BRIEF REPORT: TRANSPLANTATION OF CORD-BLOOD STEM CELLS INTO A PATIENT WITH SEVERE THALASSEMIA

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SEVERAL different kinds of thalassemia and hemoglobinopathy are prevalent in Southeast Asia.[1,2] The frequency of the α-thalassemias reaches 30 to 40 percent in northern Thailand, whereas the β-thalassemias occur at a frequency of 3 to 9 percent. Hemoglobin E, the hallmark hemoglobinopathy of Southeast Asia, occurs at a frequency of 50 to 60 percent at the junction of Thailand, Laos, and Cambodia. Mutation and gene interaction account for more than 60 different clinical syndromes. Of these, homozygous β-thalassemia and hemoglobin E–β-thalassemia disease are the most common and the most severe clinical syndromes compatible with live birth. Hemoglobin E–β-thalassemia disease is more frequent than homozygous β-thalassemia in Southeast Asia because of the much higher frequency of hemoglobin E.

Bone marrow transplantation is currently the only curative therapy for β-thalassemia.[3-7] However, less than 30 percent of patients have unaffected HLA-identical siblings to serve as donors. Cord blood contains substantial numbers of hematopoietic stem cells and may be used for transplantation.[8,9] Cord-blood stem cells have been successfully transplanted into patients with Fanconi's anemia, aplastic anemia, leukemia, and X-linked lymphoproliferative disorder.[10-15] We report the transplantation of such stem cells from an unaffected, HLA-identical sibling into a patient with hemoglobin E–β-thalassemia disease.

CASE REPORT

A 2½-year-old girl was first found to be anemic at 6 months of age. She was given a diagnosis of hemoglobin E–β-thalassemia disease. Her initial hemoglobin level was 7.4 g per deciliter; the mean corpuscular volume was 67 fl, and the reticulocyte count 4.4 percent. Hemoglobin typing revealed hemoglobin E and hemoglobin F (32.4 percent and 67.6 percent, respectively). Typing of the girl's father revealed that he was heterozygous for hemoglobin E (hemoglobin E, 34.6 percent; hemoglobin A, 65.4 percent); typing of her mother revealed that she was heterozygous for β-thalassemia (hemoglobin A, 91.8 percent; hemoglobin A_2, 8.2 percent). The patient had received 18 units of packed red cells and had been treated with iron chelation. She did not have hepatosplenomegaly. The serum ferritin level was 190 ng per milliliter (normal, 12 to 300). Bone marrow transplantation had not been considered because no donors were available.

The patient's mother became pregnant in July 1992. Prenatal tests for thalassemia were performed on October 12. DNA extracted from fetal blood was tested for the β-thalassemia mutation and the gene for hemoglobin E by hybridizing the amplified DNA with horseradish peroxidase–labeled antistreptolysin O and β^E probes, respectively.[16] The fetus was heterozygous for hemoglobin E, and no β-thalassemia mutation was found. The pregnancy was then continued. DNA typing for HLA class II antigens was performed with the UCLA technique involving sequence-specific primers.[17] The results indicated that the HLA class II haplotype of the fetus was identical to that of the patient. A boy was delivered vaginally with no complications on May 6, 1993. Typing of HLA class I antigens was performed by a serologic technique, and DNA typing of the class II HLA haplotype was rechecked. The boy and the patient were HLA-identical. Their HLA types were A2, 2; B46 (Bw6), B46 (Bw6); DRB1*0403, *0803; DQB1*0302, *05. The ABO blood group was type B in both the patient and her brother.

A 44-ml sample of cord blood was collected at the infant's birth in 20 ml of acid citrate dextrose, transferred to a freezing bag, mixed with an equal volume of cold 20 percent dimethylsulfoxide in minimal essential medium with no separation, and cooled in a controlled-rate freezer before being frozen in liquid nitrogen.

Before receiving the cord blood, the patient was conditioned with busulfan (14 mg per kilogram of body weight) and cyclophosphamide (200 mg per kilogram).[3-7] The frozen cord blood was thawed in a water bath at 37°C and given in transfusion to the patient without the removal of red cells on June 12, 1993. There were 546 million cells in all, equivalent to 39 million per kilogram; there were 229,000 granulocyte–macrophage colony-forming units, or 16,000 per kilogram. A short course of methotrexate and cyclosporine was given as prophylaxis against graft-versus-host disease.[18] Granulocyte colony-stimulating factor (10 μg per kilogram per day) was given intravenously on day 1 after transplantation and was given each day until the neutrophil count remained above 1000 per cubic millimeter for three consecutive days. Engraftment was documented by chromosome analysis and the determination of donor-type DNA by analysis of restriction-fragment–length polymorphisms (RFLPs).

METHODS

RFLP analysis was performed primarily as described elsewhere.[19] Briefly, 1.5 μg of DNA was digested with 4 units of *Ava*II and separated in 0.8 percent horizontal agarose gel. The DNA was blotted onto a nylon (Nytran) nitrocellulose membrane (Schleicher and Schuell, Dassel, Germany) and hybridized with the multilocus DNA probe hMF1,[20] labeled with digoxigenin (Boehringer–Mannheim, Mannheim, Germany). The bands were detected according to the instructions of the manufacturer. The assay for progenitor cells was carried out in methylcellulose as described elsewhere.[21] Agar-conditioned medium was used to stimulate granulocyte–macrophage colony-forming units.

RESULTS

The patient's clinical course after stem-cell transplantation was unremarkable. Fever developed on day 9, and the patient was found to have a skin infection at the site of her Hickman catheter. Vancomycin was given, but the fever persisted. Imipenem and amikacin were added to the treatment. After a positive blood culture for group B streptococcus was reported, vancomycin was discontinued and ampicillin was given (day 13). The fever subsided, and antibiotics were discontinued on day 25.

The white-cell count rose above 1000 per cubic millimeter on day 23 and above 3000 per cubic millimeter

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on day 53. The neutrophil count rose above 500 per cubic millimeter on day 23 and exceeded 1000 per cubic millimeter on day 53. The platelet count exceeded 20,000 per cubic millimeter on day 37 and exceeded 100,000 per cubic millimeter on day 44. The patient received four units of packed red cells, the last of which was given on day 19. Platelet transfusions were given eight times after transplantation; the last one was given on day 30.

On day 32 after cord-blood stem-cell transplantation, the hemoglobin level was 10.4 g per deciliter; hemoglobin types A and A_2 were present. On day 93, the hemoglobin level was 13.7 g per deciliter (11.5 percent hemoglobin E, 35.6 percent hemoglobin F, and 52.9 percent hemoglobin A). On day 117, the hemoglobin level was 11.9 g per deciliter (11.8 percent E, 38.7 percent F, and 49.5 percent A). On day 269, the hemoglobin level was 12.8 g per deciliter (21.9 percent E, 6.9 percent F, and 71.2 percent A). At follow-up on June 20, 1994 (day 373), the hemoglobin level was 14.5 g per deciliter (26.4 percent E, 3.8 percent F, and 69.8 percent A), and the reticulocyte count was 0.4 percent. The patient was clinically well, was attending kindergarten, and had no features of graft-versus-host disease.

Since the donor was male and the patient female, engraftment could be confirmed by the presence of cells with the donor's sex chromosome. Cytogenetic analysis performed at various times after transplantation showed evidence of chimerism; a study on day 373 showed that of 26 cells in metaphase examined, 24 were male and 2 were female.

RFLP analysis on day 30 of DNA from bone marrow and peripheral blood showed clear donor-specific bands and weaker recipient bands (Fig. 1). Similar results were observed on days 60, 120, and 269, with the recipient-specific bands becoming gradually weaker than those obtained on day 30.

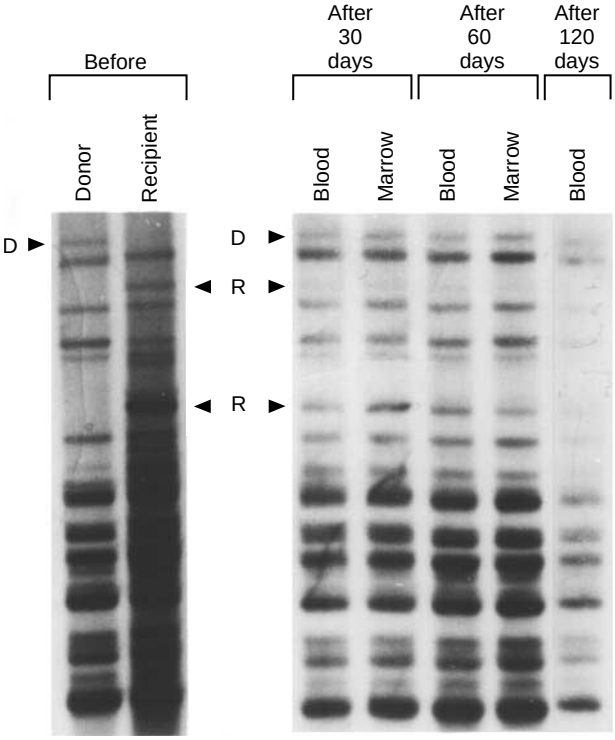

Figure 1. RFLP Analysis of Bone Marrow and Peripheral Blood from the Donor and the Recipient before and after Transplantation of Cord-Blood Stem Cells.

The donor was the HLA-identical brother of a girl with hemoglobin E–β-thalassemia disease. The blots at left show RFLPs for the donor and the recipient before transplantation. The blots at right are for the recipient at various intervals after transplantation. Arrowheads indicate donor-specific (D) and recipient-specific (R) bands.

Discussion

Transplantation of bone marrow from HLA-identical siblings offers a high probability of cure in patients with thalassemia, particularly those in Lucarelli's class 1 (no hepatomegaly and no portal fibrosis).[4-6] In Thailand a substantial number of patients treated with allogeneic bone marrow transplantation have transient engraftment and survive, but with recurrent thalassemia.[7] These patients have not undergone hypertransfusion or iron chelation; they have considerable hepatosplenomegaly, very active medullary and extramedullary erythropoiesis, and organ damage due to iron overload. In contrast, patients who undergo marrow transplantation early in the course of thalassemia have a very high probability of cure. In practice, the severity of the disease should be determined on the basis of the genotype, and bone marrow transplantation should be performed as soon as possible in children with severe disease.

Stem cells from cord blood, an alternative source of stem cells for transplantation, have been successfully transplanted into patients with Fanconi's anemia, aplastic anemia, leukemia, and lymphoproliferative disorder.[10-15] As of September 1993, 26 children had received transplantation of cord-blood stem cells; 17 had malignant diseases and 9 had nonmalignant disorders.[22] Nineteen sibling donor–recipient pairs were HLA-identical, and the remainder were not. The median volume of cord blood collected and infused was 100 ml (range, 44 to 282), with a median of 40 million nucleated cells per kilogram (range, 10 million to 160 million) and a median of 24,200 granulocyte–macrophage colony-forming units per kilogram (range, 2300 to 256,000). Engraftment was evident in 18 of 22 patients who could be evaluated. The median time to recovery (defined as a neutrophil count above 500 per cubic millimeter) was 23.5 days (range, 12 to 46), and the median time to a platelet count above 50,000 per cubic millimeter was 44.5 days (range, 15 to 105). Graft-versus-host disease was rare, occurring only in recipients of cord-blood stem-cell transplants that were mismatched for HLA antigens.

The rate of engraftment after stem-cell transplantation in our patient was slower than would be expected after bone marrow transplantation.[10-15] The absolute neutrophil count reached 500 per cubic millimeter by

day 23, and the platelet count exceeded 20,000 per cubic millimeter by day 37, despite the administration of granulocyte colony-stimulating factor after transplantation. The corresponding values for the time to hematologic recovery after bone marrow transplantation for thalassemia are usually 18 days for the absolute neutrophil count and 21 days for the platelet count. However, the rate of engraftment in our patient was similar to that in other patients undergoing cord-blood stem-cell transplantation.[13,22]

We found chimerism in our patient on day 30 that has persisted to day 373. RFLP analysis showed that recipient-specific bands decreased in intensity after day 30 after transplantation. The decrease in the production of hemoglobin F observed on days 269 and 373 also indicates a diminution in the numbers of residual host cells. Less than 10 percent of the cells observed by cytogenetic analysis on day 373 after transplantation were cells from the recipient. Previous studies showed that chimerism is common after bone marrow transplantation for thalassemia[23] and that the proportion of residual host cells two months after transplantation correlates with the occurrence of graft rejection.

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