

Brief Report

CORD-BLOOD TRANSPLANTATION FROM AN UNRELATED DONOR IN AN ADULT WITH CHRONIC MYELOGENOUS LEUKEMIA

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TO circumvent the problems inherent in allogeneic bone marrow transplantation, allogeneic cord blood has been studied as an alternative source of hematopoietic stem cells. Preliminary results with cord blood from an HLA-matched sibling are encouraging, but the experience with this procedure is limited.¹⁻⁵ Although some 200 transplantations of cord blood have been performed in children,^{6,7} few have been performed in adults. We describe an adult with chronic myelogenous leukemia who underwent the transplantation of cord-blood stem cells successfully.

CASE REPORT

A 26-year-old woman was found to have chronic myelogenous leukemia in July 1990. All the leukemic cells had the Philadelphia chromosome, and the reverse-transcriptase polymerase-chain-reaction (PCR) assay revealed two transcripts of the fused *BCR/ABL* genes, b3-a2 and b2-a2. She was treated with interferon, but the abnormal karyotype persisted. Neither a related nor an unrelated HLA-compatible bone marrow donor could be found. In October 1994 she entered the accelerated phase of chronic myelogenous leukemia and received high-dose chemotherapy (80 mg of mitoxantrone per square meter of body-surface area and 8 g of cytarabine per square meter). The disease reverted to the chronic phase. An attempt to collect autologous progenitor cells negative for the Philadelphia chromosome by leukapheresis at the time of the patient's recovery from chemotherapy-induced aplasia, after the administration of granulocyte colony-stimulating factor (filgrastim [Neupogen], Amgen-Roche, Neuilly-sur-Seine, France), was unsuccessful. By June 1995 acute myeloblastic leu-

kemia was evident, with 40 percent blast cells in the blood and 68 percent in the marrow. In an attempt to induce a second conversion to the chronic phase of myelogenous leukemia, the patient was treated with 36 g of cytarabine per square meter (3 g per square meter twice a day for six days).

Forty days after she received these high doses of cytarabine, hematopoiesis was recovered and the patient again entered the chronic phase of the disease. A unit of cord blood from the New York Blood Center was available. This unit contained 10 million nucleated cells per kilogram of the patient's body weight and 14,000 granulocyte-macrophage colony-forming units per kilogram. Other characteristics of the cord blood and the recipient are given in Table 1. The patient was prepared for transplantation of the allogeneic cord blood with antilymphocyte globulin (Thymoglobuline [IMTIX Pasteur-Mérieux, Lyon, France], 2.25 mg per kilogram per day for two days), cyclophosphamide (60 mg per kilogram per day for two days), and a single dose of total-body irradiation (10 Gy, with lung shielding at 8 Gy at the instantaneous-dose rate of 0.047 Gy per minute with a cobalt-60 teletherapy unit).

The cord blood was thawed and infused on day 0. The proportion of viable cells after thawing, as determined by the uptake of trypan blue, was 88 percent. Prophylaxis against graft-versus-host disease consisted of intravenous cyclosporine (2 mg per kilogram per day) and prednisone (1 mg per kilogram per day). Granulocyte colony-stimulating factor (10 μ g per kilogram per day intravenously) was given from day 0 to day 40, and erythropoietin (180 IU per kilogram daily; Recormon, Boehringer Mannheim, Rueil-Malmaison, France) was given from day 0 to day 35. Engraftment was documented by chromosome analysis with fluorescence in situ hybridization for the Y chromosome (the donor was male).

METHODS

Hematopoietic Progenitor Assay

Early granulocyte-macrophage colony-forming units and erythroid burst-forming units were assayed with a method based on the technique of Eaves and Eaves.⁸ The numbers of colonies of granulocyte-macrophages (containing more than 50 cells) and of erythroblasts were counted on day 14. The technique of identifying bone marrow cells capable of initiating long-term cultures was used according to the method of Sutherland et al.⁹

Immunophenotypic Analysis

Immunophenotyping was performed on bone marrow and peripheral-blood lymphocytes with monoclonal antibodies labeled with fluorescein and phycoerythrin. Double or single staining was done with the following monoclonal antibodies: CD3, CD4, CD19, CD29, CD45 RA, HLA-DR, S6F1, CD56, CD25, and CD26. The cells were analyzed with an Epics Profile flow cytometer (Coulter, Hialeah, Fla.).

Fluorescence in Situ Hybridization

Fluorescence in situ hybridization was used to ascertain engraftment of the allogeneic stem cells and detect residual leukemic cells. The probe used to find descendants of the grafted cells was a biotinylated repeat sequence (pY2.1) of the Y chromosome. To detect residual leukemia, we used two-color fluorescence in situ hybridization with a probe (Oncor, Gaithersburg, Md.) for the *BCR/ABL* translocation that identifies the t(9;22)(q34;q11) reciprocal translocation in nuclei in interphase.¹⁰

PCR to Detect Chimeric *bcr-abl* mRNA

Total cellular RNA was extracted according to the method of Chomczynski and Sacchi.¹¹ Combined reverse transcription and PCR were performed as described by Kawasaki et al., with slight modifications.¹² The limit of sensitivity of the technique, which was optimized with serial dilutions of the K562 cell line in normal bone marrow mononuclear cells, is approximately 1 leukemic cell in 100,000 normal cells.¹³

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TABLE 1. CHARACTERISTICS OF THE CORD-BLOOD GRAFT AND THE RECIPIENT.

| CHARACTERISTIC | CORD BLOOD | PATIENT |
|--|------------|-----------|
| Age (yr) | — | 26 |
| Sex | Male | Female |
| Blood group | B | A |
| Rh status | Positive | Negative |
| HLA antigens* | | |
| Class I | | |
| A | 1,2 | 1,2 |
| B | 8,57 | 51,57 |
| Class II | | |
| DR | 3,13 | 0301,1301 |
| DQB1 | | 0201,06 |
| DPB1 | | 0201,0402 |
| Dose of cells per kilogram of patient's body weight | | |
| Nucleated cells ($\times 10^{-7}$) | 1 | — |
| Granulocyte-macrophage colony-forming units ($\times 10^{-4}$) | 1.4 | — |
| Volume (ml) | 79 | — |

*All HLA antigens in the cord-blood sample and the class I antigens in the sample from the patient were determined by serologic typing. Class II antigens in the patient were determined by molecular typing.

RESULTS

The patient's course after transplantation was uncomplicated by infection. The white-cell count began to rise by day 23, and by day 38 it was 18,000 per cubic millimeter (Fig. 1). The absolute neutrophil count reached 500 per cubic millimeter on day 23 and exceeded 15,000 per cubic millimeter on day 38. The platelet count was 50,000 per cubic millimeter on day 48 and reached 100,000 per cubic millimeter on day 120. The reticulocyte count rose above 50,000 per cubic millimeter on day 27 and above 230,000 per cubic millimeter on day 37. On day 46 the lymphocyte count in the blood was 804 per cubic millimeter. At that time, almost all the lymphocytes in the blood and bone marrow had the immunophenotype of natural killer cells. The patient received 12 units of packed red cells (with the final unit given on day 25) and 17 transfusions of platelets (the final one on day 37).

On day 23 the bone marrow was moderately cellular, all three lineages were present, and there was a considerable increase in the ratio of erythroid cells to myeloid cells. Assays of hematopoietic progenitor cells in a bone marrow aspirate obtained on day 46 showed 37,868 granulocyte-macrophage colony-forming units per milliliter and 52,000 erythroblastic burst-forming units per milliliter. A long-term marrow culture initiated 1 colony-forming cell per 121,000 cultured cells.

Because the donor was male and the patient female, engraftment could be demonstrated by the presence of cells containing the donor's Y chromo-

some. Fluorescence in situ hybridization performed on peripheral-blood cells showed 100 percent male cells on day 23; complete chimerism was also found 2, 4, and 8 months after transplantation. Red cells from the donor (belonging to blood group B) were detected on day 54.

On day 46 fluorescence in situ hybridization of the bone marrow aspirate was negative for *BCR/ABL* transcripts, and the reverse-transcriptase PCR assay demonstrated a low level of the b3-a2 amplification product. On day 120 the reverse-transcriptase PCR assay for both b3-a2 and b2-a2 transcripts was negative in the marrow and blood.

Eight months after transplantation, the patient's blood counts were as follows: white cells, 3800 per cubic millimeter, with a normal differential count; hemoglobin, 10 g per deciliter; and platelets, 100,000 per cubic millimeter. Analysis of 47 cells showed that 100 percent had male karyotypes and no Philadelphia chromosome. At this writing the patient is well and has returned to work.

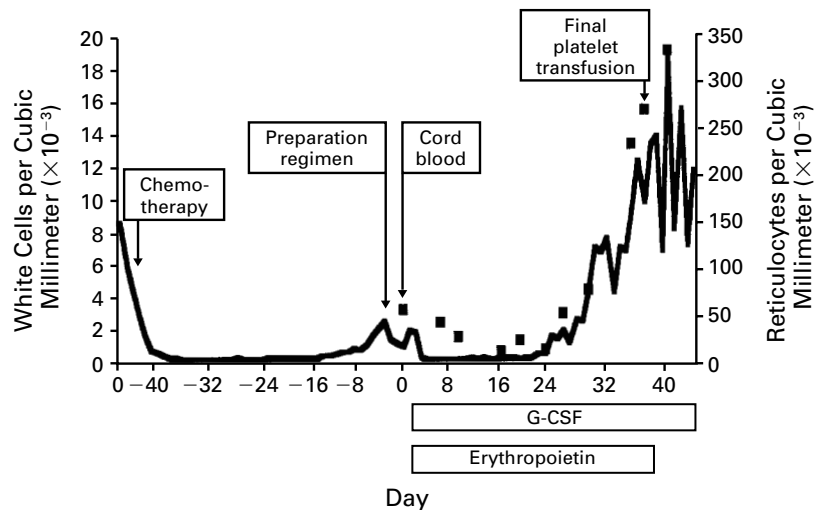
DISCUSSION

Cord blood is an attractive new source of allogeneic hematopoietic stem cells. In 1989 the first successful transplantation of cord blood was performed in a child with Fanconi's anemia.¹ Other reports of the transplantation of cord blood from siblings followed,¹⁻⁶ but recently the possibility of transplanting cord blood from unrelated donors has been studied and cord-blood banks have been established.^{7,14,15} The relative immaturity of hematopoietic stem cells in cord blood¹⁶⁻²⁰ may allow hematopoietic reconstitution with fewer nucleated cells than are required with marrow and peripheral blood. Moreover, the immaturity of lymphocytes in cord blood²¹⁻²⁴ probably reduces the risk and severity of graft-versus-host disease. This feature may permit a greater degree of HLA discrepancy between donor and host than is possible with marrow or blood.^{6,7} These advantages make cord blood an appealing source of stem cells from unrelated donors for transplantation in adults who have no HLA-identical siblings.

The number of stem cells that reconstituted hematopoiesis in our patient approximated the numbers used in children. In the 44 transplantations of cord blood from sibling donors reported by Wagner et al., the median body weight of the children who received the transplants was 18.6 kg (range, 7.5 to 50).⁶ The numbers of nucleated cells and granulocyte-macrophage colony-forming units per kilogram of the recipient's weight were 52 million (range, 10 million to 330 million) and 24,000 (range, 100 to 1 million), respectively. In the 20 cord-blood transplantations from unrelated donors reported by Kurtzberg et al., the median body weight of the patients was 19.4 kg (range, 7 to 53).⁷ The doses per kilogram were 28,500,000 nucleated cells (range,

Figure 1. The Patient's Hematopoietic Recovery after the Transplantation of Cord Blood from an Unrelated Donor.

The chemotherapy, given in June 1995, consisted of 36 g of cytarabine per square meter of body-surface area. The regimen used to prepare the patient for the cord-blood transplantation included antilymphocyte globulin, cyclophosphamide, and total-body irradiation, as described in the Case Report, and was given two days before transplantation. The solid squares denote reticulocyte counts. G-CSF denotes granulocyte colony-stimulating factor.



7 million to 98 million) and 38,000 granulocyte-macrophage colony-forming units (range, 14,000 to 404,000). Our patient weighed 55 kg and received 10 million nucleated cells and 14,000 granulocyte-macrophage colony-forming units per kilogram.

Granulocyte colony-stimulating factor, granulocyte-macrophage colony-stimulating factor, and erythropoietin have been shown to accelerate the recovery of neutrophils and reduce the need for transfusions of red cells after bone marrow transplantation, but they have not had these effects after cord-blood transplantation.⁶ We do not know whether the administration of granulocyte colony-stimulating factor and erythropoietin stimulated engraftment in our patient. The great increase in the number of leukocytes and neutrophils on day 38 and the increased ratio of erythroid cells to myeloid cells in the marrow aspirate obtained on day 23 suggest that these growth factors accelerated engraftment. Our experience indicates that the transplantation of cord-blood stem cells is feasible in adults, despite the relatively low numbers of hematopoietic precursor cells in units of cord blood.

Despite improvements in the management of chronic myelogenous leukemia, the transplantation of allogeneic stem cells is the only approach with the potential to cure the disease.²⁵ If the patient does not have an HLA-identical sibling, transplantation of marrow from an unrelated donor is an alternative. But this option is limited by the low probability of finding a suitable donor. The probability of finding an HLA-matched, unrelated donor in the international data base, which contains about 3 million potential donors, depends on the patient's HLA type. For the most common HLA types, the probability can be as high as 30 to 50 percent.²⁶ In our patient, we calculated the probability of a match as 1 in 1 million,

and indeed, a three-year search for an HLA-compatible donor was fruitless. The availability of cord blood from an unrelated donor and the ease with which this source of stem cells could be used allowed us to perform transplantation soon after the blast crisis occurred. The absence of acute graft-versus-host disease in our patient is parallel to the experience with children. This may be a disadvantage, however, because of a lack of a graft-versus-leukemia effect. We cannot rule out the possibility that the natural killer cells that predominated in the patient six weeks after engraftment had antileukemia activity.²⁷ Despite the disappearance of *BCR/ABL* transcripts from the patient's blood and marrow, long follow-up is needed to appraise the evolution of the disease.

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