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Purine Metabolizing Enzymes of Lymphocyte Cell Populations: Correlation between AMP-Deaminase Activity and dATP Accumulation in Murine Lymphocytes (42122)

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Deoxyadenosine and its nucleotides are thought to be involved in the pathogenesis of the immunodeficient state associated with inherited adenosine deaminase (ADA) deficiency (1–3) and in the lymphotoxicity that occurs during the treatment of leukemia with deoxycoformycin, an ADA inhibitor (1–3). Lymphotoxicity can be simulated *in vitro* by treating lymphoid cells with deoxyadenosine in the presence of an ADA inhibitor. Such a treatment of lymphoblasts results in intracellular accumulation of deoxy-ATP (dATP), inhibition of DNA synthesis, ATP depletion, leading to cell toxicity; different lymphoid cells display very different sensitivity to this deoxyadenosine cytotoxicity (4, 5).

The mechanism of dATP-induced cytotoxicity is still poorly understood; one of the first hypotheses was the blockade by dATP of ribonucleotide reductase which would result in an inhibition of DNA synthesis due to the shortage of deoxynucleotide precursors (4–6). More recently it was claimed that a dATP-induced inhibition of RNA synthesis could provide an alternative explanation for deoxyadenosine toxicity in lymphoid cells (7). Both hypotheses appear correlated to the cell capacity to accumulate dATP; nevertheless, as suggested by Sylwestrowicz (8), this cytotoxicity depends not only on intracellular dATP synthesis but also on the capacity of the cell to degrade accumulated dATP.

Both AMP-deaminase (AMP-DA), which deaminates AMP and dAMP, and cytosolic 5′-nucleotidase (c5′N), which hydrolyzes intracellular mononucleotides, are involved in nucleotide catabolism. Contrary to other enzymes of nucleotide metabolism, these enzymes have scarcely been studied in lymphoid cells. It was only reported that T lymphoblasts display AMP-DA activities 5- to 10-fold lower than those of B lymphoblasts and that different AMP-DA isoenzymes could be expressed in these lymphoblasts (9). It was also shown that AMP-DA activity is greatly altered during the *in vitro* differentiation of muscle cells (10). These data and the fact that the sensitivity of thymocyte subpopulations to ADA inhibitors changes with their maturation stage (11) prompted us to study AMP-DA activity in several lymphocyte subpopulations. We showed that, unlike c5′N, AMP-DA displayed activities dependent on the maturation or differentiation level. The AMP-DA activity of the cells appeared inversely related to their capacity of dATP accumulation. In resting lymphocytes the accumulation of dATP coincided with the inhibition of RNA synthesis and cell death.

Materials and Methods. *Cells.* Mouse cells were obtained from 4- to 7-week-old Swiss male mice. Separation of cortical and medullary mouse thymocytes was performed by agglutination with peanut agglutinin (PNA); T and B splenocytes were separated by agglutination with soybean agglutinin (SBA) or wheat germ agglutinin (WGA). The purification of normal peripheral blood and tonsil lymphocytes and the origin of the different cell lines have been reported elsewhere (12, 13). The cells were packed, washed twice with normal saline, and disrupted by two successive freezings; after two centrifugations (100,000*g*, 20 min, 4°C), the supernatants were dialyzed against 10 m*M* Tris–HCl, pH 7.4, and assayed for their enzymatic activities. Protein concentrations were determined by Lowry's method.

Enzymatic activities. AMP-DA was assayed by following the conversion of 5′-[^{3}H]AMP or 5′-d[^{3}H]AMP to 5′-[^{3}H]IMP or 5′-d[^{3}H]-IMP, at 37°C. The standard assay contained in 200 μl 50 m*M* sodium cacodylate, pH 6.7, 150 m*M* KCl, 0.2 m*M* EDTA, 20 m*M* α,β-methylene ADP (AOPC), 0.5 m*M* 5′-[^{3}H]-AMP, or 1 m*M* 5′-d[^{3}H]AMP, and 5 to 300 μg supernatant. AOPC, a specific inhibitor of ecto-5′-nucleotidase, and EDTA, which

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inhibits c5'N and adenosine kinase, do not affect AMP-DA activity. After 30 min incubation at 37°C the reaction was stopped at 100°C and 5 m*M* 5'-AMP and 5'-IMP or 5 m*M* 5'-dAMP and 5'-dIMP were added to the reaction mixture. 5'-IMP or 5'-dIMP were separated from 5'-AMP or 5'-dAMP by thin-layer chromatography on silica gel plates (14) and counted.

c5'N was determined at 37°C with either 5'-[^{32}P]AMP or 5'-[^{32}P]IMP as substrates. The reaction mixture contained in 200 μl, 50 m*M* imidazole, pH 6.5, 3 m*M* $MgCl_2$, 50 μM AOPC (to inhibit ecto-5'N), 0.25 m*M* substrate and 5 to 25 μg supernatant; $^{32}P_i$ produced was determined as reported by Dornand *et al.* (15). 5'-dAMP or 5'-dIMP were also used as substrates; the reaction took place in 1 ml with the same mixture, in the presence of 100 to 300 μg supernatant. After 30 min incubation at 37°C the reaction was stopped with 0.5 ml 1% TCA and P_i measured as previously reported (15).

Purine nucleoside kinase activities of cell cytosols were measured under the experimental conditions described by Lukey and Snyder (16): for adenosine kinase, 100 m*M* Tris–maleate, pH 6.0, 5 m*M* ATP, 1 m*M* $MgCl_2$, 5 μM EHNA, 45 μM [^{3}H]adenosine, 100 μg protein cytosol; for deoxyadenosine kinase: 100 m*M* Tris–HCl, pH 7.6, 1 m*M* ATP, 5 m*M* $MgCl_2$, 5 μM EHNA, 450 μM d[^{3}H]adenosine, 300 μg protein cytosol. After 30 min incubation at 37°C the reaction was stopped at 100°C, the nucleoside and the nucleotide formed were separated as described (14) and their relative concentrations determined.

All enzymatic reactions were studied under such conditions that the product formation was linear with time and with the cytosol concentration. Measurements were performed in triplicates.

Cell incubation and deoxyadenosine incorporation. Erythro-9-[3-(2-hydroxynonyl)]-adenosine (5 μM; EHNA) was added at 37°C to 10 ml RPMI 1640 containing 10^7 cells/ml. After 15 min the cultures were supplemented with 10 μM deoxyadenosine; then incubated for various times at 37°C under a 5% CO_2 wet atmosphere. The cells were sedimented (5 min, 500*g* at 4°C) and the supernatant was discarded. The cell pellets were extracted with 0.5 ml 0.4 *M* perchloric acid, neutralized, and their dATP content determined as described (17).

Uridine incorporation into thymocytes or splenocytes. Cortical thymocytes or T splenocytes (2×10^6 cells/ml) were incubated at 37°C in RPMI 1640, 5% FCS, with various deoxyadenosine concentrations, in the presence of 5 μM EHNA, for different times. [^{3}H]Uridine (1 μCi) was added to each microtitration plate well for the last 4 hr of incubation. The cells were collected on glass-fiber filter and quadruplicate determinations of uridine incorporation performed as previously described (18).

Results. *AMP-DA activity in lymphocyte subsets.* Measurements of AMP-DA activities in mouse lymphoid cells reveal that thymocytes express an activity 10- to 20-fold lower than that of splenocytes, independent of the substrate used. Lymph node lymphocytes and bone marrow cells display intermediary values (Table I).

We showed that the differences in AMP-DA levels do not result from different percentages of T and B cells in different organs. We separated T and B splenocytes by agglutination methods (12): WGA- or SBA-agglutinated fractions which represent a B-cell enriched population display activities similar to those of nonagglutinated cells (T-cell-enriched fractions) or unseparated populations.

The separation of mouse thymocytes into two broad subpopulations, cortical and med-

TABLE I. SPECIFIC ACTIVITIES OF ADENYLATE DEAMINASE IN MOUSE LYMPHOCYTE SUBSETS

Cell populations	AMP	dAMP
Lymph node lymphocytes	1800–2500	200–220
Bone marrow lymphocytes	600–800	n.d.
Unseparated thymocytes	150–200	20–25
Cortical (PNA$^+$) thymocytes	100–150	0–15
Medullary (PNA$^-$) thymocytes	850–900	70–80
Hydrocortisone-resistant thymocytes	800–1000	80–120
Unseparated splenocytes	2400–2900	250–300
T-enriched (SBA$^-$) splenocytes	2250–2700	190–250
B-enriched (SBA$^+$) splenocytes	1700–2400	200–230

Note. AMP-DA activities were measured on the cytosols of cell subpopulations as mentioned in Material and Methods, with AMP or dAMP as substrates. Reported values, obtained from four different preparations, are expressed as nmole/hr/mg.

ullary cells, was performed by a method based on the availability of binding sites for peanut agglutinin (PNA). Agglutinated cortical thymocytes (PNA^+) which are the majority of the thymic cells (80–90%) are functionally immature while the nonagglutinated medullary population (PNA^-) exhibited an immunocompetence similar to that of mature cells. PNA^- cells displayed AMP-DA activities five- to sixfold higher than those of PNA^+ cells; in two experiments over six, AMP-DA was found undetectable in PNA^+ cells when dAMP was the substrate used. We also studied medullary cells by depleting the cortical population by hydrocortisone treatment; 2 days after an intraperitoneal injection of hydrocortisone acetate, resitant thymocytes pooled from 10 mice (15×10^6 cells per thymus) displayed AMP-DA activities identical to those of PNA^- thymocytes.

The relative AMP-DA activity of lymphocyte populations could arise from the presence of an AMP-DA inhibitor in cortical cells or that of an activator in the other cells. This hypothesis was ruled out: no inhibition or activation of AMP-DA activity was observed when PNA^- thymocytes or splenocytes were incubated with cytosols of PNA^+ thymocytes (data not shown). Moreover the specific activity of a mixed population reflected the percentage of each population with its own specific activity.

The low AMP-DA activity of the thymocytes appears related to the high percentage of cortical cells, the PNA^- cells displaying higher activities which however remain two- to threefold lower than those of T splenocytes. We have already reported parallel results for ecto-5′N which can be considered as a marker of cell maturation (12, 13).

AMP-DA and dAMP-DA activities were also measured in human lymphocytes and lymphoblastoid cell lines currently used to study purine metabolism. PBL and tonsil lymphocytes displayed activities more than eightfold higher than those of thymocytes. The separation of cortical and medullary cells showed again that the low activity of thymocytes was due to the immature population. Most lymphoblastoid cell lines had abnormally low AMP-DA levels, specially T-ALL lymphoblasts with activities 10-fold lower than those of normal PBL and close to those of cortical thymocytes. Under our experimental conditions, undetectable dAMP-DA levels were found in these cell lines. AMP-DA activities of the B-cell lines tested were higher than those of T-ALL lymphoblasts (with the exception of RAJI cell line which had similar activity) but remained lower than those of normal lymphocytes. The null cell line K-562 displayed a relatively high AMP-DA level. The B and null cell lines studied, which derive from normal cells, are considered as mature cells with low terminal deoxynucleotidyltransferase (TdT) activity (12), while T-ALL lymphoblasts display high TdT levels which reflect early stages of maturation. Our results agree with those of Fishbein (9) and suggest that AMP-DA, like ecto-5′N and unlike ADA, increases during cell differentiation (Table II).

Kinetic parameters of AMP-DA in cortical thymocytes and T lymphocytes. The differences of AMP-DA or dAMP-DA activities observed among lymphoid cells at various stages of differentiation could arise from either different amounts of the same enzyme or different forms of the enzyme. AMP was found a better substrate than dAMP in both T splenocytes and cortical thymocytes, but differences in the relative substrate specificity were observed: K_m for AMP was similar for the two cell types (0.4–0.5 mM) while that of dAMP was higher for cortical thymocytes (4 mM) than for T splenocytes (1.5 mM). The maximal velocity of AMP-DA was 13-fold higher (2900 versus 220 nmole/hr/mg) in T splenocytes, while that of dAMP was 25-fold higher (380 versus 15 nmole/hr/mg) in the same cells.

Like others (19) we found that ADA inhibition by EHNA never exceeded 95%, even with EHNA concentrations higher than 10 μM. Therefore the absence of AMP-DA or dAMP-DA inhibition by EHNA (Table III) could be explained if the remaining ADA activity converted some AMP into IMP or some dAMP into dIMP. However this explanation appears rather unlikely: indeed AMP or dAMP are not deaminated by high concentrations of cell cytosol in the absence of KCl, under kinetic conditions which are optimal for ADA activity; moreover immature cells expressing the highest ADA activity (12, 13) display undetectable adenylate deaminase activities when dAMP is used as a substrate (Tables I, II). These results, consistent with

TABLE II. SPECIFIC ACTIVITIES OF ADENYLATE DEAMINASE OF HUMAN LYMPHOCYTE SUBSETS AND HUMAN LYMPHOBLASTOID CELL LINES

Cells	Origin	AMP	dAMP
Unseparated lymphocytes	blood	1200–1500	120–220
T lymphocytes	blood	1000–1200	150–190
B lymphocytes	blood	1100–1400	180–190
Unseparated lymphocytes	tonsil	1200–1700	130–140
Unseparated thymocytes	thymus	150–200	0–15
Cortical thymocytes	thymus	100–130	0
Medullary thymocytes	thymus	400–600	40–50
Cell lines			
MOLT-4	T-ALL[a]	40–60	0
MOLT-3	T-ALL	20–50	0
CEM	T-ALL	30–100	0
H-SB2	T-ALL	20–40	0
1301	T-ALL	60–70	0
RAJI	Burkitt lymphoma	70–100	0
CCRF-SB	B-cell[b]	450–600	30–50
EBV-lymphoblasts (4)	B-cell[c]	500–630	45–65
K-562	Null CML[d]	450–600	50–70

Note. Adenylate deaminase activities were measured with AMP or dAMP as substrates and expressed as nmole/hr/mg.

[a] ALL: acute lymphoblastic leukemia.

[b] CCRF-SB appears as normal B cells from a T-ALL patient (13).

[c] Four different cell lines were established after infection of normal human circulating lymphocytes with Epstein-Barr virus.

[d] Non-T non-B chronic myelocytic leukemia.

our preliminary experiments on AMP-DA purification which evidenced the actual difference in substrate specificity for AMP-DA and ADA, show that EHNA (0–50 μM) had no effect on AMP-DA of both cell types with either AMP or dAMP as substrate. Deoxycoformycin which is better inhibitor of ADA than EHNA, inhibited also dAMP-DA and to a lesser extent AMP-DA, at concentrations as low as 5 μM (Table III); it was found twice more potent for cortical thymocytes than for T splenocytes.

The kinetic data and the specificity of deoxycoformycin inhibition suggest that different forms of AMP-DA are present in the two cell populations, which is consistent with the existence, in hen skeletal muscle cells at different stages of maturation, of AMP-DA isoenzymes displaying different deamination rates for AMP and dAMP (20). The enzyme form(s) present in immature thymocytes poorly deaminates dAMP and is highly inhibited by deoxycoformycin, unlike that present in mature T cells. Such a difference in deoxycoformycin sensitivity has been reported for the enzymes expressed in T-ALL and B or null lymphoblastoid cell lines (9).

Cytosolic 5'nucleotidase, adenosine, and

TABLE III. INHIBITORY EFFECT OF EHNA OR DEOXYCOFORMYCIN ON ADENYLATE DEAMINASE ACTIVITIES FROM MOUSE LYMPHOCYTE SUBSETS

	T splenocytes		Cortical thymocytes	
Inhibitor	AMP	dAMP	AMP	dAMP
0–50 μM EHNA	100 ± 6	98 ± 5	95 ± 5	102 ± 5
10 μM deoxycoformycin	75 ± 5	78 ± 4	50 ± 5	15 ± 6
5 μM deoxycoformycin	85 ± 7	80 ± 5	61 ± 3	30 ± 5

Note. Results are expressed in percentage of AMP-DA activities measured in the absence of inhibitor with 0.5 mM 5'-AMP or 1 mM 5'-dAMP as substrate. The inhibitor was added to the incubation medium 10 min before the substrate.

deoxyadenosine kinases in lymphocyte subsets. The study of dATP accumulation in lymphocyte subpopulations led us to compare some other enzymes involved in dATP metabolism and deoxyadenosine toxicity-like cytosolic 5′-nucleotidase, adenosine, and deoxyadenosine kinases. We found only small variations for these three enzymes among the different subpopulations studied (Table IV): their activities are 1.5-fold higher in cortical thymocytes than in T splenocytes; these small differences have probably no biological implication, unlike the large changes in AMP-DA activities. c5′N activity was found twice higher in B than in T splenocytes, which is less than the difference reported for B and T lymphoblasts (21).

dATP accumulation in cortical thymocytes and T splenocytes. When incubated for 4 hr in RPMI 1640 with 10 μM deoxyadenosine, in the presence of 10 μM EHNA, both cell populations accumulated dATP. The dATP content of cortical thymocytes increased 10 times; this increase was linear during the first 3 hr then it was slower (Fig. 1); dATP accumulation was significantly higher when 10 μM deoxycoformycin substituted for EHNA. T splenocytes accumulated six- to sevenfold less dATP than cortical thymocytes; the use of deoxycoformycin did not significantly improve this accumulation.

We also investigated the ability of both subpopulations to degrade accumulated dATP. After the 4 hr incubation with deoxyadenosine and deoxycoformycin, the cells were washed and reincubated in RPMI 1640 alone, for 1 hr more. In T splenocytes the dATP content decreased almost to its initial level, while in cortical thymocytes the level remained very high (eightfold the initial level); the decrease was found lower when deoxycoformycin was used as ADA inhibitor instead of EHNA during the 4 hr incubation.

As for cortical thymocytes and T splenocytes the dATP accumulation of medullary thymocytes and B splenocytes incubated under the same conditions was inversely related to their AMP-DA activity.

Inhibition of uridine incorporation into T splenocytes and cortical thymocytes. Like human lymphocytes (7), mouse resting lymphocytes accumulated less uridine after incubation with deoxyadenosine and EHNA. This inhibition increased by increasing the incubation time and the deoxyadenosine concentrations (Fig. 2). Cortical thymocytes were found specially sensitive to this effect and their uridine incorporation was significantly reduced for incubation times as short as 4 hr (40% inhibition with 10 μM deoxyadenosine); 24 hr incubation with 3–4 μM deoxyadenosine led to half-maximal inhibition. Deoxyadenosine (25 μM) was required for the same effect on T splenocytes which are not significantly affected by 4 hr incubations. After 40 hr incubation with 5 μM deoxyadenosine, only 50% cortical thymocytes were found viable by the trypan blue exclusion test, while 25 μM deoxyadenosine was necessary to affect similarly T splenocytes (data not shown).

Discussion. It was reported that T lymphoid cell lines, incubated with deoxyadenosine in the presence of deoxycoformycin, an adenosine deaminase inhibitor, accumulated dATP, a potent inhibitor of ribonucleotide reductase, which ceased DNA synthesis

TABLE IV. CYTOSOLIC 5′-NUCLEOTIDASE AND NUCLEOSIDE KINASE ACTIVITIES OF MOUSE LYMPHOCYTE SUBSETS

Cells	Cytosolic 5′-nucleotidase				Nucleoside kinases	
					Ado	dAdo
Unseparated thymocytes	34–42	48–52	990–104	100–120	180–240	40–60
Cortical thymocytes	30–36	43–46	105–130	110–140	180–200	47–50
Medullary thymocytes	20–26	41–43	87–95	100–110	165–200	27–30
Unseparated splenocytes	42–47	50–60	110–125	130–160	120–160	22–35
T splenocytes	48–56	48–51	65–75	75–85	130–150	30–34
B splenocytes	40–44	55–75	130–154	135–145	130–140	28–32

Note. c5′N was measured with either 5′-AMP, 5′-IMP, 5′-dAMP, or 5′-dIMP as substrate, nucleoside kinases with either adenosine or deoxyadenosine as substrate. All specific activities are expressed as nmol/hr/mg.

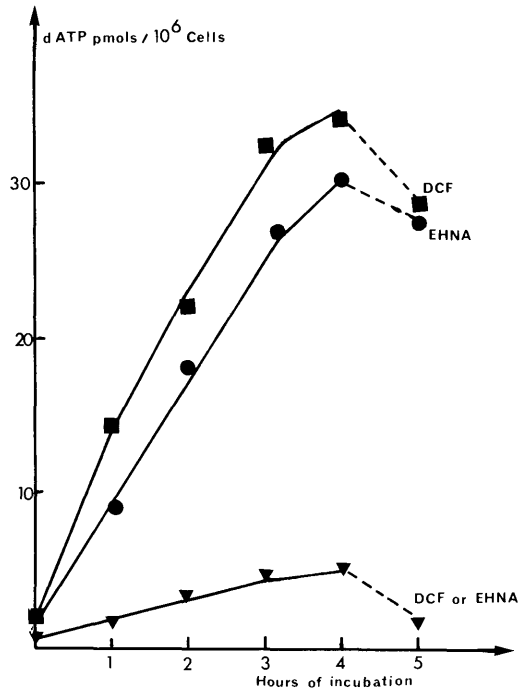

FIG. 1. Accumulation of dATP in T splenocytes and cortical thymocytes. T splenocytes (10 ml) in RPMI 1640 (10^7 cells/ml) were incubated at 37°C in the presence of 10 μM deoxyadenosine plus 5 μM EHNA or deoxycoformycin (d-CF) (▼). The same experiment was performed with cortical thymocytes (EHNA ●, d-CF ■). dATP content was determined after 0, 1, 2, 3, and 4 hr incubation. The cells were then washed and resuspended in RPMI 1640 alone for 1 hr more and their dATP content measured.

and cell division (4, 5, 21). Under the same conditions, B cell lines were found more resistant (4, 5), probably because of their ability to degrade dATP much more rapidly than T lymphoblasts. It was postulated that this difference could arise from different ecto-5'N (22) or cytosolic 5'N (21) activities. However ecto-5'N is an ecto-enzyme not involved in intracellular nucleotide degradation (23). On the other hand Carson showed that 3 over the 10 B cell lines with high c5'N activity were as sensitive to deoxyadenosine toxicity as the non-B cells (21). It was recently demonstrated that some B cell lines, RAJI for instance, accumulated dATP to the same extent as T lymphoblasts (19).

Little attention has been paid to the role of AMP-DA in the mechanism of deoxyadenosine cytotoxicity. Fishbein reported that this enzyme, involved in nucleotide metabolism, was not uniformly distributed among human lymphoblastoid cell lines (9). Bagnara *et al.* (24) suggested that AMP-DA could be involved in the deoxyadenosine-induced ATP depletion in deoxycoformycin-treated T lymphoblast, however, this event occurs a long time after dATP accumulation has reached its optimum.

The aim of the present work was to determine if AMP-DA is involved in the mechanisms of deoxyadenosine toxicity, We found that AMP-DA activity is widely different among the various human and mouse lymphocyte populations. Its distribution is similar to that of ecto-5'N (12, 13): immature lymphocytes display 10-fold lower activity than mature cells, independently of their T or B character; AMP-DA level of medullary thymocytes is two- to threefold lower than that of mature lymphocytes but fourfold higher than that of immature cortical thymocytes.

In human lymphoblastoid cell lines, our results (Table II) agree with those of Fishbein (9), but we think that AMP-DA activity correlates with the cell maturation stage rather than with their T or B character: high AMP-DA levels were found in B or null lymphoblastoid cell lines with mature characters (high ecto-5'N/ADA ratio (13) and low TdT), while immature T-ALL lymphoblasts, with low ecto-5'N/ADA ratio and high TdT, displayed low, and sometimes undetectable, AMP-DA activities.

Although the molecular characterization and purification of AMP-DA have not yet been performed, the presence of different isoenzymes in cortical thymocytes and T splenocytes is suggested by kinetics studies and by the specificity of deoxycoformycin inhibition. Such isoenzymes have been postulated in lymphoblastoid cell lines by Fishbein (9), who found AMP-DA of T-ALL lymphoblasts more sensitive to deoxycoformycin inhibition than that of B lymphoblasts. We found that dAMP is a better AMP-DA substrate in mature than in immature cells and that deoxycoformycin inhibits better the enzyme in immature cells. As the other enzymes involved in nucleotide metabolism—c5'N, adenosine and deoxyadenosine kinases (Table IV), *S*-adenosyl-homocysteine-hydrolase (25)—have similar values in cortical thymocytes and T splenocytes, the differences

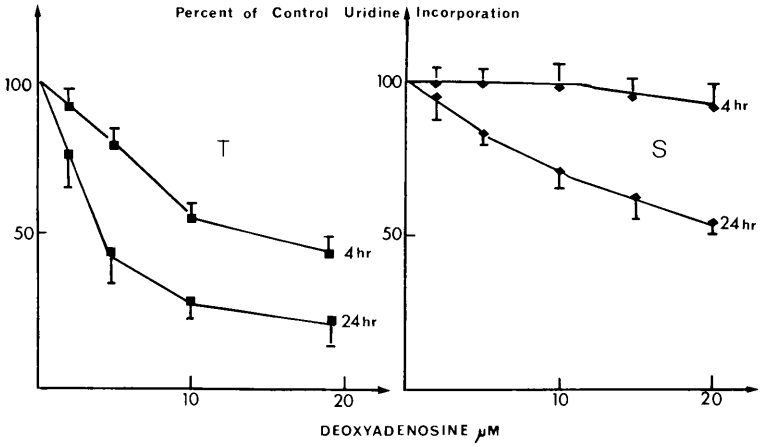

FIG. 2. Effect of deoxyadenosine plus 5 μM EHNA on uridine incorporation into T splenocytes (S) or cortical thymocytes (T). Lymphocytes were incubated with various deoxyadenosine concentrations plus 5 μM EHNA for the indicated periods of time; [^{3}H]uridine was present during the last 4 hr of incubation. The control values for [^{3}H]uridine incorporation were obtained from lymphocytes incubated without deoxyadenosine and are as follows: T splencoytes (0–4 hr) 8900 ± 720 cpm, (20–24 hr) 7800 ± 900 cpm; cortical thymocytes (0–4 hr) 6300 ± 300 cpm, (20–24 hr) 5030 ± 540 cpm.

in AMP-DA could account for the differences in dATP accumulation between the two cell types. These data suggest the hypothesis of an important role of AMP-DA in dATP detoxification. This hypothesis is consistent with the inverse correlation between the level of dATP accumulation and the AMP-DA activity and could explain why, unlike in T splenocytes, the dATP content of cortical thymocytes does not significantly drop after deoxyadenosine and EHNA have been washed off.

In rapidly dividing lymphoid cells, [^{3}H]uridine may be converted into both ribonucleotides and deoxyribonucleotides and thus be incorporated into both RNA and DNA (26); but in resting lymphocytes which display very little DNA synthetic activity, it has been established that [^{3}H]uridine incorporated mainly into RNA in such a way that this incorporation is in fact a measure of RNA synthesis (7). In resting lymphocytes the accumulation of dATP correlated with an inhibition of RNA synthesis by a mechanism which remains to be elucidated, but is independent of DNA synthesis and ATP depletion (7). When cortical thymocytes were incubated with deoxyadenosine and EHNA, which inhibits ADA but not AMP-DA, RNA synthesis was inhibited and cell viability highly decreased. Fivefold higher deoxyadenosine concentrations are required to produce the same effect on T splenocytes, which parallels the higher AMP-DA activity of these cells.

In view of our results, it appears difficult to compare the sensitivity of cells to deoxyadenosine toxicity without considering AMP-DA activity. After the publication of a preliminary report of this work (27), Ratech *et al.* (28) showed that a murine lymphoblastoid cell line, with differentiated T lymphocyte phenotype (Lyt $1^+2^-TL^-$), displayed high AMP-DA activity, did not accumulate dATP, and was insensitive to deoxyadenosine toxicity. Their conclusion agrees with ours: AMP-DA, which increases during cell differentiation, could be an important factor controlling dATP accumulation in lymphoid cells and deoxyadenosine cytotoxicity.

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