CONCISE REPORT

Molecular Analysis of Relapse in Chronic Myeloid Leukemia After Allogeneic Bone Marrow Transplantation

By T.S. Ganesan, Gao Ling Min, J.M. Goldman, and B.D. Young

Four patients with Philadelphia (Ph') positive chronic myeloid leukemia (CML) were studied before, after, and on relapse following allogeneic bone marrow transplantation (BMT). Southern analysis of DNA from cells collected before and at relapse after BMT was performed in order to investigate the origin of the leukemia at relapse. Using minisatellite probes we showed that the relapse occurred in cells of host origin in all four patients and this was confirmed with a Y chromosome specific probe in two male patients who had a female donor. Furthermore, using two probes for the breakpoint cluster region (bcr) on chromosome 22, we showed that leukemic cells at relapse bore identical rearrangements to those in the disease at time of presentation of each patient. We conclude that relapse in all four patients is due to re-emergence of the original leukemic clone.

ALLOGENEIC BONE MARROW transplantation (BMT) using HLA-identical siblings is currently the only approach that offers the possibility of cure for patients with chronic myeloid leukemia (CML). In a recent analysis performed for the International Bone Marrow Transplant Registry, the actuarial probability for survival in the chronic phase (CP) was 55% ± 5% and the actuarial probability of relapse was 19% ± 7% at 4 years.1 When leukemia recurs, it is usually in cells of host origin but in two cases of CML the relapse occurred in cells of donor origin.2,3

In this study molecular techniques have been used to examine the cellular origin of relapse in four patients with CML treated by allogeneic BMT. Minisatellite DNA probes and a probe specific for the Y chromosome were used to determine whether relapse occurred in donor or host cells.4,5 The relationship of the relapse to the leukemia at presentation was studied using probes specific for the breakpoint cluster region (bcr) on chromosome 22.6 The position of the breakpoint on chromosome 22 is different in each translocation in CML and thus the pattern of DNA rearrangement represents a unique clonal marker for each patient.

MATERIALS AND METHODS

Patients. Four patients were treated at the Hammersmith Hospital in London (Table 1). All risks of the treatment protocols were fully explained to patients, donors, and relatives. Informed consent was obtained from each patient in accordance with institutional guidelines. Each patient received chemo-radiation therapy followed by BMT from HLA identical siblings. Patients no. 2 and 4 had female donors. Three patients were in chronic phase and one patient was in blast transformation. The fourth patient presented with acute lymphoblastic leukemia (CALLA positive) in 1979 and achieved complete remission with conventional chemotherapy. In November 1980, he entered chronic phase of Philadelphia (Ph') positive CML. He was transplanted with marrow from his sister in January 1982. He was well until February 1986, when he developed a granulocytic sarcoma as an extramedullary tumour at T6 and then relapsed with myeloid blast transformation in bone marrow and peripheral blood.

Cyto genetics. Cyto genetic analysis of bone marrow cells was attempted at 3-month intervals during the first year after transplantation and at 6-month intervals thereafter. Metaphases were examined by banding techniques.7

Chemotherapy. All four patients received cyclophosphamide 60 mg/kg body weight per day (on days −6 and −5) followed by total body irradiation (10 Gy or 12 Gy in five or six fractions, on days −4 to −2), delivered by a linear accelerator at a rate of 15 cGy per minute without lung shielding. One patient received busulphan (4 mg/kg/d orally on days −8 and −7) and the other three patients daunorubicin (60 mg/m² of surface area of day −7). Patients no. 2 and 3 received donor marrow depleted of T cells by in vitro incubation with the monoclonal antibody Campath-1 and complement. Donor marrow was transfused on day 0. Cyclosporine was administered for prevention of graft vs host disease.

Southern analysis. High molecular weight DNA was prepared from peripheral blood or frozen cells. It was digested with appropriate restriction enzymes and separated on a 0.8% agarose gel by the technique of Southern.6 Blotting was on to Hybod-N (Amersham, Amersham, UK), which was treated with UV light, prehybridized and hybridized at 43°C using 50% formamide as previously described.8 Radiolabeled probes were prepared by random primer extension to a specific activity of 1 to 3 × 10⁶ cpm/µg.9 The filters were washed at 43°C in 0.1 × SSC (sodium chloride and sodium citrate, pH 7.0/0.1% sodium dodecyl sulfate) and 0.1% SDS and were exposed at −70°C for one to four days. For the minisatellite probes, prehybridization and hybridization were carried out at 64°C and the filters washed at 64°C in 1 × SSC and 0.5% SDS.

Probes

Minisatellite probes. The two probes used were 15.1.11.4 and 6.3 containing the core minisatellites from A33.15 and A33.6, respectively, cloned into M13mp19 and M13mp8 DNA, respectively.4

Y specific probe. As described previously, pHY2.1 was used as the Y specific probe.5

bcr probes. The 3' probe was a 0.6 kb fragment subcloned from a commercially available probe (Oncogene Science, Mineola, NY). The 5' bcr probe was 0.45 kb fragment from the bcr cDNA clone as published previously from the K562 cell line.10

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Table 1. Clinical and Hematologic Features

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Age/Sex</th>
<th>Spleen (cm)</th>
<th>WBC $&gt;10^9$/L</th>
<th>Duration Before BMT (mo)</th>
<th>Phase at BMT</th>
<th>Time to Relapse (mo)</th>
<th>Type of Relapse</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>30/M</td>
<td>15</td>
<td>145</td>
<td>19</td>
<td>CP</td>
<td>21</td>
<td>BT-basophilic</td>
</tr>
<tr>
<td>2</td>
<td>34/M</td>
<td>15</td>
<td>557</td>
<td>60</td>
<td>BT</td>
<td>11</td>
<td>BT-myeloid</td>
</tr>
<tr>
<td>3</td>
<td>31/M</td>
<td>14</td>
<td>195</td>
<td>13</td>
<td>CP</td>
<td>11</td>
<td>CP</td>
</tr>
<tr>
<td>4</td>
<td>30/M</td>
<td>0</td>
<td>48</td>
<td>29</td>
<td>CP</td>
<td>53</td>
<td>BT-myeloid</td>
</tr>
</tbody>
</table>

Abbreviation: BT, blast transformation; CP, chronic phase.

RESULTS

The four patients in this study relapsed with Ph' positive disease at various times after transplantation (Table 1). Three patients (no. 1, 2, 4) relapsed with acute phase disease without a preceding chronic phase, whereas one patient (no. 3) had Ph' positive cells present 3 months before a chronic phase relapse.

Southern analysis of DNA from patients at presentation and at relapse following BMT with the minisatellite probes 15.1.11.4 and 6.3 showed in each case an identical pattern of restriction fragments (Fig 1).

Patients no. 2 and 4 had female donors. Using a Y specific probe pHY2.1, the intensity of the 2.1 kb restriction fragment was the same in these two patients before and after BMT (Fig 2), indicating that relapse occurred in cells bearing a Y chromosome.

Using the 3' bcr probe, the rearrangements observed before BMT were identical to the rearrangements at relapse in patients no. 2, 3, and 4. In no. 1, only the 5' bcr probe detected the rearrangement of the bcr gene and this was identical before and after BMT (Fig 3).

DISCUSSION

Two cases of leukemia recurring in donor cells after BMT for CML have been reported. The evidence that the relapse in donor cells was sex chromosome differences demonstrated in dividing cells. It is now possible to address this problem consistently in each patient using the DNA minisatellite probes. This minisatellite region was cloned from the intron of the human myoglobulin gene and is highly polymorphic. Using a series of clones it is possible to derive a unique DNA "fingerprint" of each individual; this has been used in paternity testing and determination of twin zygosity. The probability of any two individuals having identical patterns is very low. It is therefore possible to use such an approach to distinguish host v donor origin of cells after relapse. Using two such clones as probes, 15.1.11.4 and 6.3, it was possible to show that the "fingerprints" for the original leukemias were identical to those of the relapsed leukemias in each patient, thus confirming the relapse to be in the hosts' cells. This was further confirmed by use of the Y specific probe pHY2.1 in two patients who had a female donor.

G6PD analysis of Ph' positive CML has suggested that the Ph' translocation is a secondary event preceded by clonal expansion. It is therefore possible that the Ph' positive relapse in host cells could be the result of a new genetic event. The molecular rearrangements in the bcr gene appear to be unique for each patient and hence a second independent translocation would be expected to result in a different

Fig 1. (A) Southern blot showing genomic DNA from patients no. 1, 2, and 3 digested with HinF and probed with 15.1.11.4; (B) patients no. 1, 2, 3, and 4 genomic DNA digested with HinF1, and probed with 6.3 a, pretransplant sample; b, posttransplant relapse sample.
molecular pattern. Since the bcr gene rearrangements are identical before and after BMT in each of these four patients, we conclude that the original Ph positive clone is responsible for the relapse in each case. Furthermore, it is clear that in the evolution of the disease no further alteration to the bcr gene has taken place following the initial rearrangement.

In this preliminary study all four patients unequivocally demonstrated recurrence of the original clone even 4 years later as in patient no. 4. Further, as reported by Apperley et al., 16 Ph positive metaphases can appear after BMT without hematologic relapse, only to disappear in time. 16 Again this issue can be addressed by monitoring the rearrangement after BMT. Similar investigations have been carried out in acute lymphoblastic leukemia using immunoglobulin gene rearrangement as a clonal marker. 17 In follicular lymphoma where t(14;18) is observed in the majority of cases, the breakpoints on chromosome 18 cluster within a transcriptionally active region. 18,19 Using probes from this region the question of clonality can be addressed in patients with follicular lymphoma who have relapsed after any modality of therapy.

One explanation of recurrence of leukemia in donor cells after BMT is a transfection of viral or nonviral related oncogene to transplanted stem cells. An alternative hypothesis is that the stroma may be part of the leukemic clone and being radioresistant offers an abnormal milieu to donor cells. However, donor cell recurrent leukemia after BMT accounts for <5% of relapses. 20 Possibly normal donor stem cells are naturally resistant to the development of leukemia and there is a variable efficiency in transfection. It is now possible to characterize relapse after BMT particularly in CML with a combination of bcr and minisatellite probes. This study confirms that the relapsed leukemia is in the host cell in all the four patients. The recurrence in these four patients has been proven to be due to the original leukemic clone and the failure of the conditioning regimen and/or a graft v leukemia effect to eradicate it.

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