Clin. exp. Immunol. (1976) **23**, 395–403.

Role of adenosine deaminase in lymphocyte proliferation

T. HOVI,* J. F. SMYTH,† A. C. ALLISON & S. C. WILLIAMS *Clinical Research Centre, Watford Road, Harrow, Middlesex and †Institute of Cancer Research, Royal Cancer Hospital, Clifton Avenue, Belmont, Sutton, Surrey*

(*Received* 18 *September* 1975)

SUMMARY

Activity of adenosine deaminase (ADA), an enzyme known to be deficient in some patients with severe combined immunodeficiency, increased three-fold within a 24-hr exposure of human peripheral blood lymphocytes to phytohaemagglutinin (PHA) in culture. This increase took place before the onset of DNA synthesis. Increased levels of ADA activity were also observed in lymphocytes incubated with pokeweed mitogen (PWM) for 60 hr.

DNA synthesis induced by PHA, PWM or mixed lymphocyte cultures (MLC) was strongly inhibited by adenosine at concentrations of 10^{-4}M or higher when human peripheral blood lymphocytes were cultured in a medium supplemented with horse serum, which lacks ADA. 10^{-6}–10^{-8}M coformycin, a potent inhibitor of ADA, inhibited PHA-, PWM- and MLC-induced DNA synthesis to a variable extent, whereas thymidine incorporation induced by *Salmonella* lipopolysaccharide (LPS) in mouse spleen cell cultures was strongly inhibited (by 75% or more) by 10^{-6}M coformycin. Combination of 10^{-7}–10^{-8}M coformycin and 10^{-4}–10^{-5}M adenosine synergistically inhibited mitogen- or MLC-induced DNA synthesis in human and mouse lymphocyte cultures.

These results, together with observations on children with ADA deficiency, provide evidence that adenosine deaminase is highly important for lymphocyte proliferation.

Human peripheral blood lymphocytes incubated with PHA, 10^{-5}M adenosine and 10^{-7}M coformycin showed some cytotoxicity whereas the rate of ^{51}Cr release from normal lymphocytes was not modified by the drugs. These findings suggest that *in vivo* clones of lymphocytes responding to specific antigens might be eliminated by coformycin, which may prove to be useful as a specific immunosuppressive agent.

INTRODUCTION

Adenosine deaminase (adenosine amino-hydrolase, EC 3.5.4.4.) (ADA) is an enzyme present in most mammalian tissues, the activity being highest in organs containing many lymphoid cells (Conway & Cooke, 1939; Brady, 1942; Brady & O'Donovan, 1965). The activity of ADA in lymphocytes obtained from the effluent duct of the popliteal node of sheep was found by Hall (1963) to increase after stimulating the node with antigen (chicken red cells). Recently very high levels of ADA activity were observed in malignant lymphocytes of patients with acute lymphocytic leukaemia (Smyth & Harrap, 1975). Current interest in this enzyme has been stimulated by the finding that patients with inherited deficiency of ADA

* Present address: Department of Virology, University of Helsinki, Haartmaninkatu 3, SF-00290 Helsinki 29, Finland.

Correspondence: Dr A. C. Allison, Clinical Research Centre, Watford Road, Harrow, Middlesex.

have a combined immunodeficiency (Giblett *et al.*, 1972; Dissing & Knudsen, 1972). Both humoral and cell-mediated immunity of these patients is severely impaired and they have decreased numbers of peripheral blood lymphocytes (PBL), which cannot be transformed *in vitro* by lectin mitogens (Wara & Ammann, 1975). No other biochemical lesion has been described in these patients, nor are the functions of any other tissues affected. This suggests that the activity of ADA is of particular importance for normal lymphocyte function.

To explore the role of ADA in lymphocyte proliferation we have measured ADA activity in normal human peripheral blood lymphocytes exposed to lectin mitogens and examined the effect of coformycin, a powerful inhibitor of ADA, on lymphocyte proliferation. The results indicate that the activity of ADA is increased by mitogenic stimulation and that activity of the enzyme is essential for the mitogenic responses of both human and mouse lymphocytes.

MATERIALS AND METHODS

Cell cultures. Human peripheral blood (HPB) lymphocytes were partially purified from buffy coats of healthy persons (kindly provided by the North London Blood Transfusion Service) by a modification of the Ficoll–Hypaque (Triosil) technique (Böyum, 1968) as described elsewhere (Hovi, Allison & Williams, 1975a). The final cell suspensions contained more than 90% trypan-blue-excluding mononuclear leucocytes. Fresh lymphocytes for the ADA measurements were prepared by the same technique from 10 ml of heparinized blood.

Mouse spleen cells were prepared aseptically from the T.O. strain of mice and used without fractionation. Both cell types were cultured in RPMI-1640 medium supplemented with glutamine (2 mM), penicillin (100 i.u./ml) streptomycin (50 μg/ml) and serum as indicated in the text. Except where indicated, lymphocytes were cultured in horse serum, which lacks adenosine deaminase (Ishii & Green, 1973). Sera other than autologous serum, were heat-inactivated before use.

For DNA synthesis 1×10^6 HPB or $1{\cdot}5$–2×10^6 mouse spleen cells were incubated in 1 ml of medium in flat-bottomed plastic tubes at 37°C in a humidified atmosphere (5% CO_2 in air). Mitogens, as well as the test compounds (sterilized by filtering through 450 nm Millipore-filters), were added to the cultures in small volumes (5–20 μl) of phosphate-buffered saline.

For mixed lymphocyte cultures Ficoll–Triosil-purified lymphocytes from two unrelated individuals were mixed in the culture tubes ($5 \times 10^5 + 5 \times 10^5$ cells) in a total volume of 1 ml and incubated as above.

Adenosine deaminase assay. Freshly separated or cultured lymphocytes were harvested by centrifugation, washed twice with phosphate-buffered saline and resuspended in 0·15 M phosphate buffer pH 7·1 at $0{\cdot}3$–$1{\cdot}0 \times 10^7$ cells/ml. The cells were disrupted by sonication (45 sec at 20 Kcycles/sec, MSE Ultrasonicator), centrifuged at 800 *g* for 5 min and the supernatant used for the enzyme assay.

Adenosine deaminase activity was assayed by a modification of the technique of Kalckar (1947), using a double-beam Cary 16 spectrophotometer. The reference cuvette (1 cm) contained 1·0 ml of 0·1 mM adenosine and 2·0 ml 0·15 M phosphate buffer pH 7·1. The sample cuvette (1 cm) contained 0·1 ml adenosine (0·2 mM aqueous) and 1·99–1·80 ml 0·15 mM phosphate buffer pH 7·1 equilibrated at 30°C. The reaction was started by the addition of 0·01–0·2 ml of cell extract. The reaction was followed by recording the decrease in optical density at 265 nm, which was linear for at least 5 min. One unit of adenosine deaminase activity is defined as the amount of enzyme in 10^7 cells which produces a decrease in optical density of 0·010 per minute under the conditions described (Hall, 1963).

DNA synthesis. 1 μCi of [^{3}H-methyl]thymidine (5 Ci/mmol) was added per tube at the times indicated. The cultures were incubated for a further 6 hr, if not otherwise specified in the text, and acid-insoluble radioactivity was measured as described in detail elsewhere (Hovi, Allison & Williams, 1975b).

Cytotoxicity assay. 2–5×10^7 Ficoll–Triosil-purified lymphocytes in 1–2 ml of growth medium were incubated for 60 min at 37°C with 20 μCi of sodium ^{51}Cr-chromate. After thorough washing the cells were cultured as described above. After the times indicated, tubes were centrifuged for 10 min at 600 *g* and radioactivity released from the cells was measured by counting both half of the supernatant and the rest of the culture separately.

Reagents. Purified phytohaemagglutinin type HA16 was from Wellcome Reagents Ltd, Beckenham, U.K. Pokeweed mitogen and lipopolysaccharide type W of *Salmonella marcescens* were from Difco Labs. Adenosine and inosine were from Sigma Chemical Company. [^{3}H-methyl]Thymidine and sodium [^{51}Cr]chromate were supplied by the Radiochemical Centre, Amersham, England. Coformycin was a generous gift from Professor H. Umezawa of the Institute of Microbial Chemistry, Tokyo, Japan. Ficoll was obtained from Pharmacia Fine Chemicals, Uppsala, Sweden, and Triosil from Nyegaard and Company, Oslo, Norway.

RESULTS

ADA activity in normal human lymphocytes exposed to lectin mitogens

Lymphocytes from the peripheral venous blood of thirteen healthy volunteers were separated by Ficoll–Triosil density centrifugation and their ADA activity measured. The individual variation in ADA activity was found to be relatively small (Table 1).

TABLE 1. Adenosine deaminase activity in peripheral blood lymphocytes in thirteen healthy individuals

	ADA (units/10^7 cells)*		
Assay	Duplicates		Mean
1	3·1	3·1	3·1
2	2·7	2·4	2·6
3	2·7	3·2	3·0
4	3·5	2·8	3·1
5	3·4	3·4	3·4
6	3·6	3·3	3·5
7	3·7	3·2	3·4
8	6·3	4·4	5·3
9	2·0	2·4	2·2
10	2·4	3·0	2·7
11	3·6	5·0	4·3
12	3·6	3·6	3·6
13	1·1	1·3	1·2

Group mean = 3·2
Standard deviation = ±1·0
Standard error of mean = ±0·2.

* One unit of ADA is defined as the amount of enzyme in 10^7 cells which produced a decrease in optical density of 0·010 per min under the described conditions.

Cultures exposed to PHA for 24 hr or longer showed markedly elevated levels of ADA activity (Fig. 1). The increase of ADA activity induced by PHA took place before the onset of DNA synthesis.

ADA activity in lymphocytes incubated with PWM for 60 hr was also significantly higher than that in the control cells (data not shown). ADA activity in non-stimulated cultures remained within the range of freshly separated lymphocytes over the observation period (Fig. 1, and Table 1).

Inhibition of mitogen-induced DNA synthesis in HPB lymphocytes by adenosine and coformycin

Various concentrations of adenosine and coformycin were added to cultures of purified human peripheral blood lymphocytes simultaneously with the mitogens. At adenosine concentrations of 10^{-4}M or higher there was marked inhibition of thymidine incorporation into DNA 27–33 hr after stimulation with PHA or PWM (Fig. 2). The extent of inhibition at lower concentrations of the nucleoside was variable and not linearly dose-dependent, as seen in Fig. 2. In some experiments (not shown) a slight (10–20%) enhancement of PWM-induced DNA synthesis was observed at 10^{-5}M adenosine. Combination of 10^{-5}M adenosine and 10^{-7}M coformycin inhibited synergistically DNA synthesis induced by PHA or PWM,

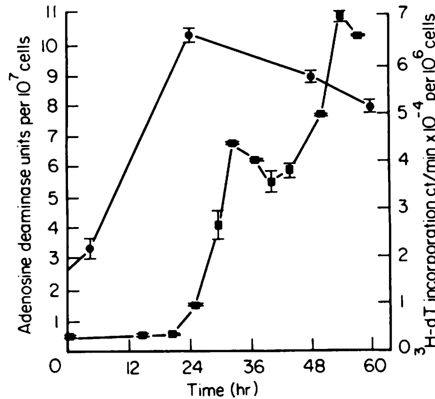

FIG. 1. Activity of adenosine deaminase in human peripheral blood lymphocytes exposed to PHA. Cultures of 6×10^6 cells in 5 ml of RPMI-1640 medium supplemented with 10% foetal calf serum were incubated at 37°C. 2 μg/ml PHA was added to cultures at various times. All cultures were harvested after 60 hr incubation and ADA activity in the cells was measured (●). The abscissa shows the time these cells have been exposed to PHA with the time point 0 representing activity in non-stimulated cells. The rate of PHA-induced DNA synthesis, studied by giving 2 hr pulses of [^{3}H]dT to cultures of 1×10^6 cells, is superimposed in the figure (■). Mean and range of duplicate assays are given.

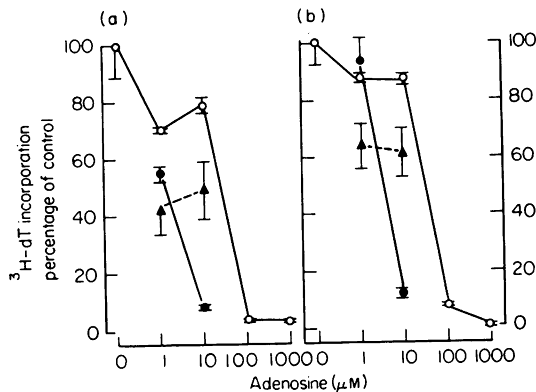

FIG. 2. Inhibition of mitogen-induced DNA synthesis by adenosine in the presence or absence of coformycin. Cultures of human peripheral blood lymphocytes were incubated with different concentrations of adenosine (o) or with adenosine and 10^{-7}M coformycin (●). Triangles represent the expected additive effect of coformycin and the concentrations of adenosine indicated, calculated from results obtained in cultures where the drugs were applied separately. Thymidine incorporation (mean and range) at 27–33 hr is expressed as a percentage of that obtained with the mitogens alone. (a) PHA; (b) PWM.

whereas the inhibition of thymidine incorporation caused by 10^{-6}M adenosine and 10^{-7}M coformycin was not even additive (Fig. 2).

10^{-6}–10^{-10}M Coformycin alone inhibited DNA synthesis to a variable extent in different experiments. In Table 2 experiments are shown with cells in 50% autologous serum, this being more closely analogous to the *in vivo* situation. In three out of five experiments coformycin at concentrations between 10^{-6} and 10^{-8}M inhibited PHA-induced DNA synthesis by more than 50%. Two out of four experiments with PWM-treated cells cultured in 50% autologous serum showed 30–50% inhibition at 10^{-6}–10^{-7}M coformycin, while lower concentrations of the drug generally slightly enhanced PWM-induced thymidine incorporation. Table 2 shows extreme results, the others being intermediate.

TABLE 2. Effect of coformycin on mitogen-induced DNA synthesis in HPB lymphocytes cultured in autologous serum

Additions	[^{3}H]dT incorporation (ratio to control)	
	PHA	PWM
Experiment 1		
Control	1·0 (1·18–0·82)	—
Coformycin 10^{-6}M	0·01	—
10^{-7}M	0·10 (0·14–0·06)	—
10^{-8}M	0·05 (0·07–0·03)	—
Experiment 3		
Control	1·0 (1·20–0·80)	1·0 (1·15–0·90)
Coformycin 10^{-6}M	0·86 (0·99–0·70)	1·20 (1·33–1·11)
10^{-7}M	0·87 (0·97–0·75)	1·08 (1·22–0·85)
10^{-8}M	0·84 (0·99–0·69)	1·37 (1·58–1·15)
10^{-9}M	0·82 (0·91–0·71)	1·45 (1·61–1·36)
10^{-10}M	0·82 (0·99–0·56)	—

0·5 or 1·0 × 10^{6} HPB lymphocytes were cultured in 0·5 or 1·0 ml of 50% (experiments 1–3) fresh autologous serum and labelled with 1 μCi [^{3}H]dT at 26–32 hr. Mean and range of duplicate or triplicate cultures are given.

In all experiments described above coformycin and adenosine were added to lymphocyte cultures immediately before the mitogen. However, inhibition of DNA synthesis in human lymphocytes assayed 26–32 hr after adding PHA was as complete if the cells were exposed to the drugs only 2 hr before starting the labelling.

Inhibition of mixed lymphocyte reaction-induced DNA synthesis by adenosine and coformycin

Like mitogen-induced DNA synthesis, thymidine incorporation resulting from mixed lymphocyte reactions were inhibited by 10^{-4}M adenosine or by a combination of 10^{-5}M adenosine and 10^{-6}–10^{-8}M coformycin (Table 3). 10^{-6}M Coformycin alone strongly inhibited MLC-induced DNA synthesis in one out of three experiments while the inhibition in the two other experiments was less marked.

Effect of coformycin and adenosine on mitogen-induced DNA synthesis in mouse spleen cell cultures

Higher concentrations of adenosine were needed to inhibit DNA synthesis by 50% in mouse spleen cell cultures than were required to inhibit lymphocytes stimulated with mitogens. 10^{-4}M concentration of the nucleoside, which was highly inhibitory to PHA-, PWM- or MLC-induced DNA synthesis in HPB cells, enhanced LPS-induced thymidine incorporation in mouse spleen cells (Table 4). PHA-induced DNA synthesis in mouse spleen cells was, however, moderately inhibited by this concentration of adenosine. 10^{-3}M adenosine also inhibited LPS-induced thymidine incorporation (Table 4). Inhibition of DNA synthesis by coformycin was again variable, being greater in cultures stimulated by LPS than in those incubated with PHA (Table 4). In two other experiments (data not shown) DNA synthesis induced by LPS was also inhibited by 10^{-7}–10^{-8}M coformycin alone. Combination of 10^{-7}–10^{-8}M coformycin and 10^{-4}M (but not 10^{-5}M) adenosine produced a synergistic inhibition of DNA synthesis induced by either mitogen in mouse spleen cell cultures (Table 4).

Toxicity of adenosine and coformycin to proliferating lymphocytes

Human peripheral blood lymphocytes labelled with ^{51}Cr were exposed to PHA, 10^{-5}M adenosine and 10^{-7}M coformycin and the percentage of ^{51}Cr released from the cells was

TABLE 3. Effect of adenosine and coformycin on DNA synthesis in human mixed lymphocyte cultures

Culture	[^{3}H]dT incorporation			
	Experiment 1		Experiment 2	
	Ct/min	Inhibition (%)	Ct/min	Inhibition (%)
A Individual 1 alone	530±70		930±250	
B Individual 2 alone	1890±180		320±50	
C 1+2	17,120±1850		21,930±1900	
D 1+2+				
Coformycin 10^{-6}M	7630±450	69	22,530±60	0
10^{-7}M	15,370±2650	24	21,310±1520	3
10^{-8}M	13,620±10	34	20,070±200	9
Adenosine 10^{-5}M	12,570±50	40	24,920±720	0
Adenosine 10^{-5}M +coformycin	3850 950	91		
10^{-6}M			1310±150	100
10^{-7}M	9660±2800	57	9580±2020	60
10^{-8}M	13,760±3500	33	13,360±140	41

Cells from two individuals, 5×10^5 of each, were mixed and incubated with the indicated additions for 5 days. DNA synthesis was then measured by labelling the cultures with 1 μCi of [^{3}H]dT for 6 hr. Mean and range of duplicate cultures are given. Percentage inhibition is calculated according to the following formula:

$$D\% = 100 - \frac{D_{ct/min} - (A_{ct/min} + B_{ct/min})}{C_{ct/min} - (A_{ct/min} + B_{ct/min})} \times 100.$$

A–D are shown in the table.

TABLE 4. Effect of adenosine and coformycin on mitogen-induced DNA synthesis in mouse spleen cell cultures

Additions	Unstimulated cells		PHA 1 μg/ml		LPS 40 μg/ml	
	Ct/min	Ratio to control	Ct/min	Ratio to control	Ct/min	Ratio to control
Control	2170±450	1·0	95,990±7420	1·0	6400±810	1·0
Coformycin 10^{-6}M	1060±10	0·49	55,840±3500	0·58	2980±240	0·47
10^{-7}M	1820±170	0·86	55,980±22,500	0·58	7140±950	1·12
10^{-8}M	1970±370	0·91	65,970±8760	0·69	10,170±40	1·59
Adenosine 10^{-3}M	220±30	0·10	1050±60	0·01	510±40	0·08
10^{-4}M	1640±170	0·75	38,200±7620	0·40	14,040±190	2·19
10^{-5}M	1640±10	0·75	86,330±17,050	0·90	12,050±990	1·88
10^{-6}M	1750±900	0·81	47,320±9850	0·49	6480±550	1·01
Adenosine 10^{-4}M+ coformycin 10^{-6}M	210±50	0·10	2240±2100	0·02	230±40	0·04
Adenosine+ coformycin 10^{-7}M	670±50	0·31	6020±910	0·06	6340±20	0·99
Adenosine+ coformycin 10^{-8}M	1010±80	0·47	14,740±9100	0·15	5960±1790	0·03

2×10^6 Mouse spleen cells were incubated with the indicated additions in 1 ml of the culture medium supplemented with 10% horse serum for 40 hr, and labelled with 1 μCi [^{3}H]dT for the following 6 hr. Mean and range of duplicate cultures are given. The percentage of inhibition of mitogen-induced DNA synthesis is calculated after subtracting the gross counts with those obtained in the respective unstimulated cultures.

measured at intervals. The drugs were not toxic to normal lymphocytes but enhanced the rate of ^{51}Cr release from PHA-stimulated cells (Fig. 3). Unlike the toxicity induced by PHA alone, ^{51}Cr release caused by the drugs was not detected during the early hours of the incubation, but, when assayed after 32 hr, the increase was seen in all three experiments (Fig. 3). Other experiments have shown that 10^{-3}–10^{-4}M adenosine alone is not toxic to small lymphocytes but caused a reproducible slight increase (10–20%) in the rate of ^{51}Cr release from PHA-stimulated cells (data not shown).

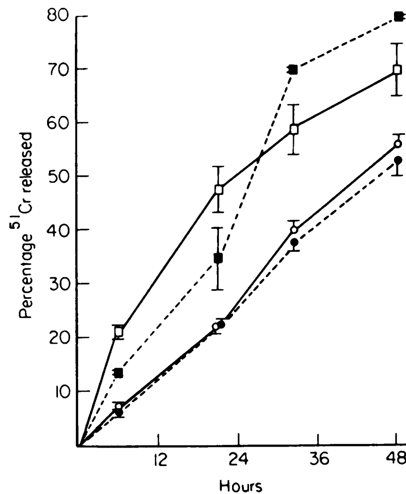

FIG. 3. Effect of adenosine and coformycin on ^{51}Cr release from PHA-stimulated human peripheral blood lymphocytes. HPB lymphocytes were labelled with ^{51}Cr and incubated with (squares) or without (circles) 1 μg/ml PHA in the presence (closed symbols) or absence (open symbols) of 10^{-5}M adenosine and 10^{-7}M coformycin. Duplicate cultures were harvested at the times indicated and the percentage of ^{51}Cr label released from the cells was measured.

DISCUSSION

We have shown that the activity of adenosine deaminase is considerably increased in human peripheral blood lymphocytes exposed to mitogenic concentrations of PHA and PWM. This increase of ADA activity, which takes place during the first cycle of proliferation, does not necessarily reflect specific enzyme induction. During the response of HPB lymphocytes to PHA there is a change of the ADA isoenzyme pattern, with a decrease in the proportion of a band showing slow electrophoretic mobility in starch gel and an increase in the proportion of more rapidly migrating forms (Hirschhorn, 1975). This change is thought to reflect the breakdown of high-molecular weight complexes ('tissue' or 'storage' form of ADA) to give rise to ADA molecules of lower molecular weight with greater specific activity. However, this change was not seen by Hirschhorn after exposure of lymphocytes to PWM, which we have also found to induce increased levels of ADA activity in HPB lymphocytes. The increase in ADA activity in cells responding to mitogens could also parallel the overall increase in cytoplasmic protein in these cells. In any case, increased ADA activity in the cells precedes the initiation of DNA synthesis and the enzyme appears to be of considerable functional importance in the transformation process. While this paper was in preparation Ochs (1975) reported an increase in ADA levels in lymphocytes incubated for several days with PHA but not in those stimulated by PWM.

Coformycin is a very potent inhibitor of ADA with a K_i of about 3×10^{-10}M (Hart & Smyth, 1976). Exposure of cultured lymphocytes to coformycin might be considered analogous to

an inherited lack of adenosine deaminase. However, in normal culture medium only LPS-induced DNA synthesis was consistently abolished by coformycin alone, whereas the combination of relatively low concentrations of the drug with exogenous adenosine produced a synergistic inhibition in all kinds of mitogenic stimulation of human or mouse lymphocytes.

The reason for the variability in effects of coformycin alone in autologous serum are unknown, but one explanation could be that during blood clotting variable amounts of adenosine or adenosine-generating nucleotides may be liberated from blood cells into serum, and thus provide a source of 'exogenous' adenosine. Adenosine has neither been detected in normal human plasma nor in that of an ADA-deficient individual (Glasky *et al.*, 1975) but the adenosine level in the immediate environment of antigen-stimulated lymphocytes in lymph nodes and spleen remains to be shown.

Inhibition of ADA by coformycin, as well as the inherited lack of this enzyme, would result in an increased intracellular concentration of adenosine, which—as shown here—can block proliferation of mitogen-stimulated lymphocytes. The inhibitory and cytotoxic properties of exogenous adenosine have been reported previously from other cell systems (Ishii & Green, 1973).

The mechanism by which increased adenosine concentration inhibits cell proliferation is still uncertain, but the observations are consistent with a single hypothesis. In mammalian cells adenosine is readily phosphorylated to 5′-AMP and then ADP and ATP. AMP, ADP and ATP are all powerful inhibitors of phosphoribosyl pyrophosphate (PRPP) synthetase (Wong & Murray, 1969; Fox & Kelley, 1971, 1972). PRPP is a common precursor of both purine and pyrimidine nucleotides and its concentration is considered to be one of the major regulatory factors for the overall activity of the *de novo* pathway of purine biosynthesis (Fox & Kelley, 1971). We have recently shown that cellular levels of PRPP increase within minutes of exposure of human lymphocytes to phytohaemagglutinin (Hovi, Allison & Allsop, 1975c). Several other lines of evidence suggest that the *de novo* pathway of purine biosynthesis is essential for lymphocyte proliferation while the salvage pathway seems to be less important. Inhibitors of the enzymes of the *de novo* pathway, such as 6-mercaptopurine and its riboside and azaserine, inhibit lymphocyte transformation (R. McKeran, personal communication, and our unpublished observations). On the other hand, lymphocytes from patients with the Lesch-Nyhan syndrome—due to a hereditary deficiency in the salvage pathway of purine biosynthesis (Seegmiller, Rosenbloom & Kelley, 1967)—show a normal mitogenic response to both PHA and PWM (R. McKeran, personal communication and our unpublished observations).

Green & Chan (1973) have suggested that the mechanism by which adenosine inhibits cell proliferation is pyrimidine starvation caused by AMP. In a recent publication in which adenosine was found to inhibit development of cytotoxic lymphocytes in culture, a cyclic AMP-mediated mode of action was suggested (Wolberg *et al.*, 1975). All these observations could, however, be explained by adenine nucleotide-induced inhibition of PRPP synthetase. In *E. coli* adenosine is known to cause a rapid decrease of PRPP levels (Bagnara & Finch, 1974).

In the course of studies of cyclic nucleotides, Hirschhorn, Grossman & Weissmann (1970) found that adenosine and AMP were more effective on a molar basis than cyclic AMP at inhibiting lymphocyte transformation. While our experiments were in progress we learned that I. Fox, E. Keystone and co-workers (personal communication) had also observed adenosine-induced inhibition of DNA synthesis in mitogen-stimulated human lymphocyte cultures.

The specificity of coformycin as a potent inhibitor of adenosine deaminase and the unique importance of this enzyme for lymphocyte proliferation, as shown by the selective effects of ADA deficiency on lymphocytes, suggest that coformycin may prove to be a powerful and relatively non-toxic immunosuppressive agent. Our observation of cytotoxicity in human

lymphocytes exposed to PHA, induced by adenosine and coformycin, raises the possibility that clones of lymphocytes responding to stimulation by specific antigens might be eliminated by treatment with coformycin. Limited availability of the drug has so far prevented direct studies of the *in vivo* effects of coformycin on immune responses.

T. Hovi was supported by a European Molecular Biology Organization Fellowship. J. F. Smyth acknowledges a Cancer Research Campaign Fellowship.

REFERENCES

BAGNARA, A.S. & FINCH, L.R. (1974) The effects of bases and nucleosides on the intracellular contents of nucleosides and 5-phosphoribosyl-1-pyrophosphate in *Escherichia coli*. *Europ. J. Biochem.* **41,** 421.

BÖYUM, A. (1968) Separation of leukocytes from blood and bone marrow. *Scand. J. clin. Lab. Invest.* **21,** supplement 97.

BRADY, T.G. (1942) Adenosine deaminase. *Biochem. J.* **36,** 478.

BRADY, T.G. & O'DONOVAN, C.I. (1965) A study of tissue distribution of adenosine deaminase in six mammal species. *Comp. biochem. Physiol.* **14,** 101.

CONWAY, E.J. & COOKE, R. (1939) The deaminases of adenosine and adenilic acid in blood and tissues. *Biochem. J.* **33,** 479.

DISSING, J. & KNUDSEN, B. (1972) Adenosine deaminase deficiency and combined immunodeficiency syndrome. *Lancet*, **ii,** 1316.

FOX, I. & KELLEY, W.N. (1971) Phosphoribosyl pyrophosphate in man: biochemical and clinical significance. *Ann. intern. Med.* **74,** 424.

FOX, I. & KELLEY, W.N. (1972) Human phosphoribosyl pyrophosphate synthetase. Kinetic mechanism and end-product inhibition. *J. biol. Chem.* **247,** 2126.

GIBLETT, E.R., ANDERSON, J.E., COHEN, F., POLLARA, B. & MEUWISSEN, H.J. (1972) Adenosine deaminase deficiency in two patients with severe impaired cellular immunity. *Lancet*, **ii,** 1067.

GLASKY, A.J., PFADENHAUER, E.H., SETTINERI, R. & GINSBERG, T. (1975) Isoprinosin a purine derivative: metabolic, immunological and antiviral effects. *Combined Immunodeficiency Disease and Adenosine Deaminase Deficiency. A Molecular Defect* (ed. by H. J. Meuwissen, R. J. Pickering, B. Pollara and I. H. Porter), p. 157. Academic Press, New York.

GREEN, H. & CHAN, T.S. (1973) Pyrimidine starvation induced by adenosine in fibroblasts and lymphoid cells: role of adenosine deaminase. *Science*, **182,** 836.

HALL, J.G. (1963) Adenosine deaminase activity in lymphoid cells during antibody production. *Aust. J. exp. Biol.* **41,** 93.

HART, L. & SMYTH, J.F. (1976) Kinetics of inhibition of adenosine deaminase by coformycin. *Biochem. J.* (In press.)

HIRSCHHORN, R. (1975) Adenosine deaminase deficiency: genetic and metabolic implications. *Combined Immunodeficiency Disease and Adenosine Deaminase Deficiency. A Molecular Defect* (ed. by H. J. Meuwissen, R. J. Pickering, B. Pollera & I. H. Porter), p. 121. Academic Press, New York.

HIRSCHHORN, R. GROSSMAN, J. & WEISSMANN, G. (1970) Effect of cyclic 3′,5-adenosine monophosphate and theophylline on lymphocyte transformation. *Proc. Soc. exp. Biol. (N.Y.)*, **133,** 1361.

HOVI, T., ALLISON, A.C. & ALLSOP, J. (1975c) Rapid increase of phosphoribosyl pyrophosphate concentration after mitogenic stimulation of lymphocytes. *FEBS Lett.*, **55,** 291.

HOVI, T., ALLISON, A.C. & WILLIAMS, S.C. (1975a) Proliferation of human peripheral blood lymphocytes induced by A23187, a *Streptomyces* antibiotic. *Exp. Cell Res.* (In press.)

HOVI, T., ALLISON, A.C. & WILLIAMS, S.C. (1975b) Mitogen-induced lymphocyte proliferation: early requirement of extracellular ionized calcium. (Submitted for publication.)

ISHII, K. & GREEN, H. (1973) Lethality of adenosine for cultured mammalian cells by interference with pyrimidine biosynthesis. *J. Cell Sci.* **23,** 429.

KALCKAR, H.M. (1947) Differential spectrophotometry of purine compounds by means of specific enzymes. *J. biol. Chem.* **167,** 416.

OCHS, H. (1975) Discussion. *Combined Immunodeficiency Disease and Adenosine Deaminaes Deficiency. A Molecular Defect.* (ed. by H. J. Meuwissen, R. J. Pickering, B. Pollara & I. H. Porter), p. 190. Academic Press, New York.

SEEGMILLER, J.E., ROSENBLOOM, F.M. & KELLEY, W.N. (1967) An enzyme defect associated with a sex-linked human neurological disorder and excessive purine synthesis. *Science*, **155,** 1682.

SMYTH, J.F. & HARRAP, K.R. (1975) Adenosine deaminase activity in leukaemia. *Brit. J. Cancer*, **31,** 544.

WARA, D.W. & AMMANN, A.J. (1975) Laboratory data. *Combined Immunodeficiency Disease and Adenosine Deaminase Deficiency. A Molecular Defect.* (ed. by H. J. Meuwissen, R. J. Pickering, B. Pollara and I. H. Porter), p. 247. Academic Press, New York.

WOLBERG, G., ZIMMERMAN, T.P., HIEMSTRA, K., WINSTON, M. & CHU, L.-C. (1975) Adenosine inhibition of lymphocyte-mediated cytolysis: possible role of cyclic adenosine monophosphate. *Science*, **187,** 957.

WONG, P.C.L. & MURRAY, A.W. (1969) 5-phosphoribosyl-1-pyrophosphate synthetase from Ehrlich ascites tumor cells. *Biochemistry*, **8,** 1608.