IV.5 GENE STRUCTURE OF A HUMAN SERUM AMYLOID A PROTEIN AND COMPARISON WITH AMYLOID A

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1. Introduction

The protein in deposits of reactive amyloidosis is amyloid A (AA) protein (1,2). Isolates of AA protein from human and various animals have similar molecular weights of about 8,000 dalton and similar amino acid sequences of 76 residues (3-5). The only serum protein which bears close biochemical and immunochemical identity with AA is serum amyloid A (SAA) protein, found mainly in the HDL fraction of plasma (6). It is a major acute phase reactant in man, rabbit, mice and other mammals (7). Its molecular weight is about 12-14,000 dalton when dissociated from HDL and is not glycosylated. Since SAA can be cleaved at the carboxy-terminus by proteolytic enzymes, it has been presumed to be the precursor for AA. Precursor-product relationship was recently demonstrated by Husebekk and colleagues (8): human SAA was shown to be incorporated into mouse AA by immunochemical methods.

Murine SAA is encoded by a family of 3 genes (9). SAA1 and SAA2 are found in equal quantities associated with HDL (10), but no polypeptide corresponding to SAA3 has been isolated. SAA3 mRNA has been detected but is apparently unstable (11). Of the two gene products, SAA2 is the sole precursor of murine AA, despite equal expression of SAA1 and SAA2 (12).

There is also a gene family for human SAA. There are two major and four minor isotypes in serum (13,14). Their molecular weights are the same, so that the differences are unlikely to be due to glycosylation. Structural studies at the amino acid, genomic DNA and mRNA levels are still incomplete. The amino acid sequence of SAA1 has been determined (15). It has two allelic forms, α and β, with double substitution of valine for alanine at residues 52 and 57. Incomplete sequence of SAA2 shows that it lacks the N-terminal arginine, but the following 30 residues are identical to SAA1 (14). Limited N-terminal analysis by Bausserman and colleagues (16) suggests that SAA4 is homologous to SAA1 and SAA5 to SAA2. Therefore these isotypes should be products of at least 3 genes. Three human SAA genomic clones have been isolated from a λ genomic library, using a cross-hybridizing mouse SAA cDNA clone, pRS48 (17). Two of these clones bear close homology to each other and the third is homologous to SAA1β. Two human SAA cDNA clones have been identified so far (18,19) and one of these (pA1) has been completely sequenced: the derived amino acid sequence is
identical to that of SAA1a (15). The total number of human SAA genes and mRNA is still unclear at present.

2. Gene structure of a human SAA genomic clone

To study the structural variants of SAA, 2 human SAA genomic clones were isolated from λL47-1 genomic library (kindly donated by S. Karathanasis) using the human specific, variable portion of the cDNA pA1: i.e. corresponding to amino acid residues 55-104 + 3' untranslated region. DNA sequence was obtained using the dideoxy chain termination method (10). The restriction maps of these two clones were identical and the gene structure of one is shown in Fig. 1.

The organization of the gene is similar to that of other apolipoprotein genes. There are three introns, one in the 5' untranslated region, one near the N-terminus after amino acid residue 12, and one in the centre of the coding region, at residue 59. DNA sequence of exon 2 and 3 is identical to that of SAA1β. However, there are nucleotide differences in exon 4 (residues 60-104 + 3' untranslated region) leading to 6 amino acid substitutions (Table I): asparagine instead of aspartic acid at residue 60, leucine for phenylalanine at residue 68, threonine for phenylalanine at residue 69, arginine for histidine at residue 71, lysine for glutamic acid at residue 84 and arginine for lysine at residue 90. All except the substitution of threonine involve a single base change.