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Severe Combined Immunodeficiency in a Child with a Healthy Adenosine Deaminase Deficient Mother

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Summary

We investigated adenosine deaminase (ADA) deficient severe-combined immunodeficiency (SCID) in an 8-month-old child with ADA deficient mother. The ADA deficiency in the child was unusual in that the thymic histology was normal. In addition, the thymocytes formed E-rosettes with sheep erythrocytes and were stimulated by T-cell mitogens. ADA activity could not be detected in the child's thymocytes.

Studies on the family indicated that the father had about one-half of the normal erythrocyte ADA activity. All the family members with detectable ADA activity appeared to have, according to starch gel electrophoresis of erythrocyte lysates, the common ADA-1 phenotype; however, rigorous identification of phenotype was not possible in this study. The mother had less than 1% of normal ADA activity in both erythrocyte and lymphocyte extracts, but her whole peripheral blood lymphocytes demonstrated about 6% of normal activity. Normal concentrations of

ATP and small amounts of dATP were found in the mother's erythrocytes. Deoxyadenosine excretion in her urine was elevated and approximately 5–10% of that excreted by individuals with ADA deficient SCID. These studies suggest that low amounts of ADA activity in erythrocytes and blood lymphocytes of certain individuals may be compatible with good immune function and longevity.

Abbreviations

ADA, adenosine deaminase
Con-A, concanavalin A
PHA-P, phytohemmagglutinin
SCID, severe-combined immunodeficiency
dATP, deoxy-adenosine triphosphate

ADA deficiency is associated with approximately 30–50% of the cases of autosomal recessive SCID (1, 6, 12). The clinical, metabolic, and biochemical aspects of this immunodeficiency have been investigated extensively (7, 8, 10, 18, 19, 21, 24). Individuals with this form of SCID invariably have profound deficiencies of ADA activity in both erythrocytes and lymphocytes (12); however, there are reports of individuals with partial deficiencies of this enzyme who apparently have normal or near normal function of the immune system (3, 11, 13). A 12-year-old boy from the !Kung tribe (25) in the Kalahari desert in Africa had 2–3% of normal ADA activity in erythrocytes and about 10–12% of normal ADA activity in blood lymphocytes (13). Similar erythrocyte and leukocyte ADA activities were reported in a black child in the United States (3, 11). In addition, heat lability of the U.S. child's leukocyte ADA was increased.

We recently studied a unique family pattern of ADA deficiency. The child and her mother both had ADA deficiency; but the mother was healthy and the child was immunodeficient. The studies suggest that the mother was homozygous for a low activity ADA gene whereas the infant had that gene and a silent ADA allele.

CASE REPORT

The child, a black female, was well until 2 months of age at which time she developed a purulent nasal discharge and a cough which did not respond to antibiotic therapy. She was hospitalized at 4 months of age with pneumonia and diarrhea. Although she appeared to improve initially, the pneumonia ultimately became worse. When she was referred to our hospital at 6 months of age, she had bilateral otitis media, maxillary sinusitis, and pneumonia. Physical examination revealed small tonsils, but absent peripheral lymphoid tissue. An immunologic investigation revealed a profound T-cell deficiency (Table 1). A diagnosis of ADA deficiency was made by a study of the child's erythrocyte lysates. Because of the continued deterioration of her lung function, an open lung biopsy was performed. No fungal, bacterial, or viral organisms were detected by direct microscopy or culture. The lung tissue demonstrated chronic inflammation with few, if any, normal alveoli. A few cells appeared to have inclusion bodies. A thymus biopsy obtained during the procedure appeared to be normal on histologic examination (Fig. 1). Afterwards, the pneumonia became worse and she died at age 8 months of respiratory failure. In order to provide genetic counseling, all available members of the family were investigated for ADA activity in their erythrocyte lysates (Fig. 2). The mother had no detectable activity.

Table 1. *Immunologic studies*[1,2]

	Patient (child)	Normal
IgG	540 mg/dl	325–875mg/dl
IgM	215 mg/dl	50–200 mg/dl
IgA	127 mg/dl	15–88 mg/dl
Lymphocytes	504/mm^3	>2000/mm^3
E-Rosettes	35/mm^3	1620–4230 mm^3
EAC-Rosettes	50/mm^3	470–2590 mm^3
PHA stimulation	124 cpm	49,912 ± 16,839 cpm

[1] Studies were performed when the child was 8 months of age.

[2] Abbreviations: EAC, erythrocyte antibody complement and PHA, phytohemagglutinin.

MATERIALS AND METHODS

Chemical and solutions. Analytical grade ion exchange resins (200/400 mesh) were obtained from Bio-Rad Laboratories (Richmond, CA). Chloroacetaldehyde was prepared from the dimethylacetal derivative as described previously (21). PHA-P and Con-A were obtained from Difco Laboratories (Detroit, MI) and were used without further modification. Starch suitable for starch gel electrophoresis was from Electrostarch Co. (Madison, WI, Lot #392). [8-^{14}C]-Adenosine (54.7 mCi/mmole) was obtained from New England Nuclear (Boston, MA). PEI-cellulose thin layer chromatography plates were purchased from J.T. Baker Chemical Co. (Phillipsberg, NJ).

Cell isolation. Heparinized venous blood was centrifuged within 15 min of collection. Plasma was removed from the erythrocytes, which were suspended in a corresponding amount of 0.154 M NaCl. The red cell suspension was deproteinized with cold trichloroacetic acid (21).

Lymphocytes were isolated by Ficoll-Hypaque density centrifugation (4). These cells were either disrupted by Dounce homogenization and subsequent sonication or used as whole cells. These cells were contaminated by as much as 30% monocytes.

Immunologic studies. Peripheral blood lymphocytes or thymocytes were cultured with PHA-P (3 μg/ml) or Con-A (100 μg/ml) for 3 days (9). The extent of stimulation was determined by measuring the incorporation of [^{3}H]-thymidine by these lymphocytes. E-rosettes were formed with sheep erythrocytes and counted according to the method of Jondal *et al.* (14). Serum immunoglobulins were quantitated by the Mancini method using commercial plates and standards (16).

ADA DETERMINATIONS

Erythrocytes lysates. Erythrocytes lysates were prepared as described previously (21). The reaction was followed spectrophotometrically by the conversion of adenosine to inosine as described by Kalckar (15).

Mononuclear cell lysates. ADA activity in lysates of peripheral blood lymphocytes or thymocytes was measured by the conversion of [8-^{14}C]-adenosine to [8-^{14}C]-inosine and [8-^{14}C]-hypoxanthine at 37°C. The reaction media consisted of 0.05 M sodium phosphate buffered saline 0.154 M, pH 7.4. The adenosine concentration was 1×10^{-4} M (specific activity, 0.54 mCi/mmole). Adenosine, inosine, and hypoxanthine were separated by one dimensional thin layer chromatography on PEI-cellulose using the solvent system ammonium hydroxide:water (20:80 v/v). Spots corresponding to each of the three compounds were cut out and counted in a liquid scintillation system.

ADA activity in whole cells. ADA activity in approximately 2×10^6 cell/ml was determined with the same assay conditions described for the leukocyte determinations. No correction was made for adenylate deaminase activity. Starch gel electrophoresis was carried out as described previously by Spencer *et al.* (23).

Erythrocyte nucleotides. Anion exchange chromatography of erythrocyte nucleotides and phosphate esters was accomplished using a multiple column technique (21). For determination of dATP in erythrocytes, an aliquot of a protein-free supernate of red cells was applied to a 1 × 3 cm AGl-X4 anion exchange column, Cl^- form. The resin was washed with 60 ml of water to remove free purines and nucleosides. The column was then placed inside a small test tube and 8 ml of 0.3 N HCl was applied

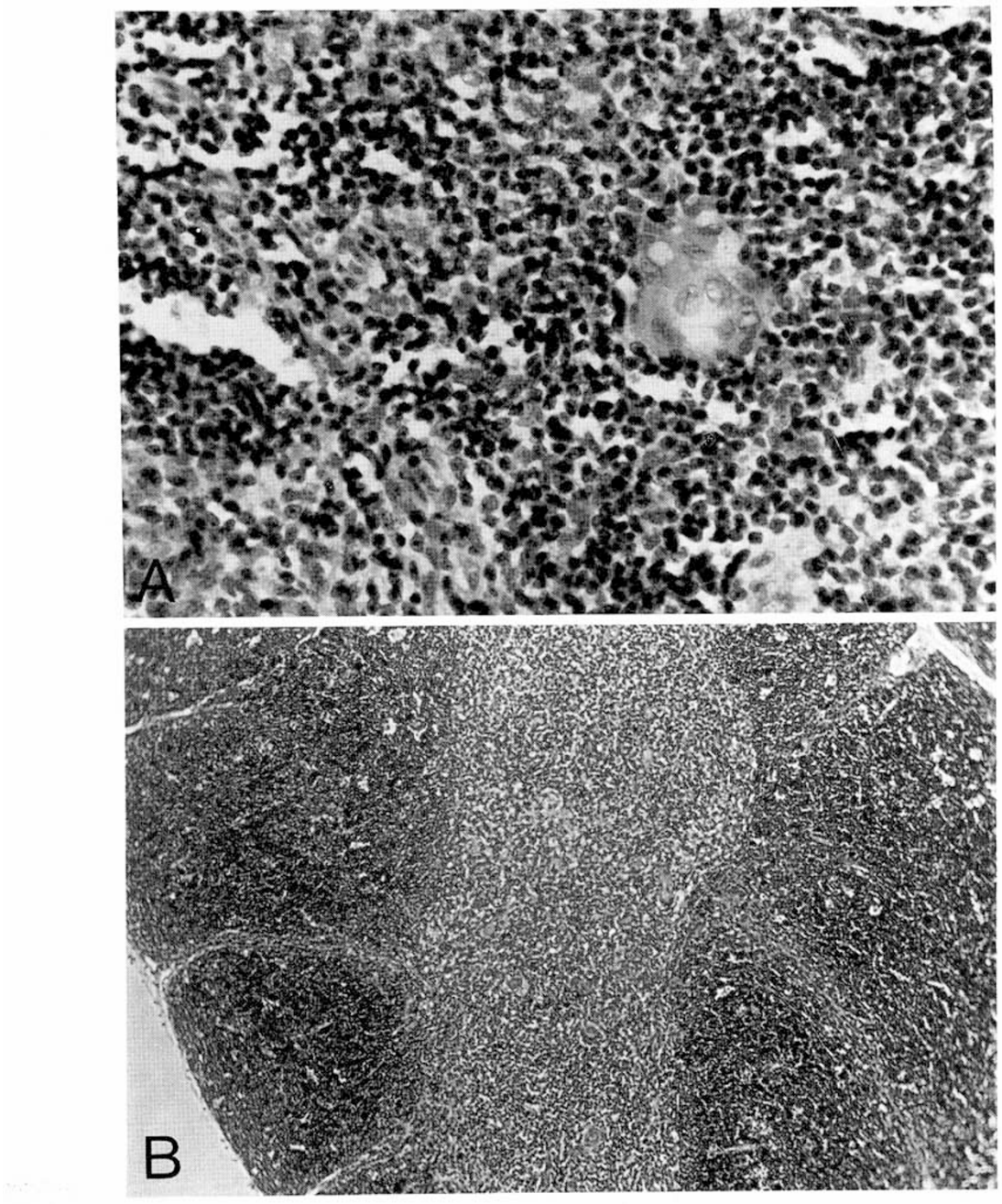

Fig. 1. Hematoxylin-eosin preparation of the AdA deficient child's thymus at 8 months of age. Cellularity and Hassal's corpuscles appeared normal (A, ×100 and B, ×400).

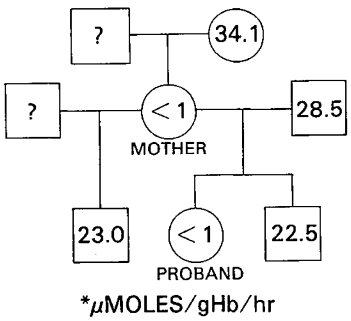

Fig. 2. Family studies of erythrocyte adenosine deaminase (normal mean, 62.5 μmole·g $Hgb^{-1}\cdot h^{-1}$).

and allowed to drip through until the liquid level was just above the resin. The flow was stopped and the column was left at room temperature for 2 h to hydrolyze the purine ribose bond of deoxyribo-nucleotides. The HCl was then allowed to drip through the column and was followed by two 25-ml water washes. The adenine in the combined HCl and water eluates was determined as the etheno derivative after separation on a cation exchange column, as described previously (17). Under these conditions, all of the dATP is converted to adenine with no significant adenine formation from ATP.

Adenosine, deoxyadenosine, and adenine in erythrocytes and urine. Because deoxyadenosine is unstable on cation exchange columns (H^+ form), initial separation of urine and protein-free erythrocyte lysates was accomplished on short anion exchange columns (1 × 5 cm, AGl-X4, Cl^- form), at pH 10. Under these

conditions, deoxyadenosine was stable and eluted from the column along with adenosine in the column wash. Adenine, which was retained, was eluted with 0.077 M sodium acetate, pH 5.2. The original column wash containing adenosine and deoxyadenosine was added to a cation exchange column (0.5 × 20 cm AG50-X4, H^+ form). Elution was accomplished sequentially with 0.77 M sodium acetate, pH 4.8 and 0.077 M sodium acetate pH 5.2, as previously described (21). Under these conditions, deoxyadenosine is converted to adenine, whereas adenosine remains unchanged. Aliquots of the separated compounds were added to 0.5 ml of 0.2 M chloroacetaldehyde and heated 40 min at 80°C to form ethenoadenine and ethenoadenosine. These fluorescent derivatives were measured quantitatively by their fluorescence at 425 nm after excitation at 280 nm.

Determination of uric acid and creatinine. Uric acid was determined by UV spectrophotometry after anion exchange separation (22). Creatinine was determined in the cation exchange HCl elution profile by its absorbance at 230 nm (17).

Table 2. *Thymic lymphocytes*[1]

	Patient (child)	Normal
E-Rosettes	98%	>90%
EAC-Rosettes	>2%	
PHA stimulation	1027 cpm	474 cpm
Con-A stimulation	985 cpm	2597 cpm

[1] Abbreviations: EAC, erythrocyte antibody complement; PHA, phytohemagglutinin; and Con-A, concanavalin A.

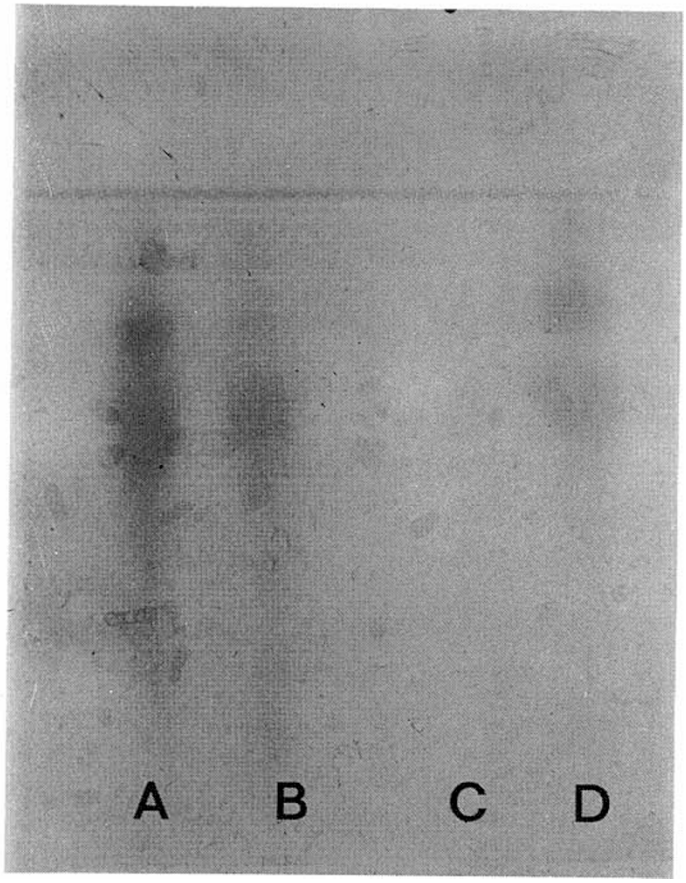

Fig. 3. Starch gel electrophoresis of erythrocyte adenosine deaminase (ADA). (*A*) Known ADA-1-phenotype; (*B*) male sibling; (*C*) mother; and (*D*) father. Because erythrocyte lysates were electrophoresed only in the absence of mercaptoethanol, definitive identification of ADA phenotypes is not possible from this study.

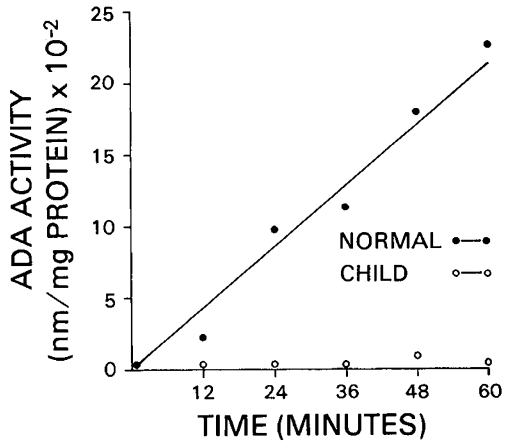

Fig. 4. Adenosine deaminase (ADA) activity in child's thymocytes. The control is from a thymic biopsy taken during cardiac surgery the same day as the patient's thymic biopsy. Manipulation of the two specimens was identical. ADA activity was determined from the conversion of [8-^{14}C]-adenosine to [8-^{14}C]-inosine and [8-^{14}C]-hypoxanthine (sensitivity of this assay was approximately 1.2 nmole·mg protein^{-1}·min^{-1}).

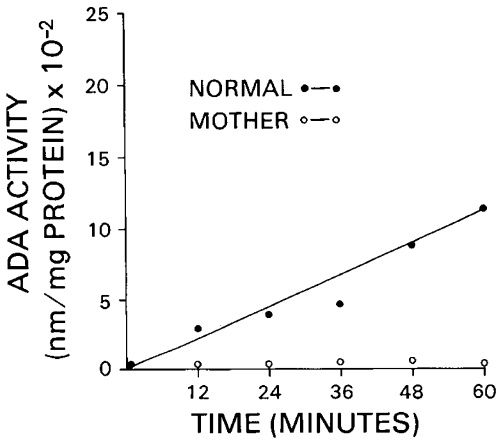

Fig. 5. Adenosine deaminase (ADA) activity in mother's peripheral blood mononuclear cell lysates. ADA activity was determined from the conversion of [8-^{14}C]-adenosine to [8-^{14}C]-inosine and [8-^{14}C]-hypoxanthine (sensitivity of this assay was approximately 1.8 nmole·mg protein^{-1}·min^{-1}).

RESULTS

Immunologic studies. Absolute numbers of blood lymphocytes, E-rosetting cells, and erythrocyte antibody complement rosetting cells were all decreased significantly as was stimulation of peripheral blood lymphocytes by PHA-P as measured by [^{3}H]-thymidine uptake (Table 1). In contrast, immunoglobulins were quantitatively normal. In addition, the patients' thymocytes demonstrated a normal number of E-rosetting cells and incorporated [^{3}H]-thymidine to a similar degree as the normal when cultured with Con-A or PHA-P (Table 2). Histologic sections of the thymus demonstrated normal architecture with numerous Hassal's corpuscles. (Fig. 1).

Erythrocyte ADA activity. Both the mother and child had less than 1% of normal ADA activity in their erythrocyte lysates. The father, a maternal grandmother, and two siblings of the child each had approximately one-half of normal activity (normal mean, μmole·g Hgb^{-1}·h^{-1} (Fig. 2). Starch gel electrophoresis of erythrocyte lysates from the same members of this family demonstrated either no activity (mother) or an apparent ADA-1 phenotype (Fig. 3).

ADA activity in mononuclear cell lysates. Using the [8-^{14}C]-adenosine ADA assay, no ADA activity as measured by the appearance of labeled inosine or hypoxanthine could be detected in lysates of the patient's thymocytes (Fig. 4). Normal thymocytes, recovered from a patient during cardiac surgery and treated exactly as the patient's thymic biopsy, showed an ADA activity of 40 nmole·mg protein^{-1}·min^{-1}. This value is approximately 20% of that reported in the literature (2, 5). The reason for this disparity is unknown; however, it is clear that the patient had less than 1% of normal activity in her thymocyte lysates.

Unexpectedly, the mother had no detectable ADA activity in her mononuclear cell lysates from peripheral blood (Fig. 5). About 6% of normal activity could be detected in the mother's mononuclear cells when they were assayed as whole cells (Fig. 6). No correction was made in this assay for adenylate deaminase activity.

Erythrocyte and urinary purines. The mother's adenine, adenosine, erythrocyte ATP, ADP, and AMP were within normal limits (Table 3). Both adenine and deoxyadenosine were elevated in the mother's urine (Table 4). Small but detectable amounts of dATP (35 μmole/liter erythrocytes) were found.

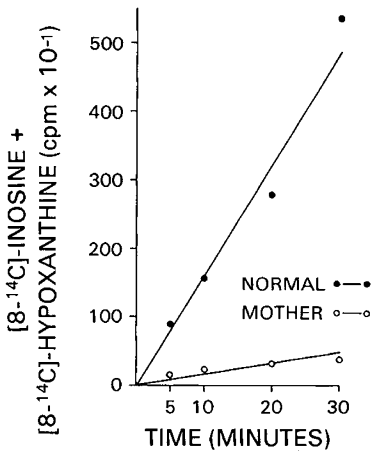

Fig. 6. ADA activity in mother's whole peripheral blood mononuclear cells. ADA activity was determined from the conversion [8-^{14}C]-adenosine to [8-^{14}C]-inosine and [8-^{14}C]-hypoxanthine. No correction was made for adenylate deaminase activity.

Table 3. *Erythrocyte purines of the mother*[1]

Compounds	Mother	Controls
AMP	24	18.6 ± 5.3
ADP	165	170 ± 25
ATP	1490	1400 ± 145
dATP	35	
Adenosine	<1.6	1.1 ± 0.4
Adenine	8.2	5.3 ± 3.2
Deoxyadenosine	<1.4	

[1] Concentrations of compounds are expressed as μmole/liter of erythrocytes. The lower limits of detection for dATP was approximately 5 μmole/liter of erythrocytes.

Table 4. *Urinary purine compounds*[1] *in the mother*

Compound	Urine (nmole/ml)	Creatinine (nmole/μmole)	
Adenine	163	1.95	(0.74 ± 0.22)
Adenosine	1.0	0.12	(0.64 ± 0.32)
Deoxyadenosine	11.8	1.41	(0.20 ± 0.17)

[1] Normal adult values (mean ± S.D.) are shown in parentheses. Similar studies in the child were not performed.

DISCUSSION

This is the first report of a healthy ADA deficient mother producing an ADA deficient infant with SCID. The low numbers and function of peripheral blood T-lymphocytes, and the clinical features of the child were consistent with other reported cases of ADA deficient SCID. The child, however, was unusual in that the thymus was histologically normal (Fig. 1). Furthermore, the thymocytes retained some functional capabilities because they were able to form E-rosettes and could be stimulated by Con-A and PHA-P. Because of the atypical features of this ADA deficiency, the erythrocyte and lymphocyte levels of ADA activity were measured in the parents and other available family members. The father displayed an intermediate level of erythrocyte ADA activity typical of reported carriers of this enzyme defect. In contrast to the usual carriers of ADA deficiency, the maternal erythrocytes lacked ADA activity; thus, it appears that the child was the product of parents with two genetic types of ADA abnormalities, the more unusual type of which presented in an asymptomatic, apparently immunocompetent individual (mother).

Erythrocyte ADA deficiency without immunodeficiency has been reported previously (3, 11, 13). The first case of ADA deficiency without immunodeficiency was found in a !Kung boy of South West Africa (13). The healthy child had 2–3% of normal enzyme activity in erythrocytes and 10–12% in lymphocytes. In this country, a black child with normal immune functions has been described with deficiencies of ADA in erythrocyte and mononuclear cell lysates and an abnormal heat sensitivity of ADA (3, 11).

The mother of the child in the present report is at least quantitatively different from previously reported healthy ADA deficient individuals in that mononuclear cell lysates and whole mononuclear cells contain less ADA activity. Despite the greatly decreased activity of ADA in erythrocytes and mononuclear cells, only small amounts of dATP were detected (Table 3). Other abnormalities in erythrocyte nucleotides and purines, such as the presence of increased concentrations of ATP or adenine reported for patients with ADA deficient SCID, were not discovered in her cells. Examination of the mother's urine revealed an increased excretion of deoxyadenosine and adenine. The excretion of deoxyadenosine (Table 4) was only 5–10% of the amount produced by another ADA deficient patient we have studied (20). The amount of deoxyadenosine excreted by the mother is probably slightly larger than that described for the previously reported child with abnormally heat sensitive ADA (3, 11). We conclude that some cell line present in this woman must have appreciable ADA activity, and that most deoxyadenosine produced endogenously was metabolized rather than excreted.

We are unable to answer the following important questions about the ADA deficiency in this family. (*1*) Was the mother homozygous or heterozygous for an altered ADA gene? (*2*) Did the ADA deficiency represent an abnormal enzyme or was it a normal enzyme present in low concentrations? Starch gel electrophoresis of erythrocyte lysates either demonstrated no activity (mother and patient) or possibly the common ADA-1 phenotype (father and two healthy siblings). Unfortunately, enzyme studies were curtailed because the family refused further study.

Although more definitive studies would have been very helpful, we postulate that the ADA deficiencies in this family were due to two discrete genetic abnormalities: a null gene from the father and a novel, low activity gene from the mother. Because of the intermediate enzyme activity in the father's erythrocytes, the profound deficiency of ADA in the mother's cells, and the relatively normal features of the thymus of the affected child, we felt that the father was heterozygous for the null gene, the mother was homozygous for the low activity gene, and the affected child possessed both the common null gene and the low activity gene. Although there may be alternate explanations, the clinical, immunologic, and enzymatic data best supported that hypothesis. If this hypothesis is correct, there must be a distinct threshold of

ADA activity necessary for normal immune function. Finally, although the question of subtle immune dysfunction in individuals with low activity ADA enzymes is not answered by these studies, the apparent good health of the mother suggests that the possible immunodeficiencies are not severe.

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25. The language employed by the !Kung people utilizes "clicks" as well as words. The exclamation point serves to indicate a click.

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