Leukocyte Purine Phosphoribosyltransferases in Human Leukemias Sensitive and Resistant to 6-Thiopurines

Martin Rosman and Hibbard E. Williams

Summary

The levels of leukocyte adenine phosphoribosyltransferase and of hypoxanthine-guanine phosphoribosyltransferase (H-GPRT) were compared in normal control subjects, patients with acute leukemia in remission, and patients with acute leukemia in exacerbation. Significant (p < 0.025) elevations of both the adenine phosphoribosyltransferase and H-GPRT activities occurred in individuals with leukemia in relapse, compared with those in remission. Altered ratios of the specific enzymatic activities of adenine phosphoribosyltransferase to H-GPRT were noted in two of eight 6-thiopurine-resistant patients. Leukemic cells from one patient with chronic myelogenous leukemia in blast crisis exhibited a marked decrease in H-GPRT activity. The H-GPRT from the second patient showed an altered affinity for purine bases and, most markedly, for 5'-phosphoribosyl-1-pyrophosphate when 6-mercaptopurine was used as substrate. The finding of these two patients with mutant H-GPRT and a reevaluation of results obtained in 6-mercaptopurine-resistant patients in a previously published series led us to the conclusion that, in human acute leukemia, resistance to 6-thiopurines may be accounted for by deficient or altered H-GPRT in a significant number of cases.

Introduction

It has been established in experimental animal tumors, bacterial systems, and in vitro human cell lines that the predominant cause of the development of resistance to 6-thiopurines is the loss of the enzyme H-GPRT, which is necessary for the activation of the drug to the inhibitory nucleotide form (2). However, there has been relatively little recent evidence that H-GPRT deficiency is the cause of the Lesch-Nyhan syndrome (20) and that it is also associated with gout in a small number of patients (24) has aroused heightened interest in this enzyme in humans. McDonald and Kelley (14) noted that a mutation affecting this enzyme need not cause complete absence of activity at saturating levels of substrate but could result in a marked decrease in effectiveness at levels of substrate commonly found in human cells. The work of Adye and Gots (1) is also significant in that several bacterial mutants resistant to 8-azaguanine apparently lost the ability to metabolize this H-GPRT substrate but retained activity with other purines tested.

In order to examine more fully the cause of resistance to 6-thiopurines in leukemic patients, we studied the levels of APRT and H-GPRT and the kinetic properties of these enzymes in leukocytes from patients with acute leukemia who were sensitive and resistant to 6-MP or 6-TG.

Materials and Methods

Patient Population. Patients with the diagnoses of active acute leukemia (regardless of type) and of acute leukemia in remission were studied. No procedure for the initial selection of patients was imposed other than that of securing the consent of the patient to undergo venipuncture for the study and the requirement that the peripheral leukocyte count exceed 3000 cells/cu mm. Of the 32 patients studied, 22 had acute leukemia in clinical and hematological exacerbation, 7 had ALL in remission, and 3 had AML in remission. Of the 22 acute leukemic patients, 9 had AML, 6 had CML in blast crisis, 3 had ALL, 2 had AMMoL, and 1 had acute promyelocytic investigation of this enzyme in leukemic patients. Davidson and Winter (3) assayed both APRT and H-GPRT activities in leukocyte lysates from normal and leukemic patients and were able to demonstrate a decrease in the activity of H-GPRT activity in only 1 of 15 6-MP-resistant cases. Subsequent work in intact leukocytes by Kessel and Hall (8) and Smith et al. (22) yielded conflicting results. Kessel and Hall noted significantly greater retention of 6-MP metabolites in vitro by leukemic leukocytes isolated from patients sensitive to 6-thiopurines, compared with patients who were drug resistant. Although decreased activity of H-GPRT would be sufficient to account for their findings, other mechanisms such as increased catabolism of 6-thioinosinic acid, proposed by Wolpert et al. (23), could also be operative. Smith et al., using a similar whole-cell system in vitro, did not find decreased uptake of labeled hypoxanthine by cells of drug-resistant patients. However, only 1 patient in this group had a blast count greater than 35%.

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leukemia, and 1 had lymphosarcoma cell leukemia. For convenience in data display, the last 4 were grouped with the AML patients. Thirteen blood samples, taken from 11 normal volunteers and patients with no known hematological abnormalities (6 female and 5 male), served as normal control preparations.

The percentage of blast cells in the patients' peripheral blood varied from 5 to 95%, with an average of 35%. There were 9 patients with greater than 50% blast cells, 5 of whom were in the 6-thiopurine-resistant category.

Patients were classified as resistant to 6-thiopurines if they had shown response (complete or partial remission) to the administered agent, either 6-MP or 6-TG, and then relapsed while still under treatment with the drug. The 6-thiopurine need not have been the sole agent used. If no remission was induced, the drug must have been administered long enough that the attending hematologist considered the patient resistant to therapy. Six of the 8 patients that were classified as resistant were studied after relapse (which followed a remission induced by a 6-thiopurine in combination with other drugs). The other 2 patients did not respond to 6-thiopurine therapy.

Materials. Adenine-14C (60 mCi/mmole) and hypoxanthine-8-14C (60.1 mCi/mmole) were purchased from American-Searle, Arlington Heights, Ill. 6-Mercaptopurine-8-14C (2.54 mCi/mmole) was purchased as a crystalline solid from New England Nuclear, Boston, Mass. Radioactive purines were separated by high-voltage electrophoresis (7) to confirm the stated purity. PRPP tetrasodium (Lot 80c-1440) was purchased from Sigma Chemical Company, St. Louis, Mo. Crystalline bovine albumin was purchased from Armour Pharmaceutical Company, Chicago, Ill. Unlabeled purines were a product of Sigma.

Cellex-D (anion-exchange cellulose) with a capacity of 0.72 mEq/g was obtained from Bio-Rad laboratories, Richmond, Calif. Dextran 100 c (M.W., 135,000) was a product of Sigma.

Methods. Leukocytes were prepared free of contaminating erythrocytes according to the method of Fallon et al. (4) with the following modifications. Ten to 20 ml of blood were collected in a heparinized plastic syringe. The cells were mixed with 1 volume of 3% dextran in 0.9% NaCl solution and allowed to sediment at room temperature for 30 to 45 min until a good separation was noted. The supernatant containing leukocytes, platelets, and some erythrocytes was decanted into a disposable plastic test tube in which all subsequent steps were performed. The cells were sedimented at 200 X g for 10 min, washed with phosphate-buffered saline, and sedimented at 150 X g for 10 min. The cells were resuspended in 0.9% NaCl solution and sedimented at 150 X g. The leukocyte pellet was suspended in 1 ml of 0.9% NaCl solution and then mixed with 6 ml of cold distilled water to lysate contaminating erythrocytes. The red-cell lysis was terminated after 30 to 45 sec (depending on the amount of red-cell contamination) with 2 ml of 3.6% NaCl solution. All remaining steps were carried out at 4°C. The leukocytes were pelleted by centrifugation at 500 X g for 10 min and suspended in 3 to 7 ml of 0.1 M Tris-HCl buffer (pH 8.0), depending on the original peripheral blood count, and then were disrupted by sonic oscillation with a Branson sonifier. Two 15-sec bursts with a 30-sec interval was sufficient to fully disrupt the cells. The resulting mixture was centrifuged at 30,000 X g for 30 min. The supernatant containing the active enzyme was stored at -70°C if not used the same day. Supernatant protein was assayed by the method of Lowry et al. (13).

The standard phosphoribosyltransferase assay contained 50 mM Tris-HCl buffer (pH 8.0), 5 mM MgCl2, 0.56 mM PRPP tetrasodium, and a radioactive purine base in a total volume of 100 μl. The final concentrations of purine bases were 0.163 mM hypoxanthine, 0.168 mM adenine, and 0.20 mM 6-MP. The reactions were initiated by the addition of enzyme (usually 40 to 80 μg protein). Appropriate control tubes in which the PRPP was omitted were included routinely. Incubations lasted 30 min at 37°C when adenine and hypoxanthine were used and lasted 2 hr when 6-MP was the substrate. The reaction was terminated by the addition of 50 μl of 0.2 M EDTA and the mixture was kept at 4°C until chromatographed. The formation of product was linear over the time interval measured and was linearly proportional to enzyme concentration. A rapid separation of reactant from products was obtained by the use of small DEAE columns. The technique is based on the method of Adye and Gots (1), as modified by Milman (G. Milman, personal communication). Columns (50 X 7 mm) were loosely packed with DEAE (Cellex-D; exchange capacity, 0.72 mEq/g) and washed with 0.01 M Tris-HCl buffer (pH 8.0). An aliquot of the reaction mixture (usually 100 μl) was applied to the column and washed with 8 ml of 0.01 M Tris-HCl buffer (pH 8.0). Unreacted purine bases were eluted by this procedure. When 6-MP was used as the enzyme substrate, 10 ml of 0.05 M Tris-HCl buffer were necessary. Nucleotides were then eluted with 3 ml of 1.0 N HCl, and the radioactivity was determined in Aquosal (New England Nuclear). The results obtained with column fractionation of the reaction were in agreement with values obtained by high-voltage electrophoresis (7).

RESULTS

Leukocyte Enzyme Characteristics. The H-GPRT was quite labile at room temperature. Thus, the enzyme decreased in activity by 20 to 30% when the preparation was stored at -70°C overnight and rethawed. Subsequent inactivation occurred more slowly, with only a 5 to 10% decrease in activity with each subsequent freeze-thaw cycle. When 6-MP was used as substrate, the decrease in activity was 40%, and was 5 to 10% for the 1st and subsequent freeze-thaw cycles, respectively. Specific activities are expressed as assayed after a freeze-thaw cycle. Negligible loss of APRT activity was produced by storage at -70°C.

The Km for hypoxanthine derived from a standard Lineweaver-Burk plot with the use of supernatant from a normal patient was 8 to 10 X 10^-6 M. The hypoxanthine concentration varied between 180 and 4 μM. This value agrees with those obtained for human erythrocytes (9). The Km for PRPP (varied between 560 and 11 μM), with hypoxanthine as the purine substrate, was 2 to 3 X 10^-4 M. This value is in agreement with those obtained by Miller and Bieber (15) in brewers' yeast and by Murray in Ehrlich ascites tumor cells (16) and mouse liver (17). It is, however, 10 times lower than...
Table 1
APRT and H-GPRT activity in patients resistant to 6-thiopurines

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age (yr)</th>
<th>Sex</th>
<th>Diagnosis</th>
<th>% blasts</th>
<th>Phosphoribosyltransferase activity (nmole of product formed per mg protein per hr)</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>B</td>
<td>18</td>
<td>F</td>
<td>AML</td>
<td>28</td>
<td>Hypoxanthine 57.2, Adenine 143.3, Mercaptopurine 19.5</td>
<td>Complete remission on prednisone, ara-C, and 6-TG; subsequent relapse treated with daunomycin, prednisone, and vincristine</td>
</tr>
<tr>
<td>G</td>
<td>43</td>
<td>M</td>
<td>AML</td>
<td>64</td>
<td>Hypoxanthine 36.9, Adenine 177.8, Mercaptopurine 3.0</td>
<td>Remission on ara-C and 6-TG; subsequent relapse treated with multi-drug combinations; currently on Adriamycin</td>
</tr>
<tr>
<td>A</td>
<td>19</td>
<td>F</td>
<td>AML</td>
<td>88</td>
<td>Hypoxanthine 305.9, Adenine 669.1, Mercaptopurine 172.9</td>
<td>Adequate course of ara-C and 6-TG without remission; subsequent treatment with multi-drug regimens, currently Adriamycin</td>
</tr>
<tr>
<td>R, 11/30</td>
<td>20</td>
<td>F</td>
<td>ALL</td>
<td>8</td>
<td>Hypoxanthine 81.3, Adenine 229.8, Mercaptopurine 22.4</td>
<td>Resistant to all multi-drug combinations, including 6-MP and 6-TG: currently on Adriamycin</td>
</tr>
<tr>
<td>R, 3/1</td>
<td>20</td>
<td>F</td>
<td>ALL</td>
<td>46</td>
<td>Hypoxanthine 244.2, Adenine 347.1, Mercaptopurine 136.8</td>
<td>Partial response to L-asparaginase; now in relapse</td>
</tr>
<tr>
<td>T</td>
<td>16</td>
<td>M</td>
<td>AML</td>
<td>88</td>
<td>Hypoxanthine 140.5, Adenine 369.5, Mercaptopurine 74.0</td>
<td>Partial remission on ara-C and 6-TG; maintained on 6-TG with subsequent relapse</td>
</tr>
<tr>
<td>A&lt;sup&gt;a&lt;/sup&gt;</td>
<td>70</td>
<td>F</td>
<td>CML-blast</td>
<td>67</td>
<td>Hypoxanthine 31.8, Adenine 352.5, Mercaptopurine 14.4</td>
<td>Partial remission of blast crisis on ara-C and 6-TG; subsequent relapse</td>
</tr>
<tr>
<td>C</td>
<td>35</td>
<td>F</td>
<td>AML</td>
<td>10</td>
<td>Hypoxanthine 66.4, Adenine 129.1, Mercaptopurine 29.1</td>
<td>Complete remission on 6-MP; subsequent relapse treated with multi-drug regimen</td>
</tr>
<tr>
<td>L</td>
<td>67</td>
<td>F</td>
<td>AMMoL</td>
<td>95</td>
<td>Hypoxanthine 639.8, Adenine 1931.2, Mercaptopurine 261.9</td>
<td>Partial response to ara-C and 6-TG; subsequent relapse</td>
</tr>
</tbody>
</table>

<sup>a</sup> Patients found to have deficient H-GPRT.
values obtained by Krenitsky and Papaioannou (9) and McDonald and Kelley (14) for human erythrocytes. The $K_m$ of PRPP with adenine as purine substrate was also 2 to $3 \times 10^{-5}$ M. The above calculations used the correction factor $\Delta$ when more than 10% of the substrate was consumed (12). The half-maximum observed rate of reaction for 6-MP (varied between 400 and 17 $\mu$M) was 3 to $4 \times 10^{-5}$ M and, for PRPP (with 6-MP at saturating levels), it was 2 to $4.5 \times 10^{-5}$ M. The H-GPRT isolated from patients with acute leukemia exhibited the same kinetic constants as did that obtained from normal control subjects.

Enzyme Activity in Leukemia. In order to investigate patients with leukemia for mutant H-GPRT, we examined the specific activities of both the APRT and H-GPRT in 6

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Chart 1. Specific activity of APRT. The values are expressed for the 6 groups noted; normal, ALL, AML, and CML-blast crisis (x) groups are represented in their respective categories. ---, mean for each group; *, patients with mutant H-GPRT.

Chart 2. Specific activity of H-GPRT, with hypoxanthine as substrate. Symbols are the same as in Chart 1.
categories of patients. The 1st 3 groups (normal, ALL in remission, and AML in remission) had normal peripheral smears and were also clinically free of hematological disease. The 4th group was comprised of 22 patients with active disease. Two subgroups were separated within this group, 1 with elevated peripheral blast cell counts and 1 that was considered resistant to 6-thiopurine therapy. All patients in the 2 subgroups were included in the parent group (acute leukemia in exacerbation), and the same patient may be represented in both subgroups. The characteristics of the resistant patients are shown in Table 1. Five of the 8 patients had blast cell counts greater than 50%. In 5 patients, an enzyme assay was performed at variable periods of time after resistance to 6-thiopurines was noted. The remaining 3 were examined during the 1st relapse after administration of the drug. All except 1 patient had received multiple medications including many combination chemotherapy regimens.

The range of enzyme-specific activities obtained from the patients is shown in Charts 1 to 3, and the means are compared in Table 2. It is evident that, although the ranges of values for the normal and remission categories are clustered close to their means with no significant difference between these groups, the acute leukemia group has a wide range of values. The difference between the means of the acute leukemia groups and the normal population is significant ($p < 0.025$), but considerable overlap is evident. Certain patients have low H-GPRT-specific activities; however, it is not clear whether these values represent individual variations within the normal range or physiologically significant abnormal enzyme function. A more exact discriminator was therefore sought that would identify significantly diminished H-GPRT activity. It has been found useful, in analysis of hair-follicle H-GPRT activity of Lesch-Nyhan heterozygotes (6), to use the ratio of APRT to H-GPRT to detect decreased enzyme activity. The calculated mean ± S.D. of this A/H ratio in our patients is shown in Table 2. The fact that the 2 patients with abnormal enzyme values are included in both the acute leukemia exacerbation and acute leukemia 6-thiopurine-resistant groups distorts the mean ± S.D. to some extent. The mean ± S.D. of the A/H ratio in the acute leukemia group is $2.195 ± 0.808$ if both of those patients are excluded. A 2nd discriminator was used to separate patients with potentially aberrant H-GPRT activity. 6-MP and hypoxanthine were used as purine substrates for H-GPRT, and the ratio of the 2 specific activities was taken (M/H ratio). It has been shown (10) that both purines are good substrates for the H-GPRT enzyme. A ratio outside the limits of the normal range might indicate a mutant enzyme with differing affinities for these 2 substrates.

A plot of the M/H to A/H ratio is shown in Chart 4. We have used the acute leukemia exacerbation group mean ± S.D. for limits in both ratios. Use of the A/H ratio mean ± S.D. of the acute leukemia exacerbation group without the 2 abnormal patients would have set the upper limit at 3.8. All
Purine Phosphoribosyltransferases in Leukemia

Chart 4. A plot of the A/H and M/H ratios of patients with active leukemia. The symbols are the same as in Chart 1. The resistant patient (●) from the study by Davidson is shown for comparison. The actual A/H ratio is shown above the 2 patients with a >9 value; *, patients with mutant H-GPRT.

Chart 5. The variation of the relative activity of H-GPRT with saturating concentrations of PRPP with the use of increasing concentrations of hypoxanthine (○, ●) and 6-MP (○, ●) as substrates. A normal (○, ●) and Patient G (●, ●) are compared.

Chart 6. The variation of relative activity of II-GPRT with increasing PRPP concentrations in normal leukocytes (○, ○) and in leukocytes of Patient G (●, ●). The substrates used at saturating concentration were hypoxanthine (○, ●) and 6-MP (○, ●).

patients in the acute leukemia exacerbation group with values for both A/H and M/H were plotted; only 2 patients fell outside the A/H lines, while 6 exceeded the M/H limits. A patient with deficient H-GPRT [from a study by Davidson and Winter (3)] is shown for comparison. One patient (A) with an A/H ratio of 11.10 was considered similar to Davidson’s patient, with a classic loss of H-GPRT, as noted in the Lesch-Nyhan syndrome and in rare cases of gout. The residual H-GPRT activity in Patient A was felt to result from the patient’s normal leukocytes. The other patients were too close to borderline values to be classified as mutant without further investigation.

Therefore, leukocyte H-GPRT was assayed in all patients with leukemia at limiting concentrations of both 6-MP and PRPP to yield one-half maximum velocity. Any patient who showed greater than the expected 50% decrease in enzyme activity was restudied with a full concentration curve. Of those tested, only 1 patient showed abnormal leukocyte enzyme kinetics (Patient G). Charts 5 and 6 show the curves for Patient G and for a normal control subject. Both hypoxanthine and 6-MP are depressed 2.5-fold, relative to the normal curve at the K_m. The greatest deviation from normal, however, is observed with the PRPP concentration curve. With the use of hypoxanthine as a substrate, the patient’s activity was about 20% of normal at the K_m, whereas with 6-MP little activity is seen at this concentration.

Charts 1 to 3 show that patients with greater than 50% blast cells have elevations of both H-GPRT and APRT but that a significant overlap with the parent group exists. More informative is the variation of these enzymes during the course of a patient’s illness. Data for 4 patients with acute leukemia and for 1 normal volunteer are shown in Table 3. As patients worsen clinically, leukocyte enzyme activities increase in contrast to those of the normal control subjects, which show little variation with time.
Table 3

Variations in APRT and H-GPRT activity during the clinical course of leukemic patients

<table>
<thead>
<tr>
<th>Patient</th>
<th>Date</th>
<th>% blasts</th>
<th>Diagnosis</th>
<th>Hypoxanthine</th>
<th>Adenine</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>D</td>
<td>9/9</td>
<td>30</td>
<td>AML</td>
<td>150.0</td>
<td>428.5</td>
<td>New patient</td>
</tr>
<tr>
<td></td>
<td>10/19</td>
<td>0</td>
<td></td>
<td>80.9</td>
<td>202.9</td>
<td>Remission on 6-TG and ara-C</td>
</tr>
<tr>
<td></td>
<td>4/19</td>
<td>0</td>
<td></td>
<td>118.9</td>
<td>193.1</td>
<td>Maintained complete remission</td>
</tr>
<tr>
<td>C</td>
<td>8/25</td>
<td>0</td>
<td>AML</td>
<td>51.7</td>
<td>65.1</td>
<td>Remission on 6-MP</td>
</tr>
<tr>
<td></td>
<td>3/28</td>
<td>10</td>
<td></td>
<td>66.4</td>
<td>129.1</td>
<td>Relapse</td>
</tr>
<tr>
<td>S</td>
<td>10/12</td>
<td>15</td>
<td>AML</td>
<td>108.8</td>
<td>432.0</td>
<td>New patient</td>
</tr>
<tr>
<td></td>
<td>10/19</td>
<td>5</td>
<td></td>
<td>66.2</td>
<td>250.6</td>
<td>Partial response to ara-C and 6-TG</td>
</tr>
<tr>
<td>L</td>
<td>2/9</td>
<td>40</td>
<td>AMMoL</td>
<td>148.0</td>
<td>260.0</td>
<td>New patient</td>
</tr>
<tr>
<td></td>
<td>4/11</td>
<td>95</td>
<td></td>
<td>639.8</td>
<td>1931.2</td>
<td>Relapse after partial response to ara-C and 6-TG</td>
</tr>
<tr>
<td>G. J.</td>
<td>7/27</td>
<td>0</td>
<td>Normal</td>
<td>137.5</td>
<td>240.4</td>
<td>Normal control</td>
</tr>
<tr>
<td></td>
<td>8/3</td>
<td>0</td>
<td></td>
<td>125.7</td>
<td>272.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1/11</td>
<td>0</td>
<td></td>
<td>126.0</td>
<td>256.8</td>
<td></td>
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</tbody>
</table>

DISCUSSION

This study demonstrates that, of 8 patients clinically resistant to 6-thiopurines, a mutation in H-GPRT may have been responsible for the resistance in 2. These data stand in contradistinction to the study by Davidson and Winter, in which 15 patients were termed 6-MP resistant. These patients had been on only 1 drug and were studied soon after development of resistance. Only 1 (No. 1) of their patients was found to have a decreased enzyme level. A reevaluation of Davidson's published data, with the 2 ratio discriminators used in our study, delineated 6 other patients with potentially mutant enzymes. The mean A/H ratio for all but 1 (No. 1) of their patients was 2.668 ± 1.226; 3 patients had ratios greater than 5. The M/H ratio was 0.528 ± 0.201; 2 patients had ratios below 0.314, and 2 others had ratios above 1.025. In all, 6 patients had potentially altered H-GPRT. Twenty-six patients were included in the Davidson study: 15 were resistant and 11 were sensitive or of unknown status. It is significant that all of the aforementioned 6 patients with potentially aberrant enzyme activity were included in the resistant category. Enzyme assays at nonsaturating levels of substrate may have uncovered mutant H-GPRT in these patients.

The infrequent occurrence of complete H-GPRT deletion as the mechanism of human resistance to the 6-thiopurines may be explained by the absence of a viable de novo purine nucleotide synthetic pathway in leukocytes and bone marrow of normal and leukemic patients. In vitro, there is little incorporation of formate into the purine ring in these cells (19, 21), and it has been proposed (11, 18) that leukocytes as well as erythrocytes rely on the preformed purines supplied by the liver for synthesis of their polynucleotides. Although there may be considerable selective pressure in a 6-thiopurine environment to mutate to the H-GPRT-negative state and thus halt further lethal synthesis of the drug, the requirement of the cell for externally supplied hypoxanthine would counteract any such pressure. A reasonable compromise for a leukemic cell with such conflicting pressures would be selective mutation, reducing the anabolism of 6-thiopurines while preserving some synthetic affinity for the other purine substrates.

The detection of an aberrant activating enzyme in patients resistant to 6-thiopurines would be important for several reasons. Therapy with drugs utilizing this pathway would be of little benefit, and the substitution of an active agent might be instituted prior to the occurrence of a clinically evident relapse. Second, the choice of a substitute agent would be affected by this information. In cells deficient in de novo purine nucleotide synthesis and mutationally H-GPRT negative, purines necessary for nucleotide synthesis must be supplied via AMP by 2 enzymes present in these cells, APRT and adenosine kinase. Advantage could be taken of this fact by administration of drugs utilizing these enzymes for lethal synthesis.

Finally, the enzyme mutation may serve as a marker of the leukemic cell much as the production of a specific paraprotein marks the myeloma cell. The malignant cell might be followed through remission and relapse with the knowledge that this protein is peculiar to the neoplastic clone. Apparently, normal end-stage cells, such as polymorphonuclear leukocytes, could be identified as arising from the malignant clone if this mutant enzyme could be detected within it. Detection might be by immunochemical means or by growth on selective media (5). Prediction of increasing leukemic potential could be assayed in the apparently normal peripheral blood and early preventative therapy could be instituted.

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