

The preparative regimen utilizing large-volume plasma exchange followed by transfusion of donor type-incompatible red cells is predominantly empiric [1, 3–7]. There has been only one report [3] of an acute hemolytic transfusion reaction and no report of delayed hemolysis following this preparation. However, *Berkman* et al. [1] described shortened survival of transfused red cells consistent with delayed hemolysis in a patient being prepared for an ABO-incompatible marrow transplant. Also, *Ockelford* et al. [19] described slight prolongation of incompatible red cell survival following plasma exchange.

The success of this method of recipient preparation in removing sufficient antibody to prevent a serious hemolytic transfusion reaction during and after marrow infusion depends on several variables, including the volume of plasma exchanged [20], the volume of incompatible donor type red cells transfused, the extent of immunosuppression, initial titer and immunoglobulin class of anti-A and anti-B antibodies, and the amount of anti-A and B transfused posttransplant. Since there is only 1 reported case in which antibody titers are related to the survival of incompatible red cells [1], the conditions under which incompatible red cells or bone marrow may be safely transfused have not been defined.

The direct antiglobulin test and the immunofluorescence studies demonstrate that IgG was responsible for the hemolysis on the

6th posttransplant day. The circulating incompatible red cells were coated with IgG; and spherocytosis, in vivo agglutination, indirect hyperbilirubinemia, and a rapid fall in the hematocrit occurred consistent with the pattern described by *Romano and Mollison* [21] and *Mollison* [22]. The red cell aggregates are thought to be loose and easily disrupted [22], producing the transient development of the striking mixed field reaction. In addition, it is possible that some of the patient's own group O red cells were transiently incorporated into these agglutinins since it has been shown that group O red cells can passively absorb A and B substance and be induced to agglutinate with the appropriate antiserum [23]. Although we could not document that this phenomenon occurred in our patient, he did receive 5 liters of group AB fresh frozen plasma, four units of group AB red cells, and group AB bone marrow, containing substantial amounts of A and B substances.

In this patient, several factors likely contributed to the development and severity of the delayed transfusion reaction. Prior to plasma exchange, the patient had a relatively high titer of IgG anti-A (1:128); it is not known if the IgG anti-B titer of 1:8 is additive. Second, the large size of the patient (105 kg) suggests the presence of a large extravascular pool of IgG which slowly re-equilibrated with the intravascular pool. Third, he was given a large volume of group AB red cells (approximately 1,625 cm^3 as red cell transfusion and in the bone marrow inoculum) during the early phase of this re-equilibration. These group AB cells constituted a significant fraction of his circulating red cell mass immediately posttransplant.

This case provided a unique opportunity to study the sequential morphologic changes occurring during a severe delayed hemolytic transfusion reaction. It supports the observation that IgG anti-A and anti-B induce spherocytosis and in vivo agglutination [23, 24]. It also suggests that the earliest observable morphologic change in delayed hemolysis is the appearance of bite cells, and demonstrates the formation of intercellular erythrocyte bridges, probably comprised of cell membrane containing IgG.

In view of the documentation of hemolysis of donor type red cells and resultant morbidity in this case, it is recommended that ABO-incompatible transplants be managed by improved methods of in vitro removal of red cells from the bone marrow rather than by removal of circulating antibody from the recipient.

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Received: August 5, 1982
Accepted: September 20, 1982

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