Genetic Expression of Adenosine Deaminase in Human Lymphoid Malignancies

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Adenosine deaminase (ADA) is an enzyme in the purine catabolic pathway that has been used as an enzymatic marker of T cell lymphoblastic malignancies due to its high specific activity in thymocytes and immature T cells. We have investigated whether the level of ADA activity in lymphoid leukemic cells correlates with the amount of ADA-specific RNA and/or immunoreactive protein in these cells as an initial step toward characterizing the nature of the genetic regulation of ADA expression during differentiation. We have found a good correlation between the steady state levels of ADA-specific RNA and ADA-immunoreactive protein in T lymphoblastic leukemic cell lines, mature T cell lines, a B lymphoblast cell line, and leukemic cells directly isolated from four patients with acute lymphoblastic leukemia and three patients with chronic lymphocytic leukemia. Southern blot analysis of DNA from these cells shows no evidence for differences in ADA gene copy number or gene rearrangement to account for the variability in ADA expression. We conclude that levels of ADA in lymphoid leukemic cells are directly related to the amount of ADA-specific mRNA present. These findings imply that ADA expression in leukemic cells reflects either the transcriptional activity of the ADA gene or the stability of ADA mRNA in these cells.

Materials and Methods

Adenosine deaminase (ADA; EC 3.5.4.4), a catabolic enzyme in the purine metabolic pathway, catalyzes the irreversible conversion of adenosine and 2'-deoxyadenosine to inosine and 2'-deoxyxynosine, respectively. The association of ADA deficiency and severe combined immunodeficiency disease in humans,2 has spurred considerable interest in the examination of this enzyme in human leukemias and lymphomas.3 5 Although ADA is a ubiquitous mammalian enzyme, its levels vary by over 50-fold in a tissue-specific manner.4 The highest levels of activity have been found in T lymphoblasts and ADA has thus been utilized as a marker of T cell malignancies.3 6 ADA activity decreases markedly during T cell maturation,8 while normal peripheral blood B cells and chronic lymphocytic leukemic cells have ADA activity that is severalfold lower than the activity in mature peripheral blood T cells.9 It has been reported that the ADA enzyme activity found in acute lymphoblastic leukemic cells has similar biochemical and kinetic properties to ADA enzyme activity in normal peripheral blood lymphocytes,10 implying that the alterations in ADA-specific activity in these disorders are due to alterations in the amount of ADA protein. However, the precise mechanism governing the differential expression of ADA activity in normal and malignant lymphoid cells is poorly understood. We have therefore investigated the expression of ADA at both the RNA and protein level in human lymphocytic leukemic cells to determine whether the specific activity of this enzyme directly correlates with changes in either or both of these parameters.

1376

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Submitted April 4, 1985; accepted December 1, 1986.

Supported by a grant from the Children’s Leukemia Foundation of Michigan and NIH grant 1 RO1 AI24012. Dr Mitchell is the recipient of a Scholar Award from the Leukemia Society of America.

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(Richmond, CA) scanning densitometer. Hybridization with the 0.65-kb ADA probe showed a distinct band at 1.6 kb and a variable band at 5.8 kb. Since the 1.6-kb transcript corresponds to the message for ADA in poly A-selected RNA and the 5.8-kb band appears to be nonspecific, we have quantitated only the 1.6-kb band for these studies. We have used the values obtained for this 1.6-kb ADA RNA band in MOLT-4 cells as a reference standard for all other cells and cell lines.

Southern blots. Genomic DNA was isolated by the method of Blin and Stafford. Ten micrograms of DNA were digested with various restriction enzymes according to the manufacturer's recommendations. The digested DNA was size-fractionated on a 0.8% agarose gel and the DNA was transferred to nitrocellulose paper. The filter was hybridized with both the full-length and 0.65-kb ADA probes for ADA, washed, and autoradiographed.

Adenosine deaminase assays. The catalytic activity of ADA in dialyzed cell lysates was determined in a radiochemical assay by measuring the conversion of [14C]adenosine to inosine. ADA immunoreactive protein (IRP) was determined by a solid-phase radioimmunoassay. Serial dilutions of commercial calf ADA, MOLT-4 standard lysate and leukemic cell lysates were spotted in 10-µL aliquots on nitrocellulose paper (1.5 cm × 1.5 cm area) that had been previously incubated with 25 mmol/L Tris-HCl, pH 7.6, containing 0.154 mol/L NaCl (Buffer A). The filter was fixed in 10% acetic acid and 25% isopropanol for 15 minutes and rinsed several times with Buffer A. The filter was then incubated with 5% BSA in buffer A overnight to block nonspecific binding of antibody, followed by a four-hour incubation at room temperature with rabbit anti-ADA serum (1:250 dilution) prepared as previously described and containing 3% BSA. The filter was then washed five times for 10 minutes each in buffer A containing 0.1% Triton X-100. Subsequently, the filter was incubated with [125I] protein A (2 x 10⁶ cpm/mL, specific activity 10 µCi/µg) in 50 mL of buffer A containing 3% BSA at room temperature for one hour. The filter was washed extensively with buffer A containing 0.1% Triton X-100, air-dried, and autoradiographed. The radioactive spots were cut out and counted in a Packard (Downers Grove, IL) gamma counter. Protein estimation was performed according to the method of Lowry.

Western blot. Cell lysates were obtained by three cycles of rapid freeze-thawing in liquid nitrogen and the supernatants obtained after centrifugation at 10,000 x g. The supernatants were electrophoresed on 10% SDS polyacrylamide gels, and the resolved proteins were transferred to nitrocellulose paper by electroelution. The nitrocellulose paper was then incubated with anti-ADA antibody, probed with [125I] protein A as described previously for the solid-phase radioimmunoassay, and autoradiographed.

RESULTS

The specificity of our polyclonal antibody, which was raised in rabbits, to human erythrocyte ADA for ADA in leukemic cell lysates was evaluated using a Western blot. As shown in Fig 1, a single radioactive band was visualized with MOLT-4 T lymphoblast crude cell lysate. This band corresponded to the molecular weight (Mₙ) of purified commercial calf ADA (Lane 5) and agrees with previously determined Mₙ values for the enzyme. For each set of assays, a standard curve was constructed using calf ADA and MOLT-4 T lymphoblast lysate as relative internal standards. We have demonstrated on other immunoblots that this antibody cross-reacts with both the human and calf enzymes at roughly equivalent concentrations. The IRP determinations were linear from 12.5 ng to 500 ng ADA protein for the calf enzyme (Fig 2) and a similar dose–response relationship was obtained with crude lymphoblast cell lysate (data not shown).

A representative Northern blot used for the quantitation of ADA mRNA levels is shown in Fig 3A. The 1.6-kb band corresponds to the ADA-specific mRNA. To control for the amount of RNA loaded onto each gel, the blots were stripped and reprobed with the β-actin cDNA probe; alternatively, duplicate nitrocellulose filters were simultaneously probed with radiolabeled ADA or β-actin cDNAs (Fig 3B). The lower 1.8-kb band in Fig 3B represents actin-specific mRNA. The ADA and actin band signals were quantitated from each autoradiograph by densitometry scanning, and a ratio of ADA to actin was obtained for each sample. MOLT-
DISCUSSION

The regulation of ADA expression in lymphoid cells has been of considerable interest because of the large variation in enzyme activity during lymphocyte maturation and among lymphoid leukemic cells and cell lines examined. One T-ALL sample repeatedly showed a twofold increase in IRP, as shown in column one. ADA catalytic activity relative to MOLT-4 cells was equivalent in T-ALL cells, but was approximately twofold lower in other cell types. Whether this discrepancy signifies exogenous factors affecting catalytic activity or is merely attributable to the limited number of samples in each group remains to be determined.

To further investigate whether the increased levels of ADA RNA in the T lymphoblasts result from amplification or rearrangement of the ADA gene, Southern blot analyses of DNA from the various cell lines and from normal lymphocytes were performed (Fig 5). Using the full-length ADA cDNA probe, digestions of genomic DNA gave similar restriction fragments in all lanes (Figure 5, A and B), making it unlikely that the ADA gene is rearranged in these cells. Furthermore, as shown in Fig 6, an Eco RI digest on DNA from five of the cell lines reveals a single band of similar intensity in all lanes, suggesting that a variation in copy number of the ADA gene is unlikely to be responsible for the variable steady state RNA levels in the lymphoid leukemic cells studied.

4 T lymphoblast RNA was run on each gel as an internal control and all mRNA data are expressed as a percentage of the concomitant MOLT-4 ADA:actin ratio. Figure 4 demonstrates that ADA mRNA levels and IRP values are in general good agreement for the lymphoid leukemic cells and cell lines examined. One T-ALL sample repeatedly showed a twofold increase in IRP, as shown in column one. ADA catalytic activity relative to MOLT-4 cells was equivalent in T-ALL cells, but was approximately twofold lower in other cell types. Whether this discrepancy signifies exogenous factors affecting catalytic activity or is merely attributable to the limited number of samples in each group remains to be determined.

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ADA EXPRESSION IN LYMPHOID MALIGNANCY

Two precedents for ADA amplification have been reported, both in rodent cells that had been subjected to selective pressure for increased ADA expression. Rat hepatoma cells grown in the presence of the ADA inhibitor 2'-deoxycoformycin and the ADA substrate adenosine demonstrated increased ADA activity and mRNA levels that were due to gene amplification. In addition, a mouse cell line with a 3,000-fold increase in ADA activity was demonstrated to have an equivalent increase in mRNA and gene copy number on Northern and Southern blots, respectively.

In this study we were unable to demonstrate any significant amplification of ADA gene sequences in human T lymphoblasts as compared to normal mononuclear cells and phenotypically more mature T₄⁺ leukemic cell lines (Hut 78 and Hut 102). This is not surprising, since the high ADA activity in malignant T lymphoblasts reflects similarly high activity in normal human thymocytes. It would be difficult to reconcile the decline in ADA activity during T cell maturation with a change in the copy number of the ADA gene.

An alternative explanation for the variability in ADA expression is that the gene is rearranged during T cell development. Gene rearrangements have been documented to account for the increased transcription of the c-myc proto-oncogene in Burkitt’s lymphoma and are known to occur for both immunoglobulins and the T cell receptor during the respective maturation of B and T lymphocytes. Our data, however, do not support ADA gene rearrangement in the lymphoid leukemic cells studied.

A third potential mechanism for changes in the transcription of specific genes during differentiation is alterations in the methylation status of cytosine residues in 5’ regulatory regions. Hypomethylation has been correlated with an increase in corresponding mRNA levels for a variety of different proteins. We have performed digestions of genomic DNA with Hpa II and Msp I restriction enzymes that show some minor differences in restriction pattern among the leukemic T cell lines when probed with the full-length ADA cDNA (data not shown). However, these changes show no consistent relationship of methylation status to ADA expression. The recent characterization of the ADA promoter and sequencing of the entire ADA gene should allow a more precise approach to the problems of regulation of ADA expression as a function of lymphocyte differentiation.

ACKNOWLEDGMENT

We thank Dale Begley for his technical assistance and Sue Siaczka for typing the manuscript.

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Genetic expression of adenosine deaminase in human lymphoid malignancies

TE Gan, PE Dadonna and BS Mitchell