7q32-q36 Translocations in Childhood T Cell Leukemia: Cytogenic Evidence for Involvement of the T Cell Receptor β-Chain Gene

By Susana C. Raimondi, Ching-Hon Pui, Frederick G. Behm, and Dorothy L. Williams

Blast cell chromosomal rearrangements involving the long arm of chromosome 7 were identified in eight of 197 cases of childhood acute lymphoblastic leukemia (ALL). Breakpoints were variable but tended to cluster in either the proximal or the terminal 7q region, depending on the immunophenotype of the cells. The 7q32-q36 region, the locus of the T cell receptor β-chain gene, was the site of breakpoints in four of 31 cases of T cell ALL but was not involved in any of the 166 cases originating from B cell precursors (P < .0004). In three of the four T cell cases it was possible to identify the chromosomal segment that had been translocated to the 7q32-q36 region: 1p32, 2p21, and 6p21. The 1p32 and 6p21 bands are particularly interesting, as they contain the sites of two known proto-oncogenes, c-L-myc and hpm2, respectively. Our findings suggest that the locus of the β-chain gene of the T cell receptor is a preferential site for certain chromosomal rearrangements in leukemic T lymphoblasts, analogous to the T cell receptor α-chain gene on human chromosome 14. Translocation of proto-oncogenes to a site near the β-chain regulatory sequences provides a potential mechanism for oncogene activation.

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MATERIALS AND METHODS

Patients. From August 1983 to December 1985, 227 consecutive children with newly diagnosed ALL were admitted to St Jude Children’s Research Hospital. Thirty-three cases had a T cell immunophenotype, as judged from surface antigen reactivity with various T cell-related monoclonal antibodies. One hundred nineteen patients, including 31 with T-ALL, had blast cell metaphase preparations adequate for cytogenetic analysis. All patients were enrolled in studies that included a cell profile analysis, approved by the institution’s clinical trials committee. All patients were advised of the procedures and attendant risks, in accordance with institutional guidelines, and informed consent was obtained in each instance.

Cytogenetic studies. Bone marrow samples, obtained from patients at the time of diagnosis, were processed according to the method of Williams et al.29 Briefly, 0.1 to 0.2 mL of heparinized bone marrow was collected in tubes containing 8 mL of RPMI-1640 with L-glutamine, antibiotics, and 30% fetal calf serum. The cells were processed immediately after a 25-minute incubation in Colcemid (final concentration, 0.06 μg/mL), rinsed twice in Hank's balanced salt solution, and exposed to a hypotonic solution (0.075 mol/L KCl) for a total of 32 minutes at room temperature, including periods of mixing, standing, and centrifugation. The cells were then

From the Departments of Pathology and Laboratory Medicine, and Hematology-Oncology, St Jude Children’s Research Hospital, Memphis, and the Division of Hematology-Oncology, Department of Pediatrics, University of Tennessee School of Medicine, Memphis.

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Address reprint requests to Susana C. Raimondi, MD, Department of Pathology and Laboratory Medicine, Cyogenetics Laboratory, St Jude Children’s Research Hospital, 332 N Lauderdale, PO Box 318, Memphis, TN 38101.

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fixed in methanol-acetic acid (3:1, vol/vol) for 15 minutes. Slides were prepared with use of a flaming technique and were allowed to age for zero to seven days. Metaphase preparations were G-banded by a modification of the trypsin method of Seabright, and chromosomes were identified and assigned according to the International System of Human Cytogenetic Nomenclature.

Immunophenotyping. Blast cells were separated on a density gradient, and only samples containing >85% blasts were assayed. Cell surface antigens were detected by a standard indirect immunofluorescence assay that relied on monoclonal antibodies to the major clusters of differentiation (CD) antigens described by the First and Second International Workshops on Human Leukocyte Differentiated Antigens. These included J5 (CD-10), anti-HLA-DR, OKT10, OKT9, 3A1 (CD-7), T101 (CD-5), T11 (CD-2), T3 (CD-3), T6 (CD-1), T4 (CD-4), T8 (CD-8), and B4 (CD-19). Two hundred cells were counted in each immunofluorescence assay; the results were considered positive if 40% or more of the blasts showed surface fluorescence. Cell surface immunoglobulin (sIg) was identified by a direct immunofluorescence assay with fluoresceinated goat anti-human immunoglobulin (Tago, Burlingame, CA). Cytoplasmic immunoglobulin (cIg) was identified by a direct method that used fluoresceinated F(ab')2 goat anti-human μ-chains (Southern Biotechnology, Birmingham, AL). Terminal deoxynucleotidyl transferase (TdT) was identified by use of an indirect immunofluorescence assay (Supertechs, Inc, Bethesda, MD). The samples were classified phenotypically according to the patterns of reactivity as T cell (T+, E rosette+, HLA-DR+), common (CALLA+, HLA-DR+, E+, T+, cIg+, sIg+), pre-B cell (cIg+, HLA-DR+, CALLA+, E+, T+), or B cell (sIg+).

RESULTS

Chromosomal rearrangements involving the 7q arm were found in blast cells from eight of the 197 cases studied. The breakpoints in four of these cases occurred in the proximal 7q region (two at 7q11 and two at 7q22), while in the remainder they were within the terminal 7q region (one at 7q32 and three at 7q36). The former group comprised three cases of common ALL and one of pre-B-ALL, whereas each of the latter four was classified as T cell ALL: common or mid-thymocyte stage (cases 1 and 3) and immature or early thymocyte stage (cases 2 and 4) (Table 1). Thus the terminal 7q region was involved in translocations within T lymphoblasts but not in those within non-T cells (four of 31 cases vs zero of 166, P < 0.0004 by two-tailed Fisher’s exact test). In T cell cases, the DNA material exchanged with the 7q32-q36 region was different in each case (Fig 1 and Table 2), and none of the additional structural or numerical chromosomal abnormalities was associated with the T cell phenotype.

Table 1. Clinical and Laboratory Data for Patients With T Cell Leukemia and 7q32-q36 Translocations

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age (yr)</th>
<th>Sex</th>
<th>Mediastinal mass</th>
<th>Spleen size (cm)*</th>
<th>Liver size (cm)*</th>
<th>Hemoglobin (g/dL)</th>
<th>Leukocytes (x 10^9/L)</th>
<th>Platelets (x 10^9/L)</th>
<th>CNS leukemia</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>17</td>
<td>M</td>
<td>Yes</td>
<td>1</td>
<td>1</td>
<td>16.2</td>
<td>12.4</td>
<td>278</td>
<td>Yes</td>
</tr>
<tr>
<td>2</td>
<td>14</td>
<td>F</td>
<td>Yes</td>
<td>10</td>
<td>9</td>
<td>12.8</td>
<td>233.5</td>
<td>45</td>
<td>Yes</td>
</tr>
<tr>
<td>3</td>
<td>9</td>
<td>M</td>
<td>Yes</td>
<td>7</td>
<td>6</td>
<td>12.8</td>
<td>233.5</td>
<td>551</td>
<td>Yes</td>
</tr>
<tr>
<td>4</td>
<td>9</td>
<td>M</td>
<td>Yes</td>
<td>10</td>
<td>6</td>
<td>12.8</td>
<td>233.5</td>
<td>92</td>
<td>No</td>
</tr>
</tbody>
</table>

*Edge palpable below the costal margin.
† Cases were considered positive if >40% of the cells reacted with the probe for a particular marker. The source of blasts was bone marrow from patients 1, 2, and 4 and pleural fluid from patient 3.

Table 2. Blast Cell Karyotypes

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Karyotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>46,XY,t(1;7)(p32;q32),del(9)(p22)</td>
</tr>
<tr>
<td>2</td>
<td>46,XY,t(2;7)(p12;q36),del(5)(p14),del(9)(p21)</td>
</tr>
<tr>
<td>3</td>
<td>47,XX,t(6;7)(p21;q36),+der(7)t(7;16)(p14;14)</td>
</tr>
<tr>
<td>4</td>
<td>44,XY,—5,—10,dup inv(1)p32p13,t(6;?)</td>
</tr>
</tbody>
</table>

Fig 1. Different abnormalities involving the 7q32-q36 region in cases of T cell leukemia. A through D show partial G-banded karyotypes of patients 1 through 4. The normal chromosomes of each pair are shown on the left and those for the rearranged chromosomes are shown on the right. Arrows indicate breakpoint sites.
numerical abnormalities were alike except for a deletion of the p arm of chromosome 9 in cases 1 and 2.

The clinical and laboratory features of patients with T cell ALL and a 7q32-q36 translocation are summarized in Table 1. These three boys and one girl ranged in age from 9 to 17 years (median, 11.5 years). All four had a mediastinal mass, and two had central nervous system leukemia at diagnosis. Patient 1 also presented with multiple scalp nodules and right facial paralysis, and patient 4 had an epidual tumor, resulting in paraparesis at diagnosis. Three of the patients entered complete remission and two remain free of leukemia for 10+ and 28+ months. Patient 3, who failed to achieve remission, was unusual in that there was a “lineage switch” from T cell ALL to acute myeloblastic leukemia after 3 weeks of induction therapy. At that time, cytogenetic analysis disclosed only normal diploid karyotypes.

**DISCUSSION**

Numerical abnormalities of chromosome 7 are common in hematologic disorders, but structural abnormalities of the 7q arm are identified much less often.3,4 Cytogenetic analysis of 197 well-banded consecutive cases of childhood ALL in this study disclosed eight cases with rearrangements of the 7q arm. Breakpoints were variable but tended to show specificity for the immunophenotype of blast cells. In cases of T cell disease, breakpoints were restricted to the terminal 7q region (one at 7q32 and three at 7q36), whereas in B cell-precursor cases, they occurred in the proximal 7q region (two at 7q11 and two at 7q22).

The 14q11-q12 region, which contains the TCR γ-chain gene,5-11 appears to be nonrandomly rearranged and presumably is involved in proto-oncogene activation in T cell neoplasias.13,18-22 Therefore, the 7qter region (to which the TCR β-chain gene has been mapped) has received less attention as an important site of chromosomal abnormalities in T cell malignancies.13,38-37 Studying the blast cell chromosomes of a large number of childhood ALL cases has allowed us to demonstrate that 7qter abnormalities represent yet another subgroup of cytogenetic abnormalities that correlates with a specific immunophenotype of ALL. In two of our cases, there was a loss of chromosome material from the short arm of chromosome 9(p21-p22), a finding previously reported in T cell lines,37 as well as a distinct subtype of ALL characterized by “lymphomatous” features and presumably of T cell origin.38 However, in a collaborative study, this chromosomal abnormality was found to bear no consistent relationship with blast cell immunophenotype or other presenting features.39

Chromosome bands involved in each of the reciprocal translocations with the 7q32-q36 region were 1p32, 2p21, and 6p21. The 1p32 belongs to the region to which c-L-myc40 has been mapped. Although no specific proto-oncogenes have been identified at 2p21, Brownell et al41 have recently isolated and localized human cellular rel sequences to chromosome 2. The related viral sequence, r-ref, was derived from the cellular genome of a turkey with leukemia induced by the reticuloendotheliosis virus. Finally, the oncogene hpim is a human homolog of a murine gene that is activated by retroviral insertion in murine T cell lymphomas. It seems reasonable to suggest that the c-L-myc, hpim, and other putative proto-oncogenes are deregulated as a consequence of their translocation to a position near the TCR β-chain gene and its regulatory sequences. Whether or not the gene encoding the β-chain of the TCR is involved in the genesis of childhood T cell ALL needs to be tested by molecular analysis of the DNA adjacent to breakpoints of the 7q32-q36 translocations.

**NOTE ADDED IN PROOF**

Since initial submission of the manuscript we have identified another T cell ALL case with a t(6;7)(q24;q36), strengthening the association of the 7qter region with T cell malignancies.

**ACKNOWLEDGMENT**

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