Two Karyotypically Independent Leukemic Clones With the t(8;21) and 11q23 Translocation in Acute Myeloblastic Leukemia at Relapse

By Yasuhide Hayashi, Susana C. Raimondi, Frederick G. Behm, Victor M. Santana, David K. Kalwinsky, Ching-Hon Pui, Joseph Mirro, Jr, and Dorothy L. Williams

Leukemic blast cells are thought to arise from clonal expansion of a single transformed hematopoietic cell. This generality is supported by the rarity of convincing reports on acute myeloblastic leukemia (AML) with two karyotypically independent clones. Relying on sequential cyogenetic analyses, we identified such clones in two children with relapsed AML. The first case, classified as M2 leukemia in the French-American-British (FAB) classification system, had a t(8;21)(q22;q22) at diagnosis; 16 months later, at relapse, the leukemic cells had uniform morphologic features similar to those observed at diagnosis, except that two independent clones were present: one with the original t(8;21) and the other with t(11;22)(p13;q13). The second case was initially classified as FAB M1 leukemia with a t(8;21)(q22;q22). At relapse, 16 months later, the blast cells appeared morphologically uniform and similar to the diagnostic specimen; however, in addition to the original t(8;21) clone, there was a t(11;11)(q23;q23). These findings suggest that separate leukemogenic events affecting different progenitor cells can occur in rare cases of AML. The presence of two karyotypically independent clones could also be explained by multistep leukemogenesis; that is, more than one cell from a common pool of preleukemic cells could be affected by the transforming event, resulting in two independent clones. Alternatively, in light of recent reports of therapy-related leukemias with an 11q23 translocation, the new independent clone in these two patients could represent a therapy-related secondary malignancy. Thus, 11q23 translocations may occur preferentially in stem cells that are more susceptible to treatment-induced malignant transformation.

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EVERAL DIFFERENT types of experimental evidence indicate that leukemic blast cells arise from clonal expansion of a single transformed hematopoietic cell. These include glucose-6-phosphate dehydrogenase (G6PD) isoenzyme studies,1 T-cell receptor and immunoglobulin gene rearrangement studies,23 restriction-fragment-length polymorphism analysis,45 rearrangement studies of DNA probes specific for the chromosomal translocation,64 and karyotype analysis.80 Each of these methods has advantages and limitations.

The t(8;21) is one of the most common chromosomal abnormalities in acute myeloblastic leukemia (AML),11,12 particularly the M2 subtype in the French-American-British (FAB) classification system.13 This abnormality is often associated with a missing sex chromosome11,12,14 and rarely coexists with the t(9;22) in the same individual leukemic cells.15 Translocations involving the 11 chromosome at band q23, other than the t(4;11), are usually associated with acute myelomonocytic leukemia (FAB M4 subtype) or acute monocytic leukemia (FAB M5 subtype).6,17 There have been several recent reports of patients with an 11q23 translocation presumably induced by chemotherapy,18-23 but to our knowledge, the presence of two coexisting independent clones, characterized respectively by t(8;21) and 11q23 translocations, has not been demonstrated in leukemia.24 In this article, we describe two cases of AML in which the original leukemic clone contained the t(8;21) and a second, apparently independent clone identified at relapse had an 11q23 translocation.

MATERIALS AND METHODS

From April 1980 to March 1987, we successfully analyzed leukemic cell karyotypes from 121 children with de novo acute nonlymphocytic leukemia (ANLL) at St. Jude Children's Research Hospital.25 Of the 14 patients with a t(8;21), five relapsed during this period, and their leukemic cell karyotypes were examined both at diagnosis and at relapse. In three patients, the original t(8;21) karyotype showed evidence of clonal evolution during the interval from diagnosis to relapse. The remaining two patients (the subjects of this report) had apparently independent leukemic clones at relapse. Informed consent was obtained from the patients or their parents, and the investigation was approved by the institution's Clinical Trials Review Committee.

Case Reports

Patient no. 1. A 12-year-old girl presented with a leukocyte count of 105.6 x 10⁹ cells/L with 58% blasts, a hemoglobin level of 5.4 g/dL, and a platelet count of 20 x 10⁹ cells/L. Her marrow aspirate (obtained on May 1, 1986) was diagnostic of AML, FAB M2 subtype. She had no antecedent myelodysplasia or history of toxic drug exposure and attained a complete remission after treatment with cytarabine, etoposide, daunorubicin, and thioguanine (July 7, 1986).28 After 16 months in complete remission (until November 16, 1987), she developed a hematologic relapse while still receiving continuation therapy. Bone marrow examination revealed 20% myeloblasts with morphologic features resembling those observed at diagnosis (FAB M2). The blast cells remained myeloperoxidase (MPO) positive. A second course of induction therapy with cytarabine, etoposide, and daunorubicin was successful, but the patient's complete remission ended 2 months later (March 2, 1988) when she developed thrombocytopenia and erythroid macrocytosis.
A bone marrow aspirate disclosed marked dyserythropoiesis and dysmyelopoiesis with <5% of blast cells, suggesting leukemic relapse or therapy-induced myelodysplastic syndrome. She was still alive in November 1988.

**Patient no. 2.** A 7-year-old boy presented with a leukocyte count of $16 \times 10^9$ cells/L with 78% blasts, a hemoglobin level of 8.5 g/dL, and a platelet count of $21 \times 10^9$ cells/L. A bone marrow aspirate was diagnostic of FAB M1 leukemia. He had no history of myelodysplasia or exposure to mutagenic drugs. A complete remission was secured with induction and continuation treatments identical to mutagenic drugs.

Cells were defined by the presence of at least two cells with the same extra chromosome or the same structural change, or at least three cells with the same missing chromosome. Multiple cytogenetic clones refer to the coexistence in the same specimen sample of at least two cell populations containing totally different numerical and/or structural chromosome changes; these were considered to be independent (or unrelated) clones. Cell populations were considered to be related if they contained an identical abnormal karyotype, or if they contained at least one chromosome change in common.

**Immunophenotyping**

Cell surface antigens were detected by a standard indirect immunofluorescence assay with monoclonal antibodies to lymphoid-associated antigens, including J5 (common ALL antigen, CALLA, CD10), T11 (CD2), T3 (CD3), T4 (CD4), B1 (CD20), and B4 (CD19); myeloid-associated antigens including MY1 (CD15), MY4 (CD14), MY7 (CD13), MY9 (CD33), and Mol (CD11b). Terminal deoxynucleotidyl transferase (TdT) was also detected by an indirect immunofluorescence assay.

**RESULTS**

There was no change in the morphologic or cytochemical features of leukemic cells (MPO*, SBB*, PAS*, ANBE*) between diagnosis and relapse in either patient. Auer rods, which are rare in leukemic cells with 11q23 translocations, were present in both cases at diagnosis and relapse. At diagnosis, the leukemic blast cells from patient no. 1 expressed CD13, CD15, CD33, and HLA-DR antigens but did not express CD14, CD19, CD10, CD20, CD2, CD3, or TdT. Follow-up immunophenotyping at relapse was inadequate because only 21% of the marrow-nucleated cells were leukemic blasts. In patient no. 2, leukemic blast cells at diagnosis were positive for HLA-DR, but were negative for CD11, CD13, CD15, CD2, CD10, and TdT. At relapse, the marrow was only partially replaced by leukemic cells (41%),

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**Table 1. Serial Cytogenetic Studies of Bone Marrow Cells from Two Patients With AML**

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Date of Examination</th>
<th>% Blasts in Bone Marrow</th>
<th>No. of Metaphase Cells Examined</th>
<th>% Cells With Normal Karyotype</th>
<th>Karyotype (% of Abnormal Metaphases)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5-1-86 (at diagnosis)</td>
<td>52</td>
<td>30</td>
<td>0</td>
<td>47,XX, +15,t(8;21)q22;q22(67%)/46,XX,t(8;21) (33%)</td>
</tr>
<tr>
<td></td>
<td>7-7-86 (1st remission)</td>
<td>0</td>
<td>ND</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td></td>
<td>9-14-87 (1st remission)</td>
<td>1</td>
<td>25</td>
<td>8</td>
<td>46,XX,t(11;22)q23;q13,t(1;18)q23;q25;q23(80%)/45,X,X,t(11;22)q23;q13,t(1;18)q23;q25;q23(12%)</td>
</tr>
<tr>
<td></td>
<td>11-16-87 (1st relapse)</td>
<td>20</td>
<td>24</td>
<td>4</td>
<td>46,XX,t(11;22)q23;q25;q23(50%)/46,XX,t(8;21),t(11;16)q23;q25;q23(46%)</td>
</tr>
<tr>
<td></td>
<td>12-1-87 (1st relapse)</td>
<td>21</td>
<td>10</td>
<td>10</td>
<td>46,XX,t(8;21),t(11;16)q23;q25;q23(80%)/48,XX,t(8;21),t(11;16)q23;q25;q23(12%)</td>
</tr>
<tr>
<td></td>
<td>1-6-88 (2nd remission)</td>
<td>1</td>
<td>15</td>
<td>100</td>
<td>46,XX</td>
</tr>
<tr>
<td></td>
<td>3-2-88 (2nd remission)</td>
<td>2</td>
<td>31</td>
<td>84</td>
<td>46,XX,t(11;22),t(11;18)q23;q25;q23(16%)</td>
</tr>
<tr>
<td>2</td>
<td>1-9-84 (at diagnosis)</td>
<td>95</td>
<td>24</td>
<td>8</td>
<td>45,XY,-9,t(8;21)q22;q22(92%)/45,XY,t(11;11)q23;q23(55%)/45,XY,-9,t(8;21)q22;q22(40%)</td>
</tr>
<tr>
<td></td>
<td>7-22-85 (1st relapse)</td>
<td>41</td>
<td>20</td>
<td>5</td>
<td>46,XY,t(11;11)q23;q23(55%)/45,XY,-9,t(8;21)q22;q22(40%)</td>
</tr>
<tr>
<td></td>
<td>9-23-86 (2nd relapse)</td>
<td>85</td>
<td>11</td>
<td>0</td>
<td>45,XY,-9,t(8;21),t(11;11)q23;q23(45%)/t(8;21)q22;q22(40%) and related abnormalities (55%)</td>
</tr>
</tbody>
</table>

ND = not done.
with an apparent CD13+, CD15+, CD33+, HLA-DR+, CD19+, CD20+, CD2+, CD3−, CD1− immunophenotype. There did not appear to be two separate leukemic populations in either patient.

All cells from patient no. 1 contained only a t(8;21) at the time of diagnosis (May 1, 1986) (Table 1). When a low percentage of blast cells was found in the bone marrow and relapse was suspected (September 14, 1987), no t(8;21)-related clone was identified but an emerging clone characterized by t(11;22) was present. At the time of clearly documented morphologic relapse (November 16, 1987), two independent clones were identified, one defined by the original t(8:21) (Fig 1A) and the other by the t(11;22) (Fig 1B). Both clones persisted for 2 weeks (December 1, 1987), although the proportions of cells with either marker varied widely (Table 1). Neither clone was detectable when the second remission was attained (January 6, 1988). A t(11;22)-related clone (Fig 2) reappeared 2 months later (March 2, 1988), at which time the patient’s marrow cells showed atypical myeloid and erythroid morphology.

Patient no. 2 had only a t(8;21)-related clone at the time of diagnosis (Table 1). At first relapse, two independent clones, one with the original t(8;21) (Fig 3A) and the other with a t(1;11) (Fig 3B), were observed in seven and 13 cells, respectively. The patient entered second complete remission after allogeneic bone marrow transplantation. At the time of second relapse, no t(1;11) cells were found, and the karyotype showed complex abnormalities including the t(8;21) (Table 1).

**DISCUSSION**

Two patients with AML, each having two independent clones at relapse, one characterized by a t(8;21) and the other by an 11q23 translocation, were identified in this study. Karyotypically independent clones have been reported in patients with acute lymphoblastic leukemia (ALL)29 and adult T-cell leukemia,30 but are rare in ANLL.16 Multiple complex chromosome abnormalities associated with -5/5q-, and/or -7/-7q- abnormalities have been observed in therapy-related ANLL,32-34 yet only rarely have these cases had two independent clones. A case of AML with both t(8;21) and t(9;22) in the same cells,13 and a case of acute myelomonocytic leukemia characterized by cells having del(7) with or without t(8;21), have been described.35 To our knowledge, there have been no reports of de novo or therapy-related AML cases with both t(8;21) and 11q23 translocations.24

The finding of two karyotypically independent clones suggest that such cases have two distinct leukemic clones, although the morphologic, cytochemical, and immunophenotypic studies do not clearly demonstrate two separate populations of leukemic cells. Possibly these cases are the result of separate de novo leukemogenic events affecting different progenitor cells in rare cases of AML. A second explanation is that two independent clones in our patients may arise from the same karyotypically normal preleukemic cell after a subsequent leukemogenic event. In this regard, studies of Philadelphia chromosome (Ph′)-negative lymphoid cell lines from G6PD heterozygous patients with Ph′-positive chronic myelocytic leukemia disclosed that chromosomal instability is a feature of the Ph′-negative lymphoid cells having the same G6PD type as the leukemic cells.36 It was suggested that another leukemogenic event may occur before acquisition of the Ph′ chromosome. Subsequent studies in AML by these investigators have supported that contention.37,38 Hirai et al,39 however, have reported that chromosomal abnormalities preceded the presence of N-ras mutation during the process of leukemic conversion in two patients with AML.

Our findings might also indicate that a completely new clone, bearing the 11q23 translocation, was induced by chemotherapy. The 11q23 region is commonly involved by chromosomal changes in the M4 or M5 subtype of ANLL.16,17 CALLA ALL,40,41 mixed-lineage leukemia,42-45 and lineage switch.46 Recently, Ratain et al46 reported two cases of t(9;11)-related secondary leukemia in adults, possibly induced by etoposide. Pedersen-Bjergaard et al19 have documented two cases of therapy-related leukemias with the t(9;11) developing over a short latent period. Therapy-
related leukemias with 11q23 translocations are well-recognized in children.\textsuperscript{20-23,46} Thus, rearrangement of the 11q23 region represents one of the nonrandom chromosomal abnormalities in therapy-related leukemia, especially in children. In this regard, 11q23 translocations have been suggested to occur preferentially in stem cells,\textsuperscript{40,45,47} which may be more susceptible than other precursor cells to treatment-induced malignant transformation. Interestingly, our induction protocol for AML included etoposide. Thus, 11q23 translocations may be induced by one or more DNA damaging agents, such as etoposide.

Finally, the second clone with an 11q23 translocation may represent selection and overgrowth of an independent drug-resistant clone that could not be detected at diagnosis. This idea seems unlikely because we could not find an 11q23 translocation in any leukemic cells at diagnosis, and there have been no reports of ANLL patients with both t(8;21) and 11q23 translocations at diagnosis.\textsuperscript{24}

Our study illustrates the importance of sequential detailed chromosomal analysis, without which the evidence of independent leukemic clones in the absence of obvious morphologic or cytochemical indications would have been missed. Moreover, the impending relapse of patient no. 1, although suspected clinically, was documented only by serial cytogenetic analysis.

A translocation similar to the t(11;22)(q23;q13) seen in patient no. 1 has been reported for Ewing sarcoma\textsuperscript{48,49} and as a constitutional anomaly,\textsuperscript{50} but has not been observed in patients with leukemia.\textsuperscript{24} The principal difference is that the breakpoint of chromosome 22(q13) in our patient lies in a more distal region of the chromosome, to which the \textit{c-sis} proto-oncogene has been mapped.\textsuperscript{51} Although t(1;11) (p32;q23) has been reported in childhood ALL,\textsuperscript{40,44} its detection in patient no. 2 appears to be the first reported instance of this abnormality in AML.

In conclusion, our data together with other reports suggest that second leukemic clones with the 11q23 translocation may be induced by chemotherapy. The possibility of therapy-related leukemia should be considered when intensive regimens of chemotherapy are being planned for pediatric patients.

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