Adenosine Deaminase Deficiency: Unanticipated Benefits from the Study of a Rare Immunodeficiency

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The serendipitous discovery of adenosine deaminase (ADA) deficiency in two patients with cellular immunodeficiency in 1972 by Dr. Eloise Giblett and colleagues (1) ushered in a new era in the investigation of the molecular mechanisms underlying primary immunodeficiency disorders. This finding led to the eventual development of novel therapies not only for ADA deficiency but also for other immunodeficiency disorders and certain leukemias.

In the early 1970s, several primary immunodeficiency diseases, including SCID, X-linked agammaglobulinemia, and Wiskott-Aldrich syndrome, were well known to pediatric immunologists and presumed to be caused by single gene defects based on patterns of inheritance. However, the gene defects responsible for these devastating disorders were unknown. In those days, the only “cure” for severe immunodeficiency diseases was bone marrow transplantation (BMT) from a histocompatible donor. In the case of one of two patients described by Giblett et al., routine HLA typing of family members failed to identify suitable donors. Thus, the patient’s physicians sent blood samples to Dr. Giblett at the King County Central Blood Bank. It was hoped that she could shed light on the relationships among the family members of the patient by examining isozyme patterns for the enzyme ADA. Much to her surprise, starch gel electrophoresis indicated that the RBCs of the patient were completely devoid of ADA enzyme activity! The parents showed detectable, but reduced, ADA activity, suggesting an autosomal recessive mode of inheritance. Subsequently, a second patient with severe cellular immunodeficiency was studied and also found to be ADA deficient. These were completely unexpected findings, as no precedent existed for ADA deficiency in humans or for ADA’s role in either the development or the function of the immune system.

ADA is part of the purine salvage pathway that includes the enzyme hypoxanthine-guanine phosphoribosyltransferase (HPRT). Mutations in the HPRT gene were known to cause the neurologic disorder Lesch-Nyhan syndrome and its associated gouty arthritis (2), but this pathway was not thought to be important for the immune system. Giblett and colleagues proposed that the two patients might have rare mutant alleles for the ADA gene. Alternatively, it was speculated that they might have a short chromosomal deletion encompassing the ADA gene and a nearby critical immune response gene. In either case, Giblett et al. concluded the following: “Since ADA anenzymia and the inherited diseases of cellular immunity are extremely rare, their coexistence in two unrelated patients seems very unlikely to be fortuitous.”

Measurements of purine metabolites in the body fluids of ADA-deficient patients showed elevated levels of adenosine (3), one of the two substrates for ADA. Investigators quickly showed that adenosine could slow the growth of lymphoid cell lines and the mitogen-induced proliferation of primary lymphocytes (3). In 1975, Giblett and colleagues (4) reported a patient with an isolated T cell immunodeficiency who lacked activity of purine nucleoside phosphorylase, an enzyme situated between ADA and HPRT in the purine salvage pathway, providing convincing evidence of the critical importance of normal purine metabolism for a functioning immune system. Although it was originally reported that ATP was elevated in the RBCs of ADA-deficient patients (5), more sensitive HPLC separation schemes in the laboratories of Drs. Mary Sue Coleman and Amos Cohen (6, 7) revealed that 2′-deoxyadenosine 5′-triphosphate (dATP) levels were elevated as well. This finding confirmed an earlier speculation by Dr. Dennis Carson et al. (8) that deoxyadenosine, the other substrate of ADA, rather than adenosine, was the toxic metabolite in this disease. Subsequent experimentation showed that deoxyadenosine is converted first to 2′-deoxyadenosine 5′-monophosphate and finally to dATP by the high levels of deoxynucleoside kinases in the thymus. A likely pathogenic mechanism is dATP-triggered cytochrome c release from mitochondria, which triggers an apoptotic cascade, leading to failure of T cell development (9). Interestingly, an understanding of this pathway led to the development of novel and successful chemotherapeutic approaches for treating hairy cell leukemia (10).

Both ADA and purine nucleoside phosphorylase are expressed in virtually every cell in the body and had been considered “housekeeping” genes. Thus, an immediate question was why the effects of ADA deficiency were focused upon the immune system. This question led to a systematic evaluation of the expression of purine-metabolizing enzymes in various human tissues and to the discovery that ADA was found at very high levels in the thymus, suggesting that this organ had evolved a mechanism to prevent the buildup of ADA sub-

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Abbreviations used in this article: ADA, adenosine deaminase; BMT, bone marrow transplantation; dATP, 2′-deoxyadenosine 5′-triphosphate; HPRT, hypoxanthine-guanine phosphoribosyltransferase; PEG, polyethylene glycol.
strates. ADA is needed because the high rate of cell death in the thymus following T cell selection events provides a source of DNA that is degraded to deoxyadenosine. This, coupled with high levels of deoxynucleoside kinases, explains why the thymi of ADA-deficient patients accumulate such high levels of dATP (8).

In addition to the normal supportive therapy given to patients with SCID, ADA-deficient patients were initially treated with packed RBC transfusions as a sort of “enzyme-replacement” therapy (5). Many patients showed significant improvement in immune function as a result, especially those with residual ADA enzyme activity. The breakthrough in the treatment of these patients came with the development of polyethylene glycol (PEG)-modified bovine ADA by the biotechnology company Enzon. PEG-ADA (Adagen) was the first FDA-approved PEG-modified protein drug. Its use as a therapy for ADA-deficient patients was championed by Dr. Michael Hershfield at Duke (11). Many patients who do not have suitable bone marrow donors have been able to lead reasonably normal lives as a result of treatment with PEG-ADA. Today, a number of protein-based drugs that are modified by pegylation to improve stability and decrease immunogenicity are on the market. These include Neulasta (Amgen) for the treatment of leukemia, IFN-β for the treatment of chronic hepatitis C, and uricase for the treatment of refractory gout (12).

ADA deficiency also played a prominent role in the development of gene therapy. It was the perfect disease for this fledgling field. As mentioned above, it was already known that patients with SCID could be cured by BMT from a histocompatible donor. It was also known that patients with only 10–12% of normal ADA enzyme activity had normal immune systems (13). Thus, it was logical to predict that autologous BMT with genetically modified bone marrow cells would be of therapeutic value even if normal levels of gene expression could not be attained. However, initial attempts were unsuccessful because the small numbers of genetically modified cells were not maintained after transplantation (14). Nevertheless, this approach was successful in patients with X-linked SCID because the genetically modified cells had a selective advantage and eventually overgrew the remaining unmodified cells (15). This realization led to the hypothesis that gene therapy for ADA deficiency was unsuccessful because patients were maintained on PEG-ADA as a sort of standard of care. This treatment removed the selective advantage that ADA gene-corrected cells would enjoy in an otherwise ADA-deficient host. Indeed, when treatment protocols were modified to remove the PEG-ADA, gene therapy for this disorder was successful, although it usually took a year or more for the number of gene-corrected T cells to reach maximal levels (16).

As with many human diseases, immunologists developed mouse models to have an experimental system in which the consequences of ADA deficiency could be studied and new treatment strategies evaluated. Much to the surprise of the investigators who made ADA-deficient mice, these mice died in the immediate perinatal period—not of immunodeficiency, but of liver failure (17, 18). At the time of death, the effect of ADA deficiency on thymus development was relatively modest. To sidestep this problem, a strain of mice was developed that was globally ADA deficient except for that controlled with a placenta-specific promoter (19). Thus, they had ADA during fetal development and became ADA deficient only after birth. Surprisingly, they had normal liver function, showing that ADA was needed in the liver during fetal development, but not thereafter. Equally surprising, these mice died of respiratory failure at ~3 wk of age (20). However, they could be maintained on PEG-ADA indefinitely. When ADA was suboptimal, they developed immunodeficiency, as expected (21). These mice have proved useful for examining the mechanisms of ADA-deficient SCID (9). In addition, owing to the accumulation of adenosine, these animals have served as a biological screen for disorders associated with aberrant adenosine receptor signaling (22). In the past 20 y, it has become increasingly apparent that adenosine regulates many important aspects of physiology through binding to four distinct, seven-transmembrane-spanning G protein-coupled adenosine receptors (23). Although adenosine is usually immunosuppressive and anti-inflammatory, work in ADA-deficient mice helped uncover novel roles for adenosine in promoting the progression of chronic diseases, including asthma, chronic obstructive pulmonary disease, and pulmonary fibrosis (22). In addition, these mice helped to define a novel role for adenosine signaling in certain manifestations of sickle cell disease (24).

In conclusion, the discovery of ADA deficiency as a cause of SCID was groundbreaking for several reasons. First, it was the first immunodeficiency disease for which the molecular defect was identified, making possible both a prenatal and a postnatal molecular diagnosis. Second, it underscored the importance of normal purine metabolism for the development of the immune system. Understanding the mechanisms of ADA-deficient SCID led to the development of ADA inhibitors and deoxyadenosine analogs for the treatment of hairy cell leukemia (10). PEG-ADA became the first PEG-modified protein to be used as a therapeutic and opened the door for the development of additional PEG-modified proteins that are in wide clinical use today. ADA deficiency was the first inherited disease to be treated by gene therapy. Finally, ADA-deficient mice became an invaluable tool for the study of adenosine receptor signaling in chronic lung diseases and sickle cell disease. Thus, the history of investigations of ADA deficiency, initiated by the startling absence of ADA bands on Eloise Giblett’s starch gel, illustrates the potential impact of serendipitous discoveries in science and medicine and the unanticipated rewards that can arise from the study of patients with rare diseases.

Disclosures
The authors have no financial conflicts of interest.

References