Philadelphia Chromosome-positive Acute Lymphoblastic Leukemia Cell Lines without Classical Breakpoint Cluster Region Rearrangement

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ABSTRACT

The Philadelphia (Ph) chromosome translocation which is classically observed in chronic myeloid leukemia (CML) is sporadically found in acute lymphoblastic leukemia (ALL). In CML the translocation breakpoint on chromosome 22 is within the breakpoint cluster region, while in childhood ALL, no detectable change in breakpoint cluster region is routinely observed. In order to investigate the nature of this difference, we have established and characterized two cell lines from a child with Ph positive ALL. The cell lines have retained the cytochemical staining pattern, enzyme activity, monoclonal antibody profile, and immunoglobulin gene rearrangements of the patient's malignant cells. The cell lines had the same Ph translocation (9;22) (q34;q11) as the child's malignant cells along with additional chromosome changes. Southern blot analysis showed that the Ph translocation did not involve the 5.8-kilobase breakpoint cluster region segment characteristically seen in CML. The cell lines reported here will be a valuable resource in ascertaining the biological significance of the Ph translocation seen in ALL.

INTRODUCTION

Cytogenetic studies have shown a characteristic translocation between chromosomes 9 and 22 leading to the Ph chromosome in greater than 90% of cases of CML (1, 2). This translocation occurs specifically between the c-abl gene on chromosome 9 and a region known as bcr on chromosome 22 (3). Cells containing this translocation produce a chimeric transcript and express a fusion protein with tyrosine kinase activity which is possibly involved in the pathogenesis of CML (4–6).

A Ph translocation is found in approximately 10% of children with ALL and is cytogenetically indistinguishable from that found in CML (7). Several cases of Ph positive ALL have been examined for the characteristic rearrangement of bcr. It has been found that, while there is considerable heterogeneity of the chromosomal rearrangements, most chromosomal translocations found in childhood Ph positive ALL do not occur within bcr (8, 9).

We report here characterization of 2 cell lines derived from a child with Ph positive ALL. The cell lines possess the same cytochemical staining pattern, biochemical enzyme levels, monoclonal antibody profile, and immunoglobulin gene rearrangements as the patient's malignant cells. While the cell line's karyotype demonstrates the Ph (and other chromosomal alterations), neither the cell lines nor the patient's malignant cells have a rearrangement at bcr. Since the cell lines carry the Ph, molecular studies of the genes located at the chromosomal translocation breakpoints may be useful in elucidating the nature of Ph positive ALL.

MATERIALS AND METHODS

Case Report. An 8-yr-old boy presented to the Children's Hospital at Stanford in August 1983 with bone pain, fever, and anemia. The bone marrow aspirate was hypercellular with 97% lymphoblasts, and the cytochemical stains and immunoperoxidase confirmed the diagnosis of acute lymphoblastic leukemia. A complete remission was induced with chemotherapy, but the child subsequently relapsed and died with progressive leukemia 26 mo after diagnosis.

Source of Malignant Cells. Malignant cells were collected from the first and second bone marrow relapse and separated into aliquots for cell culture experiments, immunophenotyping, enzyme studies, karyotyping, and immunogenotyping. The protocol procedures were approved by the Medical Committee for the Use of Human Subjects in Research at Stanford University, and informed consent was obtained from the patient's parents.

Establishment and Maintenance of the Cell Lines. Methods for cultivating lymphoblasts were a modification of our previously reported methods (10). Briefly, lymphoblasts were mixed with agar (0.3%), plated onto Petri dishes (which contained a feeder layer), and cultured at 37°C in an incubator gassed with 5% O2, 6% CO2, and 89% N2.

Characterization of Cell Antigens. Cell surface antigens were identified by the binding of monoclonal antibody as detected by indirect immunofluorescence by fluorescence-activated cell sorting (11). The presence of cytoplasmic μ was determined by a standard technique using fluorescein-conjugated goat anti-human μ antibody (Southern Biotech, Birmingham, AL) (12).

Enzyme Analysis of Malignant Cells from Patient and Cell Lines. ADA, NP, and TdT activities were measured according to established methods (13).

Molecular Genetic Studies. High-molecular-weight DNA was isolated from each cell line and bone marrow from the second relapse and treated with restriction enzymes, and Southern analysis was done (14). The 3' terminal bcr probe (Oncogene Science), which detects bcr gene rearrangements in almost all cases of Ph-positive CML (3), and an immunoglobulin JH fragment, which detects rearrangements at the heavy-chain immunoglobulin genes (15), were used as probes. DNA from the patient's marrow, the cell lines, and positive and negative controls were analyzed for the presence of the EBV genome using a probe for the BamV fragment of EBV (16).

RESULTS

Cell Culture and Cytochemical Stains. The bone marrow cells were plated in agar as a single cell suspension, and after 3 wk in culture, multiple colonies were observed (cloning efficiency, 0.002%). Colony cells were gradually weaned to suspension culture and have been growing continuously for over 18 mo. The SUP-B13 cell line was generated from the patient's first bone marrow relapse and has a doubling time of approximately 35 h. The SUP-B15 cell line was grown from the patient's second bone marrow relapse and has a doubling time of approximately 18 h.
Table 1 Reactivity of monoclonal antibodies with the patient's malignant cells and the SUP-B13 and SUP-B15 cell lines

<table>
<thead>
<tr>
<th>Antibodies</th>
<th>Bone marrow cells</th>
<th>SUP-B13</th>
<th>SUP-B15</th>
</tr>
</thead>
<tbody>
<tr>
<td>β2M</td>
<td>++++</td>
<td>++++</td>
<td>++++</td>
</tr>
<tr>
<td>HLA-DR</td>
<td>++++</td>
<td>++++</td>
<td>++++</td>
</tr>
<tr>
<td>CALLA</td>
<td>++++</td>
<td>++++</td>
<td>++++</td>
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<tr>
<td>Leu 12</td>
<td>++++</td>
<td>++++</td>
<td>++++</td>
</tr>
<tr>
<td>CB1</td>
<td>++++</td>
<td>++++</td>
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<td>OKT 9</td>
<td>-</td>
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<tr>
<td>OKT 10</td>
<td>-</td>
<td>++++</td>
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<tr>
<td>Leu M1</td>
<td>ND*</td>
<td>++++</td>
<td>-</td>
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<tr>
<td>My 7</td>
<td>ND</td>
<td>-</td>
<td>+</td>
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<tr>
<td>My 9</td>
<td>ND</td>
<td>-</td>
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<td>Slg</td>
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<tr>
<td>clg</td>
<td>++++</td>
<td>++++</td>
<td>++++</td>
</tr>
<tr>
<td>Leu 1, 2, 3, 4, 5, 6, 9</td>
<td>-</td>
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</tr>
</tbody>
</table>

*+++*, >80% of cells stain positive; ++++, 60 to 79% of cells stain positive; +++, 40 to 59% of cells stain positive; ++, 20 to 39% of cells stain positive.

ND, not done; Slg, surface immunoglobulin; clg, cytoplasmic immunoglobulin.

The cell lines as well as the patient’s diagnostic bone marrow cells stained positive for acid phosphatase, while the periodic acid-Schiff, nonspecific esterase, chloroacetate esterase, and Sudan black stains were negative. The morphology (French-American-British classification) of the bone marrow blast cells was 82% L1 and 18% L2, while both cell lines had exclusively L2 morphology.

Cellular Antigens. The cell lines were concordant with the patient’s tumor in 13 of 16 paired antigens tested (Table 1). The bone marrow and cell lines expressed multiple B-lineage markers including CALLA but lacked T-cell markers (Table 1). There was discordance between the patient’s tumor and the cell lines in the expression of CB1, OKT9, and OKT10 antigens. Cells were immunologically classified as pre-B-cells because the cells expressed cytoplasmic µ but lacked surface immunoglobulin (Table 1). Each cell line expressed some myeloid antigens; however, the cell lines differ in that SUP-B13 expressed LeuM1 and My9 but lacked My7, while SUP-B15 expressed My7 but lacked LeuM1 and My9.

Enzyme Analysis. The ADA level on the patient’s bone marrow, SUP-B13, and SUP-B15 cells was 20, 17.1, and 17.5 enzyme units/mg of protein, while NP levels were 0.35, 0.46, and 0.27 enzyme units/mg of protein, respectively. Previously, normal lymphocytes had been shown to possess ADA levels of 5.4 ± 1.2 enzyme units/mg of protein with the NP level of 0.36 ± 0.05 enzyme units/mg of protein (17). The normal NP and elevated levels of ADA are characteristic of immature B-lymphoid malignancies (13, 17). In addition, the cells stained positively for TdT.

Karyotype Analysis. Cytogenetic analysis on the patient’s bone marrow cells at the time of presentation showed: 46,XY (8 cells); 46,XY,t(9;22)(q34;q11) (2 cells). The karyotypes of the bone marrow cells at relapse showed: 46,XY (6 cells); 46,XY,q(9;22)(q34;q11)(1;4p22;q33)der(14q), 16p+.
were not infected with EBV.

Additionally, molecular probing showed that these cell lines established from a child with Philadelphia chromosome-positive ALL cell lines: SUP-B13 and SUP-B15. The cell lines were derived from the predominant malignant cells in the bone marrow and they were clonal. The cell lines did not contain a germ line DNA band, suggesting that the other copy of the J<sub>m</sub> region had been deleted. The faintly visible germ line band in the bone marrow is probably due to normal non-malignant bone marrow cells.

A Southern blot using an immunoglobulin probe showed a rearranged band in the bone marrow cells that comigrated with the band seen in the cell lines. These data demonstrate that the cell lines were derived from the predominant malignant cells in the bone marrow and that they were clonal. The cell lines did not contain a germ line DNA band, suggesting that the other copy of the J<sub>m</sub> region had been deleted. The faintly visible germ line band in the bone marrow is probably due to normal non-malignant bone marrow cells.

Although the cells in the bone marrow and the two cell lines contain a characteristic Ph translocation by cytogenetic analysis, molecular analysis with a 3' bcr probe did not show that the translocation involves the bcr region as is seen in Ph chromosome-positive CML. The bcr region is within the phi gene (formerly called the bcr gene), the full extent of which has not been mapped but is estimated to span more than 45 kilobases (19, 20). Recently, it has been shown that the 3' bcr probe fails to detect rearrangements that occur at bcr in 10 to 20% of Ph chromosome-positive leukemias. This results from deletions of the 3' end of bcr which occur during the translocation process (21, 22). Since we used only the 3' end bcr probe in this study, there is a possibility that a rearrangement at bcr was present but not detected. However, analysis of the SUP-B13 cell line by pulsed field gel electrophoresis has shown that the breakpoint in chromosome 22 is between 50 to 250 kilobases 5' of bcr (23). Therefore, the chromosome 22 breakpoint may occur within the phi gene at a site which is distinct from that found in CML, or the breakpoint may occur in a gene proximal to the phi gene. The breakpoint on chromosome 9 in the SUP-B13 cell line is within the c-abl gene which suggests a possible role for the c-abl gene in these malignant cell lines (24). One other Ph positive ALL cell line did not have involvement of c-abl as determined by analysis of c-abl mRNA (8).

Recently, several groups have demonstrated the presence of a M<sub>1</sub>, 185,000 to 190,000 protein with tyrosine-kinase activity derived from the abl oncogene in cases of Ph positive ALL which do not have molecular rearrangements of bcr (25-27). Cases of Ph positive ALL with breakpoints at bcr and producing M<sub>1</sub>, 210,000 protein kinase may be a lymphoid blast phase of a subclinal CML, while cases of Ph positive ALL without a rearrangement at bcr and producing M<sub>1</sub>, 185,000 to 190,000 kinase may represent de novo ALL (27). Okabe et al. (18) have established a cell line from a 54-yr-old patient with Ph chromosome-positive ALL; however, this cell line has a rearrangement at bcr. Since the cell lines we have established and characterized were derived from a child with Ph chromosome-positive ALL and lack the classical rearrangement at bcr, they are unlikely to have arisen from the blast crisis of a clinically silent CML. Molecular analysis of DNA, RNA, and proteins from the SUP-B13 and SUP-B15 cell lines may elucidate the biological significance of the Ph translocation in leukemic lymphoblasts which lack classical bcr rearrangements.

**DISCUSSION**

This report describes the establishment and characterization of 2 new cell lines: SUP-B13 and SUP-B15. The cell lines were derived from the predominant malignant cells in the bone marrow and that they were clonal. The cell lines did not contain a germ line DNA band, suggesting that the other copy of the J<sub>m</sub> region had been deleted. The faintly visible germ line band in the bone marrow is probably due to normal non-malignant bone marrow cells.

46,XY,t(9;22)(q34;q11) (4 cells); and 46,XY,t(9;22)(q34;q11),t(1;4)(p22;q33),der(14q), 16p+ (2 cells). The karyotype of both the SUP-B13 and SUP-B15 cell lines showed: 46,XY,t(9;22) (q34;q11), t(1;4) (p22;q33), der(14q), 16p+ in each of 10 cells analyzed (Fig. 1).

**Molecular Genetics.** The 3' bcr probe was used as a highly specific tool to determine if the breakpoint is within bcr. In a Southern analysis using the restriction enzymes BglII (Fig. 2A), BamHI, or EcoRI, the germ line control DNA gave the expected molecular weight band as did the DNA's from the cell lines and bone marrow. There were no other bands to suggest a rearrangement at bcr with this probe.

An immunoglobulin heavy chain J region probe was used in a Southern analysis of DNA from the cell lines, the patient's bone marrow, and normal germ line digested with various restriction enzymes. In the case of HindIII the probe detected one band in normal germ line DNA and one band of lower molecular weight (representing a rearrangement) in the SUP-B13, SUP-B15, and the patient's bone marrow DNA (Fig. 2B). Additionally, molecular probing showed that these cell lines were not infected with EBV.

**ACKNOWLEDGMENTS**

The authors would like to thank P. McFall, M. Cleary, E. C. Friedberg, N. Galili, and J. Sklar for technical assistance, materials, and advice.
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