Near-Haploid Acute Lymphoblastic Leukemia: A Unique Subgroup With a Poor Prognosis

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We describe two adolescent girls with acute lymphoblastic leukemia (ALL) whose leukemia cells were near-haploid. Their lymphoblasts stained in a block pattern with periodic acid Schiff and had “common ALL” surface markers confirmed by indirect immunofluorescence. Each patient had two populations of blasts, one near-haploid and one hyperdiploid, which was an exact doubling of the near-haploid karyotype. The first patient had a predominant population of cells with 26 chromosomes and a few with 52, while the second had a predominance of cells with 56 and a minority with 28. Flow cytometric analysis of DNA content initially detected the minor near-haploid population.

Despite considerable progress in treatment of childhood acute lymphoblastic leukemia (ALL), 30%-50% of patients still do not survive more than 5 yr from diagnosis. Recent emphasis has been placed on the identification of clinical and laboratory features associated with a poor prognosis so that patients at high risk of relapse can be identified and treated with potentially more effective therapy. Two cytogenetic features of leukemia cells at diagnosis have been noted to have prognostic significance: the Philadelphia chromosome (Ph'), which represents a distinct subgroup of ALL with a poor prognosis, and the 14q+ marker chromosome, which usually occurs in B-cell ALL and signifies a poor outcome. No other cytogenetically and prognostically distinct subgroups have yet been identified. We report two patients with ALL whose leukemia cells were near-haploid. Analysis of these patients, as well as four previously reported patients with near-haploid ALL, suggest that this is a cytogenetically recognizable subgroup of ALL with similar clinical characteristics and a poor prognosis.

CASE REPORTS

Patient 1

A 17-yr-old white girl was referred to St. Jude Children’s Research Hospital (SJCRH) and had a 4-wk history of fatigue, migratory bone pain, and weight loss. Physical findings included hepatosplenomegaly and submandibular lymphadenopathy. Laboratory features at diagnosis are listed in Table I. A bone marrow aspirate showed marrow replacement by a heterogeneous population of lymphoblasts of normal size, with prominent cytoplasmic vacuolization (L-2 morphology by FAB classification). Seventy-one percent of the blasts stained in a block pattern with periodic acid Schiff (PAS). The results of studies with peroxidase, Sudan black, and esterase stains were negative. Marrow blasts did not spontaneously form rosettes with sheep erythrocytes (E) or express surface immunoglobulin but did react with heteroantisera against Ia and “common ALL” antigens. A chest radiograph and cerebrospinal fluid (CSF) examinations were normal.

After a diagnosis of ALL was made, bone marrow remission was induced by a standard induction therapy with prednisone, vincristine, and daunomycin. Central nervous system (CNS) prophylaxis, consisting of 2400 rads of cranial radiation and 5 doses of intrathecal methotrexate, was then administered and the patient began maintenance therapy with daily mercaptopurine and weekly methotrexate. She did well on this treatment until the fifth month when pallor, bone pain, splenomegaly, and circulating blasts returned. The blasts were morphologically identical to those at diagnosis and reacted with the common ALL antiserum but did not react with Ia antiserum. Reinduction with conventional and experimental drugs failed, and she died 11 mo from diagnosis.

Patient 2

An 11-yr-old white girl was referred to SJCRH after a 2-wk history of pallor, fatigue, and sore throat. Physical examination revealed hepatosplenomegaly (5 cm each) without appreciable lymphadenopathy. Laboratory features at diagnosis are presented in Table I. Bone marrow studies were diagnostic of ALL; aspirates showed total marrow replacement with small homogeneous lymphoblasts (L-1 type), 95% of which stained for PAS. The results of studies with peroxidase, Sudan black, and esterase stains were negative. Surface marker studies of marrow blasts were negative for E-rosette formation and surface immunoglobulin but positive with heteroantisera against Ia and common ALL antigens. A chest radiograph and CSF studies were normal.

Bone marrow remission was achieved with a standard 4-wk regimen of prednisone, vincristine, and t-asparaginase. Since induction was complicated by hyperglycemia secondary to prednisone and t-asparaginase, the patient was hospitalized and given insulin. CNS prophylaxis with 1800 rads (12 fractions) and 5 doses of intrathecal methotrexate was administered, and this patient is now receiving daily mercaptopurine and weekly methotrexate at month 6 of remission.
Table 1. Clinical and Laboratory Findings at Diagnosis for Six Patients With Near-Haploid ALL

<table>
<thead>
<tr>
<th>Reference</th>
<th>Age (yr)</th>
<th>Sex</th>
<th>WBC (cells/µl)</th>
<th>Hb (g/dl)</th>
<th>Platelets (Cells/µl)</th>
<th>Marrow (% Blasts)</th>
<th>PAS</th>
<th>Surface Markers</th>
<th>Complete Remission (mo)</th>
<th>Survival (mo)</th>
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<tr>
<td>Our patient 1</td>
<td>17</td>
<td>F</td>
<td>34,000</td>
<td>12.5</td>
<td>155,000</td>
<td>98</td>
<td>Pos</td>
<td>Common</td>
<td>5</td>
<td>11</td>
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<td>Our patient 2</td>
<td>11</td>
<td>F</td>
<td>3,500</td>
<td>10.7</td>
<td>22,000</td>
<td>99</td>
<td>Pos</td>
<td>Common</td>
<td>6+</td>
<td>7+</td>
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<td>7</td>
<td>14</td>
<td>M</td>
<td>57,700</td>
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<td>100</td>
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<td>ND</td>
<td>0</td>
<td>5</td>
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<tr>
<td>8</td>
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<td>15,000</td>
<td>91</td>
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<td>ND</td>
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<td>9</td>
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<td>F</td>
<td>87,000</td>
<td>8.5</td>
<td>50,000</td>
<td>100</td>
<td>ND</td>
<td>Common</td>
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<td>9</td>
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<tr>
<td>11</td>
<td>17</td>
<td>F</td>
<td>13,800</td>
<td>6.7</td>
<td>37,000</td>
<td>96</td>
<td>Neg</td>
<td>Non-T, non-B†</td>
<td>16</td>
<td>31</td>
</tr>
</tbody>
</table>

*Not determined.
†No evidence of spontaneous rosette formation with sheep erythrocytes or of surface immunoglobulin detected by indirect immunofluorescence.

MATERIALS AND METHODS

Cyto genetic Studies

Bone marrow samples for cytogenetic analysis were obtained from patient 1 at diagnosis and relapse, and from patient 2 at diagnosis. Samples were prepared for cytogenetic analysis according to a modification of the technique of Tjio and Whang. A modified trypsin-Giemsa technique was used for chromosome banding.

Flow Cytometry

Samples of bone marrow from the posterior iliac crest were aspirated into a syringe that contained 3.8% sodium citrate anticoagulant. A small volume of this marrow suspension was added directly to a solution of propidium iodide (0.05 mg/ml) and 0.1% sodium citrate to achieve a final dilution of 10⁶ white cells/ml. Normal blood samples were prepared by adding 50 µl of fresh capillary blood directly to 1 ml of propidium iodide solution. The blood or marrow samples in propidium iodide solution were allowed to stain for 10 min at 4°C, and were then stored at 4°C and analyzed within 24 hr. Approximately equal numbers of stained normal blood cells and stained leukemia cells were mixed for use as a reference standard to identify the modal fluorescence of normal G0/G1-phase cells relative to that of the leukemia cells.

The relative fluorescence intensities of cells stained with propidium iodide were analyzed by a Coulter model TPS-1 cell sorter (Coulter Electronics, Inc., Hialeah, Fla.). In this instrument, single cells in suspension were exposed to a focused argon-ion laser beam (488 nm), and fluorescence from the laser excitation of the propidium iodide-DNA complex in each cell was quantitated after passing through a system of filters and photomultipliers. Measurements of the relative fluorescence intensities of all cells were recorded in the form of a frequency histogram. These data were transferred directly to a Data General Eclipse computer, and plotted as a frequency histogram with an abscissa that showed units of the DNA index (DI, or the fluorescence intensity of each cell, divided by the modal fluorescence intensity of normal G0/G1-phase blood cells). The DNA content of 50–100,000 cells was measured for each histogram, at a rate of 500 cells/sec.

RESULTS

Cyto genetic Findings

Banded chromosomes of the leukemia cells from the bone marrow of patient 1 were analyzed at diagnosis and at relapse. Of the original 28 metaphases analyzed, 24 metaphases contained 26 chromosomes, 2 had 25 chromosomes, 1 had 44 chromosomes, and 1 had 52 chromosomes. At relapse, 27 metaphases were analyzed. Twenty-six metaphases had 26 chromosomes and 1 had 46 chromosomes. The metaphases with 44 or 46 chromosomes appeared to be normal cells or cells with random chromosome loss that were derived from normal cells. At both diagnosis and relapse, an identical clone of 26 chromosomes predominated. The stem-line karyotype with 26 chromosomes differed from a haploid karyotype (one each of chromosomes 1 to 22 and X) in that chromosomes 14, 21, and X were in two copies. The metaphase with 52 chromosomes could not be completely analyzed, but probably represented a doubling of the 26-chromosome karyotype. A representative near-haploid karyotype is shown in Fig. 1.

Forty metaphases from the bone marrow of patient 2 were analyzed at diagnosis; 4 contained 46 chromosomes and 36 contained 55–56 chromosomes with the modal number 56. However, 4 additional metaphases with 28 chromosomes were found after the results from flow cytometric analysis of DNA content were known. The metaphases with 28 chromosomes had a haploid karyotype plus 5 extra chromosomes: +2C, +D, +E, +G, identified by banding as chromosomes 10, 14, 18, 21, and X. The metaphases with 56 chromosomes were an exact doubling of the near-haploid karyotype.

Fig. 1. Karyotype of the lymphoblasts from patient 1. The karyotype consists of 26 chromosomes: a haploid set (one copy each of chromosomes 1 to 22 and X) with an extra copy of chromosomes 14, 21, and X.
Flow Cytometry

DNA histograms of the leukemia cells from both patients were analyzed at relapse (patient 1) or diagnosis (patient 2). The DNA histogram of bone marrow lymphoblasts from patient 1 is shown in Fig. 2. The main G0/G1-phase peak of leukemia cells had a DI of 0.58 and represented the predominant malignant clone with only 26 chromosomes. A smaller peak of cells in this histogram had a DI of 1.0 and thus represented a small proportion of normal marrow cells in G0/G1 phase. There was no evidence of a peak of cells with a DI of 1.16, which would have indicated that another clone of leukemia cells had resulted from the duplication of the near-haploid karyotype.

Fig. 2. The DNA histogram of marrow lymphoblasts from patient 1 at relapse shows a peak with a modal DI of 0.58, corresponding to the G0/G1-phase leukemia cells with a karyotype of 26 chromosomes. Leukemia cells in the DNA synthesis phase of the cell cycle have increasing levels of DNA and are evident at DI > 0.58. The small peak of cells with a DI mode of 1.0 represents residual normal G0/G1-phase cells in the marrow.

Fig. 3. The bone marrow DNA histogram of lymphoblasts from patient 2 at diagnosis shows a small peak with a DI mode of 0.59. This peak represents the G0/G1-phase cells of the near-haploid leukemia clone with 28 chromosomes per cell. The large peak of cells with a DI mode of 1.19 represents G0/G1-phase cells from the hyperdiploid leukemia clone that resulted from doubling of the near-haploid karyotype. The skewed region of this peak (DI of 1.0) corresponds to residual normal cells in this leukemic marrow sample.
The DNA histogram from the bone marrow lymphoblasts of patient 2 at diagnosis was more complex (Fig. 3). In this histogram, the small peak with a DI of 0.59 represented the G0/G1-phase cells of the near-haploid clone. By integrating the total number of cells in the histogram, we found that this population of near-haploid cells represented 4% of the total marrow cell population. The major peak on the histogram with a DI of 1.19 represented the predominant hyperdiploid clone, which was an exact doubling of the near-haploid clone. The left skew of this peak at DI = 1.0 was due to normal G0/G1-phase cells that represented 3% of the total marrow cell population.

**DISCUSSION**

Since hypodiploidy is noted in only 1%-3% of patients with ALL, near-haploidy appears to be extremely rare.4,5 Only four cases of near-haploid ALL have been previously reported.11 The clinical, laboratory, and cytogentic features of our two patients are strikingly similar to the four patients reported so far (Tables 1, 2). Five of the six patients were girls and five were adolescents (median age 13 yr, range 5-17 yr). Morphologically, their cells were typical lymphoblasts, usually with scant cytoplasm and a coarse chromatin pattern. At least four patients had blasts that were PAS positive, and the four patients tested had a non-T, non-B (1 patient) or common ALL surface marker pattern (3 patients).

The stem line in the six patients was near-haploid with a mode of 26-28 chromosomes and had a nonrandom pattern of abnormalities. In addition to having a haploid karyotype (one each of chromosomes 1 to 22 and X), all six had disomy for chromosome 21, with or without disomy for chromosome 10, 14, 18, or X (4 cases each, see Table 2). The mechanisms that might be responsible for near-haploidy have been discussed by Oshimura et al.9 Of the several mechanisms proposed, the most feasible is multipolar mitosis.

It is remarkable that cells with a near-haploid karyotype are capable of survival and proliferation. Indeed the near-haploid cells from one patient were established as a cell line.10 This cytogenetic abnormality may not be peculiar to ALL, since there are at least three additional cases of near-haploidy in lymphoid blast crisis of Ph1-positive chronic myelogenous leukemia in adults.18-20 The karyotypes of these cases were similar to those reported here, except that all three retained the translocation between chromosomes 9 and 22.

It is not known whether malignant transformation precedes or results from the near-haploid state. However, a near-haploid genome would permit the expression of recessive genes that might result in loss of regulatory control of lymphoid growth and differentiation, leading to malignant transformation. Regardless of the sequence of events that establish the near-haploid stem line, subclones with a doubling of the near-haploid karyotype may frequently result. Five of the six patients had a second, hyperdiploid population (52-56 chromosomes) with an exact doubling of the near-haploid karyotype at some time during the disease course. That the hyperdiploid clone predominated at diagnosis in two patients suggests that the transition from near-haploidy to hyperdiploidy may be not only frequent but also early.

Since hyperdiploidy is a common finding at the time of diagnosis of ALL, it is possible that it may sometimes reflect the early clonal evolution from near-haploid stem lines and that near-haploid ALL may be more frequent than is currently recognized. The near-haploid origin of hyperdiploid stem lines could be detected by close inspection of banded preparations for homologous expression of polymorphism or for the presence of even numbers of all chromosomes, especially marker chromosomes. However, the most definitive evidence would be the presence of near-haploid and hyperdiploid clones on cytogenetic analysis or the detection of both clones by flow cytometric analysis of DNA content, as in our second patient.

The advantages of flow cytometry for the analysis of DNA content in acute leukemia cells were reported by Barlogie and coworkers for a large series of adult patients.21 These authors found that DNA flow cytometry was a sensitive and reliable means of detecting

**Table 2. Cytogenetic Features of Bone Marrow Cells at Diagnosis for Six Patients With Near-Haploid ALL**

<table>
<thead>
<tr>
<th>Reference</th>
<th>Near-Haploid Karyotype</th>
<th>Percent Near-Haploid</th>
<th>Percent Diploid</th>
<th>Percent Hyperdiploid†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Our patient 1</td>
<td>26, XX, +14, +21</td>
<td>92</td>
<td>4</td>
<td>4</td>
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<tr>
<td>Our patient 2</td>
<td>28, XX, +10, +14, +18, +21</td>
<td>9</td>
<td>9</td>
<td>82</td>
</tr>
<tr>
<td>7</td>
<td>26, XY, +D(14), +G(21)</td>
<td>98</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>8</td>
<td>27, XX, +10, +18, +21</td>
<td>58</td>
<td>42</td>
<td>0</td>
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<tr>
<td>9</td>
<td>27, X, +10, +14, +18, +21</td>
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<td>79</td>
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<tr>
<td>11</td>
<td>28, XX, +6, +10, +18, +21</td>
<td>70</td>
<td>10</td>
<td>20</td>
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</table>

*Karyotype is expressed relative to a haploid karyotype (23 chromosomes): one each of chromosomes 1 to 22 and X.
†In each case, the hyperdiploid mode represented an exact doubling of the near-haploid mode.
clones with abnormal DNA content, provided that the number of chromosomes was sufficiently abnormal (<45 or >52 chromosomes). They also noted that flow cytometry could measure abnormalities of DNA content in G0/G1-phase cells, independent of proliferation that is required for karyotyping. Further, there was surprising agreement between the number of extra chromosomes in the leukemia cell karyotype and the increase in DNA content measured by flow cytometry.

Improved detection of near-haploid ALL may be important to clinical as well as biologic studies, since this subgroup appears to be associated with a poor prognosis. Unlike the poor prognostic group of T-cell ALL, which occurs more frequently in older boys,2 near-haploid ALL is associated with the common ALL phenotype and appears to be more frequent in adolescent girls. For the five patients with adequate follow-up, the median remission duration was 6 mo and the median survival was 10 mo. Both median durations are considerably shorter than those reported for children with ALL treated with modern therapy.1

In addition, four of the six patients had a white cell count of >30,000/μl. It is possible that near-haploidy predisposes to a high proliferative capacity and an increased white count. It is not known yet whether near-haploidy or increased white count (or both) could be responsible for the early relapses that typify this subgroup. It will be necessary to identify and follow additional patients to confirm the predominant clinical and biologic features of near-haploid ALL. If such patients consistently respond poorly to conventional therapy, alternate therapy might be considered—e.g., more intensive treatment or bone marrow transplantation, as is being done for other subgroups of ALL with a poor prognosis. In one patient with the longest survival, the reappearance of a near-haploid cell was noted in a hematologically normal marrow after a 3-mo remission.11 The change in therapy at that time may have extended her remission.

Near-haploid ALL appears to be a clinically and biologically unique subgroup of ALL, detectable by karyotype analysis, similar to the subgroup of Ph'-positive ALL. Near-haploid ALL also can be detected by flow cytometry, even when the duplicated, hyperdiploid subclone is predominant. Flow cytometry is a more sensitive means of detecting small populations of aneuploid cells, and chromosome analysis is necessary to determine the specific numerical and structural rearrangements resulting in aneuploidy. These two laboratory methods are complementary and should give important information concerning the biology of near-haploid ALL and other recognizable subgroups of acute leukemia. For these reasons we are including both methods in the initial evaluation of patients with ALL.

REFERENCES

Cytometry and Sorting. New York, John Wiley & Sons, 1979, p 669


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GM Brodeur, DL Williams, AT Look, WP Bowman and DK Kalwinsky