Adenosine Deaminase Deficiency in Adults

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Adenosine deaminase (ADA) deficiency typically causes severe combined immunodeficiency (SCID) in infants. We report metabolic, immunologic, and genetic findings in two ADA-deficient adults with distinct phenotypes. Patient no. 1 (39 years of age) had combined immunodeficiency. She had frequent infections, lymphopenia, and recurrent hepatitis as a child but did relatively well in her second and third decades. Then she developed chronic sinopulmonary infections, including tuberculosis, and hepatobiliary disease; she died of viral leukencephalopathy at 40 years of age. Patient no. 2, a healthy 28-year-old man with normal immune function, was identified after his niece died of SCID. Both patients lacked erythrocyte ADA activity but had only modestly elevated deoxyadenosine nucleotides. Both were heteroallelic for missense mutations: patient no. 1, G216R and P126Q (novel); patient no. 2, R101Q and A215T. Three of these mutations eliminated ADA activity, but A215T reduced activity by only 85%. Owing to a single nucleotide change in the middle of exon 7, A215T also appeared to induce exon 7 skipping. ADA deficiency is treatable and should be considered in older patients with unexplained lymphopenia and immune deficiency, who may also manifest autoimmunity or unexplained hepatobiliary disease. Metabolic status and genotype may help in assessing prognosis of more mildly affected patients.

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RESULTS

To mitogens, tetanus toxoids, and allogeneic cells were normal.

Effect of Mutations on ADA Catalytic Activity

A215T Mutations

Human immunodeficiency virus was excluded by polymerase chain reaction at age 23. He had no history of recurrent otitis, bronchitis, pneumonia, sepsis, meningitis, or hepatitis.

DNA Amplification

PCR methods for amplifying ADA genomic and cDNA segments were as described.

Construction of ADA cDNA Containing the P126Q or X215T Mutations

P1260 cDNA. Using recombinant PCR, two primary PCR reactions were performed with wild-type ADA cDNA as template and the primer pairs (mutation underlined): (1) P126Q (+), 5'-GAA-GGGGACCTACCCCAAGACGA and primer 4; and (2) primer 3 and P126Q (-), 5'-CCACCTCCGTTCCGGTTAGGT. The two PCR products (having a central 15-bp overlap) were gel purified, cloned, annealed, and amplified with primers 3 and 4. The final product was gel purified, cut with EcoRI/HindIII, and cloned into pBluescript (Stratagene, La Jolla, CA). Sequencing identified the P126Q mutation and no other change.

A215T cDNA. Primers for the primary reactions were (1) primer 2 and A215T (+), 5'-ACTGTCACCACCCGGGAGGCTG; and (2) primer 1 and A215T (-), 5'-CCTCCCCGGGAGTTAGGCAC. The products were purified, annealed, extended, amplified with primers 1 and 2, cut with EcoRI/HindIII, and cloned into pBluescript. Sequencing showed the A215T mutation and no other change.

Effect of Mutations on ADA Catalytic Activity

As reported, mRNA transcribed in vitro from wild-type and mutant ADA cDNA subclones was translated in a rabbit reticulocyte lysate in the presence of [35S]methionine. Aliquots containing equal amounts of wild-type and mutant translation products (shown by autoradiography, as recommended by the gel manufacturer (AT Biochem, Malvern, PA). For dideoxy sequencing, a separate nested PCR product (at 25,794 to 26,169) was generated using unlabeled primers 9 and 10.

Screening cDNA Subclones for the R101Q Mutation and Retention of Exon 7

cDNA nt 96 to 872 (ATG start codon through exon 8) was amplified with primers 3 and 11. Bsg I digestion then distinguished exon 4 wild type (resistant) from R101Q mutant clones (sensitive). cDNA nt 471 to 1,126 (exons 5 to 11) was amplified using primers 12 and 13. Digestion with Nci I then distinguished clones containing exon 7 (sensitive) from those lacking exon 7 (resistant). The normal exon 7 Nci I site is unaffected by the A215T mutation.

Mutational Analysis

ADA cDNA and genomic sequences are as reported. General PCR methods for amplifying ADA genomic and cDNA segments (the latter numbered from the start of transcription, with translation starting at position 96) and for cloning and sequencing PCR products are as described. Specific target sequences amplified for this study included (primers listed in Table 1): cDNA nucleotide (nt) 16 to 1,497 (full length), primers 1 and 2; cDNA nt 96 to 1,188 (coding region), primers 3 and 4; genomic exon 7 (nt 28,376 to 29,115 spanning introns 6 to 9), primers 5 and 6; and genomic exon 5 (nt 25,794 to 26,169), primers 7 and 8.

Table 1. List of PCR Primers

<table>
<thead>
<tr>
<th>Primer Set</th>
<th>Description</th>
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<tbody>
<tr>
<td>1</td>
<td>(+) 5'TCCCAAGGTACCCGACGACGACCGACGAG</td>
</tr>
<tr>
<td>2</td>
<td>(+) 5'TCCGGAAATCTCACCAAGACGACGACGAG</td>
</tr>
<tr>
<td>3</td>
<td>(+) 5'TCCGGAAATCTCACCAAGACGACGACGAG</td>
</tr>
<tr>
<td>4</td>
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</tr>
<tr>
<td>12</td>
<td>(+) 5'TCCGGAAATCTCACCAAGACGACGACGAG</td>
</tr>
<tr>
<td>13</td>
<td>(+) 5'TCCGGAAATCTCACCAAGACGACGACGAG</td>
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Note: Underlined sequences indicate the mutation at the site of digestion by restriction enzymes.
ADA DEFICIENCY IN ADULTS

Fig 1. Analysis of ADA genotype of patient no. 1 and family. (A) SSCP analysis of genomic exon 5. Lanes 1 and 2, controls; lane 3, patient no. 1. (B) Sequencing gel showing heterozygosity of patient no. 1 for the P126Q mutation in genomic exon 5 DNA. (C) Pedigree of family 1. Arrow indicates patient no. 1 (II-1).

Patients\(^6,30\) (and unpublished data). SSCP analysis suggested that her second ADA allele had a mutation in exon 5 (Fig 1A). Sequencing of amplified genomic exon 5 showed heterozygosity for a C \(\rightarrow\) A transversion of genomic nt 25902 (mRNA nt 472; Fig 1B). This mutation, codon 126 CCA\(\text{Proline}\) to CAA\(\text{Glutamine}\) (P126Q), has not been previously reported. Patient no. 1 had inherited G216R from her mother and P126Q from her father; she transmitted P126Q to her daughter (data not shown, Fig 1C).

Patient No. 2 and Family

In the SCID proband (III-1, Fig 2A), RBC ADA was 0.1 nmol/h/mg protein, and dAXP were 508 nmol/mL. These values for patient no. 2, her healthy maternal uncle (II-3,
Fig 2A), were <0.1 nmol/h/mg and 15 nmol/ml, respectively. All other family members studied had normal or heterozygous range ADA activity and undetectable dAXP. ADA activity in cultured T cells from patient no. 2 was 170 nmol/h/mg protein versus 1,435 and 1,918 for T cells of his sister and her husband, the parents of the SCID proband (normal, 2,047 ± 1,360).

Five ADA cDNA subclones from T cells of patient no. 2 had a previously reported exon 4 mutation, codon 101 CCG{Arg} to CAG{Glu} (R101Q). Amplified genomic DNA was heterozygous for a Bsg I site created by R101Q. Three cDNA subclones lacking this Bsg I site had a deletion of exon 7, but no other mutation, suggesting that his second allele might have a deletion or splice site mutation of exon 7. Sequencing of his amplified genomic DNA showed normal exon 7 splice junctions, but heterozygosity for a previously reported exon 7 missense mutation, codon 215 GCC{Ala} to ACC{Thr} (A215T; Fig 2B). Analysis of family members indicated that patient no. 2 had inherited A215T maternally and R101Q paternally (Fig 2A). His sister had inherited a wild-type allele and R101Q, and her husband was heterozygous for G216R; they had presumably transmitted R101Q and G216R alleles to their ADA SCID daughter (who was not studied; Fig 2A).

Our initial analysis of cDNA from patient no. 2 suggested an allele-specific skipping of exon 7. To assess this further, we screened a second random group of cDNA clones from his T cells (Fig 2C). Overall, of 32 clones analyzed, 4 of 8 (50%) A215T-derived clones lacked exon 7, compared with 3 of 24 (12.5%) bearing the R101Q mutation (similar to the 10% to 15% frequency of exon 7 skipping in normal cells). In a previous report of a child homozygous for A215T, only four cDNA clones were sequenced, but one lacked exon 7 and another had missplicing of intron. Our data on a larger number of cDNA clones from a heteroallelic patient more clearly indicate that A215T is associated with both
Increased loss of exon 7 and a relative decrease in transcript level. Two other exon 7 missense mutations (G216R and R211H) did not appear to have such effects, and F.X. A.-V., I.S., M.S.H., unpublished data, Duke University.

Activity of Expressed Mutant ADA Alleles

The A215T in vitro translation product was 10% to 20% as active as the wild type; the newly identified P126Q mutant protein was inactive, as were the R101Q and G216R proteins (Fig 3). We have also expressed these cDNAs in Escherichia coli Sø3834 (an ADA deletion strain) . A215T had approximately 15% of wild-type ADA activity, compared with approximately 0.1% for P126Q and R101Q and less than 0.01% for G216R (F.X.A., I.S., M.S.H., study in progress). Because exon 7 skipping eliminates activity, the loss of ADA activity owing to the A215T mutation may be greater than is evident from the expressed cDNA, which reflects only the effect of the amino acid substitution.

Discussion

Lymphopenia and immune deficiency were appreciated in patient no. 1 at age 5, but ADA deficiency was then unknown and only aggressive treatment of infections could be offered. Remarkably, she survived and did relatively well from puberty until age 28, when she began to have serious respiratory infections, including tuberculosis. She developed chronic pulmonary and hepatobiliary insufficiency and at 40, fatal leukoencephalopathy caused by JC papovavirus, a disorder prevalent in the acquired immunodeficiency syndrome (AIDS). This history suggests that cellular and humoral immune dysfunction were not initially as complete as in SCID, which if uncorrected is fatal by age 1 to 2 years, but decreased to critical levels in the fourth decade, presumably owing to ongoing exposure to toxic metabolites. We wish to speculate that unexplained episodes of hepatitis from childhood in patient no. 1 may also have had a metabolic basis. Thus, hepatocellular degeneration is fatal in newborn ADA knockout mice, and we have reported a child with ADA SCID who presented with persistent neonatal hepatitis, apparently without infectious cause, which resolved rapidly with enzyme replacement.

Both mutations of patient no. 1, G216R and P126Q, greatly reduced ADA activity. However, her RBC dAdo level (28 nmol/mL) was lower than has been reported for immunodeficient patients (350 to >1,800 nmol/mL for SCID; 60 to 300 nmol/mL for those with a later onset). This may reflect a hypoplastic marrow, because RBC dAdo (precursor of dAXP) arises from DNA degraded during normal marrow cell turnover. However, modest dAXP elevation may reflect greater residual ADA activity in vivo than suggested from cDNA expression. G216R probably eliminates activity irreversibly by interacting with the active site residue glutamate 217; it is always associated with SCID when homozygous or combined with another severe allele (Hirschhorn et al , and unpublished data). However, P126Q occurs in a helical segment that has no contact with the active site; this protein might be stabilized or induced to fold properly in lymphoid cells, which normally express very high ADA activity (as discussed elsewhere ).

Another phenomenon that should be considered is somatic mosaicism, which was recently found in two ADA-deficient patients who were immunodeficient as children, but then, like patient no. 1, had remissions lasting at least into their second decade. Selective survival of ADA lymphocytes was postulated to explain their low RBC dAXP levels and improved immune function. Tissues from patient no. 1 were not available for study. However, her clinical improvement, although prolonged, was not sustained. If present, mosaicism may not have occurred in a stem cell, and thus might have been unstable.

The A215T allele, discovered in a healthy child with partial ADA deficiency, can plausibly explain the benign status of patient no. 2 at age 28. A215T cDNA-encoded protein had 10% to 15% of wild-type activity, 100-fold more than his R101Q cDNA product (the SCID phenotype and high RBC dAdo [precursor of dAXP] elevation may reflect greater residual ADA activity in vivo than suggested from cDNA expression. G216R probably eliminates activity irreversibly by interacting with the active site residue glutamate 217; it is always associated with SCID when homozygous or combined with another severe allele (Hirschhorn et al , and unpublished data). However, P126Q occurs in a helical segment that has no contact with the active site; this protein might be stabilized or induced to fold properly in lymphoid cells, which normally express very high ADA activity (as discussed elsewhere ).

The mechanism by which A215T alters exon 7 processing (apparently enhancing an effect that occurs with normal ADA pre-mRNA) is unclear. The mutation occurs at nt 37/72 of exon 7, distant from splice junctions, and it does not create a new splice site. Some midexonic nonsense mutations can, through unclear mechanisms, induce exon skipping and reduce mRNA abundance, eg, the R142X mutation at nt 62/116 of ADA exon 5. Skipping of exon 7 maintains reading frame, so A215T does not induce a secondary nonsense codon. Further study of the effects of A215T on splicing are warranted.

ADA deficiency is treatable, but the longer diagnosis is delayed, the less reversible is its chronic consequences.
Because of her age and clinical status, patient no. 1 was not a candidate for marrow transplantation. Local regulatory issues prevented access to polyethylene glycol-modified ADA (approved as an “Orphan Drug” in the United States and used in eight other countries for treating ADA deficiency). Earlier diagnosis would have enhanced the chance of benefitting from either therapy and might have prompted avoidance of glucocorticoids, which were also used extensively before diagnosis of ADA deficiency in the adult sisters reported previously.

There are no strict immunological parameters for identifying older ADA-deficient patients. Opportunistic infections may play a less prominent role than in patients with early onset, and older patients have manifested asthma, autoimmune, and signs of immune dysregulation, eg, IgG subclass deficiency or elevated serum IgE. The diagnosis should be considered in adults with persistent lymphopenia and immunodeficiency of unknown cause (eg, some cases of common variable immune deficiency). Once ADA deficiency is diagnosed, metabolite and genotype analysis may help in assessing prognosis and in providing genetic counseling.

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REFERENCES

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