

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

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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.^{1,2} 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.^{1,3}

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.⁴ Such depletion of erythrocytes from marrow has been achieved using sedimentation and centrifugation techniques.^{4,5} 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: 10 for severe aplastic anemia

(AA), 11 for acute lymphocytic leukemia (ALL), 3 for acute myelocytic leukemia (AML), and 1 for chronic myelocytic leukemia (CML). Sixteen BMT involved A-group incompatibilities (A donor into O recipient, or AB into B), 7 B-group incompatibilities (B into O, AB into A) and 2 both A- and B-group incompatibilities (AB into O). There were 18 male and 7 female patients. Mean age of recipients of BMT was 23 yr (Table 1).

All patients were conditioned for transplantation according to the protocol for their diagnosis current at the time of their admission. Preparative regimens included: cyclophosphamide (Cy) 50 mg/kg/day for 4 days (6 AA); Cy 60 mg/kg/day for 2 days and 800-1000 rad 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 \times 4 days (2 AML); and BU Cy TBI (1 CML) (Table 1). 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 2 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.⁶ 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.). When buffy 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 the buffy 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

From the Johns Hopkins Oncology Center, Baltimore, Md.

Supported in part by Grants 1AM-27157, CA-13596, and CA-06973 from the National Institutes of Health.

Submitted January 25, 1982; accepted March 29, 1982.

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0006-4971/82/6002-0022\$01.00/0

Table 1. Characteristics of Patients Receiving ABO Blood-Group Incompatible Bone Marrow Transplants With Erythrocyte Depletion of Marrow

| Unique Case No. | Age/Sex | Diagnosis* | ABO and Rh Type Donor/Recipient | Recipient Pretransplant Incompatible Hemagglutinin Titer(s) | Preparative Regimen† | Sustained Marrow Engraftment |
|-----------------|---------|------------|---------------------------------|---|----------------------|------------------------------|
| 103 | 13 M | ALL | A+/O+ | 1:4 | Cy TBI | Yes |
| 155 | 31 M | ALL | A+/O+ | 1:8 | Cy TBI | Not evaluable |
| 168 | 22 M | ALL | A-/O- | 1:16 | Cy TBI | Yes |
| 170 | 12 F | AML | A+/O+ | 1:16 | Cy TBI | Yes |
| 175 | 38 F | AML | A+/O+ | 1:16 | BuCy | Not evaluable |
| 186 | 37 M | CML | A+/B+ | 1:2 | BuCy TBI | Yes |
| 188 | 19 M | ALL | A+/O+ | 1:8 | Cy TBI | Yes |
| 221 | 13 F | ALL | A+/O+ | 1:8 | Cy TBI | Yes |
| 230 | 22 M | AML | AB+/B+ | 1:1 | BuCy | Yes |
| 252 | 7 F | ALL | A+/O+ | 1:8 | Cy TBI | Not evaluable |
| 262 | 7 M | ALL | A+/O- | 1:32 | Cy TBI | Not evaluable |
| 254 | 30 M | AA | A+/O+ | 1:8 | Cy TBI | Yes |
| 133 | 28 F | AA | A-/O+ | 1:32 | Cy | Yes |
| 14 | 29 M | AA | A+/O+ | 1:128 | PA-TBI | No |
| 88 | 7 M | AA | A+/O+ | 1:32 | PA-TBI | No |
| 82 | 28 F | AA | A+/O+ | 1:8 | Cy | Yes |
| 78 | 8 M | ALL | AB+/A- | 1:4 | Cy TBI | Yes |
| 87 | 19 M | ALL | B+/O+ | 1:64 | Cy TBI | Yes |
| 118 | 30 M | ALL | AB+/A+ | 1:2 | Cy TBI | Yes |
| 126 | 35 M | ALL | B-/O+ | 1:8 | Cy TBI | Yes |
| 191 | 24 M | AA | AB+/A+ | 1:4 | Cy | Yes |
| 76 | 14 F | AA | B-/O- | 1:64 | Cy | No |
| 117 | 21 M | AA | B+/O+ | 1:32 | Cy | Autologous recovery |
| 129 | 18 M | AA | AB+/O+ | 1:64 1:256 | PA-TBI | Yes |
| 132 | 19 M | AA | AB+/O- | 1:4 1:2 | Cy | Yes |

*ALL, acute lymphocytic leukemia; AML, acute myelocytic leukemia; CML, chronic myelocytic leukemia, AA, aplastic anemia.

†Cy, cyclophosphamide; TBI, total body irradiation; Bu, busulfan; P procarbazine; A antithymocyte globulin.

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 Robinson⁸ on 13 bone marrows before and after erythrocyte depletion. All patients received daily leukocyte, platelet, and hematocrit determinations. Reticulocyte and differential counts were obtained biweekly. Hemagglutinin titers were determined weekly using the method of Crawford,⁷ 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

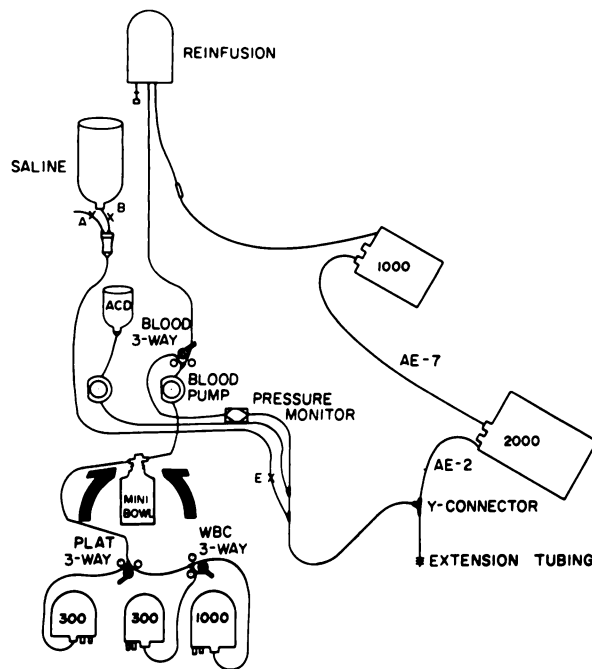


Fig. 1. Machine set-up for erythrocyte depletion of bone marrow. Marrow collection bag (2000 ml) connected to needleless harness via AE-2 and Y connector. Plasmapheresis (mini) bowl placed in adaptor and connected to marrow collection (300 ml) and plasma (1000 ml) bags. Residual processed marrow stored in refusion and overflow bag (1000 ml).

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

Table 2. Cellular Recovery Following Erythrocyte Depletion of Bone Marrow Prior to ABO Blood-Group Incompatible Transplantation

| | Initial Marrow | Fractions I and II | Fractions I and II as Percent of Initial |
|---------------------------------------|----------------|--------------------|--|
| Total leukocytes $\times 10^9$ | 20.3 ± 8.4 | 11.6 ± 6.1 | 55% |
| Total mononuclear cells $\times 10^9$ | 4.0 ± 2.4 | 3.0 ± 1.4 | 75% |
| Total CFU-GM $\times 10^4$ | 413 ± 283 | 221 ± 173 | 57% |
| Erythrocyte volume (ml) | 354 ± 115 | 21.0 ± 9.5 | 5.6% |

Values are mean \pm standard deviation.

Table 3. Posttransplant Day of Peripheral Blood Recovery Following Allogeneic Bone Marrow Transplantation

| | Posttransplant Days (± 1 SD) to Peripheral Blood Recovery | |
|-------------------------------------|--|------------------------------|
| | ABO Blood Group Compatible | ABO Blood Group Incompatible |
| Leukocyte $>500/\text{cu mm}$ (n) | 17 ± 5 (109) | 17 ± 4 (23) |
| Leukocyte $>1000/\text{cu mm}$ (n) | 20 ± 7 (106) | 20 ± 5 (21) |
| Granulocyte $>500/\text{cu mm}$ (n) | 22 ± 8 (105) | 22 ± 4 (21) |
| Reticulocyte $>1\%$ (n) | 25 ± 10 (98) | 37 ± 15 (15) |
| Platelet $>50,000/\text{cu mm}$ (n) | 32 ± 21 (65) | 31 ± 11 (13) |

(n) Number of patients.

*p from Student's t test.

Table 4. Relationship of Pretransplant Hemagglutinin Titers to Recovery of Peripheral Blood Reticulocytes Following ABO Blood Group Incompatible Bone Marrow Transplantation

| Case No. | Pretransplant Hemagglutinin Titer | | Days to Reticulocyte Count > 1% | Hemagglutinin Titer When Reticulocyte > 1% | |
|----------|-----------------------------------|--------|---------------------------------|--|--------|
| | Anti-A | Anti-B | | Anti-A | Anti-B |
| 129 | 1:64 | 1:256 | 266 | 0 | 0 |
| 87 | | 1:64 | 42 + | | * |
| 88 | 1:32 | — | 60 | 1:1 | |
| 133 | 1:32 | — | 64 | 1:1 | |
| 170 | 1:16 | — | 34 | 1:1 | |
| 168 | 1:16 | — | 27 | 1:2 | |
| 188 | 1:8 | — | 49 + | * | |
| 254 | 1:8 | — | 41 | 1:2 | |
| 126 | | 1:8 | 39 | | 1:1 |
| 221 | 1:8 | — | 34 | 1:4 | |
| 82 | 1:8 | — | 26 | 1:2 | |
| 252 | 1:8 | — | 17 | 0 | |
| 132 | 1:4 | 1:2 | 18 | 1:4 | 1:2 |
| 103 | 1:4 | — | 42 + | * | |
| 78 | 1:4 | — | 35 + | * | |
| 118 | | 1:2 | 59 | | 1:1 |
| 186 | 1:2 | — | 34 | 1:1 | |
| 230 | 1:1 | — | 17 | 1:1 | |

*Patient died prior to attaining > 1% reticulocytes.

31 ± 11 days after ABO-incompatible BMT and 32 ± 21 days after ABO-compatible BMT.

ABO-incompatible transplant patients achieved a reticulocytosis >1% 37 ± 15 days after marrow infusion. ABO blood group compatible BMT achieved >1% reticulocytes significantly earlier (25 ± 10 days) than ABO blood group incompatible BMT (*p* < 0.0001). In general, those patients with the highest pretransplant hemagglutinin titers manifested the longest delay in reticulocyte recovery (Table 4). Reticulocytosis did not occur until there had been a substantial reduction in hemagglutinins, but was sustained at >1% even in the presence of easily detectable titers of incompatible hemagglutinins of 1:1 to 1:4. Eventually, all patients who engrafted developed serologic profiles compatible with donor erythrocytes.

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,^{9,10} 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 5. Erythrocyte Morphology of Bone Marrow Aspirates Following ABO Blood Group Compatible and Incompatible Transplantation at Equivalent Stages of Peripheral Blood Recovery

| Bone Marrow Erythrocyte Morphology | Stage of Peripheral Blood Recovery | | | |
|------------------------------------|--|------------------|--------------------|------------------|
| | Leukocytes > 1000/cu mm | | Reticulocytes > 1% | |
| | ABO Compatible | ABO Incompatible | ABO Compatible | ABO Incompatible |
| Relative erythrocyte hypoplasia | 1/17 | 10/14 | 4/24 | 3/9 |
| (myeloid:erythroid ratio > 4:1) | ┌────────── <i>p</i> < 0.001* ─────────┐ | | | |
| Erythrocyte aplasia | 1/18 | 5/14 | 0/25 | 0/9 |
| | ┌────────── <i>p</i> > 0.2 ─────────┐ | | | |
| Dyserythropoiesis present | 7/18 | 4/7 | 9/24 | 5/9 |
| Megaloblastosis present | 16/18 | 8/8 | 23/25 | 9/9 |

**p* by chi-square analysis.

erythrocytes while retaining 75% of the original mononuclear cell content and greater than half of the original CFU-GM. Our current protocol leaves 8–38 ml of incompatible erythrocytes in the infusate. Undoubtedly, erythrocyte contamination could be reduced further if desired. As the toxicity of the infusion of our current concentrates has been minimal, we have not made protocol modifications to further reduce erythrocyte contamination.

Alternate techniques of erythrocyte depletion using centrifugation or gravity sedimentation in hydroxyethyl starch⁵ in plastic blood containers have been described. In order to process large volumes of marrow, such procedures require the use of multiple transfer steps. In addition, the marrows must be manipulated delicately after separation and determination of “cut points” made subjectively. We believe the use of an automated blood cell separator achieves a better closed processing system and a more objective and reproducible separation.

Depletion of recipient hemagglutinins by plasma exchange and immune absorption has been used since 1970 as an alternate approach to ABO blood group incompatible transplantation.^{1,2} The introduction of specific immune absorption columns has improved this approach by reducing the hazard of replacement solutions during plasmapheresis and obviating the need for incompatible erythrocyte transfusion.³ However, even with use of specific immune column absorption, extensive extracorporeal manipulation of recipient blood is required. Typically, 2–3 plasmapheresis-immune absorption procedures lasting 2–3 hr are required. Venous access difficulties, anticoagulant toxicity, vascular 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 incompatible BMT. ABO-incompatible erythrocytes still must be infused into diminished, but still detectable, 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 mononuclear cell (MNC) content was recovered with 57% of the CFU-GM. Unfortunately, accurate determination of the cell content of a heterogeneous mixture as

complex as bone marrow is difficult, and the variability in the CFU-GM assay is significant. Thus, accurate quantitation of MNC and CFU-GM content was difficult and calculated recoveries varied considerably. Nonetheless, sufficient stem cell content was retained as indicated by the incidence of engraftment. All AML and ALL patients who were evaluable achieved engraftment. In aplastic anemia engraftment was achieved at a rate similar to ABO blood group compatible transplants.¹² The infusion of small amounts of incompatible erythrocytes was well tolerated. There were no persistent changes in clinical status attributable to the incompatible infusion.

As shown by the rate of engraftment, hematopoietic stem cells do not appear to express sufficient A or B antigens to result in direct cytotoxicity following ABO blood group incompatible bone marrow infusion. Myeloid differentiation appeared to be unaffected following ABO blood group incompatible transplantation. Bone marrow examination revealed no morphological changes in myeloid precursors, and peripheral blood reconstitution was similar in ABO blood group compatible and incompatible transplants. Likewise, no difference in megakaryocyte–platelet differentiation was noted. Decreased numbers of bone marrow megakaryocytes were noted in both ABO blood group compatible and incompatible transplants. Peripheral blood platelet reconstitution, when present, was similar in both groups.

Erythroid differentiation was markedly reduced or absent in over 70% of ABO-incompatible transplants at a time when myeloid differentiation had achieved greater than 1000 leukocytes/cumm in the peripheral blood. Those recipients with the highest incompatible anti-A or B titers developed temporary red cell aplasia during engraftment. This may indicate that cytotoxic specificities to antigens associated with the ABO system are expressed, at least transiently, on erythroid precursors as primitive as rubroblasts. While incompatible hemagglutinins were still detectable, reticulocytes were observed in the peripheral blood, indicating that some erythroid differentiation can take place even in the presence of incompatible hemagglutinins. Presumably, these abnormalities in erythroid development following ABO blood group incompatible BMT represent the effect of humorally mediated cytotoxicity to primitive erythroid precursors. This suggests that antibody-mediated cytotoxicity can result in pure erythrocyte aplasia. However, in this setting, inhibition of erythroid development may be advantageous. The patient receiving an ABO blood group incompatible bone marrow transplant with erythrocyte depletion is in effect protected from the consequences of a major

hemolytic anemia until circulating hemagglutinins are reduced to a clinically insignificant level.

ACKNOWLEDGMENT

The authors wish to acknowledge the invaluable role of members of the medical housestaff, Oncology Nursing Service, Blood Bank,

Hemapheresis Treatment Center, and Experimental Hematology Laboratory of the Johns Hopkins Oncology Center. In particular, the contributions of Bonnie Bourn, Donald Fuller, P.A.-C., Mary O'Hare, and Wanda Novak are gratefully appreciated. The assistance of E. David Mellits in statistical evaluation is gratefully appreciated.

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