MOLECULAR FORM OF ADENOSINE DEAMINASE IN SEVERE COMBINED IMMUNODEFICIENCY[1]

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SUMMARY. The specific activity of adenosine deaminase was reduced to approximately 0.5% of normal in splenic tissue obtained from a patient with severe combined immunodeficiency. Sedimentation analysis of splenic homogenate from this patient revealed a major peak of adenosine deaminase activity which corresponded with respect to the sedimentation coefficient of one of three molecular species observed in control spleens but had markedly reduced activity. These findings suggest that the molecular heterogeneity of human adenosine deaminase is under the control of a single genetic locus and that the deficiency of adenosine deaminase activity in severe combined immunodeficiency is not due to a genetic deletion.

INTRODUCTION . A deficiency of adenosine deaminase (adenosine aminohydrolyase, E.C.3.5.4.4) activity has recently been reported in a group of patients with severe combined immunodeficiency (1,2,3). Since this represents the first association of a specific enzyme defect with congenital T and B cell dysfunction, the nature of this association is of special interest. At the present time, however, there is insufficient data to allow one to determine whether the deficiency of adenosine deaminase activity is (a) causally related to the immunologic dysfunction, (b) a reflection of an aberration of cell function, which also alters immune function, or (c) the result of a genetic deletion, which also involves loci critical to the immune response. Support for the latter possibility has been cited (1).

Based on differences in net charge and/or molecular size, multiple forms of adenosine deaminase have been described in normal human tissue (4,5,6).

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Abbreviated in the text: S_{20w} = sedimentation coefficient.

There has, however, been no attempt to characterize the molecular form of adenosine deaminase in tissues from patients with severe combined immunodeficiency and reduced activity of this enzyme. In this communication we report our findings on the activity and molecular form of adenosine deaminase in splenic homogenate obtained from a patient with severe combined immunodeficiency.

METHODS

Enzyme assay. Adenosine deaminase was assayed by the conversion of [8-^{14}C] adenosine (New England Nuclear Corporation) to [8-^{14}C] inosine. The standard adenosine deaminase reaction mixture contained [8-^{14}C] adenosine (0.16 mM, 50.4 μCi/umole or 0.4 mM, 2 μCi/umole), 50 mM Tris, pH 7.4, and enzyme (3-15 μg protein) in a final volume of 100 μl. After incubation at 37°C for 15 to 120 minutes, the reaction was terminated by the addition of 50 μl of absolute alcohol. The precipitated protein was removed by centrifugation at 1,000 X g and a 20 μl aliquot of the supernatant was spotted on Whatman 3 MM chromatography paper with appropriate carriers. Separation of the substrate from the product was achieved by high voltage electrophoresis (250 ma, 4000 to 6000 v) in 50 mM borate buffer, pH 8.5, containing 1 mM EDTA. Inosine was identified on the paper with ultraviolet light (254 nm), cut out and counted in a Packard Tri-carb liquid scintillation spectrometer at 63% efficiency. The formation of inosine was linear with time and protein concentration under these conditions.

Enzyme preparation. Splenic tissue was obtained from 5 control subjects and from one subject with severe combined immunodeficiency at the time of necropsy. The tissue from the patient with severe combined immunodeficiency and that of two control subjects had been stored at -70°C for a comparable length of time (30 to 36 months). Crude splenic homogenates were prepared by homogenizing one part of tissue with 3 to 4 volumes of 10 mM Tris, pH 7.4, and centrifuging at 6,600 X g for 30 minutes at 4°C.

Protein determination. Protein was determined using the method of Lowry et al (7) with bovine serum albumin as standard.

Sucrose gradients. 10% to 28.2% isokinetic sucrose gradients were prepared in 10 mM Tris, pH 7.4, as described previously (8). Samples (200 µl) of crude splenic homogenate and standards were layered on the gradients and then centrifuged at 40,000 RPM for 30 hours at 4°C in a Spinco SW41 rotor using a Beckman L5-50 ultracentrifuge. Fractions of 255 µl were collected with a Gilson microfractionator. Sedimentation coefficients (S_{20w}) were calculated on the basis of a linear relationship of the S_{20w} to the distance migrated in the sucrose gradient (9). Bovine serum albumin (S_{20w}, 4.3), human hemoglobin (S_{20w}, 4.1) and catalase (S_{20w}, 11.3) were used as standards for calculations.

RESULTS AND DISCUSSION. The specific activity of adenosine deaminase in homogenates prepared from splenic tissue of 5 control subjects and a patient with severe combined immunodeficiency were 7.82±2.59 and 0.03 (5,600 CPM above background) µmoles/hr/mg protein, respectively. Storage at -70°C for up to 36 months had no apparent effect on enzyme activity or on enzyme profile by sedimentation analysis. The splenic homogenates from three control subjects, when subjected to zone-sedimentation analysis, exhibited three peaks of adenosine deaminase activity (Fig. 1A) with S_{20w} values of 3.2; 7.1 to 7.4; and 10.2 to 10.4. In contrast, the splenic homogenate from the patient with severe combined immunodeficiency (Fig. 1B) displayed one molecular species of adenosine deaminase with a S_{20w} of 7.4. The total activity in this peak was approximately 2.2% of the total activity observed for the corresponding molecular species in the control preparation. The presence of adenosine deaminase activity at a low level was reproducible at the bottom of the gradient in both the control preparation and that from the patient with severe combined immunodeficiency. The nature of this activity has not been further defined.

The cause of the molecular heterogeneity of adenosine deaminase in normal human tissues has been unclear. One hypothesis proposed by Edwards et al (5) was that each molecular form of adenosine deaminase was the product of a specific gene locus. If this were the case, a mutation involving one of these loci should not result in the reduction in activity of all forms of the enzyme. Our finding

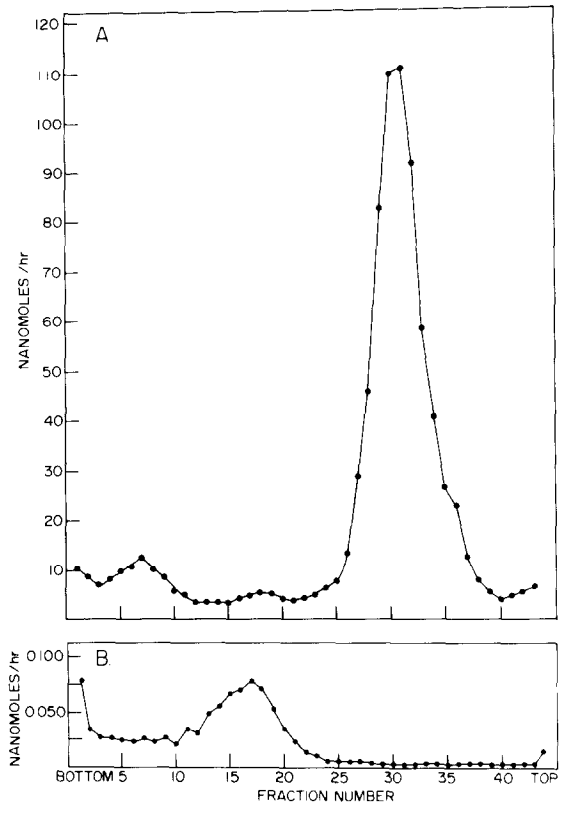

Fig. 1 - Sucrose density gradient ultracentrifugation of adenosine deaminase in splenic homogenate. A. Control Subject. B. Patient with severe combined immunodeficiency. Note different ordinates.

that two molecular forms of adenosine deaminase are absent in splenic tissue of a patient with severe combined immunodeficiency, while the form which is present, is markedly reduced in activity when compared to normal tissue, is, therefore, inconsistent with this hypothesis. The studies of Ressler (10) and Nishihara et al (11) suggest that the molecular forms of adenosine deaminase are interconvertible. One interpretation of their results is that all forms of adenosine deaminase are under the control of a single genetic locus and that the molecular heterogeneity results from post-translational events. Our observations provide further evidence for this interpretation.

The substantial residual adenosine deaminase activity in the splenic homogenate from the patient with combined immunological deficiency cannot be attributed to contamination occuring after the patient's death since

examination of another enzyme in the same tissue (hypoxanthine-guanine phosphoribosyltransferase, E.C.2.4.2.8) revealed that only the mammalian form of the enzyme was present. In addition, for the reasons cited above, the adenosine deaminase activity present cannot be attributed to another gene coding for this enzyme. These observations suggest that the residual adenosine deaminase activity in splenic tissue from the patient with severe combined immunodeficiency is due to a mutant form of the enzyme with a low level of catalytic competence.

The initial suggestion that the association of adenosine deaminase deficiency with severe combined immunodeficiency could be due to a large genetic deletion affecting both adenosine deaminase and immune function, was based on observations that the locus for adenosine deaminase appeared to be linked to the loci for HL-A (12). Recent studies using mouse-human hybrid cells in culture indicate that this is not the case since the locus for adenosine deaminase is on chromosome 20 whereas the HL-A loci are on chromosome 6 (13). However, the possibility that this association was due to a deletion involving the gene for adenosine deaminase and an important immune response gene unrelated to the HL-A loci could not be excluded by this type of evidence. In the present study we do provide evidence against this hypothesis since a large deletion involving the gene coding for adenosine deaminase would be expected to lead to the production of no enzyme protein or of a markedly aberrant molecule. In either case an absence, rather than a low level of catalytic activity, would be expected.

Although we can now suggest that adenosine deaminase deficiency is not a reflection of a large genetic deletion, it does not necessarily follow that adenosine deaminase deficiency is causally related to immune dysfunction. Our studies do not exclude the cogent possibility that the deficiency of adenosine deaminase may reflect a general disorder of cell function which also affects the recognition, transport or processing of antigens or mediators of the immune response.

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