Reconstitution after transplantation with T-lymphocyte-depleted HLA haplotype-mismatched bone marrow for severe combined immunodeficiency
(monoclonal antibody/chimerism/tolerance/alloreactivity/differentiation)

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Communicated by Eugene Braunwald, June 30, 1982

ABSTRACT—Severe combined immunodeficiency (SCID) is potentially correctable by bone marrow transplantation if a patient has a suitable histocompatible donor. In the absence of an HLA-matched donor, lethal graft-versus-host disease (GVHD), which is mediated by alloreactive donor T cells, may occur. In an attempt to prevent GVHD in one SCID patient lacking a matched donor, we treated maternal haplomismatched bone marrow with a unique nonmitogenic T-cell-specific monoclonal antibody (anti-T12) and complement to remove mature T cells. Despite the removal of >99% mature T cells, the child developed significant life-threatening GVHD, which was terminated by a 5-day course of intravenous anti-T12. Subsequently, immune reconstitution occurred by 6 wk; the mature circulating T cells proliferated in response to soluble and allo-antigens in vitro and provided help for B-cell immunoglobulin synthesis. The patient was removed from a protective environment and discharged without evidence of further infection. Both HLA and chromosomal analyses showed that the circulating cells in the patient were of maternal origin. More importantly, the maternal T cells were no longer reactive with recipient cells. Mixing experiments indicated that the state of tolerance that resulted in this chimera was not due to active suppression. We conclude that HLA-mismatched transplantation for SCID can be undertaken if mature alloreactive donor T lymphocytes are depleted before and after bone marrow grafting.

Patients with severe combined immunodeficiency (SCID) have major defects in immune function because they generally lack both T and B lymphocytes (1–3). Clinical manifestations of this defective immunity include early onset of life-threatening pulmonary infection, moniliasis, chronic diarrhea, and wasting that progressively worsens despite conservative therapies. Although the precise etiology of SCID has not been determined, previous studies indicated that the circulating lymphoid cells of SCID patients are of two major phenotypes as defined by monoclonal antibody analysis: either T10*T3*T4*T8 or T10*T3*T4*T8+ (4). The former population is derived from a precursory bone marrow or early thymocyte compartment whereas the latter represents a late stage of thymocyte differentiation (5). Only those SCID patients with circulating cells of the more mature T10*T3*T4*T8+ phenotype demonstrated immunologic function.

Corrective therapy for SCID currently requires bone marrow transplantation (6). Unfortunately, the invariably lethal graft-versus-host disease (GVHD) accompanying transplantation with histoincompatible cells has necessitated donor recipient HLA identity as a prerequisite for transplantation. This has, in effect, denied potentially corrective therapy to the majority of patients (~60%). Moreover, even among the 40% of individuals who are transplanted with donor bone marrow cells matched at HLA-A, -B, and -DR loci, there is often some degree of GVHD or post-transplant infection or both.

Recent studies in animal model systems, however, suggest a strategy to circumvent these difficulties (7, 8). In the mouse, for example, removal of immunocompetent T lymphocytes from donor marrow before transplantation allows successful haplotype-mismatched engraftment without GVHD. To explore the utility of this approach in the human, we used a monoclonal antibody to eliminate virtually all mature T cells from a haplotype-mismatched donor bone marrow inoculum before transplantation of a patient with SCID. The transient GVHD that followed was terminated by intravenous administration of the same antibody with reconstitution of mature T- and B-cell function by 6 wk after transplantation. The recipient is currently a stable chimera with circulating donor T lymphocytes that have become tolerant to recipient HLA antigens.

MATERIALS AND METHODS

Production of Monoclonal Antibodies and Cell Sorter Analysis. A series of monoclonal antibodies was used to define cell surface antigens on human lymphocytes of T- and B-cell lineage isolated by density centrifugation (9). The production and characterization of monoclonal antibodies anti-T3, anti-T4, anti-T8, anti-T6, anti-T10, anti-T11, anti-Ia, and anti-B1 have been described (10–14), and their specificities are further defined in Table 1.

In addition, we used a T-cell-specific monoclonal antibody termed anti-T12, developed through a previously reported hybridization and screening strategy (5). Although similar to anti-T3 in cellular expression (present on all peripheral T cells and mature medullary thymocytes and absent from cells of non-T lineage and immature thymocytes), it defines a unique cell surface glycoprotein of 120,000 daltons, in contrast to anti-T3, which defines a 19,000-dalton cell surface glycoprotein (15). The anti-T12 antibody was chosen for its selective binding pattern on mature T cells and medullary thymocytes and lack of mitogenicity on T lymphocytes. Anti-T12 was shown to be of the IgM type by virtue of its reactivity with a goat anti-mouse IgM antibody and lack of reactivity with a goat anti-mouse IgG antibody (Meloy Laboratories, Springfield, VA).

Abbreviations: MHC, major histocompatibility complex; SCID, severe combined immunodeficiency; GVHD, graft-versus-host disease; MLC, mixed lymphocyte culture.
Preparation of Anti-T12 for Intravenous Use. The anti-T12 monoclonal antibody was obtained in ascites form from BALB/cf mice that had been inoculated previously with the T12 hybridoma. This preparation contained >50% specific monoclonal antibody but was contaminated by albumin and normal mouse immunoglobulin. To develop a purified anti-T12 preparation, the monoclonal antibody was separated from the other ascites elements by a standard Sephadex G-200 (Pharmacia) sizing column. With this technique, the immunoglobulin of the IgM isotype was rapidly resolved into a single protein peak. Anti-T12 was obtained at ~20 mg/ml of ascites. This material was tested for endotoxin contamination and found to be negative by limulus assay (Microbiological Associates, Walkersville, MD). Routine microbiological cultures did not yield bacteria or fungal contamination. The antibody preparation was stored at -70°C in sterile 2-ml freezing vials. It was thawed, centrifuged at 100,000 x g for 20 min to remove immunoglobulin aggregates, and filtered through a 0.22-μm filter prior to clinical use. A dose of 100 μg of anti-T12/kg was administered consecutively for 5 days.

In Vitro Lysis of Bone Marrow Cells. After obtaining informed written consent by procedures approved by the appropriate ethics review committee, we took multiple bone marrow aspirates from the patient's mother under general anesthesia. Subsequently, bone marrow mononuclear cells were obtained by Ficoll-Hypaque density centrifugation and washed four times to remove residual heparin. The mononuclear cells were then placed in 15-ml sterile plastic tubes (Falcon) and centrifuged at 200 x g for 5 min. To the mononuclear pellets (20 x 10^6 cells per tube) was added 1 ml of a 1:500 dilution of purified anti-T12. The cells and antibody were spun gently in a Vortex every 10 min for 1 hr and then treated with 0.4 ml of rabbit complement (Pel-Freeze), and the mixture was placed at 37°C for 1 hr. After antibody/complement treatment, cells were washed three times to remove dead cells, and the entire procedure was repeated two times. After the third antibody/complement treatment, cell viability was >99%. Total cell loss did not exceed 30% during this procedure. Anti-T12/complement-lysed cells were placed in (final vol, 50 ml) RPMI 1640 medium (GIBCO) and reinused over a 2-hr time period.

Functional and Phenotypic Analyses of Lymphoid Cells. In vitro T-cell proliferative responses to mitogens and antigens and B-cell immunoglobulin production after pokeweed mitogen stimulation were determined as described (16, 17). Epics V cell sorter analysis of lymphoid cells with monoclonal antibodies, indirect immunofluorescence and microcytotoxicity assays, and chromosome banding used standard techniques (10, 18, 19).

Patient. The patient was a 4-month-old first female child of second cousin parents from the Cape Verde Islands with SCID (3). Erythrocytes from the child had normal activity for adenosine deaminase and nucleotide phosphorylase. Because of the lack of a related HLA-matched donor, conventional bone marrow transplantation could not be performed. Therefore, the patient was conditioned for transplantation with busulfan (5 mg/kg), cytoxan (200 mg/kg), and antilymphocytic serum (0.2 mg/kg) over 8 days. This conditioning regimen was used because two prior attempts at reconstitution with T-cell-depleted marrow without conditioning resulted in no evidence of engraftment. Maternal bone marrow was depleted of mature T cells with a monoclonal antibody, anti-T12, and complement and cells at 160 x 10^6/kg were infused into the patient 1 day after cessation of conditioning.

RESULTS

Before transplantation, ~10% of the patient's circulating lymphoid cells were reactive with anti-T10 and anti-T11 (12). The latter monoclonal antibody defines a sheep erythrocyte receptor-associated antigen (15). In contrast, there was no reactivity with anti-T3, anti-T4, anti-T8, or anti-T12 (Table 1). These findings suggest that all circulating lymphoid cells of T lineage were immature. Moreover, no B cells were detectable in peripheral blood with anti-B1, which defines a B-cell-specific antigen present throughout B-cell ontogeny (14). Expression of the HLA-D-related Ia antigen on 5% of circulating blood lymphoid cells likely correlates with a population of monocytes, precursor cells, or both (13).

The patient received a bone marrow transplant from her haplotype-mismatched mother. Before transplantation, the bone marrow specimen contained ~10% mature T lymphocytes whereas, after three cycles of anti-T12 monoclonal antibody and rabbit complement treatment in vitro, <0.1% mature T cells were detectable by indirect immunofluorescence with either anti-T12 or anti-T3. Given that this 10-kg patient received nucleated bone marrow cells at 160 x 10^6/kg, <2 x 10^6 mature maternal T cells contaminated the transplant inoculum.

Despite elimination of the majority of T cells from the maternal bone marrow, acute GVHD developed at day 10 after transplantation. The clinical signs of rash, hepatitis, diarrhea, and ascites were associated with detection of mature T cells between wk 1 and 2. At that time, 15–25% of lymphocytes were reactive with anti-T3, anti-T12, or both. Because of the severity of acute GVHD, the patient received anti-T12 (0.1 mg/kg/day) intravenously during the second week. An Epics V cell sorter analysis of circulating lymphoid cells from the patient

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Population defined</th>
<th>2 wk (treatment)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0 wk</td>
</tr>
<tr>
<td>Anti-T3</td>
<td>Mature thymocytes and T cells</td>
<td>0</td>
</tr>
<tr>
<td>Anti-T12</td>
<td>Mature thymocytes and T cells</td>
<td>0</td>
</tr>
<tr>
<td>Anti-T4</td>
<td>Helper T cells</td>
<td>0</td>
</tr>
<tr>
<td>Anti-T8</td>
<td>Suppressor T cells</td>
<td>0</td>
</tr>
<tr>
<td>Anti-T6</td>
<td>Cortical thymocytes (TL like)</td>
<td>0</td>
</tr>
<tr>
<td>Anti-T10</td>
<td>Thymocytes and precursor cells</td>
<td>10</td>
</tr>
<tr>
<td>Anti-T11</td>
<td>All thymocytes and T cells</td>
<td>10</td>
</tr>
<tr>
<td>Anti-Ia</td>
<td>HLA-D-related antigen</td>
<td>5</td>
</tr>
<tr>
<td>Anti-B1</td>
<td>B cells</td>
<td>0</td>
</tr>
</tbody>
</table>

Table 1. Development of lymphoid populations after bone marrow transplantation.
distribution of T and B cells has persisted for >8 months. Although not shown, full hematologic reconstitution appeared by wk 6 after transplantation.

The concomitant emergence of peripheral blood cells with mature T- and B-cell markers and disappearance of immature lymphoid cells suggested that the patient may have undergone normal lymphoid differentiation and acquisition of immune function. To test this possibility, T-cell proliferative responses to mitogens, antigens, and alloantigens were measured. In addition, the capacity of B cells to differentiate into immunoglobulin-secreting plasma cells in the presence of pokeweed mitogen and autologous T lymphocytes was determined. As shown in Table 2, before treatment the patient made no significant proliferative responses to the mitogen phytohemagglutinin, alloantigen, or soluble antigen (medium alone, ≈200 cpm). Her lymphoid cells did not respond to pokeweed mitogen in vitro with production of Ig. By wk 6 (Table 2), one could see excellent T-cell responses and B-cell function was evident. Taken together, our results indicate that transplantation of bone marrow after initial in vitro and then in vivo T-cell depletion can be subsequently associated with development of lymphocyte populations of mature phenotype and function.

To determine whether the circulating blood and bone marrow population after transplantation were of the maternal donor genotype, HLA and erythrocyte typing and chromosomal banding studies were performed 5 wk after transplantation. As shown in Table 3, the maternal peripheral blood HLA lymphocyte genotype was distinct from the patient’s HLA genotype since the former expressed Auc30, Bu22, and Dr5 and lacked Auc23, Bu44, and Dr4, whereas the converse was true for the latter. After transplantation, the patient’s peripheral blood and bone marrow were of the maternal HLA genotype. Further support for this idea was the finding that karyotyping of the patient’s lymphocytes after transplantation showed bright satellites on both chromosomes 22 by banding analysis that were identical to the mother’s pattern (19). Erythrocyte group analysis, likewise, demonstrated that erythroid cells were of maternal origin after transplantation.

Given the pretransplant in vitro data showing that the patient’s lymphocytes were stimulatory for maternal T cells in a mixed lymphocyte culture (MLC) as well as the development of GVHD, it was important to determine whether or not the maternal T lymphocytes in the recipient after transplantation were reactive with original recipient cells obtained and cryopreserved before transplantation. As shown in Table 4 (Exp. 1), maternal lymphocytes taken from the mother proliferated in MLC against irradiated recipient bone marrow obtained before transplantation. In contrast, lymphocytes of maternal genotype present in the recipient after transplantation (Exp. 2) were no longer responsive to the same pretransplant bone marrow. These studies indicated that the maternal T lymphocytes circulating in the child were no longer reactive with the child’s cells.
The presence of bright satellites on both chromosomes 22; −, absence of bright satellites on both chromosomes 22.

To determine whether this inability to respond to recipient major histocompatibility complex (MHC) was secondary to suppression or tolerance, a mixing study was performed in which maternal lymphocytes were combined (1:1) with recipient lymphocytes of maternal genotype and added to the pretransplant irradiated cryopreserved bone marrow specimen from the recipient in a MLC (Exp. 3). As shown, with this mixture, significant proliferation was obtained. This degree of proliferation represented slightly more than the average proliferation of experiments 1 and 2 and strongly argues against active suppression by maternal lymphocytes in the recipient as the major mechanism of proliferation inhibition. Rather, it suggests that the circulating maternal lymphocytes had lost alloreactivity or become tolerant to recipient HLA antigens.

**DISCUSSION**

In the present study, we performed a bone marrow transplantation on a patient with SCID using maternal HLA-haplotype mismatched bone marrow that had been depleted of mature T lymphocytes. T lymphocytes were eliminated from the bone marrow before infusion by using anti-T12/rabbit complement. Three cycles of in vitro anti-T12/complement treatment reduced the percentage of T cells in the inoculum by a factor of 0.001 as determined by indirect immunofluorescence. Further reduction of T cells in the donor marrow was not attempted because of the possible adverse effects in putative donor stem cells. Nevertheless, significant clinical GVHD, manifested by skin rash, hepatitis, diarrhea, and ascites, developed 10 days after transplantation. The patient was then treated by intrave-

<table>
<thead>
<tr>
<th>Cell origin</th>
<th>Peripheral blood</th>
<th>Bone marrow</th>
<th>Chromosomal banding*</th>
<th>Erythrocyte type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal</td>
<td>Au30, 32</td>
<td>ND</td>
<td>+</td>
<td>B+C+E'S+Fyb+Xg+</td>
</tr>
<tr>
<td></td>
<td>B14, Bu22</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Dr3, 5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patient</td>
<td>Au23, 32</td>
<td>Au23, 32</td>
<td>−</td>
<td>B+C'E'S+Fy b-Xg−</td>
</tr>
<tr>
<td></td>
<td>B14, Bu44</td>
<td>B14, Bu44</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Dr3, 4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>After transpl</td>
<td>Au30, 32</td>
<td>Au30, 32</td>
<td>+</td>
<td>B+C'E'S+Fy b-Xg+</td>
</tr>
<tr>
<td></td>
<td>B14, Bu22</td>
<td>B14, Bu22</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Dr3, 5</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

ND, not done.

Table 4. After parental F1 bone marrow transplantation, the chimera's T lymphocytes of maternal genotype are tolerant to recipient (F1) HLA antigens in MLC

<table>
<thead>
<tr>
<th>Exp.</th>
<th>Responder cells*</th>
<th>[3H]Thymidine, cpm</th>
<th>SI</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Maternal lymphocytes (P) in mother</td>
<td>18,046 ± 2,670</td>
<td>29</td>
</tr>
<tr>
<td>2</td>
<td>Maternal lymphocytes (P) in recipient (F1)</td>
<td>1,120 ± 101</td>
<td>1</td>
</tr>
<tr>
<td>3</td>
<td>Maternal lymphocytes in mother and in recipient (1:1 mixture)</td>
<td>13,462 ± 1,660</td>
<td>19</td>
</tr>
</tbody>
</table>

Results are mean ± SEM. SI, stimulation index.

* HLA: Au30, 32; B14, Bu22; Dr3, 5.

† HLA: Au23, 32; B14, Bu44; Dr3, 4.
may be sufficient to eliminate the alloreactive population.

T-cell-specific monoclonal antibodies offer advantages over conventional antisera or lectin-depletion techniques (20, 21). First, they can be tailored specifically to individual cell lineages and furthermore restricted to antigens expressed during either mature or immature stages of differentiation (5). Second, they can be selected for various functional virtues, including the inability to activate the immune response or induce modulation of surface antigens that they define (20). The latter is a necessary prerequisite to ensure successful complement-mediated lysis. Third, they can be derived from the appropriate immunoglobulin isotype to optimally fix complement and engage the reticuloendothelial system. In this regard, anti-T12 is a monoclonal antibody of the IgM isotype that fixes complement, detects a 120,000-dalton glycoprotein without initiating its modulation, does not induce lymphocyte proliferation or activation, and is restricted in its reactivity to mature immunocompetent T lymphocytes. Last, the quantity of monoclonal reagents is unlimited and, unlike heteroantisera, their specificity is constant.

It is clear that, after transplantation, all hematopoietic cells were of the maternal phenotype. Of note, transiently, there was the appearance of immature cells in the peripheral circulation. Nevertheless, after termination of GVHD, the subsequent mature T cells that appeared in the circulation did not mediate GVHD. These cells responded normally in an MLC, whereas, before transplantation, the child had no response. Despite this alloreactive capacity, the T lymphocytes of maternal HLA genotype did not react with the child’s cryopreserved haplotype-mismatched bone marrow. This state of tolerance within the chimera was not due to demonstrable suppressor cell mechanisms. Rather, mixing studies supported the hypothesis that cells that differentiated in the child had become tolerant to the child and were not immunosuppressed. These results are consistent with murine studies indicating that the development of recognition of “self MHC” is dependent not on the genotype of the T cell itself but rather on the MHC antigens expressed by thymic epithelial cells (22, 23).

Previous studies indicated that patients with SCID had immature cells circulating in peripheral blood (4). Whether this was due to the absence of normal precursor cells or the failure of a microenvironment to facilitate differentiation was not clear. The development of immunocompetent T lymphocytes after removal of mature maternal T lymphocytes suggests that an intracellular defect rather than a microenvironmental defect is responsible for disease in our case.

The present study represents only one experience in haplotype-mismatched transplantation. In this regard, it is evident that a series of studies will be needed to determine the general applicability of such an approach to transplantation in situations in which HLA identical donors are lacking. However, the development of functional donor T lymphocytes that are tolerant to recipient HLA antigens in our patient and a successful HLA-A, -B-mismatched transplant reported by others (24) suggest that, after initial mature donor T-cell depletion from bone marrow, HLA-mismatched transplantation is biologically rational and technically feasible. The potential for extending corrective bone marrow transplantation to individuals without previously suitable donors appears promising.

Note Added in Proof. Since the preparation of this manuscript, a second patient has been given a transplant in a similar fashion with paternal T lymphocyte-depleted HLA haplotype-mismatched bone marrow for Wiskott-Aldrich syndrome. At 3 wk after transplantation, hematologic reconstitution is evident.

This work was supported by National Institutes of Health Grants CA 19589, RO1 NS17182, and RR 128.