Adenosine Deaminase Activity in Chronic Lymphocytic Leukemia

RELATIONSHIP TO B- AND T-CELL SUBPOPULATIONS

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A B S T R A C T The level, phenotypes, and isozyme distribution of adenosine deaminase (ADA) were determined in lymphocytes from patients with chronic lymphocytic leukemia (CLL). The ADA level in lymphocytes from patients with untreated CLL was consistently lower than in lymphocytes from normal subjects. No significant differences were found in the phenotype or isozyme distribution. In untreated patients, the ADA level was inversely correlated with the lymphocyte count and the percentage of bursa-equivalent (B) cells. After therapy, a diminution in the lymphocyte count was associated with an increase of ADA activity towards normal levels. The ADA levels were slightly higher in the thymus-derived (T) than in the B lymphocytes from normal subjects. The B cells had lower activity than T cells in patients with CLL. They also had a lower activity than the B cells from normal subjects. The ADA level was 2.3-fold higher in T cells from patients with CLL than in normal T cells. The decrease in ADA levels is an anomaly that is reversible and appears to be a reflection of the proliferation of abnormal B cells in this disorder.

INTRODUCTION

Adenosine deaminase (adenosine aminohydrolase, EC 3.5.4.4), or ADA,[1] which catalyzes the deamination of adenosine to inosine is a polymorphic enzyme found in

most human tissues (1, 2). A role for ADA in the immune response has been suggested by the finding of at least 19 cases of severe combined immunodeficiency (3–5) in whose lymphocytes this activity was virtually absent. A diminished response to phytohemagglutinin stimulation and extremely low levels of circulating immunoglobulins were observed in these patients. Qualitatively similar, but less severe, findings have been documented in chronic lymphocytic leukemia (CLL); these include a sluggish response to phytohemagglutinin stimulation (6) and decreased levels of circulating immunoglobulins (7). In view of these similarities, the experiments reported below were designed to determine the level, phenotypes, and isozyme distribution in lymphocytes from patients with CLL. The results reported below indicate that decreased ADA levels occur in this disorder.

METHODS

Nucleoside phosphorylase and xanthine oxidase were purchased from C. F. Boehringer and Sons (Mannheim, West Germany). Adenosine and inosine were obtained from Sigma Chemical Co. (St. Louis, Mo.). After informed consent had been obtained from the patients according to the provisions of the Helsinki conference, their lymphocytes were isolated from heparinized blood as previously described (10).

Erythrocytes and granulocytes were separated by centrifugation through a mixture of Ficoll-Hypaque (8), while monocytes and platelets were removed on a glass wool column (9); the final preparations usually contained over 95% lymphoid cells. The criteria for selection of patients with CLL were the same as reported previously from this laboratory (10). Unless specified otherwise, the patients had never received therapy. Lymphocytes suspended in 0.05 M sodium phosphate buffer, pH 7.5, at concentrations between 5×10^7 and 2×10^8 cells/ml were disrupted by 5 cycles of freezing and thawing followed by a 5-s exposure to ultrasound generated by a Heat Systems-Ultrasonics, Inc. cell disruptor (Plainview, N. Y.). The sonicated suspension was centrifuged at 1,100 *g* for 10 min and the supernatant fluid used for enzyme assays, electrophoresis, and gel-filtration studies.

Enzyme assays. ADA activity was measured by the conversion of adenosine to uric acid, determined spectrophotometrically by the change in absorbance at 293 nm (11). In this assay ADA is linked to an excess of nucleoside phosphorylase and xanthine oxidase. In general, the assay, performed at 37°C in a total volume of 1 ml, contained the following: sodium phosphate buffer, pH 7.5, 50 mM; adenosine, 1.3 mM; nucleoside phosphorylase; 5 μg; xanthine oxidase, 50 μg; and lymphocyte supernatant fluid containing between 30 and 100 μg of protein. In the reaction blanks, an equal volume of 0.05 M sodium phosphate buffer, pH 7.5, was substituted for the lymphocyte supernatant fluid. Erythrocyte ADA activity was determined in a similar fashion, except for the cell disruption which was carried out by the lysis of 0.1 ml of packed erythrocytes with 4 ml of 0.01 M sodium phosphate buffer, pH 7.5, and the substitution of endogenous nucleoside phosphorylase. Protein concentration was determined according to Lowry et al. (12), using human serum albumin as a standard. The specific activity unit is expressed as nanomoles per hour per milligram of protein for lymphocytes, and as nanomoles per hour per milligram of hemoglobin for erythrocytes. The reaction was linear with time and protein concentration. The concentration of adenosine was saturating under the test conditions for normal and CLL lymphocytes. Quantitative conversion of inosine to uric acid was demonstrable for both cell types under the conditions of the assay. Duplicate determinations agreed within ±10%. The determination of phenotypes and the separation of ADA into erythrocyte and tissue isozymes by gel filtration were performed as previously described (2, 13).

Determination of percentage of bursa-equivalent (B) cells and thymus-derived (T) cells. The percentage of lymphocytes bearing receptors for complement (B cells) was determined from the percentage of lymphocytes forming rosettes with trypsinized sheep erythrocytes coated with amboceptor and complement by a minor modification (14) of the method of Bianco et al. (15). T lymphocytes, which have receptors for sheep erythrocytes, were measured as the percentage of lymphocytes forming rosettes with neuraminidase-treated sheep erythrocytes (16). Only lymphocytes with three or more erythrocytes attached were counted as rosettes in the determination of T and B cells. Selective depletion of B and T cells was achieved by centrifuging the B or T rosettes through a Ficoll-Hypaque mixture (17). The depleted preparation, i.e. cells which did not form rosettes, remained at the interface. They were washed three times in RPMI 1640 medium before assay. T or B cells were also recovered from the specific rosettes as follows: The cells in the pellet below the Ficoll-Hypaque layer were washed three times with phosphate-buffered saline (PBS). After the last washing, the erythrocytes were hemolyzed by the addition of 3 vol of distilled H₂O to the packed cells, followed by vigorous stirring for 30 s. Isoosmolarity was re-established by the addition of 1 vol of 0.06 M NaCl, and the lymphocytes were washed twice in PBS before assay.

RESULTS

Determination of ADA levels, phenotypes, and isozyme pattern. As shown in Table I, the specific activity of ADA in lymphocytes isolated from the blood of patients with "B"-type CLL was significantly lower than in lymphocytes from normal blood or tonsils. In contrast, cells from a patient with "T"-type CLL, with the clinical picture of Sézary syndrome, and a leukocyte count of 100,-

TABLE I
ADA Level, Phenotype, and Isozyme Distribution

Source of lymphocytes	Lymphocyte	ADA 2-1 heterozygotes	Tissue isozyme*	RBC-ADA
	nmol/h/mg protein	%	%	nmol/h/mg hemoglobin
Normal				
Blood				
Adult	3,162±866 (n = 10)	13.9 (n = 101)	14.6±8.4 (n = 9)	78.98±19.0 (n = 8)
Newborn	2,444 / 3,451			
Tonsils	3,066±251 (n = 5)	—	ND‡	—
Thymus				
Child	11,200 / 35,773	—	ND‡	—
Adult	9,876			
CLL				
"B" type	1,038±537 (n = 22)	20.9 (n = 43)	6.6±3.0 (n = 5)	90.0±20.5 (n = 8)
"T" type (Sézary)	6,945	—	—	—

* Percentage of total ADA present as high molecular weight, "tissue" ADA.
‡ Not determined.

000/mm³, had a markedly increased specific activity (Table I). Thymocytes from two infants and an adult had the highest specific activity of ADA among the cells tested. Since an association between phenotype and erythrocyte ADA levels has been reported (18), the phenotype for this polymorphic enzyme was determined in 43 patients with CLL. The results indicate that although the incidence of heterozygous 2-1 phenotype is higher than that of the control populations matched for ethnic background, this difference is not statistically significant $(0.5 > P > 0.2)$. A slight difference in the distribution pattern of the high molecular weight "tissue" form and the more active low molecular weight "erythrocyte" form of ADA found between normal and CLL lymphocytes was not statistically significant. Identical ADA levels were found in the erythrocytes from normal subjects and from patients with CLL. There was no relationship between the level of ADA and the level of 5′-nucleotidase, another enzyme activity which is frequently diminished in CLL (10).

To determine whether the decrease in enzyme activity could be attributed to the presence of an inhibitor in the CLL lymphocyte, a "mixing" experiment was performed. When homogenates from a normal or a CLL preparation with decreased ADA activity were incubated separately or in a series of mixtures of varying proportions, the specific activity corresponded to the value derived from the ratio of the homogenates in the mixture (Table II). This finding was evidence against the presence of all but a tightly bound inhibitor in CLL or an activator in the normal lymphocytes. This experiment was also performed with dialyzed homogenates with identical results.

TABLE II

Mixing Experiment

	ADA, nmol/h/ml		
	Observed	Expected	O/E*
Normal	150.3	—	—
CLL	68.8	—	—
Normal + CLL 1:3	86.5	89.0	0.97
Normal + CLL 1:1	116.2	106.7	1.09
Normal + CLL 3:1	135.1	130.1	1.04

* The homogenate mixtures were preincubated for 15 min at 37°C before addition of substrate to start the reaction. O/E = ratio of observed/expected activity.

Serial determinations of ADA activity in treated and untreated patients with CLL. ADA levels were determined on multiple occasions in 10 patients (Table III). Stable ADA levels were noted in patients 1-5 who had stable lymphocyte counts and received no therapy. Little fluctuation was also noted in two patients, 6 and 7, who received treatment but whose lymphocyte counts did not change. One patient, 8, whose lymphocyte count increased markedly while on therapy, showed a concomitant decline in ADA levels. In contrast, patients 9 and 10, whose lymphocyte count markedly decreased with therapy, showed a return in ADA level toward the normal range. These results suggested a relationship between the lymphocyte count and ADA level. They also indicate that the decreased ADA level can be a reversible abnormality. Further studies on the effect of therapy on ADA levels are required to define fully this relationship.

Studies on lymphocyte subpopulations. In patients with CLL a correlation was found between the absolute lymphocyte counts and ADA levels ($r = 0.71$) (Fig. 1). A correlation was also observed between ADA activity and the percentage of B cells ($r = 0.69$) (Fig. 2). As has been reported by others, most of these patients had an elevated percentage of B cells (14, 19), with a concurrent diminution in the percentage of T cells. As is shown in the figure, the lowest ADA levels occur in patients with the highest percentage of B cells. These were also patients who had the lowest percentage of T and null cells.

These results suggested that the decreased ADA level in lymphocyte preparations from patients with CLL reflected the higher than normal B cell/T cell ratio observed in this disorder. To determine whether the ADA activity was localized preferentially to either B or T cells, experiments were performed to deplete the purified lymphocyte preparations of these cell types. Lymphocyte populations containing T and B cells were treated as described under Methods. The results of these experiments (not shown) indicated that the ADA activity in six

preparations from CLL patients after selective depletion of B cells was 1.7 times that of preparations depleted of T cells. The same experiment performed with lymphocytes from the blood of three normal subjects revealed that the specific activity in B-depleted preparations was 1.1 times that of those which had been depleted of T cells.

TABLE III

Serial Determinations of ADA Activity

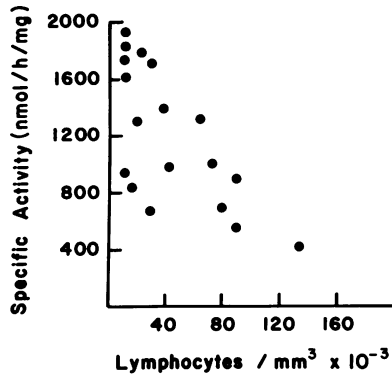| Patient no.	Date of study	Absolute lymphocyte count	ADA specific activity	Therapy
		X 10^{-3}/mm^3	nmol/h/mg	
1	6/18/74	11	2,186	None
	6/20/74	9	1,837	
	7/02/74	13	1,897	
	7/25/74	16	1,568	
2	9/31/74	75	1,018	None
	3/13/75	87	1,076	
3	6/25/74	129	689	None
	5/16/75	89	492	
4	6/26/74	12	1,760	None
	9/25/74	14	2,491	
	7/01/75	20	2,203	
5	6/26/74	43	775	None
	7/25/74	41	802	
	8/28/74	52	729	
6	7/04/74	27	564	Chlorambucil
	7/18/74	21	505	and prednisone
	7/26/74	32	530	
7	7/16/74	66	1,301	None
	4/08/75	97	1,539	Chlorambucil
	4/15/75	80	1,775	Chlorambucil
	7/09/75	70	1,274	
8	7/03/74	12	3,385	Chlorambucil
	12/04/74	68	1,140	and prednisone
	2/06/75	73	1,244	
	3/05/75	45	1,028	
9	7/08/74	127	394	None
	7/25/74	139	338	None; start Chlorambucil
	8/28/74	48	640	Chlorambucil
	9/10/74	28	1,176	Chlorambucil
	10/17/74	13	1,059	Chlorambucil
	11/20/74	12	920	Chlorambucil
	2/19/75	14	1,092	Chlorambucil
	4/01/75	12	1,293	Chlorambucil
10	4/17/74	142	1,664	None
	6/26/74	38	1,758	Chlorambucil
	7/25/74	20	3,375	Chlorambucil
	10/17/74	11	3,111	Chlorambucil
	12/12/74	14	2,241	Chlorambucil

FIGURE 1 Relationship of ADA activity to the absolute lymphocyte count in patients with CLL. None of these patients had received therapy.

The finding of higher ADA levels after B depletion suggested that a difference might exist between the ADA activity of B and T cells in CLL. An analysis of the data from this type of depletion experiment is difficult for two reasons: (a) at times the depletion was not complete, i.e. a small percent of T cells or more often a significant number of B cells (up to 18%) remained despite the depletion; and (b) a variable number of null cells remained in the "depleted" cell preparation. For this reason, the ADA activity was also determined in purified B- or T-cell preparations which were recovered from the rosettes containing B or T cells. These preparations were more homogeneous than the "depleted" samples. The results from these experiments are shown in Table IV. The ADA specific activity was slightly higher in the normal T- than in normal B-cell preparations. The CLL B cells had less than one-half the specific activity of normal B cells and one-sixth the activity of CLL T cells. Surprisingly, the T-cell preparation in CLL had a higher activity than normal T cells. The unfractionated CLL
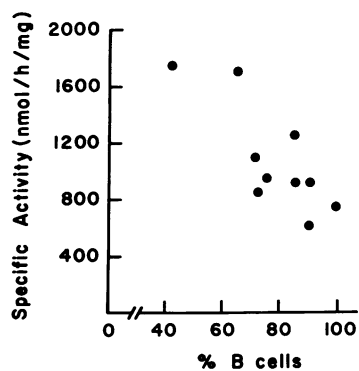

FIGURE 2 Relationship of ADA activity to the percentage of B cells in patients with CLL. The range for the percentage of B cells in normal subjects as determined in this laboratory is from 2 to 12%.

TABLE IV
ADA Activity in T and B Cells

Source of lymphocytes	Specific activity	
	T cells	B cells
	nmol/h/mg	
Normal	2,376	1,810
	2,324	2,042
	2,025	829
	2,409*	1,395*
	3,104*	
	1,542	
Average±SD	2,296±468	1,519±460
CLL	5,599	378
	4,050	983
	3,351	829
	7,637	898
Average±SD	5,159±1,645	772±234

* Tonsil lymphocytes.

cell population often contained very few T cells and as such would primarily reflect the low ADA activity of the predominant B-cell subpopulations.

DISCUSSION

The data reported above indicate that lymphocytes from untreated patients with CLL show a markedly diminished specific activity of ADA. This abnormality does not occur in their erythrocytes that have normal enzyme levels. The decreased enzyme level is not associated with an altered ratio of the high to the low molecular weight forms of the enzyme. The incidence of the 2-1 phenotype is somewhat higher in patients with CLL than in control subjects. Although the initial phases of this study suggested an increased incidence of this phenotype in CLL (20), when the number of observations was increased to 43 subjects, this difference did not appear statistically significant.

The diminution in ADA activity was closely related to the patients' increased lymphocyte count. This observation probably explains the finding of normal or low ADA levels in an earlier survey of enzymes of nucleotide metabolism in CLL (21). Other studies on the same patient population in our laboratory have found that other activities are normal (DPNH diaphorase) (22) or elevated (sialyltransferase) in CLL.[a] Additional evidence for this relationship was provided by the effect of therapy on ADA activity. In those cases where therapy lowered the lymphocyte count to normal, increases in ADA activity were observed. In contrast, the level remained low

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[a] Meyers, R., and R. Silber. Manuscript in preparation.

over a 12-mo period in subjects who were not treated or who did not respond to therapy. The normal levels of ADA in the erythrocytes of these patients, as well as the return to normal levels of ADA activity in the lymphocytes with a fall in the leukocyte count and response to therapy, suggest that in CLL the lowered ADA activity is an acquired defect. In contrast, lowered levels of ADA had been found in the lymphocytes from children with acute lymphoblastic leukemia (ALL) and from their parents, indicating a possible genetic influence (23).

In view of the well-established increase in B cells, the correlation between ADA levels and these cells was not unexpected. In addition to the observed negative correlations, two other lines of evidence suggest that the altered B cell/T cell ratio results in the decrease in enzyme activity. Experiments were performed in which B or T cells were selectively purified from lymphocyte preparations containing these subpopulations. The results of these experiments indicated that the specific activity in B cells recovered from CLL patients was significantly lower than that of their T cells and lower than that of normal B cells. Unexpectedly, the activity in T cells from CLL blood was twice that of T cells from normal blood. While the normal T-cell ADA activity was only slightly higher than the normal B-cell level, normal human thymocytes contained very high ADA activity, suggesting that the ADA level in undifferentiated T cells is markedly higher than in peripheral B or T cell. Further support for the concept that CLL T cell may differ from the normal peripheral blood T cell is the finding of a supranormal ADA level in a patient with T-cell CLL of the Sézary type.

The recent report of high ADA levels in ALL and blastic crisis of chronic myelogenous leukemia (24) may reflect a relationship between the cells proliferating in this disorder and T cell. In this respect, the CLL T cell resembles the normal thymus more closely than the normal blood T cell. A recent report of the beneficial effect of thymic irradiation in the management of CLL is of interest in view of this finding (25).

The known generalized immunological defect in CLL patients is well documented. It has been shown that a proportion of individuals with an even more profound, inherited, immunological defect (patients with severe combined immunodeficiency) exhibit an inherited deficit of ADA activity in their erythrocytes and lymphocytes. It is, therefore, tempting to speculate that the functionally abnormal B cell in CLL patients may be related to the lower levels of ADA, as appears to be the case for the lymphocytes from combined immunodeficiency patients. Experiments are underway to test the hypothesis that addition of exogenous ADA will enhance the in vitro response of CLL lymphocytes, as it does for lymphocytes from combined immunodeficiency patients (26).

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