ISOLATION AND CHARACTERIZATION OF SENILE AMYLOID-RELATED ANTIGENIC SUBSTANCE (SAS_{SAM}) FROM MOUSE SERUM

Apo SAS_{SAM} Is a Low Molecular Weight Apoprotein of High Density Lipoprotein*

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A unique amyloid fibril protein has been isolated from the liver of new inbred strains of mouse termed "SAM" (senescence-accelerated mouse) (1). SAM is a murine model of accelerated senescence developed in our laboratory and consists of P-1, P-2, P-3, and P-4 series and as a control R series (R-1, R-2, and R-3) (2). Spontaneous, age-associated, systemic amyloidosis is one of the most characteristic findings in these mice (2, 3). The unique amyloid fibril protein termed "AS_{SAM}" has a mol wt of 5,200 daltons and amino acid composition differs from amyloid protein of any other spontaneous amyloidosis in mice and murine secondary amyloidosis (1). Specific antiserum was raised against AS_{SAM} and the uniqueness of AS_{SAM} was also confirmed by immunochemical and immunohistochemical techniques (3, 4). No relationship was observed between AS_{SAM} and murine protein AA and mouse immunoglobulin component.

Circulating precursors that reacted with antisera against amyloid protein isolated from amyloid tissues were noted in several types of amyloidosis. Prealbumin in familial amyloid polyneuropathy (5–7), senile cardiac amyloidosis (8, 9), and senile plaque (10), and SAA (serum AA) in human and experimental secondary amyloidosis (11–13) are examples. Among them, SAA has been most extensively investigated and it became evident that this acute-phase reactant serum protein was transported in association with plasma high density lipoproteins (HDL) in humans (14, 15), rabbits (16), and mice (17).

Using antisera raised against AS_{SAM}, we observed that normal mouse serum has a substance that reacts with this antiserum and we termed this substance

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[1] *Abbreviations used in this paper*: GDAM, guanidine-denatured-unfractionated amyloid fibril protein; HDL, high density lipoprotein; HDL_2, HDL_3, two classes of HDL; LPS, lipopolysaccharide; SAA, serum AA; SAM, senescence-accelerated mouse; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; VLDL, very low density lipoprotein.

"SAS_{SAM}" (Serum AS_{SAM}-related antigenic substance). This substance circulates in the form of high density lipoprotein and its apoprotein, which has the same molecular weight as AS_{SAM}, was identified.

Materials and Methods

Isolation of AS_{SAM}. Murine senile amyloid protein "AS_{SAM}" was extracted from the livers of P-1 series mice of SAM and purified by gel chromatography through Sephadex G-100 in 5 M guanidine-HCl, 1 N acetic acid, as described previously by Matsumura et al. (1).

Antisera. Anti-GDAM antiserum was obtained in rabbits by immunizing with guanidine-denaturated-unfractionated amyloid fibril protein (GDAM) and absorbed thoroughly with mouse liver acetone powder, as described by Higuchi et al. (4).

Anti-AS_{SAM} antiserum was prepared by immunizing with purified AS_{SAM} and this antiserum was used in double immunodiffusion tests and for detecting protein bands transferred to nitrocellulose paper (immuno blotting test).

Antiserum to murine protein AA was also raised (4).

Source of Sera. Old (14 mo) and newborn P-1 series mice of SAM, R-1 series mouse (5 mo) and mice of several strains (A/J, CBA/St, C3H/HeN, C57BL/6J, SJL/J, B10A, S1c:1CR, and DDD) were anesthetized with ether and bled by cardiac puncture. These sera, and human and rat sera, were tested against anti-AS_{SAM} and anti-AA antiserum by the double immunodiffusion test in agar gel. For isolation of each lipoprotein fraction, sera were obtained from the old P-1 series of SAM, DDD mice, and CBA/St mice in which the serum concentration of SAA had been elevated by the subcutaneous injection of 300 μg lipopolysaccharide (LPS) in saline (18). All these sera were usually used without freezing. DDD mice serum was used for Sephadex G-200 gel chromatography.

Fractionation of Lipoprotein. Lipoprotein fractions of different density classes were prepared by preparative ultracentrifugation (19) in a Hitachi 65p ultracentrifuge. 6 ml of mouse sera in each tube were overlayed with phosphate-buffered saline (pH 7.2) and centrifuged in a RP-40 rotor for 18 h at 40,000 rpm and 10°C. The top quarter of the tube was removed by careful aspiration into a syringe with a flat-tipped needle (very low density lipoprotein: VLDL, $d < 1.006$). The remaining lipoproteins of serum were isolated by preparative ultracentrifugation (40,000 rpm, 10°C, 44 h) in a RP-40 rotor into three fractions, low density lipoprotein (LDL), $1.006 < d < 1.163$, and two classes of high density lipoprotein:HDL_2, $1.063 < d < 1.125$, HDL_3, $1.125 < d < 1.210$. Increase of density to that of the next lipoprotein fraction was carried out by appropriate additions of a high density solution ($d = 1.346$) (19). The infranatant of the finally centrifuged solution at density 1.210 g/cm^3 was separated into three fractions, serially from top to bottom. Each of four lipoprotein fractions and three infranatant fractions were dialyzed in cellulose dialyzer tubing (8,000–9,000 daltons cutoff) against 0.15 M NaCl containing 0.05% Na_2-EDTA adjusted to pH 7.0 with 0.1 N NaOH at 4°C.

Delipidation of HDL_2 and HDL_3. HDL_2 and HDL_3 fractions were dialyzed against distilled water and lyophilized. Apo HDL_2 and apo HDL_3 were obtained by delipidation with 3:2 (vol/vol) ethanol/diethyl ether at −10°C as described by Scanu and Edelstein (20).

Column Chromatography. Whole mouse serum was fractionated on a 1.5 cm × 90 cm column of Sephadex G-200 eluted with 0.1 M Tris-HCl in 1 N NaCl, pH 8.0 at 4°C. The delipidated preparation (Apo HDL_2 and apo HDL_3) was dissolved in 8 M urea in 0.01 M Tris-HCl, 1 mM EDTA pH 8.6 and fractionated on 1.5 cm × 90 cm column of Sephadex G-200 equilibrated with the same urea buffer at 20°C. Pooled effluent fractions were dialyzed exhaustively in cellulose dialyzer tubing (3,500 daltons cutoff) against distilled water and lyophilized. The urea recrystallized from ethanol or ultra pure urea (Schwarz/Mann Inc., Orangeburg, NY) was used for gel chromatography and polyacrylamide gel electrophoresis.

Quantitation of SAS_{SAM} and SAA. Quantitation of SAS_{SAM} and SAA was performed with single radial immunodiffusion test in 1% agar gel containing antibodies. 1 mg each of purified AS_{SAM} and protein AA was dissolved by heating for 2 min at 100°C in 1 ml of

0.2% SDS, 0.01 M phosphate buffer, pH 7.4, then diluted with an appropriate volume of 0.28% Tween 20, 0.01 M phosphate buffer, pH 7.4, and these protein solutions were used as standards.

Double Immunodiffusion and Immunoelectrophoresis. Double immunodiffusion and immunoelectrophoresis were performed in 1% agarose gel in 0.07% barbital buffer, pH 8.6. The plates were developed at room temperature and examined at 48 h. The plates of immunoelectrophoresis were washed in phosphate-buffered saline, pH 7.2, desalted in distilled water, immersed in 5% acetic acid in 75% ethanol, dried at 80°C, and stained for lipid with 1:4 (vol/vol) Oil Red O/Fat Red 7B solution in 60% ethanol and for protein with 1% Amide Black 10B solution.

Gel Electrophoretic Analysis. The method of Swank and Munkres (21) was used for 8 M urea-sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE). Purified AS_{SAM} and apo HDL_2 first subjected to 8 M urea-SDS-PAGE were electrophoretically transferred from the gel to nitro-cellulose paper (22). Protein bands reacting with antisera (anti-GDAM antiserum and anti-AS_{SAM} antiserum) were detected by using ^{125}I-labeled protein A (New England Nuclear, Boston, MA) and subsequent autoradiography at −80°C (22). Analytical PAGE for apolipoprotein was performed by the method of Davis (23) on the gel with 7.5% acrylamide and 8 M urea.

Other Analytical Procedures. Total protein was determined by the method of Lowry et al. Total cholesterol concentration was estimated by the 0-phthalaldehyde method (Cholesterol B-test Wako, Wako Pure Chemical Industries Ltd., Osaka, Japan).

Results

Detection of AS_{SAM}-related Antigenic Substance in Serum (SAS_{SAM}). Old P-1 series mice serum (14 mo) gave a single precipitation line, when tested by double immunodiffusion against anti-AS_{SAM} antiserum and this line fused with the precipitation line detected against purified AS_{SAM}. Absorption of anti-AS_{SAM} antiserum with purified AS_{SAM} (0.05 mg per 1 ml of antiserum) eliminated the line between the mouse serum and antiserum. All sera obtained from mice of several strains such as P-1 series, R-1 series, A/J, CBA/St, CBA/HeN, C57BL/6J, SJL/J, B10A, S1c:1CR and DDD, showed a single precipitation line with anti-AS_{SAM} antiserum in agar gel. Those precipitation lines fused with the line formed by purified AS_{SAM} and P-1 mice serum.

However, rat and human sera showed no precipitation line (Fig. 1). These mouse sera were also tested against anti-AA antisera by the double immunodiffusion test (Table I). None of the sera obtained from any strain reacted with anti-AA antiserum, however, the sera from a C57BL/6J mouse and CBA/St mice with an ulcer at the neck and amyloid deposition in the liver and spleen at autopsy, and with high levels of SAA by injection of LPS, respectively, did react with the anti-AA antiserum.

In the immunoelectrophoresis, a single precipitation line was formed at the albumin/prealbumin region between mouse serum and anti-AS_{SAM} antiserum. This precipitation line was positively stained with both Amide Black 10B and Oil Red O/Fat Red 7B solution (Fig. 2). In Fig. 2*B*, α and β lipoproteins of mouse serum were stained with Oil Red O/Fat Red 7B solution and the precipitation line of serum AS_{SAM}-related substance corresponded to the line of α lipoprotein or high density lipoprotein.

Sephadex G-200 Chromatography. Sephadex G-200 chromatography of DDD mouse whole serum is illustrated in Fig. 3. In addition to the concentration of AS_{SAM}-related substance, the amount of IgM, IgG and albumin of each fraction

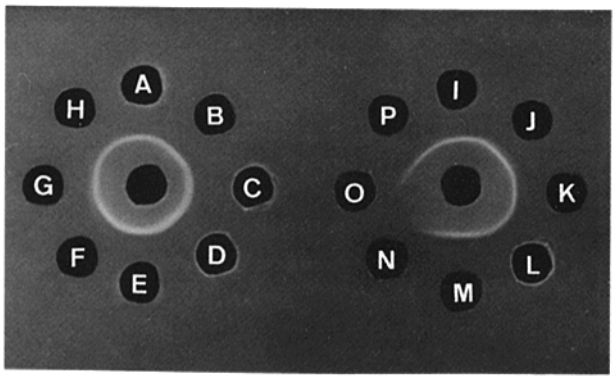

FIGURE 1. Detection of serum AS_{SAM}-related antigenic substance (SAS_{SAM}) by double immunodiffusion. Anti-AS_{SAM} antiserum in central wells was tested against purified AS_{SAM} (*H*, *D*, and *P*), old P-1 mouse (14 mo) (*A*), new born P-1 mice (*B*), R-1 mouse (5 mo) (*C*), DDD mice (*E*), S1c:ICR (*F*), CBA/St (*G*), C3H/HeN (*I*), C57BL/6J (*J*), A/J (*K*), SJL/J (*L*), B10A (*M*), rat (*N*), and human (*O*) serum.

were measured by a single radial immunodiffusion test for molecular weight standards. AS_{SAM} immunoreactivity appeared as a single peak in a position just before the peak of IgG and the molecular weight was estimated to be ~200,000 daltons (Fig. 3).

Determination of the SAS_{SAM} and SAA Concentration in Lipoprotein Fractions. The concentrations of total protein, total cholesterol, serum AS_{SAM}-related substance (SAS_{SAM}), and SAA are given in Table II. In sera obtained from old P-1 series mice and DDD mice, the largest amount of SAS_{SAM} was found in the HDL_2 fraction, 63.3% and 54.0% of total SAS_{SAM} were obtained in HDL_2 fractions of P-1 mice and DDD mice, respectively. In HDL_3 fractions of P-1 mice and DDD mice, 21.6% and 37.5% were detected, respectively. Thus, almost 90% of total SAS_{SAM} was found in high density lipoprotein fractions. The remaining SAS_{SAM} was found in the LDL fraction (DDD mice) and the second layer below the HDL_3 fractions at a density 1.21 g/cm^3 (15.1% and 6.8% in P-1 and DDD mice, respectively). Although SAA was not detected in any fraction of P-1 and DDD mice, the HDL_3 fraction of CBA/St mice serum in which SAA level was increased by injection of LPS contained 68.7% of SAA.

Chromatography of Apolipoproteins. Sephadex G-200 chromatography in 8 M urea, 0.01 M Tris, 0.01 M EDTA pH 8.6 of the delipidated HDL_2 and HDL_3 fractions (apo HDL_2 and apo HDL_3) obtained from DDD mice is illustrated in Fig. 4. Apo HDL_2 lipoproteins were separated by Sephadex G-200 into three distinctly defined ultraviolet absorbing peaks. Slight immunoreactivity to anti-AS_{SAM} antiserum was found at the first peak (void volumes), but almost all SAS_{SAM} was found at the third peak. A trace amount of SAA was observed at the third peak (Fig. 4*B*). It was more difficult to dissolve apo HDL_3 than apo HDL_2 in 8 M urea buffer and there was some difference between chromatographic patterns of apo HDL_2 and apo HDL_3. Apo HDL_3 was separated into four peaks and the first peak (void volume) was greater than apo HDL_2. A considerable amount of SAS_{SAM} was found here. However, the major peak of SAS_{SAM} was observed in the third peak, its position being the same as the third

TABLE I

Detection of SAS_{SAM} and SAA in Sera Obtained from Several Strains of Mice, Rats, and Humans by Double Immunodiffusion Testing

Antisera	Serum															
	AS_{SAM}*	AA‡	P-1 (old)	P-1 (newborn)	R-1	DDD	A/J	SJL/J	C3H/HeN	C57BL/6J	B10A	S1c:ICR	CBA/St	CBA/St§ (LPS)	Rats‖	Humans
Anti-AS_{SAM}	+	−	+	+	+	+	+	+	+	+	+	+	+	+	−	−
Anti-AA	−	+	−	−	−	−	−	−	−	+	−	−	−	+	−	−

* Purified amyloid fibril protein "AS_{SAM}."

‡ Purified amyloid fibril protein "Protein AA."

§ Serum obtained from CBA/St mice subcutaneously given lipopolysaccharide (LPS).

‖ A Wistar/MsS1c rat was used.

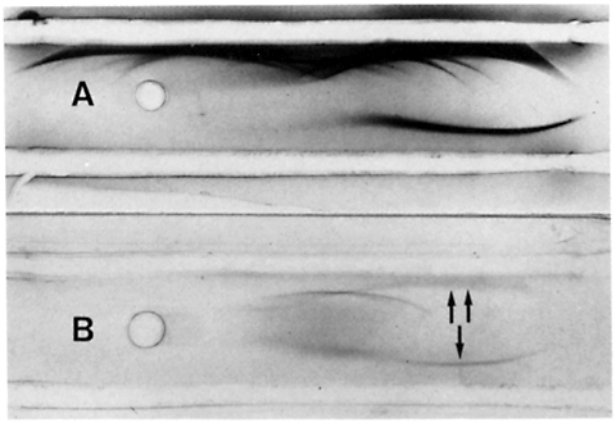

FIGURE 2. Immunoelectrophoresis in 1.0% agarose gel at pH 8.6 of DDD mice serum. After electrophoresis (anode is to the right) the upper troughs were filled with anti-mouse whole serum and the lower troughs were filled with anti-AS_{SAM} in each slide *A* and *B*. After washing and drying, slide *A* was stained with Amide Black 10B and slide *B* was stained with Oil Red O and Fat Red 7B. SAS_{SAM} precipitate (arrow) was stained with Oil Red O and Fat Red 7B and migrated to the position of α-lipoproteins of serum (double arrows).

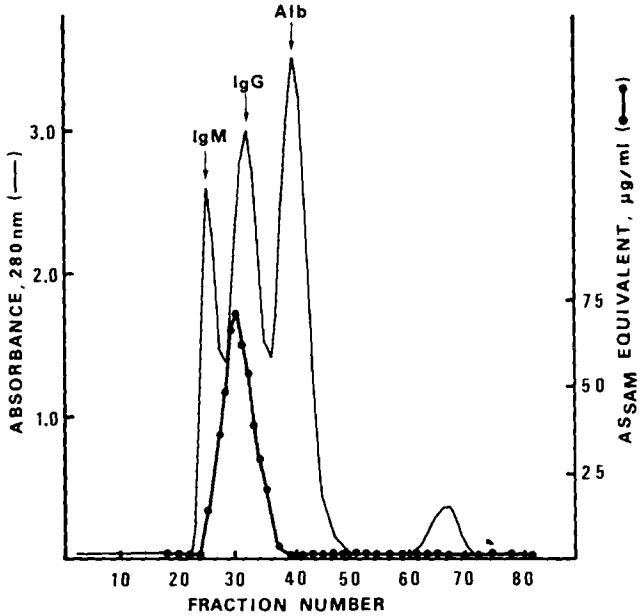

FIGURE 3. Sephadex G-200 chromatography of DDD mice serum (2.0 ml) in 1.0 M NaCl, 0.1 M Tris-HCl, pH 8.6. Fraction volume, 2.6 ml, column size, 1.5 cm × 90 cm, arrows indicate elution position of molecular weight standards (IgM; mouse IgM, IgG; mouse IgG, and Alb; mouse albumin). (——) Absorbance at 280 nm, (●——●) concentration of SAS_{SAM} represented as AS_{SAM} equivalent (μg/ml).

TABLE II

*Protein, Cholesterol, SAS_{SAM}, and SAA Concentrations in Lipoprotein Fractions**

Fraction		P-1				DDD		CBA/St		CBA/St (LPS)	
Density	Layer	Total protein	Total cholesterol	SAS_{SAM}	SAA	SAS_{SAM}	SAA	SAS_{SAM}	SAA	SAS_{SAM}	SAA
g/cm³		*mg/dl*	*mg/dl*	*mg/dl*‡	*mg/dl*§						
1.006	VLDL	25.5	10.0	ND‖	ND	0.2 (0.4)¶	ND	ND	ND	ND	ND
1.063	LDL	12.7	14.9	ND	ND	0.9 (1.4)	ND	2.0 (3.3)	ND	ND	ND
1.125	HDL_2	70.8	47.0	24.1 (63.3)	ND	35.6 (64.0)	ND	36.3 (58.9)	ND	15.2 (42.7)	14.4 (30.7)
1.210	HDL_3	45.8	14.5	8.4 (21.6)	ND	24.6 (37.5)	ND	19.7 (32.0)	ND	19.5 (54.8)	29.7 (68.7)
	2nd**	190.8	9.4	5.7 (15.1)	ND	4.5 (6.8)	ND	3.5 (5.7)	ND	0.9 (2.5)	0.3 (0.7)
	3rd	891.3	2.8	ND	ND	ND	ND	ND	ND	ND	ND
	4th	2,365.8	6.1	ND	ND	ND	ND	ND	ND	ND	ND

* Concentrations adjusted to initial serum volumes.
‡ Represented as AS_{SAM} equivalent.
§ Represented as protein AA equivalent.
‖ Not detected.
¶ Percent of the total of concentrations of each fraction.
** The 2nd layer of the infranatant of the finally centrifuged solution at a density of 1.210 g/cm³. (See text).

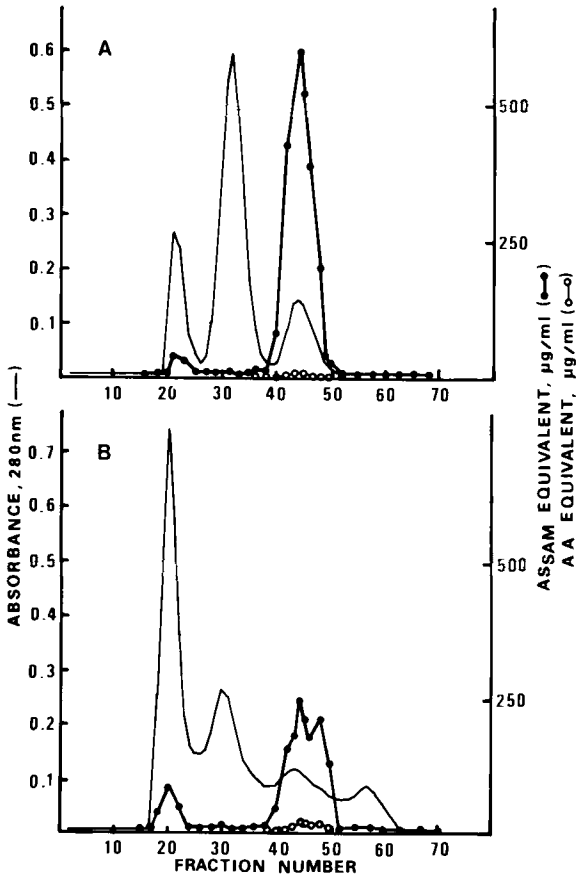

FIGURE 4. Sephadex G-200 gel chromatography in 8 M urea, 0.01 M Tris-HCl, 0.01 M EDTA, pH 8.6 of delipidated HDL fractions from DDD mice serum. (*A*) Apo HDL_2 and (*B*) apo HDL_3. Fraction volume, 3.0 ml, column size, 1.5 cm × 90 cm. (——) Absorbance at 280 nm, (●——●) concentration of SAS_{SAM}, and (○——○) concentration of SAA as protein AA equivalent (μg/ml).

peak of apo HDL_2. Immunoreactivity to anti-AA antiserum was also detected in the third peak, but the amount was considerably less than that of SAS_{SAM}. The pooled fractions of each peak were dialyzed against distilled water in cellulose tubing, lyophilized, and analyzed in the following electrophoretic procedures, as described in Materials and Methods.

Electrophoretic Analysis. 8 M urea SDS polyacrylamide gel electrophoretic patterns of apo HDL_2, peaks separated by Sephadex G-200 from apo HDL_2 are shown in Fig. 5. Apo HDL_2 contained ~12 protein bands. In these bands, the two largest had mol wt of 25,000 and 5,200 daltons, respectively. The first peak of apo HDL_2 contained several high molecular weight bands and the second peak contained a bond with a mol wt calculated to be 25,000 daltons. The third peak of apo HDL_2, which had immunoreactivity to anti-AS_{SAM}, contained mol wt 5,200 band, as a main protein band, and a few minor protein bands. Purified AS_{SAM} had a mol wt of 5,200 daltons and was the same as the main protein band

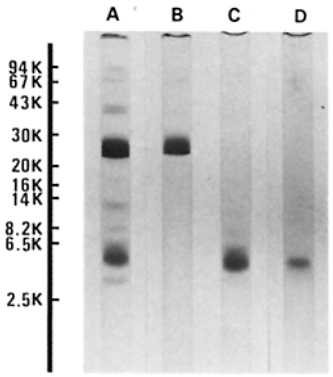

FIGURE 5. 8 M urea SDS polyacrylamide (12.5%) gel chromatography of delipidated mouse HDL. (*A*) apo HDL_2, (*B*) the second peak, and (*C*) the third peak materials isolated by gel chromatography of apo HDL_2, (*D*) purified AS_{SAM}. Molecular weight markers, phosphorylase b (94,000), albumin (67,000), ovalbumin (43,000), carbonic anhydrase (30,000), trypsin inhibitor (20,100), myoglobin (16,949), myoglobin I and II (14,400), myoglobin I (8,159), myoglobin II (6,214), myoglobin III (2,512).

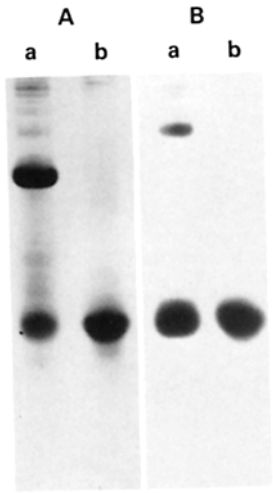

FIGURE 6. Antibody labeling of apo HDL_2 and tissue amyloid fibril proteins separated by SDS-PAGE. (*A*) Gel used for protein blot transfer to nitrocellulose stained with Coomassie Brilliant Blue. (*a*) apo HDL_2, and (*b*) purified AS_{SAM}. (*B*) Autoradiograms of protein after labeling with anti-purified AS_{SAM} and subsequent reaction with ^{125}I-labeled protein A. (*a*) apo HDL_2 and (*b*) purified AS_{SAM}. Molecular weight markers were same as Fig. 5.

of the third peak of apo HDL_2. The third peak of apo HDL_3 also had a main band of mol wt 5,200 daltons but there were also some minor bands of low mol wt proteins. The antigenic relationship of the tissue and serum constituent was demonstrated after transfer to nitrocellulose paper by labeling with antibodies specific for tissue amyloid protein (Fig. 6). Purified amyloid protein (AS_{SAM}) had only one band that reacted with the antibody. Apo HDL_2 contained two bands, the major one corresponding to a band of the third peak of apo HDL_2 and the minor one ~42,000 daltons. No other protein of apo HDL_2 reacted with anti-AS_{SAM} antiserum. In each of these experiments, the same result was obtained in

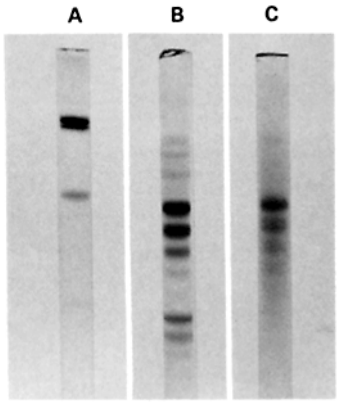

FIGURE 7. Anionic (pH 9.4) 8 M urea polyacrylamide gel (7.5%) electrophoresis of (*A*) apo HDL_2, (*B*) the third peak materials isolated by gel chromatography of apo HDL_2 and (*C*) purified AS_{SAM}.

both cases in which anti-GDAM antiserum and anti-AS_{SAM} antiserum were used as antiserum. Fig. 7 shows the anionic polyacrylamide gel electrophoretic patterns. Apo HDL_2 contained seven major apoprotein bands. The third peak of apo HDL_2 contained several proteins that migrated faster and the first three bands corresponded to purified AS_{SAM} separated into the three or four bands. The third peak of apo HDL_3 separated into many minor bands but the position of the major bands were the same as in the case of the purified AS_{SAM}.

Discussion

Purified amyloid fibril proteins have been obtained from several types of amyloidosis (24). Specific antisera were raised against these amyloid proteins and serum amyloid–related substances that reacted with the antisera were investigated. Generally these substances were considered to be precursors of amyloid proteins. To clarify the pathogenesis of amyloidosis or mechanisms of deposition of amyloid protein, the nature of these precursors has to be determined.

In humans, immunoglobulin light chain (25, 26) for the primary amyloidosis and SAA (11–13) for the secondary amyloidosis were confirmed to be precursors circulating in blood. In the case of senile amyloidosis (senile cardiac amyloidosis and senile plaque), prealbumin is considered to be amyloid-related protein (8–10). Although senile amyloidosis has long been detected in mice (27, 28, 29) little is known of the pathogenesis of amyloidosis and the biochemical nature of amyloid protein and its precursor protein.

We isolated a unique senile amyloid fibril protein from the liver of SAM, a new murine model of accelerated senescence. We found that mouse serum has a AS_{SAM}-related antigenic substance, regardless of the strain, and we termed this substance "SAS_{SAM}." SAS_{SAM} seems to be a physiological substance in contrast with acute phase reactant protein SAA (30) as demonstrated in Fig. 1 and Table 2. The data obtained in immunoelectrophoresis (Fig. 2), and gel chromatography (Fig. 3) of mouse serum and also affinity chromatography of mouse serum using CNBr-activated Sepharose 4B coupled with anti-AS_{SAM} IgG (data not shown)

revealed that SAS_{SAM} may be transported as a form of high density lipoprotein (HDL) in serum.

The determination of SAS_{SAM} concentration in lipoprotein fractions separated by ultracentrifugation provided convincing evidence that the bulk of SAS_{SAM} is associated with the HDL fractions of mouse serum, and the largest amount of SAS_{SAM} is related to the HDL_2 subfraction of HDL. SAA is a well-known precursor of protein AA and is associated with HDL. The relative amount of SAA was the largest in the HDL_3 fraction in humans (15), rabbits (16), and mice (17). In the present study, the relative amount of SAA was also highest in the HDL_3 fraction ($1.125 < d < 1.210$), 68.7% of SAA was found in HDL_3 of CBA mice serum with elevated concentrations of SAA after injection of LPS. On the other hand, the largest amount of SAS_{SAM} was found in the HDL_2 fraction ($1.063 < d < 1.125$) in DDD and SAM serum (~60% to the total), and SAA was not detected in either HDL_2 and HDL_3 fractions in DDD and SAM.

Partial purification of the protein part of SAS_{SAM} achieved by delipidation of HDL and subsequent gel chromatography in 8 M urea containing buffer revealed that the protein part of SAS_{SAM} was a low molecular weight apolipoprotein of HDL. This apoprotein was designated as apo SAS_{SAM}. Apo SAS_{SAM} was eluted as the third peak in Sephadex G-200 gel chromatography (Fig. 4) and was the same as in the case of SAA reported by Benditt et al. (17). In this study, however, AA immunoactivity was scarcely detectable in this position of both HDL_2 and HDL_3 fractions.

8 M urea SDS-PAGE of apo HDL_2 demonstrated two major components of apo HDL_2. One is an apoprotein with a mol wt of 25,000 daltons and the other is a protein with a mol wt of ~5,200 daltons. The 25,000-dalton protein corresponds to apo A-I protein of human and rat (31) and the 5,200-dalton protein corresponds to apo C proteins (32). SDS–polyacrylamide gel electrophoresis of the third peak of HDL_2 separated by Sephadex G-200 and antibody labeling of apo HDL_2 transferred to nitrocellulose paper demonstrated that apo SAS_{SAM} is a protein with a mol wt of 5,200 and this molecular weight is the same as AS_{SAM} isolated from the liver. Here, another minor band was observed in the position of 42,000 daltons and such is now under investigation. SAA is considered to be degraded by an enzyme of monocyte origin (33–36) or circulates in serum (37, 38) and deposits in tissues. In the case of SAS_{SAM}, the finding that the molecular weight of apo SAS_{SAM} was the same as AS_{SAM} supports the possibility that apo SAS_{SAM} deposits in tissues, without degradation. Further studies are underway to determine whether SAS_{SAM} is the precursor of AS_{SAM}. In anionic 8 M urea polyacrylamide electrophoresis, apolipoprotein of the third peak of HDL_2 and purified AS_{SAM} separated into three or four bands. This finding seems to be the result of polymorphism in AS_{SAM}, as is the case with protein AA and SAA (39–41).

It is noteworthy that both apo SAS_{SAM} and apo SAA are low molecular weight apolipoproteins of HDL, although there are differences in molecular weight, the distribution in HDL fractions (32) and concentration in normal serum. This class of peptides may be prone to deposition in tissues in the form of amyloid fibrils.

This idea is supported by the finding that protein AA coexists with AS_{SAM} in SAM in the presence of inflammatory lesions, as reported in a previous paper (4).

Apolipoprotein has been little investigated in mice, despite the usefulness of this species as an experimental tool. Hence, the function of apo SAS_{SAM} corresponding to apo C proteins, is poorly understood. The C apoproteins play an important role in the metabolism of lipoprotein (42–45). Whether the physiological function of apo SAS_{SAM} is related to this mode or to a heretofore unrecognized role, is the subject of ongoing study. Elucidation of this function will aid in understanding the pathogenesis of amyloidosis. SAS_{SAM} was present in the serum of all the strains we studied. Therefore, it is tempting to postulate that AS_{SAM} is a common fibril protein that originates from a common serum precursor in the case of murine senile amyloidosis.

Summary

Sera obtained from senescence-accelerated mouse (SAM) and normal mice contained a substance that reacted with antiserum raised against AS_{SAM}, a novel senile amyloid fibril protein isolated from the liver of SAM.

This physiological substance, termed "SAS_{SAM}" (serum AS_{SAM}-related antigenic substance), migrated to the albumin/prealbumin region in immunoelectrophoresis and the precipitation line formed with anti-AS_{SAM} antiserum was stained positively with both Amide Black 10 B and Oil Red O/Fat Red 7B solutions, thereby suggesting that SAS_{SAM} is an α lipoprotein. Using Sephadex G-200 gel chromatography, SAS_{SAM} was eluted as a high mol wt form of ~200,000 daltons.

Fractionation of lipoprotein from normal mouse serum by preparative ultracentrifugation disclosed that SAS_{SAM} was found mainly in high density lipoprotein, HDL (the density is between 1.063 and 1.21 g/cm^3). The largest amount of SAS_{SAM} was found in the HDL_2 fraction (the density is between 1.063 and 1.125) and in this fraction SAA was not detected. Furthermore, AS_{SAM} immunoreactivity appeared in the low mol wt proteins (below 10,000 daltons) of apo HDL separated in the buffer containing 8 M urea through Sephadex G-200.

In 8 M urea sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE), the major components of apolipoproteins in this position, possibly corresponding to apo C proteins, have the same molecular weight, 5,200 daltons, as AS_{SAM} and this component was labeled by anti-AS_{SAM} antiserum after transfer to nitrocellulose paper.

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