Purine and Phosphoribosylpyrophosphate Metabolism of Lymphocytes and Erythrocytes of an Adenosine Deaminase Deficient Immunocompetent Child

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Summary

Purine metabolism and phosphoribosylpyrophosphate content of lymphocytes and erythrocytes were studied in an immunocompetent black male child with a total deficiency of erythrocyte and partial deficiency of lymphocyte adenosine deaminase. The partial genetic deficiency of adenosine deaminase was demonstrated in intact lymphocytes, and was approximately one third of the deaminating activity of control lymphocytes. Intact lymphocytes of the patient did not incorporate adenosine at a faster rate than those of control lymphocytes. The patient's erythrocytes deaminating activity was low and adenine ribonucleotide synthesis from adenosine was increased several fold, while adenine incorporation into purine ribonucleotides was comparable to that of control erythrocytes. Transfusion with packed erythrocytes temporarily improved the deaminating capacity of circulating erythrocytes, but did not reduce the elevated incorporation of adenosine into purine ribonucleotides.

Phosphoribosylpyrophosphate content of the patient's lymphocytes and erythrocytes was not diminished. Incubation of erythrocytes with adenosine lowered phosphoribosylpyrophosphate content while incubation with phosphate increased phosphoribosylpyrophosphate content to the same extent in mutant and control erythrocytes.

Speculation

The finding that the patient's lymphocytes can deaminate adenosine to some extent may explain why the rate of adenosine incorporation into purine ribonucleotides of his lymphocytes is not increased and immune function is not impaired. Deficiency of erythrocyte adenosine deaminase results in increased adenosine incorporation, but does not appear to interfere with immune functions.

INTRODUCTION

The discovery that two inborn errors of purine metabolism, adenosine deaminase (ADA) (EC 3.5.4.4) and purine nucleoside phosphorylase (PNP) (EC 2.4.2.1) deficiency are associated with syndromes of disordered immune function highlights the importance of purine catabolism and salvage in the normal differentiation, proliferation and function of lymphocytes (8,7). Deficiency of ADA gives rise to severe combined immunodeficiency (SCID), an inherited disorder of T and B cell function, and deficiency of PNP to selective cellular immunodeficiency, an inherited abnormality of T cell function (8,5).

ADA plays an important role in the catabolism and salvage of purines. It catalyses the deamination of adenosine to inosine, which in turn is converted to hypoxanthine by PNP according to the following reactions:

1. Adenosine + H₂O → inosine + NH₃
2. Inosine ⇌ hypoxanthine + R-1-P + Pi

The two enzymes which catalyse consecutive steps in purine breakdown are also essential for the salvage of purines by providing hypoxanthine for ribonucleotide synthesis. Both enzymes play an important and as yet not clearly defined role in the normal immune functions of lymphocytes.

ADA is controlled by a single genetic locus and the enzyme deficiency is transmitted as an autosomal recessive trait from heterozygous patients (9,1,10,16). While the enzyme deficiency in SCID is clearly present at birth, failure of immune function may develop more gradually and in 15% of the patients the impairment of cellular immunity may initially be more severe than that of humoral immunity (10,16).

Phosphoribosylpyrophosphate (PP-Rib-P) is the substrate for the synthesis of phosphoribosyl-1-amine, the first intermediate in *de novo* purine biosynthesis, and also serves as a substrate for the conversion of hypoxanthine to inosinate. It is therefore an important substrate for the synthesis of purine ribonucleotides by the *de novo* and the salvage pathway. A decrease in cellular PP-Rib-P content could interfere with lymphocyte proliferation. It is known that adenosine can lower cellular PP-Rib-P (17,22). For this reason PP-Rib-P content of the patient's erythrocytes and lymphocytes was determined during his early development and the effect of adenosine on erythrocyte and lymphocyte PP-Rib-P content studied *in vitro*.

Genetic heterogeneity is to be expected in a rare autosomal recessive disorder, and to date one African child with complete ADA deficiency in lysates of erythrocytes but partial deficiency in lymphocyte lysates and apparently normal immune function has been described (15). The present study reports on the biochemical abnormalities in intact lymphocytes and erythrocytes of a black American child with total erythrocyte and partial lymphocyte ADA deficiency in whom no abnormalities in immune function have been detected during his first two years of life (8,9).

MATERIALS AND METHODS

Case history:

The patient, a black male child was the premature product (8 months gestation) of a non-consanguineous mating. At one month of age, ADA deficiency was diagnosed as part of the New York State neonatal screening program and the diagnosis confirmed by quantitative assay of hemolysates. The child was transfused for physiologic anemia at another hospital with 40 ml of packed erythrocytes. After transfer to this hospital no further transfusions have been given and the child has developed normally with no signs of graft versus host reaction. He has displayed normal humoral and cellular immune function including specific antibody production and mitogenic response to specific antigens (3,12).

Blood samples:

All studies were carried out on freshly drawn heparinized blood. Erythrocytes were harvested by centrifugation, washed in phosphate-buffered saline (0.9% NaCl in 0.01 M phosphate buffer, pH 7.3) and incubated with the appropriate radioactive tracers. Phosphoribosylpyrophosphate determinations were also carried out on unwashed erythrocytes harvested by brief (1 min) centrifugation, in a microcentrifuge. Lymphocytes were harvested by banding on a Ficoll-Hypaque gradient (Lymphoprep-Nyegaard and Co A/S Oslo, Norway); these preparations contain not only lymphocytes but also monocytes and platelets. Lymphocytes were washed (3 times) with PBS prior to incubation.

Conditions of incubation of intact cells:

Short term incubations (20 to 90 min) were carried out in air under gentle shaking in a water bath at 37°C. Longer incubations were done in an incubator in an atmosphere of 5% CO₂ and 95% air.

ADA determinations in cell lysates:

These were carried out by a modification of the method used by Hopkinson et al. (14). Hemolysates and lymphocyte lysates were prepared and assayed as previously described at 1.3 mM adenosine (11,13). Briefly, the assay (14) is based on two linked reactions, the inosine produced is converted to hypoxanthine by the action of endogenous nucleoside phosphorylase present in the hemolysates, or by adding the enzyme to lymphocyte lysates. Hypoxanthine is oxidized to uric acid by including xanthine oxidase in the reaction mixture.

A more direct assay of ADA measured the deamination of [8-¹⁴C] adenosine. For this assay hemolysates were diluted in 0.01 M Tris-HCl buffer pH 7.5 (1:81 times for control, 1:10 times for ADA⁻ samples). Hemolysates (25 µl) were incubated with [8-¹⁴C] adenosine (0.09 µM) in 0.2 ml 50 mM Tris-HCl EDTA buffer pH 7.5. Incubations were carried out for 30 to 120 min in the presence and absence of 50 µM erythro-5-2-hydroxy-3-nonyl adenine (EHNA). Reactions were terminated by the addition of ice cold perchloric acid; after centrifugation at 4°C the supernatant fraction was neutralized with KOH and the precipitate removed by centrifugation. Adenosine was separated from hypoxanthine, inosine and adenine by paper chromatography on Whatman DE 81 paper developed in 1 M ammonium formate (6). Radioactivity was counted in toluene containing 2,5 diphenyloxazole and p-bis 2-(5 phenyloxazolyl) benzene (PPO and POPOP) in a Beckman liquid scintillation counter. Values are reported as EHNA inhibitable activity. The assay is linear with time and concentration of cell lysates.

Incorporation of [8-¹⁴C] adenosine and [8-¹⁴C] adenine into purine ribonucleotides of intact cells:

Following incubation cells were harvested by centrifugation at 600 x g, all pellets were extracted with 600 µl 2N perchloric acid, supernatant fractions were neutralized with KOH and aliquots of the supernatant fractions applied to formate washed plastic backed polyethyleneimine thin layer plates (Machery Nagel and Co. distributed by Brinkman). Plates were developed stepwise in ammonium formate (7), or lithium chloride (19).

Adenosine metabolites in the media were identified by thin layer chromatography on polyethyleneimine plates which were developed in 1-butanol, methanol, water and ammonium hydroxide (60:20:20:1). Radioactivity was counted in toluene (5g PPO per liter) in a Beckman liquid scintillation counter.

PP-Rib-P content was determined in freshly harvested cells as described (23). Briefly 1.4 x 10⁶ lymphocytes or 10-20 µl packed erythrocytes were suspended in 300 µl 1 mM potassium phosphate buffer pH 7.3 containing 1 mM EDTA. Cells were heated for 45 seconds at 90°C, chilled on ice and centrifuged at 4°C. The supernatant fractions were incubated with 0.2 µCi [7-¹⁴C] orotic acid (S.A. 42.2 mCi/mmol), 4 mM MgCl₂, and 1 mg freshly prepared yeast orotidylate synthetase (P-L Biochemicals Inc) containing orotidylate decarboxylase. [¹⁴CO₂] was collected on filters wetted with KOH and counted in toluene diphenyloxazole (5g PPO/l).

Chemicals

Radioactive purine bases, nucleosides and orotic acid were purchased from New England Nuclear, [³H]-thymidine (S.A. 1.9 Ci/mM) from Schwarz Mann Co. EHNA was purchased from Burroughs Wellcome, Triangle Park, s.c. Purine nucleosides and bases were purchased from Calbiochem, tissue culture materials from Grand Island Biologicals (Gibco).

RESULTS

Adenosine deaminase activity in cell free extracts of erythrocytes and lymphocytes:

The patient was studied during his first year of life, prior to and following the transfusion of packed erythrocytes. Determinations of the specific activity of ADA are recorded in Table I. ADA activity of lymphocyte preparations was not affected by the transfusion; it was approximately 15% of normal before and after the administration of packed erythrocytes.

In hemolysates enzyme activity was initially low (1 nmol/mg Hb/hr), but increased following transfusion. After 10 days it was 28.6 nmol/mg Hb/hr; it then declined in a logarithmic fashion and reached pretransfusion levels after approximately 4 months.

Adenosine deaminase activity in intact lymphocytes and erythrocytes:

Adenosine metabolism of intact cells was initially carried out when the child was 45 days old, two weeks after he had been transfused with packed erythrocytes. Adenosine deamination in freshly harvested cells incubated in Hanks' solution was measured by following the metabolites of [8-¹⁴C] adenosine excreted into the medium. In intact lymphocytes (Table II Exp. A) deamination was abnormally low but detectable throughout the course of study. When control lymphocytes were incubated with low concentration (9 µM) of adenosine, less than 0.2% of radioactivity in the medium was recovered as adenosine, while over 99% of the radioactivity was in inosine and hypoxanthine. In the medium of the patient's lymphocytes 60-83% of the radioactivity was recovered as adenosine. At high adenosine concentration (109 µM), control lymphocytes metabolized 60% of the substrate while the patient's lymphocytes deaminated 12% of the available adenosine into hypoxanthine and inosine (Table II Exp.B).

Impairment of adenosine deamination in the child's intact erythrocytes was initially masked by the transfused donor erythrocytes. Thirty seven days after transfusion, activity in hemolysates was only 15% that of control hemolysates (Table I). Intact erythrocytes obtained from the patient at that time could metabolize almost all (97 to 99%) of the adenosine (Table II Exps. C and D). The enzyme deficiency became more readily apparent 106 days after transfusion, when donor cells were no longer present and even low concentrations of adenosine (37 µM) were only partially metabolized and approximately 80 to 90% of the radioactivity in the medium was recovered as adenosine (Table II Exp. C). Control erythrocytes deaminated adenosine completely and therefore their deaminating capacity exceeds that measured under these experimental conditions.

It is of interest to note that, at 106 days after transfusion, when the ADA⁻ erythrocytes deaminated adenosine poorly (Table II Exps. C and D), a small fraction (0.9-3.5%) of [8-¹⁴C] adenosine was metabolized to [8-¹⁴C] adenine. Less than 1% of the radioactive metabolites chromatographed as adenine in ADA⁺ erythrocytes or erythrocytes obtained from the patient 15 and 37 days following transfusion. In ADA deficient lymphocytes the appearance of radioactive adenine in the media was not as consistent (Table II Exp. A).

Incorporation of [8-¹⁴C] adenosine into lymphocytes and erythrocytes:

The incorporation of [8-¹⁴C] adenosine into acid soluble purine ribonucleotides was measured in resting and PHA stimulated lymphocytes (Table III). In resting lymphocytes (Exps. A and B) adenosine incorporation was no higher in the ADA⁻ than in the control. PHA stimulation for 3 hrs failed to increase adenosine incorporation in either the ADA⁻ or the control cells (Table III Exp. B).

In contrast to the findings in ADA⁻ lymphocytes (Table III), the incorporation of adenosine into ribonucleotides by erythrocytes obtained from the patient was several fold higher than in control erythrocytes (Table IV). Surprisingly, as early as 15 days following transfusion, adenosine incorporation of the patient's erythrocytes was high and remained so throughout the study. This difference in incorporation was equally marked at low (Exp. A) and at high (Exp. B) adenosine concentrations.

Incorporation of adenine into ribonucleotides was no higher in the patient's erythrocytes than in the control in studies carried out simultaneously (Table IV Exp. C).

The radiolabelling pattern of adenosine into adenine and guanine ribonucleotides of resting ADA⁺ and ADA⁻ lymphocytes was compared 135 days after transfusion. Adenosine incorporation into adenine and guanine nucleotides was similar in ADA deficient and control lymphocytes. In erythrocytes, despite the quantitative difference in adenosine incorporation between the patient and

the control, the distribution into adenine and guanine ribonucleotides was similar. Approximately 80% of all ribonucleotides newly synthesized were adenine nucleotides (data not shown).

Comparison of the incorporation of [8-¹⁴C] adenosine and [3H] thymidine into TCA precipitable material during the proliferative response to mitogens also failed to reveal an increase in the incorporation of adenosine relative to that of thymidine in the patient's lymphocytes (Table V). Although there appears to be an increased ratio of [3H] thymidine to [8-¹⁴C] adenosine in the patient's lymphocytes in response to PHA, this increase was not seen during the response to pokeweed mitogen (PWM) or concanavalin A (Con A).

Phosphoribosylpyrophosphate (PP-Rib-P) content of lymphocytes and erythrocytes.

The average PP-Rib-P content of the patient's ADA⁻ lymphocytes freshly harvested from a Ficoll-hypaque gradient was 28 ± 16.7 pmol/10⁶ cells, that of control lymphocytes was 42 ± 8.5 pmol/10⁶ cells (Table VI). This difference, between PP-Rib-P content of the patient's and control lymphocytes, was not statistically significant. PP-Rib-P content of washed erythrocytes did not vary in the patient between the 15th and 200th day following transfusion and did not differ significantly from the control.

In order to more closely mimic *in vivo* conditions, mutant and control lymphocytes were incubated in the patient's plasma as well as in control plasma (69 min at 37°C (Table VII Exp. 1a)). PP-Rib-P content was somewhat, but not significantly, lower when cells were incubated in the patient's plasma. Similarly, PP-Rib-P content of freshly drawn, packed erythrocytes was also determined (Table VII Exp. 1a) and again PP-Rib-P content of mutant and control erythrocytes was similar. When PP-Rib-P synthesis was stimulated by the addition of 30 mM PO₄ during incubation of erythrocytes in either Hanks' solution or control plasma (Table VII Exps. 1b, 2 and 3), PP-Rib-P accumulation was comparable in the mutant and the control erythrocytes.

The effect of adenosine, in concentrations similar to those observed in ADA deficient patients, on cellular PP-Rib-P content was tested. Erythrocytes were incubated with varying concentrations (7 to 700 μM) of adenosine (Table VIII) at low (0.8 mM) and high (30 mM) phosphate concentrations in the medium. Adenosine depressed PP-Rib-P content of ADA⁻ and control erythrocytes; this was particularly evident at high (30 mM) phosphate concentrations.

DISCUSSION

The present study provided the first opportunity to investigate adenosine metabolism and PP-Rib-P content of lymphocytes and erythrocytes of an ADA deficient child who had been transfused once, at one month of age, and who has as yet not developed clinical or laboratory evidence of immunodeficiency (3). In the patient reported here enzyme deficiency, when measured in lysates of erythrocytes (Table I) was comparable to that observed in other children with ADA deficiency but who suffer from SCID (12). In contrast, lysates of lymphocytes and lymphoid cell lines had 20% of normal ADA activity; and this residual activity was found to be abnormally labile (12). *In vivo* the enzyme deficiency was manifested by increased concentrations of adenosine in his plasma and erythrocytes. So far ADA deficiency and normal immune function has been reported only in one other instance, in a black child of the !Kung tribe (15). ADA activity of hemolysates was low (2-3%), but in lymphocyte lysates the activity was 10-15% of normal lysates; no studies were carried out in intact cells (15).

In the study of adenosine metabolism in intact lymphocytes of our patient the defect in deamination was only partial and was similar to that in lymphocyte lysates. When the patient's lymphocytes were incubated in medium containing [8-¹⁴C] adenosine approximately two thirds of the radioactivity in the medium was recovered in adenosine, while ADA⁺ lymphocytes under the same experimental conditions metabolized adenosine completely to inosine and hypoxanthine. In contrast, intact lymphocytes of an ADA⁺ patient with SCID revealed a more severe impairment of deamination, less than 5% of adenosine was deaminated and incorporation of adenosine into ribonucleotides was increased (18).

In contrast to the patient with SCID the incorporation of [8-¹⁴C] adenosine into purine ribonucleotides of the patient's lymphocytes was no higher than that of ADA⁺ lymphocytes, even when lymphocytes were stimulated with PHA for 3 hours. The distribution of radioactivity recovered in adenine and guanine nucleotides was similar in the ADA⁺ and ADA⁻ lymphocytes in spite of the defect in deamination. In ADA⁺ fibroblasts and in Lesch-Nyhan lymphoblasts adenosine incorporation into guanine nucleotides is not decreased suggesting that the deamination of AMP to IMP could contribute not only to the synthesis of guanine ribonucleotides but could also explain the observations that uric acid excretion in ADA deficiency is not unusually low (20,21,22).

In intact erythrocytes the defect in deamination was also apparent. However the relative degree of deficiency could not be accurately assessed, since ADA⁺ erythrocytes deaminated adenosine completely even at very high adenosine concentrations in the medium. Adenosine incorporation into ribonucleotides in the patient's erythrocytes was distinctly different from that of lymphocytes; it was significantly higher in the patient's erythrocytes than in the control. This finding was surprising and as yet unexplained because it occurred even when ADA deficiency was attenuated by the presence of donor erythrocytes in the blood sample (Tables I and II). While adenosine incorporation into ribonucleotides was high in the patient's erythrocytes, adenine incorporation was not elevated and was similar to that of the control throughout the time of the study (Table IV Exp. C). Most recently the intracellular concentrations of 2'-dATP of the erythrocytes of this patient were measured and found within normal limits while 2'-dATP levels of erythrocytes of patients with SCID were more than 50-fold higher than in control erythrocytes (23).

Adenosine can lower cellular PP-Rib-P content and thereby possibly impair lymphocyte proliferation (17,22). So far PP-Rib-P content of erythrocytes and lymphocytes of ADA deficient patients with SCID have not been reported. In our patient's lymphocytes the cellular PP-Rib-P content was not statistically different from that of the control nor was the PP-Rib-P content of his erythrocytes abnormally low even though they lacked ADA activity (Table VII). Incubation of erythrocytes with 30 mM PO₄, which stimulates PP-Rib-P synthesis provided further proof that ADA deficient erythrocytes unlike purine nucleoside phosphorylase deficient erythrocytes do not accumulate excessive amounts of PP-Rib-P (Tables VII and VIII) (4).

This study of purine and PP-Rib-P metabolism of lymphocytes and erythrocytes of an ADA deficient child, in whom cellular and humoral immune functions were not impaired, shows that some but not all of the biochemical abnormalities found in children with SCID were present (3,12,4). In contrast to patients with SCID we found no increase in the incorporation of [8-¹⁴C] adenosine into ribonucleotides of lymphocytes and only partial impairment of adenosine deami-

nation in intact lymphocytes. This latter observation correlates with the findings in lymphocyte lysates of this patient (Table I) (3,12). Although these findings may explain why this ADA deficient child and the children in the !Kung tribe remain immunocompetent, there may be other as yet unrecognized mechanisms which are important for the integrity of immune function.

REFERENCES AND NOTES

- Ackert, C., Pluss, H.J. and Hitzig, W.H.: Hereditary severe combined immunodeficiency and adenosine deaminase deficiency. *Pediat. Res.*, 10: 67 (1976).
- Benke, P.J. and Dittmar, D.: Purine function in cells from patients with adenosine deaminase deficiency. *Pediat. Res.*, 10: 642 (1976).
- Borkowsky, W., Gershon, A.A. and Hirschhorn, R.: Adenosine deaminase deficiency without immunodeficiency. *Pediat. Res.*, 12: 478, (1978).
- Cohen, A., Doyle, D., Martin, D.W., Jr. and Amman, A.J.: Abnormal purine metabolism and purine overproduction in a patient deficient in purine nucleoside phosphorylase. *N. Engl. J. Med.*, 295: 1449 (1977).
- Cohen, A., Hirschhorn, R., Horowitz, S.D., Rubinstein, A., Polmar, S.H., Hong, R. and Martin, D.W., Jr.: Deoxyadenosine triphosphate as a potentially toxic metabolite in adenosine deaminase deficiency. *Proc. Natl. Acad. Sci. USA*, 75: 472 (1978).
- Coleman, M.S. and Hutton, J.J.: Micro method for quantitation of adenosine deaminase activity in cells from human peripheral blood. *Biochem. Med.*, 13: 46 (1975).
- Giblett, E.R., Amman, A.J., Wara, D.W., Sandman, R. and Diamond, L.K.: Nucleoside-phosphorylase deficiency in a child with severely defective T-cell immunity and normal B-cell immunity. *Lancet*, i: 1010 (1975).
- Giblett, E.R., Anderson, J.E., Cohen, F., Pollara, B. and Meuwissen, H.J.: Adenosine deaminase deficiency in two patients with severely impaired cellular immunity. *Lancet*, ii: 1067 (1972).
- Hirschhorn, R.: Conversion of human erythrocyte-adenosine deaminase activity to different tissue specific isoenzymes. *J. Clin. Invest.*, 55: 661 (1975).
- Hirschhorn, R.: Defects of purine metabolism in immunodeficiency diseases. *Progress in Clinical Immunology III*, Robert S. Schwartz ed., Grune and Stratton, pp. 67-83 (1977).
- Hirschhorn, R., Beratis, N., Rosen, F.S., Parkman, R., Stern, R. and Polmar, S.: Adenosine deaminase deficiency in a child diagnosed prenatally. *Lancet*, i: 73 (1975).
- Hirschhorn, R., Borkowsky, W., Bajaj, S. and Gershon, A.: Adenosine deaminase deficiency in a normal child: Immunologic and biochemical studies. *Clin. Res.*, 26: 552A, (1978) (Abstract).
- Hirschhorn, R. and Sela, E.: Adenosine deaminase and immunodeficiency: an *in vitro* model. *Cellular Immunology*, 32: 350 (1977).
- Hopkinson, D.A., Cook, P.J.L. and Harris, H.: Further data in the adenosine deaminase (ADA) polymorphism and a report of a new phenotype. *Ann. Hum. Genet.*, 32: 361 (1969).
- Jenkins, T., Rabson, A.R., Nurse, G.T., Lane, A.B. and Hopkinson, D.A.: Deficiency of adenosine deaminase not associated with severe combined immunodeficiency. *J. Pediatr.*, 89: 732 (1976).
- Meuwissen, H.J., Pollara, B. and Pickering, R.J.: Combined immunodeficiency disease associated with adenosine deaminase deficiency. *J. Pediatr.*, 86: 169 (1975).
- Planet, G. and Fox, I.H.: Inhibition of phosphoribosylpyrophosphate synthesis by purine nucleosides in human erythrocytes. *J. Biol. Chem.*, 251, 5839 (1976).
- Raivita, O.K., Schwartz, A.L., Stern, R.C. and Polmar, S.H.: Adenine and adenosine metabolism in lymphocytes deficient in adenosine deaminase activity. *Purine Metabolism in Man II*, Miller, M.M., Kaiser, E., Seegmiller, J.E. eds. Plenum Press p. 448 (1977).
- Randerath, K.: Thin layer chromatography. Academic Press pp. 219-233 (1968).
- Reem, G.H.: Purine biosynthesis in mutant mammalian cells Purine and Pyrimidine Metabolism Ciba Foundation Symposium 48 (new series), Elsevier, Excerpta Medica, North Holland pp. 105-126 (1977).
- Reem, G.H.: Adenosine metabolism in permanent lymphocyte lines and in erythrocytes of patients with the Lesch-Nyhan syndrome. *Purine Metabolism in Man II*, Miller, M.M., Kaiser, E. and Seegmiller, J.E. eds., Plenum Press, pp. 384-390 (1977).
- Reem, G.H.: Regulation of phosphoribosylpyrophosphate synthesis in control and Lesch-Nyhan lymphoblasts in culture. *Human Heredity*, 27: 203 (1977) (Abstract).
- Reem, G.H. and Friend, C.: Purine and phosphoribosylpyrophosphate synthesis in differentiating murine virus-induced erythroleukemic cells *in vitro*. *J. Cell. Physiol.*, 88: 193 (1976).
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TABLE I

ADA ACTIVITY IN LYSATES OF ERYTHROCYTES AND LYMPHOCYTES OF AN ADA DEFICIENT PATIENT BEFORE AND AFTER TRANSFUSION WITH PACKED ERYTHROCYTES

Days after transfusion	Erythrocyte lysates	Lymphocyte lysates
-2	0.9	140
10	28.6	224
30	13.5	
51	10.5	
62	5.6	
76	4.4	
85	2.5	
106	2.4	
130	1.4	204
148	0.05*	166
171	0.06*	
Control	Mean 84 ± 29	1363 ± 247
Control*	Mean 69 ± 21	----

Erythrocytes were harvested by centrifugation from freshly drawn heparinized blood, and washed in phosphate buffered saline; lymphocytes were harvested by banding on Ficoll-Hypaque, washed with phosphate buffered saline three times, resuspended in buffer pH 7.5 ($2.5 - 5 \times 10^6$ cells/ml), lysed by freeze thawing (5 times) and brief sonication. ADA activity determined as described in Materials and Methods.

Results are expressed as nmol/mg Hb/hr for erythrocytes, nmol/mg protein/hr for lymphocytes (mean and standard deviation).

* denotes assays were carried out with $[8-^{14}C]$ adenosine (89 μM) as substrate and in the presence and absence of EHNA (50 μM). Activity inhibitable by EHNA is reported.

TABLE II

ADENOSINE METABOLISM OF INTACT LYMPHOCYTES AND ERYTHROCYTES ON AN ADA DEFICIENT PATIENT AND OF CONTROL SUBJECTS

Additions	Days after transfusion	Adenosine and its metabolites in media (%)				
		AR	I	Hx	A	
<u>LYMPHOCYTES</u>						
<u>Exp. A</u>						
ADA ⁻	Adenosine (9 μM)	15	61.0	23	14	2.0
		37	61.0	23	14	2.0
		106	65.0	24	9	2.0
		135	83.0	9	8	0.2
Control	1 2	--	0.17	50	50	0.2
		--	0.13	60	40	0.4
<u>Exp. B</u>						
ADA ⁻	Adenosine	106	87.0	4	8	0.6
Control	(109 μM)	--	39.0	39	21	0.6
<u>ERYTHROCYTES</u>						
<u>Exp. C</u>						
ADA ⁻	Adenosine (37 μM)	15	0.24	19	80	0.6
		37	0.18	53	47	0.3
		106	86.0	1	11	2.6
		135	63.0	1	34	2.0
Control	1 2 3	--	0.25	29	70	0.3
		--	0.28	7	93	0.2
		--	0.20	5	84	0.3
<u>Exp. D</u>						
ADA ⁻	Adenosine (137 μM)	37	3.0	47	49	0.3
		106	88.0	3	9	0.9
		106*	77.0	5	16	3.5
Control		--	0.21	21	79	0.1
		--*	0.1	31	69	0.2

Lymphocytes ($2 - 4 \times 10^6$ cells) were incubated with $[8-^{14}C]$ adenosine in 1 ml Hanks' solution buffered with Tris-Hepes to pH 7.4, containing 5.5 mM glucose and 0.8 mM phosphate. Incubations were carried out in air for 30 min at 37°. Erythrocytes ($25 \mu l$ packed cells) were incubated in a total volume of 250 μl and incubations carried out as above for 30 min at 37°. In experiment D incubations (*) were also carried out for 90 min. Results are expressed as percent radioactivity in adenosine (AR), inosine (I), hypoxanthine (Hx), and adenine (A) recovered in the media. For details see Materials and Methods.

TABLE III

COMPARISON OF THE INCORPORATION OF $[8-^{14}C]$ ADENOSINE IN RESTING AND PHA STIMULATED ADA⁻ AND CONTROL LYMPHOCYTES

	Additions	ADA ⁻	Control
Exp. A	none	324*	264*
Exp. B	none	600	850
	PHA	500	975

* pmol/ 10^6 cells

Exp. A. 3×10^6 lymphocytes were incubated for 30 min with $[8-^{14}C]$ adenosine (9 μM) under the conditions described for Table II.

Exp. B. 2×10^5 lymphocytes were incubated for 165 min in 0.2 ml RPMI 1640 medium containing 20% heat inactivated (30 min 60°) horse serum, 1 mM glutamine, 100 units penicillin and 100 μg streptomycin/ml. Phytohemagglutinin (MR 68 Burroughs Wellcome) 2 μg /ml was added as noted. Final adenosine concentration was 25 μM .

TABLE IV

COMPARISON OF THE INCORPORATION OF $[8-^{14}C]$ ADENOSINE AND ADENINE INTO ERYTHROCYTE PURINE RIBONUCLEOTIDES BEFORE AND AFTER TRANSFUSION

Additions	Days after Transfusion	CPM x 10^{-6} /ml packed cells	
		ADA ⁻	Control*
Exp. A Adenosine (37 μM)	15	28.8	5.3
	37	21.2	7.2
	106	32.1	3.0
	137	26.8	6.8
Exp. B Adenosine (137 μM)	15	13.0	2.9
	37	11.6	2.6
	137	14.6	3.2
Exp. C Adenine (37 μM)	15	--	--
	37	6.6	5.9
	106	2.9	1.4
	137	2.3	2.4

For conditions of incubation see Table II. For Exp. C $[8-^{14}C]$ adenine was substituted for $[8-^{14}C]$ adenosine.

TABLE V

INCORPORATION OF $[8-^{14}C]$ ADENOSINE AND $[^3H]$ THYMIDINE INTO TCA PRECIPITABLE MATERIAL OF MITOGEN STIMULATED LYMPHOCYTES

P R E C U R S O R				
Additions	Subject	[³ H] thymidine	[8- ¹⁴ C] adenosine	Ratio
CPM				³ H/ ¹⁴ C
PHA	ADA ⁻	57,500	1900	31
	Control 1	26,600	2800	10
	Control 2	34,800	3900	9
PWM	ADA ⁻	10,700	2870	3.7
	Control 1	12,400	1900	6.4
	Control 2	8,100	2050	4.0
Con A	ADA ⁻	20,000	3200	6.3
	Control	10,000	2500	4.0

Lymphocytes were cultured at a density of 10^6 cells/ml in 0.2 ml RPMI 1640 (see legend for Table III) with phytohemagglutinin (PHA MR 68 Burroughs Wellcome 5 μg /ml), pokeweed mitogen (PWM GIBCO) diluted to a final concentration of 1 to 40 or Concanavallin A (Con A Miles Yeda 10 μg /ml) for 72 hrs. Cells were pulsed with $[^3H]$ thymidine for the last 8 hours or with $[8-^{14}C]$ adenosine for 2 hrs. For details see Materials and Methods.

TABLE VI
PP-Rib-P CONTENT OF LYMPHOCYTES AND ERYTHROCYTES OF AN ADA⁻ PATIENT
FOLLOWING TRANSFUSION OF PACKED ERYTHROCYTES

Days after Transfusion	LYMPHOCYTES*		ERYTHROCYTES**	
	ADA ⁻	Control	ADA ⁻	Control
15	--	--	3.4	1.5
37	24	36	3.9	2.2
106	18	52	--	--
135	52	39	3.0	2.4
200	16	--	2.8	1.7
Control mean (n=8)	45 ± 8.5		2.5	
Range	(36 - 59)		1.5 - 4.0	

Freshly drawn heparinized blood was diluted 1:1 in phosphate buffered saline, erythrocytes were harvested by slow centrifugation; lymphocytes banded on a Ficoll-Hypaque gradient. Cells were washed twice with phosphate buffered saline and PP-Rib-P content determined as described in Materials and Methods. Freshly drawn blood of normal adults who had not been transfused was used as control. Control determinations were done simultaneously as noted as well as on separate occasions.

* pmol/10⁶ cells

** nmol/ml packed RBC

TABLE VII
PP-RIB-P ACCUMULATION IN ADA⁻ CONTROL LYMPHOCYTES AND ERYTHROCYTES DURING INCUBATION UNDER VARYING CONDITIONS

	Conditions of Incubation	Days after Transfusion	PP-Rib-P Content			
			ERYTHROCYTES		LYMPHOCYTES	
			ADA ⁻	Control	ADA ⁻	Control
Exp. 1a	No incubation	200	2.3	1.6	16	--
	ADA ⁻ plasma	200	2.6	2.1	12	44
	Control plasma	200	1.8	1.6	20	60
Exp. 1b	Control plasma + 30 mM PO ₄		110	140		
Exp. 2	Hanks ¹					
	+ 0.8 mM PO ₄	15	6.3	1.9		
		37	4.3	4.0		
		135	3.2	2.5		
Exp. 3	Hanks ¹					
	+ 30 mM PO ₄	15	207	160		
		37	234	281		
		106	174	162		
		135	150	181		

Erythrocytes were harvested from freshly drawn heparinized blood by centrifugation for 1 min in a microcentrifuge. PP-Rib-P content was measured immediately on unwashed cells (no incubation) and after incubation for 60 min in 1 ml Hanks¹ solution or plasma with the additions as noted. Lymphocytes (1.5 - 3 x 10⁶ cells) were incubated in 1 ml plasma. All incubations were carried out in air at 37° and determinations performed in duplicate. PP-Rib-P content was determined immediately as described in Materials and Methods.

TABLE VIII
EFFECT OF ADENOSINE OF PP-RIB-P CONTENT OF ADA⁻ AND CONTROL ERYTHROCYTES

	Additions		PP-Rib-P Content			
			ADA ⁻		Control	
	Adenosine μM	PO ₄ mM	nmol/ml cells	%	nmol/ml cells	%
Exp. 1	0	0.8	3.2	100	2.5	100
	7	0.8	1.7	53	2.0	80
	70	0.8	1.1	34	2.2	88
	700	0.8	0.9	28	1.6	64
Exp. 2	0	30	150	100	181	100
	7	30	60	33	72	40
	70	30	12	8	--	--
	700	30	5	3	14	8

PP-Rib-P content of freshly drawn packed erythrocytes (25 μl) was determined 200 days after transfusion. Erythrocytes were incubated in air for 60 min in 1 ml Hanks¹ solution at 37°C.