Biochem J. (1987) 242, 301–303 (Printed in Great Britain)

Acute-phase high-density lipoprotein in the rat does not contain serum amyloid A protein

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Serum amyloid A protein (SAA) is an acute-phase apolipoprotein of high-density lipoprotein (HDL). Its *N*-terminal sequence is identical with that of amyloid A protein (AA), the subunit of AA amyloid fibrils. However, rats do not develop AA amyloidosis, and we report here that neither normal nor acute-phase rat HDL contains a protein corresponding to SAA of other species. mRNA coding for a sequence homologous with the *C*-terminal but not with the *N*-terminal part of human SAA is synthesized in greatly increased amounts in acute-phase rat liver. These observations indicate that the failure of rats to develop AA amyloid results from the absence of most of the AA-like part of their SAA-like protein, and that the *N*-terminal portion of SAA probably contains the lipid-binding sequences.

INTRODUCTION

In reactive systemic (secondary) amyloidosis the fibrils, which constitute the bulk of the deposits, are composed of amyloid A protein (AA), which is identical with the *N*-terminal 76 residues of serum amyloid A protein (SAA) (Glenner, 1980*a*,*b*; Pepys & Baltz, 1983). SAA is a single-chain peptide of 104 residues in man (Parmelee *et al.*, 1982), and circulates in the plasma predominantly in association with high-density-lipoprotein (HDL) particles (Benditt & Eriksen, 1977). In normal healthy individuals SAA is present in only tiny amounts, but it behaves as a major acute-phase reactant, rapidly increasing in concentration after most forms of tissue injury, infection and inflammation (Pepys & Baltz, 1983). In chronic active inflammatory or infective disorders there are sustained high plasma concentrations of SAA, and these are apparently the precursor of the AA protein, which forms amyloid fibrils, presumably as a consequence of partial proteolytic cleavage followed by polymerization (Pepys, 1987).

AA amyloidosis occurs in all mammals in which it has been sought and also in Peking ducks (Jakob, 1971; Gruys, 1979), and there has been stable evolutionary conservation of the sequence of SAA (Yamamoto & Migita, 1985). However, rats seem almost never to develop amyloidosis (Dunn, 1967; Green, 1974), nor, unlike mice and other animals, can AA amyloid be induced in them by chronic infection or inflammation (Lucke & Markley, 1928; Vargas & Stephens, 1983). In all the amyloid-susceptible species SAA is an apo-HDL (Benditt *et al.*, 1979; Skogen *et al.*, 1979; Marhaug *et al.*, 1984, 1985; M. L. Baltz & M. B. Pepys, unpublished work). We now report that, in contrast, no SAA-like protein was demonstrable in rat serum HDL, and this may explain the 'resistance' of rats to induction of AA amyloidosis, as well as shedding light on structure–function relationships in the SAA molecule.

MATERIALS AND METHODS

Sources of serum

Normal human serum was from healthy laboratory volunteers, and acute-phase serum was from consenting patients at Hammersmith Hospital. Normal mouse serum was obtained from C57BI/6 mice (OLAC, Bicester, Oxon, U.K.), and acute-phase serum from MF1 outbred mice (OLAC) injected with 0.5 ml of aq. 2% (w/v) $AgNO_3$ subcutaneously at 24–48 h before they were bled. Normal and acute-phase rat sera were obtained from normal Wistar rats (Royal Postgraduate Medical School Breeding Unit) and normal Sprague–Dawley rats (OLAC), and from rats injected subcutaneously 24–48 h beforehand with 3.0 ml of 2% (w/v) $AgNO_3$, respectively. To ensure that the serum was normal or acute phase, concentrations of the acute-phase proteins human C-reactive protein, mouse serum amyloid P component and rat C-reactive protein were determined by electroimmunoassay (Pepys, 1979; de Beer & Pepys, 1982; de Beer *et al.*, 1982).

Isolation of HDL from serum

HDL (ρ = 1.063–1.21 g/ml) was isolated from fresh serum by sequential ultracentrifugation in solutions of KBr (Havel *et al.*, 1955; Lasser *et al.*, 1973). The HDL was washed once by re-centrifugation at the higher density limit and dialysed into 150 mM-NaCl, pH 7.4, containing 1 mM-EDTA.

Antiserum to HDL

Antiserum to HDL was raised in Half-Lop rabbits (Froxfield Research Rabbits, Petersfield, Hants., U.K.) by injection in complete and incomplete Freund's adjuvant of HDL isolated from acute-phase serum from man, mouse or the rat.

Abbreviations used: AA, amyloid A protein; SAA, serum amyloid A protein; HDL, high-density lipoprotein.

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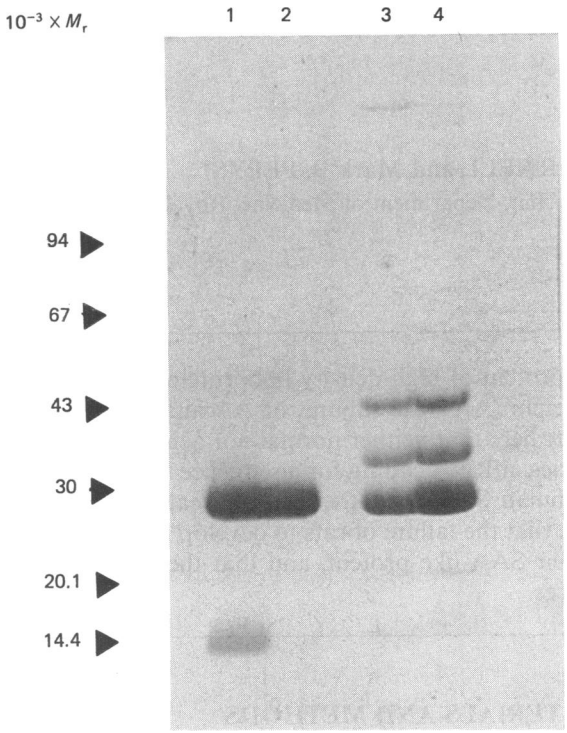

Fig. 1. SDS/4–30%-(w/v)-polyacrylamide-gradient-gel electrophoresis run under reducing conditions

Track 1, HDL isolated from acute-phase mouse serum; track 2, normal mouse serum HDL; track 3, acute-phase rat serum HDL; track 4, normal rat serum HDL. The positions of M_r markers are arrowed: rabbit muscle phosphorylase *b*, 94000; bovine serum albumin, 67000; hen's-egg ovalbumin, 43000; bovine erythrocyte carbonic anhydrase, 30000; soya-bean trypsin inhibitor, 20100; bovine milk α-lactalbumin, 14400.

Polyacrylamide-gel electrophoresis

SDS/polyacrylamide-gel electrophoresis was performed with 4–30% (w/v) gradient gels (Pharmacia, Milton Keynes, Bucks., U.K.) under reducing conditions according to the manufacturer's instructions. Homogeneous 15% (w/v) gels were run in the presence of SDS under reducing conditions by the method of Laemmli (1970). Gels were calibrated with marker proteins of known apparent M_r values (Pharmacia).

Protein blotting and immunostaining

These procedures were performed by standard methods, precisely as described elsewhere (Maudsley *et al.* 1987).

RESULTS

SDS/polyacrylamide-gradient-gel electrophoresis revealed two major polypeptides associated with HDL isolated from acute-phase mouse serum (Fig. 1), one (M_r 30000) corresponding to apolipoprotein AI, the major apolipoprotein of HDL, the other (M_r 15000) to apo-SAA. HDL isolated from normal mouse serum showed only the apolipoprotein AI band. HDL from acute-phase human serum similarly showed two major polypeptides, of M_r 30000 (apolipoprotein AI) and 15000 (apo-SAA), and only the apolipoprotein AI band was present in HDL from normal human serum. In this gel system apolipoprotein AII is not seen because, on account of its low M_r, it runs off the bottom of the gel with the marker-dye front. Although the precise M_r of human apo-SAA_1, one of the two major SAA apoproteins, is 11685, as calculated from its amino acid sequence (Parmelee *et al.* 1982), it is known that if α-lactalbumin is used as a marker on SDS/polyacrylamide-gel electrophoresis an anomalously high apparent M_r is obtained (Eriksen & Benditt, 1984).

HDL isolated from either acute-phase or normal Wistar-rat serum showed the same profile, with protein bands corresponding to known apolipoproteins of rat HDL: apolipoprotein AIV (M_r 46000), arginine-rich polypeptide (M_r 35000) and apolipoprotein AI (M_r 27000) (Swaney *et al.*, 1974). No band migrating in the position expected for apo-SAA was present (Fig. 1). HDL isolated from Sprague–Dawley rats gave identical profiles.

Normal and acute-phase rat HDLs were also subjected to electrophoresis on homogeneous SDS/15% polyacrylamide gels, in which the dye front remains in the gel and all peptides, however small, are therefore retained. The same apoprotein profile was seen as in the gradient gels, and once again there was no difference between HDLs from normal and acute-phase rat serum. Gels of this type were electroblotted on to nitrocellulose membranes, which were then immunostained with anti-(rat HDL) serum or with anti-(mouse HDL) or anti-(human HDL) antiserum. In no case was any difference between normal and acute-phase rat HDLs observed, and in particular no additional band was present in the acute-phase HDL. Although antisera to mouse and human HDL readily demonstrated their respective specific antigens, including SAA, either in immunoblots of acute-phase HDL run in polyacrylamide-gel electrophoresis or in gel immunodiffusion testing, neither of them gave any reaction with acute-phase or normal rat serum in any test system.

DISCUSSION

The present failure to detect an SAA or SAA-like protein associated with rat HDL is intriguing in view of the fact that Sipe *et al.* (1986) have demonstrated Southern-blot hybridization between rat liver genomic DNA and a cDNA probe corresponding to residues 33–45 of human SAA. This is an invariant region conserved in all known SAA sequences. Despite this evidence for the existence of SAA-related gene(s) in the rat, they failed to detect any mRNA coding for SAA in either normal or acute-phase rat liver (Sipe *et al.*, 1986).

In marked contrast, Liao & Stark (1986) have reported that acute-phase rat liver contains a 500-fold increase in concentration over normal liver of an mRNA species, 400 nucleotide residues long, which codes for a sequence, part of which shows 74% (34/46) amino acid identity with the *C*-terminal portion of human SAA. Intriguingly, the *C*-terminal 46 residues of human SAA exactly correspond to exon 4 of the SAA gene. Sipe *et al.* (1986) presumably did not detect this 'acute-phase mRNA' because it lacks the sequence for which they probed and which lies in the *N*-terminal portion of the SAA molecule. The importance of this observation derives from the fact that the part of SAA that forms AA

and thence amyloid fibrils is the *N*-terminal 76 residues, and this may explain why, despite the apparent presence of SAA-like gene(s) in the rat genome and the acute-phase expression of an SAA-like mRNA, rats fail to develop AA amyloidosis.

A further interesting deduction that flows from this analysis, assuming that the acute-phase rat liver mRNA demonstrated by Liao & Stark (1986) is indeed translated and secreted, is that the *N*-terminal part of SAA may be required for expression of its lipid-binding property. We have recently analysed the published sequences of SAA and AA proteins and have proposed a general model that includes two localized lipid-binding sites (Turnell *et al.*, 1987). One of these consists of the *N*-terminal 11 residues of human SAA_1, and of the equivalent regions in all other known sequences (Turnell *et al.*, 1987). Interestingly exon 2 of the human SAA gene codes for precisely just this hydrophobic sequence plus the signal peptide. The other putative lipid-binding site is the completely conserved sequence 48–51 (GPGG) that forms part of a predicted surface loop and that is identical with, or closely homologous with, the Ca^{2+}- and phospholipid-binding site of phospholipase A_2 molecules (Turnell *et al.*, 1987). Experimental support for these aspects of the model has already been provided by the observation that AA molecules are associated with circulating acute-phase HDL, just as are intact SAA molecules, indicating that the *N*-terminal 76 residues are sufficient for lipid-binding (Husebekk *et al.*, 1986). We now suggest that the present observations provide further support for the idea that the *N*-terminal part of the SAA molecule is required for it to become associated with HDL particles. Assuming that it is translated, the rat acute-phase protein coded for by the mRNA species that contains only the *C*-terminal SAA sequence is evidently not an apo-HDL and must behave differently from SAA in other species, quite apart from its failure to yield amyloid fibrils.

This work was supported in part by Medical Research Council Programme Grant G979/51 to M. B. P. We thank Beth Sontrop for expert secretarial assistance.

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Received 29 September 1986/2 December 1986; accepted 12 December 1986