Cytogenetic Evidence for Recurrence of Acute Myelogenous Leukemia After Allogeneic Bone Marrow Transplantation in Donor Hematopoietic Cells


A 22-yr-old man with acute myelocytic leukemia received a bone marrow transplant from a genotypically HLA-identical female sibling after cyclophosphamide preparation. He remained in complete remission for 18 mo, when he developed a chloroma in the perineum. The chloroma was treated with local radiotherapy. The chloroma recurred 8 mo later and was treated with radiotherapy followed by combination chemotherapy. At 34 mo after transplant, marrow relapse and chloroma were documented. The first chloroma contained host cells by fluorescent Y-chromatin body analyses of interphase nuclei. All metaphase cells and karyotypes from peripheral blood and marrow samples showed no evidence of host cells from 3 wk after transplant through the time of marrow relapse. Data from autosomal and sex chromosome studies indicate that the marrow relapse occurred in cells of donor origin. A new consistent chromosome abnormality [45, X, -X, t(8;21) (q22; q22)] was observed in a majority of donor cells. The patient received a second bone marrow transplant from the same donor after preparation with busulfan and cyclophosphamide and attained a complete remission with full hematologic engraftment.

The recurrence of leukemia remains one of the major problems that limits the success of allogeneic bone marrow transplantation (BMT) for acute leukemia. The recurrence rate is somewhat dependent on the intensiveness of the pretransplant cytoreductive therapy. The vast majority of these relapses, when documented by cytogenetic analyses, have occurred in host cells. There are now three reported cases of relapse in donor cells. Of course, in many relapses sex chromosome markers may be lacking such that the donor or host origin of the recurrent leukemia cannot be easily determined. Thus the frequency of relapse in donor cells cannot be accurately estimated.

We present cytogenetic evidence for a case of acute myeloblastic leukemia (AML) relapse in donor cells using both sex chromosome and autosomal chromosome markers.
MATERIALS AND METHODS

Cytogenetic analyses. Chromosome and interphase nuclei analyses were performed with the standard quinacrine fluorescent-banding technique (QFQ). Slides for examination were prepared from blood samples cultured for 2-3 days with phytohemagglutinin (PHA) and bone marrow aspirates treated briefly with colchicine. The techniques followed and the rationale of our method are essentially the same as described earlier.

Consent. The protocols for therapy and studies that this patient received were subject to approval and annual review by the Joint Committee on Clinical Investigation of The Johns Hopkins Medical Institutions. The patient and donor gave consent on forms approved by this committee.

CASE REPORT

In May 1974 R.K., a 22-yr-old white male, was referred for BMT with the diagnosis of AML in relapse. The diagnosis had been established in November 1973 and remission had been induced with cytosine arabinoside (ara-C) and 6-thioguanine (6-TG). Despite maintenance therapy relapse had occurred in 10 wk, and two reinduction attempts with ara-C and 6-TG had failed to produce complete remission.

On admission the patient's white blood count was 5650/mm³ with 52% myeloblasts on smear (Table 1). Bone marrow aspirate (Fig. 1A) showed a hypercellular marrow with 85.4% myeloblasts (Table 2), some of which contained Auer rods. A female sibling, B.K., was found to be genotypically HLA identical with the patient. The patient was prepared for BMT with cyclophosphamide (CY) and on May 23, 1974 received an infusion of 274 × 10⁶ donor bone marrow cells/kg. Eradication of the AML and hematopoietic engraftment was documented by peripheral blood counts, bone marrow aspirate and biopsy morphology, and cytogenetic studies. Low-dose CY and methotrexate (MTX) were given after BMT to prevent graft-versus-host disease (GVHD). In December 1975 the patient developed pain over the left ischial tuberosity associated with numbness on the left side of the penis. A perineal mass was palpated and excisional biopsy revealed a chloroma by touch preparation and histologic studies. Since the blood and marrow appeared free of AML, the patient received radiotherapy (3400 rads) to the perineum with resolution of the lesion. In July 1976 a relapse of the perineal chloroma occurred and a ⁶⁷Ga scan showed increased uptake over the left ischial bone and over the sacrum. Since blood and marrow again failed to reveal any evidence of AML, 4000 rads were delivered to the pelvis, but only 2000 rads were delivered to the perineal area. Following radiotherapy a ⁶⁷Ga scan showed complete resolution of lesions.

In November 1976, because of two chloroma relapses, the patient was begun on a modification of the L6 protocol maintenance phase and received vincristine, hydroxyurea, bischloroethylnitrosourea, and MTX until March 1977. In April 1977 the patient developed abdominal pain associated with a left anterior rectal mass. At this time the peripheral smear showed 5% myeloblasts and the bone marrow aspirate showed 20% 30% myeloblasts. A ⁶⁷Ga scan again showed lesions in the pelvis. In May 1977 the patient was referred again for BMT in marrow and chloroma relapse of his AML.

On admission the white blood count was 7650/mm³ with 32% myeloblasts on smear, and bone marrow aspirate (Fig. 1B) showed 76.2% myeloblasts, some of which contained Auer rods. The patient's major clinical problem was acute renal failure due to obstruction of both ureters as they enter the urinary bladder. Drainage was instituted by ureteral catheters, and cytoreductive therapy with daunomycin produced resolution of the chloroma lesion and the renal failure. The patient was then prepared for a second BMT from the same donor, who was free of any evidence of AML (Tables 1 and 2), with busulfan (Bu) and CY, and on June 3, 1977 received an infusion of 313 × 10⁶ donor bone marrow cells/kg. Eradication of the AML and engraftment were shown by peripheral blood count, bone marrow aspirate morphology, and cytogenetic studies. A brief course of low-dose CY was given to prevent GVHD. A ⁶⁷Ga scan after the second BMT showed no perineal lesions. At the time of acceptance of this communication the patient was still in complete remission, 11 mo after the second BMT.

RESULTS

Cytogenetic studies. Chromosome studies using the QFQ technique were performed on PHA-stimulated peripheral blood and nonstimulated bone marrow samples obtained from the patient while in full relapse of his AML prior to the
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Table 1. Quinacrine Fluorescence Cytogenetic Studies Performed on PHA-stimulated Peripheral Blood Cells

<table>
<thead>
<tr>
<th>Dates of Studies Performed</th>
<th>Weeks After Bone Marrow Transplants</th>
<th>DNA Content (per mm(^2))</th>
<th>Morphology: Myeloblasts and Promyelocytes (%)</th>
<th>Host Type Cells</th>
<th>Donor Cells</th>
<th>Cells With Missing Y Chromosome</th>
<th>Nuclei With Y Bodies</th>
<th>Nuclei Without Y Bodies</th>
<th>Variants/Anomalies</th>
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<tbody>
<tr>
<td>Patient (R K)</td>
<td>5/17/74 - admission</td>
<td>5650</td>
<td>52.0</td>
<td>12</td>
<td>0</td>
<td>0</td>
<td>17</td>
<td>33</td>
<td></td>
</tr>
<tr>
<td></td>
<td>6/13/74 + 3</td>
<td>1112</td>
<td></td>
<td>0</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>420</td>
<td></td>
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<tr>
<td></td>
<td>9/4/74 + 15</td>
<td>7000</td>
<td></td>
<td></td>
<td></td>
<td>0</td>
<td>0</td>
<td>150</td>
<td></td>
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<tr>
<td></td>
<td>6/11/75 + 55</td>
<td>5450</td>
<td></td>
<td></td>
<td></td>
<td>0</td>
<td>0</td>
<td>125</td>
<td></td>
</tr>
<tr>
<td></td>
<td>7/7/76 + 109</td>
<td>4700</td>
<td></td>
<td></td>
<td></td>
<td>0</td>
<td>20</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>5/8/77 + 153</td>
<td>7650</td>
<td></td>
<td></td>
<td></td>
<td>0</td>
<td>99</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5/18/77 - 2 admission</td>
<td>352</td>
<td>32.0</td>
<td></td>
<td></td>
<td>0</td>
<td>11</td>
<td>45,XX</td>
<td></td>
</tr>
<tr>
<td></td>
<td>6/21/77 + 3</td>
<td>2700</td>
<td></td>
<td></td>
<td></td>
<td>0</td>
<td>0</td>
<td>89</td>
<td>32</td>
</tr>
<tr>
<td></td>
<td>9/8/77 + 14</td>
<td>18500</td>
<td></td>
<td></td>
<td></td>
<td>0</td>
<td>1</td>
<td>45,XX</td>
<td></td>
</tr>
<tr>
<td>Donor (B K)</td>
<td>1974</td>
<td>6500</td>
<td>0</td>
<td>0</td>
<td>11</td>
<td>0</td>
<td>0</td>
<td>150</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1977</td>
<td>6700</td>
<td>0</td>
<td>0</td>
<td>11</td>
<td>0</td>
<td>0</td>
<td>100</td>
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</tbody>
</table>

* Differential count from 200 cells
† Bone marrow transplant no. 1, 5/23/74; bone marrow transplant no. 2, 6/3/77
‡ 46,XY; one QFO marker chromosome 3
§ 46,XX; two QFO marker chromosomes 3
¶ Metaphase spreads lacking Y chromosome but clearly showing either two X chromosomes or two QFO marker chromosomes 3

First BMT in May 1974. Analyses showed an apparently normal male 46,XY chromosome constitution in metaphase cells (Fig. 2). There was no evidence of constitutional chimerism or mosaicism. There was slight fluorescence on one chromosome 3 (QFO 3) and large satellites on one chromosome 21 that did not fluoresce brightly. A Y-chromatin body was detected in 11% of interphase nuclei (Table 1). This percentage is below the published range (43%-99%) for normal...
Fig. 1. (A) Photomicrograph of patient's bone marrow aspirate at time of first admission (1974) showing AML in relapse. (B) Bone marrow aspirate at the time of second admission (1977) showing AML in relapse. Wright stain. ×675.
### Table 2. Quinacrine Fluorescence Cytogenetic Studies Performed on Nonstimulated Bone Marrow Cells

<table>
<thead>
<tr>
<th>Dates of Studies Performed</th>
<th>Weeks After Bone Marrow Transplant†</th>
<th>Morphology * Myeloblasts</th>
<th>Metaphase Plate Studies</th>
<th>Interphase Studies</th>
<th>Comments</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Host Type</td>
<td>Cells With Y Chromosomes</td>
<td>Nuclei With Y Bodies</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Promyelocytes (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patient (R K)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5/17/74 (admission)</td>
<td>- 1 (no 1)</td>
<td>85.4</td>
<td>8</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>6/13/74 to 9/4/74 (+ 3)</td>
<td></td>
<td>3.2</td>
<td>0</td>
<td>4</td>
<td>174</td>
</tr>
<tr>
<td>6/11/75 (+ 55)</td>
<td></td>
<td>7.0</td>
<td>0</td>
<td>16</td>
<td>0</td>
</tr>
<tr>
<td>7/7/76 (+ 109)</td>
<td></td>
<td>3.6</td>
<td>0</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>5/6/77 (readmission)</td>
<td></td>
<td>76.2</td>
<td>0</td>
<td>110</td>
<td>4</td>
</tr>
<tr>
<td>5/18/77 to 6/21/77 (+ 153)</td>
<td></td>
<td></td>
<td>2</td>
<td>248</td>
<td>6</td>
</tr>
<tr>
<td>6/21/77 to 9/8/77 (+ 3)</td>
<td></td>
<td>4.2</td>
<td>0</td>
<td>24</td>
<td>25</td>
</tr>
<tr>
<td>9/8/77 (+ 14)</td>
<td></td>
<td>6.2</td>
<td>2</td>
<td>298</td>
<td>2</td>
</tr>
<tr>
<td>Donor (B K)</td>
<td>1977</td>
<td>2.5</td>
<td>0</td>
<td>14</td>
<td>0</td>
</tr>
</tbody>
</table>

* Differential count from 500-600 cells counted
† Bone marrow transplant no. 1: 5/23/74, bone marrow transplant no. 2: 6/3/77
‡ 46,XY, one QFQ marker chromosome 3
§ 46,XX, two QFQ marker chromosomes 3
¶ Metaphase spreads lacking Y chromosome but clearly showing either two X chromosomes or two QFQ marker chromosomes 3

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Fig. 2. Chromosome analysis of PHA-stimulated peripheral blood sample from patient prior to first bone marrow transplant prepared by quinacrine fluorescent technique. Karyotype shows 46,XY chromosome with fluorescent Y chromosome and one fluorescent marker chromosome 3.
males but inside the range (10%-70%) observed in our laboratory for males with acute leukemia.

Chromosome studies using the QFQ technique performed on PHA-stimulated peripheral blood samples obtained from the donor in 1974 showed an apparently normal female 46,XX constitution in metaphase cells (Fig. 3) with no Y chromosome detectable. There was a fluorescent marker on both chromosomes 3 and large satellites on a chromosome 21 that did not fluoresce brightly. Interphase nuclei did not show a Y-chromatin body (Table 1) but occasionally showed a small, brightly fluorescing body that lacked characteristics of a Y-chromatin body and was probably a heteropycnotic body of autosomal origin (most likely the fluorescent region of chromosome 3; see above).

Results of chromosome analyses using the QFQ technique performed on PHA-stimulated peripheral blood and nonstimulated bone marrow samples after the first BMT are presented in Tables 1 and 2, respectively. It can be seen that at no time during the period after the first BMT was there any evidence of host cells in blood or marrow samples. None of the metaphases examined had a fluorescent Y chromosome, and none of the interphase nuclei had a fluorescent Y-chromatin body. Instead, two X chromosomes and/or two QFQ 3 were invariably present. Metaphase cells having two X chromosomes and two QFQ 3 were identified as being of donor type. Metaphase cells lacking a fluorescent Y chromosome but clearly showing either two X chromosomes or two QFQ 3 were classified as cells with missing Y chromosomes. Thus these metaphase cells had either sex chromosome or autosomal chromosome markers of donor type.

Interphase nuclei analyses of the touch preparation obtained from the chloroma at biopsy in December 1975 showed the presence of the Y-chromatin body (Fig. 4).
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Fig. 4. Fluorescence photomicrograph of interphase nuclei from chloroma tissue obtained at biopsy in 1975. Note Y-chromatin body in some cells, indicating that tissue is of host origin. Y-chromatin bodies were detected in 33% of interphase nuclei.

in 33% of the nuclei. Unfortunately, no dividing cells were obtained from the chloroma. PHA-stimulated peripheral blood and nonstimulated bone marrow samples taken before (June 1975), after (July 1976), and at the time of the chloroma relapse (data not shown) failed to show any evidence of host-type cells by chromosome analysis using the QFQ technique (Tables 1 and 2).

Fig. 5. Chromosome analysis of nonstimulated bone marrow cell obtained during relapse in 1977 showing 45,X,-X,t(8;21)(q22;q22) karyotype. Both chromosomes 3 have brightly fluorescent centromeres, indicating that cell is of donor origin.
At the time of marrow relapse in May 1977, chromosome analyses using the QFQ technique performed on PHA-stimulated blood and nonstimulated marrow samples failed to show any evidence of host type cells (Tables 1 and 2). Of 47 marrow cells, 34 (May 6, 1977) had a new consistent chromosome abnormality, t(8;21)(q22;q22) (Fig. 5). The remaining cells had a normal female karyotype or were broken cells. The cells with the abnormal karyotype could be shown to be of donor origin because of the presence of two chromosomes 3, each with prominent centromere fluorescence. The majority of marrow cells obtained 5 days later had a normal female karyotype, although 8 of 47 cells had the 8q-;21q+ translocation. The decrease in abnormal cells is consistent with the clinical improvement noted in the patient after cytoreductive therapy with daunomycin.

**DISCUSSION**

We have described a male patient with AML who received a bone marrow transplant from his genotypically HLA-identical sister after preparation with high-dose cyclophosphamide therapy. Approximately 18 mo after marrow engraftment with donor cells, the patient developed a chloroma in the perineum that was found to be of host origin by fluorescence studies. This lesion responded to treatment with local radiotherapy, relapsed 8 mo later (26 mo after BMT), and was treated again with local radiotherapy followed by maintenance multiagent chemotherapy. After discontinuance of maintenance chemotherapy the patient suffered chloroma and full hematologic (marrow and peripheral blood) relapse of his AML at 34 mo after BMT. Cytogenetic evidence collected at the time of the marrow relapse indicated absence of host cells. The marrow relapse therefore occurred in donor cells despite the history of chloroma relapse in host cells.

In addition, at the time of marrow relapse after BMT a new chromosome abnormality was observed in 72% of marrow cells, all of which were of donor origin. This abnormality is of particular significance because it is the only translocation seen with any frequency in AML; it occurs in 10%–20% of patients with AML de novo who have an abnormal karyotype.\(^2\) It is frequently associated with loss of a sex chromosome, as was observed in our patient. Only one other patient\(^2\)\(^9\) has been reported in whom an 8q-;21q+ translocation developed during the course of progression of leukemia; this patient, with an initially normal karyotype, was treated with the usual chemotherapy and did not have a BMT.

The literature contains reports of relapse in donor cells in three patients with acute leukemia after BMT. All three involved relapse in male cells (46,XY) in female patients (46,XX). In two cases\(^5,6\) the relapses, at approximately 2 and 5 mo, were exclusively in donor cells in patients with acute lymphoblastic leukemia (ALL) who had been prepared with lethal doses of TBI. In the third case\(^7\) the relapse at approximately 9 mo was in both male and female cells in a patient with AML who was prepared with high-dose CY therapy. This latter patient was a long-term mixed (male and female) marrow chimera. To our knowledge none of the patients received post-BMT chemotherapy or radiotherapy except for post-BMT immunosuppression with MTX or CY to prevent GVHD.

Although an extensive discussion of the etiology of leukemia and relapse of leukemia in patients in complete remission is beyond the scope of this report, it seems relatively well established that local as well as whole-body irradiation may be associated with an increased incidence of leukemia in man.\(^24,26\) Reports have incriminated antineoplastic chemotherapy in certain cases of leukemia in man.\(^29,30\)
There is ample evidence in rodents that viruses with or without the facilitation of irradiation are etiologically associated with leukemogenesis. Relapse is generally believed to represent regrowth of remaining leukemic cells after remission induction. Relapse in donor cells may, however, be due to reinduction of leukemia by persistent leukemogenic stimuli and/or new or recurrent leukemogenic influences (see above). Alternative mechanisms to explain relapse in donor cells have been postulated, such as diploidization after the fusion of a residual host leukemic cell with an engrafted donor nonleukemic cell.

Now that two cases of AML and two cases of ALL have relapsed in donor cells with two different BMT preparative regimens with varying clinical circumstances, the potential for leukemic transformation of donor cells is undeniably demonstrated. The true incidence of this problem can be better estimated if autosomal chromosome markers are used in addition to sex chromosome markers to detect differences between patients and donors, as in this case.

Lastly, as discussed above, relapse of acute leukemia after BMT remains one of the major obstacles to successful BMT. The vast majority of patients with acute leukemia who have received bone marrow grafts have been patients considered terminal because of relapse of their disease (one or more times) or failure to achieve complete remission during the first induction attempt. Thus when a patient relapses after BMT the prognosis is generally dismal. Under certain circumstances it may be possible to use cytoreductive chemotherapy to prepare the patient for a second BMT. The case presented here illustrates that a new two-agent (busulfan and CY) chemotherapeutic cytoreductive preparative regimen has potential for patients in whom second BMT attempts appear warranted. The precise role of this regimen is the subject of another report.

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