Donor-Derived Red Blood Cell Antibodies and Immune Hemolysis After Allogeneic Bone Marrow Transplantation

By Jill Hows, Keith Beddow, Edward Gordon-Smith, Donald R. Branch, Wayne Spruce, Irena Sniecinski, Robert A. Krance, and Lawrence D. Petz

Six cases of immune hemolytic anemia attributed to donor-derived red cell antibodies after allogeneic bone marrow transplantation (BMT) are reported. In 2/6 cases, severe intravascular hemolysis was seen, 6/6 required increased red cell transfusion, and 1/6 was treated by plasma exchange. All recipients were receiving cyclosporine to prevent graft-vs-host disease. Investigations showed that in each case, the donor lacked ABO or Rh(D) red cell antigens present in the recipient. The direct antiglobulin test was positive in 6/6. Relevant serum antibody (anti-A, four cases; anti-B, one case; anti-D, one case) was first detected one to three weeks after BMT. Eluates made from recipient red cells showed the same specificity as serum antibody. Maximum hemolysis occurred nine to 16 days after BMT, suggesting that active production of antibody by "passenger" donor lymphocytes was the likely mechanism of hemolysis, rather than passive transfer of antibody.

ALLOGENEIC bone marrow transplantation (BMT) in humans involves the transfer of mature donor lymphocytes to the recipient. It is theoretically possible for transferred donor lymphocytes to produce antibodies specific for recipient red cell antigens that are lacking on the donor's own red cells. If this occurs, it could present clinically as immune hemolysis in the posttransplant period. Conceivably, complications such as renal failure could occur in association with severe hemolysis.

We report six bone marrow transplantation (BMT) cases in which the appearance of red cell antibodies was associated with clinical and laboratory evidence of immune hemolysis. The peak time for hemolysis was one to two weeks after BMT, making passive transfer of plasma antibody in the marrow infusion an unlikely cause. From the data presented, we suggest that active production of antibody by donor lymphocytes stimulated by recipient RBC antigens is the cause of the hemolysis. To determine the frequency of hemolysis due to this mechanism, we have retrospectively analyzed 97 consecutive cyclosporine-treated BMT patients from one center (Hammersmith). The association between cyclosporine and this phenomenon was strengthened by our failure to find, on retrospective analysis, a single case of immune hemolysis or donor-derived antibody production in 13 patients treated with methotrexate in the postgraft period.

MATERIALS AND METHODS

Patients

Group 1. Six patients from three transplant centers who presented clinically with immune hemolysis after BMT were investigated. Informed consent to investigations was obtained according to each of the transplant center's ethical committee guidelines. Clinical and laboratory data are shown in Table 1. Hematologic and biochemical data illustrating a typical case are shown in Fig 1. Patients 1 through 5 were transplanted from HLAl-identical sibling donors and patient 6, from an HLA phenotypically matched unrelated donor. In cases 1 through 5, the donors lacked A or B antigens present on the recipients' red cells. In case 6, the patient was A Rh(D) positive and the donor A Rh(D) negative; the donor's serum contained weak anti-D at the time of the transplant. All patients received cyclosporine after BMT to prevent graft-vs-host disease (GVHD).

Group 2. To assess the frequency of immune hemolysis, a retrospective analysis of 97 consecutive BMT patients transplanted at one center (Hammersmith) was carried out. All patients received cyclosporine in the postgraft period for the prevention of GVHD. Twenty-one patients received minor ABO mismatched marrow (ie, donor lacking ABO or Rh antigens present in the recipient) and were designated "group 2" patients. Within group 2, 14 patients received marrow from donor lacking recipient A or B antigens; this included cases 1 and 2 from group 1. Seven D-positive recipients received marrow from D-negative donors and included case 6 from group 1. Two patients were ABO and D mismatched. Group 2 patient charts were reviewed for evidence of immune hemolysis. Weekly blood bank records from the post-BMT period were searched for the occurrence of any positive direct antiglobulin tests (DAT). Sera from group 2 patients receiving minor ABO mismatched marrow, stored at −20 °C for up to 36 months, were tested for donor type IgG and IgM anti-A and anti-B at one and three months after BMT.

Serology

Standard serological methods were used. The DAT was performed using polyclonal antihuman globulin (AHG) reagents. In DAT-positive cases, testing with anti-C3 and anti-IgG reagents was carried out. Eluates were made by the chloroform or ether technique. Anti-A and anti-B were estimated by titrating the stored...
Table 1. Clinical and Laboratory Data—Patients Presenting With Immune Hemolysis—Group 1

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Diagnosis</th>
<th>ABO Group and Rh Type</th>
<th>Immunosuppressive Therapy Pre-BMT</th>
<th>Packed RBCs</th>
<th>Platelets</th>
<th>Details of Hemolysis and RBC Transfusion Requirement</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>CGL</td>
<td>17/M 15/F O+ A-</td>
<td>Cy A 60 mg/kg x 2 DN 60 mg/m² x 1</td>
<td>CyA</td>
<td>A-</td>
<td>Severe IVH. Hemoglobinuria. Maximum hemolysis day +15. Treated with plasma exchange. Nine units RBCs required day +10 to +15.</td>
</tr>
<tr>
<td>2</td>
<td>CGL</td>
<td>13/M 11/F O+ A+</td>
<td>Cy A 60 mg/kg x 2 DN 60 mg/m² x 1</td>
<td>CyA</td>
<td>A+</td>
<td>Moderate hemolysis. Maximum day +16. Six units RBCs required day +16 to +22.</td>
</tr>
<tr>
<td>3</td>
<td>SAA</td>
<td>24/M 16/F O+ B+</td>
<td>Cy A 50 mg/kg x 4 TNI 750 cGy</td>
<td>CyA</td>
<td>O+ B+</td>
<td>Severe hemolysis. Hemoglobinuria. Maximum day +10. Fourteen units RBCs required day +10 to +19 (see Fig 1).</td>
</tr>
<tr>
<td>4</td>
<td>AML</td>
<td>11/F 14/M O+ A+</td>
<td>Cy A 120 mg/kg x 1 TBI 120 x 11 cGy</td>
<td>CyA</td>
<td>A+</td>
<td>Moderate hemolysis. Maximum day +10. Seven units RBCs required day +9 to +18.</td>
</tr>
<tr>
<td>5</td>
<td>ALL</td>
<td>26/M 22/M B+ AB+</td>
<td>Cy A 120 mg/kg x 1 TBI 120 x 11 cGy</td>
<td>CyA</td>
<td>O+ B+</td>
<td>Moderate hemolysis. Maximum day +12. Six units RBCs required day +11 to +14.</td>
</tr>
<tr>
<td>6</td>
<td>SAA</td>
<td>32/F 17/F A- A+</td>
<td>Cy A 50 mg/kg x 4 ALG 12.5 mg/kg x 4 Azathioprine</td>
<td>CyA HDMP</td>
<td>A- A+</td>
<td>Severe hemolysis. Hemoglobinuria. Maximum day +13. Five units RBCs required day +9 to +16.</td>
</tr>
</tbody>
</table>

*Platelets were washed and resuspended in AB plasma. D, donor; R, recipient; CyA, cyclophosphamide; DN, daunorubicin; TBI, total body irradiation; TNI, total nodal irradiation; CyA, cyclosporine; HDMP, high-dose methylprednisone; MP, methylprednisone; ALG, anti-lymphocyte globulin.

sera against suitable test red cells. The agglutination titer at 20 °C was measured as well as the titer at 37 °C using anti-IgG AHG in the indirect antiglobulin test. Appropriate saline controls were performed. The IgG and IgM components of the antibodies were estimated by testing the sera before and after treatment with 2-mercaptoethanol or dithiothreitol. The IgM titers were measured by agglutination at 20 °C, and the IgG titers were estimated by the indirect antiglobulin test at 37 °C.

RESULTS

Group 1. Group 1 patients presented clinically with hemolysis between days 9 and 16 post-BMT (median, 12) after BMT. Antibodies against the recipient’s red cell antigens were first detectable nine to 15 days (median, 13) after BMT. In cases 3 through 6 inclusive eluates prepared from recipient RBCs contained antibody to antigens present on recipient red cells of the same specificity found in the serum. Table 2 also shows that the red cell antibodies were only transiently present in the sera of the ABO-incompatible cases. However, in the D-incompatible case (case 6), the anti-D titer continued to rise, reaching a level of 256 on day 70 after BMT.

Group 2. Relevant serological data is shown in Table 3. Six out of 16 patients receiving ABO mismatched marrow (including cases 1 and 2 from group 1) developed a positive DAT after BMT. However, only 2/6 (cases 1 and 2 in group 1) showed clinical evidence for immune hemolysis. Donor anti-A and anti-B titers pre-BMT and recipient anti-A and anti-B titers one and three months after BMT are shown. Antibodies against the recipient’s red cell antigens were first detectable nine to 15 days (median, 13) after BMT. In cases 3 through 6 inclusive eluates prepared from recipient RBCs contained antibody to antigens present on recipient red cells of the same specificity found in the serum. Table 2 also shows that the red cell antibodies were only transiently present in the sera of the ABO-incompatible cases. However, in the D-incompatible case (case 6), the anti-D titer continued to rise, reaching a level of 256 on day 70 after BMT.

DISCUSSION

Anti-A and anti-B production against donor red cells by residual recipient plasma cells after major ABO mismatched
BMT is well documented. Hemolysis of donor red blood cells at the time of the transplant can be prevented by removal of recipient antibodies by plasma exchange or by removal of donor red cells from the marrow before infusion.

More recently, cases of immune hemolysis occurring in recipients of minor ABO mismatched marrow have been reported. Some reported episodes of hemolysis were clinically severe, and two cases of acute renal failure associated with hemoglobinuria were documented. Cases of immune hemolysis occurring in patients receiving minor ABO mismatched liver and kidney transplants have also appeared in the literature.

We have investigated six patients from three transplant centers who presented with immune hemolysis after minor ABO or D mismatch BMT. From our data, active production of donor-derived red cell antibody is the most likely mechanism. Maximum hemolysis occurred one to three weeks after BMT, suggesting that passive transfer of antibody at the time of the transplant is an unlikely cause. None of the patients in group 1 received significant ABO-incompatible plasma in blood products (Table 1); therefore, transfusion of incompatible plasma does not account for the phenomenon.

Retrospective analysis of minor ABO mismatched BMT patients showed that only a minority (2/16) developed clinically significant hemolysis, despite the production of antibody against recipient red cell antigens in the majority of cases (Table 3). Six out of 16 patients developed a positive DAT, suggesting that in vivo red cell sensitization often occurs without signs of overt hemolysis. From data shown in Table 3, it can be seen that the onset of immune hemolysis cannot be predicted from either donor or recipient anti-A or anti-B titer. Most minor ABO mismatched patients were transfused with packed group O cells. However, the transfusion of group A or B red cells may have contributed to the severity of hemolytic episodes seen in patients 1, 2, 4, and 5 in group 1 (Table 1).

We have shown that production of donor-derived antibody specific for recipient ABO antigens is transient, with the relevant antibody being virtually undetectable by three months post-BMT (Table 3). These data are consistent with a report by Buckner et al in which donor-derived antibody against recipient ABO antigens was absent or weak one year after BMT. The transient nature of donor-derived antibody against recipient ABO antigens is not understood and contrasts with the more persistent donor-derived rhesus antibodies detected. Possible mechanisms are discussed below.

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Maximum Hemolysis</th>
<th>ABO Group Rh Type</th>
<th>DAT First Positive</th>
<th>Antibody in Eluate</th>
<th>Antibody in Serum</th>
<th>Day Post-BMT Serum Antibody Last Detected</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>+15</td>
<td>O+</td>
<td>C3</td>
<td>+18</td>
<td>ND</td>
<td>Anti-A</td>
</tr>
<tr>
<td>2</td>
<td>+16</td>
<td>O+</td>
<td>C3</td>
<td>+15</td>
<td>ND</td>
<td>Anti-A</td>
</tr>
<tr>
<td>3</td>
<td>+10</td>
<td>O+</td>
<td>IgG + C3</td>
<td>+10</td>
<td>Anti-B</td>
<td>Anti-B</td>
</tr>
<tr>
<td>4</td>
<td>+10</td>
<td>O+</td>
<td>C3</td>
<td>+4</td>
<td>Anti-A</td>
<td>Anti-A</td>
</tr>
<tr>
<td>5</td>
<td>+12</td>
<td>B+</td>
<td>C3</td>
<td>+9</td>
<td>Anti-A</td>
<td>Anti-A</td>
</tr>
<tr>
<td>6</td>
<td>+13</td>
<td>A−</td>
<td>IgG</td>
<td>+13</td>
<td>Anti-D</td>
<td>Anti-D</td>
</tr>
</tbody>
</table>

DAT, direct antiglobulin test; D, donor; R, recipient; ND, not done.

*Serum antibody not detectable in subsequent follow-up.
†Anti-D titer 256; later samples not tested.
§Minor ABO and rhesus (D) mismatch. No anti-D detected in recipient.
¶Case 6 from group 1.
|||
In contrast to the transient antibody production in the ABO cases, the recipients of D-incompatible marrow continued to produce anti-D for prolonged periods of up to one year after BMT. The early disappearance of donor-derived antibodies in the ABO cases may be due to high zone tolerance brought about by continued exposure of donor lymphocytes to A or B antigens in the recipients' plasma and tissues. In contrast, the rhesus mismatched BMT donor lymphocytes are only exposed to recipient rhesus antigens transiently until residual recipient red cells are eliminated from the circulation. The antigenic stimulus for donor-derived rhesus antibody production may not be strong enough to induce tolerance, but instead leads to prolonged antibody production.

The role of cyclosporine in facilitating donor-derived antibody production is uncertain. It is of interest that all cases of immune hemolysis due to donor-derived antibody so far reported have been in transplant recipients immunosuppressed with cyclosporine. Our own experience confirms this observation. We have retrospectively reviewed 13 patients transplanted in one center (Duarte) in whom methotrexate was used in the postgraft period to prevent GVHD (data not shown). We have been unable to identify a single case of immune hemolysis due to donor-derived antibody. This group of patients is now being studied prospectively to confirm our retrospective observations.

Cyclosporine is a powerful immunosuppressive agent that acts primarily by blocking proliferation of T lymphocytes in response to antigen or mitogens. However, T lymphocyte proliferation is not impaired when presensitized lymphocytes are reexposed to antigen in the presence of cyclosporine. In minor ABO mismatched BMT, the donor lymphocytes are sensitized in vivo before transfer to the recipient, so donor-derived red cell antibody production is a secondary immune response. In one D minor mismatched case (AR), we have shown that the donor was presensitized to the D antigen, with anti-D present in the donor's serum. The donor-derived anti-D produced in this recipient was, therefore, the result of a secondary immune response to the D antigen by the donor lymphocytes, analogous to the production of anti-A and anti-B in the ABO-incompatible cases described. We have no definite evidence for donor presensitization to rhesus antigens in the other two cases in which donor-derived rhesus antibodies were produced. Indeed, the donor for patient AG was a 15-year-old boy who had not been transfused, suggesting that in this case, the production of donor-derived anti-D, -C, and -E in the recipient was due to a primary immune response by the donor lymphocytes. In the third case in which donor-derived rhesus antibodies were detected (CJ), the marrow was donated by the patient's sister. Her serum did not contain detectable rhesus antibodies at the time of the transplant; however, she has two rhesus D-positive children aged 15 and 17 years. It is therefore possible that the donor became sensitized to the D antigen during pregnancy and that the production of donor-derived anti-D in the recipient was a secondary response.

Although the frequency of clinical hemolysis is low in minor ABO and rhesus mismatched BMT, it can be severe. All six group I patients required increased red cell support, two patients had evidence for intravascular hemolysis, and one patient required plasma exchange. Renal failure was not apparent in our patients; however, this remains a potential complication. It is not possible to predict which patients will hemolyze from monitoring donor and recipient anti-A and anti-B titers (Table 3). Our data suggest that significant hemolysis occurs in approximately 10% to 15% of cases receiving minor ABO or D mismatched transplants. To prevent exacerbation of hemolysis, recipients of minor ABO mismatched transplants should be transfused with packed or washed group O red cells. In D mismatched cases, D-negative red cells of the appropriate ABO group should be given. Platelet concentrates given to minor ABO mismatched BMT patients should be recipient group or group O. If group O platelets are used, transfusion of significant ABO-incompatible plasma can be avoided by using platelet preparations concentrated by centrifugation. These measures will, however, not completely prevent hemolytic episodes.

In summary, we have documented the occurrence of immune hemolysis in approximately 10% to 15% of marrow transplant recipients who received minor ABO or D mismatched marrow and were treated with cyclosporine. We believe that hemolysis is due to active production of red cell antibody by donor lymphoid cells in the early posttransplant period. Antibody production may be associated with cyclosporine immunosuppression. Further work is in progress to evaluate the role of cyclosporine in the pathogenesis of this syndrome. When hemolysis occurs, it may be severe, and we have suggested a transfusion policy for patients receiving minor mismatched transplants that we hope will prevent exacerbation of the hemolysis.

REFERENCES


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J Hows, K Beddow, E Gordon-Smith, DR Branch, W Spruce, I Sniecinski, RA Krance and LD Petz