Purine Nucleoside Phosphorylase Activity in Acute Lymphoblastic Leukemia

By Julie Blatt, Gregory H. Reaman, Neil Levin, and David G. Poplack

Purine nucleoside phosphorylase (PNP) activity has been measured in the lymphoblasts of 22 patients with acute lymphoblastic leukemia (ALL) and correlated with routine immunologic cell surface markers. Fourteen of the 22 patients were considered to have non-T, non-B-cell ALL; 8 patients had T-cell disease. The median PNP activity in 21 control samples of normal peripheral blood mononuclear cells was 83 U. The median activity of the non-T, non-B lymphoblasts was 78 U. No statistical difference in PNP activity between these two groups could be discerned (p < 0.37). In contrast, T-cell lymphoblasts demonstrated diminished PNP activity with a median of 38 U. The differences in activity between T- and non-T, non-B leukemic cells and normal peripheral blood mononuclear cells were significant (p < 0.001 and p < 0.003, respectively). Evaluation of PNP activity provides further evidence of biochemical heterogeneity among immunologic subclasses of ALL.

Recently, enzymatic differences in purine metabolism have been observed between normal mononuclear cells and acute lymphoblastic leukemia (ALL) cells. Distinct differences in enzyme activities have also been detected between immunologic subclasses of ALL: 5'-nucleotidase (5'N) has been shown to be decreased1 and adenosine deaminase (ADA) increased2 in leukemic lymphoblasts that display T-cell surface markers.

Purine nucleoside phosphorylase (PNP) acts sequentially with ADA in the purine salvage pathway, reversibly converting inosine to hypoxanthine. Like ADA and 5'N, the presence of PNP appears to be essential for normal lymphocyte maturation; the absence of each of these enzymes has been associated with immunodeficiency.3,5 In the present study we examined the activity of PNP in lymphoblasts from patients with ALL to investigate the possibility of a correlation between enzyme activity and immunologic cell surface markers.

MATERIALS AND METHODS

Cells

Leukemic cells were obtained from the peripheral blood or bone marrow of 22 patients with newly diagnosed ALL. The diagnosis of ALL was based on clinically accepted criteria.4 Leukemic cells from peripheral blood were collected by leukapheresis prior to beginning chemotherapy. Mononuclear cell suspensions were obtained by Ficoll-Hypaque (Lymphocyte Separation Medium, Litton Bionetics, Kensington, Md.) density gradient centrifugation, and cells were cryopreserved in RPMI 1640 with 10% fetal calf serum (FCS) and 10% dimethyl sulfoxide in the vapor phase of liquid nitrogen (~170°C). Leukocytes from 21 normal donors were similarly separated from preservative-free heparinized whole blood and either assayed immediately for PNP activity or cryopreserved. For use in PNP assays, frozen cells were rapidly thawed, washed three times in RPMI 1640 and 10% FCS, and resuspended in 0.05 M phosphate buffer (PB), pH 7.4. Initial studies had shown that cryopreservation of either normal or leukemic lymphoid cells results in no alteration in PNP activity. Viability, assessed by trypan blue exclusion, was greater then 90% in all instances. There was no gross contamination from erythrocytes in any of the samples.

Enzyme Preparation

Mononuclear cells, prepared as above and adjusted to a concentration of 0.5-1.0 x 10^6 cells/cc, were disrupted by alternately freezing (in an acetone-dry-ice mixture) and thawing at 37°C four times. The resulting suspensions were centrifuged at 10,000 rpm for 15 min and supernatants used in enzyme and protein assays.

PNP Assay

PNP was assayed by the coupled xanthine oxidase method of Kalckar.7 One-hundred microliters of a 6.0 mM inosine (Sigma Chemical Co., St. Louis, Mo.) solution, 10 μl of xanthine oxidase (Sigma, 50 μl, grade 1), and 0.5 cc of enzyme extract were added to 2.4 cc PB. The vortexed mixture was transferred to a 3-cc cuvette, and the reaction followed with a Gilford 250 spectrophotometer for 10 min at 37°C at a wavelength of 293 nm. The rate of the reaction, which proceeded linearly during the observation period, was calculated as the ΔOD293 over 1 min. Positive controls using known amounts of PNP (Sigma) were included with each assay. Protein determinations on enzyme extracts were determined by the method of Lowry.8 Enzyme activity was expressed in units (U), defined as ΔOD293 over 1 min divided by the protein concentration in milligrams. Assays were repeated in duplicate and found to vary by not more than 1%.

Cell Membrane Surface Markers

Leukemic lymphoblasts were examined for the presence of T-cell markers [spontaneous sheep red blood cell (SRBC) rosette (ER) formation] using neuraminidase (Vibrio cholerae) (Gibco, Grand Island, N.Y.) treated SRBC according to the method of Weiner et al.9 The presence of B-cell surface markers was assessed both by a rosetting technique designed to demonstrate complement receptors (EAC/R) using SRBC sensitized with a 19S rabbit anti-SRBC immunoglobulin (Cordis Labs., Miami, Fla.) and fresh BALB/c mouse serum as a complement sources as well as by the detection of surface immunoglobulin (SIg) as measured by direct fluorescent microscopy following incubation of lymphoblasts with a fluorescein-
conjugated polyvalent goat anti-human immunoglobulin (Cappel Labs., Downington, Pa.).

Rosette positive (ER and EAC'R) cells were enumerated by counting 200 cells in a hemocytometer. Cells binding three or more indicator erythrocytes were considered positive. Cytologic examination of the rosetted cell suspension was accomplished by the use of cytocentrifuge preparations. The number of rosettes counted in a wet preparation was multiplied by the percentage of rosettes that had morphologically identifiable lymphoblasts at their centers. Patients with ≥20% ER+ blasts were considered to have T-cell leukemia.

Statistical Methods
Tests of significance were performed using the Wilcoxon Ranked Sum test.¹⁸

RESULTS
Characteristics of the patients and immunologic surface markers of their lymphoblasts are shown in Table 1. Of the 22 patients, 8 had T-cell ALL. In these individuals, the percentage of ER-forming lymphoblasts ranged from 26% to 82%. Lymphoblasts from the remaining 14 patients expressed neither B- nor T-cell markers and were considered to be non-T, non-B lymphoblasts.

The PNP activities are shown in Fig. 1. Enzyme activity of normal mononuclear cells ranged from 10 U to 230 U, with a median of 83 U. Enzyme activity in non-T, non-B lymphoblasts ranged from 50 U to 140 U, with a median of 79 U. No statistical difference in PNP activity between these two groups could be discerned (p < 0.37). In contrast, T-cell lymphoblasts demonstrated diminished PNP activity, ranging from 10 U to 100 U, with a median of 38 U. The differences in activity between T lymphoblasts and both non-T, non-B leukemic cells and normal peripheral blood mononuclear cells were significant (p < 0.001, and p < 0.003, respectively).

DISCUSSION
Purine nucleoside phosphorylase acts sequentially with ADA in the purine salvage pathway, reversibly
converting inosine to hypoxanthine. Both partial and complete PNP deficiency have been associated with immunodeficiency in man. In particular, absence of PNP is linked to defective T-cell function. By analogy with ADA deficiency, and on the basis of enzyme reconstitution studies, this relationship is presumed to be one of cause and effect. The association of these purine enzyme deficiencies with impaired cellular immunity emphasizes their importance to normal lymphoid function.

As noted previously, ADA has been found to be increased and 5N decreased in leukemic lymphoblasts that express T-cell surface markers. Other reports have alluded to the possibility of a PNP abnormality in lymphoid malignancies. In the present study, we have observed a specific reduction in PNP activity in ALL lymphoblasts with T-cell markers. In contrast, non-T, non-B lymphoblasts had PNP levels comparable to those of normal mononuclear cells. Whether the decreased PNP represents an absolute reduction in enzyme or a functional alteration is as yet not clear.

The observed reduction in PNP activity in T lymphoblasts extends the scope of the purine metabolic abnormalities in T-cell ALL and provides further evidence of biochemical heterogeneity among immunologic subclasses of this disease.

REFERENCES

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