Feto-maternal microchimerism suggests that immunologic tolerance exists between mother and fetus. Based on this hypothesis, we performed haploidentical stem cell transplantation (SCT) without T-cell depletion (TCD) in 5 patients with advanced hematologic malignancies. HLA incompatibilities for graft-versus-host disease (GVHD) direction included 3-loci mismatches in 4 patients, and 2-loci mismatches in 1 patient. Recipient chimeric cells were detected in all patients. The prophylaxis against GVHD was tacrolimus with minidose methotrexate. Engraftment was obtained in all patients. An acute GVHD of less than or equal to grade 2 developed in all patients except one who developed tacrolimus encephalopathy. Two patients died, 1 from fungal pneumonia and 1 from disease progression. The other 3 patients survived, with one patient in complete remission. These observations suggest that haploidentical SCT based on the feto-maternal microchimerism without TCD is possible. (Blood. 2003;101:3334-3336) © 2003 by The American Society of Hematology

Introduction

The presence of fetal hematopoietic cells in the maternal blood and vice versa, which is called feto-maternal microchimerism, suggests that immunologic tolerance exists between mother and offspring. The role of feto-maternal immunologic tolerance in allologenic stem cell transplantation (SCT) was recently demonstrated. 2-4 Maternal stem cell donation was found to be better for SCT than paternal donation, based on the results of a nationwide SCT survey conducted in Japan. 2 Van Rood et al 3 also showed that the recipients of non–T-cell–depleted (TCD) maternal transplants had a significantly lower incidence of chronic graft-versus-host disease (GVHD) than the recipients of paternal transplants in haploidentical 1- or 2-antigen–mismatched transplantations. They also demonstrated a lower rate of acute GVHD in sibling transplantations mismatched for noninherited maternal antigens (NIMAs) compared with those mismatched for noninherited paternal antigens (NIPAs). NIMA-mismatched sibling donor and recipient share the inherited paternal antigens (IPAs) and are mismatched at the maternal antigens, but there are microchimeric cells expressing the NIMAs. These observations support the hypothesis that offspring may be tolerant to haploidentical relatives expressing NIMAs (mother or NIMA-mismatched siblings), and the microchimeric mother may be hyporesponsive to IPAs of the offspring.5 Recently, we successfully treated a patient with blast crisis of chronic myelogenous leukemia (CML) by transplanting a haploidentical 3-loci mismatched maternal graft without TCD. 4 This prompted us to conduct a clinical trial of non-TCD haploidentical 2- or 3-loci–mismatched SCT from mothers to their offspring and vice versa, and from NIMA-mismatched siblings of patients with advanced hematologic malignancies who lack HLA-matched donors.

Study design

This study included 5 patients with hematologic malignancies; patient characteristics are shown in Table 1. All patients had no available HLA-matched donors in family members, unrelated bone marrow, and cord blood banks. Donors were selected from healthy haploidentical family members who mutually linked with feto-maternal microchimerism, including mothers in 3 patients, 2 offspring in one patient, and 2 NIMA-mismatched siblings in one patient. HLA incompatibilities for GVHD direction included 3-loci mismatches in 4 patients, and 2-loci mismatches in one patient. The conditioning regimen consisted of 1 g/m² to 2 g/m² of cyclophosphamide (CA) twice on day −6 and once a day on day −5 and day −4, 50 mg/kg to 60 mg/kg of cyclophosphamide (CY) on day −4 and day −3, and total body irradiation of 2.0 Gy to 2.5 Gy twice on day −2 and day −1 in 4 patients, or 4 mg/kg of busulfan (BU) on day −6 to day −3 and 60 mg/kg of CY on day −2 and day −1 in one patient (Table 2). The GVHD prophylaxis consisted of 0.02 mg/kg per day of tacrolimus administered intravenously, and minidose methotrexate (5 mg/m² administered intravenously on days 1, 3, and 6). After obtaining consent from the ethical committee of Kyoto Prefectural University of Medicine and written informed consent from the patients and donors, the donors were given filgrastim at a dose of 300 μg/m² subcutaneously for 5 days. Peripheral blood stem cells (PBSCs) were collected from day 4 to day 6 using a continuous blood cell separator (CS3000; Fenwal, Deerfield, IL).

An HLA-nested polymerase chain reaction with sequence-specific primer typing (PCR-SSP) was used to detect chimeric cells. 4,7,8 Briefly, genomic DNA was obtained from peripheral blood and fingernail clippings from patients and donors. Nail DNA was used as a nonblood DNA control. The first PCR was performed with 500 ng DNA using the appropriate locus-specific primer for the target HLA antigen. For the second PCR, the target allele–specific primers were utilized. Estimates of the original cell

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concentration were based on a comparison between the bands of the $1 \times 10^{2}$-fold dilution of the first PCR product with the band produced by the positive control at $1 \times 10^{6}$- and $10^{9}$-fold dilutions.\textsuperscript{7,8}

**Results and discussion**

An HLA-nested PCR-SSP demonstrated the presence of the IPAs of the patient in the peripheral blood of the mother (cases 1, 3, and 5), and detected NIMA in the peripheral blood of the donor (cases 2 and 4), suggesting that immunologic tolerance to the recipient cells exists (Table 1). One of 2 offspring (a daughter) and one of 2 NIMA-mismatched siblings (a brother) were accepted to become a donor in cases 2 and 4, respectively. The median number of CD34 cells infused was 2.36 $\times$ 10^{6}/kg (1.26 $\times$ 10^{6}/kg to 3.57 $\times$ 10^{6}/kg). The hematopoietic recovery was rapid with an absolute neutrophil count of more than 0.5 $\times$ 10^{9}/L on day 30, examined by analyzing microsatellite polymorphism in the donor and recipient. Acute GVHD of grade 3 developed in one patient (case 3) who developed tacrolimus encephalopathy, but it subsided promptly following prednisolone administration. In the other 4 patients, acute GVHD of less than or equal to grade 2 was controlled by prednisolone and cyclosporine administration. In one patient being in complete remission (Table 2).

These observations suggest that haploidentical SCT from mother to offspring and vice versa, or from NIMA-mismatched siblings without TCD or CD34\textsuperscript{+} cell selection is possible. Sustained engraftment and the lack of severe GVHD in all patients except one suggests that immunologic tolerance to NIMA (cases 2 and 4) or IPA (cases 1, 3, and 5) exists, because the risk of graft failure and severe GVHD is more than 10% and 80%, respectively, if TCD is not performed in haploidentical SCT.\textsuperscript{9,10} The only method of determining immunologic tolerance is by the detection of microchimeric cells. To do this, we used PCR-SSP that detected these cells with an accuracy of one in 10^5 cells.\textsuperscript{4,7,8} Using this method, Maruya et al demonstrated persistent chimeric mother cells in 66% of the 76 individuals and offspring cells in 82% of the 56 mothers.\textsuperscript{8} It is uncertain whether microchimerism is an indicator of immune tolerance for successful transplantation.

Megadose of highly purified CD34\textsuperscript{+} SCT after high-dose conditioning is another approach to overcome the HLA barrier in haploidentical SCT; it diminished the risk of graft failure and severe GVHD.\textsuperscript{11,12} However, high infection-related mortality rates due to delayed immune reconstitution is still a problem, especially in advanced-stage disease. Unfortunately, we did not examine the immune recovery in detail; the risk of viral infection in this study is lower than that reported in TCD or CD34\textsuperscript{+} cell--selected SCT.\textsuperscript{11-13} It is a concern because tolerance to specific antigens might make relapse more likely.

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**Table 1. Patient characteristics**

<table>
<thead>
<tr>
<th>Case</th>
<th>Age, y</th>
<th>Sex</th>
<th>Diagnosis</th>
<th>Status at SCT</th>
<th>Donor</th>
<th>HLA mismatch</th>
<th>Microchimerism</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>17</td>
<td>M</td>
<td>CML</td>
<td>BC</td>
<td>Mother</td>
<td>3 loci</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>55</td>
<td>F</td>
<td>CML</td>
<td>BC</td>
<td>Daughter</td>
<td>2 loci</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>25</td>
<td>F</td>
<td>NHL</td>
<td>Refractory relapse</td>
<td>Mother</td>
<td>3 loci</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>23</td>
<td>M</td>
<td>ALL (L2)</td>
<td>Primary refractory</td>
<td>NIMA-mismatched sibling</td>
<td>3 loci</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>17</td>
<td>M</td>
<td>NHL</td>
<td>Primary refractory</td>
<td>Mother</td>
<td>3 loci</td>
<td>+</td>
</tr>
</tbody>
</table>

M indicates male; F, female; CML, chronic myelogenous leukemia; BC, blast crisis; NHL, non-Hodgkin lymphoma; ALL, acute lymphoblastic leukemia; NIMA, noninherited maternal antigen; SCT, stem cell transplantation; HLA, human leucocyte antigen; GVHD, graft-versus-host disease; HVG, host-versus-graft.

**Table 2. Stem cell transplantation and clinical outcome**

<table>
<thead>
<tr>
<th>Case</th>
<th>Conditioning regimen</th>
<th>CD34\textsuperscript{+} cells infused ($\times$ 10^{6}/kg)</th>
<th>Engraftment</th>
<th>aGVHD</th>
<th>cGVHD</th>
<th>Complications</th>
<th>Outcome</th>
<th>Survival after SCT (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>CA + CY + TBI</td>
<td>2.25 (PBScs + BM)</td>
<td>18</td>
<td>21</td>
<td>2</td>
<td>Eruption, diarrhea</td>
<td>Relapse at testis on day 153</td>
<td>467+</td>
</tr>
<tr>
<td>2</td>
<td>CA + CY + TBI</td>
<td>2.36 (PBScs)</td>
<td>14</td>
<td>15</td>
<td>1</td>
<td>Oral aphthia</td>
<td>CR on day 390</td>
<td>390+</td>
</tr>
<tr>
<td>3</td>
<td>BU + CY</td>
<td>2.46 (PBScs)</td>
<td>10</td>
<td>13</td>
<td>3</td>
<td>NE</td>
<td>Death on day 67</td>
<td>67</td>
</tr>
<tr>
<td>4</td>
<td>CA + CY + TBI</td>
<td>1.26 (PBScs)</td>
<td>13</td>
<td>14</td>
<td>1</td>
<td>—</td>
<td>Relapse at pericardium on day 88</td>
<td>117</td>
</tr>
<tr>
<td>5</td>
<td>CA + CY + TBI</td>
<td>3.57 (PBScs)</td>
<td>14</td>
<td>19</td>
<td>2</td>
<td>CMV antigenemia</td>
<td>PR</td>
<td>105+</td>
</tr>
</tbody>
</table>

GVHD indicates graft-versus-host disease; aGVHD, acute GVHD; cGVHD, chronic GVHD; CA, cytarabine; CY, cyclophosphamide; TBI, total body irradiation; BU, busulfan; FK506, tacrolimus; MTX, methotrexate; PBScs, peripheral blood stem cells; BM, bone marrow; BOOP, bronchiolitis obliterans organizing pneumonia; NE, not evaluable; Neut, days to reach neutrophil count >0.5 $\times$ 10^{9}/L; Plt, days to reach platelet count >20 $\times$ 10^{12}/L; CMV, cytomegalovirus; CR, complete remission; PR, partial remission; and —, not observed.
In spite of the expansion of bone marrow and cord blood banking systems, a significant number of patients lack matched donors. Non-TCD haploidentical SCT based on fetomaternal microchimerism provides patients with hematologic malignancies who lack an HLA-identical donor another chance of receiving SCT. A prospective study is required to establish this technique.

Acknowledgment
We thank Dr Hiroh Saji (HLA Laboratory, Kyoto, Japan) for pursuing HLA PCR-SSP to detect fetomaternal microchimerism, and for providing useful discussion.

References