CURRENT TOPICS IN HEMATOLOGY

Volume 4

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Genetic Deficiencies of Adenosine Deaminase and Purine Nucleoside Phosphorylase and Their Implications for Therapy of Leukemias

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I. INTRODUCTION

Immunodeficiency disorders have traditionally been classified using the conceptual framework that these disorders represent defects at different stages of differentiation of a common stem cell into either the humoral or cellular arms of the immune system [1]. During the past decade, the discovery of at least three specific inherited molecular defects, each of which gives rise to a different immunodeficiency syndrome, provides a further basis for diagnosis, classification, and delineation of primary immune disorders. Thus, inherited absence of adenosine deaminase (ADA) usually results in the clinical syndrome of severe combined immunodeficiency (SCID), genetic absence of purine nucleoside phosphorylase in a cellular immunodeficiency syndrome, and absence of transcobalamin II in agammaglobulinemia (as well as defects of other hematopoietic stem cells).

The finding of specific molecular defects with toxicity essentially limited to the lymphoid system also provides tools for further insight into the differentiation and interaction of the components of the lymphoid system and the metabolic pathways involved and for development of selective chemotherapeutic and immunosuppressive agents.

II. ADENOSINE DEAMINASE DEFICIENCY

A. Clinical, Genetic, and Pathologic Aspects

In 1972, Giblett and co-workers described two unrelated female children who presented with autosomal recessive severe combined immunodeficiency (SCID) and who both also had autosomal recessive inherited absence of the enzyme adenosine deaminase [2]. Several lines of evidence have demonstrated the genetic nature of the enzyme defect and therefore the primary and causal relationship of the enzyme defect and the immunodeficiency. Consistent with an autosomal recessive mode of inheritance, family studies demonstrated that the healthy parents of affected children (ie, obligate heterozygotes) and one of each set of grandparents had approximately one half of normal ADA activity in their erythrocytes and lymphocytes. Approximately 90% of heterozygotes for the enzyme deficiency have erythrocyte ADA which is below two

standard deviations of the logarithmic mean of normal activity. While approximately 10% of obligate heterozygotes have red blood cell (RBC) ADA within the normal range, this could reflect the wide variation seen for ADA activity in normals. An additiional marker for the presence of a deficiency allele is available because ADA is genetically polymorphic, with two common codominant alleles (ADA^1 and ADA^2) segregating in the normal population. In several families of children with ADA deficiency and SCID, abnormal segregation has been demonstrated, compatible with segregation of a deficiency allele in addition to the two normal alleles. For example, in one family, a grandmother of an affected ADA^--SCID who was phenotypically ADA 2-1, had a daughter who was phenotypically ADA 2. This can be explained if the grandmother was genotypically ADA^{1-0}, giving her daughter the null allele while the grandfather gave the daughter his ADA^2 allele. In this family, both the grandmother and daughter also had ADA activity in the heterozygous range. The daughter married a man who was also heterozygous for ADA and gave birth to a child with ADA^--SCID [2–9]. Finally, prenatal determination of adenosine deaminase in amniotic cells has allowed for the prediction of both ADA deficiency and immunodeficiency at birth. Over a dozen pregnancies at risk have been monitored and at least two affected children diagnosed correctly [9,10].

ADA deficiency has been found to account for between one third and one half of cases of SCID, not obviously transmitted in an X-linked manner. In cases where SCID is definitely autosomally recessive, the incidence of ADA deficiency has been found to be 57% with 95% confidence limits of 18%–90%. Therefore, a substantial portion of autosomal recessive SCID appears to be due to deficiency of ADA. Indeed, an informal survey of 130 patients with SCID (both autosomal recessive and X-linked) revealed 22% with ADA deficiency [11–13].

Over 30 families with ADA deficiency and immunodeficiency have been described in the literature and many more cases have been found [14]. Approximately 80%–90% of the patients are indistinguishable clinically as well as by *in vitro* tests of immune function from other patients with SCID. Normal numbers of lymphocytes and T lymphocytes as well as the presence of a response to phytohemagglutinin (PHA, albeit reduced) may be found at birth. Although this is unusual, it sugests that the diagnosis can most reliably be made at birth in a family at risk by determination of ADA activity in the red cells. The disease is progressive and any residual T-cell function found at birth rapidly disappears. Clinical manifestations usually develop in the first months of life and humoral immunity does not develop.

In 10%–15% of the cases, onset of disease may occur later than 3 to 6 months. Thus, one of the first reported patients, followed closely because of the prior death of a sib with SCID, was known to be immunocompetent and healthy until over 2 years of age, which is the latest known age of onset to date [2]. The outstanding feature in this group is the retention of immunoglobulins

and even the presence of specific antibody. These patients were undoubtedly originally classified as Nezelof syndrome. However, immunoglobulin levels eventually fall and in one case there was a preterminal monoclonal IgG [15]. Unless treated, death has occurred by 3 years of age and usually earlier. It is logical to expect that if all patients with primarily cellular immune defects were to be tested, even later age of onset and a more insidious course would be found. Indeed, such a child has been recently ascertained (see below).

During the initial studies of patients with ADA deficiency it appeared that two features might discriminate between ADA-deficient and non-ADA-deficient SCID patients. The first of these was bony abnormalities, originally appreciated radiologically [16] and subsequently studied pathologically [17]. This bony abnormality (eight of 13 families) was evident on physical examination as prominence of the costochondrial rib junction, similar to a rachitic rosary. On x-ray, cupping and flaring of the costochondral junctions was seen as well as a dysplastic pelvis. However, as initially noted, these changes are not pathognomonic and similar radiological changes, including cupping of the anterior rib ends, were observed in nonimmunodeficient patients who were severely malnourished as well as in ADA-normal immunodeficient patients. Although the radiological changes may not be specific, these bony alterations have served to alert physicians to the possibility of ADA-deficient SCID. It was initially proposed that thymic pathology differed in the ADA-deficient cases with the retention of some Hassall's corpuscles suggesting secondary atrophy of a previously differentiated thymus [18]. However, on a practical level, in patients examined from seven kindred with and without ADA deficiency we could find no such consistent correlation between thymic pathology and the presence or absence of ADA deficiency [19].

Neurological abnormalities have been reported in three cases. The most striking was a patient admitted to the hospital primarily because of neurologic abnormalities, including nystagmus, spasticity, and choreoathetoid movements [14,20]. It is obvious that these neurological manifestations could reflect a viral disease in these immunodeficient patients rather than specific pathology secondary to the enzyme deficiency. However, in two patients the neurological abnormalities cleared completely, concomitant with red cell therapy and lowering of metabolites. In one of the patients, resolution of neurologic abnormalities occurred concomitant with reduction in metabolite concentrations brought about by partial red cell exchange transfusion. Additionally, the neurologic improvement preceded by several months any alteration in immune function and occurred in the face of persistent diarrhea and malnutrition. These observations suggest that the neurologic manifestations may be an integral part of the syndrome, reflecting interaction of accumulated purines with purinergic receptors on brain cells [21]. More recent studies demonstrating an apparently dose-related incidence of neurologic abnormalities in

patients treated with deoxycoformycin, an inhibitor of ADA, would support this hypothesis [22]. An *in vitro* defect in platelet aggregation has also been reported [23,24] and it is of interest that one patient had severe gastrointestinal (GI) bleeding, albeit presumably from an ulcer.

B. Enzyme Characteristics, Assay, and Distribution in Tissues

The adenosine deaminase which is deficient in ADA$^-$-SCID exists in several molecular forms or isozymes. The catalytic unit is a 35,000-dalton monomer which can exist free (ADA_1) or as a 280,000-dalton complex (ADA_{1+CP}), which results from combination of two molecules of ADA_1 with a 200,000-dalton, enzymatically inactive glycoprotein (CP). This enzymatically inactive glycoprotein, variously termed combining protein or complexing protein, is a dimer of two approximately 100,000-dalton polypeptides. It is not clear if these two polypeptide chains are identical or differ only in the extent of glycosylation. The 35,000-dalton catalytic unit is coded for by a locus on the long arm of chromosome 20 (probably q13), while chromosomes 2 and 6 are required for expression of the combining protein. The basic polypeptide chains of both the 35,000-dalton catalytic unit and the 200,000-dalton combining protein also undergo posttranslational modifications. These posttranslational modifications result in the generation of several secondary isozymes with differing electrophoretic mobility. The posttranslational modifications of the combining protein are different in and characteristic for different tissues. Variations in the extent and type of glycosylation in different tissues appears to account for the "tissue-specific" electrophoretic differences. Both the 35,000-dalton catalytic unit and the various high molecular weight isozymes are deficient in all tissues and cultured cells from affected patients [reviewed in 25].

A minor portion of intracellular ADA in man is exhibited by an isozyme which is unrelated to the group of isozymes containing the 35,000-dalton catalytic unit. This minor component of ADA (ADA_2) has a molecular weight of 100,000 daltons, markedly different kinetic properties from, and no antigenic cross reactivity with, the major isozymes of ADA_1. This ADA_2 isozyme is controlled by a separate genetic locus, since it is present in normal amounts in tissues and cells of patients with ADA$^-$-SCID [26]. Although this isozyme is a minor component of most mammalian tissues, it may represent as much as 50% of the adenosine deaminating activity in human serum when the conditions of assay favor the kinetic properties of this isozyme (ie, high adenosine concentrations) [27]. It can most easily be differentiated from the ADA_1 which is deficient in ADA$^-$-SCID by its resistance to inhibition by EHNA, a competitive inhibitor of the more abundant form of ADA.

Adenosine deaminase has been found in all human tissues and cells studied, although the absolute specific activity varies almost 200-fold. The highest

ADA activity is found in thymus and the lowest in erythrocytes. Both of these cell types contain virtually undetectable amounts of any isozyme additional to the free 35,000-dalton ADA_1. Lymphoid tissue and some tissues of the gastrointestinal system and possibly some portion of the cerebral cortex contain the next greatest activity of ADA. All of these latter tissues contain predominantly ADA_1. Lymphoid tissue and some tissues of the gastrointestinal system and possibly some portion of the cerebral cortex contain the next greatest activity of ADA. All of these latter tissues contain predominantly ADA_1 but also contain easily detectable amounts of ADA_{1+CP}. Other tissues tested, including liver, lung, and kidney, all had similar and lower activities of ADA, but greater than that found in erythrocytes. All of these tissues predominantly exhibit the ADA_{1+CP} isozyme(s) [28–31,90].

Several different methods have been used to determine ADA activity. All methods measure, directly or indirectly, either the conversion of adenosine to inosine or the production of ammonia resulting from the deamination of adenosine. Spectrophotometric methods either directly measure the decrease in absorbance at 265 nm, which accompanies the conversion of adenosine to inosine, or utilize added "linking" enzymes (commercially available purine nucleoside phosphorylase and xanthine oxidase) to convert the inosine to uric acid, which has an absorbance maximum of 293 nm. The rate of increase in absorbance at 293 nm is then measured. This latter modification allows for greater sensitivity, use of substrate concentrations well above the K_m, and extracts with high protein content. Conversion of adenosine to inosine can also be measured with radiolabeled adenosine and separation of product and substrate by several different methods. HPLC can also be used for separation of product and substrate and allows for use of a variety of nonradiolabeled substrates. The enzyme can "deaminate" several different naturally occurring modified adenine nucleosides, including 2′-deoxyadenosine and 2′-O-methyladenosine, whose rates of deamination relative to adenosine are approximately 80% and 25%. Several artificially modified adenine nucleosides, of which adenine arabanoside (Ara A) is an example, are also deaminated [32–37].

C. Abnormal Metabolites and Pathophysiologic Mechanisms in Adenosine Deaminase Deficiency

Adenosine deaminase is an enzyme of the purine salvage pathway which catalyzes the irreversible deamination of adenosine and 2′-deoxyadenosine as well as of several naturally occurring modified adenine nucleosides (Fig. 1) [reviewed in 25,38].

Affected children accumulate and/or excrete markedly increased amounts of the substrates adenosine and deoxyadenosine in plasma, red cells, and urine. In addition, they accumulate enormous quantities of the phosphory-

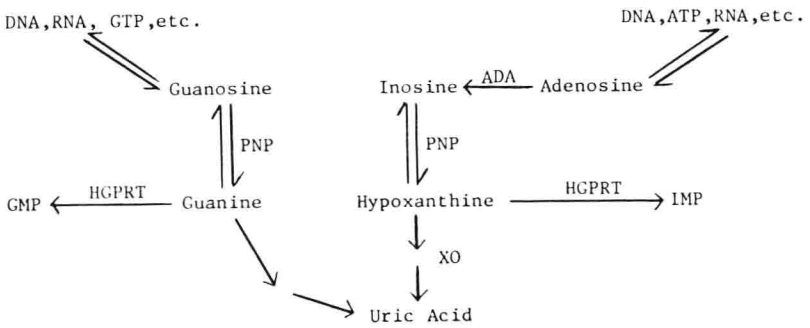

Fig. 1. Purine metabolism by adenosine deaminase (ADA) and purine nucleoside phosphorylase.

lated metabolite deoxy adenosine triphosphate (dATP) in erythrocytes and lymphocytes [21,39–48]. Affected children also uniquely excrete the modified adenine nucleoside 2′-O-methyladenosine and excrete fivefold increased amounts of another as yet unidentified modified adenine nucleoside [49]. A two- to threefold increase in lymphocyte cyclic AMP has also been reported.

Several pathophysiologic mechanisms have been proposed whereby deficiency of adenosine deaminase and the resulting accumulation of metabolites could result in immunodeficiency disease. Initial studies focused on adenosine since this compound was first reported to be increased in plasma and because earlier studies had already shown that adenosine was toxic for lymphoid cells. Following the demonstration of the marked increase in deoxyadenosine excretion and increases in intracellular deoxy ATP, attention focused upon mechanisms involving deoxyadenosine toxicity. The mechanisms proposed have included adenosine-mediated or pyrimidine starvation, adenosine-mediated increase in cyclic AMP (a known modulator of immune response), adenosine (and deoxyadenosine)-mediated increase in S-adenosyl homocysteine (SAH), an inhibitor of methylation reactions, and deoxy ATP-mediated inhibition of ribonucleotide reductase [40,41,43,50,51]. The currently most favored pathophysiologic mechanism is that involving deoxyadenosine-mediated increases in deoxy ATP since both *in vivo* as well as *in vitro* evidence support the hypothesis. Thus enormous amounts of deoxy ATP are found in cells of patients, at concentrations sufficient to be capable of inhibiting the enzyme ribonucleotide reductase. The normal functioning of this enzyme is essential to provide deoxy nucleotides for normal DNA synthesis. Second, *in vitro* experiments indicate that accumulation of deoxy ATP could account for the lymphospecific toxicity and the initially greater impairment of cellular *vs* humoral immunity. Thus, the thymus in man, compared with other tissues, has high ac-

tivity of an enzyme which converts deoxyadenosine to deoxy ATP and lower capacity to degrade deoxy ATP. *In vitro* experiments have also shown preferential accumulation of deoxy ATP by T-derived cells as compared with B-derived cells [52]. Attractive as is this hypothesis, we must inject a note of caution in extrapolating from *in vitro* to *in vivo* events. Similar enzymatic and metabolic studies in mice predict that these animals will not preferentially accumulate deoxy ATP in their thymus. Nonetheless, we have found that mice, rendered artificially ADA deficient by injection with the ADA inhibitor deoxycoformycin, preferentially accumulate deoxy ATP in the thymus [53]. The next most attractive hypothesis is that of inhibition of methylation reactions. Children with ADA$^-$-SCID have indeed been shown to have markedly diminished activity of the enzyme SAH-hydrolase, presumably as a result of "suicide inactivation" by deoxyadenosine of the SAH-hydrolase [43]. Inactivation of SAH hydrolase *in vitro* would lead to accumulation of SAH and reversal of the SAH/SAM (S-adenosyl methionine) ratio. The ratio of SAH to SAM is thought to determine the rate of SAM dependent methylation reactions crucial for normal cellular functions. *In vitro* such alterations have been found to interfere with methylation reactions in the whole cell. While elevations in SAH have yet to be demonstrated in genetically ADA-deficient patients, elevations of SAH have been found in cells of patients rendered artificially ADA deficient by treatment with deoxycoformycin, a potent inhibitor of ADA (see below).

There is, however, increasing *in vitro* data to indicate that deoxyadenosine toxicity may act at a site other than or additional to inhibition of ribonucleotide reductase. Finally, we should like to suggest that modified adenine nucleosides, which we have found to be inhibitory for *in vitro* lymphocyte responses and to be excreted uniquely or in increased amounts by ADA-deficient children, may also play a role in toxicity [49,54].

D. Therapy in Adenosine Deaminase Deficiency

Bone marrow transplantation is the therapy of choice [3]. At least seven children with ADA deficiency have been successfully transplanted. The oldest of these is over 12 years of age and clinically and immunologically normal. Bony changes originally noted have disappeared. Apparently, in addition to providing stem cells, the normal engrafted lymphocytes provide sufficient ADA activity to prevent accumulation of toxic metabolites in all cells. Indeed, Chen et al [55] have demonstrated that bone marrow-transplanted cells provided sufficient enzyme to lower red cell deoxy ATP so that it could no longer be detected by HPLC. Of even more interest, in this patient only T lymphocytes were engrafted yet metabolites appeared to have been lowered sufficiently so that the recipient's own B lymphocytes became functional. We have similarly determined metabolites in two bone marrow-transplanted ADA-de-