CONCISE REPORT

Mixed Blood Chimerism in T Cell-Depleted Bone Marrow Transplant Recipients: Evaluation Using DNA Polymorphisms

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We have used DNA sequence polymorphism analysis to document engraftment after T cell-depleted bone marrow transplantation (BMT), with a selected panel of four DNA probes. In contrast to nondepleted BMT recipients, the patients who received T cell-depleted marrow exhibited a mixed blood chimerism. This mosaicism was observed before graft failure or relapse in six patients. However, in five other patients, this mixed chimerism was not followed by these complications with a follow-up of 9 to 31 months after transplantation. Our results support the hypothesis that transplanted bone marrow T cells may help to maintain engraftment by eliminating host cells that can cause graft failure.

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and B lymphocytes by Ficoll centrifugation, E-rosetting, and plastic adherence.

RESULTS

We first estimated the sensitivity of the assay by dilution experiments in which patient and donor DNAs were mixed in varying proportions. The minor cell population is still visible at levels in the order of 1% to 5% at levels in the order of 1% to 5%.

In four recipients, the use of both St14 and HS5 probes in four fractions, days -5, -4, -3, and -2; CCNU, lomustine (150 mg/m², day -5); VP16, etoposide (200 mg/m², days -5, -4, and -3); TBI, total body irradiation (10 Gy single dose, day -1); TLI, total lymphoid irradiation (6 Gy in four fractions, days -10, -9, -8, and -7); M, mixed; T, total; NE, not evaluable; S, St14 (DXS532); L, L1.28 (DXS7); H, HS5 (DYZ1); M, Male H.

Table 1. Patients Examined Post-BMT by DNA Analysis

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Age/Sex</th>
<th>Diagnosis</th>
<th>Status at BMT</th>
<th>Donor Age/Sex</th>
<th>Preparation</th>
<th>T Cell Depletion</th>
<th>GVHD Acute/Chronic</th>
<th>Date of Study Post-BMT</th>
<th>DNA Probes</th>
<th>Chimerism Follow-up</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>40/F</td>
<td>ALL</td>
<td>1st R</td>
<td>39/F</td>
<td>+</td>
<td>0/0</td>
<td>17 mo</td>
<td>S</td>
<td>M</td>
<td>&gt;31 mo</td>
</tr>
<tr>
<td>2</td>
<td>44/F</td>
<td>CML</td>
<td>1st CP</td>
<td>46/M</td>
<td>+ + TLI</td>
<td>0/0</td>
<td>7, 10, 12, 14 mo</td>
<td>S</td>
<td>M</td>
<td>&gt;17 mo</td>
</tr>
<tr>
<td>3</td>
<td>40/M</td>
<td>ALL</td>
<td>1st R</td>
<td>36/F</td>
<td>+</td>
<td>0/0</td>
<td>8.12 mo</td>
<td>H</td>
<td>M</td>
<td>&gt;15 mo</td>
</tr>
<tr>
<td>4</td>
<td>34/M</td>
<td>CML</td>
<td>1st CP</td>
<td>51/F</td>
<td>+</td>
<td>0/0</td>
<td>1.38 mo</td>
<td>H</td>
<td>M</td>
<td>&gt;12 mo</td>
</tr>
<tr>
<td>5</td>
<td>34/M</td>
<td>CML</td>
<td>1st CP</td>
<td>32/F</td>
<td>+ + CCNU/AraC</td>
<td>2/0</td>
<td>1.3 mo</td>
<td>HS. M</td>
<td>&gt;9 mo</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>40/F</td>
<td>AML</td>
<td>2nd R</td>
<td>26/M</td>
<td>+ + VP16/CCNU</td>
<td>0/0</td>
<td>4 mo</td>
<td>S</td>
<td>M</td>
<td>Relapse at 5 mo</td>
</tr>
<tr>
<td>7</td>
<td>36/F</td>
<td>ALL</td>
<td>1st R</td>
<td>28/M</td>
<td>+</td>
<td>0/0</td>
<td>4 mo</td>
<td>S</td>
<td>M</td>
<td>Relapse at 5 mo</td>
</tr>
<tr>
<td>8</td>
<td>31/F</td>
<td>AML</td>
<td>2nd R</td>
<td>38/M</td>
<td>+</td>
<td>0/0</td>
<td>2.35 mo</td>
<td>S</td>
<td>M</td>
<td>Relapse at 4 mo</td>
</tr>
<tr>
<td>9</td>
<td>35/M</td>
<td>CML</td>
<td>1st CP</td>
<td>32/F</td>
<td>+</td>
<td>0/0</td>
<td>3.4 mo</td>
<td>HS. M</td>
<td>Graft failure at 4 mo</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>35/F</td>
<td>Preleukemic state</td>
<td>33/M + CCNU/AraC</td>
<td>0/0</td>
<td>60, 75, 103, d</td>
<td>SH</td>
<td>M</td>
<td>Graft failure at 2 mo</td>
<td></td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>39/F</td>
<td>Preleukemic state</td>
<td>37/M + TLI</td>
<td>0/0</td>
<td>3.48 mo</td>
<td>SH</td>
<td>M</td>
<td>Graft failure at 3 mo</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>18/M</td>
<td>CML</td>
<td>1st CP</td>
<td>15/M</td>
<td>+</td>
<td>0/0</td>
<td>1.2 mo</td>
<td>SLM. NE</td>
<td>8 mo</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>15/M</td>
<td>ALL</td>
<td>1st R</td>
<td>17/F</td>
<td>+</td>
<td>0/0</td>
<td>60.72 mo</td>
<td>H M</td>
<td>&gt;72 mo</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>13/M</td>
<td>AML</td>
<td>1st CP</td>
<td>14/F</td>
<td>+</td>
<td>1/0</td>
<td>30 mo</td>
<td>H T</td>
<td>&gt;36 mo</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>27/M</td>
<td>CML</td>
<td>1st CP</td>
<td>33/F</td>
<td>+</td>
<td>4/2</td>
<td>32 mo</td>
<td>H T</td>
<td>36 mo</td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>20/M</td>
<td>AML</td>
<td>1st R</td>
<td>19/F</td>
<td>+</td>
<td>1/0</td>
<td>3.15 mo</td>
<td>H T</td>
<td>&gt;15 mo</td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>17/M</td>
<td>AML</td>
<td>2nd R</td>
<td>20/M</td>
<td>+</td>
<td>1/0</td>
<td>76 d</td>
<td>L T</td>
<td>&gt;9 mo</td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>37/M</td>
<td>AML</td>
<td>1st R</td>
<td>38/M</td>
<td>+</td>
<td>3/0</td>
<td>63.3 d</td>
<td>L T</td>
<td>Deceased on 70 d</td>
<td></td>
</tr>
<tr>
<td>19</td>
<td>19/F</td>
<td>AML</td>
<td>1st R</td>
<td>19/M</td>
<td>+</td>
<td>2/0</td>
<td>30 d</td>
<td>S T</td>
<td>&gt;8 mo</td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>50/F</td>
<td>Preleukemic state</td>
<td>37/F</td>
<td>+</td>
<td>1/0</td>
<td>56 d</td>
<td>S T</td>
<td>Relapse at 6 mo</td>
<td></td>
<td></td>
</tr>
<tr>
<td>21</td>
<td>41/F</td>
<td>AML</td>
<td>1st R</td>
<td>39/M</td>
<td>+</td>
<td>2/0</td>
<td>3/0</td>
<td>S T</td>
<td>&gt;7 mo</td>
<td></td>
</tr>
<tr>
<td>22</td>
<td>31/M</td>
<td>AML</td>
<td>2nd R</td>
<td>38/M</td>
<td>+</td>
<td>2/0</td>
<td>1.2 mo</td>
<td>SLM. NE</td>
<td>&gt;9 mo</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: ALL, acute lymphoblastic leukemia; AML, acute myeloblastic leukemia; CML, chronic myeloid leukemia; R, remission; CP, chronic phase; CTX, Cyclophosphamide (60 mg/kg, days -5 and -4); AraC, cytosine arabinoside (200 mg/m², days -5, -4, -3, and -2); CCNU, lomustine (150 mg/m², day -5); VP16, etoposide (200 mg/m², days -5, -4, and -3); TBI, total body irradiation (10 Gy single dose, day -1); TLI, total lymphoid irradiation (6 Gy in four fractions, days -10, -9, -8, and -7); M, mixed; T, total; NE, not evaluable; S, St14 (DXS532); L, L1.28 (DXS7); H, HS5 (DYZ1); M, Male H.

Fig 1. Expansion of residual host cells after transplantation (patient no. 5). DNA samples were obtained from the female donor (D) and from the male recipient prior to BMT (R) and after transplantation on day 46 (d46) and on day 94 (d94). Following digestion with TaqI and hybridization with the probe St14 (DXS532) the recipient-specific allele (a) absent on day 46, was detected on day 94 post BMT (b1 and b2: donor specific alleles; c: constant fragments).
ism at 6 years, detectable only with the HS5 probe (ie, <1%).

In contrast, all T-depleted BMT recipients (patients no. 1 to 11) exhibited a mixed chimerism. This chimerism was detected in seven cases with probe St14 and in four with probe HS5. This mosaicism was observed before graft failure in three patients (no. 9, 10, and 11) at 4, 2, and 3 months, respectively or before relapse in the three others (no. 6, 7, and 8) at 5, 5, and 4 months post-BMT, respectively. In every instance the chimerism was above 10%. In five other recipients (no. 1, 2, 3, 4, and 5) (two ALL and three CML) this partial chimerism did not exceed 5% and was not followed by these complications with a follow up of 9 to 31 months after transplantation.

In five cases the leukocytes were fractionated. Mixed chimerism was observed in three recipients (no. 3, 4, and 11) (ALL, CML, preleukemic state) in the three subpopulations. The T lymphocytes of patient no. 8 (AML) were of recipient origin and the granulocytes and B lymphocytes were found to be of donor origin just before her death. In patient no. 13 (ALL), residual host cells were mainly detected in the B lymphocyte fraction at 6 years post-BMT.

**DISCUSSION**

Analysis of DNA sequence polymorphism has proved useful in studying the origin of cells after BMT and allows marrow engraftment to be documented, to detect and analyze mixed lympho-hematopoietic chimerism, and to evaluate posttransplant leukemic relapse.5,7 Cytogenetic analysis can be used to assess post-BMT chimerism but it requires dividing cells to be obtained by bone marrow puncture. DNA analysis, when performed on bone marrow cells, gave similar results to that obtained with peripheral blood cells.2 We thus studied blood cells only and DNA analysis was conclusive in 20 of the 22 patients we examined. The sensitivity of the test depends on the probe used: 1% to 5% for St14, L1.28, and MetH, and 0.1% for HS5. The clinical significance of this small amount of host residual cells is not known. Furthermore, the quantification of mixed chimerism by study of the whole blood cell population may be irrelevant since the mosaicism is often different in each hematopoietic subpopulation as observed in patients no. 8 and 13 and as previously described.

The most striking result in our study is the detection of a discernable amount of host DNA in peripheral blood of all T cell-depleted BMT recipients, even in those who had received a more ablative regimen (see patients no. 2, 5, 6, 10, and 11). Such partial chimerism has previously been observed after BMT either by DNA analysis5-7 or by cytogenetic techniques.12-14 Some were SCIDs5,6 who did not receive TBI but others received TBI-cyclophosphamide for hematological malignancies as did our patients. Among the nondepleted BMT, mixed chimerism remains uncommon.13 In contrast mixed chimerism appears very common after T cell depletion of bone marrow as shown by cytogenetic analysis14 and by DNA analysis in this report.

Although the present work is not a controlled comparative study, the observed differences between T cell-depleted and nondepleted BMT cannot be explained by mean age, diagnosis, and status at BMT or conditioning regimen. The high rate of mixed chimerism appears to be a consequence of T cell depletion of the graft. This result may be associated with the existence of radio-resistant residual host cells after TBI. BMT preparation cannot eliminate all hematological host cells as it has been described in humans5 and in dogs.16 Circulating T lymphocytes can survive intensive pretransplant conditioning regimens, proliferate after incubation with interleukin 2 and phytohemagglutinin, and function as effector cells in an in vitro model assay.13 These cells are probably destroyed by grafted T lymphocytes, explaining the rareness of mixed chimerism in nondepleted transplantation. In contrast, after T cell-depleted BMT, residual host cells survive and their growth results in partial chimerisms as observed in our study. These cells are probably involved both in the rejection episodes and in the hematological relapses. Nevertheless, the mixed chimerism does not systematically deteriorate the prognosis. Long-term cure of the disease remains a possibility when leukemic patients are grafted in complete remission (patients no. 1, 3, and 13). Recently, using a murine model of T cell-depleted BMT, Ferrara et al17 observed that mixed chimerism was frequent and could remain stable even at 1 year posttransplant. However, the persistence of the malignancy seems to be the rule in CML (patients no. 2, 4, and 5) grafted in the active phase of the disease.

In conclusion, our results demonstrate further the interest of DNA analysis techniques in the study of BMT recipients. In the future these techniques should be used to assess the quality of new protocols of T cell depletion or of appropriate conditioning regimens, designed to prevent graft rejection or relapse.

**ACKNOWLEDGMENT**

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**REFERENCES**


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