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Normal and Mutant Human Adenosine Deaminase Genes

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Adenosine deaminase (ADA) deficiency in humans is one cause of severe combined immunodeficiency. When ADA fails to catalyze the deamination of adenosine and deoxyadenosine, the levels of deoxyadenosine that accumulate are toxic to lymphoid cells. Patients with complete ADA deficiency (e.g., with less than 5% normal ADA catalytic activity) lack both B- and T-lymphocyte function. B-lymphoblast cell lines derived from patients with ADA deficiency have been analyzed at multiple levels. Blot hybridization and S1 nuclease analysis of ADA messenger RNA (mRNA) indicates that the majority of ADA-deficient cell lines have ADA mRNA in the same abundance and size as in normal cell lines. Sequence analysis of ADA cDNAs derived from these mRNAs shows that the majority of mutations are single base changes that alter the amino acid sequence. Expression analysis proves that these point mutations lead to deficiency of ADA catalytic activity. Several cell lines have mutations that alter mRNA transcription or processing. These include a point mutation in one allele of an ADA-deficient cell line that leads to deletion of exon 4 during mRNA splicing. In addition, two cell lines are homozygous for large deletions of the gene that are the result of homologous recombination. Subjects with partial ADA deficiency have undetectable ADA activity in their erythrocytes, variable activity in their lymphoid cells, and normal immunological function. Analysis of the ADA catalytic activity of partially deficient cell lines indicates that the mutations involved affect protein stability. However, the mutations causing partial ADA deficiency are as yet undefined.

Key words: severe combined immunodeficiency, point mutations, homologous recombination, splicing

Inherited deficiency of the enzyme adenosine deaminase (ADA) accounts for about one-fourth of cases of severe combined immunodeficiency disease (SCID) in children. ADA, a key enzyme in purine metabolism, catalyzes the irreversible deamination of adenosine and deoxyadenosine. The association between ADA deficiency and immunodeficiency arises because lymphoid cells are particularly sensitive to the toxic levels of deoxyadenosine that accumulate when ADA fails to function. ADA-

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deficient patients (ADA-SCID) lack both T- and B-cell function and have dramatically reduced levels of ADA catalytic activity and immunoprecipitable ADA protein. There appears to be a threshold of ADA activity required for correct function of lymphocytes, since partial ADA-deficient subjects, with 20–50% of normal ADA catalytic activity in lymphoid cells are immunocompetent. Proper regulation of ADA is also required for normal erythrocyte function. Increased ADA activity is associated with hemolytic anemia. However, the mutation(s) leading to increased ADA activity is still unknown.

Our knowledge of normal ADA mRNA and gene structure has provided a basis for examining the molecular abnormalities present in ADA-SCID patients. The investigation has also benefited from the establishment of B-lymphoblast cell lines derived from the patients. These cell lines have been the source of mutant ADA proteins, mRNAs, and genomic DNAs. Molecular analyses of normal and mutant ADA genes, reviewed here, demonstrate the following types of mutations that cause ADA deficiency: 1) point mutations that alter the protein structure and/or stability; 2) point mutations or deletions that alter transcription or splicing of ADA mRNA; and 3) still undefined mutations, which cause partial ADA deficiency and appear to affect protein stability.

THE ENZYME ADA

ADA (E.C.3.5.4.4) catalyzes the deamination of adenosine and deoxyadenosine, intermediates in the pathway of purine degradation. The amino acid sequence of ADA has been predicted on the basis of the known cDNA sequence derived from normal ADA mRNA [1–3]. The majority of this sequence (72%) has been confirmed by analysis of tryptic and CNBr fragments [2]. This single polypeptide has a molecular weight of 40,762 daltons. A soluble protein located in the cytoplasm of all cells, this ubiquitous enzyme is often classified as a "housekeeping" protein. However, this is a misnomer, as ADA levels vary over a hundred-fold among human tissues. Further, in some tissues ADA is developmentally regulated. In some cell types, but not lymphoid cells, ADA is associated on the cell membrane with a large dimeric glycoprotein called adenosine deaminase complexing protein (ADCP). This association does not affect the catalytic activity of the enzyme in vitro, however the biological significance of the association is unknown [2,4,5]. Studies of mutant human ADA proteins have been difficult because of its low abundance. B lymphoblast cell lines derived from different ADA-SCID patients have less than 5% of normal ADA catalytic activity and less than 12% of normal immunoprecipitable ADA protein [4,5].

THE ADA GENE

Our laboratory recently sequenced the entire human ADA gene and significant flanking regions [6] (Fig. 1). The gene, located on chromosome 20 [7], includes 32,040 bp, from the major transcription initiation site to the polyadenylation site. This relatively large gene has 12 exons separated by 11 introns. The exons are unevenly distributed, with exon 1, containing the entire 5′ noncoding region plus codons for the first 11 amino acids, separated from exon 2 by an intron of 15,166 bp. Exons 2 and 3 are separated by another fairly large intron of 7,052 bp, while exons 3 through 12 are all encoded within 10,000 bp. The human gene for another enzyme of

the purine salvage pathway, hypoxanthine phosphoribosyl transferase (HPRT), has a similar structure. However, in this case both exons 1 and 2 and exons 3 and 4 are separated by large introns of over 13,000 bp [8]. The ADA gene has 23 repetitive elements of the ubiquitous Alu type (Fig. 1). These sequences can provide potential sites for recombination events. Two ADA-deficient patients, to be discussed below, have large deletions that are the result of just such events [9,10].

The ADA gene lacks the characteristic eukaryotic promoter elements, the TAATA and CAAT boxes. Instead, within the 5′ flanking region of exon 1 are six GC-rich decanucleotide sequences that are highly homologous to sequences identified as functional binding sites for the transcription factor Sp1. Sp1 has been shown to bind and activate transcription from several viral and cellular promoters similar to those present in the ADA gene [for review, see reference 11]. Several of the so called "housekeeping" genes, including HPRT [12], dihydrofolate reductase [13], and glucose 6-phosphate dehydrogenase [14] have similar GC-rich promoters.

NORMAL AND MUTANT ADA mRNAs

In normal cells, mature ADA message is an approximately 1.5 kb polyA$^+$ RNA of relatively low abundance. S1 nuclease analysis of the 5′ ends of ADA mRNA in our laboratory has shown that there is a major initiation start site, base 1 of the consensus sequence, and several minor initiation sites. Variation in the site for initiation of transcription has been confirmed by our finding of sequence variations for the 5′ ends of full-length cDNAs made from ADA mRNAs [15]. The translation initiation codon ATG begins at base 96 and is followed by an open reading frame of 1,089 bp encoding a protein of 363 amino acids [1–3] (Fig. 2). ADA mRNAs from B-lymphoblast cell lines derived from ADA-deficient patients have been compared with normal ADA mRNA by several different techniques, including blot hybridization

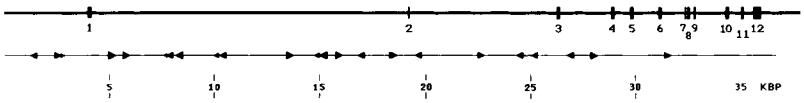

Fig. 1. Map of the human ADA gene [6]. The locations and sizes of the 12 exons are indicated by bars on the upper line. The locations, orientations, and relative sizes of the 23 Alu repetitive elements are shown as arrowheads on the lower line. The direction of transcription is from left to right.

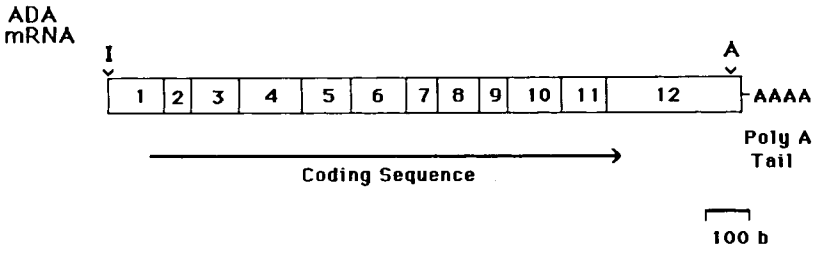

Fig. 2. Map of ADA polyA$^+$ mRNA. The relative sizes of the 12 exons are indicated by the open boxes. Within the sequence of 1,533 bases is a coding sequence of 1,089 bases indicated by the lower line. The coding sequence begins 96 bases 3′ of the primary transcription start site (**I**). The 3′ untranslated sequence contains 308 bases including a polyadenylation signal (**A**) 20 bases from the end.

or Northern analysis and S1 nuclease mapping. These analyses have shown mutations within the gene of some ADA-deficient cell lines that do affect ADA mRNA. For example, the cell line GM2825A, derived from an ADA-deficient patient, has a mutation that eliminates exon 4 during splicing. In addition, two cell lines recently derived from ADA-SCID patients were found to have no detectable ADA mRNA [9,10].

Analysis of ADA mRNA from most of the cell lines derived from ADA-SCID patients shows that they contain ADA-specific mRNAs of normal size and abundance [2,16–18]. When polyA$^+$ RNA from these cell lines is translated in vitro, the resulting protein is of the correct molecular weight and is present in equal or greater abundance than protein translated from the same amount of nonmutant polyA$^+$ RNA [4,17,18]. S1 nuclease analysis can be used to define a general map of mutant RNAs by comparing hybridization of normal ADA cDNAs with mutant ADA mRNAs. Using this technique, we have found that the ADA mRNA from most ADA-deficient cell lines, including GM2471, GM4258, GM2756, and GM2606, is indistinguishable from normal ADA mRNAs [16]. Therefore we concluded that the molecular defects in these cell lines were probably single base changes that were undetectable by S1 analysis.

POINT MUTATIONS

The sequences of eight mutant ADA cDNAs from five ADA-deficient cell lines have been reported (Table I). We have analyzed both alleles from the ADA-deficient lines GM2606 [19], GM2756, and GM2825A [15]. A single allele from the deficient cell lines GM2471 [20] and GM1715 [21] has also been reported. Comparison of these mutant ADA alleles and several normal alleles has identified mutations that result in amino acid substitutions or conservative single base changes [Table II]. All of the conservative mutations change the third base of codons and therefore do not alter the amino acids encoded. These conservative mutations are helpful in identification of independent ADA alleles. Furthermore, as some of these mutations, both conservative and detrimental, alter sites for restriction nucleases, they may be useful for restriction fragment length polymorphism (RFLP) analysis of ADA gene inheritance.

Each allele of GM2606, as determined by sequencing the corresponding cDNAs, has a single point mutation affecting the amino acid sequence. In allele 1 there is a

TABLE I. Point Mutations in the ADA Gene That Affect the Amino Acid Sequence

Base[a]		Amino acid		Location	Mutant	
No.	Change	No.	Change	(exon)	cell line	Reference
334	A to G	80	Lys/Arg	4	GM2471	20
396	C to T	101	Arg/Trp	4	GM2606	19
397	G to A	101	Arg/Gly	4	GM1715	21
727	G to A	211	Arg/His	7	GM2606	19
					GM2756	15
1,006	T to G	304	Leu/Arg	10	GM2471	20
1,081	C to T	329	Ala/Val	11	GM2756	15
					GM2825A	15

[a]Base numbers refer to the cDNA sequence with number 1 as the first base of the major initiation start site [6,15].

TABLE II. Conservative Single Base Mutations of the ADA Gene

Base[a]				
No.	Change	Location	Source	Reference
42	A to C	5′ Noncoding	Normal cDNA	1
287	G to A	Exon 3	Normal cDNA	2
			Normal cDNA	21
368	C to T	Exon 4	Normal cDNA	21
425	G to A	Exon 4	Mutant cDNA (GM2756)	15
485	G to A	Exon 5	Normal cDNA	16
629	G to A	Exon 6	Normal gene	6
			Normal cDNA	2
			Normal cDNA	21
			Mutant cDNA (GM2756, GM2825A both alleles, GM2606 both alleles)	15,19
1,483	A to G	3′ Noncoding	Normal cDNA	1

[a]Base numbers refer to the cDNA sequence, with number 1 as the first base of the major initiation start site [6,16].

change of base 396 in exon 4 from C to T. This alters the codon for amino acid 101 and results in an Arg to Trp change. In the cell line GM1715, this same amino acid is mutated from Arg to Glu as a result of a G to A change of base 397 [21]. The second allele in GM2606 has a mutation of base 727 from G to A in exon 7 that alters the codon for amino acid 211, changing it from Arg to His. We have reported the same mutation in allele 1 of the ADA-deficient cell line GM2756 [15]. These may represent the same allele in two seemingly unrelated patients, since each also has a conservative change of base 629 from G to A that does not affect the amino acid encoded. This conservative base mutation occurs in over one-half the ADA alleles so far examined (Table II).

Allele 2 of GM2756 has a base change at position 1,081, C to T, which results in an amino acid change of Ala to Val at amino acid 329 [15]. This same mutation is found in allele 2 of GM2825A [15]. However, each allele has another distinct mutation that does not alter the amino acid sequence, showing that these are independent alleles. Allele 1 of GM2825A also has a single detrimental base mutation. This mutation, discussed in the following section, affects splicing rather than the amino acid sequence.

Only one ADA allele of the deficient line GM2471 has been analyzed [20]. Unlike all the other reported mutant alleles, this allele has two point mutations that affect the amino acid sequence. Base 334 in exon 4 is mutated from A to G, altering amino acid 80 from Lys to Arg; base 1,006 in exon 10 of the same cDNA is mutated from T to G, altering amino acid 304 from Leu to Arg. Valerio et al. [20] showed by restriction nuclease mapping that GM2471 is heterozygous for this allele. The change of Lys-80 to Arg-80 seems to be functionally neutral. Valerio et al. [20] showed that the cell line HL-60, a human promyelocytic leukemic cell line, has normal levels of ADA activity but is homozygous for this Arg-80 mutation. Thus the assumption was made that the Lys-80 to Arg-80 change had no effect on the catalytic activity of the ADA protein. Rather, the mutation altering Leu-304 to Arg-304 was the cause of inactivation of the protein. To verify this assumption, Valerio et al. [20] constructed

an ADA expression minigene containing the T to G mutation at base 1,006. L cells transfected with the normal ADA minigene expressed functional human ADA, whereas cells transfected with the mutant minigene did not.

In a similar analysis, we have constructed vectors to test the expression of the ADA mutant alleles from cell lines GM2606, GM2756, and GM2825A. Of particular interest was the mutation causing the conservative amino acid change of Ala-329 to Val-329 in allele 2 of GM2756 and allele 2 of GM2825A. Also tested were the mutations affecting Arg-101 (in GM2606), Arg-211 (in GM2606 and GM2756), and the deletion of exon 4 (in GM2825A). The expression vectors were constructed using the entire coding sequence of the ADA cDNAs under transcriptional control of the Rous sarcoma virus long terminal repeat (RSV-LTR) [22]. The ADA vectors were transfected into human fibroblasts that expressed low levels of endogenous ADA. All cells transfected with mutant or normal ADA constructions had levels of ADA mRNA 15 to 25 times higher than the endogenous ADA message (Fig. 3A). Cells transfected with the mutant ADA coding sequences expressed only the low endogenous levels of ADA enzymatic activity. However, cells transfected with normal ADA coding sequences had ADA activity levels 40 times higher than the endogenous activity in cells transfected with mutant ADA sequences (Fig. 3B). This in vivo analysis of the mutant ADA alleles demonstrates that these point mutations are the cause of ADA deficiency in the cell lines GM2606, GM2756, and GM2825A.

EFFECT OF SINGLE AMINO ACID CHANGES ON ADA STRUCTURE

It is curious that the conservative amino acid change of Ala-329 to Val-329 causes a loss of ADA activity. Computer-generated analysis of protein secondary structure [23] and hydropathicity [24] shows that the Ala to Val change has virtually no effect on these parameters. While it is unlikely that this neutral amino acid plays a mechanistic role in the enzyme active site, it may be crucial for either the active site conformation or total protein conformation. A similar conservative change of Gly-51 to Val-51 has been shown by Orkin et al. [25] to eliminate ADA activity on transfection of a cloned ADA expression vector. This mutation of G to T at base 247 in exon 3 is a cloning artifact, yet it points out the role that neutral amino acids can play in ADA function.

The effects of the other amino acid changes found in the mutant alleles are more dramatic. The alteration of Arg-101 to Trp-101 found in one allele of GM2606 causes a large shift of the adjacent amino acid structure (amino acids 98–105) from relatively neutral to decidedly hydrophobic. Furthermore, this amino acid change creates the possibility for a turn in the predicted β-sheet at the adjacent Tyr-102. GM2606 and GM2756 share a common mutant ADA protein, with Arg-211 changed to His-211. With this mutation, the region from amino acid 208 to 213 makes a large hydrophobic shift. As expected, the Lys-80 toArg-80 change in allele 1 of GM2471 has no effect on predicted hydrophathicity. However, the second mutation on this allele causes a large shift in the area of the protein around the Leu-304 to Arg-304 change, so that it becomes much more hydrophilic [20].

It is difficult to study the effects of the mutations on ADA structure directly, as ADA is present at very low abundance in the deficient cell lines. The quantity of mutant ADA proteins, as detected by either immunoprecipitation [5] or Western blot analysis [26], is approximately 5% of that found in normal cells. The failure to detect

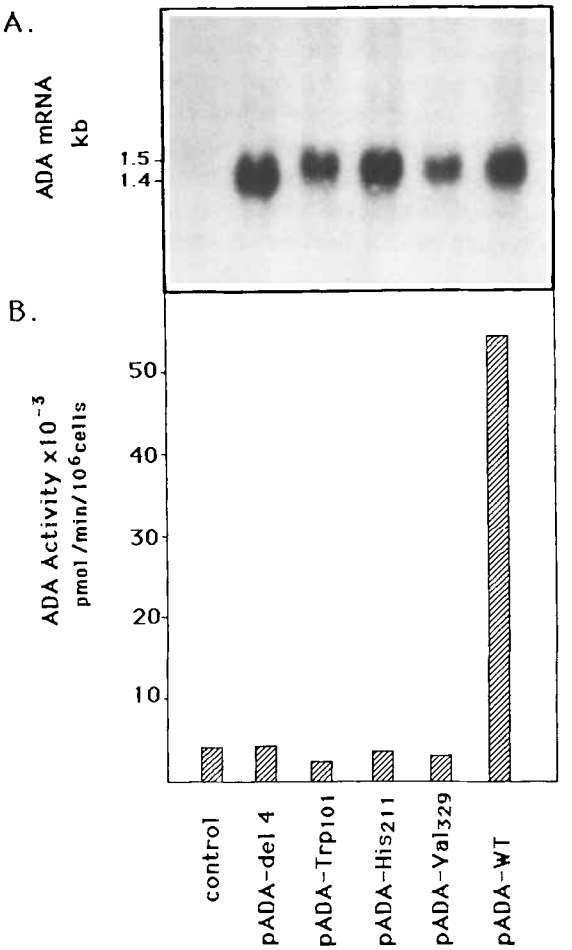

Fig. 3. Expression of ADA-specific RNA and ADA enzymatic activity in fibroblasts transfected with normal and mutant pRSV-ADA constructions. The pRSV-ADA expression vectors were constructed from normal and mutant ADA cDNAs containing the entire coding sequence and a plasmid containing the RSV-LTR promoter and polyadenylation signals [22]. An equivalent number of fibroblasts, GM4429, were transfected with 5 μg/plate of plasmid with normal, wild-type ADA sequences, pADA-WT, or ADA sequences with point mutations affecting Arg 101 (pADA-Trp 101), Arg 211 (pADA-His 211), or Ala 329 (pADA-Val 329). The plasmid pADA-del 4 corresponds to mature ADA mRNA without exon 4. Control fibroblasts were not transfected. Cell lysates were prepared 48 h after transfection. ADA mRNA was isolated [38] and characterized by Northern analysis [39], probing with a full-length ADA cDNA (**A**). ADA enzymatic activity was assayed [40] and expressed as pmole of product/min/10^6 cells (**B**).

higher levels of mutant immunoprecipitable protein may be the result of altered protein structure and/or decreased protein stability. From expression analysis of the mutant alleles, it appears that the native protein is relatively intolerant of minor structural changes. Therefore point mutations that lead to alteration of key amino acids affect ADA activity by changing the protein structure and/or stability. This finding does not exclude the possibility that the mutations may also directly affect the ADA active site.

MUTATIONS AFFECTING TRANSCRIPTION AND ADA mRNA SPLICING

We have sequenced both ADA alleles of GM2825A [15]. As discussed above, one allele represented by normal 1.5 kb message has a point mutation that alters a single amino acid. Repeated sequence analysis of cDNAs encoded by the other ADA allele revealed that exon 4 was always missing. Careful blot hybridization of genomic DNA from GM2825A showed that the absence of exon 4 could not be the result of a large deletion in the gene. We believed that it was most likely that a point mutation, or other mutation affecting only a few bases, caused exon 4 to be deleted during splicing.

To determine the type of mutation involved, we amplified the region of the ADA gene for GM2825A from the center of intron 3 to the center of intron 4 using the polymerase chain reaction [27]. Sequence analysis of amplified DNA showed a single base change of A to G within the invariant bases of the 3′ splice site of intron 3 of allele 2 [19] (Fig. 4A). Within the amplified DNA there were no other changes, compared with the normal ADA gene [6]. There are two conserved sequence elements in mammalian 3′ splice sites, a pyrimidine-rich tract and the invariant AG dinucleotide [28]. Mutation or deletion of the AG dinucleotide causes improper splicing [29]. Thus the mutation of the 3′ splice site of intron 3 of the ADA gene prevents correct splicing of the intron. As a result, introns 3 and 4 and exon 4 are eliminated during subsequent splicing events, giving an abnormal 1.4 kb message. The deletion of exon 4 does not alter the translational reading frame of the ADA message. However, the elimination

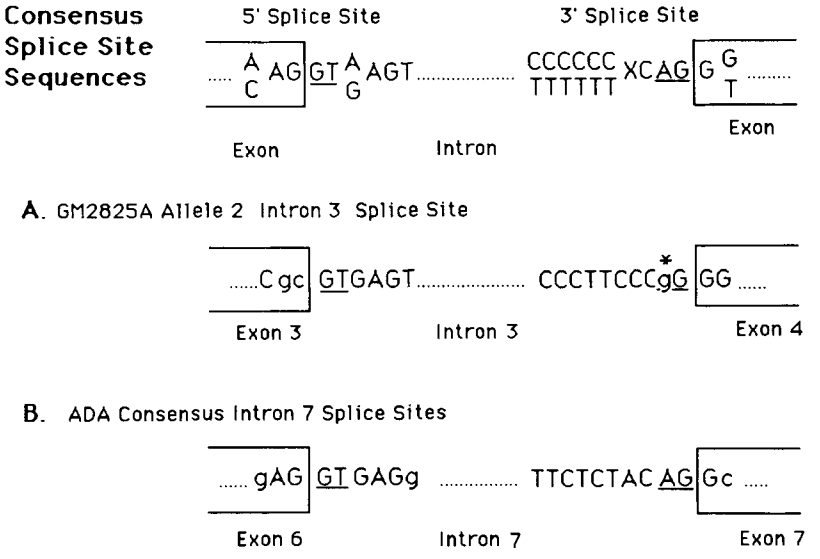

Fig. 4. Comparison of ADA splice sites to a consensus splice site sequence. The consensus splice site sequence is derived from comparison of eucaryotic nuclear mRNA precursors [28,29]. The exonic portions of the splice sites are within the boxes. The invariant GT and AG bases at the respective 5′ and 3′ ends of the introns are underlined. In the consensus sequence X stands for any base. The sequence of the intron 3 splice sites (**A**) of GM2825A was derived by polymerase chain reaction amplification [27]. Mutation of the invariant AG to gG within the 3′ splice site leads to exclusion of intron 4 during splicing of this allele of GM2825A. Intron 7 splice sites from all known normal and mutant ADA alleles have the same sequence (**B**). The noncanonical base, in lower case letters, within the 5′ splice site of intron 7 may affect the efficiency of RNA splicing.

of 48 amino acids encoded within the exon has dramatic effects on the predicted protein structure. Furthermore, amino acids encoded in exon 4 must be essential for ADA stability and/or function, as mutations of Arg-101 to Trp-101 in GM2606 and Gln-101 in GM1715 [21] occur within this region. In each case these single amino acid changes appear to cause loss of ADA activity.

Recent reports have identified two unrelated ADA-deficient patients who express no ADA-specific mRNA [9,10]. Markert et al. [9] have shown that one of these patients is homozygous for a 3.2 kb deletion that includes the entire ADA promoter region and the first exon. This deletion is the consequence of homologous recombination between two repetitive DNA sequences of the Alu family. Detailed DNA blot hybridization analysis of the second patient [10] indicates that the same or very similar deletion has occurred. These deletion mutations clearly result in absence of transcription and total ADA deficiency.

The partially deficient cell line GM3043 has unique ADA mRNA species on S1 nuclease mapping [16]. The protected species correspond to a normal, 1.5 kb message and a 1.25 kb fragment that may result from an RNA loop at the junction of exons 3 and 4. This abnormal RNA would arise from the occasional inclusion of a portion of intron 3 or the use of a cryptic splice site within intron 3. This cell line is derived from a !Kung tribesman from an isolated region of Africa. It is likely that the parents are related, and therefore the subject is probably homozygous for a single ADA allele. The question remains whether the aberrant RNA species are translated and whether the resulting abnormal protein accounts for the observed differences in enzyme activity, heat stability, and isoelectric point [5,30]. There may be mutations other than those that affect splicing that account for these protein characteristics. Only specific sequence information will provide the answer.

INEFFICIENT ADA mRNA SPLICING

The ADA mRNA undergoes inefficient splicing fairly frequently. Examples of introns remaining in mature ADA mRNAs have been shown for both normal and deficient cell lines. Several years ago we reported the sequence for a cDNA clone, ADA33 [1,18], which we now know includes the 76-bp intron 7. We have subsequently sequenced additional cDNA clones for the ADA-deficient line GM2756, which also includes intron 7 [15]. Other cDNA clones for both GM2756 and GM2825A do not include exon 7 [15]. Using S1 nuclease mapping, we found a minor band corresponding to mRNA that includes intron 7 in virtually all normal and ADA-deficient cell lines tested [16]. One partially deficient cell line, GM2294, also had the minor band corresponding to an mRNA species without exon 7. While S1 band intensities indicate that these minor mRNA species are only 10–15% of the total ADA mRNA, they must be stable, since they are readily available as template for cDNA synthesis (15).

These minor mRNA species are probably the result of inefficient splicing. Sequence analysis of the ADA gene and cDNA clones for the ADA-deficient cell lines GM2756 and GM2825A have provided some basis for understanding the abnormal splicing events involved. First, the efficiency of splicing may be influenced by the small size of intron 7. This intron contains only 76 bases and is near the minimum intron size of 60 bases required for accurate splicing [31]. Second, while the sequence of the exon 7/intron 7 splice sites in all clones analyzed was identical to the sequence

found in the normal gene [6], the sequence of the ADA splice site does not match the canonical 5′ splice site sequence [28] at two positions. The consensus sequence is (A/C)AG:GT(A/G)AGT, while the ADA intron 7 sequence is GAG:GTGAGG (Fig. 4B). Aebi et al. [32] have shown that this type of base change within the 5′ splice site of intron 3 of rabbit β-globin RNA promotes incorrect splicing, with the exclusion of exon 2. Similar inefficient use of the 5′ splice site of intron 7 in ADA may account for the aberrant exon 7/intron 7 mRNA species. It is not known whether these abnormal RNA species influence the cellular ADA activities, perhaps by decreasing the size of the effective ADA mRNA pool.

PARTIAL ADA DEFICIENCY

Partially ADA-deficient subjects are characterized as having undetectable ADA activity in their erythrocytes, variable ADA activity in their lymphoid cells, and normal immunological function [33,34]. However, these subjects may be at risk for late-onset cellular immunodeficiency syndrome [33,34]. Analysis of B-lymphoblast cell lines derived from partially ADA-deficient patients has been done at the protein level, but not at the gene level. Analysis of ADA protein, measuring electrophoretic mobility, isoelectric point, heat stability, and kinetic parameters, indicates structural mutations of ADA in many of these cell lines [30,33]. Daddona et al. [30] studied the partially deficient cell lines GM3043, GM2294, GM4396, and KS and found normal or elevated levels of ADA mRNA of the correct size. The partially deficient lines were found to have rates of degradation of ADA protein 1.5- to 3.0-fold faster than normal cell lines. These ADA alleles may have mutations that render the protein susceptible to in vivo proteolysis. The line GM4396 also has depressed rates of ADA synthesis, despite near normal mRNA levels, suggesting a mutation affecting translational efficiency. Ultimately, to determine the specific mutations involved in partial deficiency, the mutant ADA genes will need to be sequenced.

COMPARISONS WITH OTHER GENETIC DISEASES

Analogies between the molecular defects that cause ADA deficiency and those that cause other inherited diseases are of interest. Among the most common genetic diseases of man are the thalassemias. This well-studied syndrome is a heterogeneous collection of defects in organization and/or expression of the globin genes [for review, see reference 35]. Many of the thalassemias alter specific steps in transcription and RNA processing. Most of the α-thalassemia mutations are the result of deletions within the α-globin gene cluster. For example, deletions involving Alu sequences, similar to those shown for two ADA-deficient patients, have been demonstrated for the human α-globin gene cluster [36]. In contrast, the majority of β-thalassemia mutations are single nucleotide substitutions or small deletions. Analysis of β-thalassemia patients with mutations of splice junction sequences, such as the one we have shown for the ADA-deficient cell line GM2825A, has provided considerable information on the RNA sequences required for specific, selective, and efficient RNA splicing.

Other inherited metabolic diseases are less well characterized than the thalassemias. Perhaps the best analogies can be made between deficiency of ADA and deficiency of HPRT. The absence of HPRT activity is found in patients with Lesch-

Nyhan syndrome, while partial deficiency is associated with gouty arthritis. Wilson et al. [37] characterized 24 unrelated patients with HPRT deficiency. Analysis of HPRT protein and mRNA showed that the majority of patients had unique independent mutations that affected the structural gene without altering the mRNA. Only a few patients had no enzyme and no mRNA. While no specific gene sequence information is available for the HPRT mutants, Southern analysis showed that most mutations of the HPRT gene do not involve major gene rearrangements. There are exceptions. For example, one patient has an internal duplication of portions of the gene, and another has total deletion of the HPRT locus. Four known HPRT enzyme variants differ from the normal protein by single amino acid substitutions. They are probably comparable to the majority of the ADA mutants we have characterized, with point mutations leading to single amino acid protein variants.

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