The metabolism of deoxyguanosine and guanosine in human B and T lymphoblasts

A role for deoxyguanosine kinase activity in the selective T-cell defect associated with purine nucleoside phosphorylase deficiency

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Purine nucleoside phosphorylase (NP; EC 2.4.2.1) deficiency is associated with defective T-cell and normal B-cell immunity. Biochemical mechanisms were investigated by measuring deoxyguanosine and guanosine metabolism in normal T and B lymphoblasts and NP-deficient B lymphoblasts. Deoxyguanosine kinase activity was specifically measured by using an anti-NP antibody to prevent alternative-product formation. Kinase activity towards deoxyguanosine was significantly higher in T-cells, whereas NP activity was similar in both B- and T-cells. Only in T-cells was dGTP produced from exogenous deoxyguanosine, and this was prevented by the simultaneous addition of deoxycytidine, which resulted in a concomitant increase in GTP synthesis. Inhibition by 8-aminoguanosine of NP activity in T lymphoblasts increased formation of dGTP and decreased that of GTP from deoxyguanosine and decreased the formation of GTP from guanosine. These data suggest a central role for deoxyguanosine kinase activity in the T-cell selectivity of the immune defect.

NP deficiency is clinically characterized by a severely defective T-cell function, with normal B-cell immunity (Giblett *et al.*, 1975). This is in contrast with adenosine deaminase (EC 3.5.4.4) deficiency, where B- and T-cell functions are defective (Giblett *et al.*, 1972). In patients with inherited absence of NP, its substrates accumulate in the plasma (Siegenbeck Van Heukelom *et al.*, 1977; Stoop *et al.*, 1977) and are excreted in the urine (Simmonds *et al.*, 1978). dGTP has been found in a patient's erythrocytes, where in normal cells it is undetectable (Cohen *et al.*, 1978). Pathogenetic mechanisms proposed in NP deficiency have focused on the nucleoside substrates that accumulate in plasma or urine in NP deficiency, and implicate either the nucleosides themselves or their metabolic products (Giblett *et al.*, 1981; Martin & Gelfand, 1981; Kredich & Hershfield, 1983).

The dominant working hypothesis for studies of NP deficiency has involved inhibition of ribonucleotide reductase (EC 1.17.4.1) mediated by excess dGTP. The formation of dGTP from deoxyguanosine requires an initial deoxynucleoside kinase activity, and there is evidence that this activity is relatively high in lymphoid cells (Durham & Ives,

1970; Krenitsky *et al.*, 1976; Carson *et al.*, 1977; Ullman *et al.*, 1978; Gower *et al.*, 1979; Ullman *et al.*, 1981). In T-cell lines and human peripheral blood lymphocytes it has been shown that deoxyguanosine toxicity is associated with dGTP accumulation and inhibition of DNA synthesis (Mitchell *et al.*, 1978; Ullman *et al.*, 1979; Wilson *et al.*, 1979; Cohen *et al.*, 1980; Fox *et al.*, 1980). The prevention of deoxyguanosine toxicity by deoxycytidine has been used to provide further support for the involvement of ribonucleotide reductase inhibition in the pathogenesis of NP deficiency (Chan, 1978; Ochs *et al.*, 1979; Gelfand *et al.*, 1979; Ullman *et al.*, 1979; Wilson *et al.*, 1979). Cohen *et al.* (1980) have shown increases in dGTP in human T-cells. However, Tattersall *et al.* (1975) in a study of human peripheral blood lymphocytes showed increases in dGTP that produced no inhibition of DNA synthesis.

Mechanisms involving deoxynucleotide degradation have been proposed for the differential capacity of B- and T-cells to accumulate deoxynucleoside triphosphates (Carson *et al.*, 1979; Wortmann *et al.*, 1979; Carson *et al.*, 1981; Fox *et al.*, 1981). Most of the mechanisms proposed to explain the selective T-cell dysfunction in NP deficiency have been derived by analogy with results from studies of

adenosine deaminase deficiency. These studies have been significantly assisted by the availability of specific inhibitors of adenosine deaminase.

Stoeckler et al. (1982) reported that 8-aminoguanosine was a potent inhibitor of NP, and Kazmers et al. (1981) showed that this inhibitor displayed a selective toxicity towards T-lymphoblasts.

The reason for the selective T-cell dysfunction in NP deficiency is not resolved, and to investigate further the underlying mechanism(s) we have measured metabolism of guanosine and deoxyguanosine in B- and T-lymphoblasts, including B-cells derived from a patient with NP deficiency. By using a purified anti-NP antibody to prevent alternative-nucleotide formation we were able to assay kinase activity towards deoxyguanosine. The formation of guanine nucleotides in B- and T-cells was measured and also in T-cells after inhibition of NP activity by 8-aminoguanosine. The effect of deoxycytidine on dGTP formation in T-cells was evaluated.

Experimental

Chemicals

Deoxy[5-³H]cytidine (27 Ci/mmol), [8-³H]-guanosine (9.4 Ci/mmol) and deoxy[8-³H]guanosine (2.1 Ci/mmol) were obtained from Amersham. Tetrahydrouridine was a gift from the National Cancer Institute. 8-Aminoguanosine was synthesized by the method of Holmes & Robins (1965) and was over 99% pure by high-pressure liquid-chromatography. All other reagents were from Sigma.

Cell lines

The human T lymphoblastoid cell lines 8402 and HSB-2, the B lymphoblastoid cell lines 8392 and SB, and the NP⁻ B-cell line, were obtained and grown as described previously (Ochs et al., 1979; Osborne et al., 1980).

Incubation and extraction of cells

The cells were incubated in serum-free RPMI 1640 medium plus 20 mM-sodium phosphate/2 mM-MgCl₂, pH 7.4. The T- and the B-cells were incubated at densities of about 2×10^6/ml and 1×10^6/ml respectively. The cells were gently shaken with radiolabelled nucleoside at 37°C with exposure to air for 2–3 h. The formation of radiolabelled nucleotide was linear with time, and through the incubation period cell viability, as measured by Trypan Blue exclusion, was consistently 85–95%. The experiments were terminated by centrifuging the cells at $600g$ for 10 min, removing the supernatant and preparing cell extracts with HClO₄ by the method of Garrett & Santi (1979). The total cell extract was chromatographed and the distribution of

radiolabel was determined by adding 1 ml of the pooled nucleotide peak to 10 ml of Aquasol and measuring the radioactivity with a Beckman LS 7000 liquid-scintillation counter.

Nucleotide separation

In agreement with previous observations, it was found that, of the nucleotides within the cultured cells, the majority of the radiolabel was contained in the nucleoside triphosphates (Skupp et al., 1979). Therefore a simplified isocratic procedure was developed to quantify purine ribo- and deoxy-ribo-nucleoside triphosphates. These separations were performed with a Waters high-pressure liquid chromatograph, equipped with a Whatman Partosil SAX column, a u.v. detector set at 254 nm, and an Autolab IV integrator. The elution buffer was 0.25 M-KH₂PO₄/0.5 M-KCl, pH 3.5, with a flow rate of 1.5 ml/h at 23°C. The nucleotides were quantified by relating integrated peak areas to standard curves derived from pure nucleotides.

Enzyme assays

The cells were washed in 50 mM-Tris/HCl buffer, pH 7.5 and extracts prepared by sonication on ice for 30 s with a Biosonik instrument (Hill Scientific, New York, NY, U.S.A.) at 30% maximum power. Deoxyguanosine kinase and deoxycytidine kinase were assayed by the method of Ives et al. (1969). For the deoxyguanosine kinase assay 25 μl of 600 μM-deoxyguanosine, containing 0.25 μCi of [8-³H]deoxyguanosine, was added to 25 μl of cell extract (20–100 μg of protein) plus 1 μl of purified rabbit anti-(human NP) immunoglobulin (Osborne et al., 1977) and incubated for 30–60 min at 37°C. This volume of antibody was in 10-fold excess over that required to neutralize the NP activity in the cell extract. The deoxycytidine kinase was assayed in the presence of 10 μM-3,4,5,6-tetrahydrouridine, a potent inhibitor of cytidine deaminase with K_i about 3×10^{-8} M (Wentworth & Wolfenden, 1975). In this assay 50 μl of 300 μM-deoxycytidine plus 0.25 μCi of deoxy[5-³H]cytidine and 20–100 μg of protein were incubated for 30–60 min. Hypoxanthine phosphoribosyltransferase (EC 2.4.2.8) was assayed by the method of Kelley & Meade (1971), and the radiolabelled products were separated by the method of Ives et al. (1969).

Purine nucleoside phosphorylase was assayed spectrophotometrically by the method of Kalckar (1947) as previously described (Osborne, 1980).

Results

Enzyme assays

The kinase assays (Table 1) showed that the T-cells had a 2–3-fold higher kinase activity towards deoxyguanosine than did the normal B-cells, and a 10-fold higher deoxycytidine-phosphorylating ac-

Table 1. *Deoxynucleoside kinase and purine nucleoside phosphorylase activities of lymphoblastoid cells*
Values are means $\pm$ s.d. for two determinations on different cell samples, as described in the Experimental section.

Cell line	Phosphorylating activity (nmol/h per mg)		Purine nucleoside phosphorylase (μmol/h per mg)
	Deoxyguanosine	Deoxycytidine	
8402, T	52.6 ± 2.6	80.0 ± 3.4	1.02 ± 0.11
HSB-2, T	33.4 ± 0.9	47.1 ± 7.6	3.48 ± 0.33
8392, B	16.0 ± 1.1	5.4 ± 0.7	2.40 ± 0.16
SB, B	16.5 ± 1.4	4.7 ± 0.8	3.72 ± 0.28
NP$^-$, B	15.4 ± 3.3	1.6 ± 0.5	<0.02

Table 2. *Incorporation of [^3H]guanosine and [^3H]deoxyguanosine into purine nucleoside triphosphates*
The lymphoblastoid cells, 1×10^6 B-cells and 2×10^6 T-cells, were incubated with 200 μM nucleoside containing 15 μCi of radiolabel in a total volume of 1 ml for 2 h at 37°C, extracted and chromatographed as described in the Experimental section. Abbreviation: nd, not detectable (limit of detection 0.03 nmol).

Cell line	Radiolabelled nucleoside added	Nucleotide (nmol)				Radioactivity in peak (% of total)				Total radioactivity (c.p.m.)
		ATP	dATP	GTP	dGTP	ATP	dATP	GTP	dGTP	
T, 8402	None	9.90	nd	2.28	nd	–	–	–	–	–
	Guanosine	6.66	nd	3.30	nd	15.1	1.0	82.7	1.2	65 663
	Deoxyguanosine	7.75	nd	2.01	1.00	10.6	0.6	39.1	49.7	45 060
T, HSB-2	None	9.91	nd	2.58	nd	–	–	–	–	–
	Guanosine	9.52	nd	5.46	nd	10.1	0.7	88.2	1.0	108 028
	Deoxyguanosine	9.33	nd	3.24	0.66	10.0	0.5	60.9	28.6	62 069
B, 8392	None	12.20	nd	3.88	nd	–	–	–	–	–
	Guanosine	11.43	nd	7.22	nd	4.6	0.2	94.5	0.7	120 945
	Deoxyguanosine	10.59	nd	7.01	nd	5.2	0.4	93.4	1.0	86 694
B, SB	None	7.93	nd	2.05	nd	–	–	–	–	–
	Guanosine	6.68	nd	2.96	nd	7.4	0.6	91.3	0.7	50 683
	Deoxyguanosine	6.91	nd	3.43	nd	8.1	1.2	88.9	1.8	44 301
B, NP$^-$	None	4.35	nd	0.92	nd	–	–	–	–	–
	Guanosine	4.40	nd	1.05	nd	3.5	0.7	90.6	5.2	17 204
	Deoxyguanosine	3.91	nd	0.76	nd	6.2	0	86.6	7.2	11 456

tivity. The deoxyguanosine kinase activity was measured in the presence of anti-NP antibody, which prevented the alternative formation of nucleotides and gave consistently reproducible assays. Also, in the deoxycytidine kinase assay the use of a cytidine deaminase inhibitor prevented substrate depletion by this latter enzyme and gave more consistent results. The ratio of the deoxycytidine- to deoxyguanosine-phosphorylating activity was about 1.5 for the T-cells and about 0.3 for the normal B-cells (Table 1). This suggests a genetic difference in kinase or kinases between the two cell types. Deoxycytidine inhibited deoxyguanosine kinase activity in B- and T-cells. From assays in the presence of deoxycytidine (0.25 mM, 0.5 mM and 2.0 mM), the concentration giving 50% inhibition of deoxyguanosine kinase activity was 0.5 mM for T-cells and 2.0 mM for B-cells. These deoxycytidine concentrations, although not true inhibition constants, differ by 4-fold and support a genetic difference in deoxyguanosine kinase between B- and T-cells.

A survey of NP activity in several B- and T-cell lines, including those shown in Table 1, gave the following mean values $\pm$ s.d.: B-cells, 2.70 ± 0.60 μmol/h per mg ($n = 8$); T-cells, 2.39 ± 0.82 μmol/h per mg ($n = 6$). These activities are not significantly different and indicate that there is no systematic variation in NP activity between B- and T-cells. The NP$^-$ B-cell has an NP activity that is $<1\%$ of a normal B-cell, and showed undetectable NP protein by an enzyme-linked immunosorbent assay (Osborne & Scott, 1980). The hypoxanthine phosphoribosyltransferase activities of the B- and T-cells ranged from 381 to 520 nmol/h per mg of protein, with mean $\pm$ s.d. of 466.6 ± 55.8 nmol/h per mg ($n = 5$). These values are in agreement with a previous report (Hershfield et al., 1977) and showed no systematic difference between the B- and T-cells.

Nucleoside-uptake experiments

In Table 2 are listed the concentrations of purine nucleotides and the percentage distribution of

radioactive purine from incubation experiments with guanosine or deoxyguanosine using two T-cell and two B-cell lines, and a NP⁻ B-cell line. In these experiments 2×10^6 T-cells were incubated and extracted in order to produce equivalent concentrations of ATP. These results show that of the normal B- and T-cells, only T-cells accumulate dGTP from exogenous deoxyguanosine. The accumulation of dGTP by T-cells has been previously demonstrated (Mitchell *et al.*, 1978; Wilson *et al.*, 1979; Ullman *et al.*, 1979; Cohen *et al.*, 1980;

Carson *et al.*, 1980; Kazmers *et al.*, 1981). In the two T-cell lines 30–50% of the radioactivity in the purine triphosphates accumulated in dGTP after incubation with radioactive deoxyguanosine. Neither of the two normal B-cell lines or the NP⁻ B-cells accumulate measurable concentrations, or significant radioactivity, in dGTP from the labelled deoxyguanosine. In the two T-cell lines, the specific radioactivity of the dGTP was similar, with a range of 22.4×10^3 to 26.9×10^3 c.p.m./nmol of nucleotide. The specific radioactivity of GTP was similar for the
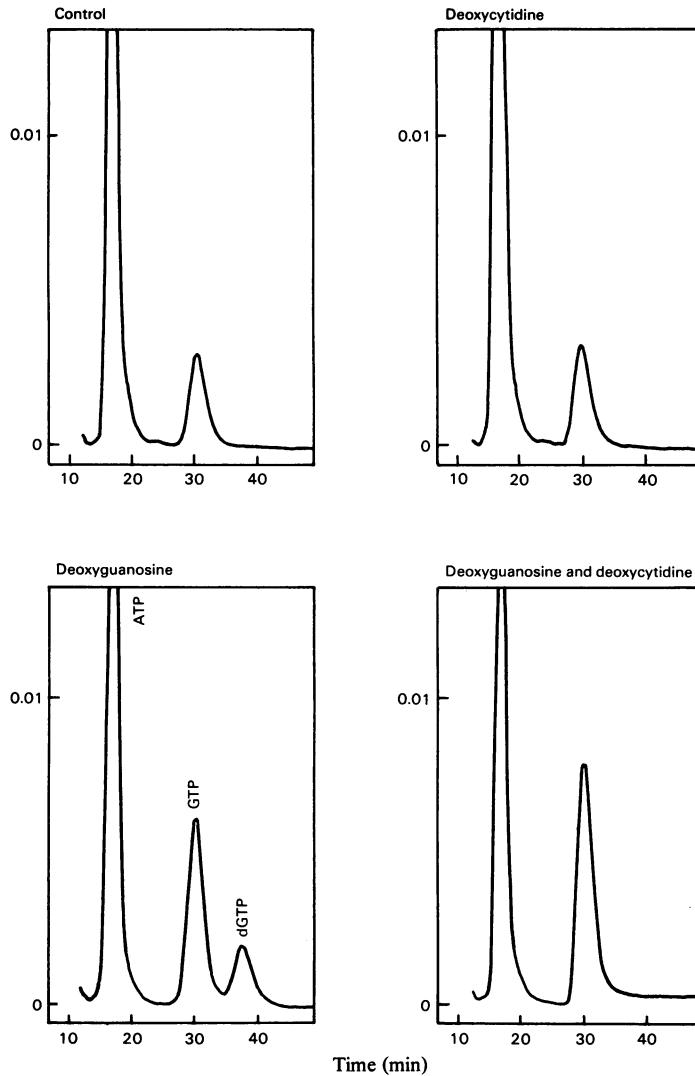

Fig. 1. *High-pressure-liquid-chromatographic elution profiles*

The T lymphoblastoid cells were incubated for 2 h at 37°C with [³H]deoxyguanosine in the presence or absence of deoxycytidine as described in Table 3, and then were extracted and chromatographed. The ordinate represents A_{254}; the detector was set at $0.02 A_{254}$ unit full-scale.

B- and T-cells, with a range of 8.7×10^3 to 11.7×10^3 c.p.m./nmol of GTP. The incubation of the normal B- and T-cells with radiolabelled guanosine resulted in an increase in GTP concentration compared with the controls, and of the purine triphosphates the GTP contained about 90% of the radioactivity. The specific radioactivity of the GTP was almost identical in the four normal lymphoblastoid cell lines, with values of 15.6×10^3–17.5×10^3 c.p.m./nmol of GTP. The uptake of radiolabelled guanosine and deoxyguanosine into purine triphosphates by the NP^- B-cell line was about 20% of that in the normal B- and T-cells. The specific radioactivity of the GTP was 14.9×10^3 and 13.1×10^3 c.p.m./nmol of GTP for the incubations with guanosine and deoxyguanosine respectively, which is equivalent to that in the normal B- and T-cells.

The experiments with deoxyguanosine incubation were repeated with the two T-cell lines with the addition of unlabelled deoxycytidine. Previous work had shown that deoxycytidine greatly decreased the growth inhibition of deoxyguanosine on T-cells (Ochs et al., 1979; Gelfand et al., 1979) and that equimolar concentrations of deoxycytidine and deoxyguanosine decreased the formation of dGTP (Mitchell et al., 1978; Wilson et al., 1979; Carson et al., 1980). From the purine triphosphate peaks obtained from an experiment with T-cell line 8402 it was evident that the simultaneous addition of deoxycytidine and deoxyguanosine prevented the formation of dGTP (Fig. 1). The quantitative data from the experiment with T-cell line 8402 and a similar one with T-cell line HSB-2 (Table 3) showed that the simultaneous addition of deoxycytidine and deoxyguanosine largely prevented the formation of radiolabelled dGTP in both cell lines and resulted in increased formation of radiolabelled GTP. For each cell line the total radioactivity in purine triphos-

phates was about the same with and without the addition of deoxycytidine, indicating that deoxycytidine did not act by merely preventing the uptake of deoxyguanosine.

The addition to T-cells of increasing concentrations of the NP inhibitor 8-aminoguanosine, in the presence of deoxyguanosine, resulted in a marked increase in dGTP formation (Table 4). The incorporation of radiolabel into the nucleoside triphosphates was decreased by a similar amount by both inhibitor concentrations. The percentage of radioactivity in dGTP increased 2-fold with the doubling of inhibitor concentration. Thus deoxyguanosine uptake was decreased and its distribution between GTP and dGTP was significantly altered in the presence of the NP inhibitor. Increased formation of dGTP after inhibition of NP has been reported by Kazmers et al. (1981). A similar experiment with radiolabelled guanosine and the NP inhibitor (Table 4) showed a marked decrease in GTP concentration with increasing inhibitor concentration, and an insignificant effect on dGTP formation. The percentage distribution of radiolabel was not greatly altered by NP inhibition, but the total incorporation of radioactivity from guanosine decreased by about 50% with the addition of 100 μM inhibitor.

Discussion

The assays of deoxyguanosine and deoxycytidine phosphorylation showed significantly higher activities in T-cells. Between B- and T-cells there were differences in the ratio of these kinase activities, and in the inhibition by deoxycytidine of deoxyguanosine kinase activity. These data suggest that there may exist genetic differences in these activities between B- and T-cells. It has been reported that B- and T-cells have similar deoxyguanosine kinase activities (North et al., 1980). However, the low

Table 3. *Incorporation of [³H]deoxyguanosine into purine nucleoside triphosphates in the presence of deoxycytidine*
The T lymphoblastoid cells (2×10^6) were incubated in 1 ml of 200 μM-deoxyguanosine plus, where indicated, 10 μCi of radiolabelled deoxyguanosine, 80 μM-deoxycytidine or both, for 2 h at 37°C, extracted and chromatographed as described in the Experimental section. Abbreviation: nd, not detected (limit of detection 0.03 nmol).

T-cell line	Deoxynucleoside added	Nucleotide (nmol)				Radioactivity in peak (% of total)				Total radioactivity (c.p.m.)
		ATP	dATP	GTP	dGTP	ATP	dATP	GTP	dGTP	
8402	None	4.08	nd	1.74	nd	–	–	–	–	–
	Deoxycytidine	4.51	nd	1.95	nd	–	–	–	–	–
	[³H]Deoxyguanosine	3.12	nd	3.83	1.54	1.8	0	64.7	33.5	38 230
	[³H]Deoxyguanosine + deoxycytidine	3.16	nd	4.85	0.10	3.7	0.2	91.5	4.7	37 203
HSB-2	None	6.94	nd	1.49	nd	–	–	–	–	–
	Deoxycytidine	8.10	nd	1.66	nd	–	–	–	–	–
	[³H]Deoxyguanosine	7.41	0.03	2.82	1.00	4.6	0.1	55.9	39.2	25 396
	[³H]Deoxyguanosine + deoxycytidine	5.13	nd	3.54	nd	7.8	0	89.6	2.7	27 687

Table 4. *Incorporation of [³H]guanosine and [³H]deoxyguanosine into purine nucleoside triphosphates in the presence of increasing concentrations of 8-aminoguanosine*

The T lymphoblastoid cells (2×10^6; line 8402) were incubated with 100 μM-guanosine or -deoxyguanosine, each containing 10 μCi of radiolabelled nucleoside, plus 0, 100 μM- or 200 μM-8-aminoguanosine in a final volume of 1 ml for 2 h at 37°C, extracted and chromatographed as described in the Experimental section. Abbreviation: nd, not detected (limit of detection 0.03 nmol).

Radiolabelled nucleoside	8-Amino-guanosine (μM)	Nucleotide (nmol)				Radioactivity in peak (% of total)				Total radioactivity (c.p.m.)
		ATP	dATP	GTP	dGTP	ATP	dATP	GTP	dGTP	
Deoxyguanosine	0	4.63	nd	1.77	0.23	12.2	0.5	68.2	19.0	29407
	100	4.96	nd	1.31	0.29	14.6	1.4	55.2	28.8	19824
	200	4.50	0.10	0.90	0.64	7.6	0.1	36.2	56.1	22009
Guanosine	0	5.06	nd	2.58	nd	13.9	0.5	84.5	1.1	56759
	100	2.69	nd	1.38	nd	14.0	2.7	80.5	2.8	28901
	200	2.74	nd	1.06	nd	11.0	0.9	87.2	0.9	24654

substrate concentrations used by those authors would probably not distinguish between low and high enzyme activities, and furthermore, by the method used (Ives *et al.*, 1969) it is not possible to measure deoxyguanosine kinase activity in the presence of NP, as there is no way of distinguishing between nucleotide product formation via kinase activity from that due to the sequential action of NP and hypoxanthine phosphoribosyltransferase. Two enzymes with activity towards deoxyguanosine have been reported in calf thymus tissue (Gower *et al.*, 1979). By using Cellogel electrophoresis and autoradiography it has been shown that human T-cells possess a deoxycytidine kinase and deoxyadenosine kinase activity not present in B-cells (S.-H. Chen, unpublished work). Thus it is probable that B-cells have a low activity of a single deoxynucleoside kinase, whereas T-cells have more than one deoxynucleoside kinase. The survey of NP activities in B- and T-cells showed that there was no significant difference between these cells. Also, similar hypoxanthine phosphoribosyltransferase activities were recorded for the two cell types. Thus the difference in deoxyguanosine metabolism between normal B- and T-cells is probably not based on differences in activity of NP or hypoxanthine phosphoribosyltransferase.

The cell-culture experiments showed a marked difference between T- and B-cells in their metabolism of deoxyguanosine. In the T-cells, the exogenous deoxyguanosine accumulates equally in GTP and dGTP, whereas in the B-cells it is almost totally incorporated into GTP. In both the B- and T-cells, exogenous guanosine accumulates as GTP, presumably via the action of NP, as there is no evidence for guanosine kinase in mammals (Henderson & Patterson, 1973). Most noteworthy were the results from the incubation of the NP⁻ B-cell line with deoxyguanosine. The deoxynucleoside is almost totally incorporated into GTP, giving further evidence for an insignificant deoxynucleoside kinase activity towards deoxyguanosine in B-cells. The increasing concentrations of the NP inhibitor in the deoxyguanosine incubations resulted in an increase in accumulation and radiolabel incorporation into dGTP and a parallel decrease in GTP. The overall radiolabel uptake was decreased by about 20% of the control. Thus inhibition of NP in a normal T-cell results in increased phosphorylation of deoxyguanosine. Similar results have been reported by Kazmers *et al.* (1981). In the incubations with guanosine, the increasing inhibitor concentration decreased the accumulation of GTP and radiolabel uptake to less than 50% of the control values. The activity of NP at the highest inhibitor concentration was <5% that of the control (results not shown). The NP⁻ B-cell line, with <1% of normal NP activity, showed a 60% decrease in guanosine uptake compared with a control. Thus at even these low NP activities there is significant metabolism of guanosine. The observation that deoxycytidine can prevent the extreme toxicity of deoxyguanosine towards T-cells (Ochs *et al.*, 1979; Gelfand *et al.*, 1979) was investigated by incubating T-cells with radiolabelled deoxyguanosine and deoxycytidine. The accumulation of dGTP from exogenous deoxyguanosine was prevented by the simultaneous addition of deoxycytidine and resulted in a concomitant increase in GTP. It was apparent from the radioactivity measured in GTP and dGTP that similar amounts of deoxyguanosine were metabolized in the presence or absence of deoxycytidine. This would suggest that the deoxycytidine did not act by merely competitively inhibiting the transport of deoxyguanosine into the cells. This observation of a lack of build-up of dGTP in the presence of deoxycytidine is noteworthy, because it has been suggested that deoxycytidine reverses the toxicity of deoxyguanosine on T-cells, not by preventing the accumulation of dGTP, but by replenishing the

dCTP pool and thus by-passing the inhibition of ribonucleotide reductase (Carson et al., 1977; Ullman et al., 1978; Gudas et al., 1978). This explanation is tenuous, because dCTP is the only product of the ribonucleotide reductase reaction that is not an allosteric effector, and thus its replenishment would still leave the enzyme inhibited by excess dGTP (Thelander & Reichard, 1979). It seems that the lack of dGTP is convincing evidence that deoxycytidine overcomes the toxicity of deoxyguanosine in T-cells by preventing the accumulation of dGTP. A possible explanation for this involves the kinetics of deoxyguanosine phosphorylation. We showed that in B- and T-cells the addition of deoxycytidine inhibited kinase action on deoxyguanosine and thus formation of dGMP and hence dGTP. The inhibition of deoxynucleoside kinase activity by deoxycytidine has been documented for deoxycytidine kinase from calf thymus (Krenitsky et al., 1976), and involves inhibition by the final product dCTP and competitive substrate inhibition.

An alternative explanation for the selective accumulation of deoxynucleoside triphosphates in T-cells has been based on their decreased capacity for nucleotide catabolism (Carson et al., 1979; Wortmann et al., 1979; Fox et al., 1981). These hypotheses have involved ecto-5'-nucleotidase (Carson et al., 1979; Wortmann et al., 1979) or ecto-ATPase (Fox et al., 1981), and are somewhat attenuated by the location of these enzymes on the external plasma membrane. A soluble 5'-nucleotidase activity with an acid pH optimum has been described, with 3-fold higher activity in B-cells (Carson et al., 1981). It is possible that this could be a factor in the differential sensitivity of T-cells to deoxyguanosine, but from the activities reported for this enzyme and its K_m of 290 μM for dGMP it would not seem to be of major significance.

These data suggest a major difference in the metabolism of deoxyguanosine between B- and T-cells, which results from a different balance between NP and kinase activities. In B-cells deoxyguanosine is primarily metabolized to give GTP formation in a reaction sequence initiated by NP. This is supported by the lack of dGTP accumulation in NP⁻ B-cells and the decrease in GTP formation without a mutual increase in the formation of dGTP when NP activity is inhibited in normal B-cells. In T-cells a major route of deoxyguanosine metabolism is initiated by kinase activity to give dGTP formation. This is supported by the higher kinase activity towards deoxyguanosine in T-cells, the formation of dGTP from deoxyguanosine and its prevention by deoxycytidine, and the increase in dGTP accumulation after inhibition of NP activity. Thus in the absence of NP activity only T-cells would accumulate the cytotoxic dGTP (Osborne, 1981).

From our conclusion that the selective T-cell defect is due to differential kinase activity towards deoxyguanosine between B- and T-cells, then an inhibitor of this activity would provide therapy for patients with this immune dysfunction. Furthermore, an inhibitor of NP should selectively regulate T-cell lymphopoiesis. In experiments with dogs we have administered 8-aminoguanosine in conjunction with deoxyguanosine and have selectively decreased peripheral blood lymphocytes by over 50%, with no effect on platelets or other white blood cells (W. R. A. Osborne, S. J. Slichter & H. J. Deeg, unpublished work).

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