

Brief Report

BONE MARROW TRANSPLANTATION IN SEVERE COMBINED IMMUNODEFICIENCY FROM A SIBLING WHO HAD RECEIVED A PATERNAL BONE MARROW TRANSPLANT

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BONE marrow transplantation to treat autosomal recessive severe combined immunodeficiency was undertaken in a one-month-old girl. The donor was the patient's HLA-mismatched six-year-old sister, who had previously received a marrow transplant from her father that was mismatched with regard to one HLA haplotype, to treat the same condition. The graft in the younger girl was not depleted of T cells, and no conditioning regimen was used before transplantation. The prompt engraftment in the girl and her uneventful course after transplantation indicated that the paternal T cells in the older sister's marrow had acquired immunologic tolerance of relevant HLA antigens and thus reconstituted the younger child's immune system without causing graft-versus-host disease.

CASE REPORTS

Patient 1

Patient 1, the first daughter of nonconsanguineous parents, was born on February 17, 1989, weighing 2.75 kg. At the age of three months herpetic skin lesions and candida dermatitis appeared. At the age of four months the infant stopped gaining weight. At the age of six months she had fever and cough, and a chest film showed bilateral pneumonia. Cefaclor was given without benefit. She was admitted to the hospital at the age of 6½ months because of progressive respiratory difficulty. Physical examination revealed thrush, the absence of lymph nodes, and bilateral rales. Tests for antibody to the human immunodeficiency virus were negative in both the patient and her mother. Other relevant laboratory findings in the patient were as follows: serum IgG con-

centration, 42 mg per deciliter (normal, 241 to 813); IgM, 51 mg per deciliter (normal, 20 to 40); IgA, below 7 mg per deciliter (normal, 10 to 46); total lymphocyte count, 2300 cells per cubic millimeter, with no detectable T cells and 98 percent B cells; and no proliferative responses of lymphocytes to stimulation with phytohemagglutinin, pokeweed, or concanavalin A mitogens. A lung biopsy revealed *Pneumocystis carinii* organisms. The patient was given mechanical ventilation and treated with pentamidine, corticosteroids, and intravenous immune globulin.

After being weaned from the respirator, she was transferred to the UCLA Medical Center at the age of seven months. Her lymphocyte counts were as follows: CD3 count, 20 per cubic millimeter (normal, 2280 to 6450); CD4 count, 10 per cubic millimeter (normal, 1670 to 4600); CD8 count, 10 per cubic millimeter (normal, 1690 to 2950); and CD20 count, 855 per cubic millimeter (normal, 500 to 900). The serum IgG concentration was 778 mg per deciliter; IgM, 15 mg per deciliter; and IgA, below 5 mg per deciliter. Concentrations of adenosine deaminase and purine nucleoside phosphorylase in erythrocytes were normal.

At eight months of age, the patient received 130 million nucleated paternal marrow cells per kilogram of body weight, which were depleted of mature T cells by an anti-CD2 monoclonal antibody and complement.² The patient was given no conditioning regimen before transplantation, but cyclosporine was administered for 100 days after transplantation to prevent graft-versus-host disease. After an uneventful course, she was discharged one month after transplantation and given trimethoprim-sulfamethoxazole as prophylaxis against *P. carinii* pneumonia and intravenous immune globulin. During the next five months she had persistent thrush and no evidence of immune reconstitution (that is, the T-cell count was persistently low and no paternal cells were detected by HLA typing).

Six months after transplantation the thrush spontaneously disappeared. The serum IgG concentration was 881 mg per deciliter (with the patient continuing to receive intravenous immune globulin); IgM, 68 mg per deciliter; and IgA, 10 mg per deciliter. The results of HLA typing were unchanged, but DNA analysis of restriction-fragment-length polymorphisms (RFLPs) revealed that 2.6 percent of circulating lymphocytes were of paternal origin. Eighteen months after transplantation, the CD3 count was 150 cells per cubic millimeter; CD4 count, 138 cells per cubic millimeter; CD8 count, 31 cells per cubic millimeter; and CD19 count, 1044 cells per cubic millimeter (normal, 500 to 1500).

Because of her persistently low T-cell count, at the age of 31 months the patient received a second transplant, without conditioning, of 160 million nucleated paternal marrow cells per kilogram, which were depleted of mature T cells by rosetting with sheep erythrocytes and agglutination with soybean lectin.³ Within four months 95 percent of her T cells were found to be of the donor's type by RFLP analysis; the CD3 count was 745 cells per cubic millimeter; CD4 count, 400 cells per cubic millimeter; CD8 count, 430 cells per cubic millimeter; and CD19 count, 620 cells per cubic millimeter. There was no graft-versus-host disease. Her serum immunoglobulin concentrations had risen: the IgG concentration was 1160 mg per deciliter; IgM, 106 mg per deciliter; and IgA, 97 mg per deciliter. Two years after the second transplantation, the treatment with intravenous immune globulin was stopped. After vaccination against diphtheria, pertussis, tetanus, *Haemophilus influenzae* type B, and pneumococcus, the patient had protective titers of antibodies to tetanus, *H. influenzae* polysaccharide, and type 3 pneumococcus. She also had iso-hemagglutinins and anticytomegalovirus antibodies. One year after the intravenous immune globulin was discontinued, her serum immunoglobulin concentrations were as follows: IgG, 786 mg per deciliter; IgM, 127 mg per deciliter; and IgA, 113 mg per deciliter. At this writing she is asymptomatic, in the 25th percentile of height and weight, and attending school. The in vitro responses of her lymphocytes to stimulation with phytohemagglutinin, allogeneic cells, and tetanus antigen are normal.

At the age of 6½ years, when Patient 1 donated bone marrow

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TABLE 1. RESULTS OF SEROLOGIC HLA TYPING OF THE FATHER AND HIS TWO CHILDREN WITH SEVERE COMBINED IMMUNODEFICIENCY, BEFORE AND AFTER BONE MARROW TRANSPLANTATIONS.*

	AGE	HLA-A	HLA-B	HLA-DR
Father	—	28	35, 39	4, 8
Patient 1				
Before transplantation	7 mo	3, 28	35, 44	7, 8
6 mo after 1st transplantation	14 mo	3, 28	35, 44	7, 8
4 mo after 2nd transplantation	35 mo	28	35, 39	7, 8
B-cell line	6 yr	2†, 3, 28	35, 44	7, 8
Patient 2				
Before transplantation	1 mo	3, 28	35, 44	7, 8
After transplantation				
1 mo	2 mo	3, 28	35, 39, 44	7, 8
8 mo	9 mo	28	35, 39	7, 8
14 mo	15 mo	28	35, 39	7, 8
B-cell line	9 mo	2†, 3, 28	35, 44	7, 8

*The father was the marrow donor for Patient 1, and Patient 1 was the donor for Patient 2. HLA-A and HLA-B types were determined from isolated T cells, and HLA-DR typing from isolated B cells.

†The appearance of HLA-A2 types in the B-cell lines is probably due to cross-reaction with HLA-A3.

to her sister, her CD3 count was 1010 cells per cubic millimeter; CD4 count, 400 cells per cubic millimeter; and CD8 count, 500 cells per cubic millimeter. Table 1 shows the results of HLA typing of her lymphocytes. Her T cells have the HLA type of her father, whereas her B cells are unchanged from the type present before transplantation. The presence of T-cell engraftment without B-cell engraftment was confirmed by chromosomal analysis of peripheral-blood cells, which revealed that all the cells had an XY (donor) karyotype, and by the study of a B-cell line (established by in vitro infection with the Epstein-Barr virus), which was found to have an HLA type identical to that observed before transplantation.

Patient 2

Patient 2, the third child of the parent of Patient 1, was born on June 4, 1995, after an uncomplicated pregnancy, weighing 3.07 kg. (An unaffected second daughter had been born two years earlier.) A chest film of Patient 2 at birth showed no thymic shadow. The white-cell count was 17,000 cells per cubic millimeter with 2 percent lymphocytes. The serum IgA concentration was below 7 mg per deciliter; IgM, 8 mg per deciliter; and IgG, 1560 mg per deciliter. The CD3 count was 110 cells per cubic millimeter; the CD4 count, 2 per cubic millimeter; the CD8 count, 7 per cubic millimeter; and the CD20 count (B cells), 1140 per cubic millimeter. The patient was transferred to UCLA on day 2, at which time her physical examination was normal except for the absence of tonsils and lymph nodes. Concentrations of adenosine deaminase and purine nucleoside phosphorylase in erythrocytes were normal. Because of maternal fever and birth under nonsterile conditions, the infant was treated with gentamicin and ampicillin. Prophylaxis with trimethoprim-sulfamethoxazole was started at one month of age.

Repeated immunologic testing showed no T cells but normal numbers of B cells. At an effector:target ratio of 25:1, the natural-killer-cell activity against K562 cells was 8 percent (mean [\pm SD] normal value, 37 ± 12 percent), and there were 728 CD16/56 natural killer cells per cubic millimeter (normal, 338 to 1170).¹ The results of HLA typing and mixed-lymphocyte culture are shown in Tables 1 and 2. Her HLA haplotypes were identical to

those of Patient 1 before that patient received the marrow transplant from her father. Patient 2 and her unaffected sister had different HLA haplotypes. Patient 1's current T cells (which originated from her father's transplanted marrow) did not react to Patient 2's cells in mixed-lymphocyte culture, although the father's peripheral-blood T cells were reactive to cells from Patient 2. This finding was confirmed by a second mixed-lymphocyte culture. We therefore decided that Patient 1's T cells were tolerant of Patient 2's HLA antigens and could be used in transplantation. At five weeks of age Patient 2 was given 480 million marrow cells from Patient 1 per kilogram without a conditioning regimen or T-cell depletion. After transplantation, she was given cyclosporine, ganciclovir, trimethoprim-sulfamethoxazole (as prophylaxis against *P. carinii* pneumonia), and intravenous immune globulin. Except for slight facial edema, her clinical course after transplantation was unremarkable.

By week 4 after transplantation, engraftment was evidenced by the detection of a noninherited paternal HLA antigen (Table 1). The white-cell count was 4400 per cubic millimeter; CD3 count, 129 cells per cubic millimeter; CD4 count, 89 cells per cubic millimeter; CD8 count, 45 cells per cubic millimeter; CD19 count, 660 cells per cubic millimeter; and CD16/56 count, 71 cells per cubic millimeter. Immunologic studies at seven months of age revealed the following: serum IgG concentration, 992 mg per deciliter; IgM, 42 mg per deciliter; IgA, 45 mg per deciliter; CD3 count, 193 cells per cubic millimeter; CD4 count, 178 cells per cubic millimeter; CD8 count, 277 cells per cubic millimeter; CD16/56 count, 138 cells per cubic millimeter; and CD19 count, 1344 cells per cubic millimeter; a nonreactive response to allogeneic cells in mixed-lymphocyte culture; a weak lymphoproliferative response to phytohemagglutinin; and an XY (donor) karyotype in 95 percent of cells on chromosomal analysis of peripheral blood. HLA typing 8 and 14 months after transplantation indicated that Patient 2's T cells, but not her B cells, were of paternal (donor) origin (Table 1). Treatment with immune globulin was discontinued when she was 15 months old, six weeks after the last infusion, since the serum IgG concentration was 765 mg per deciliter. At 17 months she was asymptomatic and growing normally.

DISCUSSION

The inheritance of identical HLA haplotypes by two sisters with autosomal recessive severe combined immunodeficiency and the successful transplantation of haploidentical bone marrow in Patient 1 provided an opportunity to perform transplantation in Patient 2 with marrow from Patient 1. As with most haploidentical transplantations in patients with severe combined immunodeficiency, B-cell engraftment did not occur.⁴⁻⁶ Indeed, serum immunoglobulin concentrations return to normal in fewer than half of patients who receive transplants of haploidentical marrow.⁷

The very rapid engraftment of HLA-disparate T cells without a conditioning regimen or T-cell depletion of the graft, the absence of graft-versus-host disease, and the subsequent uneventful clinical course in Patient 2 indicate that the lymphocytes in the marrow from Patient 1 were tolerant of HLA antigens from Patient 2. This state of tolerance must have arisen in the population of paternal T cells that reconstituted the immune system of Patient 1. Patient 1 is positive for HLA-DR7. It was possible to show that the paternal T cells she received in transplantation were unresponsive to cells from an unre-

TABLE 2. RESULTS OF MIXED-LYMPHOCYTE CULTURES BEFORE AND AFTER THE TRANSPLANTATION OF BONE MARROW FROM PATIENT 1 TO PATIENT 2.*

SOURCE OF RESPONDING CELLS	SOURCE OF IRRADIATED STIMULATING CELLS				
	PATIENT 1	PATIENT 2	FATHER	UNRELATED DONOR 1	UNRELATED DONOR 2
	stimulation index†				
Before transplantation					
Patient 1	1.0	0.6	0.9	55	37
Patient 2	2.2	1.0	4.7	6.4	11
Father	7.0	29	1.0	40	65
Unrelated donor 1	86	46	34	1.0	50
Unrelated donor 2	73	71	49	79	1.0
7 mo after transplantation					
Patient 1	1.0	1.5	0.8	27	22
Patient 2	0.4	1.0	0.6	0.5	0.6
Father	1.5	4.0	1.0	25	25
Unrelated donor 1	20	21	17	1.0	22
Unrelated donor 2	25	25	16	26	1.0

*The father had donated bone marrow to Patient 1 five years earlier.

†The stimulation index was calculated as the number of counts per minute in cells cultured with irradiated stimulating cells divided by the number of counts per minute in cells cultured with irradiated autologous stimulating cells.

lated person homozygous for HLA-DR7, whereas T cells from her father were stimulated by cells from this donor. Nonreactivity, or tolerance, of this kind has been demonstrated by several investigators.⁷⁻¹² Schiff and Buckley¹⁰ showed that in three of eight cases the transplanted cells reacted vigorously to the donor's original mononuclear cells, but such reactivity was not noted in our Patient 1 (Table 2).

The two paternal marrow transplants given to Patient 1 resulted in selective T-cell engraftment; nevertheless, there was complete restoration of B-cell function, including normal antibody responses. Similar results indicating successful interaction between B cells and T cells, despite differences in HLA antigens, have been noted in other haploidentical transplantations.⁴⁻⁶ The overall success rate of HLA-haploidentical marrow transplantation in severe combined immunodeficiency is about 60 percent.¹³

The slow engraftment in Patient 1 after the first transplantation may have been due to the lack of conditioning with immunosuppressive drugs.¹⁴ There was prompt engraftment of a second transplant from the father (again without a conditioning regimen before transplantation). This "booster" effect, noted in seven other patients with severe combined immunodeficiency,³ may be due to the administration of a large number of cells to patients whose first transplantations were only partially successful.

Direct chromosomal analysis of the marrow cells in Patient 1 at the age of 6½ years (performed at the time of her donation of marrow to Patient 2) revealed only hematopoietic cells with an XX (female) karyotype, suggesting that myeloid engraftment was

TABLE 3. FLOW-CYTOMETRIC STUDY OF PHENOTYPICALLY DEFINED SUBPOPULATIONS OF CELLS IN BONE MARROW FROM PATIENT 1 TO IDENTIFY XX/XY CHIMERISM.*

CELLS STUDIED	TOTAL CELLS	NO. WITH MALE KARYOTYPE	% WITH MALE KARYOTYPE
Myeloid, lymphocyte precursors, and activated T cells (CD34+, 33+, 38+)	614 (310+314)	31 (10+21)	5
Monocytes, polymorphonuclear leukocytes, and activated T cells (CD34-, 33+, 38+)	604 (301+303)	190 (72+118)	32
Mature lymphocytes, erythroid cells, and nonmyeloid cells (CD34-, 35-, 38+)	250 (30+220)	147 (12+135)	59
Entire cell population	522 (210+312)	114 (38+76)	22

*The numbers in parentheses are the results obtained in separate marrow samples.

limited or absent, as was also the case in other successful marrow transplantations in patients with severe combined immunodeficiency.¹⁵ However, paternal (XY) cells were identified in the marrow of Patient 1 by the analysis reported in Table 3, in which XX and XY cells were identified in myeloid and lymphoid subpopulations by fluorescence in situ hybridization.¹⁶ Marrow cells enriched in lymphoid precursors had an XY karyotype, confirming the presence of paternal T cells.

The immunologic reconstitution in Patient 2 has

been slow but she has remained clinically well, has grown and developed normally, and is not unduly susceptible to infection. Four months after transplantation, HLA typing revealed both paternal and autologous HLA-A and HLA-B antigens, but at 8 and 13 months only paternal T cells were present. Her T cells are still unresponsive to allogeneic cells in mixed-lymphocyte culture.

This kind of immunologic pas de trois has also been executed successfully by Friedrich et al. in two children with leukocyte-adhesion defect type 1.¹⁷ The first child, who had received a haploidentical transplant from the mother, served as a marrow donor for his sister. The sister, unlike our patient, received conditioning with busulfan and cyclophosphamide and had a different maternal haplotype from the donor. Prompt engraftment ensued, without graft-versus-host disease. In 1975 Geha¹⁸ performed two marrow transplantations in a child with severe combined immunodeficiency, using as a donor the patient's HLA-identical sibling, who had the same disease and had previously undergone a successful transplantation of marrow from her HLA-identical father. However, T-cell engraftment was only transient and the patient died.

Rosenkrantz et al.¹⁹ have suggested that both specific, ongoing immune suppression and the elimination of T-cell clones contribute to the tolerance that follows haploidentical transplantation. Tolerance induced by transplanted bone marrow may decrease the likelihood of rejection of solid organs from the same donor.^{20,21} The use of marrow from an HLA-identical sibling who has successfully undergone transplantation with normal bone marrow will of necessity be limited to very unusual situations, but it is ideal for the treatment of patients with congenital immunodeficiencies who have immunologically tolerant lymphocytes.

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