Hemolytic anemia is a common complication following bone marrow transplant when there is "minor" ABO blood group incompatibility between donor and recipient. This has been attributed to destruction of the patient's incompatible erythrocytes by donor-derived anti-A and/or anti-B antibody produced from "passenger" immunocompetent donor lymphocytes. Extraordinary transfusion requirements of group O erythrocytes in a series of patients receiving unrelated minor ABO-incompatible marrow grafts led us to investigate whether this mechanism could account for the extent of hemolysis observed. In seven consecutive minor ABO-incompatible unrelated donor bone marrow transplant recipients receiving cyclosporine without posttransplant methotrexate, we observed excessive hemolysis. For cases in this index group, a strongly reactive donor-derived ABO blood group antibody was identified coincident with development of hemolysis. Transfusion requirements in the first three patients (26 U of group O erythrocytes each) greatly exceeded the recipient's volume of incompatible erythrocytes, indicating that lysis of transfused group O erythrocytes was occurring. Pretransplant erythrocyte exchange transfusion with group O erythrocytes performed in the four subsequent patients decreased the severity of hemolysis, but did not prevent it. Among minor ABO-incompatible marrow graft recipients, an analysis of variance demonstrated effects on transfusion requirements due to donor-recipient relationship being unrelated ($P < .002$) and the use of posttransplant methotrexate ($P = .0001$), and there was interaction between these two factors ($P < .001$). Bone marrow transplants from unrelated donors resulted in an exaggerated immune response to ABO blood group antigens, which was associated with hemolysis of transfused group O erythrocytes, as well as the patient's ABO-incompatible erythrocytes. This serious complication may be prevented by posttransplant immunosuppression with methotrexate.

A LLOGENEIC bone marrow transplantation may be successfully performed despite ABO incompatibility between the donor and the recipient. Minor ABO incompatibility—where donor-derived antibody is directed against antigens on the recipient's erythrocytes—can cause delayed hemolysis of recipient erythrocytes 1 to 2 weeks posttransplant. This phenomenon is not due to passive transfer of antibody during marrow infusion, but rather is caused by transient antibody production from "passenger" immunocompetent donor lymphocytes. Passenger lymphocyte-mediated hemolysis has been described in solid organ transplant recipients particularly since the use of cyclosporine for prevention of graft rejection. The donor origin of the antibodies has been confirmed by studies of immunoglobulin allotypes. Delayed hemolysis associated with minor ABO incompatibility has most frequently been described in genotypically matched sibling bone marrow recipients given posttransplant immunosuppression with cyclosporine for prevention of acute graft-versus-host disease. Other immunologic complications of allogeneic bone marrow transplants such as acute or chronic graft-versus-host disease, graft rejection, or delayed engraftment have not been associated with major or minor ABO incompatibility, except in isolated case reports.

Since first performing unrelated-donor bone marrow transplants in 1987, we have observed clinically significant hemolysis in seven patients transplanted with unrelated, closely matched HLA marrow grafts with minor ABO incompatibility. The transfusion requirements needed in the first three patients greatly exceeded the recipient's own erythrocyte volume, indicating destruction of transfused group O erythrocytes. The hemolysis was documented by posttransplant development of a strongly reactive donor-derived ABO blood group antibody in association with an excessive erythrocyte transfusion requirement and supporting laboratory evidence of hemolysis. These seven consecutive patients received posttransplant immunosuppression with cyclosporine alone or in combination with methylprednisolone and anti-CD-5-ricin-A immunotoxin. To better explore factors associated with this hemolysis, the transfusion requirements in these seven patients, "the index group," were compared with those of 17 recipients of unrelated or related, genotypic HLA-matched marrow grafts from minor ABO-incompatible donors transplanted during the same 31-month period. Because of the difficulty distinguishing hemolysis from baseline transfusion requirements in thrombocytopenic bone marrow transplant patients, transfusion requirements were also analyzed in 59
recipients of ABO-identical marrow grafts from related or unrelated donors transplanted during the same 31-month period.

**MATERIALS AND METHODS**

**Patient population.** Between November 1987 and June 1990, 83 consecutive patients, older than age 10 years, received unrelated, closely matched HLA allogeneic marrow grafts or genotypic HLA-matched (related) marrow grafts at UCLA Medical Center (Table 1). All patients or their parents or guardians signed informed consent forms approved by the UCLA Human Subjects Protection Committee. Recipients of unrelated marrow grafts were required to have serologic phenotypic identity with the donor for at least five of six HLA-A, -B, and -DR antigens. Patients received bone marrow transplants for acute leukemia, chronic myelogenous leukemia, aplastic anemia, or myelodysplasia. Because patients with documented bleeding, graft failure, and those dying early without engraftment from infection would be difficult to assess for immunohematologic reactions, they were excluded from the analysis. All recipients of second allogeneic marrow grafts were also excluded from analysis. Recipients of marrow grafts with major (recipient v donor) ABO incompatibility, who did not also have minor (donor v recipient) ABO incompatibility were not analyzed because they have different immunohematologic problems.19,20

Leukemia and myelodysplasia patients received the following preparative regimens: total body irradiation 1,125 cGy in five fractions, cyclophosphamide 120 mg/kg, and cytarabine 2 to 8 g/m² or mitoxantrone 36 mg/m², busulfan 16 mg/kg, and cyclophosphamide 120 mg/kg; or total body irradiation 1,200 cGy in six fractions, etoposide 60 mg/kg, and cyclophosphamide 90 mg/kg. Aplastic anemia patients received 300 to 750 cGy total lymphoid irradiation plus 200 mg/kg cyclophosphamide21 (one mismatched aplastic anemia patient received an additional 600 cGy total body irradiation). The donor bone marrow was infused within 48 hours after completion of the preparative regimen.

Unrelated marrow graft recipients were entered into three consecutive single-arm acute graft-versus-host disease prophylaxis trials studying cyclosporine plus posttransplant short-course methotrexate,22 cyclosporine, methotrexate, and methylprednisolone,23 and cyclosporine, methylprednisolone, and anti-CD-5-ricin-A immunotoxin.24 During the study of cyclosporine and short-course methotrexate, three unrelated marrow graft recipients inadvertently received cyclosporine alone without posttransplant methotrexate. Two of these patients received minor ABO-incompatible grafts and were the first two patients in the index group. Ex vivo T-lymphocyte depletion of unrelated marrow was not performed. Related marrow graft recipients were entered into a prospective randomized trial of cyclosporine alone versus cyclosporine plus ex vivo depletion anti-CD8-positive T lymphocytes25 or, when not entered into that study, these patients usually received cyclosporine and posttransplant short-course methotrexate.

Cyclosporine was started at day −2 at a loading dose of 3 mg/kg over 12 hours and was administered by continuous infusion from day −2 until oral intake could be used postengraftment. Dosing was adjusted to maintain plasma levels of approximately 150 to 200 ng/mL with modifications as necessary for liver and renal functions. Methotrexate was administered 15 mg/m²/d on day +1 and 10 mg/m²/d on days +3 and +6. Methylprednisolone was administered at 0.25 mg/kg/d starting on either day −3 or day +3.

Table 1. Patient Characteristics

<table>
<thead>
<tr>
<th></th>
<th>ABO-Idenphatible Patients</th>
<th>ABO-Identical Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Unrelated</td>
<td>Related</td>
</tr>
<tr>
<td></td>
<td>MTX (n = 7)</td>
<td>MTX (n = 6)</td>
</tr>
<tr>
<td>Median age (range)</td>
<td>34 (21-46)</td>
<td>35 (22-46)</td>
</tr>
<tr>
<td>Diagnosis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ALL</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>AML</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>CML</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>AA</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>MDS</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>AGVHD prophylaxis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CsA</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td>CsA + IT + steroids</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>CsA + T-cell depletion</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>CsA + MTX</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>CsA + MTX + steroids</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>ABO mismatch</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Minor</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Major-minor</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Donor-recipient sex relationship</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male-male</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>Male-female</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Female-male</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>Female-female</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Prophylactic IV immunoglobulin given</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>No</td>
<td>6</td>
<td>6</td>
</tr>
</tbody>
</table>

Abbreviations: MTX, methotrexate; ALL, acute lymphocytic leukemia; AML, acute myelogenous leukemia; CML, chronic myelogenous leukemia; AA, aplastic anemia; MDS, myelodysplasia; AGVHD, acute graft-versus-host disease; CsA, cyclosporine A; IT, anti-CD5-ricin-A immunotoxin.
Anti-CD5-ricin-A immunotoxin was started on either day -3 or day +3, and was administered at 0.1 mg/kg for 7 days and then on alternating days for 7 days.

For those patients receiving marrow grafts with major and minor ABO incompatibility (eg, a group A donor into a group B recipient), density gradient separation of the red blood cells (RBCs) from the marrow graft using hetastarch was performed to prevent hemolysis with infusion of the marrow graft.26

Four recipients of unrelated marrow grafts had major and minor ABO-incompatibility with their donors and 10 had only minor ABO incompatibility, the other 14 received ABO-identical marrow grafts. Among recipients of unrelated marrow transplants given minor ABO-incompatible marrow grafts, acute graft-versus-host disease prophylaxis was cyclosporine alone (n = 2 patients); methylprednisolone, cyclosporine, and anti-CD5-ricin-A immunotoxin (n = 5); and cyclosporine and short-course methotrexate (n = 7). Of the latter seven patients, two also received methylprednisolone. Among 14 evaluable recipients of unrelated ABO-identical marrow grafts, five patients were given cyclosporine with short-course methotrexate, and nine patients received cyclosporine, methylprednisolone, and anti-CD5-ricin-A immunotoxin.

During this 31-month period, 10 recipients of related marrow grafts received minor ABO-incompatible or major and minor incompatible marrow grafts, and 45 patients received related ABO-identical marrow grafts. Acute graft-versus-host disease prophylaxis was cyclosporine alone (n = 22 patients), cyclosporine with ex vivo CD8-positive T-cell depletion (n = 21), or cyclosporine with short-course methotrexate (n = 12). Cytomegalovirus (CMV)-seronegative patients received CMV antibody-negative blood products and were entered into a randomized trial of prophylactic intravenous (IV) immunoglobulin, which was administered weekly at a dose of 1,000 mg/kg/wk.27,28 CMV-seropositive recipients were ineligible for this trial (Table 1).

Transfusions. Since hemolysis in the index group was demonstrated to occur between days +5 and +20, we used this period to compare transfusion requirements in various groups of patients. During the posttransplant period, recipients were transfused to maintain the hematocrit at or greater than 27%. For recipients of minor ABO-mismatched marrow grafts, all transfused erythrocytes were group O, washed with 1 L saline on the COBE 2991 Blood Cell Processor (COBE Laboratories, Inc, Lakewood, CO) to avoid passive infusion of incompatible ABO antibody. When available, platelet transfusions were apheresis products of the recipient's original ABO group. Blood components were irradiated with 1,500 cGy (using the Gammacell 1000 irradiator; Isomedix, Inc, Parsippany, NJ) before transfusion to avoid transfusion-associated graft-versus-host disease.31,32

In an attempt to reduce the risk of hemolysis, four patients in the index group received prophylactic exchange transfusions with 8 U of group O erythrocytes before bone marrow transplantation. No related recipients or patients receiving posttransplant methotrexate received a prophylactic exchange transfusion.

Sero logical testing. Routine ABO, Rh, direct antiglobulin test, erythrocyte elution, and antibody identification procedures were performed using standard techniques and licensed reagents.33 When a recipient's direct antiglobulin test was positive using a polyspecific antiglobulin reagent, the proteins coating the patient's erythrocytes were characterized with monospecific anti-IgG and anti-C3. Anti-IgM serum was not used. When the direct antiglobulin test was positive with circumstances demonstrating hemolysis, antibody was eluted from recipient erythrocytes by heat elution or the Lui freeze elution procedures. For antibody detection, the recipient's serum or erythrocyte eluate was incubated with A and B erythrocytes at room temperature for 15 minutes, with a low ionic strength saline (LISS) additive, followed by an indirect antiglobulin test.

To detect absorbed A or B antigens on transfused group O erythrocytes, the specimens of one patient (A.M.) in the index group were tested with licensed anti-A, anti-B, and anti-A,B reagents and serum from a group O donor for reactivity at immediate spin, after 37°C incubation, and at the antiglobulin phase. Such an analysis was performed after the patient's blood type had converted to group O by routine testing on days +18, +22, and +27.

Determination of ABO-incompatible recipient erythrocyte volume. The erythrocyte mass before the onset of hemolysis was estimated using each patient's weight and hematocrit on day +5 and standard formulae for calculating blood and plasma volume.25,24 For patients receiving pretransplant erythrocyte exchange transfusions, the circulating mass of remaining ABO-incompatible cells was determined using their calculated erythrocyte volume, calculating in the dilution of exchange transfusion.35

The percentage of circulating recipient erythrocytes before or during hemolytic episodes was estimated in three index patients (A.M., R.B., N.T.) using diluted anti-A or anti-B reagents by comparing the reactivity of mixed-field agglutination in patient specimens to control dilutions of 2%, 5%, 10%, and 20% group A or B erythrocytes in group O erythrocytes (N. Postoway, personal communication).

Comparison of transfusion requirements. To compensate for differences in intravascular volume among recipients of different body mass, transfusion requirements were compared using ratios of transfused erythrocyte mass on day +5 to day +20 to recipient erythrocyte mass on day +5.

Statistics. Comparisons of two means were performed using a two-sample t test. A preliminary analysis of variance (ANOVA) of transfusion requirements, which included ABO compatibility, donor-recipient relationship, and short-course methotrexate treatment as factors, demonstrated a three-way interaction; therefore, two-factor analyses were conducted separately for ABO-identical and minor incompatible patients. Because there was significant inequality of variances among the ABO-identical patients, the Brown-Forsythe ANOVA procedure was used.36,37 Age, diagnosis, donor-recipient sex relationship, use of IV immunoglobulin, and type of minor ABO incompatibility (minor v major-minor) were also evaluated for their effect on transfusion requirements individually and in a multivariate linear model along with donor-recipient relationship and use of methotrexate.38

RESULTS

Evidence of immune hemolysis. The seven index patients (Table 2) with unrelated minor ABO-mismatched marrow grafts developed immune-mediated hemolysis posttransplant, detected by a decreasing hematocrit despite transfusions in association with an increased bilirubin and lactate dehydrogenase (LDH) and a conversion of the direct antiglobulin test from negative to positive (Fig 1). Rare fragmented erythrocytes were observed on peripheral smears in two patients. None of the patients had evidence of bleeding during their hospital course. There was no evidence for disseminated intravascular coagulation in these patients as documented by a normal prothrombin time and a normal partial thromboplastin time tested every week. Acute renal failure occurred in four of the seven patients shortly after the onset of hemolysis. The first three patients each received 26 U of erythrocytes during days +5 to +20, 15 minutes with a low ionic strength saline additive.
HEMOLYSIS AFTER MINOR ABO-INCOMPATIBLE BMT

Table 2. Patient Characteristics of the Index Group

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age</th>
<th>Disease</th>
<th>Degree of HLA Matching</th>
<th>Recipient ABO Type</th>
<th>Donor ABO Type</th>
<th>AGVHD Prophylaxis</th>
<th>Grade of AGVHD</th>
<th>Renal Insufficiency</th>
<th>Cause of Death</th>
</tr>
</thead>
<tbody>
<tr>
<td>R.L. 35</td>
<td>35</td>
<td>CML</td>
<td>5/6</td>
<td>A</td>
<td>O</td>
<td>CsA</td>
<td>IV</td>
<td>No</td>
<td>CMV IPN</td>
</tr>
<tr>
<td>W.H. 45</td>
<td>45</td>
<td>CML</td>
<td>6/6</td>
<td>A</td>
<td>O</td>
<td>CsA</td>
<td>0</td>
<td>Yes</td>
<td>Infection</td>
</tr>
<tr>
<td>R.B. 47</td>
<td>47</td>
<td>CML</td>
<td>6/6</td>
<td>B</td>
<td>A</td>
<td>CsA</td>
<td>I</td>
<td>Yes</td>
<td>Hepatic VOD</td>
</tr>
<tr>
<td>A.M.* 21</td>
<td>21</td>
<td>ALL</td>
<td>6/6</td>
<td>B</td>
<td>A</td>
<td>CsA</td>
<td>II</td>
<td>Yes</td>
<td>Disseminated aspergillosis</td>
</tr>
<tr>
<td>T.P.* 22</td>
<td>22</td>
<td>AML</td>
<td>6/6</td>
<td>B</td>
<td>O</td>
<td>CsA</td>
<td>II</td>
<td>Yes</td>
<td>Septic emboli</td>
</tr>
<tr>
<td>R.T.* 35</td>
<td>35</td>
<td>AML</td>
<td>6/6</td>
<td>A</td>
<td>O</td>
<td>CsA</td>
<td>0</td>
<td>No</td>
<td>CMV IPN</td>
</tr>
<tr>
<td>N.T.* 25</td>
<td>25</td>
<td>CML</td>
<td>5/6</td>
<td>A</td>
<td>O</td>
<td>CsA</td>
<td>III</td>
<td>No</td>
<td>CMV IPN</td>
</tr>
</tbody>
</table>

Abbreviations: IPN, interstitial pneumonitis; VOD, veno-occlusive disease.
*Recipients received 8-U pretransplant exchange transfusion with group O erythrocytes before marrow graft infusion.

which encompassed the period for all patients during which there was evidence supporting hemolysis. Erythrocyte consumption in the four recipients who received a prophylactic exchange transfusion was 16 U, 10 U, 10 U, and 6 U, respectively, during this period. The last patient, N.T., received a single dose of 20 mg methotrexate when she developed hemolysis.

Transfusion requirements. A comparison of transfusion requirements for all groups is shown in Table 3. In 59 patients receiving an ABO-compatible marrow, the estimated “baseline” transfusion requirement from day +5 to +20 ranged from 0 to 1.96 times erythrocyte volume on day +5 (overall mean ± SE, 0.85 ± 0.07).

Transfusion requirements in the three patients (R.L., W.H., R.B.) in the index group who did not receive a pretransplant erythrocyte exchange transfusion were far greater than could be accounted for by lysis of recipient-derived erythrocytes (Table 4). These three patients each received 26 U of erythrocytes (~4,680 mL) during the period of hemolysis (day +5 to +20). Their incompatible erythrocyte volume at the onset of hemolysis ranged from 1,592 to 2,039 mL and their estimated “baseline” transfusion requirement (0.85 times their RBC volume on day +5) varied from 1,353 mL to 1,733 mL. These patients required transfusions ranging from 2,947 to 3,327 mL of erythrocytes above their estimated baseline requirement. Their transfu-
Transfusion requirements were greater for recipients of minor ABO-incompatible marrow grafts than ABO-identical marrow grafts, suggesting hemolysis was occurring in addition to baseline transfusion requirements. Because of difficulties comparing transfusion requirements between those receiving pretransplant exchange transfusions to those not being pretreated with an exchange transfusion, an ANOVA was performed on recipients of ABO-incompatible marrow grafts including only those patients not receiving a pretransplant exchange transfusion. The ANOVA demonstrated significant effects on transfusion requirements due to donor-recipient relationship being unrelated ($P < .002$) and the use of posttransplant methotrexate ($P = .001$), and there was a significant interaction between these two factors ($P < .001$). Thus, in the setting of a minor ABO-incompatible marrow transplant, unrelated transplant recipients not receiving posttransplant methotrexate had the greatest transfusion requirements. Recipients of minor ABO-incompatible marrow grafts receiving posttransplant methotrexate in addition to cyclosporine had transfusion requirements similar in magnitude to ABO-identical marrow graft recipients (Table 3). In the multivariate model, which simultaneously examined in ABO-incompatible marrow graft recipients the effect on hemolysis of donor-recipient relationship, use of posttransplant methotrexate, diagnosis, use of prophylactic IV immunoglobulin, type of ABO incompatibility (minor v major-minor), donor-recipient sex relationship, and recipient age, only the use of methotrexate was significant ($P = .02$). Because all four aplastic anemia patients received posttransplant methotrexate, a separate analysis was performed excluding these patients. In this analysis, the effect of posttransplant methotrexate remained significant ($P = .02$) and donor-recipient relationship being unrelated had borderline significance ($P = .09$). ANOVA for ABO-identical recipients demonstrated a borderline minor reduced transfusion requirement in the unrelated group ($P = .04$) and no significant effect or interaction with the use of posttransplant methotrexate.

**Use of ABO-incompatible plasma products.** Plasma that was ABO incompatible with the patient's original blood

### Table 3. Posttransplant Transfusion Requirements

<table>
<thead>
<tr>
<th>Minor ABO Incompatible</th>
<th>Unrelated recipients</th>
<th>Related recipients</th>
<th>ABO Identical</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>No MTX (n = 5)</strong></td>
<td>2.70 (±0.20)</td>
<td>1.43 (±0.06)</td>
<td>0.62 (±0.23)</td>
</tr>
<tr>
<td><strong>MTX (n = 7)</strong></td>
<td>0.78 (±0.14)</td>
<td>0.82 (±0.23)</td>
<td>0.36-1.45</td>
</tr>
</tbody>
</table>

| A.M.                   | 0.43 (±0.10)         | 0.91 (±0.22)      |
| N.T.                   | 0.70 (±0.19)         | 0.00-1.70         |

Abbreviation: MTX, posttransplant methotrexate.

*Recipients of pretransplant exchange transfusion excluded.

### Table 4. RBC Volumes (mL) for the Index Group

<table>
<thead>
<tr>
<th>Patient</th>
<th>Total RBC Volume</th>
<th>Recipient Type</th>
<th>Estimated Baseline Requirement (mL)</th>
<th>Volume Transfused Group O RBC Above Estimation Requirement (mL)</th>
<th>Ratio of Volume Transfused Group O RBC in Excess of Baseline Transfusion Requirement</th>
<th>Volume Transfused Group O RBC in Excess of Baseline Transfusion Requirement Plus Recipient Type RBC Volume (mL)</th>
<th>Ratio of Volume Transfused Group O RBC Days +5 to +20 to Recipient RBC Volume on Day +5 (mL)</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
<th>F</th>
<th>G</th>
</tr>
</thead>
<tbody>
<tr>
<td>R.L.</td>
<td>2,039</td>
<td>2,039</td>
<td>4,680 (26)</td>
<td>1,733</td>
<td>2,947</td>
<td>908</td>
<td>2.30</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>W.H.</td>
<td>1,592</td>
<td>1,592</td>
<td>4,680 (26)</td>
<td>1,353</td>
<td>3,327</td>
<td>1,735</td>
<td>2.94</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R.B.</td>
<td>1,635</td>
<td>1,635</td>
<td>4,680 (26)</td>
<td>1,389</td>
<td>3,291</td>
<td>1,656</td>
<td>2.86</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A.M.</td>
<td>1,779</td>
<td>600*</td>
<td>4,680 (26)</td>
<td>1,512</td>
<td>1,368</td>
<td>728</td>
<td>N/A</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T.P.</td>
<td>1,585</td>
<td>538*</td>
<td>1,800 (10)</td>
<td>1,347</td>
<td>453</td>
<td>(–85)</td>
<td>N/A</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R.T.</td>
<td>1,357</td>
<td>379*</td>
<td>1,800 (10)</td>
<td>1,153</td>
<td>647</td>
<td>268</td>
<td>N/A</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N.T.</td>
<td>1,480</td>
<td>459*</td>
<td>1,080 (6)</td>
<td>1,258</td>
<td>(–178)</td>
<td>(–637)</td>
<td>N/A</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Calculated volume of incompatible RBC remaining following exchange RBC transfusion with group O RBC before bone marrow transplant.

†Measured volume of incompatible RBC remaining following exchange RBC transfusion with group O RBC.
HEMOLYSIS AFTER MINOR ABO-INCOMPATIBLE BMT

Fig 2. Flow chart of index group patient receiving pretransplant exchange transfusion with biochemical and serologic evidence for hemolysis. MF, mixed field agglutination. Other abbreviations as in Fig 1.

group was transfused as part of platelet transfusion support to most patients who received an ABO-incompatible marrow graft. The seven index patients with hemolysis received a mean of 2.3 U of platelet products (range, 0 to 5.8 U) containing ABO-incompatible plasma (each platelet product contains ~ 250 mL of plasma) during days +5 to +20. The seven recipients with unrelated minor ABO-incompatible marrow grafts who received posttransplant methotrexate and had no documented hemolysis received a mean of 1.8 U of platelet products (range, 0 to 6) (P = NS).

Serologic findings. Serologic findings for recipients of minor ABO-incompatible marrow grafts are reviewed in Table 5. All seven patients in the index group with hemolysis developed erythrocyte antibody reacting against the A or B antigen of the recipient. These antibodies were detectable during hemolysis, but were not detectable immediately after the marrow graft infusion (see Figs 1 and 2), indicating infused lymphocytes produced antibody posttransplant in the recipient against recipient A or B antigens. The anti-A or anti-B antibodies caused a 3 to 4+ agglutination reaction in all patients in the index group. No other erythrocyte antibodies were observed.

The direct antiglobulin test was positive during hemolysis in all cases in the index group. In two patients, positive results were obtained with polyspecific antiserum, anti-IgG, and anti-C3; in two patients with polyspecific antisera and anti-IgG; in one patient with polyspecific antiserum and C3; and in two patients with polyspecific antisera only. In four patients, eluates were prepared from the patient’s erythrocytes during the period of hemolysis and tested with group A or B erythrocytes, with positive reactions consistent with donor-derived antibody in three patients.

Positive direct antiglobulin tests and positive reactions with the erythrocyte eluate were more common in patients not receiving short-course methotrexate. Generally, those patients receiving posttransplant methotrexate had negative or weakly reacting tests for serum anti-A or anti-B.

In the one index group patient (A.M.) tested, we could not identify absorbed A or B antigen on the transfused group 0 erythrocytes. Testing for secretor status could not be determined posttransplant because severe mucositis prevented collection of saliva.

DISCUSSION

Cases of immune-mediated hemolysis attributed to donor-derived anterythrocyte antibodies after allogeneic bone marrow transplant have been previously described. Most
commonly, “minor” ABO blood group incompatibility is present, that is, the donor possesses anti-A and/or anti-B which can react with the corresponding antigens present on recipient erythrocytes. Since the antibody is not detected immediately posttransplant, but is detected at times of hemolysis, usually beginning 5 to 10 days posttransplant, it must be produced by lymphocytes transfused with the marrow graft, rather than by antibody infused with the marrow graft.5

Unexplained by the “passenger lymphocyte” mechanism of hemolysis is the excessive transfusion requirement for group O erythrocytes during the period of hemolysis for the index group not receiving a pretransplant exchange transfusion. These three patients each required 26 U (~4,680 mL) of group O erythrocytes during a 16-day period, which far exceeded the patients' volume of ABO-incompatible erythrocytes, thus indicating that transfused group O erythrocytes were also hemolyzed. Serologic findings only indicated anti-A or anti-B antibodies in the patients' serum and in an eluate from these patients' erythrocytes.

Prophylactic pretransplant exchange transfusions with group O erythrocytes in subsequent index group patients lessened the transfusion requirements, but did not prevent the development of hemolysis.

Patients receiving minor ABO-incompatible marrow from related donors, who did not receive posttransplant methotrexate, also had increased transfusion requirements compared with patients receiving an ABO-identical marrow graft. This was accompanied by development of posttransplant anti-A or anti-B antibodies, consistent with immune hemolysis, although of a lesser degree than in the index group. None of the recipients of minor ABO-incompatible marrow grafts receiving cyclosporine and posttransplant methotrexate had evidence for hemolysis, and their mean transfusion requirement was similar to that observed in the ABO-identical marrow graft recipients.

Our analyses comparing the index group with other groups suggest that when methotrexate is not part of the posttransplant treatment, an exaggerated immune response to ABO blood group antigens occurs in marrow grafts from unrelated donors compared with transplants from related donors. Serologic findings offer qualitative support for this conclusion. All seven index patients with hemolysis had anti-A or anti-B serum antibodies, which caused a 3 to 4+ agglutination reaction using group A or B erythrocytes, respectively. The agglutination reaction was also noted, but was generally weaker in the related recipients of minor ABO-incompatible marrow grafts. All patients receiving methotrexate had weakly positive or negative tests for anti-A or anti-B antibodies.

This hemolysis occurred before engraftment or any evidence of acute graft-versus-host disease. Prior studies in HLA-matched sibling bone marrow transplants have not demonstrated an association between graft-versus-host disease and ABO incompatibility.15,16 Unrelated-donor bone marrow transplants have been typically associated with an increased risk of immunologic complications, including acute graft-versus-host disease and early graft failure.21,39-42 The unrelated donor and recipients are only phenotypically matched for the major histocompatibility antigens. Biologically relevant genotypic differences may exist. This inherent immunogenetic disparity in unrelated-donor bone marrow transplants may stimulate exaggerated immune responses in antigen-responsive cells, such as passenger lymphocytes.

Cyclosporine may stimulate this exaggerated immune response by permitting a B-lymphocyte proliferation with the suppression of T-helper lymphocytes.41 Recipients of minor ABO-incompatible marrow grafts who received posttransplant methotrexate in addition to cyclosporine had similar transfusion requirements to recipients of ABO-identical marrow grafts. The low incidence of passenger lymphocyte-mediated hemolysis in related-donor minor ABO-incompatible bone marrow transplants was reported in a group of patients receiving posttransplant immunosuppression with methotrexate without concomitant cyclosporine.5 Our observations indicate that posttransplant immunosuppression with methotrexate may prevent the passenger lymphocyte syndrome from occurring even in patients receiving cyclosporine. Although the mechanism remains to be determined, methotrexate has been shown to be cytotoxic for B lymphocytes and may prevent the antibody production necessary for passenger lymphocyte syndrome to occur.44,45

The “bystander” hemolysis of the transfused group O erythrocytes in the index group could be accounted for by reactive hemolysis, a form of erythrocyte lysis differing from classical complement-mediated hemolysis because it occurs in the absence of antibody on erythrocytes.46-48 Complement activation may produce a C5b7 complex which can attach to normal erythrocyte membranes, creating the condition necessary for lysis by C8 and C9. In the index patients, the hemolysis of group O erythrocytes could have occurred as a result of activation of complement by the immune reaction between donor-derived antibody and the recipient’s A or B erythrocyte antigens. Such immunemediated hemolysis of antigen-negative bystander cells may account for the fatal case of a hemolytic transfusion reaction with acute autohemolysis.49 A similar phenomenon may occur in paroxysmal nocturnal hemoglobinuria, in which reactive lysis has been demonstrated in vitro and lysis of autologous erythrocytes may occur following transfusion of homologous blood.50-53

Complement sensitization of antigen-negative erythrocytes has been previously reported. Salama et al54,55 and Ness et al56 reported that direct antiglobulin tests with anti-C3d usually yield positive results in delayed hemolytic and delayed serologic transfusion reactions, and that the agglutination observed is generally not of the mixed-field type. These data suggest that components of complement bind not only to the transfused antigen-positive erythrocytes, but also to autologous antigen-negative erythrocytes.

Another possible mechanism to account for the lysis of group O erythrocytes in our patients could be the absorption of A or B antigen onto the transfused group O erythrocytes57 with subsequent hemolysis by the antibody produced by the passenger lymphocytes. We could not find evidence for absorption of A or B antigen on erythrocyte cell membranes in the one patient tested. Renton and
Hancock have reported that group O erythrocytes trans-
fused to group A or B patients become agglutinable by
selected group O sera. Blood type A or B patients
receiving marrow from group O donors generally type as
group O after engraftment, but their erythrocytes may yield
weak agglutination reactions using group O sera. Blood
group A substance has been demonstrated on erythrocytes
of recipients of group O marrow grafts who were originally
group A and were secretors. Similarly, immune complexes
formed between A or B antigens in plasma and anti-A or
anti-B produced by the donor marrow could absorb to
bystander erythrocytes and cause hemolysis by a mecha-
nism analogous to that proposed for lysis of P1A1-negative
platelets by anti-P1A1 antibodies in posttransfusion pur-
pura. These mechanisms are not likely to be operative
except in patients who are secretors. Because of severe
mucositis in the index patients, determination of their
secretor status could not be performed. Since only about
75% of subjects are secretors, it is unlikely that these
seven would be secretors and the patients receiving post-
transplant methotrexate would not be secretors.

A and B glycosyltransferases have been demonstrated to
persist in plasma after bone marrow transplantation. Per-
sistent glycosyl transferase could transform H substance
on group O erythrocytes into A or B antigens; but this is an
unlikely mechanism because glycosyl transferases ordi-
narily react intracellularly and require specific substrate.

The index group had a high incidence of renal failure.
However, these patients were also receiving nephrototoxic
drugs such as amphotericin and cyclosporine. Hemolytic-
uremic syndrome has been observed in bone marrow
transplant recipients, but only after recovery of hema-

topoesis. The development of a strongly positive direct
antiglobulin test and donor-derived antibody coincident
with the development of hemolysis makes hemolytic-uremic
syndrome an unlikely mechanism for the hemolysis in the
index group patients.

High-dose IV immunoglobulin has previously been asso-
ciated with a brief episode of hemolysis occurring imme-
diately after infusion of the IV immunoglobulin, but only one
patient in the index group received IV immunoglobulin and
the hemolysis observed was of a longer duration than that
observed secondary to IV immunoglobulin.

Our data indicate that immune-mediated hemolysis may
be an important complication of minor ABO-incompatible
marrow grafts, especially in patients transplanted from
unrelated donors. The hemolysis may not be limited to
group A or group B erythrocytes, but immune hemolysis of
bystander transfused group O erythrocytes may also occur.
Determining the mechanism of this hemolysis will require
further investigation. This serious complication may be
prevented by posttransplant treatment with methotrexate.

ACKNOWLEDGMENT

The authors gratefully acknowledge the assistance of Stephen D.
Nimer in reviewing the manuscript and Pamela Stephens in typing
the manuscript.

REFERENCES

1. Petz LD: Immunohematologic problems associated with bone
2. Bensinger WI, Buckner CD, Thomas ED, Clift RA: ABO-
incompatible marrow transplants. Transplant 33:427, 1982
3. Lasky LC, Warkentin PI, Kersey JH, Ramsay KC, McCabe
PB, McCullough J: Hemotherapy in patients undergoing bone
marrow transplant able bone marrow transplantation. Transfusion
23:277, 1983
4. Hershko C, Gale RP, Ho WG, Fitchen J: ABH antigens and
5. Howes J, Beddow K, Gordon-Smith E, Branch DR, Spruce W,
Sniecinski I, Krance RA, Petz LD: Donor-derived red blood cell
antibodies and immune hemolysis after allogeneic bone marrow
6. Hazlehurst GRP, Brenner MK, W形peris JS, Knowles SM,
Prentice HJ: Haemolysis after T-cell depleted bone marrow
transplantation involving minor ABO incompatibility. Scand J
Haematol 37:1, 1986
7. Rowley S, Braine H: Probable hemolysis following minor and
incompatible marrow transplantation (IMT). Blood 60:171a, 1982
(suppl, abstr)
8. Mangal AK, Growe GH, Sinclair M, Stillwell GF, Reeves CE,
Naiman SC: Acquired hemolytic anemia due to auto anti-A, or
auto anti-B induced by group O homograft in renal transplant
recipients. Transfusion 24:201, 1984
9. Ramsey G, Nusba cher J, Starzl TE, Lindsay GD: Isohemag-
glutinins of graft origin after ABO-unmatched liver transplantation.
10. Ramsey G, Israel L, Lindsay GD, Mayer TK, Nusba cher J: Anti-Rh0(D)
in two Rh-positive patients receiving kidney grafts from an Rh-immunized donor. Transplantation 41:67, 1986
11. Nyberg G, Sandberg L, Rydberg L, Gabel H, Persson H,
Wedel N, Ahlmen J, Brynser H: ABO-autoimmune hemolytic
anemia in a renal transplant patient treated with cyclosporine: A
case report. Transplant 37:529, 1984
12. Swanson J, Sebring E, Sastamoinen R, Chepok M: Gm
allotyping to determine the origin of the anti-D causing hemolytic
anemia in a kidney transplant recipient. Vox Sang 52:228, 1987
13. Ramsey G: Red cell antibodies arising from solid organ
transplants. Transplantation 31:76, 1991
RE: Hemolytic anemia resulting from autoantibodies produced by
the donor's lymphocytes after renal transplantation. Transplantation
43:163, 1986
blood group system and bone marrow transplantation. Blood
50:185, 1977
16. Buckner CD, Clift RA, Sanders JE, Williams B, Gray M,
Storb R, Thomas ED: ABO incompatible marrow transplants.
Transplantation 26:233, 1978
17. Blacklock HA, Katz F, Michalewicz R, Hazlehurst GRP,
Davis I, Prentice HG, Hoffbrand AV: A and B blood group antigen
expression on mixed colony cells and erythroid precursors: Rele-
ance for human allogeneic bone marrow transplantation. Br J
Haematol 58:267, 1984
18. Ockelford PA, Hill RS, Nelson L, Blacklock HA, Woodfield
DG, Matthews JHD: Serologic complications of a major ABO
incompatible bone marrow transplantation in a Polynesian with
aplastic anemia. Transfusion 22:62, 1982
Metaxas M: Pure red cell aplasia of long duration complicating
major ABO-incompatible bone marrow transplantation. Blood 75:290, 1990


37. Brown MB, Forsythe AB: The small sample behavior of some statistics which test the equality of several means. Technometrics 16:129, 1974


