ISOLATION AND CHARACTERIZATION OF SENILE AMYLOID-RELATED ANTIGENIC SUBSTANCE (SASsAM) FROM MOUSE SERUM

Apo SASsAM 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 "ASsAM" 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 ASsAM and the uniqueness of ASsAM was also confirmed by immunochemical and immunohistochemical techniques (3, 4). No relationship was observed between ASsAM 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 ASsSAM, we observed that normal mouse serum has a substance that reacts with this antiserum and we termed this substance

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† Abbreviations used in this paper: GDAM, guanidine-denatured-unfractionated amyloid fibril protein; HDL, high density lipoprotein; HDLc, HDL2, 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\textsubscript{SAM}" (Serum AS\textsubscript{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\textsubscript{SAM}, was identified.

Materials and Methods

Isolation of AS\textsubscript{SAM}. Murine senile amyloid protein "AS\textsubscript{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-denatured-unfractionated amyloid fibril protein (GDAM) and absorbed thoroughly with mouse liver acetone powder, as described by Higuchi et al. (4).

Anti-AS\textsubscript{SAM} antiserum was prepared by immunizing with purified AS\textsubscript{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, 11c1CR, and DDD) were anesthetized with ether and bled by cardiac puncture. These sera, and human and rat sera, were tested against anti-AS\textsubscript{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 \( \mu \)g 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 < \( d < 1.25 \), 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 (3,500 daltons cutoff) against distilled water and lyophilized. The urea recrystalized from ethanol or ultra pure urea (Schwarz/Mann Inc., Orangeburg, NY) was used for gel chromatography and polyacrylamide gel electrophoresis.

Delipidation of HDL\(_2\) and HDL\(_3\). HDL\(_2\) and HDL\(_3\) fractions were delipidated 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 \( \times \) 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\(_2\)) was dissolved in 8 M urea in 0.01 M Tris-HCl, 1 mM EDTA pH 8.6 and fractionated on 1.5 cm \( \times \) 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 recrystalized from ethanol or ultra pure urea (Schwarz/Mann Inc., Orangeburg, NY) was used for gel chromatography and polyacrylamide gel electrophoresis.

Quantitation of SAS\textsubscript{SAM} and SAA. Quantitation of SAS\textsubscript{SAM} and SAA was performed with single radial immunodiffusion test in 1% agar gel containing antibodies. 1 mg each of purified AS\textsubscript{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 ASsAM and apo HDL2 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-ASsAM antiserum) were detected by using 125I-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 (29) on the gel with 7.5% acrylamide and 8 M urea.

Results

Detection of ASsAM-related Antigenic Substance in Serum (SASsAM). Old P-1 series mice serum (14 mo) gave a single precipitation line, when tested by double immunodiffusion against anti-ASsAM antiserum and this line fused with the precipitation line detected against purified ASsAM. Absorption of anti-ASsAM antiserum with purified ASsAM (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/HcN, C57BL/6J, SJL/J, B10A, 1sc:1CR and DDD, showed a single precipitation line with anti-ASsAM antiserum in agar gel. Those precipitation lines fused with the line formed by purified ASsAM and P-1 mouse serum.

However, rat and human sera showed no precipitation line (Fig. 1). These mouse sera were also tested against anti-AA antiserum 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-ASsAM antiserum. This precipitation line was positively stained with both Amide Black 10B and Oil Red O/Fat Red 7B solution (Fig. 2). In Fig. 2B, α and β lipoproteins of mouse serum were stained with Oil Red O/Fat Red 7B solution and the precipitation line of serum ASsAM-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 ASsAM-related substance, the amount of IgM, IgG and albumin of each fraction
were measured by a single radial immunodiffusion test for molecular weight standards. \$\text{AS}_{\text{SAM}}\$ immunoreactivity appeared as a single peak in a position just before the peak of IgG and the molecular weight was estimated to be \( \sim 200,000 \) daltons (Fig. 3).

**Determination of the \$\text{SAS}_{\text{SAM}}\$ and SAA Concentration in Lipoprotein Fractions.** The concentrations of total protein, total cholesterol, serum \$\text{AS}_{\text{SAM}}\$-related substance (SAS$_{\text{SAM}}$), and SAA are given in Table II. In sera obtained from old P-1 series mice and DDD mice, the largest amount of SAS$_{\text{SAM}}$ was found in the HDL$_2$ fraction, 63.3\% and 54.0\% of total SAS$_{\text{SAM}}$ 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$_{\text{SAM}}$ was found in high density lipoprotein fractions. The remaining SAS$_{\text{SAM}}$ 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$_{\text{SAM}}$ antiserum was found at the first peak (void volumes), but almost all SAS$_{\text{SAM}}$ was found at the third peak. A trace amount of SAA was observed at the third peak (Fig. 4B). 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$_{\text{SAM}}$ was found here. However, the major peak of SAS$_{\text{SAM}}$ was observed in the third peak, its position being the same as the third...
### TABLE 1

Detection of $\text{AS}_{\text{SAM}}$ and SAA in Sera Obtained from Several Strains of Mice, Rats, and Humans by Double Immunodiffusion Testing

| Antisera    | $\text{AS}_{\text{SAM}}^*$ | $\text{AA}^\dagger$ | P-1 (old) | P-1 (new-born) | R-1 | DDD | A/J | SJL/J | C3H/HeN | C57BL/6J | B10A | S1c:ICR | CBA/St | CBA/St* (LPS) | Rats† | Humans |
|-------------|-----------------------------|----------------------|-----------|----------------|-----|-----|-----|-------|---------|----------|------|---------|--------|---------------|-------|---------|
| Anti-$\text{AS}_{\text{SAM}}$ | +                            | -                    | +         | +              | +   | +   | +   | +     | +       | +        | +    | -       | -      | +             | -     | -       |
| Anti-$\text{AA}$    | -                            | +                    | -         | -              | -   | -   | -   | -     | -       | -        | -    | -       | -      | -             |       | -       |

* Purified amyloid fibril protein "AS$_{SAM}$."  
† Purified amyloid fibril protein "Protein AA."  
* Serum obtained from CBA/St mice subcutaneously given lipopolysaccharide (LPS).  
† A Wistar/MsStc 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-ASSAM 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. SASSAM 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 SASSAM represented as ASSAM equivalent (μg/ml).
| Density | Layer | Total protein | Total cholesterol | SASSAM | SAA | SASSAM | SAA | SASSAM | SAA | SASSAM | SAA |
|---------|-------|---------------|-------------------|--------|-----|--------|-----|--------|-----|--------|-----|
| 1.006   | VLDL  | 25.5          | 10.0              | ND     | ND  | 0.2    | ND  | ND     | ND  | ND     | ND  |
| 1.063   | LDL   | 12.7          | 14.9              | ND     | ND  | 0.9    | ND  | 2.0    | ND  | ND     | ND  |
| 1.125   | HDL₂  | 70.8          | 47.0              | 24.1   | ND  | 35.6   | ND  | 36.3   | ND  | 15.2   | 14.4|
| 1.210   | HDL₃  | 45.8          | 14.5              | 8.4    | ND  | 24.6   | ND  | 19.7   | ND  | 19.5   | 29.7|
|         | 2nd** | 190.8         | 9.4               | 5.7    | ND  | 4.5    | ND  | 3.5    | ND  | 0.9    | 0.3 |
|         | 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 SASSAM 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).
peak of apo HDL₂. Immunoreactivity to anti-AA antiserum was also detected in the third peak, but the amount was considerably less than that of SAS₅₅. 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 HDL₂, peaks separated by Sephadex G-200 from apo HDL₂ are shown in Fig. 5. Apo HDL₂ 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 HDL₂ contained several high molecular weight bands and the second peak contained a band with a mol wt calculated to be 25,000 daltons. The third peak of apo HDL₂, which had immunoreactivity to anti-AS₅₅, contained mol wt 5,200 band, as a main protein band, and a few minor protein bands. Purified AS₅₅ had a mol wt of 5,200 daltons and was the same as the main protein band.
of the third peak of apo HDL₂. The third peak of apo HDL₃ 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 (ASSAM) had only one band that reacted with the antibody. Apo HDL₂ contained two bands, the major one corresponding to a band of the third peak of apo HDL₂ and the minor one ~42,000 daltons. No other protein of apo HDL₂ reacted with anti-ASSAM antiserum. In each of these experiments, the same result was obtained in
both cases in which anti-GDAM antiserum and anti-AS\textsubscript{SAM} antiserum were used as antiserum. Fig. 7 shows the anionic polyacrylamide gel electrophoretic patterns. Apo HDL\textsubscript{2} contained seven major apoprotein bands. The third peak of apo HDL\textsubscript{2} contained several proteins that migrated faster and the first three bands corresponded to purified AS\textsubscript{SAM} separated into the three or four bands. The third peak of apo HDL\textsubscript{3} separated into many minor bands but the position of the major bands were the same as in the case of the purified AS\textsubscript{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\textsubscript{SAM}-related antigenic substance, regardless of the strain, and we termed this substance "SAS\textsubscript{SAM}." SAS\textsubscript{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\textsubscript{SAM} IgG (data not shown).
revealed that SASsAM may be transported as a form of high density lipoprotein (HDL) in serum.

The determination of SASsAM concentration in lipoprotein fractions separated by ultracentrifugation provided convincing evidence that the bulk of SASsAM is associated with the HDL fractions of mouse serum, and the largest amount of SASsAM 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 SASsAM 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 SASsAM achieved by delipidation of HDL and subsequent gel chromatography in 8 M urea containing buffer revealed that the protein part of SASsAM was a low molecular weight apolipoprotein of HDL. This apoprotein was designated as apo SASsAM. Apo SASsAM 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 SASsAM is a protein with a mol wt of 5,200 and this molecular weight is the same as ASSAM 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 SASsAM, the finding that the molecular weight of apo SASsAM was the same as ASSAM supports the possibility that apo SASsAM deposits in tissues, without degradation. Further studies are underway to determine whether SASsAM is the precursor of ASSAM. In anionic 8 M urea polyacrylamide electrophoresis, apolipoprotein of the third peak of HDL$_2$ and purified ASSAM separated into three or four bands. This finding seems to be the result of polymorphism in ASSAM, as is the case with protein AA and SAA (39–41).

It is noteworthy that both apo SASsAM 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 ASSAM 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 SASSAM 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 SASSAM 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. SASSAM was present in the serum of all the strains we studied. Therefore, it is tempting to postulate that ASSAM 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 ASSAM, a novel senile amyloid fibril protein isolated from the liver of SAM.

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

Fractionation of lipoprotein from normal mouse serum by preparative ultracentrifugation disclosed that SASSAM was found mainly in high density lipoprotein, HDL (the density is between 1.063 and 1.21 g/cm³). The largest amount of SASSAM was found in the HDL2 fraction (the density is between 1.063 and 1.125) and in this fraction SAA was not detected. Furthermore, ASSAM 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 ASSAM and this component was labeled by anti-ASSAM antiserum after transfer to nitrocellulose paper.

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References

1. Matsumura, A., K. Higuchi, K. Shimizu, M. Hosokawa, K. Hashimoto, K. Yasuhira, and T. Takeda. 1982. A novel amyloid fibril protein isolated from senescence-accelerated mice. Lab. Invest. 47:270.
2. Takeda, T., M. Hosokawa, S. Takeshita, M. Irino, K. Higuchi, T. Matsushita, Y. Tomita, K. Yasuhira, H. Hamamoto, K. Shimizu, M. Ishii, and T. Yamamuro. 1981. A new murine model of accelerated senescence. Mech. Ageing Dev. 17:183.

3. Takeshita, S., M. Hosokawa, M. Irino, K. Higuchi, K. Shimizu, K. Yasuhira, and T. Takeda. 1982. Spontaneous age-associated amyloidosis in Senescence Accelerated Mouse (SAM). Mech. Ageing Dev. 20:13.

4. Higuchi, K., A. Matsumura, A. Honma, S. Takeshita, K. Hashimoto, M. Hosokawa, K. Yasuhira, and T. Takeda. 1983. Systemic senile amyloid in Senescence Accelerated Mouse (SAM): a unique fibril protein (ASAM) demonstrated in tissues from various organs by the unlabeled immunoperoxidase method. Lab. Invest. 48:231.

5. Costa, P. P., A. S. Figueira, and F. R. Bravo. 1978. Amyloid fibril protein related to prealbumin in familial amyloidotic polyneuropathy. Proc. Natl. Acad. Sci. USA. 75:4499.

6. Tawara, S., S. Araki, K. Toshimori, H. Nakagawa, and S. Ohtaki. 1981. Amyloid fibril protein in type-I familial amyloidotic polyneuropathy in Japanese. J. Lab. Clin. Med. 98:811.

7. Skinner, M., A. S. Cohen. 1981. The prealbumin nature of the amyloid protein in familial amyloid polyneuropathy (FAP)-Swedish variety. Biochem. Biophys. Res. Commun. 99:1326.

8. Sletten, K., P. Westermark and J. B. Natvig. 1980. Senile cardiac amyloid is related to prealbumin. Scand. J. Immunol. 12:503.

9. Cornwell, G. G. III, P. Westermark, J. B. Natvig, and W. Murdoch. 1981. Senile cardiac amyloid: evidence that fibril contains a protein immunologically related to prealbumin. Immunology. 44:447.

10. Shirahama, T., M. Skinner, P. Westermark, A. Rubinow, A. S. Cohen, and T. L. Kemper. 1982. Senile cerebral amyloid. Prealbumin as a common constituent in the neuritic plaque, in the neurofibrillary tangle, and in the microangiographic lesion. Am. J. Pathol. 107:41.

11. Levin, M., M. Pras, and E. C. Franklin. 1973. Immunological studies of major nonimmunoglobulin protein of amyloid. I. Identification and partial characterization of a related serum component. J. Exp. Med. 138:373.

12. Linke, R. P., J. D. Sipe, P. S. Pollock, T. F. Ignazak, and G. G. Glenner. 1975. Isolation of a low molecular weight serum component antigenically related to an amyloid fibril protein of unknown origin. Proc. Natl. Acad. Sci. USA. 72:1473.

13. Anders, R. F., J. B. Natvig, T. E. Michaelson, and G. Husby. 1975. Isolation and characterization of amyloid-related serum protein SAA as a low molecular weight protein. Scand. J. Immunol. 4:397.

14. Benditt, E. P., and N. Eriksen. 1977. Amyloid protein SAA is associated with high density lipoprotein from human serum. Proc. Natl. Acad. Sci. USA. 74:4025.

15. Eriksen, N., and E. P. Benditt. 1980. Isolation and characterization of the amyloid-related apoprotein (SAA) from human high density lipoprotein. Proc. Natl. Acad. Sci. USA. 77:6860.

16. Skogen, B., A. L. Borresen, J. B. Natvig, K. Berg, and T. E. Michaelsen. 1979. High density lipoprotein as carrier for amyloid related protein SAA in rabbit serum. Scand. J. Immunol. 10:39.

17. Benditt, E. P., N. Eriksen, and R. H. Hanson. 1979. Amyloid protein SAA is an apoprotein of mouse plasma high density lipoprotein. Proc. Natl. Acad. Sci. USA. 76:4049.

18. Gorevic, P. D., Y. Levo, B. Frangione, and E. C. Franklin. 1978. Polymorphism of tissue and serum amyloid A (AA and SAA) protein in the mouse. J. Immunol. 121:138.

19. Scanu, A. M. 1966. Forms of human serum high density lipoprotein protein. J. Lipid
20. Scanu, A. M., and C. Edelstein. 1971. Solubility in aqueous solution of ethanol of small molecular weight peptides of serum very low density and high density lipoproteins: relevance to the recovery problem during delipidation of serum lipoproteins. *Anal. Biochem.* 44:576.

21. Swank, R. T., and K. D. Munkres. 1971. Molecular weight analysis of oligopeptides by electrophoresis in polyacrylamide gel with sodium dodecyl sulfate. *Anal. Biochem.* 39:462.

22. Towbin, H., T. Staehelin, and T. Gordon. 1979. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheet: procedure and some application. *Proc. Natl. Acad. Sci. USA.* 76:4350.

23. Davis, B. J. 1964. Disc electrophoresis-II. Method and application to human serum protein. *Ann. NY Acad. Sci.* 121:404.

24. Glenner, G. G. 1980. Amyloid deposits and amyloidosis, the β-fibrilloses. *N. Engl. J. Med.* 302:1283.

25. Isersky, C., D. L. Page, M. Harada, and G. G. Glenner. 1972. Immunochemical cross-reactions of human amyloid proteins with immunoglobulin light polypeptide chains. *J. Immunol.* 108:486.

26. Glenner, G. G., D. Ein, E. D. Eanes, H. A. Bladen, W. Terry, and D. L. Page. 1971. Creation of “Amyloid” fibrils from Bence Jones proteins in vitro. *Science (Wash. DC).* 174:712.

27. Scheinberg, M. A., E. S. Cathcart, J. W. Eastcott, M. Skinner, M. Benson, T. Shirahama, and M. Benditt. 1976. The SJL/J mouse: a new model for spontaneous age-associated amyloidosis. I. Morphologic and immunochromic aspects. *Lab. Invest.* 35:47.

28. Thung, P. J. 1957. Senile amyloidosis in mice. *Gerontologia (Basel).* 1:259.

29. Chai, C. K. 1976. Reticular cell hyperplasia and amyloidosis in a line of mice with low leukocyte counts. *Am. J. Pathol.* 85:49.

30. McAdam, K. P. W. J., and J. D. Sipe. 1976. Murine model for human secondary amyloidosis: genetic variability of the acute-phase serum protein SAA response to endotoxins and casein. *J. Exp. Med.* 144:1121.

31. Swaney, J. B., H. Reese, and H. A. Eder. 1974. Polypeptide composition of rat high density lipoprotein: characterization by SDS gel electrophoresis. *Biochem. Biophys. Res. Commun.* 59:513.

32. Hoffman, T. S., and E. P. Benditt. 1982. Changes in high density lipoprotein content following endotoxin administration in the mouse: formation of serum amyloid protein rich subfractions. *J. Biol. Chem.* 257:10510.

33. Lavie, G., D. Zucker-Franklin, and E. C. Franklin. 1978. Degradation of serum amyloid A protein by surface-associated enzymes of human blood monocytes. *J. Exp. Med.* 148:1020.

34. Lavie, G., D. Zucker-Franklin, and E. C. Franklin. 1980. Elastase type proteases on the surface of human blood monocyte: possible role in amyloid formation. *J. Immunol.* 125:175.

35. Silverman, S. L., E. S. Cathcart, M. Skinner, A. S. Cohen, and L. Burnett. 1980. A pathogenetic role for polymorphonuclear leukocytes in the synthesis and degradation of SAA protein. In *Amyloid and Amyloidosis.* G. G. Glenner, P. P. Costa, and A. F. Freitas, editors. Excerpta Medica. Amsterdam-Oxford-Princeton. p. 420.

36. Skogen, B., L. Thorsteinsson, and J. B. Natvig. 1980. Degradation of protein SAA to an AA-like fragment by enzymes of monocytic origin. *Scand. J. Immunol.* 11:533.

37. Skogen, B., J. B. Natvig, A. L. Borrensen, and K. Berg. 1980. Degradation of amyloid-related serum protein SAA by a component present in rabbit and human
38. Skogen, B., and J. B. Natvig. 1981. Degradation of amyloid protein by different serine protease. Scand. J. Immunol. 14:389.
39. Baussmann, L. L., P. N. Herbert, and K. P. W. J. McAdam. 1980. Heterogeneity of human serum amyloid A proteins. J. Exp. Med. 152:641.
40. Marhang, G., and G. Husby. 1981. Characterization of human amyloid-related protein SAA as a polymorphic protein: association with albumin and prealbumin in serum. Clin. Exp. Immunol. 45:97.
41. Westermark, P. 1982. The heterogeneity of protein AA in secondary (reactive) systemic amyloidosis. Biochim. Biophys. Acta. 701:19.
42. Herbert, P. N., H. G. Windmueller, T. P. Bersot, and R. S. Shulman. 1974. Characterization of the rat apolipoproteins. I. The low molecular weight protein of rat plasma high density lipoproteins. J. Biol. Chem. 249:5718.
43. La Rosa, J. C., R. I. Levy, D. N. Herbert, S. E. Lux, and D. S. Fredrickson. 1970. As specific apoprotein activator for lipoprotein lipase. Biochim. Biophys. Res. Commun. 41:57.
44. Brown, W. V., and M. L. Baginsky. 1972. Inhibition of lipoprotein lipase by apoprotein of human very low density lipoprotein. Biochem. Biophys. Res. Commun. 46:375.
45. Jackson, R. L., H. N. Baker, E. B. Gilliam, and A. M. Gotto, Jr. 1977. Primary structure of very low density apolipoprotein C-II of human plasma. Proc. Natl. Acad. Sci. USA. 74:1942.