Proc. Nat. Acad. Sci. USA
Vol. 73, No. 1, pp. 213–217, January 1976
Immunology

Characterization of residual enzyme activity in fibroblasts from patients with adenosine deaminase deficiency and combined immunodeficiency: Evidence for a mutant enzyme

(severe combined immunodeficiency/isoenzyme/genetic defect)

ROCHELLE HIRSCHHORN*, NICHOLAS BERATIS†, AND FRED S. ROSEN‡

* New York University School of Medicine, Department of Medicine, New York, N.Y. 10016; † Mt. Sinai School of Medicine of the City University of New York, Department of Pediatrics, New York, N.Y. 10029; and ‡ Harvard University Medical School, Boston Children's Hospital, Boston, Massachusetts 02115

Communicated by K. Frank Austen, October 20, 1975

ABSTRACT A proportion of patients suffering from the autosomal recessive form of severe combined immunodeficiency have an inherited deficiency of adenosine deaminase (EC 3.5.4.4; adenosine aminohydrolase) (erythrocyte isoenzyme). We have, however, found residual adenosine deaminase activity in fibroblasts derived from four such patients. The enzyme responsible for this activity is biochemically homologous with the high-molecular-weight tissue isoenzyme of adenosine deaminase found in normal fibroblasts and tissues other than erythrocytes. The residual adenosine deaminase has an altered electrophoretic mobility, increased heat stability as compared to normals, and can be detected in fibroblasts of obligate heterozygotes. Our previous studies have indicated that the tissue and erythrocyte adenosine deaminase isoenzymes contain a common catalytic unit controlled by the gene affected in severe combined immunodeficiency with absent adenosine deaminase (erythrocyte isoenzyme). This residual adenosine deaminase therefore represents, most likely, a "mutant" enzyme in fibroblasts of patients with severe combined immunodeficiency. The data support the hypothesis that, in these patients, severe combined immunodeficiency is due to a mutation at the adenosine deaminase locus.

Severe combined immunodeficiency (SCID) is a group of inherited disorders of infancy characterized by defects of both cellular and humoral immunity, which are uniformly fatal if untreated. A deficiency of the red blood cell (RBC) enzyme, adenosine deaminase (EC 3.5.4.4; adenosine aminohydrolase) (ADA), has been reported in a proportion of patients with the autosomal recessive form of the disease (1–4). Children who are as severely affected clinically, but have the X-linked form of the disorder, as well as approximately 50% of those with the autosomal recessive form, have normal levels of RBC-ADA (4). Deficiency of ADA, therefore, appears to be a primary genetic defect, rather than one resulting from the disease state. Additionally, diminished levels of RBC-ADA, transmitted as an autosomal Mendelian trait, are found among the family members of children with SCID and RBC-ADA deficiency (2, 4–6). The inherited nature of the enzyme deficiency has further been confirmed by the demonstration of transmission of a "null" or "silent" gene for ADA, as determined by studies of its polymorphism in two of these families (6, 7). It has, therefore, been suggested that the enzyme deficiency and the disease state are causally related. Alternatively, it has been suggested that the association of the two defects (RBC-ADA deficiency and SCID) could represent a frame shift mutation or a deletion fortuitously involving the locus for ADA and for a closely linked gene controlling immune differentiation (1). The demonstration of residual enzyme activity or of a crossreacting material would favor a causal role for the enzyme deficiency in SCID.

ADA activity resides in several different isozymes in erythrocytes and in other tissues (8, 9). RBCs contain a relatively small molecular weight (33,000) ADA which migrates rapidly on starch gel electrophoresis and exhibits a major band of activity with two secondary isozymes. Tissues other than RBCs contain, in addition to the RBC isozyme, several other forms of ADA. These additional forms of ADA are usually larger (>200,000 molecular weight), migrate more slowly during electrophoresis on starch gel, and vary in electrophoretic mobility from tissue to tissue in a characteristic manner. The electrophoretic patterns of RBC-ADA in most human populations differ according to the inheritance of two autosomal allelic genes, ADA^1 and ADA^2, whereas the tissue isozymes reportedly do not exhibit this genetic polymorphism. In spite of these differences, we have previously demonstrated that the tissue isozymes, as well as the RBC isozyme, were deficient in tissue extracts from a child who lacked ADA activity in her RBCs (10). Furthermore, the RBC-ADA isozyme could be converted to each of five different tissue specific isozymes of ADA by coincubation of RBC-ADA with tissue extracts which did not themselves contain detectable ADA activity (11). This conversion of RBC-ADA to tissue isozymes presumably results from interaction with a family of previously described 140,000 molecular weight proteins present in tissues (12). The tissue isozymes generated *in vitro* had the biochemical characteristics of the tissue isozymes found naturally in each of the different tissues. The genetic polymorphism of the RBC-ADA isozymes could also be detected in the tissue isozymes that had been generated *in vitro* (10). These findings suggest that the tissue and RBC-ADA enzymes contain a common catalytic unit coded for by the same gene as that altered in SCID with RBC-ADA deficiency. Therefore, alterations in tissue isozyme could represent mutations at the structural locus for RBC-ADA.

We have found residual ADA activity in fibroblasts from children with SCID and RBC-ADA deficiency. This ADA activity resides in material of the same estimated molecular weight as the tissue isozyme of ADA found in normal fibroblasts, but is different in electrophoretic mobility and heat stability. Moreover, other properties of the residual ADA enzyme activity suggest that this enzyme is a product of a mutation at the structural locus for RBC-ADA.

MATERIALS AND METHODS

Fibroblasts were grown in RPMI-1640 containing 10–20% of various sera as indicated. Cells were harvested at late con-

Abbreviations: ADA, adenosine deaminase; SCID, severe combined immunodeficiency; RBC, red blood cell.

fluency by trypsinization (0.25% trypsin GIBCO), washed three times in phosphate-buffered saline, suspended in 0.05 M Na/PO_4, pH 7.5, and lysed by sonication in a Heat Systems Sonifier. Whole lysates were assayed at 37° for ADA activity, essentially as described by Hopkinson *et al.* (13). Reaction mixtures contained 1 ml of 1.5 mM adenosine in 0.05 M Na/PO_4, pH 7.5, 50 μl containing 0.2 unit of xanthine oxidase and 0.25 unit of nucleoside phosphorylase (Boerhinger-Mannheim, New York), and 50 μl of fibroblast extract (50–200 μg of protein). The inosine produced by the deamination of adenosine was converted to uric acid by the added enzymes, nucleoside phosphorylase and xanthine oxidase, and the rate of appearance of uric acid measured at 293 nm in a Gilford recording spectrophotometer. The rate of appearance of uric acid was simultaneously measured in the absence of fibroblast extracts, since the commercial nucleoside phosphorylase appears to be contaminated with ADA (Method 1).

Fractions from Sephadex columns, as well as whole lysates, were also assayed using a previously described modification of this method (14). Lysates were incubated with substrate for 1–3 hr at 37° and chilled in ice water; the amount of inosine present was determined by adding 0.25 unit of nucleoside phosphorylase and 0.2 unit of xanthine oxidase and measuring uric acid at 293 nm (Method 2). The modified assay was linear with incubations of up to 3 hr and with enzyme concentrations which produced between 10^{-5} and 10^{-4} M inosine. At higher concentrations, inosine inhibits nucleoside phosphorylase activity, but linearity can be extended to 5×10^{-4} M inosine by diluting the incubation mixture just prior to the addition of nucleoside phosphorylase and xanthine oxidase. Inosine was not appreciably converted during incubation under these conditions to products that could not be measured, since inosine (5×10^{-5} M) added at the start of incubation of the fibroblasts was completely recovered at the end of the incubation period. Protein was determined by the method of Lowry *et al.* (15). A unit of ADA enzyme activity is defined as 1 nmol of uric acid produced/mg of protein per min at 37°.

Molecular weight was estimated by chromatography of fibroblast lysates on a Sephadex G-200 column, 100 × 1.5 cm, eluting with 0.05 M Na/PO_4 buffer, pH 7.5. The column was calibrated by elution of aldolase, ovalbumin, cytochrome *c*, blue dextran, and Dnp-lysine. Cell lysates were centrifuged at 15,000 × *g* for 20 min; the supernatant was applied to the column and 1-ml fractions were collected and assayed for ADA activity. Gel electrophoresis and staining for enzyme activity were performed as described by Spencer *et al.* (8).

For determination of rates of heat inactivation, fibroblast extracts were suspended in 0.1 M Na/PO_4 pH 7.5, since lower molarities have been reported to affect the rate of heat inactivation of RBC-ADA (16).

RESULTS

ADA activity studied in ten normal fibroblast cultures was found to vary with the degree of confluency of the fibroblasts, showing an increase in activity from "early" to "late" confluency (10.19 ± 3.39 compared with 16.40 ± 3.32). The fibroblasts described in this report were therefore harvested at very late confluency (approximately 2–3 weeks after plating), in order to facilitate the study of any residual enzyme in mutant fibroblasts.

ADA activity was determined in fibroblasts derived from nine additional normal subjects, four children affected with SCID and RBC-ADA deficiency ("mutant"), and two obligate heterozygote parents of two of the patients. The RBCs of the two obligate heterozygotes were known to contain ADA activity which was more than 2 SD below the mean ADA activity of 44 normal subjects (2). Each of the fibroblast strains was assayed in duplicate on two to four separate harvests, and the values for individual fibroblast strains represent the average of these separate determinations. Normal human fibroblasts harvested at very late confluency contained 25.92 ± 6.02 units of ADA activity (Table 1, Method 1). There appeared to be a moderately skewed distribution of ADA activity similar to that seen for ADA activity of normal red blood cells (5, 6). Fibroblasts from four children with SCID and RBC-ADA deficiency contained an average residual ADA enzyme activity of 5.42 ± 1.15 units of ADA activity or 20.9% of normal. Fibroblasts from the two obligate heterozygotes contained enzyme activity of 19.7 and 26.1 units, which is within one SD of the average ADA activity found in the normal fibroblasts; therefore, in contrast to determinations on RBCs, heterozygotes could not be distinguished from the normals. Similar but slightly higher levels of ADA activity were found when the same fibroblasts were assayed using Method 2. The mean ADA activity of nine normal fibroblast strains was 35.7 ± 7.30 units, with a range of 28.0–49.8 units. Fibroblast lines from children with SCID and RBC-ADA deficiency contained an average ADA activity of 6.74 ± 0.46 units, or 18.9% of normal activity. Fibroblasts from two obligate heterozygotes contained ADA activity of 23.7 and 27.9 units of ADA activity. Although the average ADA activity of fibroblasts from the two obligate heterozygotes was less than the lower range of ADA activity of fibroblasts from normals by this method, single determina-

Table 1. ADA activity* of fibroblasts

	Method 1	Method 2
Normals (n = 9)	25.9 ± 6.0 (21.3–38.2)	35.7 ± 7.3 (28.0–49.8)
Patients (n = 4)	5.42 ± 1.15 (4.3–6.96)	6.7 ± 0.46 (6.22–6.89)
Obligate heterozygotes (n = 2)	22.9 (19.7–26.1)	25.8 (23.7–27.9)

* 1 unit = 1 nmol of uric acid produced/mg of protein per min. Values are given ± SD; ranges are in parentheses.

Table 2. ADA activity of fibroblasts cultured in media containing different amounts of exogenous ADA

	ADA activity		
A.	*20% FCS**		*20% heated FCS**
Mutant	4.35		6.86
Normal 1	27.1		26.6
Normal 2	18.8		21.2
B.	*10% FCS**	*10% Horse serum**	*10% Human serum**
Mutant	5.88	4.59	4.33
Normal 1	23.8	19.0	28.2

* The ADA activity of fetal calf serum (FCS) = 20.26 nmol/ml per min compared to 4.8 nmol/ml per min for the heated serum. Horse serum did not contain detectable levels of ADA activity. Human serum contained 8.0 nmol/ml per min.

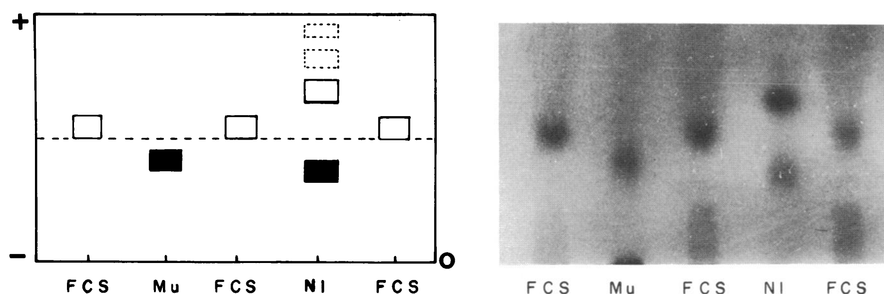

FIG. 1. Increased electrophoretic mobility of ADA tissue isozyme in "mutant" fibroblasts. Lysates of normal and "mutant" fibroblasts were electrophoresed in starch gel and stained for adenosine deaminase activity as described in the *text*. Fetal calf serum (FCS) was electrophoresed in alternate channels as a marker of electrophoretic mobility, as designated by the horizontal dotted line. The normal fibroblasts (N1) exhibited two areas of ADA activity, one with the faster anodal mobility of RBC-ADA with its two minor secondary isozymes, and a slower "tissue" isozyme. (The respective molecular weights of the two isozymes were 33,000 and 260,000.) The mutant fibroblasts (Mu) exhibited only the "tissue" isozyme (260,000 molecular weight). This isozyme was faster in electrophoretic mobility than the corresponding tissue isozyme of the normal fibroblasts (both indicated in black in the diagram). Additional activity was seen on occasion in mutant fibroblasts at the origin (O) and may correspond to the very high molecular species seen in Fig. 3.

tions of ADA activity in fibroblasts derived from heterozygotes overlapped with normal values.

Experiments were performed to determine if the residual ADA activity might represent enzyme endocytosed from the surrounding medium, since the fetal calf serum used in the tissue culture medium contained considerable ADA activity. Normal and mutant fibroblasts were grown for 1 week in media containing either 20% fetal calf serum or 20% fetal calf serum heated for 2.5 hr at 56°. Unheated fetal calf serum contained 22 nmol/ml per min of ADA activity, while heated serum contained only 4.8 nmol/ml per min. Cultures were also grown in horse serum, which contained no ADA activity, and in human serum, which contained 8.0 nmol/ml per min. There was no significant difference between the ADA activity of fibroblasts grown in the presence of high or low levels of exogenous ADA (Table 2).

Lysates of mutant and normal fibroblasts were electrophoresed in starch gel and the areas of ADA activity visualized. Normal fibroblasts showed either the RBC isozymes alone, a "tissue" isozyme alone, or both together as previously reported (9). Additionally, a more slowly moving "tissue" isozyme could sometimes be detected when cells were very confluent. In order to identify the various isozymes more accurately, the mobility of the fibroblast isozymes was compared with that of tissue isozyme in spleen extracts. The more commonly observed, normal fibroblast tissue isozyme migrated slightly ahead of spleen tissue isozyme, with the slowly moving isozyme migrating slightly behind the spleen isozyme. Mutant fibroblasts did not demonstrate any activity with the mobility of RBC isozyme. However, concentrated lysates of all four mutant fibroblasts did contain an ADA activity that migrated more rapidly to the anode than did the usual tissue isozyme of normal fibroblasts (Fig. 1). Occasionally, at confluence, as with the normals, a slower isozyme was also seen (Fig. 2). This slower isozyme also migrated more rapidly than did the slow isozyme seen occasionally in normal fibroblasts.

Fibroblast extracts from two obligate heterozygotes were also examined by starch gel electrophoresis. The major portion of the ADA activity had a mobility indistinguishable from that of the usual tissue isozyme of normal fibroblasts. However, fibroblasts from heterozygotes also contained ADA activity with an increased mobility like that of the enzyme found in mutant fibroblasts (Fig. 2).

In order to confirm that this enzyme activity did not represent endocytosed enzyme, fibroblasts were grown in

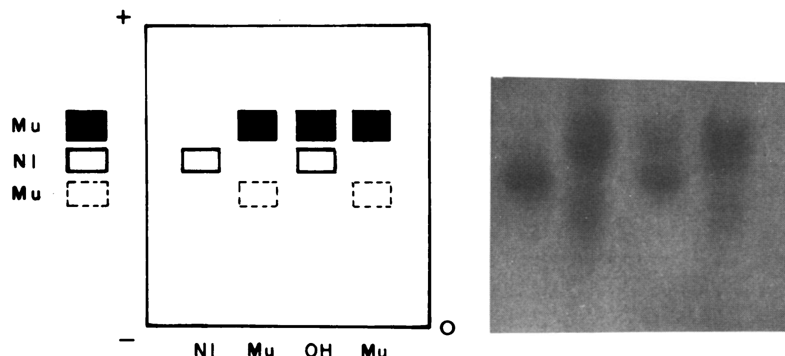

FIG. 2. ADA activity of normal, obligate heterozygote and "mutant" fibroblasts after electrophoresis in starch gel: demonstration of "mutant" enzyme in fibroblasts from obligate heterozygotes. Lysates of fibroblasts from normals (N1), obligate heterozygotes (OH), and an affected child (Mu) were electrophoresed in starch gel and stained for ADA activity. Gels were allowed to develop for longer than usual in order to detect minor components. The normal fibroblasts exhibited only one tissue isozyme of ADA without the RBC-ADA isozyme seen in Fig. 1. This variation in expression of RBC-ADA has been reported previously (9). The "mutant" fibroblasts exhibited a major band of enzyme activity that had a faster electrophoretic mobility than the corresponding tissue isozyme seen in normal fibroblasts (as also seen in Fig. 1). The major area of enzyme activity in the obligate heterozygote (OH) was of the same electrophoretic mobility as the normal tissue isozyme, but there was an additional area of enzyme activity with the faster electrophoretic mobility of the mutant isozyme. An additional area of activity of slower mobility was sometimes found in very confluent cultures of both normal and mutant fibroblasts. On this occasion (designated by dotted rectangles) it is seen only in the mutant extracts. This isozyme also exhibited an increased electrophoretic mobility when compared with the corresponding normal slow isozyme when present. indicates origin.

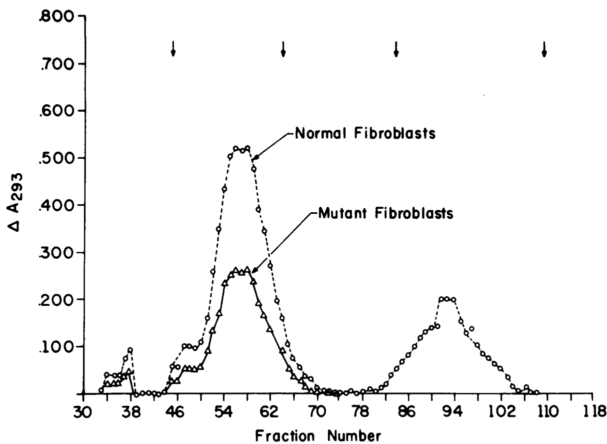

FIG. 3. Molecular weight of ADA activity in normal and mutant fibroblasts. Lysates of normal and mutant fibroblasts were centrifuged at 15,000 × g for 20 min and applied to a Sephadex G-200 column. The column was calibrated with blue dextran, aldolase, ovalbumin, and cytochrome c, indicated by the arrows from left to right. One-milliliter fractions were collected and assayed for ADA activity by Method 2 as described in the *text*, using a 1-hr incubation for normal fibroblasts and 3 hr for mutant fibroblasts. The normal fibroblasts contained peaks of activities with molecular weights of 33,000 and 260,000 and a shoulder of activity of 480,000 molecular weight. There was also activity early in the void volume, possibly representing aggregates of even greater molecular weight. Mutant fibroblasts contained the same molecular weight species as did the normals, except that of 33,000, which is always absent in the mutant but which may or may not be present in the normals.

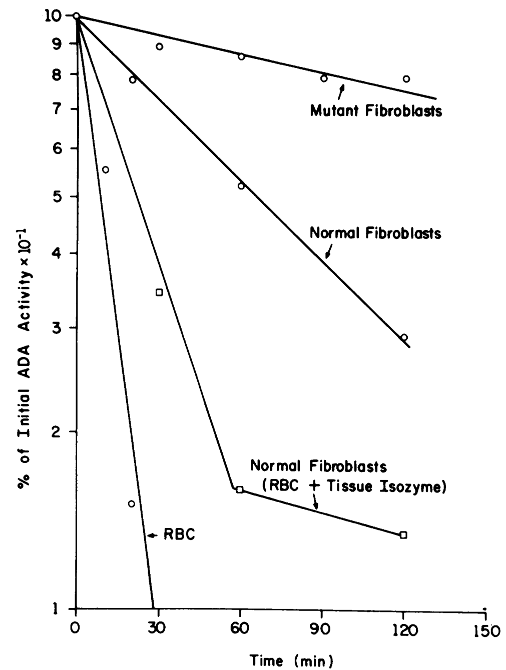

FIG. 4. Inactivation of ADA activity at 56° of normal and ADA deficient (Mutant) fibroblasts and of RBC-ADA. Mutant fibroblasts were more heat-stable than normal fibroblasts whether containing the "tissue" isozyme only or both tissue and RBC isozymes. RBC-ADA, either from erythrocytes or lymphoblastoid lines, was most rapidly inactivated. The results in this figure represent typical experiments.

media containing fetal calf serum, human serum, or horse serum. Fetal calf serum and human serum contain ADA activities that differ in electrophoretic mobility from each other and from the enzyme seen in either normal or mutant fibroblasts. Horse serum does not contain ADA activity detectable on gel electrophoresis by this assay. Mutant fibroblasts demonstrated ADA with the same electrophoretic mobility, after culturing in any of the three sera, and did not contain ADA of mobility typical of that found in human (not shown) or calf serum (see Fig. 1).

The molecular weight of normal and mutant fibroblast ADA was estimated by chromatography on Sephadex G-200 (Fig. 3). Most normal, but no mutant fibroblasts, contained detectable ADA activity of 33,000 molecular weight, representing the RBC-ADA isozyme, as confirmed by gel electrophoresis. This activity was never detected in the mutant, but may or may not be present in the normal. Normal and mutant fibroblasts also contained a high-molecular-weight ADA activity with an estimated molecular weight of about 260,000. These values agree with previous estimates of 280,000 molecular weight for the tissue isozyme of ADA (9). Both mutant and normal fibroblasts also contained a shoulder of activity of molecular weight of approximately 480,000.

The Michaelis constant (K_m) for adenosine of the mutant and normal fibroblasts was compared. The K_m for the enzyme from mutant fibroblasts was 6.47×10^{-5} M compared with a K_m of 6.18×10^{-5} M for ADA (16). Both normal and mutant fibroblast ADA showed a broad pH optimum between pH 6.5 and 8.0.

Lysates of normal and mutant fibroblasts, as well as of RBCs and lymphoid line cells, were subjected to heating at 56°, and residual enzyme activity was determined at different time intervals. As seen in Fig. 4, extracts of tissues such as RBCs and lymphoid line, which contain only RBC-ADA isozymes (as determined by gel electrophoresis), were rapidly inactivated after heating at 56°. Less than 5% of the initial activity was detectable at 30 min. Enzyme from normal fibroblasts, which contained only the tissue isozyme, was more heat-stable than the RBC isozyme, with 29.7 ± 9.9% (n = 5) remaining after 2 hr at 56°. Normal fibroblasts containing both RBC and tissue isozymes showed a two-component inactivation profile. Initially there was rapid inactivation, although somewhat slower than that of the RBC isozyme alone, with a later phase of slow inactivation. The enzyme from mutant fibroblasts demonstrated the greatest heat stability, with 82 and 84% of activity remaining at 2 hr, which is considerably more than that seen in tissue isozyme from normal fibroblasts.

Since the tissue isozyme appears to result from interaction, of RBC-ADA with tissue factors, it was possible that the altered mobility of the tissue isozyme reflected a defect in these tissue factors. RBC-ADA was incubated with fibroblast lysates from affected children and the mixtures were subjected to electrophoresis. The isozyme generated by interaction of normal RBC-ADA with mutant fibroblasts now demonstrated a component with the slower mobility of the normal fibroblast tissue isozyme, in addition to the pre-existing mutant isozyme with faster mobility. Thus, interaction of "tissue factor" from mutant fibroblasts with normal RBC-ADA generated a tissue isozyme of normal electrophoretic mobility.

DISCUSSION

We have found residual ADA activity in the fibroblasts derived from patients with SCID and RBC-ADA deficiency. While no enzyme was found with the physicochemical characteristics of RBC-ADA, the residual activity resembled the

tissue isozyme found in normal fibroblasts in molecular weight, K_m, and pH optimum, but differed in electrophoretic mobility and heat stability. This enzyme activity detected in "mutant" fibroblasts did not appear to be endocytosed from the medium, since the level of activity, as well as its electrophoretic mobility, was independent of the level of ADA in the culture medium or the electrophoretic mobility of the exogenous ADA. We have also previously found residual, if lower, levels of ADA activity in uncultured mononuclear cells (6% of normal) (2, 6), as well as in tissues (0.02–2.0% of normal) from two patients. Residual enzyme activity has also been described by other authors in spleen and fibroblasts from two other patients (17, 18).

We have previously demonstrated that the catalytic unit responsible for ADA activity in erythrocytes and tissues is coded by the same gene (10, 11). Therefore the residual catalytic activity of the tissue isozyme in mutant cells is most likely a product of the same locus. The simplest explanation for the observed alteration in its electrophoretic mobility is a mutation leading to an amino-acid substitution in the catalytic unit, which results both in the observed charge difference and diminished activity. Although the tissue isozyme results from interaction of RBC-ADA with a tissue factor, a primary mutation in the tissue factor, as suggested by Van der Weyden and Kelly (19), cannot account for the results observed here. First, the patient's RBCs, which do not contain tissue factor, demonstrated the most profound deficiency of ADA (<2% of normal). Second, we have shown here that the tissue or conversion factor present in "mutant" fibroblasts converts normal RBC-ADA to a tissue isozyme of normal electrophoretic mobility. The detection of the electrophoretically altered ADA tissue isozyme, in addition to the normal enzyme, in fibroblasts from heterozygotes lends further support to the hypothesis that the isozyme found in patients consists of a "mutant" form of the ADA catalytic unit bound to normal tissue factor.

The apparent increase in heat stability could similarly have resulted from a substitution that conferred greater stability to the molecule. However, alternative explanations for the increased heat stability must be considered. Since the tissue isozyme is normally more heat-stable than the RBC isozyme, the increased heat stability of the mutant tissue isozyme could result from a difference in interaction with the previously reported conversion factor responsible for the appearance of the tissue isozyme. Thus, a mutation resulting in a greater affinity of the catalytic unit for conversion factor could maintain the catalytic activity in the heat-stable, high-molecular-weight form. Alternatively, a mutation that gives rise to an ADA catalytic unit with a shorter half-life in the free form (RBC-ADA) would result in a greater ratio of conversion factor to free (RBC) catalytic unit, thus favoring the equilibrium for the existence of the more heat-stable tissue form.

The finding of a residual, altered or mutant ADA activity in fibroblasts from children with SCID and RBC-ADA deficiency would argue strongly against a chromosomal deletion and support a causal role for this enzyme deficiency in SCID. The pathophysiologic mechanism for this, as for many genetic disorders, is as yet uncertain. Evidence has been presented to suggest that increased concentrations of adenosine and AMP inhibit *de novo* pyrimidine biosynthesis (20), and inhibit *in vitro* lymphocyte response (21), indicating a possible mechanism for the suppression of differentiation and expression of immune function. It can also not be determined from the results presented whether the residual enzyme activity represents a catalytically less efficient ADA enzyme or an unstable protein. The finding of 6–20% residual activity in cells such as fibroblasts and mononuclear cells, actively engaged in protein synthesis, as compared with no detectable activity in RBCs, would suggest that the enzyme is unstable in the cases studied here. A quantitation of cross-reacting material as compared to ADA activity may resolve this question.

We thank Dr. P. Brunel and R. Scott for their gift of fibroblasts from two patients, and Dr. R. Parkman for his generous and invaluable aid. This work was supported by NIH Grants AI-10343, GM-19443, AI-05877, FR-128, and the National Foundation.

1. Giblett, E. R., Anderson, J. E., Cohen, F., Pollara, B. & Meuwissen, H. J. (1972) *Lancet* **2**, 1067–1069.
2. Parkman, R., Gelfand, E. W., Rosen, F. S., Sanderson, A. & Hirschhorn, R. (1975) *N. Engl. J. Med.* **292**, 714–719.
3. Pollara, B., Pickering, R. J. & Meuwissen, H. J. (1973) *Pediat. Res.* **7**, 362–364.
4. Pickering, R. J., Pollara, B. & Meuwissen, H. J. (1974) *Clin. Immunol. Immunopathol.* **3**, 301–303.
5. Scott, C. R., Chen, S-H. & Giblett, E. R. (1974) *J. Clin. Invest.* **53**, 1194–1196.
6. Hirschhorn, R., Beratis, N., Rosen, F. S., Parkman, R., Stern, R. & Polmar, S. (1975) *Lancet* **1**, 73–75.
7. Chen, S-H., Scott, C. R. & Giblett, E. R. (1974) *Am. J. Hum. Genet.* **26**, 103–107.
8. Spencer, N., Hopkinson, D. A. & Harris, H. (1968) *Ann. Hum. Genet.* **32**, 9–14.
9. Edwards, Y. H., Hopkinson, D. A. & Harris, H. (1971) *Ann. Hum. Genet.* **35**, 207–219.
10. Hirschhorn, R., Levytska, V., Meuwissen, H. J. & Pollara, B. (1973) *Nature New Biol.* **246**, 200–202.
11. Hirschhorn, R. (1975) *J. Clin. Invest.* **55**, 661–667.
12. Nishihara, H., Ishikawa, S., Shinkai, K. & Akedo, H. (1973) *Biochim. Biophys. Acta* **302**, 429–442.
13. Hopkinson, D. A., Cook, P. J. L. & Harris, H. (1969) *Ann. Hum. Genet.* **32**, 361–367.
14. Hirschhorn, R. & Levytska, V. (1974) *Cell Immunol.* **12**, 387–395.
15. Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275.
16. Osborne, W. R. A. & Spencer, N. (1973) *Biochem. J.* **133**, 117–123.
17. Van der Weyden, M. B., Buckley, R. H. & Kelley, W. N. (1974) *Biochem. Biophys. Res. Commun.* **57**, 590–595.
18. Chen, S-H., Scott, C. R. & Swedberg, K. R. (1975) *Am. J. Hum. Genet.* **27**, 46–52.
19. Van der Weyden, M. B. & Kelley, W. N. (1974) *J. Clin. Invest.* **53**, 81a.
20. Green, H. & Chan, T-S. (1973) *Science* **182**, 836–837.
21. Hirschhorn, R., Grossman, J. & Weissmann, G. (1970) *Proc. Soc. Exp. Biol. Med.* **133**, 1361–1365.