

MINIREVIEW

ADENOSINE DEAMINASE DEFICIENCY AND SEVERE COMBINED IMMUNODEFICIENCY DISEASE

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SUMMARY

The nature of the association of adenosine deaminase deficiency and severe combined immunodeficiency disease is reviewed. The basis for the molecular heterogeneity exhibited by adenosine deaminase in human tissue and the mechanisms whereby a deficiency of this activity results in the extreme perturbation of the immune system as observed in severe combined immunodeficiency are critically discussed.

Severe combined immunodeficiency (SCID) is a disorder of infancy characterized by defects of both cellular and humoral immunity with a uniformly fatal outcome if untreated. In a group of patients with the autosomal recessive form of SCID, a deficiency of adenosine deaminase activity (adenosine aminohydrolase EC 3.5.4.4, ADA) has been extensively documented (1-4). Since the deficiency of this activity, which catalyses the hydrolytic deamination of adenosine to produce inosine, represents the first association of a specific enzyme defect with an inherited disorder of T and B cell function, the nature of this association is of some significance. It has been suggested that the enzyme deficiency and the disease state are causally related. Alternatively, this association could reflect a deletion fortuitously involving the locus for ADA and a closely linked locus controlling immune function (1).

The purpose of this communication is to review the characteristics of ADA occurring in human tissue and to consider the possible mechanisms whereby a deficiency of this activity in patients with SCID could be related to the observed immunological dysfunction.

Nature of the Molecular Heterogeneity of ADA in Human Tissue

In human tissue ADA exhibits considerable molecular heterogeneity (5-11). The ADA activity of human erythrocytes displays several forms which were first demonstrated by their differing electrophoretic mobility

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Abbreviations: ADA, adenosine deaminase; SCID, severe combined immunodeficiency; T cell, thymus derived lymphocytes; B cell, bursa derived lymphocyte; PRPP, phosphoribosylpyrophosphate; EHNA, erythro-9 (2-hydroxyl-3-nonyl) adenine.

in starch gel (5). The predominance of one of several electrophoretic phenotypes in human erythrocytes was attributed to a genetic polymorphism due to two allelic genes on an autosome termed ADA<sub>1</sub> and ADA<sub>2</sub>. Human tissues other than erythrocytes were reported to have several additional electrophoretic forms of ADA designated a to e, and termed "tissue specific" isoenzymes. Isoenzyme a was noted only in lung and liver extracts, isoenzyme e in kidney and intestinal extracts while isoenzymes b, c and d were widely distributed. While the molecular weight of human erythrocyte ADA was reported to range from 30,000 to 35,000 (9, 10), the molecular weight of ADA in other tissues was reported to be from 280,000 to 440,000 (7).

More recent studies on the molecular weight and interconvertibility of the various forms of human ADA have helped to clarify the nature of the molecular heterogeneity of the enzyme (8, 12-15). Akedo and his coworkers reported two forms of ADA with molecular weights of 47,000 and 230,000. The large form occurred only in lung and liver extracts, while the small form was found in leukocyte preparations; in gastric tissue a mixture of the two species was present (8). An interrelationship between the two forms of ADA was suggested by their demonstration that the small form of the enzyme could be converted to the large species by a specific protein which they termed "conversion factor" (12). Subsequently, Van der Weyden and Kelley (15) noted four species of ADA with different molecular weights in human tissue. One form of the enzyme in human tissue was particulate and appeared to be associated with the plasma membrane (15-17). Three forms of the enzyme were soluble and interconvertible with apparent molecular weights of 36,000 (small), 114,000 (intermediate) and 298,000 (large). As observed by Akedo et al, the small form of ADA, which represents the protomer (18), was convertible to the intermediate and large form only in the presence of another protein, which had a molecular weight of 200,000 and exhibited no ADA activity. This conversion of the small form to the large occurred at 4<sup>o</sup>, exhibited a pH optimum of 5.0 to 8.0 and was associated with a loss of "conversion factor" (15). In the absence of "conversion factor" the large species disaggregated to the intermediate and small form (15). In accordance with these findings, the small form of ADA predominates in tissue preparations exhibiting the higher enzyme specific activities but no detectable "conversion factor" while the large form predominates in tissue extracts exhibiting the lower enzyme specific activities and abundant "conversion factor". The kinetic characteristics of the three soluble species are identical except for pH optimum which is 5.5 for the intermediate species and 7.0 - 7.4 for the large and small form.

Elucidation of the interrelationship of the different molecular forms of ADA has also helped to partly explain the electrophoretic heterogeneity. With isoelectric focusing the small form shows two to four electrophoretic variants. The large form exhibits the electrophoretic properties of the small form plus three to four additional variants. However, in contrast to the findings with starch gel electrophoresis (7), no tissue specificity of the isoenzymes is evident other than that attributable to the presence or absence of conversion factor.

#### Nature of the Association of SCID and ADA Deficiency

Several observations provided strong evidence that the deficiency of ADA in patients with SCID was genetically determined. Reduced erythrocyte ADA activity was noted among relatives of children with SCID and ADA deficiency (3, 19, 20, 21) with a pattern suggesting transmission of the defect as an autosomal mendelian trait. In addition, the electrophoretic

mobility of ADA in fibroblast extracts from apparent heterozygotes corresponded to that observed in fibroblast extracts of the relevant homozygote (22, 23). ADA activity, however, has been noted to be normal in red cell lysates of the parents of at least two children with SCID and ADA deficiency (24, 25). In one of these families (25), but not the other (24), the deficiency of ADA appears to be due to the presence of an inhibitor. These observations suggest that different mutations and perhaps even different molecular mechanisms may lead to a deficiency of ADA in patients with SCID.

One of the initial proposals to account for the association of ADA deficiency and SCID was a genetic deletion or a frameshift mutation involving both the locus for ADA and a linked locus critical for immune function (1). This hypothesis was based on the observations that the loci for ADA and HLA might be linked (26) and that one type of immune responder gene was linked to HLA (27). The subsequent assignment of the loci for ADA and the HLA complex to human chromosome 20 and chromosome 6, respectively (28), rendered this proposition less likely. Although identification of these loci to different chromosomes did not exclude the possibility that ADA deficiency was due to a large deletion or frameshift mutation, strong evidence against this was provided by the finding of residual ADA activity in tissue (24) and fibroblast cell strains (22, 23) from patients with ADA deficiency and SCID. In the splenic tissue of one patient with SCID, 0.5% of normal ADA activity was noted (24). The bulk of this residual activity was found to have the molecular and kinetic properties of the intermediate form of the enzyme. Thus, in contrast to the normal enzyme, this mutant form of ADA did not aggregate to the large form nor dissociate to the small form (24). Residual ADA activity has now been reported in fibroblasts from five additional patients with SCID and ADA deficiency (22, 23). Although the kinetic characteristics and molecular weights of the ADA in each of these cell strains were indistinguishable from normal (22, 23), the electrophoretic mobility and heat lability of the ADA from these cells differed from that displayed by the wild-type enzyme. The demonstration and characterization of residual ADA activity in various tissues of patients with SCID thus not only provided evidence against the deletion hypothesis, but led to the suggestion that the mutations involved a gene or genes which affect the structure of ADA. Thus the deficiency of ADA appears to be the primary genetic defect.

#### Mechanisms Whereby the Deficiency of ADA Activity Results in the Expression of SCID

Several observations suggest that ADA may be of importance in cell proliferation and/or differentiation. Exogenous adenosine and/or inhibitors of ADA activity depress the viability and function of lymphoblasts (29-32), macrophages (33, 34) and fibroblasts (29) in cell culture. In addition, exogenous ADA activity stimulates the depressed *in vitro* response of mononuclears from a patient with SCID and ADA deficiency to specific mitogens (35). The toxicity of adenosine accumulation has been postulated to be secondary to an induced state of "pyrimidine starvation" since (a) there is a reduction of the intracellular pyrimidine di- and tri-phosphate pool and (b) the toxicity can be prevented by the addition of the pyrimidine nucleosides, uridine or cytidine. Inhibition of pyrimidine biosynthesis *de novo* in this situation has been postulated to be due to a block in the conversion of orotic acid to orotidine 5'-monophosphate secondary to adenosine mediated depletion of phosphoribosylpyrophosphate (PP-ribose-P) (36). These findings have led to speculation that the immune dysfunction associated with ADA deficiency may be improved by uridine administration.

There are several major questions which remain to be resolved. Are the effects of adenosine specific or just a reflection of nonspecific toxicity and cell death? Are immunocytes particularly sensitive to this effect of adenosine? If the immune dysfunction in ADA deficiency is due to inhibition of pyrimidine biosynthesis de novo then how does one account for the following observations: (a) immune deficiency is not a hallmark of congenital orotic aciduria secondary to orotate phosphoribosyltransferase and orotidine 5'-phosphate decarboxylase deficiency (37) or of PP-ribose-P synthetase deficiency (38), (b) megaloblastic hemopoiesis occurs with inhibition of pyrimidine biosynthesis while the bone marrow is normoblastic in SCID due to ADA deficiency, (c) orotic aciduria does not occur in SCID and ADA deficiency (39, 40), and (d) uridine fails to augment the depressed response to specific mitogens of mononuclears from patients with SCID and ADA deficiency (3, 35)?

A possible unifying concept for the effect of ADA deficiency, whether due to a mutation or metabolic inhibition, is a disturbance in cAMP metabolism. Firm evidence exists that adenosine and cAMP metabolism are intricately related (41-44) and a marked increase in cAMP levels has been shown in fibroblast extracts of a patient with SCID and ADA deficiency (45). Furthermore, cAMP accumulation has a negative effect on both lymphocyte proliferation and PP-ribose-P synthetase activity (46, 47). Wolberg et al (33) have demonstrated that the destruction of murine ascites leukemia cells *in vitro* by specifically sensitized lymphocytes can be inhibited by adenosine. This phenomenon is potentiated by metabolic inhibitors of ADA and is accompanied by an increase in cAMP levels in the effector cells. In the light of these findings it has been suggested that the absence of ADA activity may render lymphoid cells particularly sensitive to inhibition of an effector mechanism mediated through adenosine - cAMP accumulation. Complimenting these findings has been the demonstration that monocyte - macrophage maturation is accompanied by a 2 to 9 fold increase in ADA activity (34); incubation of monocytes with cAMP blunts the monocyte-macrophage maturation process.

In conclusion, many studies are beginning to suggest a potentially important relationship between ADA and the immune system. The further elucidation of the precise role of this enzyme in immune function promises to be a most exciting chapter in human immunobiology.

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