IMP: and AMP:Pyrophosphate Phosphoribosyltransferase in Leukemic and Normal Human Leukocytes[1] (35480)

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(Introduced by M. Lipkin)

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The functions of IMP:pyrophosphate phosphoribosyltransferase (EC 2.4.2.8) (hypoxanthine-guanine phosphoribosyltransferase) and AMP:pyrophosphate phosphoribosyltransferase (EC 2.4.2.7) (adenine phosphoribosyltransferase) in cell purine metabolism are not fully understood. These enzymes have been found in mouse, rat, monkey, and man, in varying amounts, depending on the tissue examined (1–3). In man, an absence of hypoxanthine phosphoribosyltransferase and raised levels of adenine phosphoribosyltransferase (4) are associated with a disease characterized by neurologic dysfunction, destructive behavior, over-production of urate (5), and abnormal purine excretion (6).

The possible roles of these enzymes in the interconversion of purine nucleotides (3) suggested that these enzymes may be abnormal in disease states which respond to therapy with purine analogs such as 6-mercaptopurine and 6-thioguanine. In fact a wide variation in relative and absolute amounts of hypoxanthine and adenine phosphoribosyltransferases have been observed in a small number of leukemic patients (3).

While attempts to correlate other leukocyte enzyme functions in leukemia with drug therapy have not been completely successful (7), the present study was undertaken to investigate the levels of purine transfer enzymes in intact leukocytes from acute leukemic patients in remission and relapse, and to investigate changes in these enzyme levels during drug therapy. In the course of these studies leukocytes from patients clinically resistant to purine analogs were investigated for changes in enzyme levels compared with normal cells.

Materials and Methods. Leukocyte preparations. Leukocytes were isolated from venous blood collected directly into heparinized tubes, to which was added 0.3% dextran (clinical grade, Mann Research Laboratory, New York). Erythrocytes were allowed to sediment for 1 hr at 37°. The supernatant was drawn off and the leukocytes were washed twice with Hanks' balanced salt solution (BSS) by centrifugation at 800*g* for 10 min before final suspension in culture medium (Eagle's medium) (8), supplemented with "nonessential" amino acids as described by Ambrose (9). The final suspension was dispersed in aliquots containing 1–4 $\times$ 10^6 cells into 10-ml culture tubes. Final culture volume was 1 ml.

All glassware used for cell preparation was siliconized.

Enzyme assay. Adenine-8-^{14}C and hypoxanthine-8-^{14}C were included in the culture medium at a concentration of 100 mμ moles/ml and containing 2–5 $\times$ 10^5 cpm/ml. The incorporation of precursor into nucleotide was measured by incubating the cells for 30 min at 37° in a shaking water bath. At the end of the incubation period the cells were chilled in an ice bath, washed twice with BSS at 4° and stored at —20°. Further determination was made by resuspending the frozen pellet in 0.2 ml of water and heating in a boiling water bath for 2 min. After centrifugation of the samples, 0.1 ml of the supernatant was spotted onto squares of

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TABLE I. Erythrocyte Enzyme Activity.[a]

Preparation	Erythrocytes ($\times 10^6$/ml)	Lymphocytes ($\times 10^6$/ml)	Product (mμmoles/10^6 lymphocytes/hr)	
			Ad	Hx
Untreated	114.8	16.4	0.088	0.022
Lysed	60.0	12.0	0.102	0.028
Untreated	68.0	3.4	0.108	0.042
Lysed	13.5	2.7	0.114	0.038

[a] Preparations of lymphocytes were lysed and the activities of the lysed suspensions were compared to the controls. The numbers of erythrocytes and lymphocytes in the original suspensions are given. Assays were done with 1–3 $\times 10^6$ lymphocytes; (Ad, adenine nucleotides; Hx, hypoxanthine nucleotides).

DEAE-cellulose paper. The papers were air dried and washed twice with 10^{-3} *M* ammonium formate, once with distilled water, and twice with ethanol to remove precursors, as previously described (10). Nucleotide formation was determined by counting the dried papers in a Packard liquid scintillation counter with toluene scintillant (4.2 g of PPO(2,5-diphenyloxazole) and 53 mg of POPOP(2,2-phenylenebis(5-phenyloxazole))/liter of toluene).

Results. Substrate concentration. Leukocytes were incubated with substrate (adenine-8-^{14}C or hypoxanthine-8-^{14}C) at concentrations in the range of 2.0 $\times 10^2$ μM. Both the adenine and hypoxanthine phosphoribosyltransferases are saturated with substrate at concentrations greater than 50 μM. Substrate concentrations of 100 μM were chosen for further assays.

The incorporation of substrate at concentrations of 100 μM in the medium was linear over the range 0–6 $\times 10^6$ leukocytes/ml. In routine assays determinations were made with cultures containing 1–3 $\times 10^6$ leukocytes/ml and the incorporation of substrate was expressed as millimicromoles of product formed per 10^6 leukocytes/hr.

Erythrocyte contaminant. Leukocyte preparations from normal donors were contaminated with 5$\times$ as many erythrocytes as leukocytes. Preparations from very leukopenic patients sometimes contained 15$\times$ as many erythrocytes as leukocytes.

To determine the effect of erythrocytes in the preparations, lymphocyte suspensions were prepared from normal blood by cotton wool filtration (11). Erythrocytes in an aliquot of the suspension were lysed by adding 1.5 ml of water to 1.0 ml of cells in medium at 37°. After 1 min, isotonicity was restored with 1.5 ml of 1.7% saline and the cells were washed with BSS once, before resuspension in the medium. The enzyme activity of the lymphocytes in treated suspensions was compared to the activity of the untreated control. Reducing the number of erythrocytes in the suspension by four times did not alter the activity of the adenine and hypoxanthine transferases in these suspensions (Table I).

Enzyme levels in normals and patients. The activities of leukocyte adenine and hypoxanthine transferases from leukemic patients in relapse and others are shown in Tables II, III, and IV. The activities of the cells from chronic myelogenous leukemics were in the normal range.

The mean peripheral blood leukocyte hypoxanthine transferase activity of nine pa-

TABLE II. Mean Values of Leukocyte Enzyme Activities.[a]

Diagnosis	No. of donors	Product (mμmoles/10^6 leukocytes/hr)	
		Hx	Ad
Normal	8	0.044 ± 0.011	0.182 ± 0.061
AML	9	0.129 ± 0.059	0.297 ± 0.161
ALL	5	0.067 ± 0.052	0.257 ± 0.187

[a] Values are from normals and from leukemic patients in relapse shown with their standard deviations. These means were obtained from one observation with each patient and represent the mean of the number of individuals shown.

TABLE III. Enzyme Activities of Leukocytes from Adult Patients with Acute Myeloblastic Leukemia.[a]

Patient	Date	% Leukemic cells in blood	Bone marrow	Current treatment	Purine analog resistance[b]	Peripheral cell count × 10^3/mm^3	Product (mμmoles/10^6 leukocytes/hr) Hx	Product (mμmoles/10^6 leukocytes/hr) Ad	Ratio, Ad/Hx
A	12/11	50	rel	Hu	—	10.0	0.164	0.304	1.7
Z	8/30	10	rel	—	+	2.5	0.140	0.376	2.7
	9/3	30	rel	Ca + TG		0.7	0.092	0.194	2.1
	9/9	30	rel	—		0.5	0.112	0.228	2.0
S	9/4	50	rel	—	+	1.2	0.112	0.230	2.1
	9/10	10	rel	Asp		1.0	0.176	0.224	1.3
	9/25	0	rem	Asp		0.7	0.056	0.134	2.5
	10/4	0	rem	Asp		0.7	0.032	0.238	7.5
L	10/4	50	rel	Ca + TG	—	8.5	0.240	0.660	2.7
V	8/30	5	rel	—	+	3.0	0.232	0.410	1.7
	10/11	4	rel	Ca + TG		2.5	0.096	0.364	3.8
F	9/24	68	rel	Pred	—	30.0	0.128	0.338	2.6
	9/27	75	rel	Pred		46.0	0.118	0.372	3.1
	10/2	85	rel	Pred + Asp		38.0	0.168	0.372	2.2
	10/9	90	rel	Pred + Asp		42.0	0.138	0.258	1.9
R	10/30	30	rel	—	+	2.5	0.086	0.240	2.9
J	2/5	35	rel	Ca + TG	±	0.7	0.44	0.138	2.9
B	9/12	85	rel	—	—	130.0	0.066	0.124	1.9

[a] Values are from adult patients with acute myelogenous leukemia. % Leukemic cells in blood includes blasts, promyelocytes and myelocytes or monocytes when appropriate. (HU = hydroxyurea; Ca = arabinosyl cytosine; TG = 6-thioguanine; Asp = L-asparaginase; Pred = prednisone: rel = relapse; rem = remission.)

[b] Resistance: see Discussion.

tients with acute myelogenous leukemia [(AML) includes myeloblastic and myelomonocytic leukemics] in bone marrow relapse was significantly greater than normal ($0.01 > p > 0.001$). The mean activity of this enzyme in leukocytes from patients with acute lymphoblastic leukemia (ALL) in marrow relapse was higher, but not significantly, than normal levels (Table II).

Leukocytes from AML and ALL patients in relapse had increased mean levels of adenine transferase. However, the activities were not significantly greater than normal (AML: $0.1 > p > 0.05$; ALL: $0.50 > p > 0.30$).

The activity of adenine phosphoribosyltransferase was 2.8–7.2 times as great as hypoxanthine phosphoribosyltransferase in normal leukocytes. In leukocytes from patients with leukemia, the adenine enzyme was 0.7–7.5 times as active as the hypoxanthine enzyme. One ALL patient's leukocytes had a ratio of 22.5 on one occasion (Tables III, IV).

Relation of leukocyte enzyme activity to patient status. The enzyme activities of patients with acute myelogenous leukemia and acute lymphoblastic leukemia have been tabulated together with clinical data in Tables III and IV. The activities of the cells do not appear to be correlated to drug therapy or the peripheral cell counts of the patients studied. Leukocytes from patients judged clinically resistant to purine analogs did not show impairment of enzyme activities.

A fall in leukocyte hypoxanthine transferase activity was observed in one patient (S) with AML, who reached bone marrow remission during investigation. Nevertheless, a strict correlation between leukemic cells and abnormal enzyme activities could

TABLE IV. Enzyme Activities of Leukocytes from Adult Patients with Acute Lymphoblastic Leukemia.

Patient	Date	% Leukemic cells in blood	Bone marrow	Current treatment	Purine analog resistance	Peripheral cell count $\times 10^3$/mm^3	Product (mμmoles/10^6 leukocytes/hr)		Ratio, Ad/Hx
							Hx	Ad	
F	1/23	0	rel	Pred	—	7.9	0.082	0.360	4.4
G	2/4	4	rel	VCR[a] + Pred	—	0.9	0.046	0.148	3.2
	2/12	0	rem?	Pred	—	0.7	0.048	0.228	4.7
M	2/4	0	rel	—	—	4.5	0.152	0.543	3.5
McC	8/29	0	rem	Asp	—	4.0	0.092	0.354	3.8
	9/4	0	rem	Asp	—	2.5	0.540	0.358	0.7
	9/10	0	rem	Asp	—	4.0	0.160	0.592	3.7
	11/4	0	rem	—	—		0.082	0.332	3.7
B	9/18	0	rel	Asp	+	2.8	0.034	0.102	3.0
V	1/23	0	rel	VCR + Pred	—	3.0	0.036	0.114	3.2
	1/29	0	rel	Pred		4.3	0.004	0.090	22.5
T	1/23	0	rem	—	—	6.5	0.036	0.144	4.0
	1/29	0	rem	—		6.0	0.032	0.124	3.9

[a] VCR = vincristine.

not be made. In fact, none of the cell preparations from ALL patients contained significant numbers of leukemic cells and only 8 out of 18 preparations from AML patients had 50% or more leukemic cells. While the mean activity of hypoxanthine transferase was not significantly raised among the ALL patients investigated, all observations in three patients were above the normal range, suggesting that normal cells in these patients had increased activity. In the AML group the increased enzyme activity in seven preparations from four patients correlated with a predominance of normal cells; the increased activity in five preparations from two patients correlated with a predominance of leukemic cells while in three preparations an increase was observed with preparations containing 50% leukemic cells.

Discussion. An assay has been developed in which the activities of the adenine and hypoxanthine phosphoribosyltransferase enzymes can be measured successfully in intact leukocyte preparations from normal and diseased subjects. Cells for such an assay are readily prepared from blood and can be cultured successfully for short periods of time. In contrast, assays with lysed preparations of leukocytes have the inherent difficulty of obtaining pure preparations of cells for lysis without loss or impairment of cells during preparation. Cell lysis, too, may release subcellular fractions which inhibit or otherwise affect enzyme function. An assay with intact cells is a measure not only of the enzyme activity but the transport of the purine substrate across the membrane and the endogenous supply of phosphoribosylpyrophosphate (PRPP).

The principle of the present assay depends on the fact that although purine bases such as hypoxanthine and adenine can cross cell membranes, when they are converted within the cell to their respective nucleotides, inosinic acid (IMP) and adenylic acid (AMP), they no longer can leak out of the cell. Suitable washing removes the substrate, leaving the radioactive nucleotide product for counting.

Erythrocytes did not incorporate significant amounts of substrate compared with leukocytes, a fact previously reported by Berman *et al.* (12); as a consequence these cells were not eliminated from preparations. However, in lysed preparations with added PRPP erythrocytes incorporate both adenine and hypoxanthine into nucleotide. Normal leukocytes tested *in vitro* contain more adenine

phosphoribosyltransferase enzyme activity than the corresponding hypoxanthine enzyme. The ratio of the activities of the two enzymes is similar to that reported for lymph node lysates prepared from rhesus monkey (2). However, this ratio varies significantly from human erythrocyte lysates which contain more hypoxanthine than adenine enzyme (10).

Because of the clinical circumstances of most of these patients it was not possible to achieve all of the objectives of this study in a systematic way. Some observations can, nevertheless, be made. The level of hypoxanthine transferase activity was significantly increased in AML leukocytes compared with normal, and in both AML and ALL patients, the activities of the adenine and hypoxanthine transferases were more variable than normal. The cause of these findings is obscure.

In bacteria, transplanted tumors and mammalian cells in culture, a loss of hypoxanthine transferase activity is associated with resistance to 6-mercaptopurine (13). In contrast, of the nine patients who had received at some time, therapy with purine analogs, five were clinically resistant to this treatment, yet none of these showed reduced enzyme activities, a finding consistent with earlier reports (14).

Other workers have suggested that "normal" leukocytes in leukemic patients may be different from leukocytes obtained from normal donors (15). As noted above, increased activities of these enzymes sometimes occurred in preparations of cells where "normal" cells predominated as well as in cases where leukemic cells predominated. It is clear that in these patients the normal cells must be the source of increased enzyme activity, and either the morphologic critieria of normals are inadequate or these cells are responding to extracellular stimuli.

Summary. An *in vitro* assay measuring AMP: and IMP: pyrophosphate phosphoribosyltransferase (EC 2.4.2.7 and EC 2.4.2.8) levels in intact leukocytes has been described. Intact erythrocytes incorporate insignificant amounts of precursor compared with leukocytes. The mean level of IMP:pyrophosphate phosphoribosyltransferase in leukocytes from nine patients with acute myelogenous leukemia in relapse was significantly higher ($0.01 > p > 0.001$)) than in normal leukocytes. The levels of leukocyte AMP: pyrophosphate phosphoribosyltransferase were higher in leukemics but not significantly so compared with normal values. No significant reduction of IMP:pyrophosphate phosphoribosyltransferase activity was observed in leukocytes from leukemics clinically resistant to purine analogs. No correlation could be found between leukocyte enzyme activity and relapse, remission or therapy of leukemia. Enzyme levels in leukocytes isolated from patients with other malignancies were in the normal range. However, in a number of leukemics with apparently normal differential blood smears, the enzyme activities of the nonleukemic cells were greater than normal.

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