Transplantation for Severe Combined Immunodeficiency With HLA-A,B,D,DR Incompatible Parental Marrow Cells Fractionated by Soybean Agglutinin and Sheep Red Blood Cells

By Yair Reisner, Neena Kapoor, Dahlia Kirkpatrick, Marilyn S. Pollack, Susanna Cunningham-Rundles, Bo Dupont, Marquis Z. Hodes, Robert A. Good, and Richard J. O'Reilly

Three patients with severe combined immunodeficiency (SCID) received transplants of HLA haplotype-mismatched parental bone marrow depleted of T lymphocytes by differential agglutination with soybean agglutinin (SBA) and subsequent E-rosette depletion. Two patients achieved durable engraftment with reconstitution of both humoral and cell-mediated immunity. Neither of these patients developed graft versus host disease (GVHD). The third patient achieved only a transient engraftment with concomitant development of mitogen-responsive lymphocytes of paternal origin. Our experience indicates that depletion of T lymphocytes by this technique can abrogate the potential of histoincompatible marrow grafts to induce lethal GVHD without limiting immunologic reconstitution. It also provides further evidence of nonimmune mechanisms of graft resistance that may necessitate preparative treatment of patients with SCID before transplantation with HLA-mismatched marrow cells.

Recently we described a technique for removal of T lymphocytes from large volumes of human bone marrow aspirates. This technique involves selective removal of cells agglutinable by soybean agglutinin (SBA) and subsequent removal of residual T lymphocytes from the unagglutinated marrow fraction by differential sedimentation of cells forming rosettes with sheep red blood cells (SRBC). Using this technique, we demonstrated that hematopoietic cells from parental marrow were capable of reconstituting full and durable hematopoietic function without graft versus host disease (GVHD) in a lethally irradiated, cyclophosphamide-treated, HLA-A,B,C haplotype nonidentical child with leukemia. The full potential of this technique for prevention of GVHD, however, could not be determined from this case because lymphocytes of the paternal donor, who was HLA-DR2 heterozygous, did not respond in vitro in mixed lymphocyte culture (MLC) to the HLA-DR2 homozygous lymphocytes of the patient.

We report here the outcome of transplants of marrow from HLA-haploidentical, MLC-incompatible, parental donors into three children with severe combined immunodeficiency (SCID). For two of the three patients, these transplants have resulted in sustained engraftment and immunologic reconstitution without GVHD. The third patient experienced early transient engraftment.

MATERIALS AND METHODS

Fractionation of Bone Marrow Cells with SBA and SRBC

Fractionation of bone marrow cells with SBA and SRBC is carried out as described. Briefly, there are four main steps to this procedure: (1) selective removal of red blood cells by gravity sedimentation in Hetastarch solution; (2) agglutination with SBA and differential sedimentation of the agglutinated (SBA⁺) cells; (3) removal of E-rosette-forming T cells from the SBA cell fraction by centrifugation over Ficoll-Hypaque; and (4) repetition of the E-rosetting step using neuraminidase-treated SRBC to eliminate residual rosetting cells remaining in the SBA E fraction. The nonrosetting cells collected from the second rosetting step (SBA E) cells were washed four times in sterile physiologic saline containing 1% human serum albumin for intravenous injection. Whereas the final recovery of SBA E cells averaged approximately 5% of the nucleated cells in the original marrow collected from the donors, more than 80% of the colony-forming cells (CFU-C) were retrieved. The SBA E marrow cell fractions did not contain T lymphocytes at concentrations detectable by E-rosette formation with SRBC, in vitro transformation responses to phytohemagglutinin (PHA) or allogeneic cells, and cytofluorimetric analyses for cells reactive with a panel of T-cell-specific monoclonal antibodies.

In vitro transformation responses to mitogens, antigens, and allogeneic cells were assayed by standard microtiter plate assays using 5 x 10⁵ Ficoll-Hypaque separated mononuclear cells per culture and quantitated by ³H-thymidine incorporation. Analyses of lymphocyte subpopulations for receptors for sheep red blood cells, for surface immunoglobulins, and for reactivity with the T-cell-specific monoclonal antibodies anti-leu-4 (pan-T), anti-leu-2a (suppressor/killer), and anti-leu-3a (helper) were performed according to methods previously described. Immunoglobulins G, A, and M were quantitated by radial immunodiffusion. Isoagglutinins and antibody responses to killed polio-virus vaccine were quantitated by standard serologic methods and Antitoxins developed following.

From Memorial Sloan-Kettering Cancer Center, New York, N.Y.

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tetanus and diphtheria toxoid immunization were quantitated by hemagglutinating antibodies as previously described.10 Adenosine deaminase (ADA) and nucleoside phosphorylase levels were quantitated using techniques previously described.11,12

Histocompatibility testing for HLA-A, B, C, and DR specificities were performed on all family members using standard serologic techniques,13 and inheritance of HLA genotypes thereby established. Chimerism was evaluated by determining the HLA phenotype and chromosome banding characteristics of circulating lymphocytes separated according to their capacity to form SRBC rosettes (E+ E-) as previously described.14 Karyotypic analyses were performed using standard techniques.

The patients were tested for GVHD by clinical evaluation and skin biopsies according to the clinical criteria of Gaudine, et al.,15 and the histologic criteria of Slavin and Woodruff.16 Transfer factor used in case 1 was prepared according to the method of Ascher et al.17 and administered subcutaneously in unit doses equivalent to the diastole of 2 x 10^6 lymphocytes.

CASE REPORT

Case 1

The patient [assigned unique patient number (UPN) 75 in our transplant series], a white male, was born at term on 7/16/78 and developed normally until 4 mo of age, when, after a course of antibiotics for a respiratory tract infection, he developed refractory oral moniliasis. At age 6 mo, he was presented to the Pediatric Clinic "La Paz" in Madrid (Spain) with a history of persistent thrush, impetigo, recurrent fever, diarrhea, and failure to thrive. The diagnosis of SCID was made on the basis of absence of tonsils and lymph nodes, numerical deficiency of T and B lymphocytes, failure of his lymphocytes to transform in vitro to mitogens, antigens, or allogeneic cells, anergy to Candida and streptokinase/streptodornase (SKSD) skin tests, and profound hypogammaglobulinemia (Table 1).

In May 1979, the patient was referred to Memorial Sloan-Kettering Cancer Center. Reevaluation of lymphoid function confirmed the diagnosis of SCID. ADA and nucleoside phosphorylase levels were normal. In vitro coculture of marrow mononuclear cells on a normal thymic epithelial monolayer failed to induce development of lymphoid populations carrying SRBC receptors or responsiveness to mitogens.18 No histocompatible bone marrow was available. Accordingly, a series of transplants of fetal tissue were administered in an attempt to correct his immunodeficiency.

On 7/24/79, an irradiated (800 rad) thymus from a 16-wk fetus was implanted intramuscularly to restore thymic secretory functions. Immunologic deficiencies persisted. A transplant of liver cells from an 8-wk fetus administered on 9/19/79 failed to engraft. A subsequent intramuscular implantation of thymus and intravenous infusion of 2 x 10^6 liver cells from a fetus of 12 wk gestation on 11/13/79 achieved a durable engraftment detected exclusively in the SRBC rosette-positive lymphocyte fraction, which now comprised up to 30% of peripheral blood lymphocytes. The engrafted fetal lymphocytes, identified by their HLA phenotype (HLA-A2,w30; B17,w35) were SRBC receptor positive, but were negative for the leu-1,4,2a,3a antigens, and failed to respond in vitro to mitogens, antigens, or allogeneic cells. Thus, despite persistent engraftment of fetal-derived "T" lymphocytes from 3/13/80 to 4/13/81, immunologic deficiency persisted. The patient suffered episodes of sepsis due to Candida albicans, pseudomonas, enterobacteriaceae, and Staphylococcus epidermidis, and experienced chronic diarrhea and malnutrition necessitating prolonged administration of parenteral antibiotics and initiation of total parenteral nutrition. In December 1980, he was transferred to a laminar flow isolator and treated with nonabsorbable antimicrobials for skin and mucosal decontamination.

The HLA genotypes of the patient and his father are presented in Table 2. The patient and his paternal donor were incompatible for one HLA haplotype. The paternal lymphocytes tested prior to the fetal liver transplant responded in vitro to the patient's lymphocytes in the MLC (relative response, 124%). Immediately prior to the first administration of SBAEEN marrow cells, the patient's lymphocytes were shown not to respond in vitro to the patient's lymphocytes in the MLC (relative response, 124%). Immediately prior to the first administration of SBAEEN marrow cells, the patient's lymphocytes were shown not to respond in vitro to the patient's lymphocytes in the MLC (relative response, 124%).

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Table 1. Immunologic Development Before and After Transplantation

<table>
<thead>
<tr>
<th>Case 1 Upn 75</th>
<th>Case 2 Upn 30</th>
<th>Case 3 Upn 226</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>At Admission</strong></td>
<td><strong>Pre-BMT</strong></td>
<td><strong>Post-BMT</strong></td>
</tr>
<tr>
<td><strong>Pallable lymph nodes</strong></td>
<td>Absent</td>
<td>Absent</td>
</tr>
<tr>
<td><strong>Tonsils</strong></td>
<td>Absent</td>
<td>Absent</td>
</tr>
<tr>
<td><strong>Lymphocyte counts</strong></td>
<td>2,000</td>
<td>544</td>
</tr>
<tr>
<td><strong>% E+T lymphocytes</strong></td>
<td>20</td>
<td>19</td>
</tr>
<tr>
<td><strong>% S IgG lymphocytes</strong></td>
<td>17</td>
<td>36</td>
</tr>
<tr>
<td><strong>Response to</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Medium</strong></td>
<td>43</td>
<td>98</td>
</tr>
<tr>
<td><strong>PltA</strong></td>
<td>93</td>
<td>162 (&lt;1%)</td>
</tr>
<tr>
<td><strong>Con-A</strong></td>
<td>68</td>
<td>60 (&lt;1%)</td>
</tr>
<tr>
<td><strong>Candida albicans</strong></td>
<td>75</td>
<td>163 (&lt;1%)</td>
</tr>
<tr>
<td><strong>E. coli</strong></td>
<td>75</td>
<td>175 (&lt;1%)</td>
</tr>
<tr>
<td><strong>PDD</strong></td>
<td>68</td>
<td>170 (&lt;1%)</td>
</tr>
<tr>
<td><strong>LMIF production to mitogen (Con-A)</strong></td>
<td>Absent</td>
<td>Absent</td>
</tr>
<tr>
<td><strong>Delayed-type hypersensitivity (DNCB)</strong></td>
<td>NT</td>
<td>Absent</td>
</tr>
<tr>
<td><strong>Granulocyte formation (M. anth):</strong></td>
<td>NT</td>
<td>Absent</td>
</tr>
<tr>
<td><strong>Immunoglobulin (mg/dl):</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>lgG</strong></td>
<td>121*</td>
<td>252*</td>
</tr>
<tr>
<td><strong>lgM</strong></td>
<td>8</td>
<td>22*</td>
</tr>
<tr>
<td><strong>lgA</strong></td>
<td>4.4</td>
<td>7</td>
</tr>
<tr>
<td><strong>IgG levels</strong></td>
<td>NE</td>
<td>NE</td>
</tr>
<tr>
<td><strong>Antibody response to tetanus toxoid:</strong></td>
<td>0.35</td>
<td>None</td>
</tr>
<tr>
<td><strong>Antibody response to diphtheria toxoid:</strong></td>
<td>0.09</td>
<td>None</td>
</tr>
</tbody>
</table>

*Post gamma globulin injection.

NT, not tested; NE, not evaluable due to plasma therapy; ND, not detected.
Table 2. Genetic Analysis of Donor/Recipient Pairs

<table>
<thead>
<tr>
<th>Patient</th>
<th>HLA Genotypes</th>
<th>MLC Donor Response</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Parental Donor</td>
<td>Recipient x Relative Response</td>
</tr>
<tr>
<td>UPN 75</td>
<td>A2,B37, (w4), DRw10</td>
<td>A1,B37,(w4), DRw10</td>
</tr>
<tr>
<td>UPN 30</td>
<td>A2,B84,4,Cw5,DRw4</td>
<td>A2,B84,4,Cw5,DRw4</td>
</tr>
<tr>
<td>UPN 226</td>
<td>A29,B84,4(DR7(MT3))</td>
<td>A29,B84,4(DR7(MT3))</td>
</tr>
</tbody>
</table>

The clinical course is presented in Fig. 1. On 12/18/80, the patient received an intravenous infusion of SBA E E Es marrow cells from his HLA-A, B, DR haploidentical father at a dose of 40 x 10^6 nucleated cells/kg. Methotrexate was administered at doses of 10, 15, and 10 mg/sq m on days, 1, 3, and 7 for GVH prophylaxis. In the following 14 wk, no evidence of engraftment was observed.

On November 25, 1980, prior to the initial transplant of lecutin-separated marrow, the patient experienced the first of a series of nocturnal febrile episodes that were originally attributed to parenteral antimicrobial therapy. During January 1981, following the transplant, he developed marked hepatosplenomegaly and mild leukopenia and thrombocytopenia. On February 12, 1981, sputum smears revealed acid-fast bacilli. Subsequent cultures of sputum, gastric aspirates, stool, blood, and bone marrow were positive for a Mycobacterium avium strain moderately sensitive only to Rifamycin, Cycloserine, Ethionamide, and Clofazamine. Marrow biopsy revealed large numbers of both intracellular and extracellular acid-fast bacilli without granulomas. The patient was treated with Rifamycin, Clofazamine, Ethionamide, and Amikacin. Over the subsequent 3 mo, blood, stool, sputum, and marrow remained strongly culture positive; fever and hepatosplenomegaly remained unchanged.

In March 1981, the patient received a course of transfer factor derived from leukocytes of his PPD reactive father, but failed to reverse skin test anergy or to form granulomas in the marrow.

On 3/27/81, the patient received a second lectin-separated, SRBC-rosette-depleted marrow graft from his father at a dose of 41 x 10^6 nucleated cells/kg. No methotrexate prophylaxis was administered. By 4/14/81, HLA typing of isolated T lymphocytes revealed, for the first time, a population of cells bearing both HLA-B37 and B17. By 5/1/81, all T-cells expressed the father's HLA phenotype, A1,2; B37,17. The distinctive fetal determinants, HLA-Aw30, Bw35 could no longer be detected. Marrow biopsies from 4/17/81 demonstrated granulomatous responses to M. avium. Thereafter, lymphocyte transformation responses to mitogens, allogeneic cells, and microbial antigens increased. On 5/21/81, the patient became afebrile, splenomegaly gradually resolved, leukocyte and platelet counts normalized. Smears and cultures of sputum and marrow for M. avium reverted to negative on 7/13/81 and 10/13/81, respectively. The patient was discharged on antituberculous therapy on 6/8/81. He remains well at home without intercurrent infection 13 mo after the second transplant. At no time in the posttransplant period did the patient develop clinical evidence of GVHD. Blind skin biopsies also failed to show evidence of GVH pathology.

Case 2

The patient, UPN 30, is a male born on 6/24/77 originally diagnosed to have SCID at the age of 7 mo, who was hospitalized in sterile laminar flow isolation from age 10 mo until discharge, following transplantation, at 53 mo. This unique patient has been described previously.14 The immunologic criteria establishing the diagnosis of SCID are identical to those described for patient UPN 75 and are detailed in Table 1. However, prior to transplantation,
this patient had been found to be persistently and selectively engrafted with T lymphocytes possessing the HLA phenotype and karyotype (documented in rare spontaneous mitoses) of his histoincompatible mother, yet had no evidence of GVHD. The engrafted E-rosette ‘+’ maternal ‘+’T’ cells were not responsive to stimulation with mitogens, antigens, or allogeneic cells, nor could they be induced to respond to appropriately incubated with thymopectin, thymic epithelium, or interleukin-2. These cells also failed to alter the strong allogeneicity of the mother’s own lymphocytes against patient-derived or paternal non-T-cells in MLC.

Between 8/18/78 and 1/31/80, the patient received a series of three fresh transplants of liver cells from fetuses of 11 wk gestation, the third in combination with thymus. Despite immunosuppression with antithymocyte globulin, which was used prior to the first graft, no engraftment or reconstitution was observed. An irradiated fetal thymus graft (15.5 wk gestation) administered 8/30/78 restored serum factor thymic serum (FTS) and thymopoietin levels, but failed to alter immune function.

On 1/28/81, the patient (HLA-Aw32, B8, DRw3/A2, Bw44, Dw5, DRw4) received a transplant of SBA E Eα marrow cells at a dose of 3.16 x 10^7 cells/kg from his HLA haplo-type-mismatched, MLC-reactive father (HLA-Aw32, B8, DRw3/Aw32, B15, Dw3, DRw2) 10 days after receiving a course of antithymocyte globulin (30 mg/kg x 3) and cyclophosphamide (50 mg/kg x 4) designed to eliminate maternal cells. Maternal cells were only partially and transiently suppressed; engraftment was not achieved.

On 5/7/81, the patient received a transplant of SBA E Eα marrow cells at a dose of 7.8 x 10^6 cells/kg from his HLA haplo-type-mismatched, MLC-reactive mother (HLA-A2, Dw44/A2, Dw44, Dw5, DRw4). No pretransplant immunosuppression was administered. Two units of irradiated (2000 rads) maternal leukocytes were concurrently infused to potentiate engraftment.

Two weeks posttransplant, the patient developed a generalized maculopapular rash. A skin biopsy was performed, and the patient was immediately treated with prednisone, 2 mg/kg/day, for presumptive GVHD. Biopsy of the rash failed to document GVHD; the rash resolved within 2 days of initiating therapy. Prednisone was tapered and discontinued after 7 days of treatment. No hepatic or enteric dysfunction was observed.

Engraftment of cells possessing the maternal HLA phenotypes was first detected in the non-T-cell fraction 8 wk posttransplantation. Lymphocyte transformation responses to PHA, first detected 12 wk postgrafting, increased to that of normal controls by 10/22/81. Responding cells are exclusively female in karyotype. The patient’s clinical course has since been uncomplicated. He was discharged on 10/27/81 and continues to be well at home, 12 mo after the second transplant, with no recurrent infections limited to one transient and mild upper respiratory tract infection. There has been no clinical evidence of GVHD.

Case 3

Patient UPN 226, a white male, presented to the University of Rochester Medical Center at the age of 4 mo, with oral thrush resistant to topical therapy, pharyngeal ulcerations and purulent skin rash, and a history dating from age 1 mo of chronic rhinorrhea and cough, and recurrent otitis media. The diagnosis of SCID was made on the basis of T lymphopenia (575/mL), absence of in vitro lymphocyte transformation responses to PHA, concanavalin-A (Con-A), and pokeweed mitogen, and severe hypogammaglobulinemia. Adenosine deaminase and nucleoside phosphorylase levels were normal. In addition, the patient suffered from persistent severe neutropenia (<1000/μL) with hematologic findings suggesting either an arrest of myeloid maturation or enhanced destruction of bands and neutrophils not associated with the presence of a neutrophil antibody. Histocompatibility testings (Table 2) failed to identify a suitably matched donor for marrow transplantation.

At age 4.5 mo, he received a thymic epithelial implant at Toronto Sick Children’s Hospital. Although the patient gained weight briefly, thereafter, immunologic deficiencies were not altered. Subsequently, the patient developed chronic diarrhea, worsening oral moniliasis, poor oral intake, and progressive weight loss. A second thymic implant was administered at age 7 mo. Again, no improvement in lymphocyte function was observed. Thereafter, the patient was treated at the University of Rochester with hyperalimentation, parenteral gammaglobulin injections, and intensive treatment with Nystatin, Clotrimazole and 5-fluorocytosine, which achieved control of moniliasis, weight gain, and clinical improvement. Diarrhea, reflecting enteritis due probably to both Candida and rotavirus infections, abated.

On 11/4/81, at age 10 mo, the patient was referred to Memorial Sloan-Kettering Cancer Center for a lectin-separated marrow graft. Pretransplant evaluation again reflected severe combined immunodeficiency (Table 1). Neutropenia persisted (500-1200/cu mm). Marrow aspirates and biopsy again revealed a deficiency of granulocytic elements beyond the myelocyte stage of development.

On 12/24/81, the patient received an intravenous infusion of SBA E Eα marrow cells from his father at a dose of 4.3 x 10^6 cells/kg body weight. The SBA’ marrow fraction and 2 U of paternal peripheral blood leukocytes, were irradiated (2000 rads) and administered concurrently. Engraftment of paternal lymphocytes bearing HLA-B7 was first detected on 1/12/82, were prominent in both E+ and E- fractions by 2/9/82, and persisted through 3/2/82. During this period, neutrophil counts rose to 1000-2200/sq mm. However, lymphocyte counts and the proportion of E-rosette-forming cells did not change significantly. Increments in in vitro transformation response to PHA were also detected by 2/3/82 and reached the maximal levels recorded on 2/24/82 (Table 1). Thereafter, PHA responses rapidly decreased, returning to pretransplant levels by 3/17/82, at which time the paternal HLA-B7 could no longer be detected on lymphocytes in the peripheral blood. Throughout this course, the patient was clinically stable, free of oral moniliasis and enteritis, and gaining weight with oral and total parenteral nutrition. A second transplant to be administered after immunosuppressive treatment is planned.

RESULTS

Characteristics of Engraftment

The genetic markers distinctive for donor and host in each of the three cases are summarized in Table 2. Following engraftment in cases 1 and 2, lymphocytes forming spontaneous SRBC rosettes have exclusively expressed the parental donor’s HLA phenotype. Distinctive HLA antigens of both the host and the engrafted cells of the marrow donor are detected in the nonrosetting, SIg’ lymphocyte populations, indicating their mixed genetic origins. In cases 1 and 3, neither fetal nor maternal cells are detected. Maternal cells are present posttransplant in non-T-cell fractions in case 2. Maternal cells were not detected in this fraction pretransplant. In each case, lymphocytes replicating in response to PHA exhibit the chromosomal banding pattern of the parental donor.
Immunologic Reconstitution

The immunologic status of each patient before administration of SBA E E marrow, and at defined intervals after successful engraftment, are summarized in Table 1. Results are presented both in absolute values or as a percent of values detected in healthy adult volunteers. As can be seen, following transplantation, the number of SRBC-rosette-positive T lymphocytes and Slg lymphocytes in cases 1 and 2, are near normal. The pan-T reagents, anti-leu-4 and anti-leu-1, also stain a normal proportion of the lymphocytes. In vitro transformation responses to the mitogens PHA and Con-A, to allogeneic cells, and to antigenic preparations from C. albicans, E. coli, and S. aureus are 90%-100% of those mounted by normal control lymphocytes. Both of these patients have developed delayed-type hypersensitivity reactions following secondary challenges with dinitrochlorobenzene (DNCB). Patient UPN 75 also developed in vivo granulomatous responses to M. avium after engraftment and initial development of transformation responses to mitogens. However, in vitro transformation and in vivo delayed-type hypersensitivity responses to PPD have remained nonreactive.

The peak alterations in cell-mediated immune function detected in case 3 during the period of engraftment are also presented in Table 1. As can be seen, transient but significant increments in both the number and proportion of E-rosettes and the level of lymphocyte responses to in vitro stimulation with mitogens were realized during this period.

The reconstitution of humoral immunity achieved in cases 1 and 2 is presented in Table 1. Both patients maintain normal levels of IgG, IgM, and IgA without supplementation. Antibody responses to immunization have also been recorded in each case.

Patient UPN 75 (case 1) experienced no cutaneous, hepatic, enteric, or hematopoietic evidence of GVHD. A blind skin biopsy revealed no pathology. Patient UPN 30 (case 2) did develop a transient maculopapular rash 2 wk posttransplant, but biopsies failed to reveal any perivascular or subepidermal mononuclear cell infiltration to suggest GVHD. Neither hepatic nor enteric dysfunction was observed. In these cases, the host-specific tolerance of engrafted parental lymphoid elements is also demonstrable in MLC, as illustrated for patient UPN 75 in Table 3. As can be seen, the engrafted paternal T lymphocytes fail to respond to host-type non-T-cells, yet respond to both maternal and third-party lymphocyte populations. In contrast, the father’s own lymphocytes respond strongly to each of these stimulants.

DISCUSSION

This article documents the application of HLA-A, B, D, DR haploidentical parental marrow, fractionated by differential agglutination with SBA and secondary separation of SRBC rosettes for transplantation of three children with severe combined immunodeficiency. Two of these patients have enjoyed durable engraftment with full reconstitution of immune function. In the third patient, only transient engraftment was achieved.

We previously demonstrated the potential of SBA E E marrow fractions for restoring full hematopoietic function in a leukemic patient who had been pretreated with lethal total body irradiation and cyclophosphamide. That patient did not develop GVHD. However, this event may have reflected the partial (HLA-D) compatibility existing between the patient and her paternal donor, rather than depletion of alloreactive T cells from the graft. The absence of GVHD in the present cases, in which donor and recipient were incompatible for a full HLA haplotype, provides more conclusive evidence that depletion of T cells from human marrow by our technique will prevent or abrogate this process.

In murine models, studies in which lymph node T cells were mixed in increasing proportions with antibody-treated, T-cell-depleted bone marrow preparations and thereafter transplanted into lethally irradiated allogeneic recipients, indicated that T-cell contamination in the range of 0.3%-1% was sufficient to produce lethal GVHD. Requirements for T-cell

<table>
<thead>
<tr>
<th>Table 3. MLC Posttransplantation of Patient UPN 75 (In Vitro Incorporation of ³²C-Thymidine [net cpm])</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stimulators</td>
</tr>
<tr>
<td>Responders</td>
</tr>
<tr>
<td></td>
</tr>
</tbody>
</table>

*Non-T lymphocytes express HLA phenotype of the patient.
depletion may be more or less stringent, depending upon the genetic disparities of the strain combinations used. If such quantitative information can be extrapolated to man, the fractionation procedure described would be expected to significantly abrogate GVHD. The T-cell depletion achieved by our technique is such that no T lymphocytes detectable by SRBC rosette formation could be found in counts of 1000 cells in the SBA E E_n cell fraction. T cells reactive with the monoclonal antibodies anti-leu-4 (pan-T), anti-leu-2a (suppressor/killer T), and anti-leu-3a (helper T) were also not detected in this fraction.

Obviously, more extensive application of this approach in human marrow transplantation will be required to determine whether the fractionation procedure as currently formulated will provide a degree of T-cell depletion adequate to avoid development of GVHD in every case. Additional depletion steps may ultimately be found to be necessary for effective T-cell depletion of marrow from certain donors, e.g., allo-sensitized or maternal donors or donors exhibiting certain HLA haplotypes. The possibility that the previously engrafted fetal and maternal lymphocytes detected prior to transplantation in cases 1 and 2, respectively, also contributed to prevent GVHD must also be considered. In each case, these lymphocytes were phenomenally immature and could not be induced to proliferate or to generate lymphokines or cytolytic effectors in response to allogeneic cells, mitogens, or antigens. As previously reported, case 2 was also extensively examined for the presence of nonspecific or allospecific suppressor cells capable of modulating the reactivity of the mother's own T lymphocytes in MLC and B lymphocytes in the production of antibody in vitro. Suppressors of these types could not be detected; however, it is still possible that these cells exerted alternative GVH-inhibiting functions operative in vivo. Further transplants in patients who have received no prior therapeutic manipulations should clarify this issue.

The immunocompetence of the engrafted donor T cells must be ascertained for meaningful interpretations of the significance of the GVH abrogation observed. In two of our patients, engraftment and development of parental lymphoid cells was associated with normalization of T-cell numbers, full reconstitution of in vitro transformation responses to mitogens and antigens, development of the capacity to generate lymphokines in vitro, and in vivo delayed-type hypersensitivity responses to DNCB. Patient UPN 75 (case 1) also generated granulomas in response to M. avium during the course of his reconstitution. Control and recovery from a disseminated M. avium infection is rare due to the insensitivity of this agent to antituberculous chemotherapy. In this case, culture reversion rapidly followed development of an effective immune response.

The engrafted paternal lymphocytes were also capable of allospecific response, as indicated by their strong reactivity to third-party cells in MLC. Despite this capacity to mount alloreactions, reactivity of engrafted parental T cells against host cells in vitro was not observed. Whether this host-specific tolerance represents the result of active suppression of host-directed responses, or deletion of host-reactive clones, remains to be determined.

Selective development of donor T lymphocytes and their precursors, with persistence of host-type B lymphocytes is a common finding in patients transplanted for SCID irrespective of the type of transplant administered. In our series, over half of the patients transplanted with unfractionated marrow failed to develop donor-type B cells. Thus, the apparent split lymphoid chimerism detected in the two reconstituted patients cannot be attributed to the type of transplant used.

The function of host lymphocytes in patients with SCID following engraftment of T lymphocytes from an HLA-identical or HLA-D-compatible donor has been restored to normal in most reported cases. This suggests that host B cells, when detected, are intrinsically normal and capable of antigen-specific responses if provided with appropriate help by T cells, and possibly other accessory cells from the normal donor. In contrast, SCID patients who have developed normal cell-mediated immunity following selective engraftment of T-cell precursors from an HLA-disparate, fully allogeneic fetal liver have, in all but one case, failed to recover humoral immunity. We and others have suggested that this residual deficiency of humoral immunity might be due to a failure of cooperation between genetically disparate fetal donor T cells and host B cells. Based on existing data from murine models, transplants of T-cell depleted, HLA-haploidentical marrow would be expected to circumvent functional restrictions imposed by genetic disparity between donor and host. The development of normal levels of IgG, IgM, and IgA and detection of isoagglutinins and antibodies to tetanus and diphtheria toxoid immunizations in our patients, suggests this to be the case. However, selective elution of the antibody formed and evaluation of its genetic origin by allotyping will be required to ascertain whether the antibody detected is indeed the product of an effective cooperation between donor T and host B cells or between donor T cell and a small population of donor B-lymphocyte precursors in the blood or lymphoid organs of the host.
It is likely that in the first patient described, the administration of methotrexate prophylaxis compromised engraftment of the first marrow transplant, since a secondary transplant from the same donor engrafted unevenly, without the pretreatment of the host. Whether the fractionation procedure in some way alters the sensitivity of marrow cells to antimetabolites, such as methotrexate, remains to be determined. In case 3, neither methotrexate nor other potentially myelosuppressive agents were administered. Insofar as degree of genetic disparity, marrow fractionation procedures, and transplant cell doses were comparable, it seems more likely that the graft failure observed was due to host-mediated resistance mechanisms not reflected by our immunologic analysis. Transplants of partially matched marrow or fully allogeneic fetal liver in patients with SCID have often failed to engraft, despite the immunoincompetence of the host.26 Pre-treatment of the host with cyclophosphamide has abrogated this resistance.28 In mice, resistance to allogeneic marrow grafts not mediated by conventional immunologic mechanisms is well described30 and can be abrogated by prior administration of cyclophosphamide, antilymphocyte globulin, silkia, or BCG.31,32 It is thus also conceivable that in case 1, the development of a disseminated Mycobacterium avium infection, late after the first graft, may have suppressed nonimmune forms of graft resistance and thereby potentiated engraftment of the second transplant. Further experience will determine whether resistance mechanisms of this type constitute a common obstacle to transplants of T-cell-depleted marrow. Definition of the cellular basis of such resistance may permit identification and selection of patients with SCID who will require preparative treatment to ensure engraftment.

In summary, this report clearly demonstrates the potential of HLA-haploidentical marrow depleted of T cells to engraft and reconstitute immunologic function in SCID patients without GVHD. Further experience will be necessary to define the general applicability of this approach to patients with SCID or other lethal congenital or acquired blood disorders.

NOTE ADDED IN PROOF

In the interval between initial acceptance of this report and its publication, patient UPN226 (case 3) received a transplant of HLA haplotype-mismatched, maternal SBA E E marrow after immunosuppression and has been engrafted and reconstituted. Three further patients with SCID have been engrafted with restoration of immune function in two of the three cases. None of these patients has experienced GVHD. These later cases will be reported separately.

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