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*Review*

# Purinogenic Lymphocytotoxicity: Clues to a Wider Chemotherapeutic Potential for the Adenosine Deaminase Inhibitors

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## Introduction

Inherited deficiencies of the purine catabolic enzymes adenosine deaminase (ADA) and PNP produce selective accumulation of purine deoxynucleotides in lymphocytes, resulting in immunodeficiency disease in children. The finding that resting lymphocytes are as susceptible to accumulated dATP as their dividing precursors has extended the potential clinical role for the ADA inhibitor deoxycoformycin to include immunosuppressive therapy, modification of graft-versus-host disease, and the possible treatment of human lymphoid cancers. Attempts to account for purinogenic lymphocytotoxicity and the special selectivity of this toxicity towards cultured T lymphoblasts have revealed some differences between lymphocyte subpopulations in their purine metabolic pathways, and allow speculation about the role of these biochemical differences in lymphocyte regulation and interaction.

## Adenosine Deaminase Deficiency

Inherited deficiency of ADA is an important cause of severe combined immunodeficiency disease. ADA mediates the first step in the catabolism of Ado and dAdo (Fig. 1). It is widely distributed in human tissues, with high levels being detected in the lymphoid system. Since the first description of ADA deficiency by Giblett et al. in 1972 [17], more than 70 children with the syndrome have been reported [35]. Affected patients have thymic hypoplasia, a predominantly T cell lymphopenia, and a variable defect in B cell numbers and function. Elevated dATP levels have been found in erythrocytes and lymphocytes form ADA-deficient children [12, 13], and in some patients transfusion of ADA-containing, normal erythrocytes produced a fall in dATP levels to normal associated with return of normal immune function [35].

## Cultured Cells and Model Systems

The availability of potent ADA inhibitors (coformycin, deoxycoformycin and EHNA) (Fig. 2) led to the use of cultured lymphocytes in investigating the lymphospecific toxicity associated with ADA deficiency or inhibition. Early interest was focused on adenosine as a possible lymphotoxin (Fig. 1). However, in the presence of ADA inhibitors, cultured human T leukaemic lymphoblasts and PHA-stimulated lymphocytes were more sensitive to micromolar concentrations of dAdo, while EBV-transformed B lymphoblasts were resistant [5, 16, 36, 44, 48]. Sensitivity to dAdo correlated with the ability of cultured T cells to elevate their dATP pools on exposure to micromolar concentrations of dAdo, while EBV-transformed B cells required 100–300 times higher concentrations of dAdo [5, 16, 36].

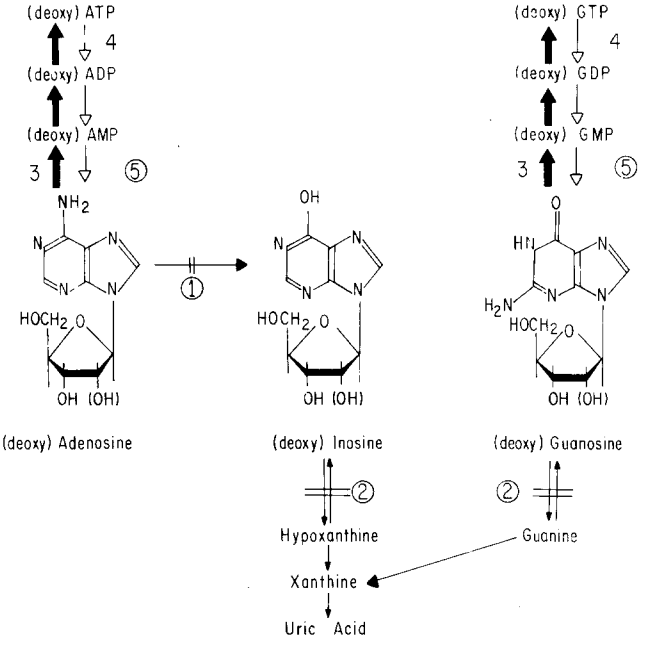


**Fig. 1.** Purine metabolic pathways associated with immunodeficiency. 1, adenosine deaminase; 2, purine nucleoside phosphorylase; 3, (deoxy)nucleoside kinases; 4, (deoxy)nucleotide triphosphatase; 5, 5′-nucleotidase. *Circled figures,* enzyme defects associated with immunodeficiency

## Biochemical Mechanism of Deoxyadenosine Lymphotoxicity

### *a) Nucleotide-Dependent Toxicity*

High deoxyadenosine kinase activity in human lymphoid tissues permits the selective accumulation of dATP in

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*Abbreviations used in this paper:* ADA, adenosine deaminase; PNP, purine nucleoside phosphorylase; dATP, 2′-deoxyadenosine 5′-triphosphate; Ado, adenosine; dAdo, 2′-deoxyadenosine; EHNA, erythro-9-(2-hydroxy-3-nonyl)adenine; PHA, phytohaemagglutinin; EBV, Epstein-Barr virus; PBL, peripheral blood lymphocytes; CLL, chronic lymphocytic leukaemia; dGTP, 2′-deoxyguanosine-5′-triphosphate, dGuo, 2′-deoxyguanosine

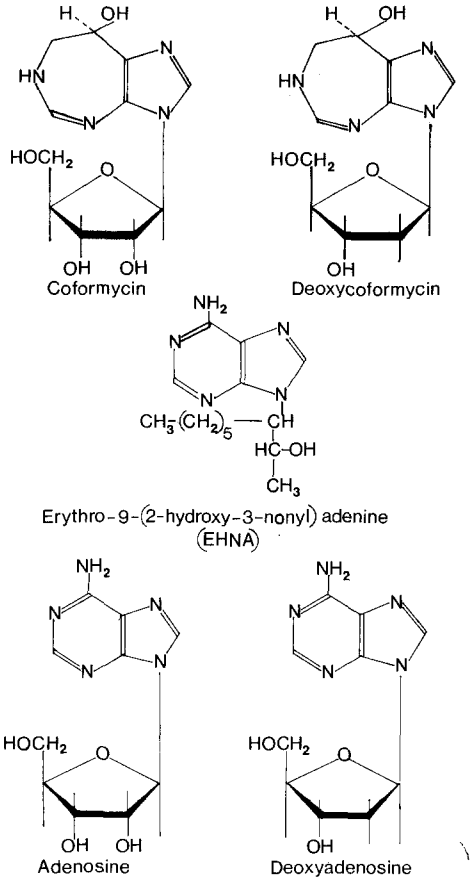


**Fig. 2.** Structure of adenosine, deoxyadenosine, and the adenosine deaminase inhibitors, coformycin, deoxycoformycin, and EHNA

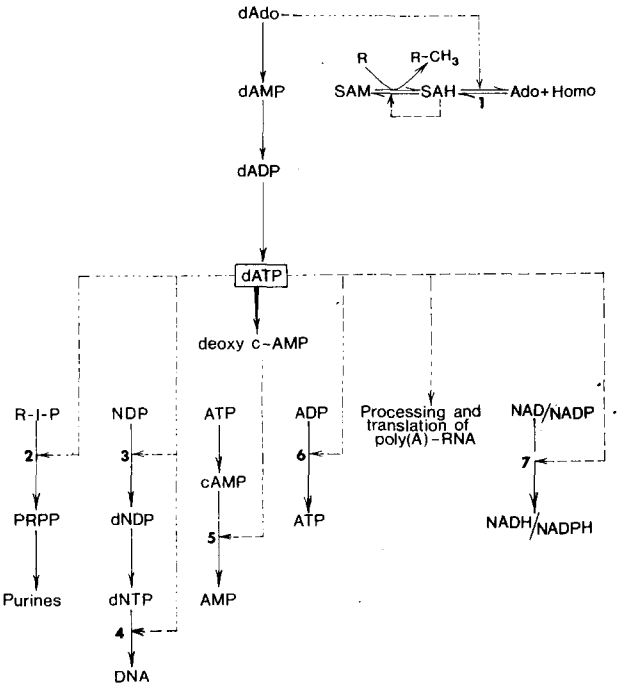


**Fig. 3.** Possible biochemical targets for deoxyadenosine and dATP toxicity. 1, *S*-adenosylhomocysteine hydrolase; 2, phosphoribosylpyrophosphate synthetase; 3. ribonucleotide reductase; 4, DNA polymerase; 5, phosphodiesterase; 6, ATP metabolism; 7, dehydrogenases. *Abbreviations:* dAdo, 2′-deoxyadenosine; Ado, adenosine; Homo, homocysteine; SAM, *S*-adenosylmethionine; SAH, *S*-adenosylhomocysteine; R-I-P, ribose-1-phosphate; PRPP, phosphoribosylpyrophosphate; NDP, nucleotide diphosphates; dNTP, deoxynucleotide triphosphates. *Dotted lines,* postulated inhibitory effect

lymphocytes when the catabolic pathway for dAdo is blocked either pharmacologically or genetically (Fig. 1). Accumulated dATP is unable to cross the plasma membrane and is 'trapped' intra-cellularly. Non-lymphoid tissues, with the presumable exception of erythrocytes, lack the necessary kinase enzymes and do not accumulate dATP [4]. The failure of EBV-transformed B lymphoblasts to accumulate dATP at low dAdo concentrations, despite the presence of deoxynucleoside kinase activity, may relate to enhanced catabolic pathways for deoxynucleotides in these cells (discussed later). dATP is a powerful allosteric inhibitor of ribonucleotide reductase in vitro. This enzyme catalyses the reduction of UDP, CDP, GDP, and ADP to their respective deoxynucleotides, and is the rate-limiting step in the formation of these DNA precursors. The high levels of dATP accumulating in lymphocytes have been postulated to inhibit ribonucleotide reductase, resulting in the cessation of dCDP, dGDP, and dUDP production, inhibiting DNA synthesis in cycling cells (Fig. 3).

There is now evidence that dAdo, presumably via accumulated dATP, is toxic to lymphoid cells independently of effects on ribonucleotide reductase or DNA replication. Resting PBL are killed by micromolar concentrations of dAdo in the presence of ADA inhibitors, and, like replicating cultured T lymphoblasts, these non-dividing lymphocytes elevate their dATP pools at micromolar concentrations of dAdo [9, 28]. Resting peripheral blood T cells are more sensitive to dAdo than non-T mononuclear cells by an order of magnitude (Fig. 4). A similar difference has recently been

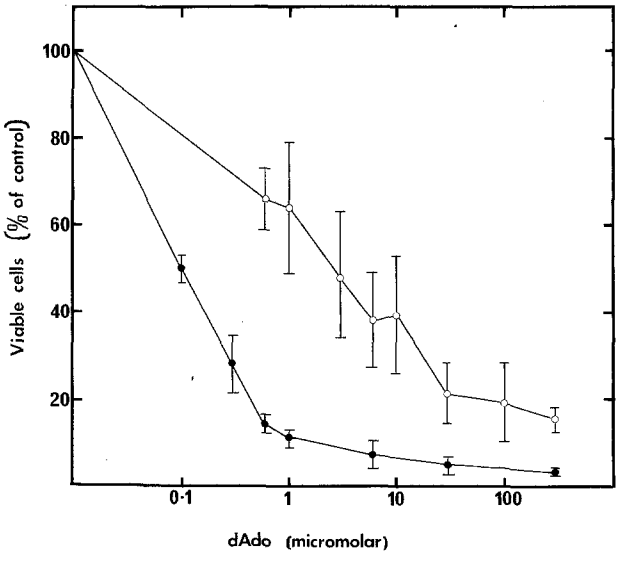


**Fig. 4.** Lymphotoxicity of deoxyadenosine in resting peripheral blood lymphocytes at 96 h. T cells (●) and non-T cells (○) were prepared from peripheral blood mononuclear cells and incubated as previously described [28] in the presence of 5 μ*M* EHNA and various concentrations of deoxyadenosine. After 96 h, the percent viable non-adherent cells was determined by trypan blue exclusion and phase contrast microscopy. Controls contained 5 μ*M* EHNA alone and were 88%–94% viable at 96 h. Data *points* are means and *error bars* 1SE of values obtained in cells from three separate donors

reported by Carson et al. [9]. While this suggests a degree of relative resistance in resting B cells, the non-T mononuclear fraction from these cell separation procedures routinely contains 30%–40% monocytes. The finding that non-dividing PBL from patients with B cell CLL are also an order of magnitude less sensitive to dAdo than T cells lends some support to the view that B cells have an inherent relative resistance to dAdo [29]. Both T and non-T PBL have an equivalent capacity to sustain elevations in dATP pools [28], and this capacity is enhanced in B CLL cells [29]. This suggests that the capacity for dATP elevation is not the only determinant of dAdo lymphotoxicity.

Clearly, DNA synthesis is an unlikely target for dAdo or dATP in these cells. A lymphocytotoxic effect independent of inhibition of DNA synthesis is also supported by the early toxicity (less than 20 h) induced by dAdo in mitogen-stimulated PBL [48].

Furthermore, DNA flow cytometric studies of dAdo toxicity in cultured human T leukaemic lymphoblasts suggest a cytotoxic mechanism independent of inhibition of ribonucleotide reductase. After exposure of these cells to cytostatic concentrations of dAdo (3 μ*M*) in the presence of an ADA inhibitor, a $G_1$ phase arrest was observed. Cells in S phase completed that round of DNA replication, passed through $G_2$-M, and accumulated in $G_1$. This contrasted with the S phase block seen in these cells following exposure to thymidine or hydroxyurea, agents known to inhibit ribonucleotide reductase [14]. In contrast, EBV-transformed B cell lines, when exposed to high concentration of dAdo, arrest in S phase.

The biochemical mechanism of this $G_0$-$G_1$ toxicity is not understood. It is possible that elevated dATP pools may interfere with ATP metabolism [2, 35, 43, 51], altering the adenylate energy charge or ATP-dependent processes critical in $G_1$ or $G_0$, e.g., processing of mRNA or cyclic AMP metabolism (Fig. 3).

*b) Nucleotide-Independent Toxicity*

dAdo is a potent inhibitor of the enzyme *S*-adenosylhomocysteine hydrolase, which converts *S*-adenosylhomocysteine to homocysteine and Ado [22]. Accumulated *S*-adenosylhomocysteine inhibits its own formation from *S*-adenosylmethionine, inhibiting essential transmethylation reactions (Fig. 3). Inhibition of *S*-adenosylhomocysteine hydrolase has been observed in erythrocytes and lymphocytes from patients with ADA deficiency [24], in leukaemic lymphocytes of patients treated with ADA inhibitors [23, 29, 37], and in cultured EBV-transformed B lymphoblasts exposed to high dAdo concentrations [22].

However, this mechanism negates the association between dAdo sensitivity and the capacity for sustained dATP pool elevation in sensitive cells, at present the only satisfactory explanation for the lymphoselectivity of dAdo toxicity. Furthermore, a lack of correlation between *S*-adenosylhomocysteine hydrolase inhibition and lymphocytotoxicity of dAdo in cultured T and B lymphoblasts and resting PBL has been recently demonstrated [31].

## Deoxyadenosine Toxicity in Lymphocyte Subpopulations

Experiments on cultured leukaemic lymphoblasts suggested that, in the presence of ADA inhibitors, T cells were considerably more sensitive to dAdo than transformed B cells [5, 16, 36]. EBV-transformed B lymphoblasts possess a cytoplasmic 5′-nucleotidase while cultured T lymphoblasts lack this enzyme, correlating with an enhanced sensitivity of these cells to the toxic effects of purine deoxynucleosides [8]. EBV-transformed B lymphoblasts also have high levels of an ecto-[$Ca^{2+}$, $Mg^{2+}$-dependent] nucleotide triphosphatase, which breaks down both ribo- and deoxyribonucleotide triphosphates [15]. This enzyme has low activity in resting peripheral T and B cells, but is expressed after mitogen transformation of B cells but not T cells (R. M. Fox, unpublished observations). It is also deficient in cultured T and null leukaemic lymphoblasts [15], dividing and non-dividing human thymocytes [30], and B CLL lymphocytes (R. M. Fox, unpublished observations). Thus, deficiency in nucleotide catabolising enzymes 'distal' to ADA (4 and 5 in Fig. 1) correlates with the selective capacity of dAdo-sensitive lymphoid cells to accumulate toxic levels of dATP. Enhanced catabolism of dATP by B lymphoblasts seems the most likely explanation for the relative dAdo resistance of these cells, since there is very little difference between T and B lymphoblasts in the activity and kinetics of dAdo kinase activity [6, 26].

Lymphocyte subpopulations also show marked differences in the distribution of ADA and PNP. In the rat, the ADA activity in peripheral lymphoid tissues roughly correlates with the percentage of T cells. The ratio of ADA to PNP activities is high in cortical thymocytes, but low in medullary thymocytes and in splenic and bone marrow lymphocytes [3]. This ratio seems to vary inversely during T cell differentiation and has been proposed as a method for characterising specific stages of T cell development [3]. Immature, dividing human thymocytes are equivalent to mature, non-dividing thymocytes in their capacity to elevate dATP and dGTP pools to potentially toxic levels [28], so that differences in ADA and PNP levels could provide for a possible cytotoxic effect of physiological levels of deoxyribonucleosides. Such a possibility deserves investigation in relation to the large numbers of thymocytes which apparently undergo intra-thymic death without entering the peripheral circulation [42].

## Purine Nucleoside Phosphorylase Deficiency

The other major purine deoxynucleoside, dGuo, is catabolised by the enzyme PNP (Fig. 1). Congenital deficiency of PNP also results in immunodeficiency, with T cell lymphopoenia and defective cell-mediated immunity [18], and has been the subject of a recent review [35]. dGuo is toxic to cultured T leukaemic lymphoblasts, while EBV-transformed B lymphoblasts are resistant [16, 36, 38]. The biochemical correlate of lymphoblast sensitivity to dGuo is the capacity of cells to accumulate high levels of dGTP which, like dATP in ADA deficiency, is probably the toxic effector molecule in PNP deficiency. Recently, however, non-dividing lymphoid cells have been reported to be resistant to dGuo [9], despite their capacity to elevate dGTP pools [9, 28]. Perturbations in the deoxynucleotide triphosphate pools of dGuo-treated cultured T lymphoblasts suggest that dGTP may be exerting its toxic effect in these cells by allosterically inhibiting the reduction of pyrimidine ribonucleotides by ribonucleotide reductase, thus inhibiting DNA synthesis in dividing cells [11]. The recent description of the potent PNP inhibitors 1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamidine (TCNR) [49] and 8-aminoguanosine [27], and their capacity for marked potentiation of

T-lymphoblast sensitivity to dGuo in vitro [27] will encourage further investigation into the suitability of these agents for clinical application alongside the ADA inhibitors, as a related class of purinogenic lymphotoxins.

## Clinical Use of Adenosine Deaminase Inhibitors

### *1. Anti-Leukaemic Chemotherapy with ADA Inhibitors*

Both deoxycoformycin and EHNA have been used in vivo to induce ADA inhibition, but human trials have been confined to deoxycoformycin which has a lower Ki for ADA. The clinical pharmacology of deoxycoformycin has been reviewed [46]. Early experiments on mice treated with deoxycoformycin and EHNA suggested their use as potential immunosuppressants [10, 34]. However, these results were clouded by toxicity, as the mouse is a poor model for studying these drugs since murine dAdo kinase activity is widely distributed in other non-lymphoid tissues [7]. Early phase 1 trials in humans showed profound lymphopenia even at very low doses of deoxycoformycin, with little effect on other bone marrow elements [45]. However, until recently, clinical use has been directed at acute T-cell leukaemia, a condition resistant to conventional cytotoxic chemotherapy, and remissions have been reported (Table 1). Toxicity attributed to deoxycoformycin treatment has included haemolytic anaemia, keratoconjunctivitis, urate nephropathy and acute tubular necrosis, pulmonary oedema, and transient hepatic and neurological abnormalities. Most of these effects have been reversible. It is not clear which may be direct drug reactions and which may reflect high levels of dATP accumulating in non-lymphoid tissues in the presence of high plasma dAdo concentrations. A biochemical monitor for impending tissue toxicity has been proposed using daily measurement of the erythrocyte dATP/ATP ratio during therapy [32]. Reports of successful remission induction with deoxycoformycin in patients with end-stage CLL of B cell type [19, 29] support the in vitro demonstration of the toxicity of dAdo to non-cycling, non-T lymphoid cells. These studies support the further trial of ADA inhibitors in lymphoproliferative diseases of T and non-T phenotypes, and of low proliferative index.

### *2. Immunosuppression with ADA Inhibitors*

The cytolytic effect of dAdo on non-dividing normal PBL suggests the possible extension of the clinical role of the ADA inhibitors to that of therapeutic immunosuppression in humans. The sensitivity of resting T lymphocytes to micromolar concentrations of dAdo in vitro suggest that only low doses of deoxycoformycin would be necessary in this form of therapy, just sufficient to inhibit serum and lymphocyte ADA and allow the 'natural' accumulation of dATP from serum

**Table 1.** Deoxycoformycin in lymphoproliferative disease

| Disease[a] | No. of patients | Dose | Outcome[a] | Toxicity[a] | Reference |
|---|---|---|---|---|---|
| ALL<br>Hodgkin's<br>NHL | 7<br>1<br>1 | Various (0.1–1.0 mg/kg/day) in phase 1 trial | Reduction in blasts in 3<br>NR<br>NR | Urate nephropathy | 45 |
| T ALL | 1 | 0.25 mg/kg, D 1–4 | Reduction in blasts | Sepsis. Death on day 20 | 51 |
| Null ALL | 1 | 0.25 mg/kg, D 1–5 repeated weekly | Reduction in blasts | | 33 |
| T ALL | 2 | 0.25 mg/kg, D 1–5 repeated 2nd weekly | 2 CR | Nil | 40 |
| T ALL | 1 | 0.001–0.15 mg/kg escalation D 1–6 | CR | Pulmonary oedema<br>Acute tubular necrosis | 37 |
| T ALL | 1 | 1 mg/kg | CR | | 25 |
| Sezary syndrome | 1 | 0.01–0.25 mg/kg, D 1–5 | PR | Pulmonary oedema<br>Haemolytic anaemia | 43 |
| T CLL | 1 | 0.25 mg/kg, D 1–5 | PR | N/A | 52 |
| CLL | 7 | 4 mg/m$^2$/wk, wks 1–5 or longer | 1 CR, 1 PR | Nil | 20 |
| ALL (T and non-T) | 26 | 0.5–1.0 mg/kg, D 1–3 | 2 CR, 6 PR | Encephalopathy (high dose only)<br>Acute tubular necrosis (high dose only)<br>Keratoconjunctivitis | 39 |
| ALL<br>CML/BT<br>NHL<br>CLL | 6<br>2<br>4<br>3 | Various doses in phase 1 trial | Reduction in blasts in 5<br>Reduction in blasts in 2<br>1 PR<br>1 PR | Conjunctivitis<br>Renal insufficiency<br>Hepatic transaminitis | 19 |
| CLL | 1 | 0.1 mg/kg, D 1–5 | PR | Keratoconjunctivitis<br>Neutropenia<br>Hepatic transaminitis | 29 |
| T ALL<br>Non-T ALL | 12<br>5 | 0.15–0.5 mg/kg, D 1–7 | 7 CR, 2 PR<br>NR | Renal dysfunction<br>Hepatic transaminitis<br>Keratoconjunctivitis<br>Heamolytic anaemia | 41 |

[a] ALL, acute lymphoblastic leukaemia; NHL, non-Hodgkin's lymphoma; CLL, chronic lymphocytic leukaemia; CML/BT, chronic myeloid leukaemia in blastic transformation; CR, complete response; PR, partial response; NR, no response; N/A, not available

dAdo, which in this setting probably derives largely from the turnover of nucleated erythrocytes in the bone marrow [21]. With careful biochemical monitoring it is likely that selective lymphotoxicity could be achieved without serious toxicity to other tissues.

ADA inhibitors may also have application in the extracorporeal lymphocyte depletion of human bone marrow prior to grafting to prevent graft-versus-host disease. This would require exogenous dAdo in combination with an ADA inhibitor. There is some confirmatory evidence that bone marrow haemopoeitic cells are unaffected by dAdo/deoxycoformycin combinations. Mouse and human haemopoietic stem cells are unaffected by high concentrations of deoxycoformycin alone [1, 47], and normal human bone marrow shows no inhibition of DNA synthesis by 10 $\mu M$ dAdo/20 $\mu M$ deoxycoformycin [51]. The effect of dAdo and deoxycoformycin on human bone marrow in vitro awaits further study, but peripheral blood neutropenia and thrombocytopenia have only rarely been reported as complications of deoxycoformycin therapy (Table 1).

## Conclusion

The resistance of replicating B cells to dAdo toxicity compared with the sensitivity of non-dividing lymphocytes and replicating T cells is intriguing. Two fundamental biochemical observations underly these phenomena: (a) the ability of sensitive cells to elevate their dATP pools at micromolar concentrations of dAdo, and (b) toxicity to $G_0$ cells, or $G_1$ phase arrest in replicating T cells.

While the mechanism of these observations, at a molecular level, remains to be elucidated, they justify investigations into a wider application of the ADA inhibitors to include possible chemotherapy of a wide range of lymphoproliferative disorders, immunosuppressive therapy, and lymphocyte depletion of human bone marrow in vitro prior to grafting.

## References


1. Aye MT, Dunne JV (1981) Effect of 2′-deoxycoformycin on erythroid granulocytic and T-lymphocyte colony growth. Blood 58: 1043
2. Bagnara AS, Hershfield MS (1982) Mechanism of deoxyadenosine-induced catabolism of adenosine ribonucleotides in adenosine deaminase-inhibited human T lymphoblastoid cells. Proc Natl Acad Sci USA 79: 2673
3. Barton RW, Goldschneider I (1979) Nucleotide-metabolizing enzymes and lymphocyte differentiation. Mol Cell Biochem 28: 135
4. Carson DA, Kaye J, Seegmiller JE (1977) Lymphospecific toxicity in adenosine deaminase deficiency and purine nucleoside phosphorylase deficiency: Possible role of nucleoside kinase(s). Proc Natl Acad Sci USA 74: 5677
5. Carson DA, Kaye J, Seegmiller JE (1978) Differential sensitivity of human leukemic T cell lines and B cell lines to growth inhibition by deoxyadenosine. J Immunol 121: 1726
6. Carson DA, Kaye J, Matsumoto S, Seegmiller JE, Thompson L (1979) Biochemical basis for enhanced toxicity of deoxyribonucleosides toward malignant human T cell lines. Proc Natl Acad Sci USA 76: 2430
7. Carson DA, Kaye J, Wasson DB (1980) Differences in deoxyadenosine metabolism in human and mouse lymphocytes. J Immunol 24: 8
8. Carson DA, Kaye J, Wasson DB (1981) The potential importance of soluble deoxynucleotidase activity in mediating deoxyadenosine toxicity in human lymphoblasts. J Immunol 126: 348
9. Carson DA, Wasson DB, Lakow E, Kamatani N (1982) Possible metabolic basis for the different immunodeficient states associated with genetic deficiencies of adenosine deaminase and purine nucleoside phosphorylase. Proc Natl Acad Sci USA 79: 3848
10. Chassin MM, Chirigos MA, Johns DG, Adamson RH (1977) Adenosine deaminase inhibition for immunosuppression. N Engl J Med 296: 1232
11. Cohen A, Gudas LJ, Ammann AJ, Staal GEJ, Martin DW Jr (1978) Deoxyguanosine triphosphate as a possible toxic metabolite in the immunodeficiency associated with purine nucleoside phosphorylase deficiency. J Clin Invest 61: 1405
12. Cohen A, Hirschorn R, Horowitz S, Rubinstein A, Polmar SH, Hong R, Martin DW Jr (1978) Deoxyadenosine triphosphate as a potentially toxic metabolite in adenosine deaminase deficiency. Proc Natl Acad Sci USA 75: 472
13. Coleman MS, Donofrio J, Hutton JJ, Hahn L, Daoud A, Lampkin B, Dyminski J (1978) Identification and quantitation of adenosine deoxynucleotides in erythrocytes of a patient with adenosine deaminase deficiency and severe combined immunodeficiency. J Biol Chem 253: 1619
14. Fox RM, Kefford RF, Tripp EH, Taylor IW (1981) $G_1$-phase arrest of cultured human leukemic T cells induced by deoxyadenosine. Cancer Res 41: 5141
15. Fox RM, Piddington SK, Tripp EH, Tattersall MHN (1981) Ecto-ATPase deficiency in cultured human T and null leukemic lymphocytes. A biochemical basis for thymidine sensitivity. J Clin Invest 68: 544
16. Gelfand EW, Lee JJ, Dosch HM (1979) Selective toxicity of purine deoxynucleosides for human lymphocyte growth and function. Proc Natl Acad Sci USA 76: 1998
17. Giblett ER, Anderson F, Cohen B, Pollara B, Meuwissen HJ (1972) Adenosine deaminase deficiency in two patients with severely impaired immunity. Lancet II: 1067
18. Giblett ER, Ammann AJ, Wara DW, Sandman R, Diamond LK (1975) Nucleoside phosphorylase deficiency in a child with severely defective T-cell immunity and normal B-cell immmunity. Lancet I: 1010
19. Grever MR, Siaw MFE, Jacob WF, Neidhart JA, Miser JS, Coleman MS, Hutton JJ, Balcerzak SP (1981a) The biochemical and clinical consequences of 2′-deoxycoformycin in refractory lymphoproliferative malignancy. Blood 57: 406
20. Grever MR, Wilson HE, Kraut EH, Neidhart JA, Balcerzak SP (1981b) Deoxycoformycin in the treatment of refractory chronic lymphocytic leukemia. (Abstract) Proc AACR/ASCO C-605: 487
21. Henderson JF, Smith CM (1981) Mechanisms of deoxycoformycin toxicity in vivo. In: Tattersall MHN, Fox RM (eds) Nucleosides and cancer treatment. Academic Press, Sydney, p 208
22. Hershfield MS (1979) Apparent suicide inactivation of human lymphoblast *S*-adenosylhomocysteine hydrolase by 2′-deoxyadenosine and adenine arabinoside. J Biol Chem 254: 22
23. Hershfield MS, Kredich NM (1981) Effects of adenosine deaminase inhibition on transmethylation. In: Tattersall MHN, Fox RM (eds) Nucleosides and cancer treatment. Academic Press, Sydney, p 161
24. Hershfield MS, Kredich NM, Ownby DR, Ownby H, Buckley R (1979) In vivo inactivation of *S*-adenosylhomocysteine hydrolase by 2′-deoxyadenosine in adenosine deaminase deficient patients. J Clin Invest 63: 807
25. Hershfield M, Kredich N, Falletta J, Kinney T, Mitchell B, Koller C (1981) Effects of deoxyadenosine and adenosine arabinoside during deoxycoformycin therapy. (Abstract) Proc AACR/ASCO. 621: 157
26. Hershfield MS, Fetter JE, Small WC, Bagnara AS, Williams SR, Ullman B, Martin DW Jr, Wasson DB, Carson DA (1982) Effects of mutational loss of adenosine kinase and deoxycytidine kinase on deoxyATP accumulation and deoxyadenosine toxicity in cultured CEM human T-lymphoblastoid cells. J Biol Chem 257: 6380
27. Kazmers IS, Mitchell BS, Dadonna PE, Wotring LL, Townsend LB, Kelley WM (1981) Inhibition of purine nucleoside phosphor-

ylase by 8-aminoguanosine: selective toxicity for T-lymphoblasts. Science 214: 1137
28. Kefford RF, Fox RM (1982) Purine deoxynucleoside toxicity in nondividing human lymphoid cells. Cancer Res 42: 324
29. Kefford RF, Fox RM (1982) Deoxycoformycin-induced response in chronic lymphocytic leukemia: Deoxyadenosine toxicity in non-replicating B lymphocytes. Br J Haematol 50: 627
30. Kefford RF, Taylor IW, Fox RM (1981) Deoxyribonucleoside triphosphate pool changes in human thymocytes exposed to deoxynucleosides. In: Tattersall MHN, Fox RM (eds) Nucleosides and cancer treatment. Academic Press, Sydney, p 190
31. Kefford RF, Helmer MA, Fox RM (1982) *S*-adenosylhomocysteine hydrolase inhibition in deoxyadenosine treated human T lymphoblasts and resting peripheral blood lymphoblasts. Cancer Res 42: 3822
32. Koller CA, Mitchell BS (1981) 2′-Deoxycoformycin toxicity correlates with red cell dATP/ATP ratios, (Abstract) Proc AACR/ASCO 875: 221
33. Koller CA, Mitchell BS, Grever MR, Mejias E, Malspeis L, Metz EN (1980) Treatment of acute lymphoblastic leukemia with 2′-deoxycoformycin: Clinical and biochemical consequences of adenosine deaminase inhibition. Cancer Treat Rep 63: 1949
34. Lum CT, Sutherland DER, Najarian JS (1977) Adenosine deaminase inhibition for immunosuppression. N Engl J Med 296: 819
35. Mitchell BS, Kelley WN (1980) Purinogenic immunodeficiency diseases: Clinical features and molecular mechanisms. Ann Intern Med 92: 826
36. Mitchell BS, Mejias E, Daddona PE, Kelley WN (1978) Purinogenic immunodeficiency diseases – selective toxicity of deoxyribonucleosides for T-cells. Proc Natl Acad Sci USA 75: 5011
37. Mitchell BS, Koller CA, Heyn R (1980) Inhibition of adenosine deaminase results in cytotoxicity to T lymphoblasts in vivo. Blood 56: 556
38. Ochs UH, Chen S-H, Osborne WRA, Scott CR (1979) Purine nucleoside phosphorylase deficiency: A molecular model for selective loss of T cell function. J Immunol 122: 2424
39. Poplack DG, Sallan SE, Rivera G, Holcenberg J, Murphy SB, Blatt J, Lipton JM, Venner P, Glaubiger DL, Ungerleider R, Johns D (1981) Phase I study of 2′-deoxycoformycin in acute lymphoblastic leukemia. Cancer Res 41: 3343
40. Prentice HG, Ganeshaguru K, Bradstock KF, Goldstone AH, Smyth JF, Wonke B, Janossy G, Hoffbrand AV (1980) Remission induction with adenosine deaminase inhibitor 2′-deoxycoformycin in Thy-lymphoblastic leukemia. Lancet II: 170
41. Prentice HG, Lee N, Blacklock H, Smyth JF, Russell NH, Ganeshaguru K, Piga A, Hoffbrand AV (1981) Therapeutic selectivity of and prediction of response to 2′-deoxycoformycin in acute leukemia. Lancet II: 1250
42. Scollay RG, Butcher EC, Weissman IL (1980) Thymus cell migration. Quantitative aspects of cellular traffic from the thymus to the periphery in mice. Eur J Immunol 10: 210
43. Siaw MFE, Mitchell BS, Koller CA, Coleman MS, Hutton JJ (1980) ATP depletion as a consequence of adenosine deaminase inhibition in man. Proc Natl Acad Sci USA 77: 6157
44. Simmonds HA, Panayi GS, Corrigall V (1978) A role for purine metabolism in the immune response: adenosine deaminase activity and deoxyadenosine catabolism. Lancet I: 60
45. Smyth JF (1979) Selective treatment of lymphoid malignancy with adenosine deaminase inhibitors. In: Enzyme defects and immune dysfunction. Excerpta Medica, Amsterdam, p 263 (Ciba Foundation Symposium no. 68)
46. Smyth JF, Paine RM, Jackman AL, Harrap KR, Chassin MM, Adamson RH, Johns DG (1980) The clinical pharmacology of the adenosine deaminase inhibitor 2′-deoxycoformycin. Cancer Chemother Pharmacol 5: 93
47. Trotta PP, Tedde A, Ikehara S, Pahwa R, Good RA, Balis ME (1981) Specific immunosuppressive effects of constant infusion of 2′-deoxycoformycin. Cancer Res 41: 2189
48. Uberti J, Lightbody JJ, Johnson RM (1979) The effect of nucleosides and deoxycoformycin on adenosine and deoxyadenosine inhibition of human lymphocyte activation. J Immunol 123: 189
49. Willis RC, Robins RK, Seegmiller EJ (1980) An in vivo and in vitro evaluation of 1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamidine: an inhibitor of human lymphoblast purine nucleoside phosphorylase. Mol Pharmacol 18: 287
50. Yu AL, Kung FH, Bakay B, Nyhan WL (1979) In vitro and in vivo effect of deoxycoformycin in human T cell leukemia. Adv Exp Med Biol 122B: 373
51. Yu AL, Bakay B, Kung FH, Nyhan WL (1981) Effects of 2′-deoxycoformycin on the metabolism of purines and the survival of malignant cells in a patient with T cell leukemia. Cancer Res 41: 2677
52. Yu AL, Bakay B, Matsumoto S, Nyhan W, Green M, Royston I (1981) (Abstract) Proc AACR/ASCO 894: 226

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