Lymphoblast Purine Pathway Enzymes in B-Cell Acute Lymphoblastic Leukemia

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Activities of enzymes of the purine metabolic pathway, adenosine deaminase (ADA), purine nucleoside phosphorylase (PNP), and 5'-nucleotidase (5'-N), were investigated in the lymphoblasts of a patient with B-cell acute lymphoblastic leukemia. These lymphoblasts exhibited increased ADA activity and diminished activities of both PNP and 5'N as compared to normal lymphocytes as well as non-T, non-B leukemia cells. This enzymatic pattern is identical to that which has been described in T-cell leukemic lymphoblasts and differs from that which has been observed in the malignant cells of undifferentiated B-cell lymphomas. These data suggest that there is biochemical heterogeneity within the spectrum of B-cell malignancies. Furthermore, inhibitors of ADA may be of use in those B-cell lymphoid neoplasms that exhibit increased ADA activity.

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) activities when compared with either non-T, non-B leukemia 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-CELL 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/sq m) 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 cytocentrifuge 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-
tinging technique designed to demonstrate complement (C') receptors by use of sheep erythrocytes sensitized with a 19s rabbit antiserum to sheep erythrocyte immunoglobulin (Cordis Laboratories, Miami, Fla.) and fresh BALB/c mouse serum as a source of complement. 11 The presence of B-cell markers was also assessed by the detection of surface immunoglobulin, as measured by direct fluorescence microscopy after incubation of lymphoblasts with a fluorescein-conjugated polyvalent [F(ab),] goat anti-human immunoglobulin (Cappel Laboratories, Downington, Pa.).

5'-Nucleotidase Activity

5'-Nucleotidase was measured by a modification of the method of Shenoy and Clifford, 2-4 x 10^6 mononuclear cells (leukemic lymphoblasts or normal lymphocytes) were incubated with 0.1 ml of a 0.1 mM solution of U-14C-adenosine 5'-monophosphate (479.7 mCi/mmole, New England Nuclear, Boston) and phosphate-buffered saline (pH 6.8) in a total reaction mixture of 0.5 ml for 30 min at 37°C, with constant gentle shaking. After incubation, 25 l of the reaction mixture was precipitated by mixing with 1 ml of 0.5 M lanthanum chloride at 4°C for 4 hr and centrifuging at 2000 g for 10 min. An aliquot (0.5 ml) of the supernatant containing 14C adenosine was placed in 12.5 ml of Aquasol (New England Nuclear) and counted in a liquid scintillation counter (Packard Instruments, Downers Grove, Ill.). Control tubes containing only labeled substrate (both precipitated and unprecipitated) were run concurrently, and the results were expressed as nanomoles of adenosine 5'-monophosphate degraded per 10^6 cells per minute. All experiments were performed three times, and the results are the mean values obtained.

Specificity of this assay for 5'-nucleotidase was confirmed by demonstration of complete inhibition of enzymatic activity by 0.01 M methylendiaminotetraacetate and by equimolar concentrations of the a, b-methylene phosphonate analogue of adenosine diphosphate, a potent competitive inhibitor of 5'-nucleotidase. No inhibition of enzymatic activity was observed with equimolar concentrations of either p-nitrophenyl phosphate, a substrate for nonspecific phosphatases, or B-glycerophosphate, which inhibits the nucleotidase effect of alkaline phosphatase.

Preparation of Cell Extracts for Enzyme Assay

Mononuclear cells (0.5-1.0 x 10^7), 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 centrifuged at 4000 g for 15 min and supernatants used in enzyme and protein assays.

Purine Nucleoside Phosphorylase Assay

PNP was assayed by the coupled xanthine oxidase method of Kalckar. 7 Reaction mixtures contained 100 M of 6.0 M inosine (Sigma Chemical Co., St. Louis, Mo.) solution, 10 M of xanthine oxidase (Sigma, 50 M, grade 1), 0.5 M of cell extract, and 2.4 M of Na phosphate buffer (pH 7.2). The vortexed mixture was transferred to a 3-m I 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 OD 293 over 1 min divided by the mg of protein in the cell extract as determined by the method of Lowry. 12

Positive controls using known amounts of PNP (Sigma) were included with each assay.

Adenosine Deaminase Assay

ADA activity was assayed as previously reported. 8 The sample cuvette contained 0.2 ml adenosine (2 mM) and 2.3 ml 0.15 M phosphate buffer (pH 7.1). The reaction was started by the addition of 0.5 ml cell extract, and followed spectrophotometrically by recording the initial decrease in OD at 265 nm at 30°C, which was linear for at least 5 min. Results were expressed as OD 265 over 1 min divided by mg protein determined as above.

RESULTS

Forty-three percent of the patient's lymphoblasts expressed C' receptors and 40% demonstrated surface membrane immunoglobulin. Because of the high percentage of leukemic cells expressing both C' receptors and surface immunoglobulin and their failure to form rosettes with sheep erythrocytes, this patient was considered to have B-cell ALL. Enzyme studies are shown in Table 1. For comparison, mean values and ranges of 5'-N, PNP, and ADA activities, which have been previously reported for several lymphoid malignancies, are given. 15 These B-cell lymphoblasts displayed extremes in activity with respect to each of the purine pathway enzymes studied. The ADA level was one of the highest observed, well above the range seen in either normal lymphocytes or in the malignant B-cells of patients with Burkitt's lymphoma and was closer to the levels observed in T-cell rather than non-T, non-B leukemic lymphoblasts. PNP and 5'-N activities were markedly diminished, similar to the patterns observed in T-cell ALL.

DISCUSSION

Although more than 50% of children with ALL now remain in continuous first remission for periods exceeding 5 yr, 13 many do poorly despite current therapy. Identification of poor prognostic factors has helped to separate this group of patients with the expectation that more effective therapy will be developed for them. Although B-cell ALL is rare, several reports have suggested that children with this subtype do not respond favorably to currently accepted therapy. 5,14

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

<table>
<thead>
<tr>
<th>Cell Type/Disease</th>
<th>ADA (units)*</th>
<th>PNP (units)*</th>
<th>5'-N (unit)†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal peripheral blood lymphocytes</td>
<td>150(50-58)</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>410(4-9)</td>
</tr>
<tr>
<td>ALL (non-T, non-B)</td>
<td>60(10-110)</td>
<td>70(10-140)</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.
†nanomoles AMP degraded/10^6 cells/min x 10^3.
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
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