Proc. Natl. Acad. Sci. USA
Vol. 76, No. 3, pp. 1074–1078, March 1979
Biochemistry

Isolation and characterization of purine-nucleoside phosphorylase-deficient T-lymphoma cells and secondary mutants with altered ribonucleotide reductase: Genetic model for immunodeficiency disease

(ribonucleoside-diphosphate reductase/dGTP/feedback resistance/deoxycytidine kinase/deoxyguanosine)

BUDDY ULLMAN, LORRAINE J. GUDAS, SHIRLEY M. CLIFT, AND DAVID W. MARTIN, JR.*

Howard Hughes Medical Institute Laboratory and Division of Medical Genetics, Department of Medicine and Department of Biochemistry and Biophysics, University of California, San Francisco, California 94143

Communicated by Arthur B. Pardee, December 6, 1978

ABSTRACT **The inherited deficiency of purine-nucleoside phosphorylase (PNPase; purine-nucleoside:orthophosphate ribosyltransferase, EC 2.4.2.1) in humans is associated with a severe deficiency of the T lymphocytes of the immune system. Because of the unsatisfactory nature of previously described model systems, we have selected, cloned, and characterized a mutant mouse T cell lymphoma (S49) completely deficient in PNPase. Of the four substrates of PNPase, only deoxyguanosine at low concentrations is toxic to the PNPase-deficient (NSU-1) cells.**

In order to delineate the biochemical processes necessary for the sensitivity of the NSU-1 cells to deoxyguanosine, we have isolated a series of secondary mutants resistant to deoxyguanosine from the PNPase-deficient line. One of these mutants is defective in its ability to transport deoxyguanosine into the cell. A second type of mutant cannot phosphorylate the deoxyguanosine and is totally deficient in deoxycytidine kinase activity. A third type of mutant (NSU-1-dGuo-L) can both transport and phosphorylate deoxyguanosine and accumulates dGTP. However, unlike its parent, NSU-1-dGuo-L does not become depleted of dCTP and TTP when exposed to exogenous deoxyguanosine. This observation is accounted for by the fact that the reduction of CDP to dCDP by the ribonucleotide reductase (ribonucleoside-diphosphate reductase, 2′-deoxyribonucleoside-diphosphate:oxidized-thioredoxin 2′-oxidoreductase, EC 1.17.4.1) of NSU-1-dGuo-L cells is not normally sensitive to feedback inhibition by dGTP.

Thus, in order to exert its toxicity deoxyguanosine must be transported into the cell, be phosphorylated by deoxycytidine kinase, and be accumulated as dGTP. By inhibiting ribonucleotide reductase, dGTP depletes the cell of dCTP and to some extent TTP, thus preventing the synthesis of DNA, a process necessary for any proliferation-dependent function of T cells.

The recent advances in immunology, particularly the conceptualization of the T and B cell limbs of the immune system, have aided greatly in the understanding of normal immune function as well as the classification of human immune diseases. More recently, some immunodeficiency diseases have been recognized as being inborn errors of metabolism, and these observations have introduced the discipline of biochemical genetics to immunology (1–5). The use of biochemical genetics has allowed the elucidation of several metabolic processes that seem to be required for normal immune function (6–10).

Recently, the techniques of biochemical genetics have progressed to the stage in which *in vitro* cell culture models, especially those cell models that take advantage of somatic cell genetics, have aided in an understanding of inborn errors of metabolism (11–15). By using a cell culture model, we have applied the techniques of somatic cell genetics to a biochemical genetics problem in immunology and have characterized the mechanism by which a known specific inherited defect in purine metabolism severely impairs immune function.

The absence of either adenosine deaminase (ADase;adenine aminohydrolase, EC 3.5.4.2) or purine nucleoside phosphorylase (PNPase;purine-nucleoside:orthophosphate ribosyltransferase, EC 2.4.2.1) in humans is associated with a severe impairment of the immune system. Patients lacking ADase have severe B and T cell dysfunction (1). ADase converts Ado and 2′-dAdo to Ino and 2′-dIno, respectively. In patients who lack ADase it appears that the toxic metabolite is 2′-dATP, the ultimate phosphorylated product of 2′-dAdo (16, 17). A number of investigations using cell culture systems have suggested that dAdo can be toxic by virtue of dATP-mediated inhibition of ribonucleotide reductase (ribonucleoside-diphosphate reductase, 2′-deoxyribonucleoside-diphosphate:oxidized-thioredoxin 2′-oxidoreductase, EC 1.17.4.1) (9, 18–20).

Patients lacking PNPase have specific T cell abnormalities with apparently normal B cells (3). PNPase converts Guo, 2′-dGuo, Ino, and 2′-dIno to the appropriate purine bases and either ribose- or 2′-deoxyribose-1-phosphate. All four of these nucleoside substrates of PNPase accumulate in millimolar concentrations in the urine of patients who lack this enzyme (21). In addition, dGTP accumulates in the erythrocytes of patients who lack PNPase but not in the erythrocytes of normal controls or immunodeficient patients possessing normal PNPase activity (22).

In order to provide a valid cell culture model for a biochemical genetic study of PNPase deficiency, we have selected and characterized a PNPase-deficient cell line from a mouse T cell lymphoma (S49) in continuous culture. By introducing additional specific mutations into cells of this PNPase-deficient line, we have analyzed biochemically and genetically the metabolic mechanisms responsible for the loss of T cell proliferative capacity and function in this inborn error of metabolism.

MATERIALS AND METHODS

Chemicals and Reagents. [8-^{14}C]Adenosine (55 mCi/mmol) (1 Ci = 3.7 × 10^{10} becquerels), [2-^{14}C]cytidine (40 mCi/mmol), [^{14}C(U)]CDP (468 mCi/mmol), [5-^{3}H]deoxycytidine (30 Ci/mmol), [8-^{14}C]deoxyguanosine (56 mCi/mmol), [8-^{3}H]deoxyguanosine (1.9 Ci/mmol), [*U*-^{14}C]glycine (99.6 mCi/mmol),

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U. S. C. §1734 solely to indicate this fact.

Abbreviations: PNPase, purine-nucleoside phosphorylase; ADase, adenosine deaminase; EHNA, *erythro*-9-[3-(2-hydroxynonyl)]adenine; MNNG, *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine; EC_{50}, concentration at which 50% of the desired effect is achieved.

* To whom reprint requests should be addressed at: Department of Medicine, Division of Medical Genetics, University of California, San Francisco, CA 94143.

[8-^{3}H]guanosine (7.6 Ci/mmol), [8-^{14}C]hypoxanthine (42.4 mCi/mmol), and [2-^{14}C]uridine (53 mCi/mmol) were all obtained from Amersham, New England Nuclear, or Schwarz/Mann. *Erythro*-9-[3-(2-hydroxynonyl)]adenine (EHNA) was obtained from Burroughs-Wellcome (Research Triangle Park, NC). All other materials, chemicals, and reagents were of the highest qualities commercially available (6, 15).

Cell Culture. The immunological properties and growth characteristics of the wild-type S49 and mutant mouse T cell lymphoma cell lines have been described in detail (6, 23–25). Cells were grown in Dulbecco's modified Eagle's medium containing 10% heat-inactivated horse serum with undetectable levels (<0.5 nmol of product hr^{-1} ml^{-1} of serum at 200 μM Ino) of PNPase activity. Cell growth experiments were performed as described (9, 10).

Mutant Selections. Techniques for selection of S49 cell variants using semisolid agarose overlying mouse embryo fibroblast feeder layers have been described (24, 25). To isolate a PNPase-deficient S49 cell line, we carried out mutagenesis of approximately 10^8 wild-type cells with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG) at 2 μg/ml for 3 hr, after which the cells were centrifuged and resuspended in Dulbecco's modified Eagle's medium containing 10% horse serum. To allow for phenotypic expression, we grew cells for 7 days in complete medium containing 0.5 mM Hyp, 0.4 μM amethopterin, and 16 μM dThd. After the preliminary selection, cells were selectively cloned in the presence of 7 μM 6-thioguanosine and 100 μM dCyd.

To isolate the secondary mutants from the PNPase-deficient line, we carried out mutagenesis of (≈10^8) NSU-1 cells with MNNG as above. The cells were grown for 7 days nonselectively and then selectively cloned in either 60 nM 5-fluorouridine (FUrd) or 200–500 μM dGuo. The transport-defective NSU-1-FUrd-60-5 cells were isolated from the selection in FUrd, an agent known to select for transport-defective mutants in S49 cells (unpublished observations).

Enzyme and Uptake Assays. The preparations of cell-free extracts for enzyme assays were performed as described (9). PNPase was measured by the method of Kalckar (26) as modified by Zannis *et al.* (27), and deoxycytidine kinase was assayed by the method of Ullman *et al.* (9). Ribonucleotide reductase activity was measured as described by Lewis *et al.* (28). The radioactive dCyd formed was measured as described by Steeper and Steuart on borate Dowex-1 columns (29). Under these conditions, recovery of dCyd was complete, whereas no radioactivity was recovered after addition of 0.2 μCi of [^{14}C]Cyd to a simulated assay mixture. The identity of the final deoxynucleoside product of the ribonucleotide reductase assay, 2'-dCyd, was confirmed by high performance liquid chromatography.

The rates of ribonucleoside and deoxyribonucleoside uptake in all cell lines were determined by a modification of the method of Cohen *et al.* (30). The uptake of all nucleosides was linearly proportional to time and cell number in the ranges examined.

Measurements of Intracellular Levels of Ribo- and Deoxyribonucleosides and Their Phosphorylated Derivatives. After the appropriate incubations, cells were harvested by centrifugation at ambient temperature, washed once with 1.5 ml of chilled phosphate-buffered saline, and extracted with 0.5 M $HClO_4$/0.1 M potassium phosphate, followed by neutralization with KOH as described (8). For the determinations of ribo- and deoxyribonucleoside levels, aliquots of the neutral extracts were chromatographed on a Waters C_{18} μBondapak analytical column in 2 mM potassium acetate, pH 7.5, containing 0.5% methanol, at a flow rate of 2 ml/min. The elutions were monitored at 254 and 280 nm with an Altex high performance system as described (21).

The ribonucleoside triphosphate concentrations in the extracts were determined by high performance liquid chromatography as described (9). The triphosphates of the deoxyribonucleosides were measured by first destroying the ribonucleoside phosphates with periodate† and then separating them by high performance liquid chromatography using a SAX column with 0.45 M ammonium phosphate plus 1.5% acetonitrile (pH 3.45) at 2.0 ml/min as the mobile phase.

RESULTS

Isolation of a PNPase-Deficient Mutant. Of 15 colonies isolated from the clonings in 7 μM 6-thioguanosine, 13 were partially or totally deficient in hypoxanthine phosphoribosyltransferase, 1 survived the selective conditions by virtue of its inability to transport the toxic purine nucleoside analog, and 1 clone, NSU-1, was deficient in PNPase. The levels of PNPase activity in extracts of NSU-1 cells were undetectable—i.e., less than 0.1% of wild-type enzyme levels (10 nmol of product min^{-1} mg^{-1} of protein at 200 μM Ino). Mixing experiments indicated the absence of any diffusable inhibitor of PNPase in NSU-1 cell extracts. In suspension culture the NSU-1 cells were resistant to 10 μM 6-thioguanosine, were normally sensitive to killing by 6-thioguanine, and could salvage as a sole source of purines 0.5 mM Hyp but not equimolar amounts of Ino.

Growth Phenotype and Biochemical Phenotype of NSU-1 Cells. We have compared intracellular nucleoside concentrations of NSU-1 cells with those of wild-type cells. All four substrates of PNPase were present in higher concentrations in the enzyme-deficient cells (Table 1). However, the growth rates of wild-type and NSU-1 cells in the absence of exogenous nucleosides were comparable, indicating that the PNPase deficiency *per se* is not intrinsically lethal to this T cell model.

In order to determine which of the four PNPase substrates were toxic to NSU-1 cells, we measured the rates of NSU-1 cell growth in the presence of various concentrations of each PNPase substrate. It is clear from Fig. 1 that, to NSU-1 cells, dGuo was the most cytotoxic of the four nucleosides, as has been shown for wild-type cells (10). dGuo inhibited NSU-1 cell growth by 50% (EC_{50}) at 19 μM; at much higher concentrations dIno (EC_{50} = 280 μM) was also cytotoxic. Neither Guo nor Ino at concentrations below 1 mM exhibited much growth inhibitory effect towards NSU-1 cells.

Even though NSU-1 cells are more resistant to Guo than are wild-type cells, the EC_{50} value for dGuo is lower in NSU-1 cells than in wild-type cells (10). The enhanced sensitivity to dGuo indicates that nucleoside and deoxynucleoside transport is intact in the PNPase-deficient mutant. However, due to the impaired

† C. Garrett and D. Santi, unpublished results.

Table 1. Intracellular nucleoside concentrations in wild-type and PNPase-deficient (NSU-1) cells

	nmol per 10^9 cells			
	Ino	dIno	Guo	dGuo
Wild type	2.2	<1	8.5	<1
NSU-1	16.0	3.1	10.8	2.2
Wild type plus dGuo	3.1	<1	9.7	6.8
NSU-1 plus dGuo	18.4	3.0	11.8	53.3
NSU-1 plus dIno	16.0	191.0	10.3	2.7

Wild-type (WT) and NSU-1 cells were grown in the absence or presence of 100 μM dGuo or 500 μM dIno for 5 hr at 37°C. The intracellular nucleosides were extracted with perchloric acid, and the extracts were neutralized. The levels were determined by high performance liquid chromatography and quantitated by comparing peak heights with those of known standards. The values are means of four determinations which differed by less than 15%.

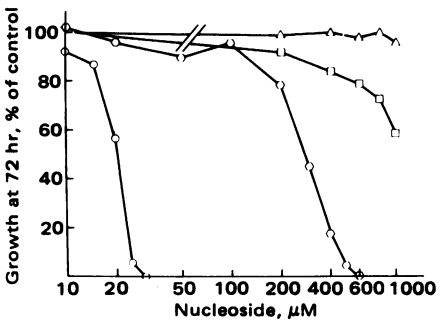

FIG. 1. The sensitivity of PNPase-deficient (NSU-1) cells to growth inhibition by increasing concentrations of the four substrates of PNPase. The percentage of control growth at 72 hr was determined as described (8, 9); growth during that time was logarithmic and cell density increased 16- to 25-fold. The substrates of PNPase, 2′-dGuo (○), 2′-dIno (○), Guo (□), and Ino (△), were added at zero time at the indicated concentrations.

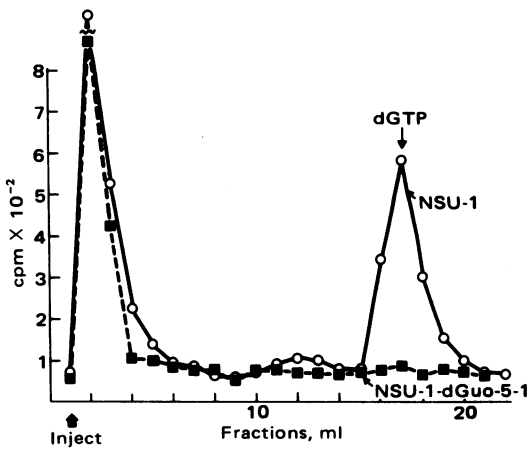

FIG. 2. The abilities of NSU-1 cells and NSU-1-dGuo-5-1 cells to phosphorylate exogenous dGuo to dGTP. Logarithmically growing NSU-1 cells (○) and NSU-1-dGuo-5-1 cells (■) were incubated for 7 hr in the presence of 100 μM [³H]dGuo (10 mCi/mmol), and the intracellular nucleotides were then extracted with perchloric acid. After periodation the extracts were subjected to high performance liquid chromatography on an anion exchange column along with a known standard of dGTP. The eluates were fractionated and assayed for radioactivity in a liquid scintillation system. The dGTP standard ran at the indicated position in the elution.

metabolism of the four substrates of PNPase in NSU-1 cells, the uptake of these substrates is diminished. The transport and subsequent metabolism (as measured by uptake) of other nucleosides—e.g., Ado—in NSU-1 cells is equivalent to that in wild-type cells.

The cytotoxicity of dGuo in wild-type cells has been shown to be accompanied by the intracellular accumulation of dGTP and depletion of dCTP (10). Experiments with NSU-1 cells demonstrated that, when exposed to exogenous dGuo, they accumulate dGTP, as do wild-type cells under similar conditions. No accumulation of dITP or dGTP was observed after similar incubation of NSU-1 cells with 0.5 mM dIno.

The Generation of Secondary Mutations in NSU-1 Cells. Having isolated PNPase-deficient S49 cells which accumulate the PNPase substrates intracellularly and having demonstrated the enhanced and specific sensitivity of the mutant cells to exogenous dGuo, we introduced secondary somatic mutations into the NSU-1 cells in order to delineate the biochemical mechanisms by which the inherited loss of PNPase is lethal to this model of T cells of the immune system.

We have shown in earlier studies with S49 cells that a frequently occurring mutation confers resistance to cytotoxic purine or pyrimidine nucleosides as a consequence of a defective cellular transport of these compounds (30). Accordingly, we isolated from the PNPase-deficient cells a line of cells that also lacks this transport function. This cell line cannot transport exogenous Urd or Ado, and its growth is resistant to FUrd and Ado (in the presence of the ADase inhibitor, EHNA). The cells of this double mutant lacking PNPase and the transport function have an EC_{50} for dGuo which is 4-fold greater than that of the NSU-1 parent cells. Thus, as expected (30), the toxicity of dGuo in PNPase-deficient cells requires the transport of this deoxynucleoside into the cells.

To determine which other cellular functions were necessary for the PNPase-deficient cells to be sensitive to the cytotoxicity of dGuo, we selected clones from remutated NSU-1 cells for their resistance to exogenous dGuo. Cells of one of these clones, NSU-1-dGuo-5-1, were more extensively characterized. These mutant cells possess an EC_{50} for dGuo of 280 μM, 15-fold higher than that of the NSU-1 parent cells. The intact NSU-1-dGuo-5-1 cells were not capable of phosphorylating dGuo to dGTP (Fig. 2). These mutant cells also contained less than 1% of the wild-type level (7 pmol min^{-1} mg^{-1}) of deoxycytidine kinase activity, an activity which we have demonstrated to be responsible for the phosphorylation of both dCyd and dGuo in S49 cells (10). Thus, the accumulation of dGTP is required for the toxicity of dGuo.

As mentioned above and described in detail elsewhere (10), the accumulation of dGTP in wild-type cells exposed to dGuo is accompanied by depletion in intracellular dCTP. NSU-1 cells exposed to dGuo also accumulated dGTP and were depleted of dCTP and, to some extent, TTP (Fig. 3). Furthermore, the toxicity of dGuo to both wild-type (10) and NSU-1 cells could be reversed by the addition of dCyd to the culture medium. This latter observation is consistent with the depletion of dCTP

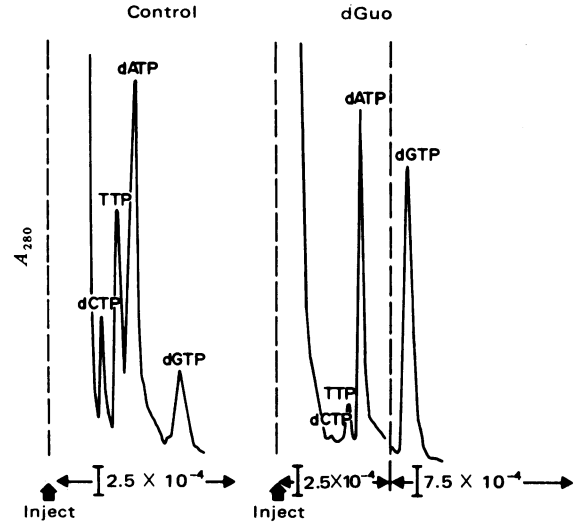

FIG. 3. The effect of exogenous dGuo on the concentrations of intracellular deoxynucleoside triphosphates in PNPase-deficient (NSU-1) cells. Logarithmically growing NSU-1 cells were incubated with or without 200 μM dGuo in the culture medium for 6 hr and extracted with perchloric acid, and their intracellular deoxynucleoside triphosphate concentrations were determined by high performance liquid chromatography after periodation of the neutralized extract. The point of injection of extract into the chromatography system is indicated, and the scales of the absorbance at 254 are indicated by the bar under the tracings. In the case of the extract from the NSU-1 cells incubated with dGuo, the scale has been reduced 1:3 after the elution of dATP in order to keep the peak of absorbance of the dGTP on the chart. Extracts from equal numbers of cells (5×10^6) were injected for the two determinations. The tracings are representative of at least two experiments, the values of which differed by less than 10%.

being the toxic effect caused by exogenous dGuo. However, dCyd could circumvent the presumed starvation of wild-type cells for dCTP as well as serve as a preferred substrate over dGuo for phosphorylation by the same deoxynucleoside kinase. Because of the latter property, the presence of dCyd prevents the accumulation of dGTP in cells exposed simultaneously to dGuo (10). Thus, these data do not prove that the depletion of dCTP is responsible for the toxicity of dGuo in the PNPase-deficient cells; dGTP might exert its toxic effect through some other mechanism, while the depletion of dCTP is merely coincidental.

The biochemical characterization of cells of a second dGuo-resistant, PNPase-deficient clone provides additional insight to the mechanism of dGuo toxicity. NSU-1-dGuo-L cells, which were isolated by virtue of their resistance to 200 μM dGuo, were resistant to dGuo (EC_{50} = 130 μM) but could phosphorylate exogenous dGuo and accumulate it intracellularly as dGTP (Fig. 4 *left*). Interestingly, NSU-1-dGuo-L cells had abnormally elevated intracellular concentrations of dCTP (Fig. 4 *center*) and dGTP, as well as TTP (data not shown). Furthermore, after exposure to exogenous dGuo, they did not become depleted of dCTP or TTP upon accumulation of dGTP at concentrations that severely depleted NSU-1 (and wild-type) cells of their dCTP (Fig. 4 *right*) and TTP (data not shown).

The mechanism proposed for the depletion of dCTP by exogenous dGuo involves the inhibition of ribonucleotide reductase activity by accumulated dGTP (10, 20, 31). Therefore, we compared the ribonucleotide reductase-specific catalytic activities of NSU-1-dGuo-L and NSU-1 cells. The specific catalytic activities for the reduction of CDP are within 30% of each other (0.26 nmol of dCDP formed hr^{-1} per 10^7 NSU-1 cells and 0.37 nmol of dCDP formed hr^{-1} per 10^7 NSU-1-dGuo-L cells), and the K_m values for CDP are 50 and 33 μM for NSU-1 and NSU-1-dGuo-L, respectively. Accordingly, we examined the sensitivity of the CDP reduction activity to dGTP in each of the two mutants. As shown in Fig. 5, whereas dGTP almost completely inhibited the reduction of CDP in both NSU-1 and wild-type cells, the CDP reductase activity in NSU-1-dGuo-L

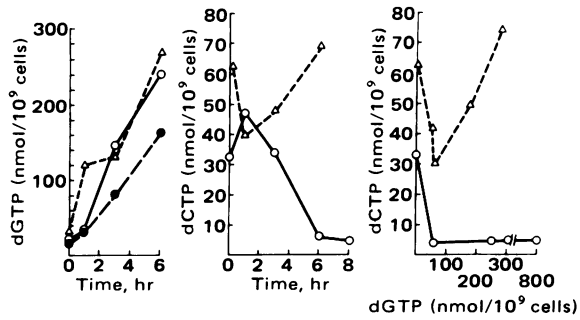

FIG. 4. dGTP and dCTP levels in wild-type, NSU-1, and NSU-1-dGuo-L cells. (*Left* and *Center*) Wild-type (●) and NSU-1 (○) cells at a density of 7×10^5 cells per ml were grown in the presence of 100 μM dGuo; NSU-1-dGuo-L cells (△) were grown in the presence of 200 μM dGuo. At various times, 50-ml volumes were removed and extracted with perchloric acid, and the neutralized extracts were periodated and subjected to high performance liquid chromatography. dGTP and dCTP were measured by comparing peak heights to known quantities of standards. dGTP and dCTP levels were also measured by the DNA polymerase assay (10) with very similar results (data not shown). (*Right*) NSU-1 (○) and NSU-1-dGuo-L (△) cells were grown in the presence of varying concentrations of exogenous dGuo (20 μM, 50 μM, 100 μM, and 200 μM) for 6 hr. At various times, 50-ml volumes were treated as described in *Left* and *Center*, and samples were analyzed for dGTP and dCTP levels by high performance liquid chromatography. The levels of dGTP and dCTP in cells not exposed (zero time) to exogenous dGuo are averages of 12 separate experiments, in which the values differed by less than 15% from the mean. The other values are those from a single representative experiment.

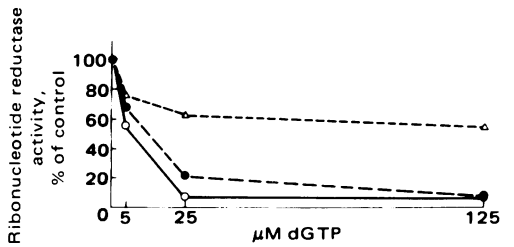

FIG. 5. The sensitivities of ribonucleotide reductase activity (CDP reduction) from wild-type, NSU-1, and NSU-1-dGuo-L cells to feedback inhibition by dGTP. The sensitivities of CDP reduction to dCDP were determined in permeable wild-type (●), NSU-1 (○), and NSU-1-dGuo-L (△) cells at increasing concentrations of dGTP at 50 μM CDP. Each point on the graph represents a rate of accumulation of dCyd nucleotides at 37°C determined from three different time points. The ordinate is normalized to the control value in the absence of dGTP, because there was as much as 30% variation in the activities of CDP reduction per 10^7 cells among the three cell types examined. The experiment is a representative one which has been repeated on three occasions with essentially identical results.

cells was maximally inhibited by only about 50%. This feedback resistance of the ribonucleotide reductase to dGTP in NSU-1-dGuo-L cells is clearly sufficient to confer resistance to exogenously added dGuo, even in the absence of PNPase activity.

DISCUSSION

Among the heterogeneous group of inherited human immune disorders are two diseases that are associated with the inherited absence of two purine salvage enzymes. Defects in either one of these two enzymes, ADase or PNPase, result in severe impairment of the immune system. Although these enzymes act metabolically in a sequential fashion, they are genetically unlinked (32, 33), stressing that an inherited defect in either the ADase or the PNPase locus is responsible for both the enzymological and immune defects. Studies with cultured S49 cells have implied that ADase and PNPase deficiencies have a common basis of pathogenesis—i.e., inhibition of ribonucleotide reductase with subsequent depletion of intracellular deoxycytidylate nucleotides to levels inadequate for proper DNA synthesis (9, 10). The organ specificity of these diseases seems to be related to tissue-specific distribution of deoxynucleoside kinase activities (34–36).

Cell culture models for studying ADase deficiencies have been greatly facilitated by the existence of potent specific inhibitors of ADase (37, 38), which permits pharmacologic simulation of the enzyme defect. There are no known potent specific inhibitors of purine nucleoside phosphorylase; therefore, T cell culture models have involved either wild-type or hypoxanthine phosphoribosyltransferase-deficient cells that contain normal PNPase activity (10, 36, 39), and studies in PNPase-deficient cells from patients have utilized cell types unaffected by the PNPase deficiency (21, 22, 40).

In order to study PNPase deficiency, we have isolated a PNPase-deficient cell line (NSU-1) which has provided a T cell genetic model for the study of PNPase associated immunological disease. Because the growth rate of NSU-1 cells in the absence of exogenous dGuo is the same as that of wild-type cells, it is apparent that the genetic loss of PNPase is not intrinsically lethal to this T cell model. The NSU-1 cells do accumulate all four substrates of the missing PNPase, but these endogenously generated concentrations must be supplemented with exogenously provided dGuo for the enzyme defect to inhibit cell proliferation. Thus, it is likely that PNPase-deficient immune defects *in vivo* are caused by circulating dGuo, most of which is probably generated by purine-secreting organs such as the liver (41).

Of the four naturally occurring PNPase substrates, only dGuo at low concentrations is toxic to PNPase-deficient S49 cells. With the aid of secondary somatic cell mutations in the PNPase-deficient cells, we have elucidated the mechanism by which dGuo is metabolized to a toxic compound, the nature of the toxic compound, and its target. The cell must transport dGuo into the cytoplasm, phosphorylate it with deoxycytidine kinase, and accumulate it as dGTP. dGTP inhibits the synthesis of dCyd nucleotides by the enzyme ribonucleotide reductase; mutant cells in which the ribonucleotide reductase activity is insensitive to dGTP are resistant to the toxic effects of dGuo. Thus, the cellular ribonucleotide reductase is the target of the toxic metabolite of dGuo, dGTP.

The response of the CDP reductase activity in NSU-1-dGuo-L cells to dGTP suggests that the reductase activity has two components. About half of the CDP reductase activity appears to be normally sensitive to feedback inhibition by dGTP, but the other half appears to be refractory to inhibition by dGTP even at concentrations in which the normal activity is inhibited by greater than 90%. One would anticipate that a feedback-resistant ribonucleotide reductase would be dominantly expressed in the biochemical phenotype of a mutant cell. Thus, it is likely that NSU-1-dGuo-L is heterozygous, possessing one normal allele for ribonucleotide reductase and one allele that confers resistance of its reductase activity to feedback inhibition by dGTP.

NSU-1-dGuo-L cells are also resistant to the cytotoxic effects of exogenous dThd but normally sensitive to dAdo. As predicted from these observations, the CDP reductase activity of dGuo-L cells, as compared to that of parental NSU-1 cells, is less sensitive to feedback inhibition by TTP but normally sensitive to dATP. We have other independently derived mutants from NSU-1 cells that also possess ribonucleotide reductase activities with altered regulatory properties. These mutants will likely provide much useful information about the properties and functions of this important mammalian enzyme. Other laboratories have also reported cultured mammalian cells containing ribonucleotide reductases with abnormal properties (20, 42, 43).

By using a cell culture model we have applied a somatic cell genetic approach to a biochemical genetics problem in immunology to characterize the mechanisms by which known specific inherited defects in PNPase cause severely impaired immune function. The understanding of these biochemical mechanisms allows us to use our cell culture model to propose that repletion of deoxynucleoside triphosphates by the parenteral administration of deoxyribonucleosides might be used clinically to reverse the lethal effects of this inborn error of metabolism.

This work was supported by Grant PCM77-04494 from the National Science Foundation and by Grant AM20428 from the National Institute of Arthritis, Metabolism, and Digestive Disease. B.U. is supported by National Institutes of Health Program Project Grant, Clinical Pharmacology-Pharmacokinetics (GM16496). L.J.G. is supported by a fellowship from the National Institute of Child Health and Human Development. D.W.M. is an Investigator of the Howard Hughes Medical Institute.

1. Giblett, E. L., Anderson, J. E., Cohen, F., Pollara, B. & Meuwissen, H. J. (1972) *Lancet* ii, 1067–1069.
2. Hitzig, W. H., Dohmann, U., Pluss, H. J. & Vischer, D. (1974) *J. Pediatr.* **85**, 622–628.
3. Giblett, E. L., Ammann, A. J., Wara, D. W., Sandman, R. & Diamond, L. K. (1975) *Lancet* i, 1010–1013.
4. Johnson, S. M., Osherson, R., Watts, W. E., North, M. E., Allsop, J. & Webster, A. D. B. (1977) *Lancet* i, 168–170.
5. Edwards, N. L., Magilavy, D. B., Cassidy, J. T. & Fox, I. H. (1978) *Science* **201**, 628–630.
6. Ullman, B., Cohen, A. & Martin, D. W., Jr. (1976) *Cell* **9**, 205–211.
7. Kredich, N. M. & Martin, D. W., Jr. (1977) *Cell* **12**, 931–938.
8. Gudas, L. J., Cohen, A., Ullman, B. & Martin, D. W., Jr. (1978) *Somat. Cell Genet.* **4**, 201–219.
9. Ullman, B., Gudas, L. J., Cohen, A. & Martin, D. W., Jr. (1978) *Cell* **14**, 365–375.
10. Gudas, L. J., Ullman, B., Cohen, A. & Martin, D. W., Jr. (1978) *Cell* **14**, 531–538.
11. Sato, K., Slesinski, R. S. & Littlefield, J. W. (1972) *Proc. Natl. Acad. Sci. USA* **69**, 1244–1248.
12. Lever, J. E., Nuki, G. & Seegmiller, J. E. (1974) *Proc. Natl. Acad. Sci. USA* **71**, 2679–2683.
13. Chasin, L. A. (1974) *Cell* **2**, 37–41.
14. Spector, E. B., Hershfield, M. S. & Seegmiller, J. E. (1978) *Somat. Cell Genet.* **4**, 253–264.
15. Green, C. & Martin, D. W., Jr. (1973) *Proc. Natl. Acad. Sci. USA* **70**, 3698–3702.
16. Cohen, A., Hirschhorn, R., Horowitz, S. D., Rubinstein, A., Polmar, S. H., Hong, R. & Martin, D. W., Jr. (1978) *Proc. Natl. Acad. Sci. USA* **75**, 472–476.
17. Coleman, M. S., Donofrio, J., Hutton, J. J., Hahn, L., Daoud, A., Lampkin, D. & Dyminski, J. (1978) *J. Biol. Chem.* **253**, 1619–1626.
18. Reichard, P., Canellakis, Z. N. & Canellakis, E. S. (1961) *J. Biol. Chem.* **236**, 2514–2519.
19. Klenow, H. (1962) *Biochim. Biophys. Acta* **61**, 885–896.
20. Meuth, M. & Green, H. (1974) *Cell* **3**, 367–374.
21. Cohen, A., Doyle, D., Martin, D. W., Jr. & Ammann, A. J. (1976) *N. Engl. J. Med.* **295**, 1449–1454.
22. Cohen, A., Gudas, L. J., Ammann, A. J., Staal, G. E. J. & Martin, D. W., Jr. (1978) *J. Clin. Invest.* **61**, 1405–1409.
23. Horibata, K. & Harris, A. W. (1970) *Exp. Cell Res.* **60**, 61–77.
24. Coffino, P., Baumal, R., Laskov, R. & Scharff, M. D. (1972) *J. Cell. Physiol.* **79**, 429–440.
25. Daniel, V., Litwack, G. & Tomkins, G. M. (1973) *Proc. Natl. Acad. Sci. USA* **70**, 76–79.
26. Kalckar, H. M. (1947) *J. Biol. Chem.* **167**, 429–443.
27. Zannis, V., Doyle, D. & Martin, D. W., Jr. (1978) *J. Biol. Chem.* **253**, 504–510.
28. Lewis, W. H., Kuzik, B. A. & Wright, J. A. (1978) *J. Cell. Physiol.* **94**, 287–298.
29. Steeper, J. R. & Steuart, C. D. (1970) *Anal. Biochem.* **34**, 123–130.
30. Cohen, A., Ullman, B. & Martin, D. W., Jr. (1979) *J. Biol. Chem.* **254**, 112–116.
31. Moore, E. C. & Hurlbert, R. B. (1966) *J. Biol. Chem.* **241**, 4802–4807.
32. Ruddle, F. (1973) *Nature (London)* **242**, 165–169.
33. George, D. L. & Francke, U. (1976) *Science* **194**, 851–852.
34. Durham, J. P. & Ives, D. H. (1969) *Mol. Pharmacol.* **5**, 358–375.
35. Krenitsky, T. A., Tuttle, J. V., Koszalka, G. W., Chen, S. I., Beachem, L., Rideout, J. L. & Elion, G. B. (1976) *J. Biol. Chem.* **251**, 4055–4061.
36. Carson, D. A., Kaye, J. & Seegmiller, J. E. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 5677–5681.
37. Schaeffer, H. J. & Schwender, C. F. (1974) *J. Med. Chem.* **17**, 6–8.
38. Woo, P. W. K., Dion, H. W., Lange, S. M., Dahl, L. F. & Durham, L. J. (1974) *J. Heterocycl. Chem.* **11**, 641–643.
39. Chan, T. S. (1978) *Cell* **14**, 523–530.
40. Thompson, L. F., Willis, R. C., Stoop, J. W. & Seegmiller, J. E. (1978) *Proc. Natl. Acad. Sci. USA* **75**, 3722–3726.
41. Lajtha, L. G. & Vane, J. R. (1958) *Nature (London)* **182**, 191.
42. Lewis, W. H. & Wright, J. A. (1974) *Biochem. Biophys. Res. Commun.* **60**, 926–933.
43. Lewis, W. H. & Wright, J. A. (1978) *J. Cell. Physiol.* **97**, 73–85.