Deficiency of Methylthioadenosine Phosphorylase in Human Leukemic Cells In Vivo

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Cells from 20 patients with leukemia and 9 with solid tumors were assayed for the enzyme methylthioadenosine phosphorylase, which functions in both purine and polyamine metabolism in rapidly dividing cells. As determined by autoradiography of viable cells, and by direct enzymatic analysis, samples from one individual with pre-T-cell acute lymphoblastic leukemia and one with common acute lymphoblastic leukemia were methylthioadenosine phosphorylase deficient. In contrast, other leukemias of similar antigenic phenotype, as well as normal peripheral blood lymphocytes, thymic lymphocytes, and normal bone marrow cells, had substantial methylthioadenosine phosphorylase activity. This evidence suggests that the complete absence of methylthioadenosine phosphorylase distinguishes some leukemic cells in vivo from their nonmalignant counterparts.

THE SEARCH FOR specific metabolic pathways that distinguish normal from malignant cells has been relatively unrewarding. However, in 1977, Toohey described four murine tumors that lacked a recently described purine metabolic enzyme, methylthioadenosine phosphorylase (MTAse). The substrate for this enzyme, 5'-deoxy-5'-methylthioadenosine (MTA), is produced stoichiometrically during the synthesis of the polyamines spermidine and spermine by dividing mammalian cells. Methylthioadenosine phosphorylase cleaves MTA to adenine and 5-methylthioribose 1-phosphate, which are reconverted to adenosine nucleotides and methionine, respectively (Fig. 1).

We recently reported that 23% (7 of 31) of human malignant tumor cell lines had no MTAse, at least at detectable levels. The enzyme-deficient cell lines included 5 leukemias, 1 melanoma, and 1 breast cancer. In contrast, all of 16 cell lines of nonmalignant origin, derived from lymphoblasts, fibroblasts, and epithelial cells, contained the enzyme. One explanation for the absence of MTAse among established human malignant tumor cell lines was that the enzyme disappeared during the prolonged maintenance of the malignant cells under artificial growth conditions in tissue culture. It was therefore important to determine the incidence, if any, of MTAse deficiency among human leukemias in vivo. In the present experiments, malignant cells from 20 patients with leukemia were analyzed for MTAse. As determined by two independent methods, malignant cells from one individual with a pre-T-cell acute lymphoblastic leukemia and one with a common acute lymphoblastic leukemia were MTAse deficient.

MATERIALS AND METHODS

Specimens Analyzed

Mononuclear cells were isolated from heparinized peripheral blood or bone marrow by isopycnic sedimentation through Ficoll-Hypaque, followed by two washes in RPMI-1640 medium (Flow Labs, Rockville, Md.). In some cases, the cells were suspended in 10% fetal calf serum, 10% dimethylsulfoxide in RPMI-1640 at a density of 5 x 10^6 cells/ml, and then were cryopreserved in liquid nitrogen for up to 3 mo. Maximum care was taken to keep the cells viable during the freezing and thawing procedure. Those leukemia specimens (A) with viability less than 80%, as determined by trypan blue dye exclusion immediately before testing, or (B) with more than 40% nonleukemic cells, were excluded from autoradiographic or enzymatic analysis. Thymic lymphocytes obtained during cardiac surgery were processed similarly to peripheral blood and bone marrow. Solid tumors and adjacent normal tissues removed during surgery were dissected free from each other. The specimens were either tested immediately or frozen at -80°C for up to 3 mo and then thawed just before analysis. MTAse activities in the fresh and frozen-thawed specimens were equivalent.

Peripheral blood lymphocytes were stimulated with phytohemagglutinin and analyzed for MTAse according to our previously described protocol.

Autoradiographic Detection of MTAase

To assay for MTAase activity, the incorporation into nucleic acid of radioactive adenine cleaved from [2-3H]5'-chloroadenosine by MTAse was determined by autoradiography. The incorporation of [2-3H]adenine was used as an indicator of overall nucleic acid synthetic activity in the same cells.

Fresh or thawed cells were washed twice in RPMI-1640 medium immediately before use, and approximately 3.75 x 10^6 cells were then incubated with either 0.375 μCi [2-3H]adenine (0.117 Ci/mmol., ICN, Irvine, Calif.) or [2-3H]5'-chloroadenosine (0.117 Ci/mmol) in RPMI-1640 medium supplemented with 2 mM glutamine and 10% horse serum (Flow Labs) in a volume of 0.15 ml in a humidified atmosphere of 95% air and 5% CO2 at 37°C. The [2-3H]5'-chloroadenosine, prepared as previously described, was used as an indicator of overall nucleic acid synthetic activity in the same cells.

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Supported in part by Grants CA31497 and CA27740 from the National Cancer Institute and Grant GM23000 from the National Institutes of Health. N.K. is a recipient of a Human Cancer Research Fellowship from the Damon Runyon-Walter Winchell Cancer Research Foundation (DRG-0-1).

Submitted May 14, 1982; accepted July 26, 1982.

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0006-4971/82/6006-0022$01.00/0

Blood Vol. 60, No. 6 (December), 1982

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were processed for autoradiography. All but one sample (from patient L.A.) incorporated at least 51 grains [2-3H]adenine per cell (Figs. 2 and 3), indicating that the cells were active in the synthesis of nucleic acids. The sample from patient L.A. incorporated 3-5 grains [2-3H]adenine per cell, and a similar number of [2-3H]5'-chloroadenosine grains. Among all the leukemias, save the two MTAse-deficient tumors, the numbers of [2-3H]5'-chloroadenosine and [2-3H]adenine grains per individual cell correlated well with each other (Fig. 3).

The leukemic cells from two patients, R.H. and M.R., did not incorporate [2-3H]5'-chloroadenosine into nucleic acid under conditions in which [2-3H]adenine uptake was abundant. The uptake of [2-3H]5'-氯oadenosine was described previously. MTase activities in the extracts are expressed as nmole/min/mg protein. The protein content was determined by Lowry's method.

RESULTS

MTase Detection by Autoradiography

Twenty-four specimens from 20 leukemic patients were processed for autoradiography. All but one sample (from patient L.A.) incorporated at least 51 grains [2-3H]adenine per cell (Figs. 2 and 3), indicating that the cells were active in the synthesis of nucleic acids. The sample from patient L.A. incorporated 3-5 grains [2-3H]adenine per cell, and a similar number of [2-3H]5'-chloroadenosine grains. Among all the leukemias, save the two MTase-deficient tumors, the numbers of [2-3H]5'-chloroadenosine and [2-3H]adenine grains per individual cell correlated well with each other (Fig. 3).

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Fig. 3. Distribution of grains per cell in autoradiogram. Abscissa shows the number of grains per cell (median of the counts from 25 leukemic cells) after incubation with [2-3H]adenine. Ordinate shows the grain number calculated similarly after incubation with [2-3H]5'-chloroadenosine. The samples were from 9 common acute lymphoblastic leukemia (O), 2 acute myelocytic leukemia (★), 1 chronic myelocytic leukemia in blast crisis (▲), 3 T-cell acute lymphoblastic leukemia (△), 1 pre-T acute lymphoblastic leukemia (▽), 3 pre-B acute lymphoblastic leukemia (□), and 1 T-cell chronic lymphocytic leukemia (●) patients. The antigenic phenotypes were determined by Dr. I. Royston.11,12 Arrows indicate samples from M.R. (A) and R.H. (B).

Chloroadenosine, but not [2-3H]adenine, into nucleic acid depends on MTase activity. This result therefore indicates that the leukemic cells were MTase deficient.

Patient R.H. (Fig. 2, C and D, Fig. 3B) was a 13-yr-old male in a relapse of acute lymphoblastic leukemia. Surface marker analysis of his leukemia cells, performed by Dr. Ivor Royston as previously described,11,12 revealed a pre-T-cell phenotype (T101-positive, E-rosette-negative). Previous chemotherapy had included vincristine, prednisone, L-asparaginase, methotrexate, adriamycin, thioguanine, cytosine arabinoside, and cyclophosphamide. Leukemic bone marrow cells were obtained on two occasions, at 31 and 32 mo after diagnosis. All the leukemic cells in each sample were MTase negative.

Patient M.R. (Fig. 2, E and F, Fig. 3A) was a 28-yr-old male in a relapse of acute lymphoblastic leukemia of non-T, non-B surface phenotype. All his lymphoblasts bore the common acute lymphoblastic leukemia antigen.13 He had been previously treated with vincristine, prednisone, adriamycin, cyclophosphamide, methotrexate, L-asparaginase, cytosine arabinoside, and hydroxyurea. As determined by autoradiography, the leukemic lymphoblasts in two bone marrow aspirates obtained 12 and 13 mo after diagnosis were all MTase deficient. In contrast, his phytohemagglutinin-stimulated normal peripheral blood lymphocytes were all enzyme-positive. Hence, the patient was not himself MTase deficient.

It should be noted that many of the patients whose leukemic cells were MTase positive had received extensive prior chemotherapy with the same drugs used to treat patients R.H. and M.R.

MTase Activity in Cell Extracts

The MTase activities in extracts of normal and malignant cells are plotted on a logarithmic scale in Fig. 4. The levels in normal peripheral lymphocyte extracts ranged from 0.230 to 0.447 nmole/min/mg protein. Thymic lymphocytes had a higher activity (0.628 nmole/min/mg protein). The values for normal solid tissues (breast, lung, and ovary) varied from

Fig. 4. MTase levels in 9 leukemia (2 acute myelocytic leukemia, 2 T-cell acute lymphoblastic leukemia, 1 chronic myelocytic leukemia in blast crisis, 1 common acute lymphoblastic leukemia, 1 pre-B acute lymphoblastic leukemia, and 1 pre-T acute lymphoblastic leukemia) (★), 6 breast (□), 2 lung (△), and 1 ovary (○) cancer tissues or 6 peripheral blood lymphocytes (▲). 1 thymus (▽), 3 breast (▽), 2 lung (◇), and 1 ovary (●) tissues. Arrows indicate the samples from M.R. (A) and R.H. (B).
0.298 to 0.869 nmole/min/mg protein. With one exception, tumors from the same sites as the normal tissues had proportionately higher MTAse activities (0.750–1.953 nmole/min/mg protein).

Extracts prepared from 7 leukemic specimens had MTAse levels equivalent to solid tumors and normal thymocytes (0.459–1.670 nmole/min/mg protein). In contrast, the leukemic cell extracts prepared from patients R.H. (Fig. 4B) and M.R. (Fig. 4A) had MTAse levels of only 0.005 and 0.015 nmole/min/mg protein, respectively. Repeat analysis of fresh leukemic bone marrow specimens obtained approximately 1 mo later from the same patients yielded similar results. Considering that MTAse is found in normal bone marrow cells and peripheral blood lymphocytes, the minimal activities detected in the two patients’ pooled bone marrow extracts almost certainly may be attributed to a minor admixture of nonmalignant cells. Indeed, as noted earlier, the normal lymphocytes from patient M.R. were clearly MTAse positive.

DISCUSSION

Malignant lymphoblasts from 2 of 20 patients with acute lymphoblastic leukemia were deficient in methylthioadenosine phosphorylase (MTAse). The deficiency was established by autoradiography of individual viable tumor cells and by direct enzymatic assay of extracts prepared from leukemic bone marrow. Hence, the absence of MTAse is not an attribute solely of leukemic cells maintained in tissue culture. Rather, MTAse deficiency also occurs among human leukemias in vivo.

MTAse activity in enzyme-positive leukemias, in normal thymic lymphocytes, and in extracts of nine solid tumors distributed in the same narrow range as the multiple enzyme-positive human tumor and normal cell lines previously studied in detail. The levels of the enzyme in resting peripheral blood lymphocytes and in normal solid tissues were generally lower than in the malignant cells, although still far above the levels in the enzyme-deficient leukemic cells.

Among the possible causes for the absence of MTAse deficiency in a proportion of human leukemias in vivo, the following trivial explanations need to be ruled out: (1) derivation of the malignant cells from preexisting MTAse-deficient normal cells, (2) the association of the enzyme with a particular state of cellular differentiation, (3) the selection of MTAse-deficient clones secondary to drug treatment, and (4) the loss of the enzyme consequent to a gross karyotypic abnormality. The accumulated experimental evidence renders each possibility unlikely.

To date, we and others have not found a normal rodent tissue or human tissue that lacks detectable MTAse, and Carson, D., unpublished data). Moreover, at least 98% of dividing human bone marrow cells, which should include cells of diverse phenotype and states of differentiation, contain the enzyme as detected by autoradiography. Normal thymocytes and peripheral blood lymphocytes also have abundant MTAse activity. Other than lacking MTAse, enzyme-deficient malignant cells do not fall into any unique phenotypic category. Thus, not only leukemic lymphoblasts, but also some malignant plasmoblasts, erythroblasts, mammary cells, melanoma cells, and undifferentiated carcinomas lack detectable MTAse. In contrast, other malignant cells of the same type may contain the enzyme. As demonstrated in the present report, leukemias of similar antigenic phenotype can be either MTAse positive or negative. Cells with intermediate levels of the enzyme were not found. Taken together, these data render the notion that MTAse-deficient leukemias arose from enzyme-deficient normal cells of a particular lineage or state of differentiation highly improbable.

The two MTAse-deficient leukemias described here came from patients who had received extensive prior chemotherapy. The loss of an enzyme in purine or pyrimidine metabolism following chemotherapy with antimetabolites is a well described, albeit uncommon, phenomenon. However, no chemotherapeutic agents currently in use are known to affect the MTAse enzyme. Antileukemic therapy would not be expected to select for MTAse-deficient cells. Certainly, MTAse-deficient leukemia cell lines (e.g., CCRF-CEM and L-1210) are not naturally resistant to purine or pyrimidine antimetabolites. Finally, to the best of our knowledge, at least two MTAse-deficient mouse tumor cell lines (L-1210 and L cells, Carson, D., unpublished data) developed in experimental animals in the absence of selective chemotherapy.

There is no evidence to suggest that the MTAse-deficient phenotype is associated with a particular gross karyotypic abnormality. Thus, the well characterized MTAse-deficient human leukemic cell line CCRF-CEM has a diploid or near diploid karyotype, while other deficient lines have a variety of chromosomal abnormalities. Our preliminary experiments indicate that the MTAse-deficient phenotype behaves as an autosomal recessive characteristic in somatic-cell hybrids (Kamatani, N., Willis, E., and Carson, D., manuscript in preparation). It seems unlikely that a deletion encompassing the gene for MTAse would similarly affect homologous chromosomes.

Considering the improbability that MTAse-deficient leukemias arose from normal cells lacking the enzyme, or that the enzyme loss was secondary to prior
chemotherapy or a gross karyotypic abnormality, how can one explain the absence of this particular enzyme in the malignant cells? This question cannot yet be answered with any certainty. As diagrammed in Fig. 1, however, MTAse is related to the polyamine biosynthetic pathway and also to a pathway for the recycling of methionine necessary for S-adenosylmethionine synthesis. A diverse literature indicates that changes in polyamine synthesis and the methylation of nucleic acids are important in the control of mammalian cell replication and gene expression. It is conceivable that the loss of MTase might alter the regulation of both pathways and thereby promote the transformation, growth, or systemic spread of the leukemic clones. Future experiments will be directed at determining the exact consequences of MTase deficiency for polyamine synthesis and methylation in intact cells. Initial results suggest that MTase-deficient cells exposed to micromolar concentrations of the nucleoside accelerate their rate of putrescine synthesis.

The absence of an enzyme potentially important in polyamine and S-adenosylmethionine metabolism among human leukemias, but not in normal dividing cells, may provide a prime target for selective chemotherapy. The cleavage of MTA is the only source of endogenously synthesized adenine in human cells, and the flux through this pathway is substantial. By inhibiting de novo purine synthesis with azaserine or with methotrexate-thymidine in the presence of exogenous MTA, one can selectively kill MTase-deficient malignant cell lines without harming normal cells. An important issue now is to ascertain if this or an alternative strategy will be effective in treating MTase-deficient neoplasms in vivo.

ACKNOWLEDGMENT

We thank Drs. S. Rosenthal and J. Vangrov for providing clinical specimens; Dr. S. Matsumoto, L. Frincke, E. Willis, and L. Bleeker for technical assistance; and Anna Milne and Beth Morgan for typing the manuscript.

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