Acute Lymphoblastic Leukemias With Deletion of 11q23 or a Novel Inversion (11)(p13q23) Lack MLL Gene Rearrangements and Have Favorable Clinical Features


Balanced translocations affecting the 11q23 region are among the most frequent chromosomal abnormalities in childhood acute lymphoblastic leukemia (ALL), comprising 5% to 6%. These cases consistently have a rearranged MLL gene and are associated with high-risk presenting features, hyperleukocytosis and younger age, and a poor treatment outcome. To assess the clinical and biologic significance of 11q23-associated structural chromosomal abnormalities other than translocations, we studied 17 cases of childhood ALL with del(11)(q23) and 3 with inv(11)(p13q23) that were identified among 785 cases with successful chromosome analysis. In contrast to reported cases with 11q23 and MLL gene rearrangement, our series was characterized by relatively low leukocyte counts (median, 15.1 × 10⁹/L), expression of CD10 antigen but not myeloid-associated CD15 and CDw65 antigens, a relatively high frequency of T-cell immunophenotypes, and a generally favorable prognosis. All 13 cases with interpretable molecular analysis lacked MLL gene rearrangements. We suggest that most cases with deletions or inversions affecting the 11q23 region represent clinically and biologically different entities as compared with those defined by 11q23 translocation.

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CHROMOSOME BAND 11q23, the location of the MLL gene, is a region of recurrent translocations in human malignancies. The usual consequence of 11q23 translocations is the fusion of N-terminal sequences of the MLL gene to a variety of partners producing chimeric products with putative transforming activities.1-3 Cases of childhood acute lymphoblastic leukemia (ALL) with rearrangements of this gene are generally characterized by a young age, high white blood cell counts, organomegaly, central nervous system (CNS) involvement, a B-precursor cell immunophenotype without CD10 expression but with coexpression of the myeloid associated antigens CD15 and CDw65, and a poor prognosis.4-6 Such cases largely account for the dismal prognosis ascribed to infants with ALL.7-9 Molecular studies of the cases with 11q23 chromosomal translocation have consistently shown involvement of the MLL gene1-5,9-10,12-17 and application of these molecular approaches has identified additional cases that were missed or not appreciated by cytogenetic analysis.5,9,10,12-14,17-21 Whether rearrangements of the 11q23 regions by mechanisms other than balanced translocations similarly affect MLL is unknown. We describe here findings in 17 consecutive cases with either a deletion or inversion affecting the q23 band on chromosome 11.

MATERIALS AND METHODS

Patients. From December 1979 to December 1993, 1,161 consecutive children with newly diagnosed ALL were admitted to St Jude Children’s Research Hospital (SJCRH) and enrolled in one of four successive Total Therapy studies (X-XIII).22-24 The diagnosis of ALL was based on morphologic criteria of the French-American-British (FAB) Cooperative Group, including negative staining for myeloperoxidase and nonspecific esterase.25 We successfully analyzed the leukemic cell karyotypes in 785 cases, including 14 with a deletion of chromosome 11 at band q23 and 3 with an inversion of chromosome 11 with breaks at p13 and q23. These patients are the subjects of the present report. Informed consent was obtained from all patients or their guardians, and the investigations were approved by the institution’s Clinical Trials Review Committee.

Cytogenetic evaluation. Bone marrow samples were prepared by a direct method,26 with or without short-term (24-hour) culture. A modified trypsin-Wright technique was used for chromosome banding. Chromosomes were described according to conventions of the International System of Human Cytogenetic Nomenclature (ISCN-91).27

Immunophenotyping studies. Leukemic cell surface antigens were detected by standard indirect immunofluorescence assays with monoclonal antibodies to lymphoid and myeloid-associated antigens. Blast cells were also tested for surface (slg) and cytoplasmic (clg) Ig as previously described.28 Depending on reactivity patterns, cells were classified as T (CD7*, CD5*, E-rosette*), B (slg*), pre-B (clg*), early pre-B (clg*, slg*, HLA-DR*, CD19*, CD10*), or B-precursor (slg*, CD19*, DR*, CD10*, but unknown clg status).28

Southern blot analysis. Genomic DNA was extracted from the bone marrow of leukemic patients at diagnosis. Aliquots (5 to 8 µg) of high molecular weight DNA were digested with BamHI, HindIII, and SacI restriction endonucleases, separated by electrophoresis in 0.8% agarose gels, and blotted onto nylon membranes. All blots were hybridized with a 3P-labeled probe (Oncor, Gaithersburg, MD) derived from 0.74-kb BamHI cDNA fragment of the MLL gene. This MLL probe will detect the common breakpoints of MLL between exons 5 through 11 with BamHI digests and breaks between exons 2 and 16 approximately on Sac I digest band size (Fig 1). The membranes were then sequentially probed with p98.40, 4.2E, and ps4 probes.29,30 These three probes will detect breaks between exons 2 and 18 in BamHI- and HindIII-digested DNA (Fig 1). Membranes were stripped in sodium hydroxide between hybridizations with each probe. The 4.2E probe required the inclusion of 300 µg/mL total human placental DNA (SignaChemical Co, St Louis, MO).

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Submitted April 12, 1995; accepted April 16, 1995.
Supported in part by Grants No. CA 20180, CA 21765, and CA 49721 from the National Cancer Institute, by the American Lebanese Syrian Associated Charities (ALSAC), and by Children’s Cancer Research Fund.

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0006-4971/95/8605-0039$3.00/0


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RESULTS

Of the 14 cases with a del(11)(q23), the deletion was the only chromosomal abnormality in 7 (nos. 1 through 7); the remainder had additional changes (Table 1), including a t(9;22)(q34;q11) in 2 and a 12p abnormality in 3. The modal number was 46 in all but 3 cases (1 with 45 and 2 with 47 chromosomes). In none of these 14 cases was there cytogenetic evidence of an 11q23 translocation or an interstitial deletion. Although the variability in the breakpoint assignment within this region cannot be defined further with any degree of certainty, 2 cases (no. 3 and 8) might have a more proximal breakpoint at 11q22-23.

The median age of the 8 boys and 6 girls with a del(11)(q23) was 7.5 years at diagnosis (range, 2.3 to 14.7 years). Initial leukocyte counts ranged from 1.2 to 85.7 × 10^9/L (median, 7.5 × 10^9/L). Of the 13 del(11)(q23) cases with complete immunophenotyping, 8 were classified as early pre-B, 2 as pre-B, and 3 as T-cell ALL. CD10 was expressed in all but 2 cases with T-cell ALL. CD15 was expressed in all 3 cases, and CD15 and CDw65 were expressed in the T-cell case. The infant with T-cell ALL had a hematologic relapse and has
Deletion

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Age (yr)</th>
<th>Race</th>
<th>Leukocyte Count (&lt;10^9/L)</th>
<th>Karyotype</th>
<th>MLL Status</th>
<th>Immunophenotype</th>
<th>CD10</th>
<th>CD15</th>
<th>CDw65</th>
<th>Remission Duration (mo)</th>
<th>Type of Failure</th>
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<tbody>
<tr>
<td>1</td>
<td>4.9/W</td>
<td>W</td>
<td>6.4</td>
<td>46,XY,del(11)(q23)(6)/46,XY(14)</td>
<td>ND</td>
<td>Early pre B</td>
<td>+</td>
<td>ND</td>
<td>ND</td>
<td>149+</td>
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<tr>
<td>2</td>
<td>12.9/W</td>
<td>W</td>
<td>11.4</td>
<td>46,XY,del(11)(q23)(8)/46,XY(12)*</td>
<td>ND</td>
<td>T</td>
<td>+ -</td>
<td>ND</td>
<td>37</td>
<td></td>
<td>Kidney</td>
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<tr>
<td>3</td>
<td>2.3/W</td>
<td>W</td>
<td>3.8</td>
<td>46,XY,del(11)(q22)(3)/46,XY(13)*</td>
<td>G</td>
<td>Early pre B</td>
<td>+</td>
<td>-</td>
<td>90+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>4.7/W</td>
<td>W</td>
<td>6</td>
<td>46,XX,del(11)(q23)(9)/46,XX(16)*</td>
<td>G</td>
<td>Pre B</td>
<td>+</td>
<td>-</td>
<td>30+</td>
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</tr>
<tr>
<td>5</td>
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<td>W</td>
<td>52</td>
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<td>+</td>
<td>-</td>
<td>27+</td>
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<tr>
<td>6</td>
<td>14/W</td>
<td>W</td>
<td>18.9</td>
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<td>G</td>
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<td>+</td>
<td>-</td>
<td>20</td>
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<td>CNS</td>
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<tr>
<td>7</td>
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<td>W</td>
<td>76.7</td>
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<td>G</td>
<td>T</td>
<td>-</td>
<td>+</td>
<td>18+</td>
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<td>8</td>
<td>14.7/W</td>
<td>W</td>
<td>85.7</td>
<td>45,XX,inv(5)(p13q14),del(10) (q22),del(11)(q22-23), dic(12;17)(p11;11)(q23)(q21),del(11)(q23)(9)/46,XX(8)*</td>
<td>ND</td>
<td>Early pre B</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>54+</td>
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Inversion

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<tr>
<th>Patient No.</th>
<th>Age (yr)</th>
<th>Race</th>
<th>Leukocyte Count (&lt;10^9/L)</th>
<th>Karyotype</th>
<th>MLL Status</th>
<th>Immunophenotype</th>
<th>CD10</th>
<th>CD15</th>
<th>CDw65</th>
<th>Remission Duration (mo)</th>
<th>Type of Failure</th>
</tr>
</thead>
<tbody>
<tr>
<td>9</td>
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<td>W</td>
<td>7.5</td>
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<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>43+</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>7.5/W</td>
<td>W</td>
<td>3.5</td>
<td>46,XX,del(11)(q23)(2)/46,XX(13)</td>
<td>G</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>38+</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>6.8/W</td>
<td>W</td>
<td>1.2</td>
<td>46,XX,inv(11)(p13q23)(25)</td>
<td>G</td>
<td>Early pre B</td>
<td>+</td>
<td>-</td>
<td>9</td>
<td></td>
<td></td>
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</tbody>
</table>

Abbreviations: G, germline; ND, not done; NA, inadequate sample for markers; W, White; B, Black; kidney, kidney relapse; CNS, CNS relapse; IF, induction failure; H, hematologic relapse.

* Karyotype previously reported.

died, whereas the 2 other children remain in remission for 7+ and 102+ months.

DNA was available for Southern blot hybridization for 11 of 14 cases with del(11) and 2 of 3 cases with inv(11). All 13 cases lacked evidence of a rearranged MLL gene using the cDNA MLL probe with BamHI, HindIII, and Sac I digests (Fig 3). To further exclude an MLL break outside of the region detected by the 0.74-kb MLL probe, membranes with BamHI- or HindIII-digested DNA were stripped and reprobed with 4.2E, p98.40, and ps4. Studies with these three probes likewise showed only germline MLL bands. Slight differences in the amounts of loaded DNA for each patient sample precluded qualitative assessment of the number of MLL copies detected by Southern blot. Densitometric Southern blotting using other 2 copy genes as baseline controls was not performed to search for evidence of deletions of an MLL locus.

**DISCUSSION**

The clinical characteristics of the 14 patients with a del(11)(q23) and the 3 with an inv(11)(p13q23) differed from those of patients with 11q23 translocations. The leukemic cell burden was relatively small; only 4 cases had an initial leukocyte count greater than 50 x 10^9/L and 1 each had CNS involvement or splenomegaly. Moreover, expression of the myeloid-associated CD15 and CDw65 antigens was limited to single cases with a deletion or inversion. T-cell ALL, which is unusual among cases with 11q23 translocations and structural abnormalities affecting the 11q23 region do not appear to confer a poor prognosis unless there is molecular evidence of MLL rearrangement.
Previous molecular studies of cases with a deletion or inversion of the 11q23 region have yielded discrepant results regarding breaks in an MLL locus. Rearrangements of MLL with BamHI digests and probes as used in our cases were reported in 2 infants with M4 and M5 AML and a del(11)(q23) and del(11)(q21), respectively; in 2 cases of acute leukemia with an interstitial deletion of 11q2; in an infant and a 5-year-old child with a del(11)(q23); in an adult with secondary AML and a del(11)(q23.3-q24); in an infant with ALL and del(11)(q23); and in a de novo ALL patient with a del(11)(q23). By contrast, MLL rearrangements were not found in 3 leukemias and 17 lymphomas with other 11q23 abnormalities that included deletions and duplications of 11q23 in one study, 2 infants with an 11q23 cytogenetic alteration, 2 adults with therapy-related AML and a del(11)(q13q23) or del(11)(q14q23), and an inv(11)(p11q23) in a case of Philadelphia-positive chronic myelogenous leukemia. Although it is difficult to reconcile the apparent higher frequency of involvement of MLL in patients in the literature, in some instances these cases may represent undetected translocations. This was demonstrated by Yamamoto et al in a cell line with a del(11)(q23) using conventional cytogenetic analysis, but determined to have an MLL-LTG9 transcript using reverse transcription-polymerase chain reaction. Likewise, Kobayashi et al, using fluorescence in situ hybridization, have shown that 11q23 deletions are mostly caused by undetected translocations.

The molecular probes (MLL, p98.40, ps4, and 4.2E) used in this study are sufficient to detect all described breaks between exons 2 and 18 of MLL. This study does not exclude the possibility of breaks proximal and distal to exons 2 and 18, respectively. It is also possible that the entire MLL locus is lost or that a locus other than MLL is involved in the cases with chromosome 11q23 deletions. Similarly, genes other than MLL within band q23 may be rearranged in the cases with inversions of chromosome 11. Fluorescence in situ hybridization studies with probes to distal and proximal regions of MLL will help in further delineating where the breaks are occurring in these cases.

Infants with ALL and rearrangements of MLL have a poor clinical outcome. The frequency of MLL gene involvement and its effect on prognosis in older children with 11q23 translocations or other 11q23 abnormalities is uncertain. Recent studies have suggested that such rearrangements occur not only in association with the common 11q23 translocations but in other 11q23 anomalies as well. By contrast, we found that childhood ALL with the deletion of 11q23 or a novel inversion (1)(p13q23) lacked MLL gene rearrangement. Furthermore, patients with B-lineage ALL and del(11)(q23) or inv(11)(p13q23) with no molecular evidence of MLL gene rearrangements do not appear to share the unfavorable prognosis of 11q23 translocations that involve MLL.

ACKNOWLEDGMENT

We thank T. Huddleston, P. Mardis, and R.O. Moore for technical assistance; J. Gilbert for scientific editing; and T. McGhee and V. Turner for manuscript preparation.

REFERENCES


Fig 3. Southern blot of BamHI-digested DNA of leukemic marrow cells from patients with del(11)(q23) or inv(11)(p13q23). Lanes labeled “N” and “RS” contain DNA from cell lines with normal 11q23 and t(4;11)(q23), respectively. Other lanes are labeled with the case number according to Table 1. Blots were hybridized with a cDNA probe (MLL) that recognizes sequences between exons 5 and 11 of the MLL gene. The 8.5-kb germline band from normal MLL is indicated by the arrowhead. None of the patient samples shows a rearranged MLL gene. The faint extra band in lane 4 is due to partial digestion of DNA.


arrangement exhibits B lineage and monocytic characteristics. Blood 65:21, 1985
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SC Raimondi, JL Frestedt, CH Pui, JR Downing, DR Head, JH Kersey and FG Behm