2'-Deoxyguanosine Toxicity for B and Mature T Lymphoid Cell Lines Is Mediated by Guanine Ribonucleotide Accumulation

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Abstract. Inherited deficiency of the enzyme purine nucleoside phosphorylase (PNP) results in selective and severe T lymphocyte depletion which is mediated by its substrate, 2'-deoxyguanosine. This observation provides a rationale for the use of PNP inhibitors as selective T cell immunosuppressive agents. We have studied the relative effects of the PNP inhibitor 8-aminoguanosine on the metabolism and growth of lymphoid cell lines of T and B cell origin. We have found that 2'-deoxyguanosine toxicity for T lymphoblasts is markedly potentiated by 8-aminoguanosine and is mediated by the accumulation of deoxyguanosine triphosphate. In contrast, the growth of T_4^+ mature T cell lines and B lymphoblast cell lines is inhibited by somewhat higher concentrations of 2'-deoxyguanosine (ID_{50} 20 and 18 μM, respectively) in the presence of 8-aminoguanosine without an increase in deoxyguanosine triphosphate levels. Cytotoxicity correlates instead with a three- to fivefold increase in guanosine triphosphate (GTP) levels after 24 h. Accumulation of GTP and growth inhibition also result from exposure to guanosine, but not to guanine at equimolar concentrations. B lymphoblasts which are deficient in the purine salvage enzyme hypoxanthine guanine phosphoribosyltransferase are completely resistant to 2'-deoxyguanosine or guanosine concentrations up to 800 μM and do not demonstrate an increase in GTP levels. Growth inhibition and GTP accumulation are prevented by hypoxanthine or adenine, but not by 2'-deoxycytidine. 8-Aminoguano-sine appears to effectively inhibit extracellular PNP activity; thus, it prolongs the extracellular half-life of 2'-deoxyguanosine and guanosine, but does not completely inhibit intracellular PNP activity in these lymphoid cells. As a result, 2'-deoxyguanosine and guanosine are phosphorolyzed and actively salvaged within the cell, accounting for the accumulation of GTP. Partial inhibition of PNP activity in vivo, therefore, may lead to nonselective cellular toxicity by a mechanism independent of dGTP accumulation.

Introduction

Inherited deficiency of the enzyme purine nucleoside phosphorylase (PNP)[1] results in a severe depletion of T lymphocytes and loss of cellular immunity (1, 2). The immunodeficiency disease appears to result from the selective metabolism of the PNP substrate, 2'-deoxyguanosine, to the deoxyribonucleotide, deoxyguanosine triphosphate (dGTP), in the T cells of affected individuals (3). This hypothesis has been given added credence by the observation that cultured human lymphoblasts of T cell origin accumulate large amounts of dGTP when exposed to 2'-deoxyguanosine in vitro (4, 5). dGTP inhibits the enzyme ribonucleotide reductase, which catalyzes the reduction of nucleoside diphosphates to their corresponding 2'-deoxy derivatives. Thus, accumulation of dGTP leads to depletion of other deoxynucleoside triphosphates, inhibition of DNA synthesis, and cell death (6, 7). Human B lymphoblasts and mature peripheral blood lymphocytes are both relatively resistant to the toxic effects of 2'-deoxyguanosine alone and do not accumulate significant amounts of dGTP under in vitro culture conditions (8-10).

As a consequence of these observations, there has been considerable interest in the development of PNP inhibitors as potentially selective T cell cytotoxic agents. We have previously reported that the guanosine analogue, 8-aminoguanosine, is a

1. *Abbreviations used in this paper:* dGTP, deoxyguanosine triphosphate; HPRT, hypoxanthine guanine phosphoribosyltransferase; GDP, guanosine diphosphate; GMP, guanosine monophosphate; GTP, guanosine triphosphate; PNP, purine nucleoside phosphorylase; PP-ribose-P, 5-phosphoribosyl 1-pyrophosphate.

competitive inhibitor of PNP which markedly potentiates both the toxicity of 2'-deoxyguanosine and the accumulation of dGTP in T lymphoblasts (9). We now have evidence that 8-aminoguanosine also markedly enhances the toxic effects of 2'-deoxyguanosine for cultured B lymphoblasts and mature T cell lines. The inhibition of growth in these cells is mediated not by dGTP, but by the accumulation of guanine ribonucleotides.

Methods

Materials. 2'-Deoxyguanosine, guanosine, guanine, hypoxanthine, adenine, and 2'-deoxycytidine were purchased from Sigma Chemical Co. (St. Louis, MO). 8-Aminoguanosine was a gift of Dr. L. Townsend, Department of Medicinal Chemistry, University of Michigan, Ann Arbor, MI. Horse serum and RPMI 1640 medium were purchased from Gibco Laboratories (Grand Island, NY).

Cell lines. Human T lymphoblast lines Molt 4 and RPMI 8402 were derived from patients with T cell acute lymphoblastic leukemia and obtained from HEM Research, Rockville, MD. The B lymphoblast lines GM 558, GM 1899 (hypoxanthine guanine phosphoribosyltransferase [HPRT$^-$]), and GM 2292 (HPRT$^-$) were obtained from the Human Genetic Mutant Cell Repository, Bethesda, MD. Line MGL 8 was obtained from the laboratory of Dr. John Littlefield, Johns Hopkins University, Baltimore, MD. The mature T cell lines HUT 78 and HUT 102 were obtained from Dr. Paul Bunn at the National Cancer Institute and have been described previously (11, 12). These cells were originally obtained from the peripheral blood or lymph node of patients with cutaneous T cell lymphomas and have surface antigens reactive with OKT4 monoclonal antibodies. The HUT 102 line is known to possess human T cell leukemia virus (12). All cell lines were propagated in RPMI-1640 medium with 10% horse serum at 37°C in 5% CO_2.

Cell growth experiments. Exponentially growing cells were suspended at a concentration of 2×10^5 cells/ml in RPMI-1640 medium, 10% horse serum, and the additives indicated in a final volume of 100 μl in flat-bottom microtiter plates (Corning Medical and Scientific, Corning, NY). Each experiment was performed in triplicate and control growth was exponential over 96 h. Cell counts were performed at 72 h by hemocytometer, using trypan blue dye exclusion to identify viable cells. Counts were expressed as viable cells per milliliter and related to the growth of the cells designated as controls.

Nucleotide pool measurements. Cell incubations were performed at the final concentration of 2×10^5 cells/ml in RPMI-1640 medium with 10% horse serum and the additives indicated. 8-Aminoguanosine was added 30 min before the addition of other additives. Following the incubations, cells were sedimented at 400 g, the supernatants carefully aspirated, and the cells extracted in 1 ml 60% methanol at −20°C for 24 h. The percentage of viable cells was determined before each extraction and 0.5–2 $\times 10^7$ cells were extracted for each determination. Ribo- and deoxyribonucleotides were separated by high pressure liquid chromatography on a Partisil-10 SAX anion-exchange column (Whatman Laboratory Products, Inc., Clifton, NJ), using an ammonium phosphate elution gradient (0.3–0.45 M, pH 3.3–3.6) over 35 min at a flow rate of 2 ml/min. Eluted nucleotides were detected by their absorbance at 254 nm and quantitated by comparison to calibration curves of nanomole amounts of pure standards. This method resulted in the complete separation of cytidine triphosphate, uridine triphosphate, ATP, deoxyadenosine triphosphate, GTP, and dGTP.

dGTP levels in unincubated T lymphoblasts and in B lymphoblast and mature T cell lines required quantitation by the more sensitive DNA polymerase assay, which was performed as previously described (9).

Extracellular 2'-deoxyguanosine or guanosine levels were measured in cell culture supernates which were deproteinized by the method of Koller et al. (13). 0.96 ml H_2O and 0.4 ml of 0.15 M $Ba(OH)_2$ were added to 1 ml of supernate. After 2 min, 0.64 ml of 0.15 M $ZnSO_4$ was added, and the resultant sediment was discarded. The supernate was run on a reverse phase C-18 μBondapak column (Whatman Associates, Milford, MA) at a flow rate of 2 ml/min and eluted with 0.05 M K_2HPO_4 and 5% methanol, pH 2.6. The recovery of 2'-deoxyguanosine and guanosine by this procedure consistently ranged between 55 and 62%.

Results

Cell growth experiments. The effects of 2'-deoxyguanosine and guanosine in the absence and presence of 8-aminoguanosine on the growth of T lymphoblasts, B lymphoblasts, and mature T cell lines are summarized in Table I and representative growth curves in the presence of 8-aminoguanosine are shown in Fig. 1. 8-Aminoguanosine at a concentration of 100 μM markedly potentiates 2'-deoxyguanosine toxicity for T lymphoblasts, as has been previously described (9). In addition, it enhances the toxicity of both 2'-deoxyguanosine and guanosine for B lymphoblasts and for mature T cell lines. B lymphoblasts deficient in the enzyme HPRT are completely resistant to 2'-deoxyguanosine or guanosine concentrations up to 800 μM in both the absence and presence of 8-aminoguanosine. Fig. 1 demonstrates that, in the presence of 8-aminoguanosine, 2'-deoxyguanosine and guanosine have equivalent growth inhibitory effects on B and mature T cell lines, whereas T lymphoblasts are both more sensitive to 2'-deoxyguanosine and more resistant to guanosine. Guanine inhibits the growth of B and mature T cell lines to a similar degree as 2'-deoxyguanosine or guanosine alone, but guanine toxicity is not potentiated by 8-aminoguanosine (Table I).

2'-Deoxycytidine at a concentration of 100 μM markedly reduces the toxicity of 2'-deoxyguanosine and 8-aminoguanosine for T lymphoblasts (Fig. 2 A), but has a far less marked rescuing effect on 2'-deoxyguanosine-mediated growth inhibition of B lymphoblasts (Fig. 2 B) and mature T cells (Fig. 2 C). Uridine at concentrations up to 100 μM does not prevent 2'-deoxyguanosine-induced growth inhibition of either B lymphoblasts or mature T cells (data not shown). In contrast, both adenine (100 μM) and hypoxanthine (100 μM) completely prevent the inhibition of growth in B lymphoblasts (Fig. 3 B) and mature T cell lines (Fig. 3 C), but have no effect on the toxicity of 2'-deoxyguanosine for T lymphoblasts (Fig. 3 A).

Nucleotide pool determinations. The effects of incubations of the various cell lines with 100 μM 8-aminoguanosine and 50 μM 2'-deoxyguanosine on GTP and dGTP pools are shown in Fig. 4. T lymphoblasts did not accumulate any GTP, whereas HPRT$^+$ B lymphoblasts and mature T cell lines

Table I. The Effect of 8-Aminoguanosine on 2'-Deoxyguanosine, Guanosine, and Guanine Toxicity for Lymphoid Cell Lines

Cell lines		ID$_{50}$ (μM)					
		2'-Deoxyguanosine		Guanosine		Guanine	
		−8AGuo	+8AGuo	−8AGuo	+8AGuo	−8AGuo	+8AGuo
T lymphoblasts	Molt 4	20	1.3	210	152	350	400
	RPMI 8402	22	0.9	300	240	ND	ND*
B lymphoblasts	MGL 8	60	18	160	12	160	150
	GM 558	160	17	250	14	ND	ND
	GM 2292 (HPRT⁻)	>800	>800	>800	>800	>600	>600
	GM 1899 (HRPT⁻)	>800	>800	>800	>800	ND	ND
Mature T cells	HUT 78	75	20	80	15	200	150
	HUT 102	280	30	600	70	ND	ND

Cell growth experiments were performed at a starting concentration of 2×10^5 cells/ml in RPMI-1640 medium plus 10% horse serum in the absence or presence of 100 μM 8-aminoguanosine (8AGuo) and increasing concentrations of either 2'-deoxyguanosine, guanosine, or guanine. Each experiment was performed in triplicate and repeated on at least two occasions. The SEM on triplicate determinations was <15%. Values represent the mean concentrations of 2'-deoxyguanosine, guanosine, or guanine resulting in 50% inhibition of growth at 72 h. * ND, not done.

increased their GTP pools from three- to fivefold. HPRT-deficient B lymphoblasts did not accumulate any GTP under identical conditions. In contrast, T lymphoblasts accumulated large amounts of dGTP, as has been previously reported (9), whereas B and mature T cell lines were both resistant to dGTP accumulation.

The resistance of HPRT-deficient B lymphoblasts to both 2'-deoxyguanosine-mediated toxicity and GTP accumulation in the presence of 8-aminoguanosine led us to postulate that guanine ribonucleotide accumulation depended first upon the conversion of 2'-deoxyguanosine to guanine by PNP activity and, secondly, on the salvage of guanine to guanine nucleotides by HPRT activity (Fig. 5). The conversion of guanine to guanosine monophosphate (GMP) also requires 5-phosphoribosyl 1-pyrophosphate (PP-ribose-P) (Fig. 5). Since both adenine and hypoxanthine are also actively salvaged by phosphoribosylation and, hence, would compete with guanine for intracellular PP-ribose-P pools, we examined the effects of these compounds on intracellular GTP accumulation (Fig. 6). Both adenine (100 μM) and hypoxanthine (100 μM) completely prevented the GTP accumulation potentiated by 8-aminoguanosine at 24 h. We, then, compared the effects of 2'-deoxyguanosine in the absence and presence of 8-aminoguanosine with those of guanosine and guanine on GTP pools (Table II). 2'-Deoxy-guanosine and guanosine caused very similar elevations in GTP pools, which were markedly potentiated by 8-aminoguanosine. Equimolar concentrations of guanine, on the other hand, did not increase GTP levels and the addition of 8-aminoguanosine had no enhancing effect.

To examine the mechanism by which 8-aminoguanosine potentiates GTP accumulation, the levels of both GTP (Fig. 6 A) and extracellular 2'-deoxyguanosine (Fig. 6 B) were moni-

tored simultaneously over a 48-h culture of B lymphoblasts. In the absence of 8-aminoguanosine, GTP accumulated rapidly and reached a peak level of 4,000 pmol/10^6 cells within 3 h, with a return to base-line levels by 24 h, while extracellular 2'-deoxyguanosine concentrations dropped precipitously over a 12-h period. The addition of 8-aminoguanosine markedly prolonged the half-life of 2'-deoxyguanosine in the medium (Fig. 7 B), and the intracellular GTP concentration remained elevated at a level more than three times the base-line concentration over the entire 48-h incubation period (Fig. 7 A). Similar results were obtained using guanosine. 8-Aminoguanosine alone in concentrations up to 400 μM did not cause change of GTP concentrations at any time point. However, in the presence of 50 μM 2'-deoxyguanosine, increasing the 8-aminoguanosine concentration from 100 μM to 400 μM effectively prevented both the accumulation of GTP and inhibition of growth in B and mature T cell lines (Fig. 8). We conclude from these experiments that 8-aminoguanosine at lower concentrations competitively inhibits the phosphorolysis of 2'-deoxyguanosine and of guanosine by extracellular PNP activity, thereby prolonging the availability of the nucleosides for transport. 2'-Deoxyguanosine and guanosine are then cleaved to guanine by intracellular PNP activity and salvaged by HPRT. Higher concentrations of 8-aminoguanosine, on the other hand, more completely inhibit intracellular PNP activity, thereby preventing guanine ribonucleotide-related toxicity.

Discussion

We have established that the toxicity of 2'-deoxyguanosine for human B lymphoblast and mature T cell lines is mediated by the accumulation of guanine ribonucleotides. The evidence to
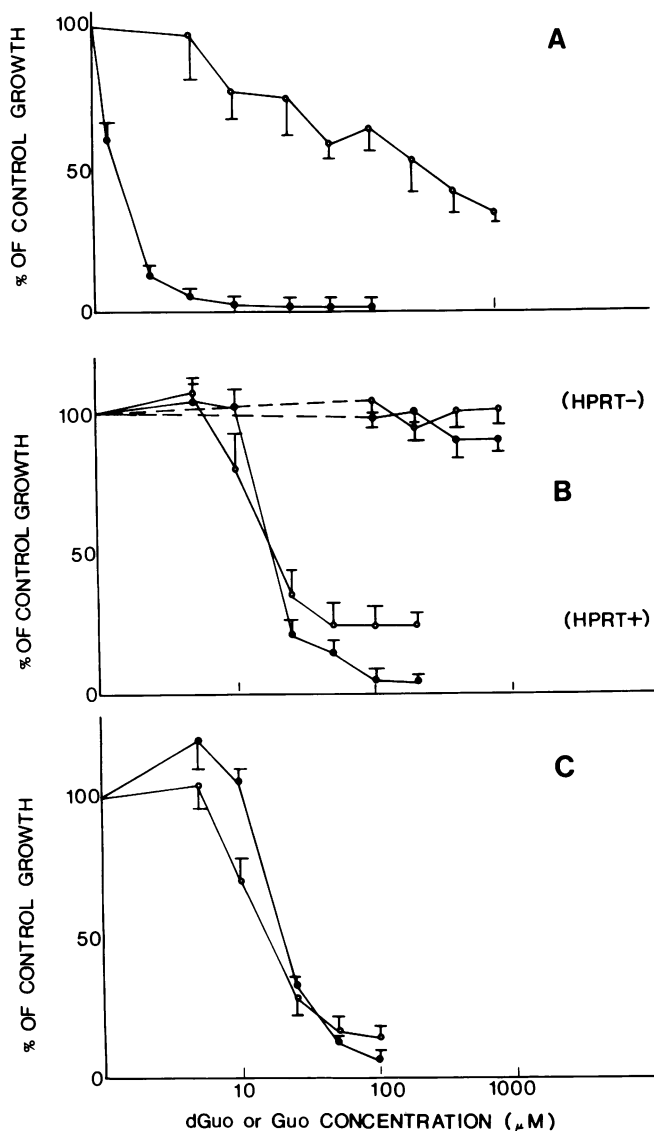
Figure 1 (left panel)

% OF CONTROL GROWTH (A)

100

50

0

A

% OF CONTROL GROWTH (B)

100

50

0

(HPRT−)

B

(HPRT+)

% OF CONTROL GROWTH (C)

100

50

0

C

10   100   1000

dGuo or Guo CONCENTRATION (μM)

Figure 1. Relative effects of 2'-deoxyguanosine (dGuo) and guanosine (Guo) plus 8-aminoguanosine on the growth of T and B cell lines. (*A*) T lymphoblasts (Molt 4); (*B*) HPRT⁺ B lymphoblasts (GM 558) and HPRT⁻ B lymphoblasts (GM 1899); (*C*) mature T cell line (HUT 78). Cells were grown in microtiter plates as described in Methods in the presence of the indicated concentrations of 2'-deoxyguanosine (solid symbols) or guanosine (open symbols). The concentration of 8-aminoguanosine was 100 μM in all experiments. Cell counts were performed after 72 h. Values represent cell counts in the presence of additives relative to control cultures containing 8-aminoguanosine alone. 8-Aminoguanosine (100 μM) alone had no effect on the growth of any cell line. Each point represents the mean±SD of three experiments done in triplicate.

support this statement comes from the following observations: (*a*) HPRT-deficient B lymphoblasts which are incapable of the synthesis of guanine ribonucleotides from guanine are resistant
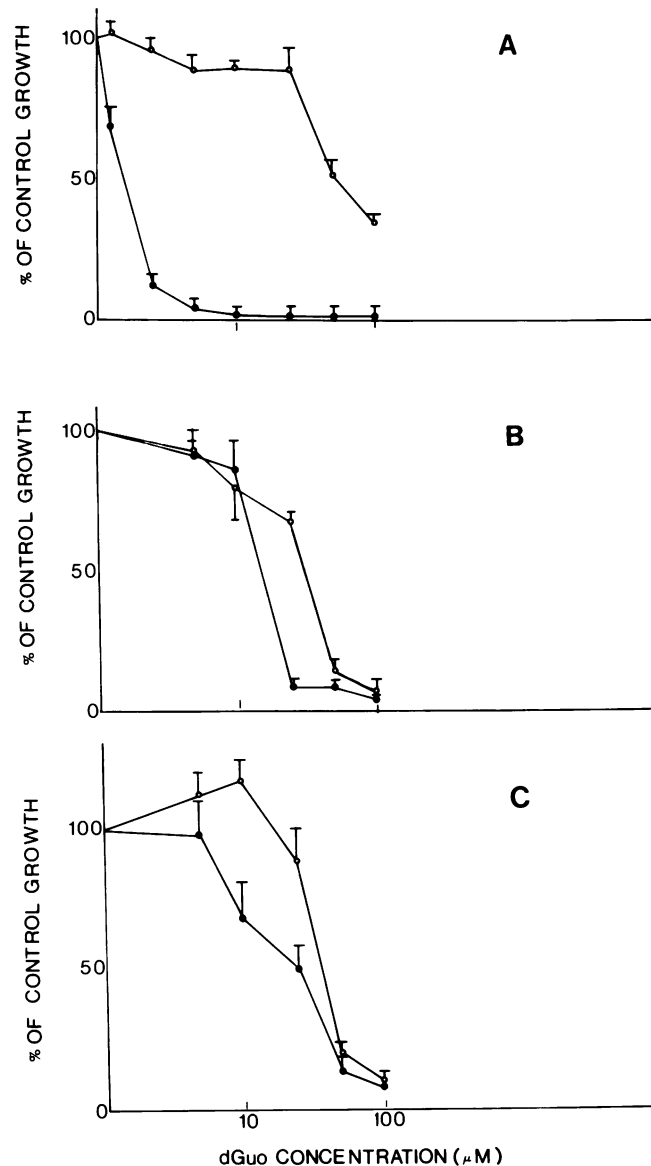
Figure 2 (right panel)

% OF CONTROL GROWTH (A)

100

50

0

A

% OF CONTROL GROWTH (B)

100

50

0

B

% OF CONTROL GROWTH (C)

100

50

0

C

10   100

dGuo CONCENTRATION (μM)

Figure 2. Effects of 2'-deoxycytidine on 2'-deoxyguanosine (dGuo) toxicity for T and B cell lines. (*A*) T lymphoblasts (Molt 4); (*B*) B lymphoblasts (GM 558); (*C*) mature T cell line (HUT 78). Cells were grown in the presence of 2'-deoxyguanosine at the concentrations shown plus 100 μM 8-aminoguanosine in the absence (— ● —) or presence (— ○ —) of 100 μM 2'-deoxycytidine. Values represent the mean±SD of three experiments performed in duplicate.

to both GTP accumulation and 2'-deoxyguanosine or guanosine cytotoxicity; (*b*) the addition of hypoxanthine or adenine rescues these cells and prevents GTP accumulation, presumably as a consequence of competing for and depleting PP-ribose-P pools; and (*c*) incubation of these lymphoid cells with either guanosine or 2'-deoxyguanosine and 8-aminoguanosine leads to a three- to fivefold increase in GTP levels at 24 h which is
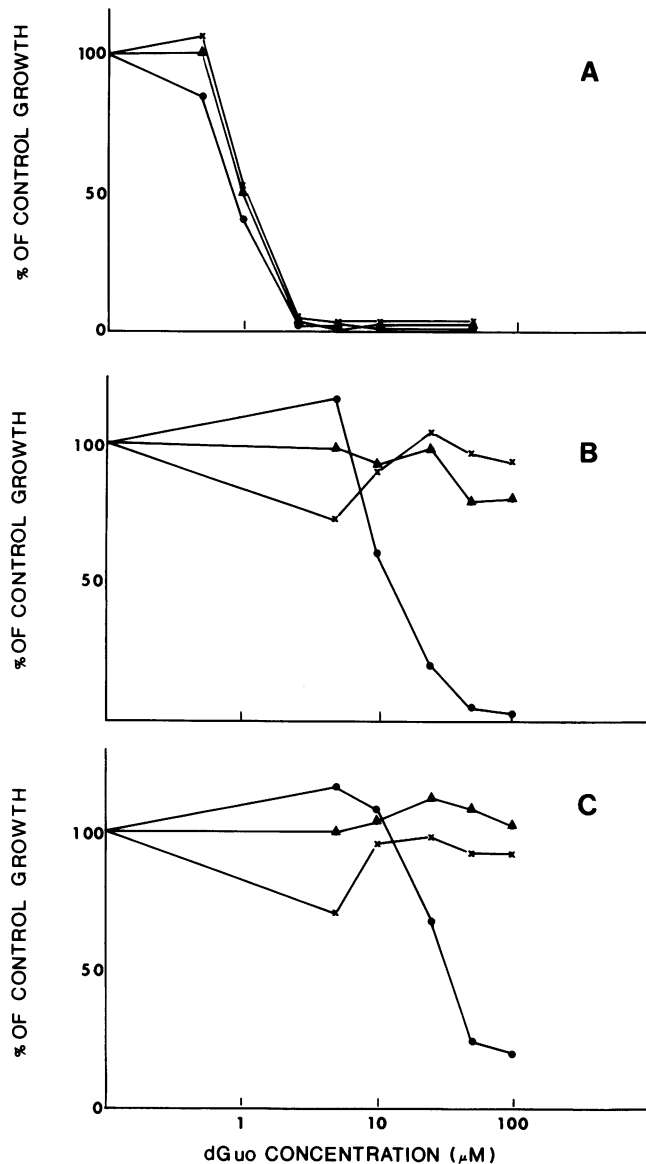
Figure 3. The effects of hypoxanthine or adenine on 2'-deoxyguano-sine (dGuo) toxicity for T and B cell lines. (*A*) T lymphoblasts (Molt 4); (*B*) B lymphoblasts (GM 558); (*C*) mature T cell line (HUT 78). Cells were grown in the presence of 2'-deoxyguanosine at the concentrations shown plus 100 μM 8-aminoguanosine in the absence of adenine or hypoxanthine (— ● —), in the presence of 100 μM hypoxanthine (— × —), or in the presence of 100 μM adenine (— ▲ —). Each point represents the mean of triplicate determinations from a single experiment.
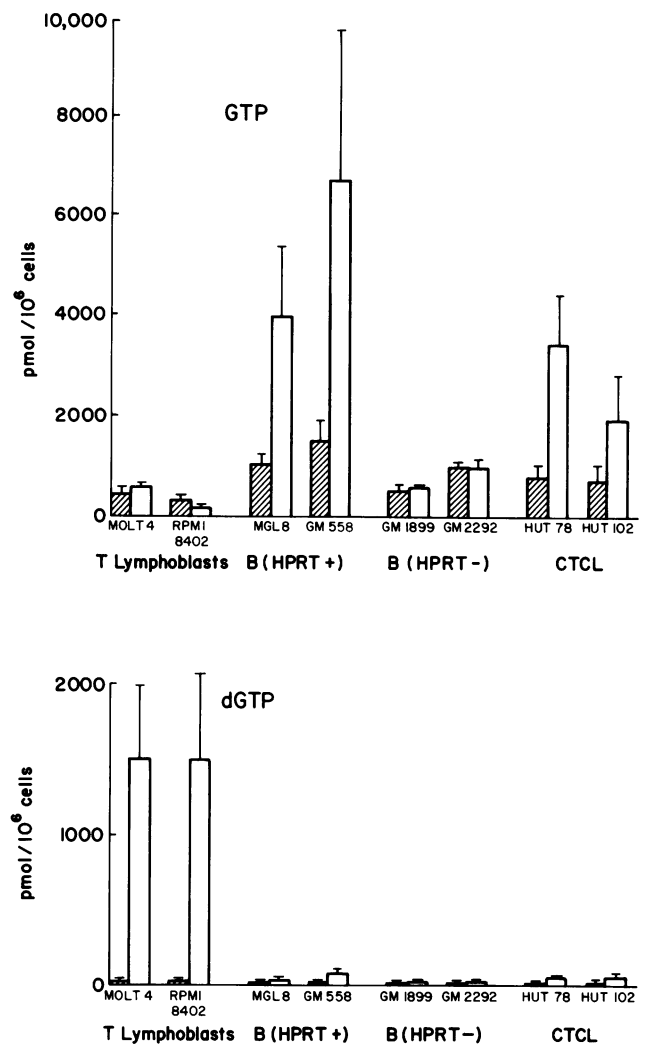
Figure 4. GTP and dGTP pools in T and B cell lines after incubations with 8-aminoguanosine with and without 2'-deoxyguanosine. Each bar represents the mean±SD of GTP or dGTP pools extracted from cells incubated for 24 h in the presence of 100 μM 8-amino-guanosine alone (hatched bars) or 50 μM 2'-deoxyguanosine plus 100 μM 8-aminoguanosine (open bars). Values were calculated from between three and nine separate experiments. B lymphoblast cell lines MGL 8 and GM 558 have normal HPRT activity (HPRT⁺), whereas lines GM 1899 and GM 2292 are deficient in this enzyme activity (HPRT⁻). CTCL represents cutaneous T cell lymphoma cell lines of mature phenotype.

directly associated with a marked reduction in overall cell growth.

The evidence that the toxicity of 2'-deoxyguanosine for T lymphoblasts is mediated by guanine deoxyribonucleotide rather than ribonucleotide accumulation is equally compelling: (*a*) GTP levels fail to rise in T lymphoblasts in the face of large increases in dGTP; (*b*) adenine and hypoxanthine have no salvaging effect; and (*c*) 2'-deoxycytidine rescues T lymphoblasts, but not B lymphoblasts or mature T cells, from 2'-deoxy-guanosine toxicity. The rescue effect of 2'-deoxycytidine is presumed to result from the repletion of deoxycytidine tri-phosphate pools which are reduced as a consequence of dGTP accumulation and ribonucleotide reductase inhibition. In ad-
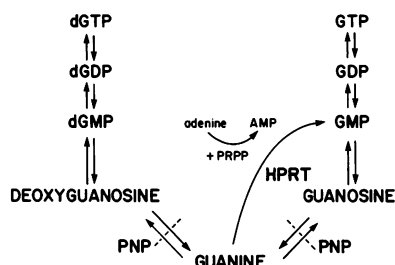
Figure 5. The metabolism of 2'-deoxyguanosine and guanosine.

dition, 2'-deoxycytidine reduces the extent of 2'-deoxyguanosine phosphorylation and, hence, of dGTP accumulation by competing for deoxycytidine kinase activity (2). Thus, it is clear that the toxicity of 2'-deoxyguanosine for B and mature T cell lines is mediated by a completely different mechanism than that which has been previously established for T lymphoblasts. Differences between the metabolism of 2'-deoxyguanosine and guanosine by B and T lymphoblasts have also recently been reported by Osborne and Scott (14).

The role of 8-aminoguanosine as a potentiator of both 2'-deoxyguanosine and guanosine toxicity remains to be fully
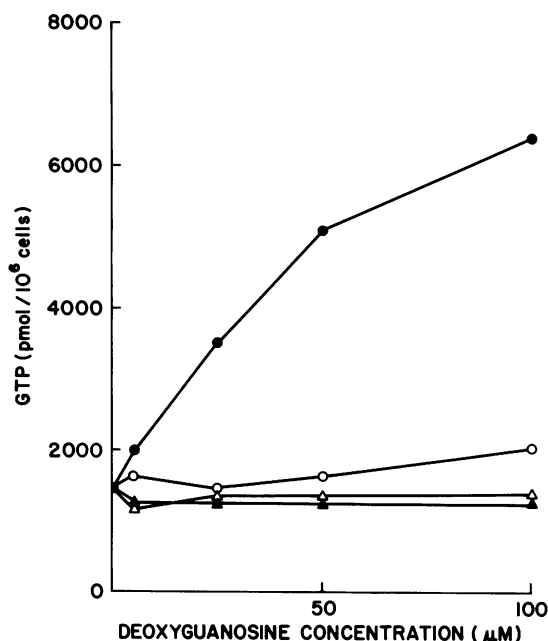

Figure 6. The effects of hypoxanthine and adenine on GTP accumulation in B lymphoblasts. GTP pools were measured in extracts of GM 558 cells after 24 h incubations with 50 μM 2'-deoxyguanosine (— o —), 50 μM 2'-deoxyguanosine plus 100 μM 8-amino-guanosine (— ● —), 50 μM 2'-deoxyguanosine plus 100 μM 8-aminoguanosine plus 100 μM hypoxanthine (— △ —), or 50 μM 2'-deoxyguanosine plus 100 μM 8-aminoguanosine plus 100 μM adenine (— ▲ —). Values represent the results of a single representative experiment. Similar results were obtained with the HUT78 and MGL8 cell lines.

Table II. Comparative Effects of 2'-Deoxyguanosine, Guanosine, and Guanine on GTP Pools in B Lymphoblasts

Concentration of additive (μM)	2'-Deoxyguanosine		Guanosine		Guanine	
	−8AGuo	+8AGuo	−8AGuo	+8AGuo	−8AGuo	+8AGuo
10	1,538	1,990	1,711	1,411	1,615	1,043
25	1,788	3,561	1,629	3,000	1,618	1,204
50	1,776	5,078	1,803	4,321	1,690	1,254
100	2,075	5,126	2,045	4,777	1,354	1,616

GTP pools were measured in GM 558 B lymphoblasts which had been incubated for 24 h with the indicated additives in the absence or presence of 100 μM 8-aminoguanosine (8AGuo). Cells were extracted in 60% methanol and nucleotide pools determined by high pressure liquid chromatography, as described in Methods. Values are representative of those obtained in two additional experiments. GTP levels in the absence of any additives were 1673±345 pmol/10⁶ cells and in the presence of 8-aminoguanosine alone were 1525±390 pmol/10⁶ cells.

elucidated. This compound has been reported to be a relatively weak competitive inhibitor of PNP-catalyzed phosphorolysis of inosine with an inhibition constant (K_i) of 8 μM (9, 15). It has been demonstrated that 8-aminoguanosine can be readily phosphorolyzed to 8-aminoguanine, also a competitive PNP inhibitor, by low amounts of PNP activity, which are equivalent to that present in horse serum, and that it prolongs the half-life of both guanosine and 2'-deoxyguanosine in tissue culture medium free of cells (15, 16). Our present experiments confirm that 8-aminoguanosine markedly prolongs the half-life of 2'-deoxyguanosine in tissue culture medium containing cells, again presumably by inhibiting PNP activity. The prolongation in 2'-deoxyguanosine half-life correlates temporally with a far more prolonged accumulation of intracellular GTP. Paradoxically, however, both guanosine and 2'-deoxyguanosine would have to be cleaved to guanine by PNP to explain the complete resistance of HPRT-deficient B lymphoblasts to GTP accumulation. We, therefore, must postulate that 8-aminoguanosine does not completely inhibit intracellular PNP activity, but permits 2'-deoxyguanosine and guanosine to be transported intact into the cell where they are phosphorolyzed to guanine.

It is clear that guanine itself is less toxic to these lymphoid cell lines than is guanosine or 2'-deoxyguanosine in the presence of 8-aminoguanosine and does not cause prolonged elevations of GTP at 24 h at concentrations up to 100 μM. Two possible explanations for this differential effect must be considered. First, guanine could be less readily transported into the cell than are the guanine nucleosides. This appears unlikely since all available data indicate that the transport of guanine, analagous to that of hypoxanthine in cultured mammalian cells, is extremely rapid with uptake limited only by the rate of phosphoribosylation within the cell (17). Secondly, guanine could be rapidly depleted from the extracellular medium, making it unavailable for the sustained synthesis of GTP. Although we were unable to measure guanine in the extracellular medium due to its loss during the deproteinization
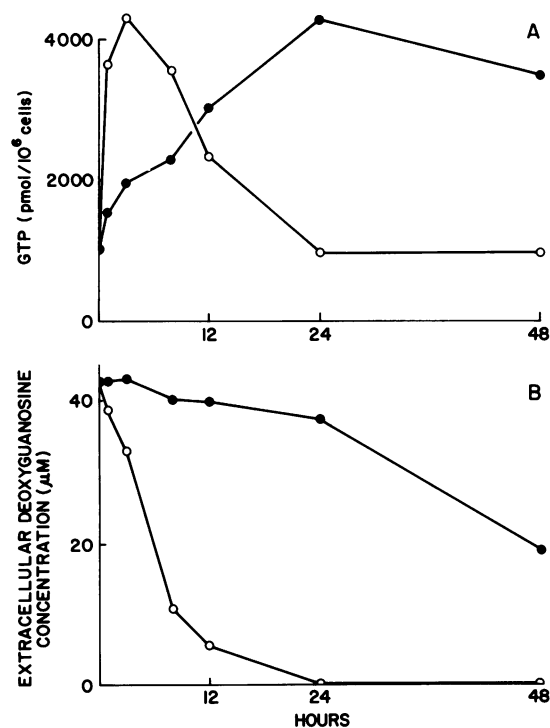
Figure 7. The effect of 8-aminoguanosine on extracellular 2'-deoxyguanosine concentrations and intracellular GTP pools in B lymphoblasts. Extracellular 2'-deoxyguanosine concentrations were measured in deproteinized supernates from cultures of MGL8 B lymphoblasts incubated in the presence of 50 μM 2'-deoxyguanosine (— o —) or 50 μM 2'-deoxyguanosine plus 100 μM 8-aminoguanosine (— • —) by high pressure liquid chromatography on a C-18 μBondapak reverse phase column, as described in Methods. Values are corrected for the 60% recovery of 2'-deoxyguanosine from the deproteinization procedure. GTP pools were measured in methanol extracts of cells sampled at the same time points. Values are those from a representative experiment; similar results were obtained in two additional experiments.

procedure, we favor this explanation. The addition of guanine to B lymphoblasts produced a very transient increase in GTP with a return to base-line levels by 5 h. 8-Aminoguanosine did not prolong the time course of guanine-induced GTP accumulation. Therefore, it seems likely that guanine is transported into the cell and rapidly depleted from the medium in the process of generating guanine nucleotides over a short time period. We conclude from these observations that the prerequisite for guanine ribonucleotide accumulation which is sufficient to inhibit cell growth is the prolonged availability of intracellular guanine, leading to sustained elevations of intracellular guanine ribonucleotides.

Several previous studies with mutant cell lines have also implied that guanine ribonucleotide accumulation may have a role in inhibiting cell growth. Novikoff hepatoma cells selected for resistance to high concentrations of guanosine were found to be strikingly deficient in HPRT activity, indi-
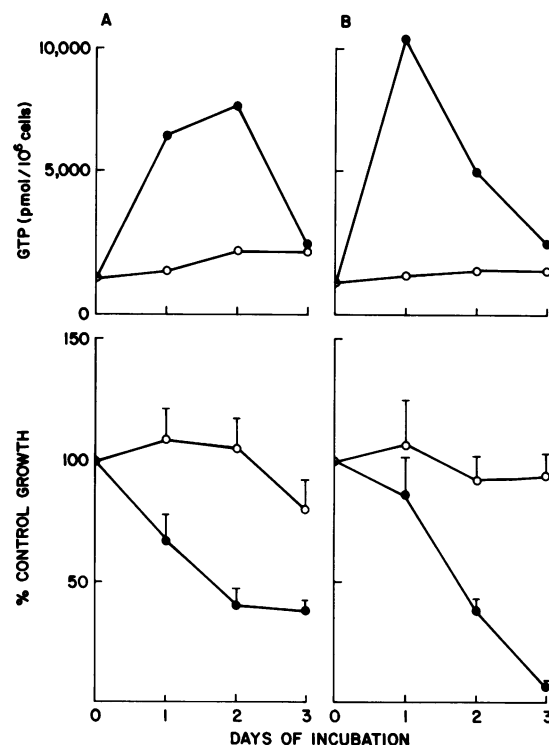

Figure 8. The effect of 8-aminoguanosine concentrations on GTP accumulation and cell growth. (A) B lymphoblasts (GM 558); (B) mature T cell line (HUT 78). Cells were grown in the presence of 50 μM 2'-deoxyguanosine and 100 μM (— • —) or 400 μM (— o —) 8-aminoguanosine. GTP values represent the mean of two experiments and the cell growth data the mean±SD of five determinations.

cating that nucleotide synthesis was important in mediating the sensitivity of wild type cells to guanosine (18). Guanine and guanosine in 100 μM concentrations have also been reported to inhibit the growth of human lymphoblasts in association with GTP accumulation, while HPRT-deficient cells were unaffected (19). In addition, Chinese hamster ovary cells and mouse T lymphoma cells deficient in the enzyme adenylosuccinate synthetase, an enzyme which is rate limiting for the conversion of inosine monophosphate into the adenylate nucleotide pool, have been shown to be highly sensitive to both guanine and guanosine (20, 21). Although the effects of exogenous nucleosides on nucleotide pools were not measured, the mutant T lymphoma line contained three times more GTP and GMP than did the wild type cells. It was implied that the block in adenine nucleotide synthesis facilitated the accumulation of guanine nucleotides within these cells and that growth inhibition resulted from the imbalance between the two pools. Finally, mouse T lymphoma cells deficient in both HPRT and deoxycytidine kinase are more resistant to 2'-deoxyguanosine toxicity than are mutants deficient in deoxycytidine kinase alone (22), while 2'-deoxyguanosine toxicity for these cells is more completely reversed by the addition of

both 2'-deoxycytidine and hypoxanthine than by 2'-deoxycytidine alone (23). Again, these data support the importance of guanine salvage as one component of the cytotoxic effect of 2'-deoxyguanosine.

Although we have measured only the nucleoside triphosphate pools in these experiments, it is clear that elevation of GTP levels may be associated with concomitant increases in GMP and guanosine diphosphate (GDP). The mechanism whereby one or more of these guanine ribonucleotides inhibit growth remains a subject for some speculation. One major possibility is that they inhibit *de novo* purine biosynthesis, with consequent depletion of the adenine nucleotide pools. The exact mechanism by which guanine nucleotides inhibit purine biosynthesis in intact cells in unclear. Ullman et al. observed an increase in PP-ribose-P pools and a threefold reduction in purine synthetic rates in their adenylosuccinate synthetase-deficient cells and concluded that guanine nucleotides inhibit glutamine PP-ribose-P-amidotransferase, which catalyzes the first reaction specifically concerned with purine biosynthesis (20). This enzyme is known to be allosterically regulated by a number of purine ribonucleotides including GMP and, to a lesser extent, GDP and GTP (24, 25). However, others have been unable to demonstrate inhibition of this enzyme under conditions yielding a threefold increase in GTP levels by guanine and inhibition of purine biosynthesis (26). Depletion of PP-ribose-P by guanine appears an unlikely mechanism, since the addition of hypoxanthine or adenine, which effectively reduced PP-ribose-P levels to the point of preventing guanine ribonucleotide synthesis, salvaged the cells rather than producing any growth inhibitory effects. Finally, pyrimidine starvation can be excluded since the addition of uridine did not rescue the cells.

Although the mechanism remains somewhat uncertain, the observation that a competitive inhibitor of PNP can potentiate the toxicity of 2'-deoxyguanosine and guanosine for lymphoid cells other than T lymphoblasts has important implications for the use of such agents as immunosuppressive drugs. Inherited PNP deficiency is known to result in the overproduction of all four PNP substrates—inosine, 2'-deoxyinosine, guanosine, and 2'-deoxyguanosine (27). It is clear that the use of enzyme inhibitors in vivo can lead to increases in substrate concentrations to levels which far surpass those found in the congenital deficiency state (28). Thus, the pharmacologic use of PNP inhibitors which are not tight-binding inhibitors and/or which result in only partial inhibition of intracellular PNP activity might well be associated with significant and prolonged elevation of both guanosine and 2'-deoxyguanosine concentrations in the plasma and with guanine ribonucleotide accumulation which is not limited to the target T cell population. In addition, the observation that T lymphoblasts remain about 10-fold more sensitive to 2'-deoxyguanosine plus 8-aminoguanosine than do mature T cell lines raises the question as to whether ablation of differentiated T cells can be achieved in the absence of more generalized guanine ribonucleotide accumulation and nonspecific cellular toxicity.

These alternate metabolic effects do not, however, appear to play a role in inherited PNP-deficiency disease. In fact, erythrocytes from a PNP-deficient child have been found to have very low levels of GTP, while peripheral blood lymphocyte GTP levels are apparently within the normal range (29). It remains possible that a selective depletion of immature T cells can be achieved by more potent PNP inhibitors than are currently available.

Note added in proof. Subsequent to the submission of this work, Spaapen et al. have demonstrated that guanine ribonucleotide accumulation contributes to inhibition of mitogenic response in peripheral blood lymphocytes cultured with guanosine or 2'-deoxyguanosine plus 8-aminoguanosine (30).

Acknowledgments

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