Bone Marrow Transplantation With Major ABO Blood Group Incompatibility Using Erythrocyte Depletion of Marrow Prior to Infusion

By Hayden G. Braine, Lyle L. Sensenbrenner, Susan K. Wright, Peter J. Tutschka, Rein Saral, and George W. Santos

Twenty-five patients with major ABO blood group incompatibility between donor and recipient underwent allogeneic bone marrow transplantation using erythrocyte depletion of the bone marrow infusate prior to administration. Over 95% of the original erythrocyte content of the marrows was removed, while retaining 75% of the mononuclear cell content and 57% of the granulocyte-monocyte colony-forming units. Recipients, well hydrated and premedicated with corticosteroids, diphenhydramine, and mannitol, tolerated infusions well. The frequency of engraftment, rate of recovery of peripheral blood leukocytes, granulocytes, and platelets, and the incidence of graft-versus-host disease was similar to that observed following ABO blood group compatible bone marrow transplantation. Erythroid development following ABO blood group incompatible transplantation was significantly impaired until hemagglutinins fell to 1:4 or lower, at which time recovery of erythrocytes was detected in the peripheral blood. The erythrocyte hypoplasia associated with incompatible hemagglutinins was temporary. Erythrocyte purging is a safe and effective technique to perform bone marrow transplantation across major ABO blood group incompatibilities.

SUCCESSFUL BONE MARROW transplantation between donor and recipients with major ABO blood group incompatibilities was first reported in 1978. Hemolysis of the incompatible erythrocytes in the bone marrow infusate was minimized by depleting the recipient of hemagglutinins by plasmapheresis and absorption with appropriate erythrocyte transfusions. The introduction of perfusion columns to specifically absorb anti-A and anti-B hemagglutinins has reduced some of the toxicities of this approach, but it is still difficult. Plasmapheresis of recipients is still required and hemagglutinins are rarely removed completely. Since 1976 we have employed the alternate approach of removing the bulk of erythrocytes from the harvested bone marrow and infusing the concentrated nucleated cell fraction without attempting to lower recipient hemagglutinins. Such depletion of erythrocytes from marrow has been achieved using sedimentation and centrifugation techniques. Here we report our experience with 25 consecutive ABO blood group incompatible allogeneic bone marrow transplants (BMT) using the technique of erythrocyte depletion.

MATERIALS AND METHODS

Patient Population

From 1976 through 1980, 25 patients underwent ABO blood group incompatible allogeneic BMT for severe aplastic anemia. Successfully transplanted patients would receive total body irradiation (TBI) (11 ALL, 1 AA, 1 AML; antithymocyte globulin, procarbazine, and TBI (1 AA); busulfan (BU) 4 mg/kg p.o. for 4 days followed by Cy 50 mg/kg/day × 4 days (2 AML); and BU Cy TBI (1 CML) (Table I). Posttransplantation immunosuppression included either Cy or methotrexate for graft-versus-host disease (GVHD). Prophylactic platelet transfusion for platelet counts less than 20,000/cumm and 5-day transfusion series of leukocyte concentrates were generally employed. Type O erythrocytes were transfused to maintain the hematocrit over 25% until serologic testing indicated donor type red cells were compatible.

Bone Marrow Collection and Processing

Bone marrow was collected, anticoagulated with heparin (10 U/ml), and diluted with TC-199 using standard technique. The final marrow–tissue culture mixture totaling 600–2000 ml was filtered through stainless steel screens (final 0.0055 in.). The Haemonetics Model-30 cell separator (Haemonetics Corp., Braintree, Mass.) was prepared using the needleless harness (no. 6630) and 100 ml pediatric plasmapheresis bowl (no. 7641) and adaptor (Fig. 1). The marrow collection bag was entered with a transfer set and connected to the harness with a Y connector. The marrow was kept well mixed by gentle hand agitation. Bowl filling was started at 40 ml/min. Marrow was mixed in a 8:1 ratio with acid citrate dextrose NIH formula A (Fenwal, Deerfield, Ill.). Whenuffy coat was appreciated at the top of the bowl, the collection rate was slowed to 20 ml/min. Two 40-sec (13 ml) collections were sequentially channeled into 300-ml collection bags attached to the usual platelet (fraction I) and leukocyte (fraction II) ports. Timing was begun when theuffy coat was observed at the initial solenoid. The erythrocyte-rich bowl contents and plasma/TC-199 waste were returned to a reservoir bag. Repetitive cycles were performed until all marrow was processed. The leukocyte concentrates (fractions I
and II) were then diluted to approximately 100 ml with residual plasma/TC 199 in order to allow a sufficient volume for transfusion.

**Marrow Infusion**

For 12 hr prior to and following marrow infusion, recipients were hydrated with half normal saline and diuresed with intravenous furosemide to maintain a urine flow greater than 100 ml/hr. Thirty minutes prior to infusion, all patients were premedicated intravenously with 50 mg of diphenhydramine, 250 mg hydrocortisone sodium succinate, and 50 ml of 20% mannitol. Fraction I, containing the majority of the nucleated cells and the fewest erythrocytes, was infused over 15–30 min. If no untoward effects were noted, fraction II was then infused.

**Laboratory Testing**

Granulocyte-monocyte colony-forming cells (CFU-GM) were determined in agar cultures using a modification of the technique of Pike and Robinson on 13 bone marrows before and after erythrocyte depletion. All patients received daily leukocyte, platelet, and hema-
tocrit determinations. Reticulocyte and differential counts were obtained biweekly. Hemagglutinin titers were determined weekly using the method of Crawford, which measures both IgM and IgG. All patients who lived 21 days or longer were tested weekly until appropriate chromosomal, immunogenetic, or erythrocyte typing documented engraftment.

Bone marrow aspirates were obtained weekly and examined to determine the effect of ABO blood group incompatibility on bone marrow development. The day posttransplant to peripheral blood recovery of 1000 leukocytes/cumm and a reticulocytosis of 1% were determined in each case. A marrow aspirate obtained within 7 days of achieving each stage of peripheral blood reconstitution was then simultaneously blindly evaluated by two observers. Technically adequate bone marrow aspirates were available on 14/19 ABO blood group incompatible patients when peripheral blood leukocytes exceeded 1000/cumm and on 9/19 patients when reticulocytes exceeded 1%. Bone marrow aspirates obtained from ABO blood group compatible patients matched for preparative regimen and diagnosis were evaluated at equivalent stages of peripheral blood leukocyte differentiation (leukocytes >1000; n = 18) and erythroid differentiation (reticulocytes >1%; n = 25). Erythroid development was assessed as absent (aplasia) or hypoplastic relative to myeloid differentiation [myeloid:erythroid (M:E) ratio >4:1]. In two cases a M:E ratio could not be determined because of marked hypocellularity. Dyserythropoiesis and megaloblastic erythroid differentiation were scored (present or absent) when the blinded observers felt there were adequate numbers of erythroid forms to evaluate.

**RESULTS**

**Erythrocyte Depletion and Administration of ABO Blood-Group Incompatible Marrow**

Processing of marrow with the Haemonetics Model-30 was easily accomplished. Definition of the buffy coat from the pink supernatant required skill but was learned easily. A mean of 5.6% (range 2.7%–8.8%) of...
The original red cell volume was contained in the final infusate (Table 2). Fraction I contained a mean of 9.2 ± 5.0 ml of erythrocytes and fraction II a mean of 12.6 ± 5.6 ml. Seventy-five percent of the original marrow mononuclear cell content with 57% of the original CFU-GM were contained in fractions I and II (2/3 in fraction I and 1/3 in fraction II).

Fifteen patients tolerated the infusion of the marrow concentrate without adverse effects. Nine adequately premedicated patients experienced symptoms attributable to the marrow infusion: 3 episodes of fever (rise in temperature greater than 1°C), 2 of hypertension (maximum 190/120), 3 of chills, 2 of hemoglobinuria, and 1 of bradycardia and confusion. One patient who was not premedicated developed acute shortness of breath, wheezing, back pain, hemoglobinuria, and hypertension (230/150) during the infusion of fraction I. After standard premedication, fraction II was subsequently given without adverse effect.

**Engraftment**

Four patients (3 ALL, 1 AML) died prior to day 21 and were not evaluable for sustained marrow engraftment. Of the 21 evaluable cases, 4 (19%) failed to show evidence of sustained marrow engraftment. One of these 4 failures developed complete autologous reconstitution. All failures were transplanted for AA. Three of the 4 were B-group mismatches and one an A-group mismatch. All others allografted as demonstrated by at least one chromosomal, immunogenetic, or erythrocyte marker.

**Time Course of Hematologic Recovery**

The kinetics of leukocyte recovery following ABO-incompatible BMT was essentially the same as in other allogeneic transplants (Table 3). Total leukocytes exceeded 1000/cumm by 20 ± 5 days post ABO blood group incompatible transplantation and 20 ± 7 days post ABO blood group compatible transplantation. Eight of 21 patients who survived greater than 3 wk had not achieved platelet counts greater than 50,000/cumm at the time of their death. Failure to achieve platelet recovery was also observed commonly in ABO blood group compatible transplants and was usually associated with GVHD and/or systemic viral, fungal, or bacterial infections. When platelet recovery was observed, counts exceeded 50,000/cumm a mean of 32 ± 21 days post transplant.
Hemagglutinin Titer When Reticulocyte Count > 1% Anti-A Anti-B

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Pretransplant Hemagglutinin Titer</th>
<th>Days to Reticulocyte Count &gt; 1%</th>
<th>Hemagglutinin Titer When Reticulocyte &gt; 1%</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Anti-A</td>
<td>Anti-B</td>
<td>266</td>
</tr>
<tr>
<td>129</td>
<td>1:64</td>
<td>1:256</td>
<td>266</td>
</tr>
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<tr>
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<td>-</td>
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</tr>
<tr>
<td>133</td>
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<td>-</td>
<td>64</td>
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<td>-</td>
<td>27</td>
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<td>188</td>
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<td>-</td>
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<td>1:2</td>
<td>-</td>
<td>34</td>
</tr>
<tr>
<td>230</td>
<td>1:1</td>
<td>-</td>
<td>17</td>
</tr>
</tbody>
</table>

*Patient died prior to attaining > 1% reticulocytes.

Bone marrow development was consistent with the pattern of peripheral blood reconstitution. No differences in granulocyte or megakaryocyte development were observed. There was a marked decrease in all identifiable stages of erythrocyte development, including rubroblasts (Table 5). At equivalent stages of myeloid recovery (peripheral blood leukocytes >1000/cumm), 5/14 ABO blood group incompatible transplants manifested no identifiable erythrocyte precursors and 10/14 showed a M:E ratio greater than 4:1 (p < 0.001). Serial hemagglutinin titers were available in 18/21 patients with sustained marrow engraftment. Patients manifesting the greatest deficits in erythroid differentiation tended to have higher pretransplant titers of incompatible hemagglutinins. When marrows were compared at an equivalent stage of peripheral blood erythroid development (reticulocytosis >1%), there were no significant differences between ABO blood group compatible and incompatible BMT.

**Graft- Versus-Host Disease**

Fatal GVHD was observed in both ABO blood group compatible and incompatible transplant recipients. Using a modification of a standard staging system of GVHD,8,9 8/18 (44%) incompatible ABO blood group transplants developed stage 2 or worse acute GVHD compared to 55/166 (33%) following ABO blood group compatible transplantation. Chronic GVHD was observed in 4/18 (22%) of incompatible transplants and 27/152 (17%) of compatible transplants.

**DISCUSSION**

Erythrocyte depletion of the bone marrow prior to infusion is a safe and convenient approach to BMT across major ABO blood group incompatibilities. Using the Haemonetics Model-30 cell separator, bone marrows can be depleted of 94% of the incompatible

<table>
<thead>
<tr>
<th>Stage of Peripheral Blood Recovery</th>
<th>Leukocytes x 1000/cumm</th>
<th>Reticulocytes x 1%</th>
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<tr>
<td>Bone Marrow Erythrocyte Morphology</td>
<td>ABO Compatible</td>
<td>ABO Incompatible</td>
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<tr>
<td>-----------------------------------</td>
<td>------------------------</td>
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</tr>
<tr>
<td>Relative erythrocyte hypoplasia</td>
<td>1/17</td>
<td>10/14</td>
</tr>
<tr>
<td>(myeloid:erythroid ratio &gt; 4:1)</td>
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<tr>
<td>Erythrocyte aplasia</td>
<td>1/18</td>
<td>5/14</td>
</tr>
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<td>*p by chi-square analysis.</td>
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of the CFU-GM. Unfortunately, accurate determina-
tion of the cell content of a heterogeneous mixture as
acceptable. Seventy-five percent of the original mono-
stem cell loss using erythrocyte depletion seems
stem cell loss during processing and the hazard of
able, hemagglutinins with the risk of acute hemolysis.
still must be infused into diminished, but still detect-
antibody depletion techniques of ABO blood group
ular volume changes, mild platelet depletion, and the
ive extracorporeal manipulation of recipient blood is
with use of specific immune column absorption, exten-
tions during plasmapheresis and obviating the need for
compatible separation.

Depletion of recipient hemagglutinins by plasma
exchange and immune absorption has been used since
1970 as an alternate approach to ABO blood group
compatible transplantation.1,2 The introduction of
pecific immune absorption columns has improved this
approach by reducing the hazard of replacement solu-
tions during plasmapheresis and obviating the need for
incompatible erythrocyte transfusion.1 However, even
with use of specific immune column absorption, extensive
tracorporeal manipulation of recipient blood is
required. Typically, 2–3 plasmapheresis-immune ab-
sorption procedures lasting 2–3 hr are required.
Venous access difficulties, anticoagulant toxicity, vas-
cular volume changes, mild platelet depletion, and the
risk of infection must be addressed. Such risks may be
untoward, particularly in severely neutropenic and
 thrombocytopenic patients with aplastic anemia. In
addition, the risk of hemolysis remains generic to all
antibody depletion techniques of ABO blood group
compatible BMT. ABO-incompatible erythrocytes
still must be infused into diminished, but still detect-
able, hemagglutinins with the risk of acute hemolysis.
A delayed hemolysis secondary to a “rebound” of
recipient hemagglutinins following plasmapheresis has
been reported also.11

The major risks of erythrocyte depletion include
stem cell loss during processing and the hazard of
infusing small amounts of incompatible erythrocytes.
Stem cell loss using erythrocyte depletion seems
acceptable. Seventy-five percent of the original mono-
nuclear cell (MNC) content was recovered with 57%
of the CFU-GM. Unfortunately, accurate determina-
tion of the cell content of a heterogeneous mixture as
hemolytic anemia until circulating hemagglutinins are reduced to a clinically insignificant level.

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REFERENCES


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