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Partial adenosine deaminase deficiency: another family from southern Africa

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Summary. Adenosine deaminase (ADA) from a partially ADA-deficient Xhosa man has been characterized. This is only the second such case described in southern Africa, the previous one being a Kalahari San ("Bushman"). Red blood cell ADA levels were found to be only 6–9% of normal whereas his white cell ADA levels were much higher at 30% of normal. The stability of the enzyme at 57°C was shown to be greatly decreased indicating a mutation resulting in an enzyme with decreased stability in vivo. The Michaelis constant (K_m) for adenosine was found to be normal. Deoxy-ATP levels in the red cells were elevated 2- to 3-times above normal, although this appears to be of no immunological consequence. Starch gel electrophoresis of red cell ADA from family members of the index case, in conjunction with red cell ADA activity levels, suggested that both parents carried a gene for partial ADA deficiency. Isoelectric focusing studies suggested that the two parental partial ADA-deficiency genes were not the same. Electrophoretic studies also revealed that another rare allele of ADA, possibly *ADA*5*, was segregating within the same family although this event appears to be unconnected with the ADA partial deficiency. A Xhosa population sample was assayed for red cell ADA activity. The results suggested a frequency of 0.015 ± 0.010 for ADA partial-deficiency alleles, although the number of different alleles involved is not known.

Introduction

Adenosine deaminase (ADA_1) (EC 3.5.4.4) catalyses the irreversible deamination of adenosine and deoxyadenosine to inosine and deoxyinosine, respectively (Conway and Cooke 1939). These are important steps in the purine salvage pathway and profound deficiency of this enzyme, which is normally distributed throughout all tissues, results in one of the inherited forms of severe combined immunodeficiency (SCID) (Giblett et al. 1972; Martin and Gelfand 1981). Several cases of "partial" ADA deficiency (3–30% of normal RBC activity) have, however, been described and in these cases the immune systems appear to function normally (Jenkins et al. 1976; Borkowsky et al. 1980; Hirschhorn et al. 1979; Perignon et al. 1979; Dadonna et al. 1983; Hirschhorn and Ellenbogen 1986).

Individuals with SCID due to ADA deficiency have less than 1% of normal ADA activity in their red blood cells, mononuclear cells, and lymphocytes. The consequences of such a severe ADA deficiency are the marked accumulation and increased excretion of the substrates, adenosine and deoxyadenosine (Hirschhorn and Ratech 1983). These substrates are potentially toxic when present at high concentrations and it is believed that the immune system dysfunction which occurs in ADA-related SCID stems from this toxicity (van Laarhoven and de Bruyn 1983). We report here investigations carried out on a family in which two cases of partial ADA deficiency have resulted from the inheritance of two different ADA-deficiency alleles.

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Materials and methods

Subjects

The proband, an apparently healthy Bantu-speaking male in his early thirties, was discovered during routine ADA phenotyping by starch gel electrophoresis. Subsequent enzyme assays confirmed that he had abnormally low red and white blood cell ADA activities. Further biochemical and genetic studies aimed at comparing the subjects' residual ADA to those of previously described cases of partial ADA deficiency were then undertaken and segregation of the abnormal allele was followed in members of the family. In addition blood samples were collected from 68 volunteer blood donors who were members of the Xhosa chiefdom, and assayed for red cell ADA activity and electrophoretic mobility.

Escherichia coli DNA polymerase I, (U-^{14}C) adenosine (500–600 mCi/mmol), (U-^{14}C) deoxyadenosine (>450 mCi/mol), and (methyl-^{3}H) thymidine 5′-triphosphate (40–60 Ci/mmol) were purchased from Amersham; "cold" thymidine 5′ triphosphate from Sigma; erythro-9-(2-hydroxy-3-nonyl) adenine (EHNA) was generously donated by Dr. Rochelle

Hirschhorn, and poly(dA-dT) was obtained from Pharmacia P-L Biochemicals and Boehringer-Mannheim.

Blood collection and processing

Blood was collected by venepuncture into acid citrate dextrose (ACD anticoagulant). The samples arrived in the laboratory within 24 h of collection and white cells were immediately separated by the dextran method described by Skoog and Beck (1956) and the remaining red cells mixed with a glycerol-based preserving fluid. The separated white cells were stored at −70°C and the preserved red cells at −20°C.

Preparation of cell lysates

Preserved red blood cells and thawed white blood cell pellets were disrupted by sonication. The white cell lysates were centrifuged at 10,000 × *g* for 10 min at 4°C and the clear supernatants used as a source of ADA. Haemolysate haemoglobin concentrations were measured by standard methods (Beutler 1975). The protein levels of white cell lysate supernatants were measured by the method of Lowry et al. (1951).

ADA activity

Two techniques were used, the first was the spectrophotometric method described by Hopkinson et al. (1969). The second method used to measure ADA activity was the radiochemical method of Coleman and Hutton (1975) as modified by Hirschhorn et al. (1979). This method enables measurements to be made of the rates at which ADA catalyses the conversion of ^{14}C-labelled adenosine or alternatively deoxyadenosine to ^{14}C inosine or ^{14}C deoxyinosine, respectively.

This method is sufficiently sensitive to measure the small amounts of a second ADA, ADA_2 (Schrader et al. 1978) which is present in normals as well as in cases of partial or severe ADA deficiency. When measuring ADA_2 activity, erythro-9-(2-hydroxy-3-nonyl) adenine (EHNA), a specific and irreversible inhibitor of ADA (Schrader et al. 1978; Dadonna and Kelley 1981) was included in the assay. Since ADA_2 exhibits its greatest activity at high substrate concentrations, assays performed at low substrate concentrations, in the presence and then in the absence of 50 μ*M* EHNA, yield measurements of ADA activities which are more precise than those obtained by the spectrophotometric method.

Michaelis constant (K_m) determination

The ADA activities of samples from the subjects and controls were measured by the radiochemical method at substrate concentrations of between 50 and 175 μ*M*. Michaelis constants were then estimated from standard Lineweaver-Burke plots.

Heat stability measurements of red cell ADA

The method described by Hirschhorn et al. (1979) was followed and heat stability expressed as a half-life.

Starch gel electrophoresis

Isozyme patterns were determined by starch gel electrophoresis according to the method of Spencer et al. (1968).

Isoelectric focusing (IEF)

Isoelectric focusing of ADA was carried out in 1 mm thick gels containing 4.85% w/v acrylamide, 0.15% w/v bis-acrylamide, 2% v/v ampholine, pH 4–6 (LKB 1809-116), glycine (0.122 *M*) and riboflavin (0.88 μ*M*) using an LKB Multiphor apparatus. Gels were stained for ADA activity using the method described under starch gel electrophoresis.

dATP metabolite measurements

Red blood cell dATP levels were determined by the method of Solter and Handschumacher (1969) with the modification that poly (dA-dT) was used instead of calf thymus DNA as the template (R. Hirschhorn, personal communication, 1985).

Results

Family study

It was found that the proband's sister (II-2 in Fig. 1) also had partial ADA deficiency and that the father (I-1), in addition

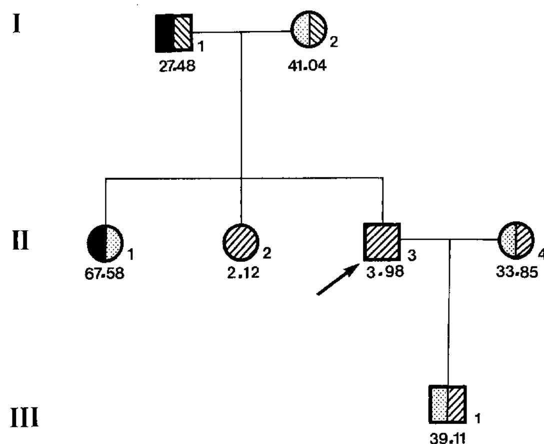

Fig. 1. Pedigree of the proband's family. Haemolysate ADA activities (nmol/mg Hb/h) appear below the symbols. The normal activity level is 67.0 ± 13.5 nmol/mg Hb/h. ▨, ADA-1 (normal); ■, ADA-5; ▨, ADA-partial deficiency

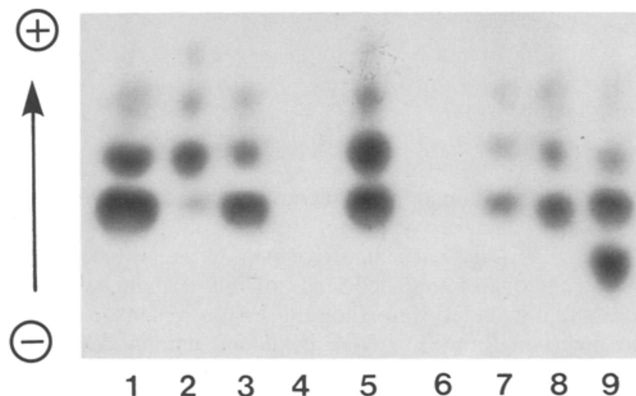

Fig. 2. Electrophoresis of haemolysate ADA on starch gel. From *left to right* samples were *(1)* ADA-1, control, *(2)* I-1, *(3)* I-2, *(4)* II-2, *(5)* II-1, *(6)* II-3, *(7)* III-1, *(8)* II-4, *(9)* ADA 2-1, control

Table 1. ADA activity in RBC at adenosine substrate concentrations of 0.1 m*M* for the spectrophotometric method and 0.09 m*M* for the radiochemical method. Activity expressed in nmol/mg Hb/h. Numbers in *brackets* refer to the number of observations on the same individual or on different normal individuals

		Spectrophotometric method	Percent of normal activity	Radiochemical method	Percent of normal activity
Normals		67.0 ± 13.5 (27)	100.0	54.57 ± 8.2 (5)	100.0
II-3	KJ	3.98 ± 1.32 (5)	5.9	4.97	9.1
II-2	VJ	2.12 ± 0.54 (3)	3.2	2.75	5.0
I-1	AJ	27.48	41.0	26.99	49.5
I-2	JJ	41.04	61.2	32.03	58.7
II-1	EJ	67.58	100.0	88.14	161.6
II-4	PR	33.85	50.5	–	–
III-1	Vuz	39.11	58.4	–	–

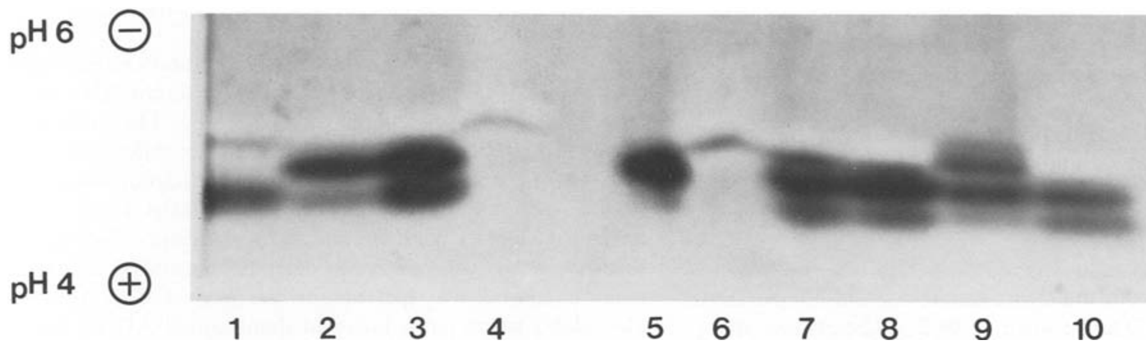

Fig. 3. Isoelectric focusing (IEF) of haemolysate ADA. From *left to right* samples were *(1)* I-1, *(2)* I-2, *(3)* II-1, *(4)* II-2, *(5)* ADA-1, control, *(6)* II-3, *(7)* III-1, *(8)* II-4, *(9)* ADA 2-1, control, *(10)* ADA-1, control

to being an obligatory carrier of the partial ADA-deficiency allele, possessed another rare allele, probably *ADA*5* (Fig. 2). The proband's other sister (II-1) also inherited the *ADA*5*-like allele from her father as well as the *ADA*1* allele from her mother (I-1). The residual ADA of the proband and her sister did not appear to migrate at a different rate to the ADA of individuals with the normal ADA-1 phenotype when electrophoresed in starch gels.

Activity measurements (Table 1) with adenosine as substrate confirmed that both the proband and his sister (II-2) had deficiencies of red cell ADA activity. (Their respective levels were approximately 3 and 5% of normal using deoxyadenosine as substrate.) Their sibling (II-1), who had the ADA 5-1 phenotype, was found to have normal levels of activity. The parents (I-1 and I-2) had, as expected, intermediate levels of ADA activity (≅46 and ≅61% of normal, respectively). The probands wife (II-4) also has intermediate levels of activity which suggests that she too carries an allele for partial ADA deficiency. In contrast to his low red blood cell ADA activity (6–9% of normal), the proband's white cells were found to have almost 30% of normal activity.

K_m determination

The proband's residual ADA was found to have a K_m for adenosine of 49.9 ± 15.8 μ*M*, a value which is not significantly different from that estimated for normal ADA (51.7 ± 11.4 μ*M*). The K_m for deoxyadenosine was estimated to be 39.9 ± 11.4 μ*M*, a value which is similar to that found for normal ADA (50.7 μ*M* ± 22.2).

Heat stability

The half-life measurements suggest that the proband and his sister produce an ADA which is much less stable than that of individuals with the ADA-1 phenotype. Under the specified conditions, the proband's red cell ADA had a half-life of 13.5 ± 3.5 min compared with a value of 58 ± 18 min for ADA from ADA-1 normal controls.

Red cell dATP levels

Red cell dATP levels were measured to determine whether or not the residual enzyme activity was sufficient to prevent accumulation of metabolites in vivo. Normal red blood cells were found to contain between 1.4 and 4.4 nmol of dATP/ml packed red blood cells (3.0 ± 1.2). The proband in this study was found to have slightly elevated red blood cell dATP (8.5 nmol dATP/ml packed red blood cells). The proband's sister (II-2), who has similar ADA levels, also appears to have only slightly elevated dATP levels in her red cells (5.2 nmol dATP/ml packed red blood cells).

Isoelectric focusing

Isoelectric focusing revealed that the residual red cell ADA enzyme activity of both the proband and his sister, II-2, had a higher pI than the usual ADA (Fig. 3). Their father's ADA produced two bands on IEF, a cathodal one corresponding to enzyme coded by his ADA-deficiency allele and an anodal

band due to his purported *ADA*5* allele. The ADA-partial deficiency allele of the proband's mother, however, does not express the cathodal band, indicating heterogeneity of ADA partial-deficiency alleles. However, the proband's wife, who has approximately half the normal level of red cell ADA activity, shows the cathodal band which indicates that she too is heterozygous with the same partial ADA deficiency allele as her father-in-law. The proband's son has the cathodal component due to an allele which must have come from his father, as well as the usual pattern due to the *ADA*1* allele from his mother.

Population study

Red cell ADA levels of the 68 individuals were plotted as a histogram (data not shown) from which it was apparent that a sharp fall in level occurred at about 40 nmol/mg Hb/h. A modal activity of 67.01 nmol/mg Hb/h was evident and if one accepts 40 nmol/mg Hb/h as a cut-off point the sample contained two individuals who were heterozygous for an ADA partial-deficiency allele.

Starch gel electrophoresis of these 68 samples did not reveal any phenotypic variation in isozyme patterns; all showed the ADA-1 pattern.

Discussion

Some confusion about the direct causal relationship of ADA deficiency and SCID arose when, in 1973, a 12-year-old !Kung ("Bushman") boy was discovered who at first appeared to be deficient in adenosine deaminase activity but, in contrast to the ADA-deficient SCID patients, was perfectly healthy (Jenkins 1973). Subsequent studies revealed, however, that his deficiency was not as severe as that observed in SCID patients. He had 2–3% of normal enzyme activity in his red cells and 10–33% of normal activity in leukocytes and cultured skin fibroblasts (Jenkins et al. 1976), levels which appeared to be adequate for efficient functioning of the immune system. This form of partial ADA deficiency was considered to constitute a recessively inherited entity mediated by an allele not identical with those associated with SCID. Population studies on the !Kung suggested that this allele, designated *ADA*8*, occurred in that population at polymorphic frequencies (Jenkins et al. 1976, 1979). Several other cases of partial ADA deficiency have since been described and their residual enzyme characterised (Hirschhorn et al. 1983; Dadonna et al. 1983; Hirschhorn and Ellenbogen 1986). Enzyme, DNA, and mRNA studies of Epstein-Barr virus transformed B-lymphocyte cell lines derived from several partially ADA-deficient individuals as well as ADA-deficient SCID patients have revealed much genetic heterogeneity (Hirschhorn et al. 1983; Adrian et al. 1984; Dadonna et al. 1985; Valerio et al. 1984; Hirschhorn and Ellenbogen 1986).

The cell lines derived from the !Kung boy, GM3043, had adenosine deaminase with a greatly reduced half-life ($t_{1/2}$) normal electrophoretic mobility, and a normal isoelectric point (pI 4.9) (Hirschhorn et al. 1983). Studies of hybridization between a cDNA probe and adenosine deaminase mRNA from this cell line, revealed that the ADA mRNA concentration was elevated twofold above normal which may explain why his enzyme activity levels was so much higher (47.5%) than that found in the other partially ADA-deficient cell lines (Dadonna et al. 1985). The ADA of another cell line, GM2294, derived from a black American individual, differed considerably from that of the !Kung cell line, GM3043, in spite of the likelihood that both variant alleles arose in Africa. Enzyme activity was lower in GM2294, the $t_{1/2}$ was lower, and the isoelectric point was higher (pI 5.0) than in cell line GM3043 (Hirschhorn et al. 1983). Furthermore, the hybridizable mRNA of GM2294 was present at about 85% of normal compared with 210% in GM3043 (Dadonna et al. 1985).

Adenosine deaminase derived from red and white cells of our proband appears to resemble the enzyme from GM2294 rather than that from GM3043. The ADAs produced by the proband as well as by GM2294 both have decreased heat stability, an increased isoelectric point, but apparently normal electrophoretic mobility in starch gels. In the more recent study of Hirschhorn and Ellenbogen (1986) in which ADAs from transformed cells of five cases of partial ADA deficiency are described, one cell line, GM5816, in particular produces an ADA which appears to be similar to that of cell line GM2294 and that of our proband.

The isoelectric focusing study carried out on samples from the proband's family, suggests that two different ADA-deficiency alleles are segregating in this family. The proband's father (I-1) and mother (I-2) appear to be obligate heterozygotes for partial adenosine deaminase deficiency since they both have significantly reduced levels of ADA activity and have two children with partial ADA deficiency. Isoelectric focusing of red cell adenosine deaminase from I-1 reveals a major band of activity (presumably the product of his *ADA*5*-like allele) which has a lower pI than normal ADA-1 and a weakly staining band with a higher isoelectric point than ADA-1 (although one which is not as high as that of ADA-2). This weak band appears to be the product of his ADA-deficiency allele whose inheritance can be traced in this way through two successive generations. The proband, his sister, II-2, and his son, III-1, all produce this isozyme. In all three individuals, the presence of the band correlates with low ADA activity: in the proband and his sister II-2, very low levels of ADA activity result from the combination of this allele with their mother's type of deficiency allele while in III-1, intermediate levels of ADA activity occur due to the combination of this allele with a normal *ADA*1* allele from II-4. Isoelectric focusing of material from the proband's mother does not reveal this weakly staining band and only the major band of activity corresponding to enzyme coded for by her *ADA*1* allele is evident. The enzyme, if any, which is coded for by the deficiency allele of I-2, must be present at levels which are much lower than that coded for by the deficiency allele of her husband.

Apart from two ADA "deficient alleles" in this family, starch gel electrophoresis has indicated the presence of a third allele, one which produces a similar isozyme pattern to that due to the *ADA*5* allele found by Detter et al. (1970) in two unrelated Afro-Americans. A similar pattern was also observed after electrophoresis of ADA from an African from Mozambique (Renninger and Bimboese 1970). Since the family under investigation is also African (being part of the Xhosa tribe), it is possible that the *ADA*5* allele is more common in African populations than in others. Individual I-1 is very unusual because he is heterozygous for a deficiency allele and an *ADA*5*-like allele which makes him in effect hemizygous for the *ADA*5*-like allele.

Adenosine deaminase from the transformed partially ADA-deficient cell line GM4396 (Hirschhorn et al. 1983), has a similar electrophoretic mobility to that of ADA-5. The find-

ing of an *ADA*5*-like allele in the family of the proband in association with a partial ADA deficiency allele is therefore of great interest, although the possible significance of this finding is not apparent. The residual ADA of the proband has Michaelis constants for adenosine and deoxyadenosine which are very similar to the corresponding values of normal ADA. These findings suggest that the mutational event which results in low red cell ADA activity does not affect the active site of the enzyme.

It has previously been reported that ADA-deficient SCID individuals have red cell dATP levels which are several hundred-fold higher than those in normals. In contrast, red cells of partially ADA-deficient individuals have only slightly elevated dATP levels. In the present study, the proband's red cells were found to have 2–3 times the normal level of dATP and the red cells of his sister, II-2, who has the same ADA phenotype, also showed a slight, although less marked, elevation in the level of this metabolite. These two partially ADA-deficient individuals have sufficient residual ADA activity to prevent the in vivo accumulation of adenosine and deoxyadenosine to toxic levels. It has been suggested (Hirschhorn 1983) that the red cell dATP levels as well as ADA activity levels should be determined in order to assess the susceptibility of the individual to immune system failure since adenosine deaminase activities in red and white cells from partially deficient individuals show a very wide range which, at its lower end, may overlap with levels found in ADA-deficient SCID patients. Furthermore, Hirschhorn (1983) has also suggested that this metabolite should be monitored in ADA-deficient newborns as an aid to the early detection of immunodeficiency.

A population sample, drawn from the Xhosa chiefdom to which the proband and his family belong, was screened for individuals with low red cell ADA activity in order to investigate the possible prevalence of the gene(s) for partial ADA deficiency in this group. Two out of the 68 individuals tested had levels which were similar to those of the obligatory heterozygotes for partial ADA deficiency in the family of the proband and were more than two standard deviations below the sample mean. If these two individuals are carriers of the partial ADA-deficiency state then the combined frequency of such alleles in this population sample is about 0.015 ± 0.010, a frequency at which a polymorphism is deemed to be present. A partial ADA-deficiency allele (called *ADA*8*) occurs at a polymorphic frequency of about 0.11 in the !Kung San ("Bushmen") of the Kalahari (Jenkins et al. 1979).

Until recently the !Kung lived in small family groups, hunting game and gathering plant foods. The Bantu-speaking Negro peoples are agriculturalists known to occasionally take San wives, with the result that the gene flow between these two groups has occurred, mainly from the hunter-gatherers to the agriculturalists. It is, therefore, possible that an allele such as one resulting in partial ADA deficiency could have attained polymorphic frequencies in the San due to genetic drift and from them have been transmitted to the Bantu-speaking tribesmen. There is no evidence, however, that the same ADA-deficiency alleles are present in these two groups. There is phenotypic evidence from studies on 11 other partially ADA-deficient individuals that at least eight different partial ADA-deficiency alleles exist in the nine individuals of African origin. Such variety does not support the genetic drift-gene flow hypothesis but would be in favour of selection by an environmental agent acting over a large area, and malaria would be a possible agent but other intraerythrocytic parasites could be responsible. It is also possible that some of the differences observed between the products of some of the partial ADA-deficiency alleles may reflect experimental error.

Nine of the 11 previously described partially ADA-deficient individuals are of African origin (Hirschhorn and Ellenbogen 1986), providing circumstantial evidence that natural selection operating in a tropical environment might be responsible for the apparent high frequency of the alleles. It is hoped that DNA-sequencing data will elucidate the problem by revealing the true degree of genotypic diversity in the gene for ADA as well as the relationship between mutation at the DNA level and structural effects on the enzyme.

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