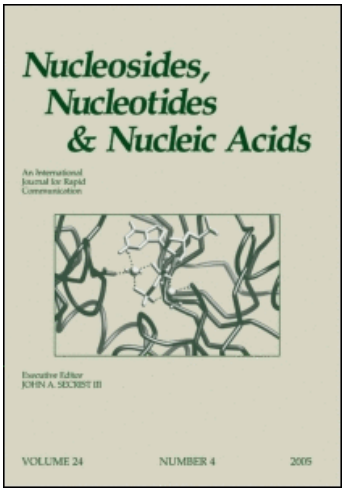

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

Differential Sensitivities of Normal and Malignant Murine Lymphocytes to Purine Nucleosides

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To cite this Article Nelson, J. Arly , Richie, Ellen R. , Kandil, Osama , Vidale, Elena and Pan, Bih Fang(1989) 'Differential Sensitivities of Normal and Malignant Murine Lymphocytes to Purine Nucleosides', Nucleosides, Nucleotides and Nucleic Acids, 8: 4, 569 — 586
To link to this Article: DOI: 10.1080/07328318908054199
URL: http://dx.doi.org/10.1080/07328318908054199

NUCLEOSIDES & NUCLEOTIDES, 8(4), 569-586 (1989)

DIFFERENTIAL SENSITIVITIES OF NORMAL AND MALIGNANT MURINE LYMPHOCYTES TO PURINE NUCLEOSIDES

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ABSTRACT

Inherited human immunodeficiences associated with loss of adenosine deaminase or purine nucleoside phosphorylase are thought to result from accumulation of the nucleoside substrates. This work attempts to identify lymphocyte subpopulations that are uniquely-sensitive to the endogenous substrates or their analogs.

INTRODUCTION

The first observations that certain purine enzyme deficiencies were associated with immune defects were made over 15 years ago.[1,2] Specifically, deficiency of ADA[3] was found to be associated with SCID and deficiency of PNP was noted to be associated with the loss of cellular immunity. In either case, the immune dysfunction has been postulated to have as its basis loss of normal T cell function.[4] dAdo and Ado (ADA substrates) and dGuo (a PNP substrate) are known to be particularly cytotoxic and/or growth-inhibitory toward cells under certain conditions of culture.[5,6] In the absence of ADA or PNP in humans, these nucleoside substrates accumulate.[7-11] Accumulation of the nucleosides is currently considered to play the primary role in the immune deficiencies; however, the precise mechanisms for the immune deficiencies remain unknown. Because these nucleosides are active against numerous cell types, it is important to determine whether particular cell populations that are

uniquely sensitive to purine nucleosides exist. If such subpopulations do exist, by inference they would represent populations likely to be most severely influenced by the enzyme deletions. This report summarizes our efforts using endogenous nucleosides and their analogs to identify such lymphocyte subpopulations in mice. A preliminary presentation of some of these data has appeared.[12]

MATERIALS AND METHODS

Preparation of spleen and thymus lymphocytes. AKR/J male mice, 5 to 13 weeks of age, from the Jackson Laboratory (Bar Harbor, ME) were used as a source of lymphocytes. The mice were sacrificed by CO_2 asphyxiation, and spleen and thymus cell suspensions were prepared by passing the organs through stainless steel mesh screens. Red blood cells were removed either by treatment with 0.83% ammonium chloride[13] or by density gradient centrifugation in Ficoll-Hypague [14] (Pharmacia Fine Chemicals Inc., Piscataway, NJ).

Separation of Lymphocyte Subpopulations. The method described by Reisner, et al.[15] was employed to obtain PNA-positive and PNA-negative thymocyte populations. A modification of the "panning" technique described by Wysocki and Sato[16] and Reinherz, et al.[17] was utilized to separate lymphocyte suspensions into subsets based on cell surface antigens. Affinity-purified polyvalent rabbit anti-rat Ig or rabbit anti-mouse Ig (Accurate Chemicals, Westbury, NY) were precoated onto 100 x 15 mm polystyrene bacteriological Petri dishes (Fisher Chemical Co., Houston, TX). The dishes were incubated with 100 µg of the polyvalent antibodies in 10 ml of 0.05 M Tris buffer, pH 9.5 at room temperature for 60 min or at 4° overnight. Plates were washed four times with 10 ml of cold PBS and then filled with PBS supplemented with 1% fetal calf serum. This latter PBS medium was removed immediately prior to addition of the lymphocyte suspensions.

To separate B cells and non-B cells from spleen suspensions, 5×10^7 spleen cells in 5 ml RPMI medium were incubated at 37° for 90 min in an uncoated Petri dish to remove macrophages that attached to the dish. The non-attached cell suspensions were then placed into a Petri dish that had been coated with rabbit anti-mouse Ig. The dish was then incubated at room temperature for 90 min. The nonadherent cells were removed by decanting, and the Petri dish was gently washed twice with 5 ml of RPMI medium. The nonadherent fraction and the washings were combined (non-B

cell population). RPMI (5 ml) medium was then added to the Petri dish, and the solution was vigorously and repeatedly pippeted into the dish to dislodge the adherent B cells. The detached cells were then collected by centrifugation, and the Petri dish was examined under an inverted microscope to determine the degree of cell removal. The procedure was repeated until all visible cells were removed. To obtain T helper and T suppressor/cytotoxic subpopulations, anti Lyt-2 (Cedarlane Laboratory, Hornby, Ont., Canada) and anti-L_3T_4[18] monoclonal antibodies were used. Spleen T lymphocytes (3×10^7 cells) were washed three times with PBS and incubated with 200 µl of 1:10 diluted anti-Lyt-2 or anti-L_3T_4 monoclonal antibody at room temperature for 20 min. After incubation, the cells were washed three time in PBS and resuspended in 5 ml of RPMI medium. Since anti-Lyt-2 monoclonal antibody was obtained from the mouse, anti-Lyt-2 bound lymphocytes were incubated in anti-mouse Ig precoated Petri dishes. Similarly, because anti-L_3T_4 antibody came from the rat, anti-L_3T_4 bound lymphocytes were incubated in anti-rat Ig precoated Petri dishes. After incubation at room temperature for 90 min, nonadherent cells were collected and the Petri dish was gently washed twice with 5 ml of RPMI medium. The nonadherent fractions and washings were pooled as Lyt-2^- or $L_3T_4^-$ populations. The adherent fractions (Lyt-2^+ or $L_3T_4^+$ populations) were collected by pipeting medium up and down vigorously to remove the cells from the Petri dishes. Partial purification of the spleen T lymphocyte subpopulations was confirmed by direct and indirect immunofluorescent microscopy. Lyt-2^+ and Lyt-2^- populations were 74% and 17% positive for Lyt-2 (anti-Lyt-2 FITC; Becton Dickinson, Mountain View, CA), whereas they were 24% and 74% positive for L_3T_4 (anti-L_3T_4 plus rabbit anti-rat Ig-FITC; Accurate Chemical and Scientific Corp. Westbury, NY). Likewise, $L_3T_4^+$ and $L_3T_4^-$ populations were 88% and 7% positive for L_3T_4 and 3% and 66% positive for Lyt-2.

Inhibition of Mitogen-Stimulated DNA Synthesis in Lymphocytes. Spleen and thymus lymphocytes (2.5×10^6/ml) or their subpopulations were cultured in the presence of 5 µg/ml ConA (Pharmacia Fine Chemicals, Inc.), PHA (Difco Laboratories, Detroit, MI) or LPS (Difco) in a 96-well microtiter plate (200 µl/well) at 37°C, 5% CO_2. Various concentrations of nucleosides were added to the cultures at the time mitogen was added. After a 44-hr incubation, the cultures were pulsed with [^{3}H]-TdR (62 Ci/mmole; ICN Radiochemicals, Irvine, CA) at 1 µCi/well for 4 hr. Cells

were then harvested onto a glass microfiber filter (Whatman 934-AH, Bodman Chemicals, Doraville, GA) using a cell harvester. Cells collected on the filter were lysed with ice-cold 10% trichloroacetic acid and washed with ice-cold 80% ethyl alcohol. [^{3}H]-TdR incorporation was determined by measuring radioactivity on each filter in a liquid scintillation counter. The results shown are the average values for at least three separate experiments, each performed in quadruplicate.

Inhibition of the growth of AKR lymphomas. Five lymphomas that developed spontaneously in AKR mice were adapted to tissue culture in RPMI 1640 media containing 10% fetal calf serum.[19,20] After inoculation of 10^5 cells/ml, the lymphomas grew in an exponential fashion over the 72-hr interval tested. The numbers of tumor cells were evaluated at 0, 24, 48, and 72 hr, and treated culture cell growth was compared to that of controls to estimate the concentration of nucleosides required to inhibit growth by 50%. The lymphomas were characterized during early cultures for surface antigens and terminal deoxynucleotidyl transferase activities.[19] Based on established criteria, the initial isolates were defined as representing T-cell, B-cell, "immature" or "mature" T-cell origin. To evaluate the effects of Tub on lymphoma cell viability, cells were removed from tissue culture and implanted IP into untreated, healthy AKR male mice. The survival time of the mice was then used as a measure of the lymphoma cell viability.

HPLC Methods. The metabolism of radiolabeled compounds was followed by chromatographic separation of nucleosides and bases (reverse-phase, μBondapak C_{18} column; Waters Assoc., Milford, MA) or of nucleotides (strong anion exchange column; Partisil-SAX, Whatman, Inc., Clifton, NJ). The reverse phase system employed a gradient from 5 mM KH_2PO_4, pH 5.5, to 20% methanol in 15 min at a flow rate of 2 ml/min. The anion-exchange separation was achieved with a gradient from 5 mM $NH_4H_2PO_4$, pH 2.8 to 750 mM $NH_4H_2PO_4$, pH 3.7, in 30 min at a flow rate of 2 ml/min. These separations have been illustrated in previous publications from this laboratory.[21]

Enzyme Analyses. PNP and ADA were assayed in whole cell homogenates by ultraviolet spectrophotometry as previously described.[22] Nucleoside kinase activities were determined using radiolabeled nucleoside substrates by a weak-anion exchange disc assay.[23]

RESULTS

As shown in Table 1, mitogen-induced DNA synthesis in murine thymocytes is generally more sensitive to inhibition by endogenous purine

TABLE 1
INHIBITION OF MITOGEN RESPONSES OF NORMAL SPLEEN AND THYMUS LYMPHOCYTES BY PURINE NUCLEOSIDES[a]

LYMPHOCYTE SOURCE	NUCLEOSIDE	MITOGEN		
		PHA	ConA	LPS
Spleen:				
	dAdo[b]	4.5	2.0	3.3
	Ado[b]	70	83	110
	dGuo	300	300	280
	Guo	480	500	750
Thymus:				
	dAdo[b]	1.2	0.7	1.0
	Ado[b]	29	32	45
	dGuo	42	18	23
	Guo	180	130	68

[a]The values given are the concentrations (μM) that produce 50% inhibition of control mitogen-induced DNA synthesis as described in Methods.
[b]Incubations were performed in the presence of DCF (1 μM) to inhibit ADA, a treatment that alone had no effect on mitogen-induced DNA synthesis.

nucleosides than that of splenocytes. There is no apparent marked selectivity for T cell (ConA and PHA) versus B cell (LPS) mitogen, i.e., the ED_{50} values are similar for a given nucleoside and lymphocyte source regardless of the mitogen used. The thymus represents a predominantly immature population of T cells, whereas the spleen is a source of mature T cells, B cells and other cells. The differential sensitivity (thymus versus spleen) is most marked for dGuo (Table 1). This differential sensitivity appears to be a reflection of increased degradation by PNP in the spleen cells, as illustrated in Figure 1. The disappearance of dGuo from the medium upon incubation with the cells is accompanied by a corresponding formation of guanine, indicative of PNP activity in the cells. In the absence of DCF, both thymus and spleen lymphocytes rapidly degrade dAdo to deoxyinosine (ADA activity) and hypoxanthine (PNP activity); however, in the presence of DCF, degradation is impaired and occurs at about the same rate in the cells from these two organs (data not shown). In these experiments, degradation was associated with the presence of cells, i.e., nucleosides incubated in medium in the absence of cells were not degraded.

To investigate whether the greater sensitivity of thymus lymphocytes to dGuo is due to the presence of relatively more immature T cells than

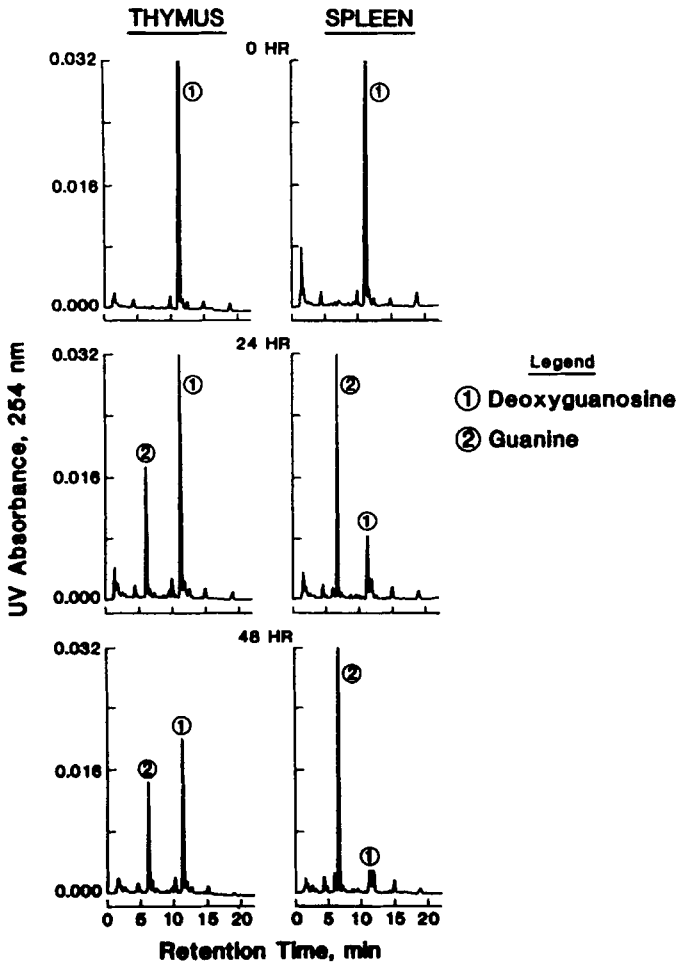

Figure 1: Disappearance of dGuo from medium incubated in the presence of mouse spleen or thymus lymphocytes. Lymphocytes ($1x10^6$/ml) were incubated in the presence of 100 µM dGuo. Samples of the medium were subjected to HPLC separation after 24-and 48-hr incubation, using the reverse-phase system described in Methods.

occurs in spleen, populations were enriched for mature (PNA^-) and immature (PNA^+) T cells from the thymus. When the cells were stimulated with ConA, DNA synthesis in the more immature T cells was inhibited at lower concentrations of dGuo, i.e., the ED_{50} values were 15 µM and 55µM for PNA^+ and PNA^- populations, respectively. Again, a likely explanation for this differential effect of dGuo relates to the observation that the

immature T cells (PNA^+) were much less efficient in converting dGuo to guanine than were the more mature (PNA^-) cells (data not shown). As observed in Figure 1, disappearance of dGuo from the culture media of thymus cell subpopulations was accompanied by a quantitative appearance of guanine. Although not shown, the PNP activity measured in broken cell homogenates paralleled the whole cell activity as measured in Figure 1.

These experiments using various mitogens, mature and immature lymphocytes, and endogenous nucleosides, failed to disclose clearly a population that might be uniquely-sensitive to one or more of the nucleosides. As illustrated with dGuo, degradation of these nucleosides by ADA and PNP renders interpretation of the results difficult. To circumvent this difficulty, we have employed analogs that are poor substrates for ADA or PNP. The analogs used are: Tub, an Ado analog; CldAdo and FaraAMP, dAdo analogs; and araGua, a dGuo analog. The effects of these selected nucleosides on the mitogenic responses of thymus and spleen lymphocyte subpopulations are summarized in Table 2. Tub was most active toward the "suppressor/cytotoxic" phenotype ($Lyt\text{-}2^+$ in Table 2), whereas there was no marked selectivity for any subpopulation toward CldAdo. FaraAMP was considerably more active toward "immature" T-cells (thymus) than "mature" T-cells (spleen non-B cells), and this nucleoside was much less active in inhibiting the mitogenic response of B-cells. Among the analogs tested, araGua demonstrated unique selective inhibition toward mature vs. immature T-cells (i.e., the ED_{50} values were 12 and 1.2 μM for thymus and spleen T-cells, respectively). Compared to normal mature T-cells (non-B cells from spleen), the B-cell population in spleen was less sensitive to araGua (9.7 vs. 1.2 μM in Table 2).

To better understand the basis for the greater sensitivity of mature T-cells to araGua, metabolism of the drug was evaluated in thymus and non-B cells from spleen (Figure 2). During the incubation interval studied, formation of araGua nucleotides was most marked in the mature T-cells from spleen. Further, the degradation of araGua nucleotides occurred at a faster rate in the thymus cells (Table 3). The higher activity of araGua kinase in the spleen Non-B cells is reflected in broken cell homogenates (Figure 3); however, product inhibition of the enzyme from both cell populations is similar (incubations performed in the presence of 0.02 and 0.1 mM araGTP in Figure 3). Thus, both an increased rate of nucleotide formation and a decreased rate of nucleotide degradation appear associated with the greater sensitivity of the spleen non-B cells toward araGua.

TABLE 2
INHIBITION OF MITOGEN-INDUCED DNA SYNTHESIS IN LYMPHOCYTE POPULATIONS BY PURINE NUCLEOSIDE ANALOGS[a]

NUCLEOSIDE	THYMUS[b]	SPLEEN[c] "NON-B"	SPLEEN[c] LYT-2$^+$	SPLEEN[c] L3T4$^+$	SPLEEN[c] "B"
Tub	0.046	0.11	0.027	0.078	0.060
CldAdo	0.42	0.88	0.53	0.53	0.80
FaraAMP	1.0	9.8	7.2	2.9	27
araGua	12	1.2	1.4	0.80	9.7

[a]The values shown are the μMolar concentrations of nucleoside required to inhibit mitogen-induced DNA synthesis by 50% (ED_{50}). DNA synthesis was measured 44-48 hr after ConA (T-cells) or LPS (B-cells) addition using ^{3}H-TdR as described in Methods.
[b]Whole thymus cell suspensions were used as a source of "immature" T-lymphocytes.
[c]Spleen cells were prepared by "panning" for surface antigens as described in Methods.

TABLE 3
DISAPPEARANCE OF ARAGUA NUCLEOTIDES FROM THYMUS AND SPLEEN NON-B CELLS[a]

Time After Removal of [^{3}H]-AraGua, Hr	AraGua Nucleotides, nanomoles/10^9 cells[b] Thymus	Spleen Non-B
0	1.64 ± 0.18	2.76 ± 0.23
4	0.66 ± 0.25	1.08 ± 0.07
24	<0.05 ± 0[c]	0.54 ± 0.08

[a]Cells were prepared and incubated as described in Table 2. After 24 hr incubation in the presence of 10 μM [^{3}H]-araGua and 5μg/ml ConA, the cells were washed and "chased" with non-radioactive 10μM araGua and 5 μg/ml ConA. Cells were harvested 0, 4, and 24 hr later and nucleotides were measured in the cold acid-soluble fraction.[23]
[b]The results shown are mean values ± range for two separate experiments.
[c]Minimal detectable concentration under the experimental conditions.

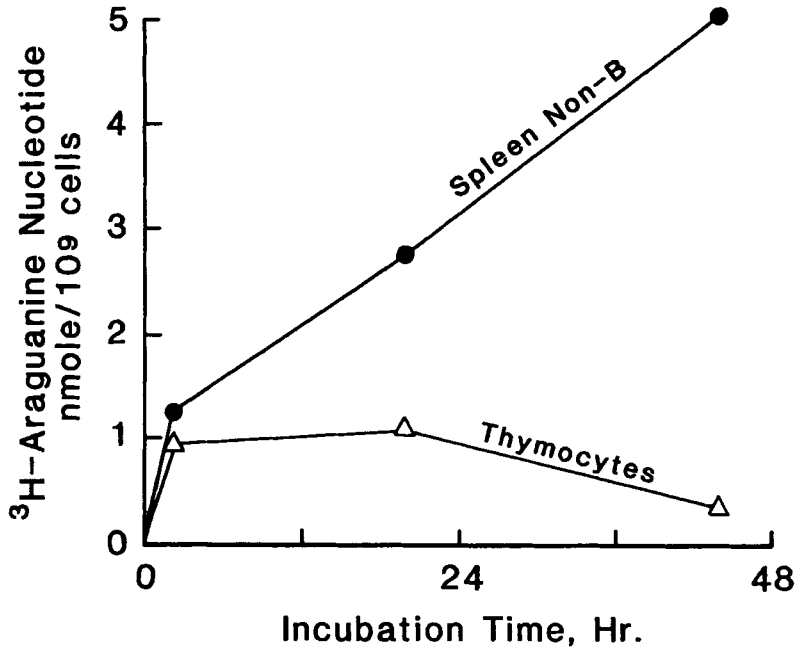

Figure 2: Formation of araGua nucleotides during culture of mouse lymphocytes with mitogens. The separated murine lymphocytes described in Table 2 were cultured with ConA in the presence of 10 μM [^{3}H]-araGua. At the indicated times, cells were harvested and extracted with cold 5% perchloric acid followed by neutralization of the acid-soluble material with KOH. Nucleotide formation was measured by the weak anion exchange disc method described by Ives, et al.[23] The experiment shown is representative of two separate experiments, each performed in duplicate.

Another method to test for the existence of a lymphocyte subpopulation uniquely sensitive to purine nucleosides is to use lymphomas that arise spontaneously in aged AKR mice. The thymomas are thought to represent the malignant expansion of a particular cell type, arrested during differentiation. We have evaluated five such lymphomas for their sensitivities toward the growth-inhibitory effects of the nucleoside analogs (Table 4). One spontaneous lymphoma was of B-cell origin (cell line 225 in Table 4). This unusual lymphoma in the AKR mouse model was generally less sensitive to the nucleosides tested than were the T-cell lines. An interesting exception was araGua, for which the B-cell tumor was equally-sensitive. It is interesting to note that

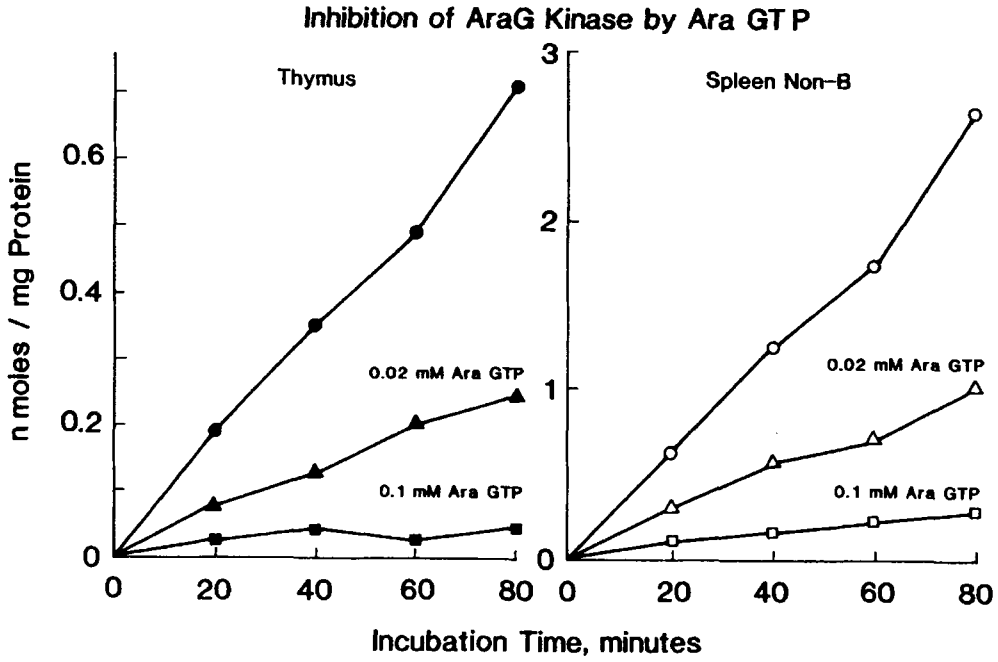

Figure 3: Inhibition of araGua kinase in murine T cells by araGTP. The araGua kinase activity in whole cell homogenates was determined in the presence or absence of araGTP at the indicated concentrations. The incubation conditions were: [^{3}H]-araGua, 10 µM; ATP, 5mM; MgCl2, 5mM; Tris-HCl, 100mM, pH 7.4. Samples of the incubation mixture were removed at the times shown and nucleotide formation was determined by the method of Ives, et al.[23]

the most sensitive lymphoma toward araGua was the more "mature" T-cell line, number 920. Among the T-cell lymphomas tested, cell line 411 was the most sensitive toward Tub. Compared with the sensitivities of normal lymphocyte subpopulations (Table 2), this T-cell lymphoma would be predicted (based on the in vitro data contained herein) to exhibit a favorable therapeutic index. This assumes, naturally, that the cells tested represent the population that becomes dose-limiting with regard to the host. Further, it assumes that antiproliferative responses to Tub reflect "cell killing" potential. Therefore, we evaluated the effects of Tub on the viability of cell line 411 (a "relatively sensitive cell") and cell line 1210 (a "relatively refractory cell"). In agreement with the growth-inhibitory effects of Tub as shown in Table 4, the 411 tumor is

TABLE 4

INHIBITION OF THE GROWTH OF SPONTANEOUS AKR LYMPHOMA CELL LINES IN TISSUE CULTURE BY PURINE NUCLEOSIDE ANALOGS[a]

Cell Line	Phenotype[b]	TUB	CldAdo	Para AMP	AraG	Doubling Time (Hr)
1210	"T" Immature	0.039	0.12	5.3	22	18
920	"T" Mature	0.010	0.049	6.8	13	16
411	"T" Immature	0.005	0.17	14	23	12
316	"T" Immature	0.023	0.040	3.2	22	20
225	"B"	0.10	0.40	42	23	24

[a]The thymomas, initially-isolated from AKR male mice, were grown in RPMI 1640 media in the presence or absence of the nucleosides shown. The values given are the concentrations of nucleosides (μM) inhibiting growth by 50% compared to controls during a 72-hr incubation. The average results for three or more separate experiments, each performed in duplicate, are shown.

[b]Established by cell surface and enzyme markers.[19,20]

TABLE 5
IN VITRO CYTOTOXICITY OF TUBERCIDIN TOWARD T-CELL LYMPHOMAS[a]

Cell Line	Time (hr) After Adding Tubercidin	% of Control Cell Growth[b]	Median Survival Time (days) in Bioassay[c]
411	Control	100	18
	4	---	19
	24	49	23
	72	2	>50[d]
1210	Control	100	23
	4	---	25
	24	80	24
	72	94	21

[a]The AKR lymphomas were incubated in vitro as described in the legend to Table 4.

[b]Cell growth was measured in the presence or absence of Tub, 0.05μM.

[c]At the indicated times following Tub, the cells were injected IP into AKR mice (10^5 cells/mouse). Survival time of the mice was used as a bioassay for viability of the lymphomas.

[d]Statistically different from control, $P<0.01$ (2-sample rank test).

also more sensitive toward the cell-killing effect (Table 5). Specifically, a 72-hr exposure to Tub (0.05 μM) was highly-effective in reducing the viable tumor cell population of the 411 lymphoma, but not that of the 1210 lymphoma.

These results predict a greater sensitivity of 411 to the cell-killing potential of Tub, and that continuous exposure to the drug may be required to achieve a chemotherapeutic effect. In agreement, preliminary experiments (data not shown) in AKR mice bearing the 411 tumor have failed to demonstrate an increase in survival when Tub was administered once daily.

DISCUSSION

Following the initial observations of Giblett and her coworkers that ADA and PNP deficiencies were associated with SCID and a selective T cell dysfunction in humans,[1,2] considerable enthusiasm spread amongst immunologists that a rapid solution to the relationship between a specific enzyme deletion and immunodeficiency would be forthcoming. Unfortunately, and in spite of extensive laboratory efforts, the precise

role of ADA and PNP deletions in the associated immunodeficiencies are still unclear. However, it is generally accepted that the accumulation of purine nucleoside substrates, rather than a depletion of products or other aspect of the enzymes' roles, is causally related to the induction of immunodeficiency in either case. For both ADA and PNP deficiencies, a number of interesting and plausible hypotheses for purine nucleoside toxicities toward lymphoid cells have been proposed.[5] Since any of these mechanisms may play a role in the defects, it appeared important to first identify the particular cell types that may be more intimately associated with the diseases. The underlying hypotheses that led to the present study are, therefore: 1) accumulation of purine nucleosides in ADA or PNP deficiency is responsible for the immune defects observed, and 2) a particular lymphocyte subpopulation is uniquely sensitive to one or more of these purine nucleosides. The nucleosides that accumulate and that have been demonstrated to have cytotoxic or growth-inhibitory activity are Ado, dAdo, Guo and dGuo. To test these hypotheses, we separated normal populations of lymphocytes with regard to stages of maturation and cell surface markers, and we attempted to identify among these subpopulations a lymphoid cell that is particularly sensitive to one or more of the above nucleosides that accumulate in ADA or PNP deficiency. Mitogen-induced DNA synthesis in relatively immature lymphocytes obtained from the thymus of AKR mice is more sensitive to inhibition by endogenous nucleosides than is the synthesis from more mature spleen cells (Table 1). This differential sensitivity was most marked for dGuo; however, it seems likely that the higher PNP in the spleen cells[24,25] simply degrades the dGuo (Figure 1) so that the concentration and time of exposure of spleen cells to the dGuo is inadequate to effect inhibition. The greater sensitivity of relatively immature lymphocytes to the other endogenous nucleosides of interest was not as marked as that seen with dGuo (Table 1). Accordingly, when DCF is present at a maximally effective concentration of 1 μM,[26] there is no preferential degradation of dAdo by spleen cells (data not shown). There was no major difference, in terms of the ED_{50}, for these nucleosides when relatively selective T cell mitogens (PHA or ConA) or a B cell mitogen (LPS) were used (Table 1). Thus, this in vitro model would not predict a selective effect of these endogenous nucleosides on T versus B cells.

In the above experiments, cellular ADA (in spite of high concentrations of DCF) and PNP degraded the purine nucleosides studied,

rendering interpretation of ED_{50} values complex. In an effort to obviate this problem, we employed nucleoside analogs that are poor substrates for ADA and PNP. An Ado analog (Tub), two dAdo analogs (CldAdo, FaraAMP), and a dGuo analog (araGua) were selected. Although Tub is among the most toxic purine nucleosides known, its mechanism of action is not clear.[27] Like Ado, it is a good substrate for Ado kinase and, with exception to ADA, it is generally metabolized like its endogenous counterpart. Tub might mimic the effects of ADA deficiency since treatment of AKR mice with Tub[28] or DCF[29] produced qualitatively-similar effects on primary antibody response. CldAdo and FaraAMP are also poor substrates for ADA and their mechanisms of action are currently being investigated.[30,31] Like dAdo, the enzymes activating these compounds may differ between cell types. For example, 2-fluoroarabinosyladenine (the nucleoside for which FaraAMP is a prodrug), seems to be activated by a deoxycytidine kinase rather than an Ado or dAdo kinase.[32,33] Although not investigated, sensitivities of various cell types to FaraAMP may differ due to differed abilities to dephosphorylate this nucleotide. araGua has marked selectivity in inhibiting the growth of human T compared with B lymphoblasts in culture, the basis for which seems to be its enhanced metabolism in T lymphoblasts to the corresponding araGTP.[34-37] Similar to the results obtained with the endogenous nucleosides Ado and dAdo, the Ado analog (Tub) and the dAdo analogs (CldAdo and FaraAMP) were more active toward thymus-derived T cells than those from spleen (Table 2). In contrast, the dGuo analog, araGua, was more active toward spleen T cells than it was toward thymus T cells. The spleen non-B cells (mature T cells) were capable of forming and maintaining higher levels of the araGua nucleotides during the 48 hr incubation than were the thymus cells (Figure 2). This ability to maintain higher levels of araGua nucleotides appears to be due to both a greater activity of an araGua kinase (Figure 3), and a decreased araGua nucleotide degradative activity in the spleen non-B compared to thymus cells (Table 3). Although spleen T cells are more sensitive to araGua than are spleen B cells (Table 2), the differential activity (about tenfold) is considerably less than that reported[34-37] for the inhibition of proliferation of human malignant T and B lymphoblasts (i.e., differences of 100-fold or greater).

With the exception of dGuo, the endogenous nucleosides were equally effective in inhibiting the mitogen-induced DNA synthesis of spleen T

lymphocytes expressing the Lyt-2 as those that express the L_3T_4 antigens (data not shown). The greater sensitivity of the Lyt-2 phenotype to dGuo (ED_{50}=10μM vs. >100μM) may be indicative of a subpopulation uniquely sensitive to the nucleoside. In agreement with this observation, dGuo inhibited spleen suppressor cell activity in mice *in vivo*.[38] Tub demonstrated some selectivity toward the Lyt-2 phenotype compared with the L_3T_4 subpopulation (Table 1). Accordingly, we found that Tub inhibited lymphocyte-mediated cytotoxicity (natural and immune-stimulated) at doses that did not inhibit primary antibody production in mice.[28] Among the analogs evaluated *in vitro*, FaraAMP is of interest since it demonstrated selectivity for the L_3T_4 and "immature" T cell subpopulations. *In vivo*, FaraAMP and CldAdo inhibited primary antibody response at doses lower than those that inhibited cell-mediated lymphotoxicity.[28]

Malignant lymphoma cell lines derived from the AKR mouse also demonstrated differential sensitivity toward the nucleoside analogs. Of the compounds studied, Tub and CldAdo would be anticipated to have the more favorable chemotherapeutic indexes, i.e., the ED_{50} values for the most sensitive lymphoma lines (Table 4) were less than those of the most sensitive normal lymphocyte subpopulations (Table 2). Initial experiments to test this possibility using Tub suggested that the drug should be given continuously to obtain a chemotherapeutic response (Table 5).

In summary, these data revealed several murine lymphocyte subpopulations that were differentially sensitive toward purine nucleosides that accumulate in ADA or PNP deficiencies. For example, the preferential effect toward immature T (versus mature T or B) cells has been observed with endogenous nucleosides. Also, correlations concerning *in vivo* effects on cytotoxic T cells or the antibody-producing function has been obtained with dGuo, Tub and FaraAMP. Other investigators have attempted to use mice as models for ADA and/or PNP deficiencies *in vivo*[39] and to use mouse cells *in vitro*.[40,41] Limited success has been obtained *in vivo* using DCF to inhibit ADA. The difficulty of this approach may lie in the fact that mice have a high plasma level of Ado compared to humans (i.e., 10-15 μM versus 0.1 μM,[42]) even in the absence of DCF administration. Thus, mice may have developed a tolerance toward this or related nucleosides. Another possible explanation for the difficulty in establishing a murine SCID model using DCF may relate to the observation that SCID results only when a profound deletion (or inhibition, i.e, 90%

or greater) of ADA occurs.[43] These findings, coupled with the current observations, suggest that the use of nucleosides or enzyme inhibitors in mice may be poor models for these human diseases.[44,45]

ACKNOWLEDGMENTS

Supported by grant HD-13951 from the Institute for Child Health and Human Development and grant CA-28034 from the National Cancer Institute, National Institutes of Health.

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3. The abbreviations used are: ADA, adenosine deaminase; Ado, adenosine; araGua, 9-β-D-arabinosylguanine; CldAdo, 2-chlorodeoxyadenosine; ConA, concanavalin A; dAdo, deoxyadenosine; DCF, 2′-deoxycoformycin; dGuo, deoxyguanosine; FaraAMP, 9-β-D-arabinosyl, 2-fluoroadenine 5′-monophosphate; LPS, E. coli lipopolysaccharide; PBS, 0.9% sodium chloride in 10 mM phosphate buffer, pH 7.4; PHA, phytohemaglutinnin; PNA, peanut (Arachis Hypogaea) agglutinin; PNP, purine nucleoside phosphorylase; SCID, severe combined immunodeficiency disease; TdR, deoxythymidine; Tub, tubercidin or 7-deazaadenosine.

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Received June 29, 1988.