

BRIEF REPORT: HEPATIC DYSFUNCTION AS A COMPLICATION OF ADENOSINE DEAMINASE DEFICIENCY

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COMplete deficiency of adenosine deaminase causes severe combined immunodeficiency that is inherited as an autosomal recessive trait. The patients present in infancy with recurrent infections, lymphopenia, defective proliferative responses to mitogens, hypogammaglobulinemia, and an inability to mount specific antibody responses. Patients with a low level of residual adenosine deaminase activity have a later onset of clinical disease owing to a slower and sometimes less complete loss of immune function.¹⁻³

Unlike other primary immunodeficiencies caused by defects in lymphocyte signaling pathways,⁴ adenosine deaminase deficiency is a systemic metabolic disorder. The enzymatic defect is expressed in all cells, and therefore the substrates for the enzyme, adenosine and 2'-deoxyadenosine, accumulate in cells of all types.³ Immunodeficiency is thought to occur because immature lymphoid cells are particularly sensitive to the toxic effects of adenosine and 2'-deoxyadenosine. In addition, some patients have neurologic abnormalities that are thought to be due to adenosine deaminase deficiency.⁵ Unlike humans, mice that express no adenosine deaminase die perinatally of severe hepatocellular degeneration.^{6,7} Hepatotoxicity in humans has not previously been attributed to adenosine deaminase deficiency. We report a neonate with this deficiency and prolonged hyperbilirubinemia with hepatitis that resolved after the institution of adenosine deaminase-replacement therapy.

CASE REPORT

The patient was born to a healthy mother who was seronegative for the human immunodeficiency virus (HIV) and hepatitis B virus. He was normal at birth, but by the age of three weeks he had thrush, hepatomegaly, and jaundice. The patient's serum total bilirubin concentration was 5 mg per deciliter (86 μ mol per liter), and his direct bilirubin concentration was 2.9 mg per deciliter (50 μ mol per liter). The serum enzyme activities were as follows: aspartate aminotransferase, 561 IU per liter; alanine aminotransferase, 109 IU per liter; alkaline phosphatase, 528 IU per liter; and lactate dehydrogenase, 1997 IU

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Supported by a grant (DK20902) from the National Institutes of Health (to Dr. Hershfeld) and by Enzon, Inc. Dr. Hershfeld is a consultant to Enzon, Inc.

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per liter. The white-cell count was 5100 per cubic millimeter, with an abnormal differential count (61 percent neutrophils, 18 percent monocytes, 16 percent eosinophils, and 5 percent lymphocytes). Diagnostic studies revealed no anatomical explanation for the jaundice; viral and bacterial cultures were negative; and no antibodies to cytomegalovirus, Epstein-Barr virus, or hepatitis viruses A, B, and C were detectable. The sweat chloride concentration was normal. Hepatic radionuclide imaging with megrofenin isotope (Squibb Pharmaceutical, Princeton, N.J.) revealed rapid uptake with delayed excretion.

At the age of seven weeks, the patient weighed 3100 g (less than at birth), and his length and head circumference were below the fifth percentile. He was icteric and had a diffuse, truncal erythematous macular rash, oral and perineal candidiasis, and erythema of the palms and soles. He had tachypnea with scattered rales. The liver was palpable 4 cm below the costal margin; the tip of the spleen was just palpable. No tonsillar tissue was visible, and no lymph nodes were palpable at any site.

The lymphocyte count was 126 cells per cubic millimeter, with low percentages of T cells, B cells, and natural killer cells. The serum immunoglobulin concentrations were low (IgG, 240 mg per deciliter; IgA, 19 mg per deciliter; and IgM, 15 mg per deciliter). There was no *in vitro* proliferation of peripheral-blood mononuclear cells in response to a panel of mitogens. A skin biopsy revealed no evidence of graft-versus-host disease or infection. In erythrocytes, adenosine deaminase activity was undetectable (normal mean [\pm SD] activity, 52.3 \pm 38.8 nmol per hour per milligram of protein), adenosylhomocysteinase activity was reduced (0.17 nmol per hour per milligram of protein; normal, 4.2 \pm 1.9), and the concentration of total deoxyadenosine nucleotides was elevated (709 nmol per milliliter of packed cells; normal, <2). These findings established the diagnosis of adenosine deaminase deficiency.

A percutaneous liver-biopsy specimen showed early giant-cell transformation, with enlarged foamy hepatocytes and portal and lobular eosinophilic infiltrates (Fig. 1). There was no evidence of graft-versus-host disease, and no viral inclusions were seen. Immunostaining for herpes simplex virus and cytomegalovirus was negative. Viral cultures of liver, cerebrospinal fluid, nasopharyngeal secretions, stool, urine, and blood were negative, as were serum tests for cytomegalovirus and hepatitis B antigens and a polymerase-chain-reaction (PCR) test for hepatitis C.

METHODS

Adenosine deaminase complementary DNA (cDNA) and genomic sequences were identified,^{8,9} and methods of amplifying the cDNA and genomic segments and of cloning and sequencing PCR products

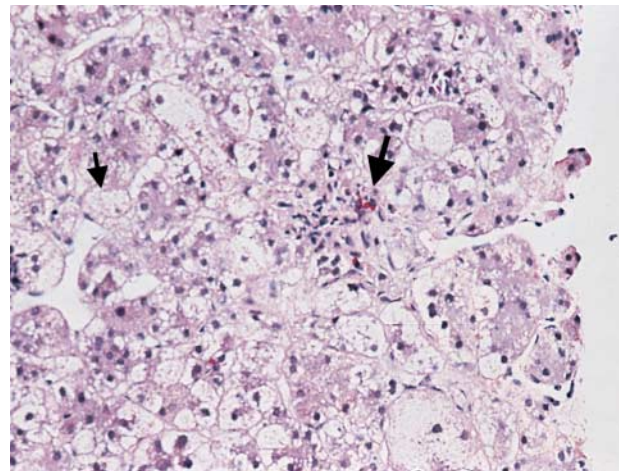


Figure 1. Liver-Biopsy Specimen from the Patient with Adenosine Deaminase Deficiency, Showing Eosinophilic Infiltrates (Large Arrow), Enlarged Foamy Hepatocytes (Small Arrow), and Bile Stasis (Hematoxylin and Eosin, \times 160).

were performed as described previously.¹⁰⁻¹³ The DNA fragments and PCR primer pairs used were as follows: adenosine deaminase cDNA coding region (base pairs [bp] 96 to 1188), 5'CGCGCGAATTCA-TGGCCAGACGCCCGCTTCGAC and 5'GCGCAAAGCTTCA-GAGGTTCTGCCCTGCAGAGGC; genomic exon 4 (bp 24,787 to 25,481), 5'GTATGCACTTCCAAAGTAGAGCTG and 5'CAGTTAT-GAAGTTAGAGCAGGACC; and genomic exons 10 and 11 (bp 30,319 to 31,340), 5'AGGCTGCTGTGAGGATCAAAGCGGGTGAA and 5'TGCTAGAAGTCCCACAGAAAGCCACACTGG.

The effect of mutations on adenosine deaminase activity was assessed as described elsewhere.^{10,12,13} Messenger RNA (mRNA) transcribed in vitro from cDNA subclones was used to prime a rabbit reticulocyte lysate translation system in the presence of methionine labeled with sulfur-35. Aliquots of these reactions containing equal amounts of wild-type translation products and translation products from cDNA samples from the patient (as determined by 10 percent sodium dodecyl sulfate-mercaptoethanol polyacrylamide-gel electro-

phoresis and fluorography) were subjected to electrophoresis on cellulose acetate and stained for adenosine deaminase activity in situ.¹⁴

RESULTS

The patient had no HLA-matched sibling and was considered a poor candidate for bone marrow transplantation because of hepatitis. Enzyme-replacement therapy was begun with pegademase bovine (polyethylene glycol-modified adenosine deaminase [PEG-ADA], Adagen, Enzon, Piscataway, N.J.; 30 U per kilogram of ideal body weight twice weekly).^{3,15-19} Plasma adenosine deaminase activity rose rapidly to therapeutic levels. Within days after the start of the replacement therapy, the patient's serum aminotransferase and bilirubin

concentrations began to fall, paralleling a decrease in the concentration of deoxyadenosine nucleotides in erythrocytes as a percentage of all adenine nucleotides (Fig. 2A). The serum bilirubin concentration became normal by day 55 of therapy and remained so subsequently. The improvement in the serum aminotransferase and bilirubin concentrations was followed by improvement in the lymphocyte count and the distribution of subgroups (Fig. 2B). The patient is 23 months old at this writing, and his height, weight, and development are normal. He has had no infections other than a few episodes of otitis media. The in vitro proliferative responses of lymphocytes to mitogens and tetanus toxoid have been normal.

Sequencing of adenosine deaminase cDNA subclones prepared from a B-lymphoblastoid cell line identified two missense mutations (data not shown). One group of subclones had a novel guanine-to-thymidine (G-to-T) mutation at position 316 (221 bp from the starting site of translation) that changed a glycine (GGC) to a valine (GTC) at codon 74 and eliminated a *BbvI* restriction site in exon 4. All the cDNA subclones with the wild-type Gly74 had a previously reported^{20,21} cytidine-to-thymidine (C-to-T) mutation at position 1081 (986 bp from the starting site of translation), which causes a substitution of valine for alanine at position 329 and introduces a new *BalI-MscI* restriction site in exon 11. Heterozygosity for each mutation (Fig. 3A) was confirmed by digesting PCR-amplified fragments of genomic DNA containing exons 4 and 11 with *BbvI*

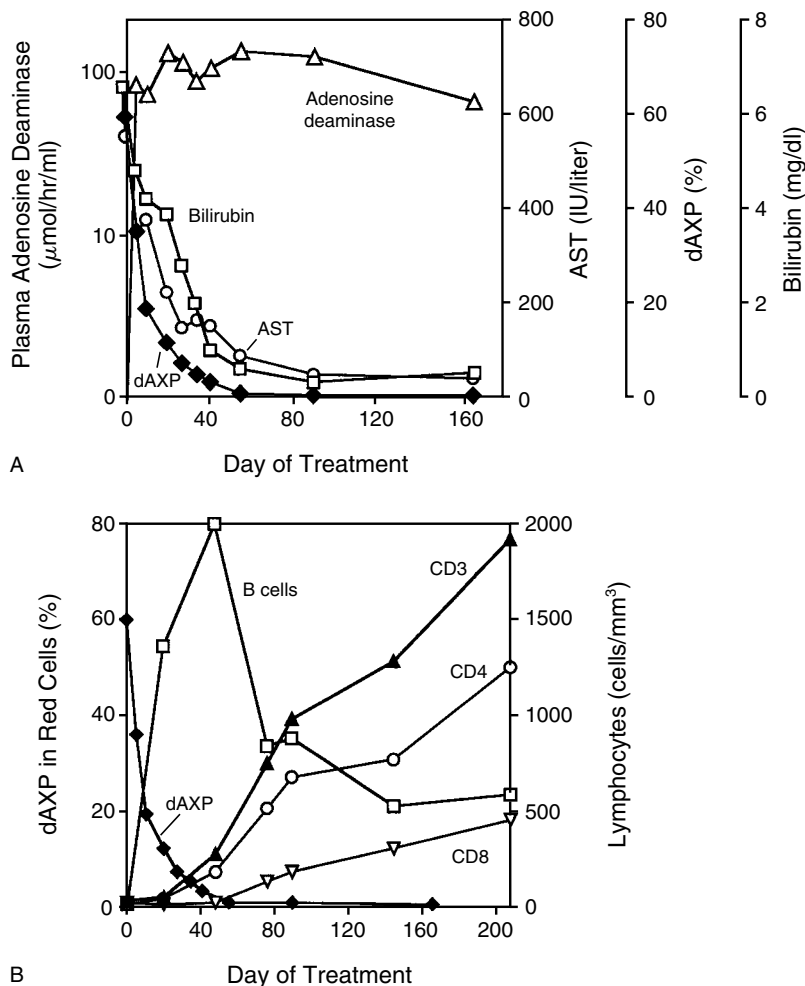


Figure 2. Responses to Treatment with Pegademase (PEG-ADA).

Panel A shows the increase in plasma adenosine deaminase activity and the declines in the proportional concentration of total deoxyadenosine nucleotides (dAXP) in erythrocytes and in concentrations of bilirubin and aspartate aminotransferase (AST) in serum after the start of pegademase therapy. Values for deoxyadenosine nucleotides are expressed as a percentage of all adenine nucleotides (adenosine plus deoxyadenosine) in red cells, determined as described elsewhere.^{10,15} To convert values for bilirubin to micromoles per liter, multiply by 17.1.

Panel B shows the recovery of lymphocyte counts during treatment. For comparison, the proportional decline in deoxyadenosine nucleotides in erythrocytes is plotted as in Panel A.

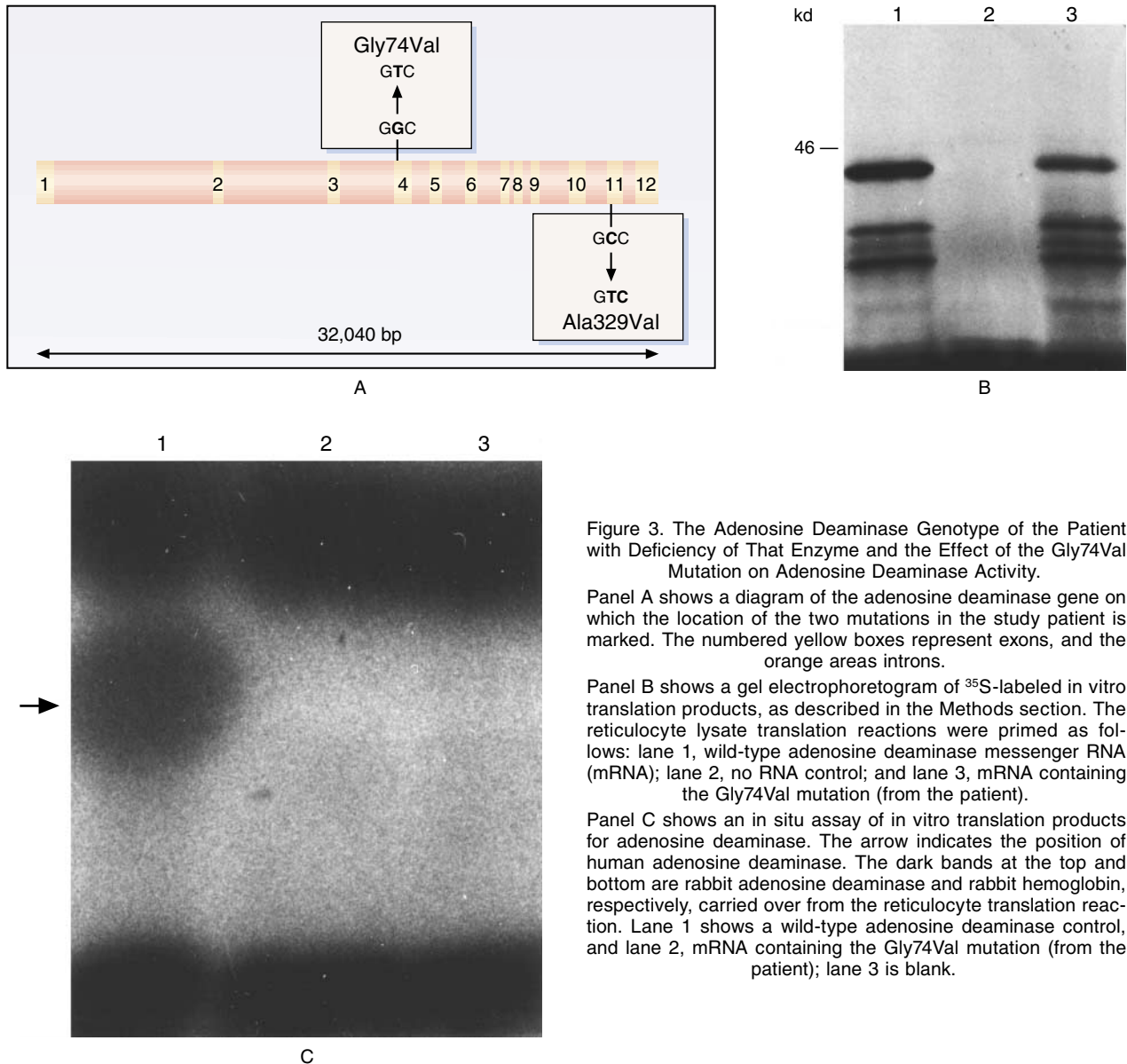


Figure 3. The Adenosine Deaminase Genotype of the Patient with Deficiency of That Enzyme and the Effect of the Gly74Val Mutation on Adenosine Deaminase Activity.

Panel A shows a diagram of the adenosine deaminase gene on which the location of the two mutations in the study patient is marked. The numbered yellow boxes represent exons, and the orange areas introns.

Panel B shows a gel electrophoretogram of ^{35}S -labeled in vitro translation products, as described in the Methods section. The reticulocyte lysate translation reactions were primed as follows: lane 1, wild-type adenosine deaminase messenger RNA (mRNA); lane 2, no RNA control; and lane 3, mRNA containing the Gly74Val mutation (from the patient).

Panel C shows an in situ assay of in vitro translation products for adenosine deaminase. The arrow indicates the position of human adenosine deaminase. The dark bands at the top and bottom are rabbit adenosine deaminase and rabbit hemoglobin, respectively, carried over from the reticulocyte translation reaction. Lane 1 shows a wild-type adenosine deaminase control, and lane 2, mRNA containing the Gly74Val mutation (from the patient); lane 3 is blank.

and *MscI*, respectively (data not shown). The Ala329Val mutation is known to abolish the catalytic activity of adenosine deaminase.²² The in vitro translation product containing the novel Gly74Val mutation also lacked detectable enzymatic activity (Fig. 3B and 3C).

DISCUSSION

Moderately elevated serum hepatic-enzyme concentrations whose cause and importance are uncertain are found in some patients with adenosine deaminase deficiency. In a review of autopsy findings in eight patients, severe bridging portal fibrosis was found in two.²³ In our patient, prolonged neonatal jaundice and elevated serum aminotransferase concentrations were the dominant features at presentation, obscuring signs of primary immunodeficiency. Once lymphopenia and the absence of lymphoid tissues were recognized and aden-

osine deaminase deficiency was diagnosed, an extensive evaluation failed to implicate known causes of hepatic dysfunction. No infectious agent was identified, nor was there evidence of graft-versus-host disease due to the transfer of maternal T cells across the placenta. However, the findings of enlarged hepatocytes and biliary stasis, though nonspecific, were similar to changes found in the livers of adenosine deaminase-deficient mice.^{6,7} The patient's serum aminotransferase and bilirubin concentrations declined rapidly to normal after the start of adenosine deaminase-replacement therapy. These changes occurred in parallel with the correction of metabolic abnormalities induced by the accumulation of adenosine deaminase substrates, but before the numbers and function of T lymphocytes improved.

No biochemical studies of the patient's hepatocytes were conducted. However, in studies of adenosine de-

aminase-deficient mice⁷ (and unpublished data), the increase in deoxyadenosine triphosphate (the major cause of lymphopenia^{3,24}) in the liver was slight. The activity of adenosylhomocysteinase, which is inactivated by deoxyadenosine,²⁵ was reduced by 85 percent, resulting in an increase in hepatic adenosylhomocysteine by a factor of five to six.⁷ Partial inactivation of adenosylhomocysteine may contribute to the lymphopenia in adenosine deaminase deficiency.^{3,25-29} Among the mechanisms responsible for these effects are the inhibition by adenosylhomocysteine of *S*-adenosylmethionine-dependent transmethylation reactions^{26,27,30} and possibly the depletion of methionine and folate pools. Limiting transmethylation by various means is hepatotoxic in rodents.³¹⁻³³

On the basis of these findings, we suggest that adenosine deaminase deficiency can cause hepatitis in some patients, who may have unknown predisposing factors or carry certain adenosine deaminase alleles, more than 40 of which have been identified.^{3,10-13,20-22,34} The specificity of the alleles is most likely related to their effect on adenosine deaminase activity, and hence to the degree of metabolic abnormality. Both our patient's missense mutations, Gly74Val and Ala329Val, reduce catalytic activity greatly. His very high erythrocyte deoxyadenosine nucleotide value at diagnosis indicates severe adenosine deaminase deficiency in all tissues.^{3,10} Alternatively, specific adenosine deaminase alleles may act like the alpha₁-antitrypsin Z allele, which causes hepatotoxicity by being deposited as aberrantly processed inclusions in hepatocytes.³⁵ No inclusions were found in our patient's liver-biopsy specimen, and adenosine deaminase replacement would not be expected to correct dysfunction due to an intracellular accumulation of improperly processed enzyme.

The metabolic effects of adenosine deaminase deficiency may cause morbidity unrelated to immunodeficiency, even though the latter is the overriding clinical problem. This may explain why adenosine deaminase-deficient patients with severe combined immunodeficiency have generally fared worse than others undergoing transplantation of haploidentical bone marrow.³⁶ Two centers, for example, reported overall survival rates of 56 and 58 percent among a total of 74 patients who underwent this procedure, but none of the 8 with adenosine deaminase deficiency survived; the adenosine deaminase-deficient patients were also more likely than others to die before transplantation could be performed.^{37,38} The hepatotoxicity of adenosine deaminase substrates may be additive with the effects of cytotoxic agents used to prepare these patients for marrow transplantation.

We are indebted to Mr. Stephan Toutain for expert technical assistance.

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