Establishment of Erythropoiesis Following Bone Marrow Transplantation in a Patient With Congenital Hypoplastic Anemia (Diamond-Blackfan Syndrome)

By Charles S. August, Elena King, John H. Githens, Kenneth McIntosh, James R. Humbert, Arnold Greensheer, and Franklin B. Johnson

Marrow transplantation was attempted in a 13-yr-old boy with congenital hypoplastic anemia who had never responded to corticosteroid therapy. Prior to the transplant, he had received 238 transfusions, at least 12 of which were from his father. He was prepared for grafting with anti-lymphocyte globulin, procarbazine, and total body irradiation (1000 rads). The patient, whose red cells were Group B, then received marrow cells from his Group O, histocompatible, sister. Thereafter, reticulocytes, Group O erythrocytes, and female leukocytes appeared in the peripheral blood. Erythroid precursors were seen in the patient’s marrow for the first time in his life, and all lacked fluorescent Y chromosomes. Dividing cells were all female. After initially progressing well, the patient developed interstitial pneumonia and died 55 days after the transplant. The successful erythroid graft suggested that this patient’s failure to produce red blood cells was due to a defective stem cell rather than to a humoral defect, plasma inhibitor, or abnormal marrow microenvironment. It suggested further that sibling marrow may be engrafted in patients who have received multiple transfusions, even from a parent.

CONGENITAL HYPOPLASTIC ANEMIA (CHA), first described by Diamond and Blackfan in 1938,1 is characterized by a failure of the bone marrow specifically to produce red blood cells. Children with the syndrome usually develop reticulocytopenia and anemia in the first few months of life and are found to have virtual or complete absence of erythroid precursors in their marrows. Fortunately, therapy with corticosteroids induces remission in most patients.2 The children who do not respond to corticosteroids require regular transfusions. A fraction of these may remit spontaneously, but the majority will develop iron overload and die in the second or third decade of life.3

It has been postulated4 that the underlying hematologic defect in CHA could be either (1) a defective erythroid stem cell, such that it fails to differentiate, (2) an abnormal microenvironment within the marrow, (3) a defective erythropoietin mechanism, or (4) a humoral inhibitor of red cell differentiation.

This report describes a bone marrow transplant undertaken in a boy with steroid-resistant CHA. Although the patient died 55 days after transplanta-
tion, erythroid marrow was successfully grafted, thus suggesting that this patient's failure to produce red blood cells was due to a defective stem cell.

CASE REPORT

The patient, a 13½-yr-old boy, was born in 1959 and found to be severely anemic at 1 mo of age. Bone marrow examination disclosed erythroid hypoplasia, and monthly transfusions were begun. Prednisone therapy, in doses ranging from 1 to 3 mg/kg/day, failed to stimulate erythropoiesis. Combinations of prednisone with testosterone, vincristine (which was thought at the time to stimulate erythropoiesis), pyridoxine, oxymetholone, and even phenobarbital were employed all without success. Plasma infusions from both normal and polycythemic individuals similarly failed to improve red cell production.

At 4 yr of age, the patient began to experience febrile transfusion reactions, which were found to be due to leukoagglutinins. At 8 yr of age a liver biopsy disclosed hemosiderosis. The patient then received desferrioxamine in a dose of 500 mg/day for the next 2½ yr. A second liver biopsy revealed an increase in the iron deposits, and the desferrioxamine was discontinued. At age 10, he suffered his first episode of congestive heart failure (CHF) during an influenza-like illness. In 1972, at 12½ yr of age, he was found to have a diabetic glucose tolerance test. Liver function tests became abnormal, with elevations of transaminases and lactic dehydrogenase. Later that year, the patient developed severe congestive heart failure and required both digitalis and diuretic therapy for the rest of his life. At age 13, he began to experience episodes of chest pain and had yet another bout of congestive failure. He developed overt diabetes mellitus during a final attempt to treat his CHF with prednisone.

In 1971 it was determined the he and an older sister were HLA identical and unreactive in mixed lymphocyte culture (stimulation index of 0.46). The patient was blood Group B and his sister was Group O. Although the patient had both agglutinating and cytotoxic antibodies against the leukocytes of his father and virtually all unrelated individuals, his serum showed no reactions against his sister's leukocytes by the microcytotoxicity test (performed by Dr. Terasaki) and by the leukoagglutinin test.

After the third episode of cardiac failure, the decision was made to attempt marrow transplantation in the hope of establishing erythropoiesis and eventually eliminating the need for
transfusions. At the time of the transplant the patient had received 238 transfusions, at least 12 of which were from his father. No other family member had ever been used as a blood donor. Informed consent was obtained from the parents, the donor, and the patient himself.

The patient was then placed in reverse isolation in a room under slight positive air pressure. Topical orificial antibiotics and antiseptics, oral nonabsorbable antibiotics, and a diet low in viable bacteria were given according to the protocol of Levine and associates. Immunosuppressive therapy was administered using a protocol devised by Storb and associates for use in sensitized dogs. Antilymphocyte globulin (3.2-3.9 ml) was given intramuscularly 10, 8, 6, and 4 days before transplantation. Procarbazine (10 mg/kg) was given intravenously 9, 7, and 5 days before the transplant. Total body irradiation (TBI) was administered on the day before the transplant.

The boy then received $1.3 \times 10^{10}$ nucleated marrow cells ($4.3 \times 10^{9}$/kg) intravenously. Methotrexate was given intermittently according to the Seattle protocol until the 25th day. Following the transplant the patient was given 2 units of irradiated buffy coat (containing platelets and leukocytes) twice weekly and irradiated packed red blood cells (RBC) whenever needed (Fig. 1). Thirty-three days after grafting the absolute granulocyte count was normal (Fig. 2) and the nonabsorbable antibiotics and the special diet were discontinued. Thirty-eight days after grafting the boy developed interstitial pneumonitis and rapidly required ventilatory assistance. In spite of therapy directed against both bacteria and Pneumocystis carinii, the patient could not be weaned from his respirator, and he died suddenly 55 days after the transplant with bilateral pneumothoraces and a cardiac arrest. At no time in his course did the patient manifest any of the dermal, hepatic, or gastrointestinal signs of a graft-versus-host reaction (GVHR).

**MATERIALS AND METHODS**

Routine hematologic, immunologic, chemical, and microbiologic studies were carried out by standard methods in the clinical laboratories of the Children’s Hospital of Denver and at the University of Colorado Medical Center. Bone marrow biopsies were fixed in Zenker formol, embedded in methacrylate, cut as 2-µm sections, and stained with hematoxylin eosin azure II according to the method of Zambernard et al.

HL-A typing and cytotoxicity testing were performed in the laboratory of Dr. Paul Terasaki, University of California, Los Angeles. Leukocagglutinins were studied by the method of Thompson et al. Mixed lymphocyte cultures were performed by the method of Bach and Voynow.
Bone marrow chromosomes were stained with Giemsa following 24 hr in culture according to the method of Tjio. Ethanol-fixed smears of bone marrow and peripheral blood were stained with quinacrine mustard to demonstrate fluorescent Y chromosomes according to the method of Pearson et al.

The equine antilymphocyte globulin used to treat the patient was obtained from Dr. Thomas Starzl of the Department of Surgery, University of Colorado Medical Center. Total body x-ray from a single cobalt-60 source was administered at a distance of 200 cm at a rate of 18 rads/min over 80 min to a midline dose of 1000 rads. All blood products were irradiated to a dose of 1500 rads prior to transfusion.

Approximately 24 hr after the patient's total body irradiation, the donor was taken to the operating room and given general anesthesia. Bone marrow was aspirated from multiple sites along both of her posterior iliac crests and then injected through 23-gauge needles into a plastic blood administration bag (Fenwal) containing 100 ml of heparinized (40 U/ml) Ringer's lactate solution. The contents of the bag were kneaded manually and passed twice through nylon mesh blood filters (Fenwal) before being administered intravenously.

RESULTS

Hematologic Studies

Twenty-two days after the transplant, Group O red cells were identified serologically in the peripheral blood and the Coombs' test became positive. This development was followed by brisk hemolysis, and transfusions of packed red blood cells were required for 2 wk. Antibody eluted from the patient's RBC was identified as anti-B. As shown in Fig. 1, 25 days after the transplant, reticulocytes began to appear in the peripheral blood. The requirement for packed RBC transfusions ceased 35 days after the transplant. Forty days after the transplant, the reticulocyte count rose to a maximum of 7%, following which it declined markedly.

White blood cells began to appear in the peripheral blood 20 days after the transplant, and the white blood count rose to 10,000/cu mm after the onset of the patient's interstitial pneumonitis. Figure 2 shows the total WBC and the absolute granulocyte count. Lymphocytes disappeared prior to the transplant, following administration of antilymphocyte globulin, procarbazine, and the TBI. After the transplant, absolute lymphopenia persisted until the patient developed interstitial pneumonitis.

Although the platelet count never rose above 35,000/cu mm, the patient ceased to require platelet transfusions 30 days after the transplant (Fig. 2), and bleeding never occurred.

Fig. 3. Erythroid precursors (basophilic and polychromatophilic normoblasts) found in the patient's bone marrow aspirate 30 days after his transplant. Giemsa. ×338.
Prior to the transplant, numerous bone marrow aspirates and biopsies had failed to show any erythroid precursors. A marrow aspirate obtained 30 days after the transplant showed megakaryocytes, granulocyte precursors, erythroblasts, and normoblasts at all stages of maturation (Fig. 3). The ratio of myeloid to erythroid precursors (M:E ratio) was 1:2. The erythroid series was represented by: erythroblasts and pronormoblasts 8%, basophilic normoblasts 16%, polychromatic normoblasts 19%, and orthochromic normoblasts 20%. At the time of death, 55 days after transplant, the marrow biopsy showed continued increase in cellularity with persistence of erythroid precursors (Figs. 4A and 4B). The M:E ratio was estimated to be 1:1. The erythroid series showed erythroblasts and normoblasts 5%, basophilic normoblasts 10%, polychromatic normoblasts 18%, and orthochromic normoblasts 5%. The granulocytic series showed increasing maturation.

Cytogenetic Studies

Prior to the transplant the patient’s bone marrow cells showed a normal male karyotype, and 88% of interphase cells had fluorescent Y bodies (Table 1). Following the transplant, the percentage of cells showing fluorescent Y bodies in both the marrow and the peripheral blood declined. Moreover, inspection of marrow preparations by a combination of phase contrast and fluorescence microscopy revealed that all erythroid precursors lacked nuclear fluorescence. In the marrows obtained 20 and 55 days after the transplant, all cells spontaneously undergoing mitosis were female.

Autopsy Studies

At autopsy, marrow obtained from iliac crest and vertebral bodies showed nearly normal cellularity with persistence of erythroid precursors (Fig. 5), thus confirming the findings of the marrow biopsy done immediately after death.

The lungs showed diffuse interstitial fibrosis, edema, hyaline membranes, diffuse hemorrhage, and alveolar macrophages containing hemosiderin. Methenamine silver stain failed to demonstrate Pneumocystis carinii, and no viruses were isolated. Heavy iron deposition was found in the liver (which was cirrhotic), spleen, lymph nodes, thymus, pancreas, thyroid, and parathyroid
Table 1. Cytogenetic Studies

Bone Marrow Chromosomes

<table>
<thead>
<tr>
<th>Day After Grafting*</th>
<th>No. of Mitoses</th>
<th>Karyotype</th>
<th>No. of Interphase Cells With Nuclear Y Fluorescence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient (†)</td>
<td>–67</td>
<td>25</td>
<td>46,XY</td>
</tr>
<tr>
<td></td>
<td>+20</td>
<td>10</td>
<td>46,XX</td>
</tr>
<tr>
<td></td>
<td>+55</td>
<td>17</td>
<td>46,XX</td>
</tr>
</tbody>
</table>

Peripheral Blood Leukocyte Nuclear Y Fluorescence

<table>
<thead>
<tr>
<th>Day After Grafting*</th>
<th>No. of Cells</th>
<th>Cells With Nuclear Y Fluorescence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Donor (0)</td>
<td>50</td>
<td>4</td>
</tr>
<tr>
<td>Patient (†)</td>
<td>–9</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>+6</td>
<td>21</td>
</tr>
<tr>
<td></td>
<td>+18</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>+41</td>
<td>25</td>
</tr>
</tbody>
</table>

*The day of the transplant is designated "0."

glands. Moderate amounts of iron were found in the heart, adrenal glands, and testes. There was no histologic evidence of GVHR in the skin, intestinal mucosa, liver, or other organs.

DISCUSSION

To our knowledge, this was the first patient with congenital hypoplastic anemia (Diamond-Blackfan syndrome) in whom bone marrow transplantation had ever been attempted. The transplant was undertaken late in the course of the patient's illness at a time when iron chelation therapy had been demonstrated not to be effective and when cirrhosis, diabetes, and heart failure were far advanced. Because some patients with this syndrome apparently recover spontaneously after puberty, every attempt was made to delay the transplant. However, after the third bout of severe congestive heart failure, it was considered unlikely that the patient would live long enough to undergo puberty, and thus marrow transplantation was attempted.

The evidence for a successful erythropoietic graft in our patient seems to us compelling. Following the transplant, reticulocytes and erythroid precursors

Fig. 5. A section of iliac crest marrow obtained at postmortem examination 55 days after bone marrow infusion. Cellularity is nearly normal. Hematoxylin and eosin. ×41.6.
appeared in our patient's peripheral blood and marrow for the first time in his life. Group O (donor) red cells were identified serologically in the peripheral blood prior to any transfusions of Group O blood. Moreover, when quinacrine mustard-stained smears of marrow aspirates were inspected by one of us (CSA), all red cell precursors, which were easily identified in the preparations, lacked the nuclear fluorescence characteristic of Y chromosomes.

At the time of his death our patient's peripheral reticulocyte count had fallen precipitously. This change raised the question of (1) possible graft rejection, (2) specific failure of erythropoiesis (and thus a return to his original disease state), or (3) temporary suppression of red blood cell production occurring as a consequence of the interstitial pneumonitis or its therapy in the presence of liver damage from hemosiderosis. Treatment included pentamidine, sulfadiazine, and the folic acid antagonist pyrimethamine. Finding erythroid and granulocytic precursors, near normal cellularity, and 100% (17 of 17) female (donor) mitotic figures in the postmortem marrow argued strongly against graft rejection. The persistence of erythroid precursors in the postmortem marrow probably ruled out erythropoietic failure. Thus, the third mechanism seemed most probable. It must be conceded, however, that our patient's limited survival precluded complete certainty in these regards.

The engraftment of erythropoietic cells in our patient has significant implications for the pathogenesis of CHA. Within the limits of our patient's survival, the successful graft implied that the microenvironment of the marrow could support erythropoiesis and that there was no humoral inhibitor of erythropoietin or of stem cell differentiation. Ortega et al. have recently reported that CHA serum inhibited erythropoietin-stimulated heme synthesis in cultured marrow cells. However, Geller and associates have recently reported their failure to find erythropoietic inhibitory activity in vivo and in vitro when the sera of patients with CHA were incubated with normal rat, mouse, and human bone marrow cells. Our experience complements the latter data and suggests that the underlying hematologic defect in CHA is a primary failure of the erythroid stem cell to differentiate.

The successful engraftment of marrow in our patient is noteworthy in view of the evidence from Thomas' group that transfusions given prior to grafting sensitize a recipient and make rejection much more likely to occur. Our patient had received over 200 transfusions and it was documented that in the 5 yr before the transplant, his father had served as donor on at least 12 occasions. Therefore, it was presumed that the patient was sensitized to minor or weak paternal histocompatibility antigens which might have been shared by his sister-donor. Thus, procarbazine and antilymphocyte globulin were employed in addition to total body irradiation for immunosuppression.

Finally, our experience in this patient with terminal iron overload is relevant to any consideration of the possibility of transplanting bone marrow in the much more common disorder, beta-thalassemia. At the outset, the fact that our patient had chronic congestive heart failure ruled against using cyclophosphamide as an immunosuppressant and thus single source total body irradiation was employed. This mode of immunosuppression is more complicated to administer and associated with more severe gastrointestinal and dermal side effects than is immunosuppression with cyclophosphamide. In addition, our
patient’s diabetes proved difficult to control and his insulin requirement fluctuated wildly, ranging from 15 to 110 U/day. Our patient’s cirrhosis may also have complicated the management of his diabetes and increased toxic side effects of drugs metabolized by the liver. The existing liver damage might have added to the hepatic abnormalities of a GVHR if this had developed. Thus, we concluded that the timing of our transplant was not optimal and that future attempts at marrow transplantation in patients with iron overload should be made when recipients show only minimal evidence of organ dysfunction.

ACKNOWLEDGMENT

The authors thank Dr. Louis K. Diamond, who participated in the decision to undertake the transplant; Dr. E. Donnall Thomas, Dr. Rainer Storb, and their associates for encouragement and advice; and Dr. Matthew Block for assistance in interpreting the marrow biopsies. Sheila Cox and Kathy Hoyer provided invaluable technical and secretarial assistance. The authors also acknowledge the dedicated service provided by the nursing staff of the Pediatric Clinical Research Center of the University of Colorado Medical Center.

REFERENCES

Establishment of erythropoiesis following bone marrow transplantation in a patient with congenital hypoplastic anemia (Diamond-Blackfan syndrome)

CS August, E King, JH Githens, K McIntosh, JR Humbert, A Greensheer and RB Johnson