References


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Prenatal diagnosis for adenosine deaminase deficiency

SUMMARY Amniocentesis was performed in two successive pregnancies of the mother of a child with adenosine deaminase (ADA) deficient severe combined immunodeficiency. Assay of ADA in amniotic fluid fibroblasts showed the pregnancies to be normal and homozygous deficient, respectively. These findings were confirmed by the demonstration of normal ADA activity in cord blood of the healthy male born of the first pregnancy and by the demonstration of undetectable ADA activity in cord erythrocytes, spleen, liver, and kidney of the abortus of the second pregnancy. Prenatal diagnosis of ADA deficiency appears to be a reliable procedure.

Deficiency of the purine catabolic pathway enzyme ADA represents the first inborn error of metabolism to be associated with an immunodeficiency disorder. The clinical significance of this association has been exploited in a number of ways. Where a family has had a child with severe combined immunodeficiency, the finding of ADA deficiency can be useful for genetic counselling, since it indicates an autosomal recessive mode of inheritance. Enzyme replacement appeared feasible since the enzyme is abundant in erythrocytes and successful correction has been achieved, though such success is not invariable. Intrathecal injection is possible since ADA is widespread in tissues. Hirschhorn et al reported the diagnosis of this deficiency in a 28-week gestation fetus. The pregnancy proceeded and the deficiency was confirmed at delivery. The purpose of this communication is to report that prenatal diagnosis has been performed successfully in two pregnancies of a woman who had previously born an ADA deficient child.

Case report

The case history, clinical and laboratory findings, and details of management of the proband have been reported elsewhere. Briefly, a first-born male was referred at 14 weeks with failure to thrive, recurrent bacterial infection, thrush, diarrhoea, and cough with respiratory distress. Tonsillar and peripheral lymphoid tissue were deficient. X-rays showed absent thymic shadow, interstitial pneumonitis, and skeletal findings suggestive of ADA deficiency. Immunological studies showed severe combined immunodeficiency, ADA deficiency was shown in red blood cells and leucocytes, and the parents were found to be heterozygous deficient. A family study has been reported elsewhere. Therapy included gammaglobulin replacement, erythrocyte and plasma infusions, and fetal liver transplants, but was unsuccessful and the patient died at age 17 months of a parainfluenza pneumonitis. The mother of this child has since had two pregnancies and prenatal diagnosis was offered for both (see below). The first of these proceeded to term and produced a healthy infant. The second was terminated at 22 weeks’ gestation.

Methods

Amniotic fibroblast cultures were performed as described. Amniotic fluid was collected at 14 to 16 weeks' gestation and set up in 25 cm² flasks (Falcon Plastics) in Ham’s F10 medium buffered with 25 mmol/l Hepes. For ADA assay, cells were lysed directly from the flask.

ADA assay on all tissues examined was determined by an isotopic method previously described in detail. Tissue homogenate or cell suspensions in 10 mmol/l

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tris (pH 7.4) were rapidly frozen and thawed, and centrifuged at 10,000 g for 20 minutes. The supernatants were dialysed against 100 vol 10 mmol/l tris (pH 7.4) at 4°C for 24 hours. A 50 μl quantity of cell extract in 100 μl of 50 mmol/l tris (pH 7.0) with 0.4 mmol/l 14C-adenosine (2 mCi/mmol) was incubated at 37°C, and reactions terminated by addition of 50 μl absolute alcohol. This was then incubated at 95°C for 2 minutes and clarified by centrifugation at 2000 g for 5 minutes. Conversion to 14C-inosine was determined by chromatography on Whatman CM-82 paper.

Results

The adenosine deaminase activities in amniotic fluid fibroblasts obtained from 30 pregnancies for indications other than potential immune deficiencies are shown in the figure. The specific activity of ADA showed a non-bimodal distribution with an arithmetic mean of 803 nmol/h/mg protein and a range of 130 to 2250 nmol/h/mg protein, values corresponding favourably with those reported by others. 

Results of ADA determinations on fibroblasts taken from the two pregnancies under study are also shown. Two separate fibroblast cultures were maintained after amniocentesis at 14 weeks in the first of these pregnancies and ADA assay gave results of 738 and 1740 nmol/h/mg protein, values compatible with normal ADA status. The pregnancy proceeded and a healthy male child was born at term. ADA level on cord erythrocytes was 115 nmol/h/mg protein (normal range 53 to 120). This infant, when reviewed at age 22 months, was growing and developing normally with no history of infections of note.

With the next pregnancy, cells obtained at 15 weeks’ gestation grew poorly and were inadequate for enzymatic assay. A second amniocentesis was performed at 18 weeks. Again these cells grew poorly and exhibited an ADA activity of 15 nmol/h/mg protein. The pregnancy was terminated at 22 weeks and ADA activity was undetectable in cord erythrocytes, kidney, liver, and splenic extracts of the abortus.

Discussion

These experiences appear to validate the role of prenatal diagnosis in families with ADA deficiency. One cautionary note is the wide range of ADA activity in ADA positive amniotic fluid fibroblasts, a phenomenon noted by others and suggested to be related to cell cycle. For clinical purposes we suggest that ADA deficiency should be considered if the measured activity approximates 10% that of the lower normal range. Our difficulty in growth of sufficient numbers of ADA negative amniotic fluid fibroblasts may have been purely coincidental. However, although ADA negative human lymphoblasts proliferate without difficulty in vitro, the metabolic consequences of this deficiency may have different implications for human amniotic fluid fibroblasts, and difficulty in growth in the absence of obvious causes may be another indicator of ADA deficiency.

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References

Propionyl coenzyme A carboxylase deficiency presenting as non-ketotic hyperglycinemia

**SUMMARY** A 4-month-old girl presented with myoclonic seizures and an electroencephalogram showing hypsarrhythmia. Hyperglycinuria and a cerebrospinal fluid to plasma glycine ratio of 0.2 suggested the diagnosis of non-ketotic hyperglycinemia. Propionic acid and methyl citric acid were present in the urine, and propionyl coenzyme A carboxylase was deficient in leukocytes and fibroblasts. The ketotic and non-ketotic hyperglycinemia cannot be differentiated by CSF: plasma glycine ratios.

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Hyperglycinemia syndromes in children may assume either ketotic or non-ketotic forms. Children with non-ketotic hyperglycinemia frequently exhibit myoclonic seizures and an absence of voluntary muscle movement. Although few survive the recurrent acute crises in infancy, those who do usually become spastic and profoundly delayed developmentally. On the other hand, ketotic hyperglycinemia is clinically characterised by intermittent attacks of ketoacidosis and hyperammonaemia, which may cause vomiting, hypotonia, and lethargy, progressing to coma or ultimately death in the neonatal period. The glycine cleavage system, which converts glycine to serine, is deficient in the cerebral tissue of children with non-ketotic hyperglycinemia. Raised brain glycine content and a cerebrospinal fluid glycine to plasma glycine ratio exceeding 0.03, in conjunction with clinical differences, was proposed as a discriminator of non-ketotic from ketotic hyperglycinemia. Ketotic hyperglycinemia is the consequence of deficient propionyl coenzyme A carboxylase, methylmalonyl coenzyme A mutase, β-ketothiolase, or isovaleryl coenzyme A dehydrogenase activity, none of which appears to affect CSF glycine concentration.