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Research Article

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Overproduction of Adenine Deoxynucleosides and Deoxynucleotides in Adenosine Deaminase Deficiency with Severe Combined Immunodeficiency Disease

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ABSTRACT The deoxynucleotide, dATP, is elevated 50- to 1,000-fold above normal in erythrocytes, lymphocytes, and bone marrow from a child with adenosine deaminase deficiency and severe combined immunodeficiency disease. The child, when 17 mo of age, was also excreting ~30 mg of deoxyadenosine per day in urine (normal is <0.1 mg/day). Urinary excretion of uric acid was decreased. Elevated dATP levels in lymphocytes and bone marrow, and increased urinary excretion of deoxyadenosine, persisted despite hypertransfusion of the child with irradiated erythrocytes from a donor with normal adenosine deaminase. Overproduction of deoxynucleotides by increased salvage of adenosine appears to be the primary metabolic abnormality in patients with adenosine deaminase deficiency.

INTRODUCTION

A deficiency of adenosine deaminase (adenosine aminohydrolase, EC 3.5.4.4) activity is associated with the clinical syndrome of severe combined immunodeficiency with impairment of both cellular and humoral immunity (1). The nature of the metabolic abnormalities resulting from the enzyme defect and the causes of the selective effects on lymphoid cells are under investigation. Deoxyadenosine 5'-triphosphate and deoxyadenosine 5'-diphosphate are the most abundant nucleotides in erythrocytes from children with adenosine deaminase (ADA)[1] deficiency, in striking contrast to normal erythrocytes where deoxynucleotides are not detectable (2–4). We wish to report increased levels of dATP in the lymphocytes and bone marrow and increased excretion of deoxyadenosine in the urine of a patient with ADA deficiency. These abnormalities persisted despite efforts to replace the missing enzyme by hypertransfusion of the patient with normal erythrocytes. Our data suggest a generalized overproduction of deoxyadenosine, perhaps by increased salvage of adenosine, as the primary metabolic abnormality in ADA deficiency with severe combined immunodeficiency.

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[1] *Abbreviation used in this paper*: ADA, adenosine deaminase.

METHODS

Patient. The black male infant is the first child of healthy parents with no family history of immunologic disease. The child was admitted to the Children's Hospital (Cincinnati, Ohio) at 4 mo of age and diagnosed as having severe combined immunodeficiency. Less than 3% of the normal activity of ADA was present in erythrocytes, lymphocytes, and granulocytes (3). Both parents were heterozygous with less than normal levels of ADA activity. For the past 18 mo the child has been treated with regular transfusions of irradiated erythrocytes in an effort to replace the missing enzyme (5).

Chemicals. Radiolabeled adenosine and deoxynucleoside 5'-triphosphates were obtained from New England Nuclear, Boston, Mass. All other nucleosides and nucleotides were purchased from Sigma Chemical Co., St. Louis, Mo. *Escherichia coli* DNA polymerase I was from Boehringer Mannheim Biochemicals, Indianapolis, Ind. Activated DNA was prepared in our laboratory (6). All other chemicals were reagent grade from commercial sources.

Cell separations and preparations of extracts. Samples of peripheral blood and bone marrow were anticoagulated with EDTA. Purified lymphocytes from each were prepared on Ficoll-Hypaque gradients (Pharmacia Fine Chemicals, Div. of Pharmacia Inc., Piscataway, N. J.) (3). Nucleotides and nucleosides were extracted from cells by suspension in 60% methanol overnight at −20°C. The samples were centrifuged and the supernatant fraction was dried under a stream of nitrogen gas. The residue was resuspended in a small volume of water.

Preparation of urine samples. Nucleosides were isolated

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from urine by anion-exchange chromatography (7). Recoveries of nucleosides were >95% as estimated by use of internal standards. The nucleoside fraction was lyophilized to dryness and reconstituted in an appropriate volume of water.

Assay of deoxynucleotides. Deoxynucleotides were estimated using DNA polymerase (8). Standard curves were prepared over the range of 0.2–1.0 nmol of the appropriate deoxynucleotide. The reactions were carried out for 45 min to assure total nucleotide incorporation into DNA. A 25-μl aliquot of the reaction mixture was then spotted on glass fiber disks and processed (9).

High performance liquid chromatography. Analyses of nucleoside pools from urine were carried out using a Varian model 8530 high pressure liquid chromatograph (Varian Associates, Palo Alto, Calif.) with a UV detector set at 254 nm. For nucleoside resolution, a strong cation-exchange column (2 mm × 25 cm) packed with Aminex A-7 resin (Bio-Rad Laboratories, Richmond, Calif.) was utilized at 52°C under isocratic conditions. The buffer consisted of 0.2 M ammonium formate, pH 4.55, with a flow rate of 5 ml/h and a pressure maximum of 2,000 psi (10).

RESULTS

We have reported greatly increased levels of adenine deoxynucleotides in the erythrocytes of this child with ADA deficiency and severe combined immunodeficiency (2, 3). Because of the child's young age and lymphopenia we were unable to obtain sufficient number of lymphocytes to measure nucleotide pool sizes. The child is now older and his immune status has improved somewhat, perhaps because of repetitive transfusion with irradiated erythrocytes over the past year and a half. Specimens of lymphocytes obtained from peripheral blood when the child was 17 and 21 mo of age have both shown tremendous elevations of the intracellular concentration of dATP (Table I). Normal lymphocytes contain 2–4 pmol of dATP/10^6 cells. Lymphocytes from the patient contained 770 and 1,760 pmol of dATP/10^6 cells, despite the fact that regular transfusions of irradiated erythrocytes containing normal levels of ADA had been administered since the child was 5-mo old. Nucleated cells in the lymphoid fraction from bone marrow contained 270 pmol of dATP/10^6 cells which is much higher than the 20 pmol of dATP/10^6 cells in marrow from a normal child of similar age.

Samples of urine from our patient were examined for nucleosides by high performance liquid chromatography after chromatography on an anion-exchange column to remove nucleotides and free bases. Excretion of deoxyadenosine is normally so low (<0.1 mg/day) that it cannot be detected. Large quantities of deoxyadenosine were excreted in the urine of our patient (Table II). Values of 27.6 and 31.1 mg deoxyadenosine excreted/24 h were obtained on two occasions, 4 mo apart. Excretion of adenosine may also be elevated in the patient, 1.5 mg/day as compared with 0.5 mg/day in a normal child (Table II). Uric acid excretion by our patient was lower than expected (Table II) and the uric acid/creatinine ratio was lower in the patient than in normal adults or children. Plasma from our patient was examined for deoxyadenosine and adenosine and none was detected.

Identification of deoxyadenosine in the urine was proved in several ways. Fig. 1A shows the chromatogram of the patient's urine compared to urine from a normal person (Fig. 1C). Urine from the patient, but not from the normal control, contains material eluting in the same position as deoxyadenosine. The positions of several nucleosides are indicated in Fig. 1. The pool of nucleosides obtained from the patient's urine was treated with purified ADA from calf intestine. The treated urine was then examined by high performance liquid chromatography (Fig. 1B). The peak previously seen in the position where deoxyadenosine elutes (Fig.

TABLE I
dATP Concentration in Normal Cells and Cells from a Patient with ADA Deficiency

Cell type	Subject	Age	dATP	Comment*
		mo	*pmol/10⁶ cells*	
Erythrocytes	Normal	All‡	<0.1	
	Patient	4	157	Before transfusion
	Patient	20	<0.1	After transfusion
Lymphocytes	Normal	All	2–4	
	Patient	17	770	After transfusion
	Patient	21	1,760	After transfusion
Bone marrow	Normal	16	20	
	Patient	13	270	After transfusion

* Hypertransfusion with irradiated erythrocytes was begun when the patient was 5-mo old.
‡ The concentration of dATP has been measured in erythrocytes and lymphocytes from normal children and adults. There are no major changes in concentration with age of the individual, so the amounts given in the table apply at all ages.

TABLE II

Deoxyadenosine, Adenosine, and Uric Acid Excretion in Normal Urine and in Urine from a Patient with ADA Deficiency

Source	Adenosine	Deoxyadenosine	Uric acid
		mg/day	
Normal child*	0.5	<0.1	300
Normal adult*	—	<0.1	780
Patient (specimen 1)‡	1.5	27.6	177
Patient (specimen 2)	—	31.1	180

* These values fall within the normal ranges as recorded in Mabry and Tietz (11).

‡ Specimens 1 and 2 were obtained when the patient was 17 and 21 mo old, respectively. Hypertransfusion with irradiated erythrocytes was begun when the patient was 5 mo old.

1A) disappears after treatment of urine with ADA (Fig. 1B). Final confirmation of deoxyadenosine as the abnormal metabolite in urine was obtained by gas chromatography-mass spectrometry. Samples of the deoxyadenosine isolated from urine by high performance liquid chromatography were derivatized and examined as described previously for our laboratory (3). The molecular ions produced coincided with those formed from a derivatized standard of deoxyadenosine.

DISCUSSION

Severe impairment of thymus-derived and bone marrow-derived cell immunity has been well established as the usual consequence of hereditary deficiency of ADA (1, 12). The specific nature of the metabolic defect resulting from a deficiency of ADA activity has been puzzling. The recent identification of dATP as the principal abnormal metabolite in erythrocytes from several patients identified one metabolic consequence of inadequate levels of ADA. We have now shown that lymphocytes and bone marrow from an affected child also contain large quantities of dATP. The lymphocytes were obtained after the child had been treated for over a year by hypertransfusions with irradiated erythrocytes from a donor with normal ADA. Activity of ADA was normal and dATP was absent from erythrocytes of the patient at the time the lymphocytes were obtained. This shows that frequent transfusion of normal erythrocytes does not constitute a form of enzyme replacement therapy that corrects the metabolic abnormality in lymphocytes.

Our immunodeficient child is excreting large quantities of deoxyadenosine. Other investigators have reported that little or no deoxyadenosine is excreted by children with ADA deficiency (4, 7). The reason for the discrepancy between our and their results is not known. ADA deficiency is clinically heterogeneous. The level of ADA activity in cells does not always correlate with the severity of immunodeficiency disease. Whether the severity of the metabolic defect, e.g. level of excretion of deoxyadenosine, correlates better with severity of disease is not yet known.

Since deoxyadenosine is excreted in measurable quantities and accumulated in cells, deficiency of ADA

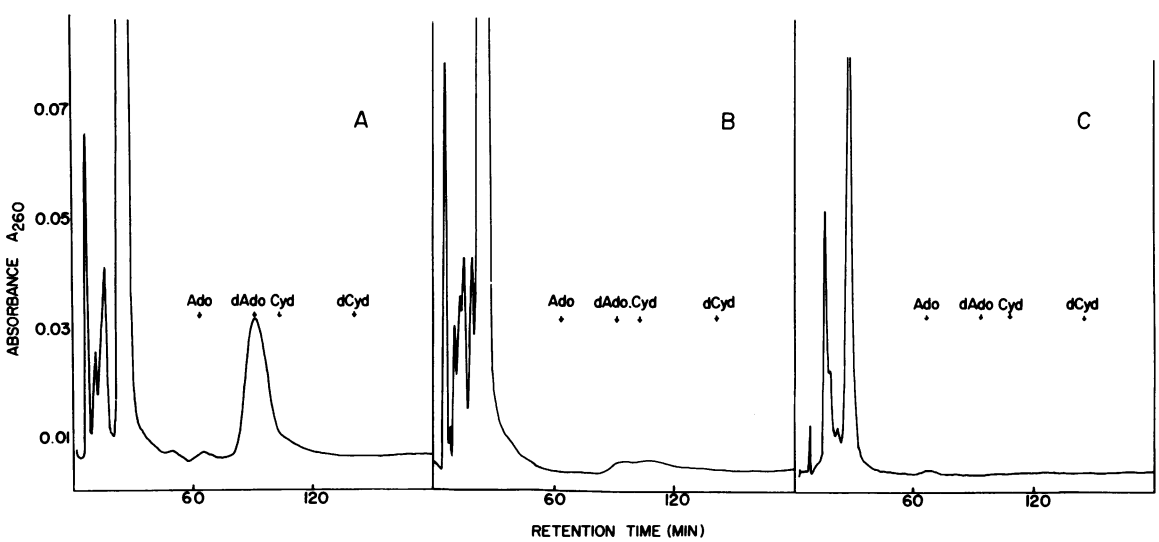

FIGURE 1 Separation of nucleosides in urine by high performance liquid chromatography. The elution positions of adenosine, deoxyadenosine, cytidine, and deoxycytidine are shown. (A) Nucleosides in the urine of a patient with ADA deficiency showing the large amount of deoxyadenosine. (B) Urine from the patient with ADA deficiency was treated with purified ADA and then nucleosides were chromatographed as shown in panel A. The deoxyadenosine has disappeared. (C) Nucleosides in normal urine showing the absence of deoxyadenosine.

must cause overproduction of adenine deoxynucleosides and deoxynucleotides. Uric acid excretion seems reduced so the overproduction probably results from decreased catabolism of adenosine. This can be explained by the lack of ADA. What cannot be explained is the conversion of adenine ribonucleosides and nucleotides to deoxyribonucleosides and nucleotides. Generally the concentrations of adenine deoxynucleotides in cells are two orders of magnitude lower than adenine ribonucleotides. Ribonucleotide reductase is thought to be the major pathway for conversion of ADP to dADP (13). Although the human enzyme has not been thoroughly characterized, its activity is generally regulated by the concentration of deoxynucleotides and is relatively insensitive to the concentration of ribonucleotides. Because of feedback inhibition of the reductase, one would not expect large quantities of dADP to be synthesized.

Variation in the clinical and biochemical characteristics of ADA deficiency could be explained if genes at more than one genetic locus control ADA activity. These genes may differ in their effects on other metabolic and immunologic processes. For example, ADA may be a complex molecule with one polypeptide chain unique to ADA and one shared by ADA and ribonucleotide reductase. Two types of genetic mutation would be possible, one affecting ADA but the second affecting both ADA and ribonucleotide reductase.

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