Although significant advances have been made in the treatment of childhood acute lymphoblastic leukemia (ALL), the long-term outlook for some patients remains poor. Identification of these patients has been accomplished to some extent through the demonstration of leukemia cell surface markers by conventional immunologic methodology. Patients whose lymphoblasts manifest T-cell markers, and the yet smaller group whose leukemic cells express B-cell markers, have less favorable outcomes when compared to children whose leukemic lymphoblasts lack such markers (non-T, non-B ALL).

One additional approach to the identification of leukemia subtypes that has already shown diagnostic and therapeutic promise is that of purine pathway enzyme quantitation. T-cell lymphoblasts have reduced 5' nucleotidase (5'-N) and purine nucleoside phosphorylase (PNP) and increased adenosine deaminase (ADA) activities when compared with either non-T, non-B leukemic lymphoblasts or normal peripheral blood lymphocytes. Inhibitors of ADA, such as 2'-deoxycoformycin, have been reported to have chemotherapeutic activity in T-cell ALL.

To date, there is little information regarding the status of purine pathway enzymes in B-cell ALL. In the present report we describe the pattern of 5'-N, PNP, and ADA activity in the lymphoblasts of a patient with B-ALL.

CASE REPORT

J.B. is a 12-yr-old black male with acute lymphoblastic leukemia who presented with symptoms of fatigue and epistaxis. Physical examination at that time revealed a pale, ill-appearing child with a temperature of 40°C, marked lymphadenopathy and hepatosplenomegaly, and petechiae. Laboratory data included a white blood cell count of 1,000,000/cu mm with 95% lymphoblasts (L2 morphology, PAS positive, peroxidase negative, terminal deoxynucleotidyl transferase negative) platelet count 6000/cu mm, hemoglobin 6 g/dl, uric acid 12.8 mg/dl, LDH 4400 units; chest x-ray revealed no mediastinal mass; bone marrow aspiration confirmed the diagnosis of acute lymphoblastic leukemia. Lumbar puncture performed at diagnosis revealed the presence of meningeal leukemia. Initial induction therapy consisted of vincristine, prednisone, methotrexate, and 6-mercaptopurine, which resulted in the achievement of hematologic remission within 14 days. Specific central nervous system (CNS) therapy consisted of 2400 rads cranial irradiation and intraventricular methotrexate (12 mg/m²) by Ommaya reservoir. The patient subsequently did well with systemic and CNS maintenance therapy until 2 yr later when he experienced testicular relapse. Treatment at that time consisted of 2400 rads to both testes and systemic reinduction therapy with vincristine, asparaginase, prednisone, and daunomycin followed by systemic maintenance therapy. Two years later the patient developed hematologic relapse with concurrent meningeal relapse. Systemic reinduction therapy at that time included the combination of vincristine, prednisone, asparaginase, and daunomycin and treatment of the central nervous system consisted of intraventricular methotrexate. At the present time the patient is doing well and remains in complete remission on maintenance therapy.

MATERIALS AND METHODS

Leukemic lymphoblasts were obtained by leukopheresis prior to therapy. Mononuclear cell preparations were obtained by Ficoll-Hypaque (LSM, Litton Bionetics, Kensington, Md.) density centrifugation. Cells were washed 3 times with Roswell Park Memorial Institute (RPMI) 1640 medium and resuspended in RPMI 1640. Examination of Wright-stained cytospin preparations of the cell suspension revealed that greater than 95% of the cells were leukemic lymphoblasts.

Cell Membrane Surface Markers

Leukemic lymphoblasts were studied for the presence of T-cell markers (spontaneous sheep erythrocyte rosette formation) by use of neuraminidase-treated Vibrio cholerae; Grand Island Biological Co., Grand Island, N.Y.) sheep erythrocytes according to the method of Weiner et al.

The presence of B-cell surface markers was assessed by a roset-
Preparation of Cell Extracts for Enzyme Assay

Mononuclear cells (0.5–1.0 x 10⁶), prepared as above, were disrupted by alternately freezing (in an acetone-dry ice mixture) and thawing at 37°C four times. The resulting suspensions were disrupted by alternately freezing (in an acetone-dry ice mixture) and thawing at 37°C four times. The resulting suspensions were centrifuged at 4000 g for 15 mm and supernatants used in enzyme and protein assays.

Purine Nucleoside Phosphorylase Assay

PNP was assayed by the coupled xanthine oxidase method of Kalckar. Reaction mixtures contained 100 μl of 6.0 mM inosine (Sigma Chemical Co., St. Louis, Mo.) solution, 10 μl of xanthine oxidase (Sigma, 50 μl, grade 1), 0.5 ml of cell extract, and 2.4 ml of Na phosphate buffer (pH 7.2). The vortexed mixture was transferred to a 3-m1 cuvette, and the reaction followed with a Gilford 250 spectrophotometer for 10 min at 37°C at a wavelength of 293 nm. Results are expressed as Δ OD₂₆₅ over 1 mm divided by mg protein determined as above.

Table 1. Purine Pathway Enzyme Activity in Normal and Malignant Lymphoid Cells

<table>
<thead>
<tr>
<th>Cell Type/Disease</th>
<th>ADA Activity Mean (Range)</th>
<th>PNP Activity Mean (Range)</th>
<th>5'-N Activity Mean (Range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal peripheral lymphocytes</td>
<td>15(0–58)</td>
<td>90(60–130)</td>
<td>39(23–58)</td>
</tr>
<tr>
<td>ALL (T cell)</td>
<td>130(40–420)</td>
<td>38(10–100)</td>
<td>4(0.4–9)</td>
</tr>
<tr>
<td>ALL (non-T, non-B)</td>
<td>60(10–110)</td>
<td>79(20–140)</td>
<td>29(12–50)</td>
</tr>
<tr>
<td>Burkitt’s lymphoma (B cell)</td>
<td>26(4–130)</td>
<td>100(30–200)</td>
<td>ND</td>
</tr>
<tr>
<td>Patient (B-cell ALL)</td>
<td>410(386–434)</td>
<td>20(17–23)</td>
<td>6(3–9)</td>
</tr>
</tbody>
</table>

ND, not determined.
*Described in text.
†nmols AMP degraded/10⁶ cells/min x 10⁶.
Additional characterization of these poor prognosis patients is necessary for the development of potentially more effective therapy in this particular subgroup of patients. The purine pathway enzyme abnormalities that were observed in this patient’s B-cell lymphoblasts are of interest in that they are similar to those which have been previously described in leukemic cells that manifest T-cell surface markers (Table 1). Furthermore, the enzymatic characteristics of this patient’s B-cell lymphoblasts were different than those of the malignant cells of individuals with undifferentiated B-cell lymphomas. A previous study reported that ADA levels in the lymphoblasts of three patients with B-cell ALL were lower than those seen in normal lymphocytes. Morphologically, the lymphoblasts of all three of these patients resembled Burkitt’s lymphoma cells. It is of note that our patient’s lymphoblasts were morphologically of the L2 variety and not of the L3 (Burkitt’s type) subclass. These data suggest that there may be biochemical heterogeneity within the spectrum of B-cell malignancies.

As previously noted, inhibitors of ADA have demonstrated in vitro and in vivo activity against malignant T-cells that manifest increased ADA activity. The finding of increased ADA activity in this patient’s lymphoblasts suggests that ADA inhibitors may also be useful for some patients with B-cell ALL. It is unclear at present whether the relative deficiencies of 5’N and PNP may afford similar therapeutic exploitation. Enzyme characterization of other cases of B-cell ALL will be of interest.

REFERENCES

Lymphoblast purine pathway enzymes in B-cell acute lymphoblastic leukemia

GH Reaman, J Blatt and DG Poplack