Establishment and Characterization of a Common Acute Lymphoblastic Leukemia Cell Line with a Deletion of Chromosome 3 Band q26*

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ABSTRACT

This paper describes the establishment and characterization of a new cell line (SUP-B7) which was established from a child with "common" acute lymphoblastic leukemia. The SUP-B7 cells (and the patient's tumor) have been characterized by cytochemical staining, monoclonal antibodies, enzyme analyses, gene rearrangement studies, and karyotype analysis. The SUP-B7 cells are periodic acid-Schiff positive, common acute lymphoblastic leukemia antigen positive, and terminal deoxynucleotidyl transferase positive, and they lack the Epstein-Barr virus genome. In addition, the SUP-B7 cells lack cytoplasmic and surface immunoglobulins, and the immunoglobulin gene rearrangement studies showed rearranged heavy chain genes with germ line light chain genes. Concordance between the cell line and the patient's tumor was established by the immunoglobulin gene rearrangement studies. Using Southern blot analysis of the DNA from the patient's tumor and the SUP-B7 cells, there was comigration of the bands representing the rearranged immunoglobulin heavy chain gene.

In addition, the SUP-B7 cells possess a single chromosome abnormality: del(3)(q26q28), with the chromosome breakpoint at or near the transferrin receptor gene. Since the SUP-B7 cell line is concordant with the patient's malignancy and since these cells possess a single chromosomal abnormality, the SUP-B7 cell line may be a useful tool in determining the biological significance of the chromosomal deletion: del(3)(q26q28).

INTRODUCTION

A new clinicopathological syndrome has been described in adults with a variant of myeloid leukemia (or dysmyelopoietic syndrome) which is associated with thrombocytosis, aberrant megakaryopoiesis, and a poor response to chemotherapy (1–5). In over 50% of the cases, a chromosomal structural rearrangement on chromosome 3 involving Bands q21 and q26 has been identified. These chromosomal rearrangements are near the site of the transferrin gene (3q21) and the transferrin receptor gene (3q26) (6, 7). Molecular analysis of malignant myeloid cells possessing abnormalities on chromosome 3 Bands q21 and q26 suggests that the break at q21 splits the transferrin gene and that both genes (transferrin and transferrin receptor) are altered in these cells (8).

We report here characterization of a new cell line (SUP-B7) which was established from a child with acute lymphoblastic leukemia and thrombocytopenia. This cell line is unusual, because it possesses a single chromosome abnormality: del(3)(q26q28). The SUP-B7 cells possess the same cytochemical staining pattern, biochemical enzyme levels, monoclonal antibody profile, immunoglobulin gene rearrangements, and karyotype as the patient's malignant cells. In cell lines with single chromosomal abnormalities, molecular studies on the genes located at or near the chromosome breakpoints may define cellular events leading to malignant transformation.

MATERIALS AND METHODS

Case Report. A 2-yr-old girl presented in September 1983 with malaise, pallor, hepatosplenomegaly, and pancytopenia. The peripheral blood count showed a hemoglobin of 8.0 g/dl, a platelet count of 8,000/mm³, and a WBC of 54,500/mm³ with 80% lymphoblasts. The bone marrow was hypercellular with 95% lymphoblasts, and the cytochemical stains and immunophenotyping confirmed the diagnosis of acute lymphoblastic leukemia (Tables 1 and 2). A complete remission was induced with chemotherapy, but this child subsequently relapsed and died of progressive leukemia 24 mo after diagnosis.

Source of Malignant Cells. Malignant cells were collected from the initial diagnostic bone marrow aspirate and separated into aliquots for cell culture experiments, immunophenotyping, enzyme studies, and karyotype determination. 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 Line. The technique for culturing ALL lymphoblasts was a modification of our previously reported methods (9, 10). Briefly, separated cells were mixed with agar (0.3%) and plated onto Petri dishes which contained a feeder layer consisting of medium, normal human serum (10%), and agar (0.5%). The Petri dishes were incubated at 37°C in an incubator gassed with 5% O₂, 6% CO₂, and 89% N₂.

Characterization of Cell Antigens. Monoclonal antibodies to Leu-1 (Pan T), Leu-2a (T-cytotoxic/suppressor), Leu-3a (T-helper), Leu-4 (Pan T), Leu-5 (sheep erythrocyte receptor), Leu-6 (thymocyte), Leu-9 (Pan T), CALLA, Leu-10 (B-cell lineage), Leu-12 (B-cell lineage), Leu-M1 (myeloid lineage), Leu-M3 (myeloid lineage), and HLA-DR were generously provided by the Becton Dickinson Corporation (Mountain View, CA). Monoclonal antibodies to BA-1 (B-cell lineage) and BA-2 (monocytoid, lymphoid lineage) were purchased from Hybritech, Inc. (La Jolla, CA). OKT₂ (transferrin receptor) and OKT₆ were purchased from Ortho Pharmaceuticals (Raritan, NJ). Surface immunoglobulin was detected by the F(AB')₂ fragment of fluorescein isothiocyanate-conjugated goat anti-human immunoglobulin (TAGO, Burlingame, CA). Cell surface antigens were identified by the binding of monoclonal antibody as detected by indirect immunofluorescence according to established methods (11).

Malignant cells were evaluated for antigen expression by the IHCS technique (12) to add supplemental data to the fluorescence-activated cell sorter analysis, since the IHCS detects cytoplasmic as well as cell surface antigens. This technique was used when antigen discordance between the tumor and the cell line was found. Also, the ability of cells from the bone marrow and cell line to form rosettes with sheep RBC was determined (13).

*The abbreviations used are: ALL, acute lymphoblastic leukemia; CALLA, common acute lymphoblastic leukemia antigen; IHCS, immunohistochemical staining; ADA, adenosine deaminase; NF, nucleoside phosphorylase; TdT, deoxyribonuclease; EBV, Epstein-Barr virus; FAB, French-American-British; FACS, fluorescence-activated cell sorter; AML, acute myelogenous leukemia.
Enzyme Analysis on Malignant Cells from Patient and Cell Lines. ADA and NP activities as well as protein determinations were performed according to established methods (14–17). Enzyme activity was expressed in units defined as change in absorbance over 1 min divided by the protein concentration. In addition to these purine enzymes, TdT activity was assayed utilizing a testing system from Bethesda Research Laboratories, Bethesda, MD.

Gene Rearrangement Studies. High-molecular-weight DNA from the leukemic blasts from the bone marrow aspirated and SUP-B7 cell line was digested with appropriate restriction enzymes, and the products were electrophoresed on a 0.8% agarose gel. DNA fragments separated by electrophoresis were transferred out of the gels onto activated nylon membranes (Plasco, Inc., Woburn, MA) by the method of Southern (18). Filters were hybridized with human immunoglobulin gene DNA fragments which were 32P radiolabeled by the random hexamer priming method. The hybridization probe for heavy chain gene rearrangements is specific for the joining, or J-region, of the heavy chain gene and detects heavy chain gene rearrangements regardless of the expressed heavy chain class. The constant region light chain hybridization probes detect κ and λ light chain gene rearrangements (19). Hybridization reactions were carried out under conditions described elsewhere (20).

In addition, DNA from the patient’s bone marrow and from the SUP-B7 cell line was analyzed for the presence of the EBV genome using a probe for the BamV fragment of EBV (21).

Cytogenetic Analysis. The patient’s bone marrow cells were shipped to The Genetics Center (Scottsdale, AZ) for chromosomal analysis. The SUP-B7 cell line was analyzed after 9 mo in culture using techniques described previously (22, 23).

Table 1 Morphology and cytochemical stain on the patient’s malignant cells and SUP-B7 cell line

<table>
<thead>
<tr>
<th>Bone marrow cells</th>
<th>SUP-B7 cell line</th>
</tr>
</thead>
<tbody>
<tr>
<td>Morphology (FAB classification)</td>
<td>85% L1</td>
</tr>
<tr>
<td>Periodic acid-Schiff</td>
<td>+</td>
</tr>
<tr>
<td>Acid phosphatase</td>
<td>+*</td>
</tr>
<tr>
<td>Nonspecific esterase</td>
<td>–</td>
</tr>
<tr>
<td>Chloroacetate esterase</td>
<td>–</td>
</tr>
<tr>
<td>Sudan black</td>
<td>–</td>
</tr>
</tbody>
</table>

* Six % of the lymphoblasts had positive activity limited to the Golgi area.

Table 2 Reactivity of monoclonal antibodies with the patient’s malignant cells and the SUP-B7 cell line

<table>
<thead>
<tr>
<th>Bone marrow cells</th>
<th>Cell line</th>
</tr>
</thead>
<tbody>
<tr>
<td>β2M</td>
<td>++</td>
</tr>
<tr>
<td>HLA-DR</td>
<td>+++</td>
</tr>
<tr>
<td>CALLA</td>
<td>+++</td>
</tr>
<tr>
<td>BA-1</td>
<td>+</td>
</tr>
<tr>
<td>BA-2</td>
<td>++</td>
</tr>
<tr>
<td>Leu-10</td>
<td>+</td>
</tr>
<tr>
<td>Leu-12</td>
<td>++</td>
</tr>
<tr>
<td>Slg</td>
<td>–</td>
</tr>
<tr>
<td>clg</td>
<td>–</td>
</tr>
<tr>
<td>OKT3</td>
<td>–</td>
</tr>
<tr>
<td>OKT1b</td>
<td>–</td>
</tr>
<tr>
<td>Leu-M3</td>
<td>+</td>
</tr>
<tr>
<td>Leu-1, 2a, 3a, 4, 5, 6, 9</td>
<td>–</td>
</tr>
</tbody>
</table>

* β2M, Beta 2 microglobulin; Slg, surface immunoglobulin; clg, cytoplasmic immunoglobulin.

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 in the middle of the plate (cloning efficiency = 0.006%). Colony cells were gradually weaned to suspension culture and have been growing continuously for over 30 mo. The SUP-B7 cell line had a doubling time of 7 days. The cell line was designated SUP- in recognition of its establishment at the Stanford University Pediatrics Department.

Cytochemical stains were performed on cytospin preparations of the SUP-B7 cell line and compared to the smears made of the patient's diagnostic bone marrow (Table 1). While 85% of the bone marrow lymphoblasts had an L1, FAB classification, 80% of the SUP-B7 cells had L2 morphology. Both the cell line and the patient’s lymphoblasts were periodic acid-Schiff positive, and both lacked reactivity with the nonspecific esterase, chloroacetate esterase, and Sudan black. A small subpopulation of the patient’s lymphoblasts were acid phosphatase positive, while 100% of the SUP-B7 cells were positive with this stain.
Characterization of a Calla Cell Line

Cellular Antigens. Both the bone marrow cells and the SUP-B7 cell line expressed high levels of HLA-DR, CALLA, BA-1, BA-2, and Leu-12 but lacked cytoplasmic immunoglobulin, surface immunoglobulin, and T-cell antigens (Leu-1, 2a, 3a, 4, 5, 6, 9), and were erythrocyte rosette negative. There was concordance for 18 of the 19 paired antibody tests performed (Table 2). While the SUP-B7 cell line expressed moderate OKT3 (transferrin receptor) antigens, this antigen was not found on the patient's cells when analyzed by FACS analysis. However, IHCS showed the transferrin receptor antigen to be present in the cytoplasm on approximately 2% of the patient's bone marrow cells. Thus, the discordance of OKT9 expression may be due to antigen loss (from the tumor cells), antigen gain (by the SUP-B7 cells), or selective growth of the OKT9-positive subpopulation.

Enzyme Analysis. ADA and NP were assayed in malignant cells from the patient and the SUP-B7 cell line. In the malignant cells from both the patient and the cell line, ADA was markedly elevated, and NP was unaltered by comparison with normal lymphocytes (Table 3). Activity for TdT was present in more than 90% of malignant cells from the patient and the cell line. These results are consistent with the usual biochemical profile of immature B-cells of common ALL (24-27).

Molecular Genetics. Using the JH probe for immunoglobulin heavy chain, the patient's bone marrow showed two germ line bands (one major and one cross-hybridizing band) and one rearranged band with each of the two (BamHI, EcoRI) restriction enzymes used (Fig. 1). The SUP-B7 cell line showed absence of the major germ line band and comigration of the rearranged band with the rearranged band of the patient's bone marrow cells. Using the constant region probes for the immunoglobulin light chains, the patient's bone marrow and the SUP-B7 cells both showed germ line bands only. The presence of two germ line bands (and one rearranged band) suggests that at least one germ line is due to DNA from normal cells in the bone marrow.

Karyotype Analysis. The results of the cytogenetic analysis of the patient's bone marrow cells showed: 46,XX (10 cells); 46,XX,del(3)(q26q28) (2 cells); 46,XX,del(3)(q26q28),18q- (2 cells). The karyotype of the SUP-B7 cell line showed: 46,XX,del(3)(q26q28) (Fig. 2).

Discussion

This paper describes the establishment and characterization of a new cell line: SUP-B7. The cell line was established from a child with acute lymphoblastic leukemia (non-T, non-B, CALLA positive), and it represents one of a small number of cell lines established from childhood lymphoblastic leukemia (common ALL of childhood or c-ALL) (28–33). To establish authenticity of a new cell line, we determined how the cell line's characteristics compared to the patient's tumor. The SUP-B7 cells possessed morphological characteristics of lymphoblasts and had the same basic cytochemical staining as the patient's...
tumor cells (Table 1). The cell line (and the patient’s tumor) possessed high levels of HLA-DR, CALLA, and BA-1 antigens, and lacked cytoplasmic immunoglobulin, cell surface immunoglobulin, and EBV genome.

Enzyme levels have been helpful in defining the maturity and lineage of lymphoid cells, for specific enzyme levels often change with cellular differentiation. For instance, ADA levels are high in c-ALL and normal in mature peripheral blood lymphocytes, NP activity is normal in c-ALL while low levels are found in T-ALL, and TdT activity is high in immature B-cell malignancies and normal in more mature B-cell tumors (25–27, 34). The presence of TdT, the high level of ADA, and normal NP activity of the SUP-B7 cell line corresponded well to the patient’s bone marrow levels and support the diagnosis of c-ALL.

Recently, immunoglobulin gene rearrangement studies have shown that the migration levels of the rearranged bands are uniquely characteristic of a given patient’s tumor (20, 35). In the present study, immunoglobulin gene rearrangement analysis showed comigration of a rearranged band (using JH probe). These data lend strong support to the hypothesis that the SUP-B7 cell line represents the patient’s malignancy. The absence of the major germ line band in the cell line indicates that this cell line, and possibly the tumor, contained a rearranged heavy chain gene together with a deletion of the other heavy chain allele. The major germ line band in the bone marrow DNA probably comes from contamination of the tumor cells with nontumorous marrow elements. In addition, germ line light chains existed in both the patient’s cells and cell line, supporting the incomplete maturation of this B-lineage malignancy.

However, concordance between the patient’s tumor and the SUP-B7 cells was not complete. The morphology of the cell line had shifted from predominantly L1 to predominantly L2 and was more reactive with acid phosphatase than the bone marrow lymphoblasts (Table 1). In comparing the patient’s bone marrow lymphoblasts to the SUP-B7 cell line, the monoclonal antibody profile showed concordance for 18 of 19 antibodies tested. The only discordant result was obtained with the OKT9 antigen (transferrin receptor) which was present on the cell line but lacking (by FACS analysis) on the surface of the patient’s cells. However, by IHCs technique, cytoplasmic OKT9 was observed in 2% of bone marrow lymphoblasts.

Collectively these data suggest that at least two populations of malignant lymphoblasts existed in the patient’s bone marrow. The major population of the bone marrow lymphoblasts was FAB L1, acid phosphatase negative, and OKT9 antigen negative and did not grow long term in vitro. A minor subpopulation of the bone marrow lymphoblasts was FAB L2 (20%), acid phosphatase positive (6%), and possessed OKT9 antigen (2%). These cells grew in agar and were the cells from which the SUP-B7 cell line was established. However, the gene rearrangement studies indicate that only one clone of cells made up this patient’s tumor, despite the mixed phenotype. We therefore hypothesize that this patient had a single clonal malignancy which contained two populations of cells: a major, nonproliferating, differentiated group which lacked the transferrin receptor (OKT9 negative) and a minor, proliferating subpopulation which possessed the transferrin receptor (OKT9 positive).

The chromosomal abnormality in the SUP-B7 cell line is unique for it involves a single chromosome change: del(3)(q26q28). While this chromosome breakpoint may be at or near the site observed in the AML cases with inv 3q21q26, several clinical differences occurred. Inv 3q21q26 has been reported in adults with AML with thrombocytopenia, while the SUP-B7 cell line was established from a child with ALL with thrombocytopenia. If the breakpoint on chromosome 3 Band q26 is at the same site in these two clinical diseases, this suggests that the 3q26 site may not be involved in the abnormal megakaryopoiesis and thrombocytosis. Specific genes have been mapped to the distal end of the long arm of chromosome 3 (i.e., transferrin receptor, ceruloplasmin), and molecular studies are continuing to determine if these genes are altered in the SUP-B7 cells (12, 13, 36).

**REFERENCES**

CHARACTERIZATION OF A CALLA CELL LINE


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