Reconstitution of Cell-mediated Immunity in Severe Combined Immunodeficiency Following Fetal Liver Transplantation

S. SETO, T. MIYAKE and T. HIRAO

Division of Allergy, Immunology and Infectious Diseases, Shizuoka Children's Hospital, Shizuoka, Japan

A male infant X-linked, adenosine deaminase-positive severe combined immunodeficiency underwent partial immunological reconstitution by fetal liver transplantation at twenty months of age. Reconstitution of T lymphocytes was observed from sixteen weeks after transplantation, in the increase of T lymphocytes, positive delayed-type skin reaction and in vitro responsiveness to mitogens and allogeneic cells, sequentially. Chimerism was defined by chromosomal analysis and HLA typing, in which one haplotype seemed to be shared between the donor and the host by chance. However, defective immunoglobulin production has not yet been corrected. The assay on T-B lymphocyte interaction in vitro suggested that the failure might be attributed to an intrinsic defect of B lymphocytes, which were cells of donor origin after transplantation. Three years following transplantation, the patient is free of severe infections, although he requires regular injections of gamma globulin.

CASE PRESENTATION

Clinical course before transplantation

A male infant, whose stepbrother from the same mother died of overwhelming infection in early infancy, was born with normal weight and height after an uneventful pregnancy. Since birth, he had oral moniliasis and cutaneous candidiasis, and further suffered from frequent diarrhea and bronchitis. At 9 months of age, he was hospitalized by reason of the above problems and failure to thrive.

At that time, no lymphoid apparatus was observed and chest X-rays did not reveal a thymus shadow. Laboratory data showed moderate lymphopenia (1100/cmm) with almost complete absence of T cells which was less than 2% as E-rosette forming cells (E-RFC). There was no proliferative response to mitogens or allogeneic cells. In addition, delayed-type hypersensitivity (DTH) skin reactions were negative to phytohemagglutinin (PHA) and candida antigen. Serum immunoglobulin levels were extremely low although B cells accounted for 38%, consisting of surface-IgG (15%), IgA (6%) and IgM (8%) bearing cells. Values of serum IgG, IgA, IgM and IgD were 20, 2, 5 and 2 mg/dl respectively at admission. Later, those immunoglobulins became undetectable. Intravenous replacement therapy with S-sulfonated gamma globulin was continued to maintain IgG levels at over 200 mg/dl. Terminal differentiation of the patient's B cells into plasma cells could not be induced by normal allo-T cells in pokeweed mitogen (PWM)-stimulated cultures (data not shown). Both adenosine deaminase and purine nucleoside phosphorylase activity were within normal limits.

Though lymphocyte counts fluctuated between 500 and 1500 per cu mm, T cells remained almost absent except during therapy by transfer factor, which raised E-RFC transiently to 13%. The incidence of diarrhea increased progressively, which resulted in severe weight loss and profound lymphopenia. Furthermore, candida granuloma ineffectively responsive to antibiotics developed all over the body surface.

Fetal liver transplantation and clinical improvement

At 20 months of age, fetal liver transplantation...
tion (FLT) was performed because no histocompatible donor was available among his relatives. A ten-week-old fetus was obtained by vaginal delivery from a woman healthy except for hypertension. Fetal liver was minced in to small fragments and placed in a sterile dish containing Hank's balanced medium. After gross particles of connective tissue were removed, the suspension was passed through successively thinner needles until a single-cell suspension was obtained. Then, $1.1 \times 10^7$ cells (2.0 $\times 10^6$ per kg of body weight) were given intravenously within two hours of delivery. No immunosuppressive treatment was given during, pre- and post-transplantation.

Fig. 1 shows the clinical course one year after FLT. For about four months after FLT, the diarrhea progressed, accompanied by a marked depletion of lymphocytes. These episodes appeared to correspond to a mild graft-versus-host (GvH) reaction, but neither liver dysfunction, skin rash, eosinophilia nor fever was observed. Sixteen weeks after FLT, the patient showed reversal of wasting and became free of candida infection. An increase of peripheral lymphocytes was also noted. However, on the way to recovery, a mild GvH reaction reoccurred for a short period of time, after which both lymphocytes and body weight increased very rapidly thereafter. Although the patient was recontaminated at 8 months after FLT, he has been rid of deleterious infections despite mild but recurrent bowel malfunctions and upper respiratory infections. Currently, at three years post-transplantation, he exhibits good psychomotor development, but his physical growth remains below normal.

Immunological reconstitutions

Fig. 2 shows the changes of cell-mediated immunity following FLT. Eighteen weeks after FLT, DTH skin reaction by 2 $\mu$g PHA turned positive, coinciding with the rise of E-RFC to 46%. One week later, responsiveness of the lymphocytes to mitogens was also recognized in vitro. The stimulation index in $^3$H-thymidine (Tdr) incorporation was 8.8 by PHA and 19.9 by concanavalin A, which then increased toward normal levels. Lymphocytes could respond weakly to candida antigen in vivo at 56 weeks after FLT. Lymphocyte responsiveness to allogeneic cells also improved (Table 1). The patient's lymphocytes were able to respond well to unrelated lymphocytes as well as lymphocytes from his uncle, but reactivity to his father's lymphocytes remained weak. On the other hand, natural killer cell activity to K562 cells was observed in the patient's post-transplant mononuclear cells at 37% of control activity which was determined by a chromium release assay. In summary, every parameter here indicates that cell-mediated immunity was remarkably improved by FLT.

Nevertheless, serum immunoglobulin has as yet been undetectable except for the IgG being supplemented continually. After FLT, regulatory T cell subsets, involved in B cell differentiation, with phenotypes OKT4$^+$ (32%) and OKT8$^+$ (24%) were detected. For these reasons, the patient's B cells were examined post-transplantation to test their ability to differentiate with the help of normal allogeneic T cells (Fig. 3). While the patient's T cells could induce the differentiation of control B cells in PWM-stimulated cultures, the patient's B cells did not get into plasma cells with the assistance of control T cells. These results suggest that the patient's B cells are not capable of responding to B cell differentiation factor in vitro and the sustained defects of immunoglobulin production might be attributed to intrinsic B cell defects, not to the function of T cells per se besides a problem of T-B interaction in vivo.

Chimerism

Analyses of karyotype were performed three times at 6, 24 and 36 months post-transplantation. The karyotype of peripheral lymphocytes was 46XX at all times, while the analysis of bone marrow cells revealed 46XY. These indicated that chimerism had been established and that all the PHA-responsive lymphocytes were derived from the donor.

The data on HLA typing are shown in Table 2. HLA-A26 and HLA-DRw9 were newly defined after FLT. Both antigens, which are the donor's father's antigens, were located in the same haplotype. HLA-A2 and HLA-DR4 of the patient pretransplantation were not detected except at 39 weeks post-transplantation. These results would allow us the following presumption. One haplotype of the donor probably consisted of HLA-A26, Bw61 and DRw9. Another haplotype of the donor should be derived from
donor's mother, and HLA-All and Bw22 of her antigens may be excluded from the fetus' HLA variety. Therefore it seems likely that one haplotype of the fetus (donor) was HLA-Aw24, Bw52, DR2 and was inherited from her mother, which was shared with the patient (recipient) by chance.

COMMENTS

Fetal liver transplantation is usually from an HLA-incompatible donor. Hematopoietic stem cells of fetal liver are less capable of repopulating in the recipient than those of bone marrow (4). Actually the rate of engraftment by FLT is lower than by bone marrow transplantation (2, 6). Moreover, in this case, the time of FLT was late for the patient's age and the dose of transplanted cells was small, compared to other cases (2, 5). The successful outcome in our patient, despite these unfavorable factors (9), might have been due to the coincidence of one common haplotype between the donor and host.

Even in successful engraftment of fetal liver cells, B cell functions often fail to be reconstituted, especially in the cases of SCID with B cell phenotypes (1, 6, 7). It was described by Zinkernagel that a fetal donor and a recipient should share at least one HLA-D haplotype plus one of the HLA-A or HLA-B antigens to achieve competent lymphocyte interactions (10). However, in a case reported by Touraine et al., T and B cells were seen to cooperate between complete HLA mismatches to produce antibodies (8). After FLT our patient had cells originating from the donor defined by the appearance of new HLA-DR antigen. Furthermore, this case is consistent with the hypothesis put forward by Zinkernagel. Notwithstanding, B cell reconstitution has not been established as in many other cases. Considering the results shown in Fig. 3, the lack of B cell functions post-transplant may be attributed to defects of the B cell itself, and not to a failure of T-B interaction in vivo. Conceivably, that is a characteristic of hematopoietic stem cells in fetal liver.

From the clinical point of view, a problem is a lack of secretory IgA which plays a major role in the initial defence mechanism of the mucosa. Indeed, the patient has had recurrent bronchitis and malnutrition after FLT. These may lead to more serious diseases such as bronchiectasia or malnutrition in the future even if the T cells work well. With the availability of secretory IgA as a medicament in the near future this problem can then be easily solved.

ACKNOWLEDGMENTS

We thank Dr. Noraki Shinomiya and Dr. Nobuo Sakura for performing the B cell differentiation assay and the enzyme determination.

REFERENCES

Table 1  Response of patient's lymphocytes to allogeneic cells

<table>
<thead>
<tr>
<th>Stimulator</th>
<th>Before FLT</th>
<th>After FLT</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>26 wk</td>
<td>39 wk</td>
</tr>
<tr>
<td>Father</td>
<td>1.1</td>
<td>1.0</td>
</tr>
<tr>
<td>Mother</td>
<td>1.2</td>
<td>NT</td>
</tr>
<tr>
<td>Uncle</td>
<td>2.2</td>
<td>13.7</td>
</tr>
<tr>
<td>Unrelated</td>
<td>NT</td>
<td>18.0</td>
</tr>
</tbody>
</table>

Lympocyte responsiveness was examined in one-way mixed-lymphocyte-cultures.
Data are expressed as stimulation indices.
FLT: fetal liver transplantation, NT: not tested.

Table 2  HLA phenotypes before and after fetal liver transplantation

Before transplantation
Patient  Aw24, Bw52, DR2 / A2, Bw61, DR4
Father    Aw24, Bw52, DR2 / - , Bw61, -
Mother    A2, Bw61, DR4 / - , Bw16, -
Fetus' mother  A11, Aw24, Bw22, Bw52, DR2

After transplantation
26 weeks  Aw24, A26*, Bw52, Bw61,
39 weeks  A2, Aw24, A26*, Bw52, Bw61,
51 weeks  Aw24, A26*, Bw52, Bw61, DR2, DRw9*
3 years   Aw24, A26*, Bw52, Bw61, DR2, DRw9*

Designations for the HLA antigens are based on the report from the 8th International Histocompatibility Testing Workshop.
* Newly detected HLA antigens after transplantation.

Fig. 1  Clinical course after fetal liver transplantation in a patient with severe combined immunodeficiency.
GVH: graft-versus-host (reaction).
Reconstruction of Cell-mediated Immunity in Severe Combined Immunodeficiency Following FLT - 237

Fig. 2  Development of cell-mediated immunity after fetal liver transplantation. In delayed-type hypersensitivity (DTH) skin tests, diameters of erythema or induration are expressed as the average of the longest and the shortest. In this test, 2 μg PHA and a 1:10000 dilution of candida broth antigen were used. In $^{3}$H-thymidine (TdR) incorporation, the average count of unstimulated cells was 130 cpm per $2 \times 10^5$ cells.
Fig. 3 Differentiation of B cells in pokeweed mitogen-stimulated cultures. The assay was performed by an immunofluorescent method as described previously (3). cB; control B cell-enriched fraction, pT; patient’s T cell-enriched fraction. Each fraction contained $2 \times 10^6$ cells in the assay.