# A Serum Component Related to Nonimmunoglobulin Amyloid Protein AS, a Possible Precursor of the Fibrils

G. Husby and J. B. Natvig

*From the Institute of Immunology and Rheumatology, Rikshospitalet University Hospital, Oslo, Norway*

Abstract A nonimmunoglobulin protein with the molecular weight of 9,145 (protein AS) has been shown to be a principal component of the amyloid fibrils in different clinical types of amyloidosis. A protein component, antigenically closely related to protein AS, was detected in human sera. The protein AS-related component (protein ASC) was found in the sera of many groups of patients, including 48 out of 55 patients with various clinical types of amyloidosis. No structural relationship of protein ASC to the plasma component of amyloid was found. Protein ASC was also present with high frequency in the serum of diseases known to be frequently complicated by amyloidosis. In some cases, ASC was found in the sera of patients 2–3 yr before the diagnosis of amyloidosis was established. Protein ASC was also frequently found in hypogammaglobulinemia. Among normal individuals, protein ASC was seldom detected in the serum by our techniques, but there was a noticeable increase with age and during pregnancy. Moreover, a more sensitive technique, immunoelectro-osmophoresis, revealed protein ASC in a higher number of sera from both patients and normal controls. Thus protein ASC was suggested to be a normal serum constituent, usually present only in minor quantities. Under certain conditions, protein ASC increases considerably in serum, and may in such instances act as a precursor for the deposition of amyloid fibrils in the tissues.

## INTRODUCTION

Two main components of amyloid substances are known. One is the plasma component, called the P-component,[1] or periodic rods (1, 2). This P-component has been found to be antigenically identical to an α-globulin present in normal human plasma (3).

The second main component is the amyloid fibril or F-component which is regarded as a unique component of all amyloid substances. Recently developed procedures for the isolation and solubilization of amyloid fibrils have enabled the chemical and antigenic characterization of the protein components of amyloid fibrils (4–6). We reported previously that a nonimmunoglobulin low molecular weight protein AS (amyloid subunit) can be isolated from amyloid fibril preparations derived from patients with different clinical types of amyloidosis involving primary and myeloma/macroglobulinemia-associated as well as secondary amyloidosis (7–9). Protein AS was thought to be an essential part of amyloid fibrils in most amyloid substances. Recently, a protein in serum (we call protein ASC), antigenically related to protein AS, was described independently by Levin, Pras, and Franklin (10) and by Husby, Michaelsen, Sletten, Natvig, and Höst (11, 12).

In the present communication, further studies on protein ASC in serum will be presented. The component is a protein with electrophoretic mobility in the α–β-region and a molecular weight of about 100,000. The protein ASC was found in nearly all of the sera from patients with amyloidosis, but also with high frequency in patients with other diseases, particularly those prone to be complicated by amyloidosis. No antigenic cross-reaction of protein ASC with the P-component was seen. In contrast to the P-component and its antigenically related α-globulin found in normal serum, the serum correlate to the amyloid fibril protein AS was detected in only 3% of the normal control sera with the immunodiffusion techniques used. However, there was an increase of protein ASC in the serum of individuals advanced in age and during pregnancy. More sensitive techniques revealed smaller amounts of protein ASC in the serum of several nor-

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[1] *Abbreviations used in this paper:* DAM, degraded amyloid fibrils; F-component, amyloid fibril; P-component, plasma component; protein AS, amyloid subunit; protein ASC, protein AS-related component.

mal individuals, indicating that protein ASC is a normal serum constituent, but usually present in very small quantities. This study also includes quantitation experiments demonstrating a marked variability in the concentration of protein ASC in serum among individual patients. The protein AS-related serum component is supposed to be a circulating, soluble precursor of the amyloid fibril. Its detection and quantitation in patients' sera may be a valuable tool for the detection of precursor state and for the early diagnosis of amyloidosis.

## METHODS

*Preparation of amyloid fibrils, protein AS, and P-component.* Amyloid fibrils were isolated using extraction with distilled water (4, 5) as described previously (8, 13). In this study, preparations derived from two patients with amyloidosis associated with juvenile rheumatoid arthritis (T. H., amyloid I) and Waldenström's macroglobulinemia (J. B., amyloid XVIII) were used (9, 13). For separation of protein AS, the "native" amyloid fibrils (10–20 mg/ml) were treated with 6 M guanidine-HCl in 0.55 M Tris-HCl buffer pH 8.5 containing 0.1 M dithiothreitol, or with 0.1 N NaOH for 2–24 h before gel filtration on a 3.2 × 96.5-cm Sephadex G-100 column. Elution effluent was 5 M guanidine-HCl in 1 N acetic acid. Elution rate was 8.1 ml/h. For further purification of protein AS, gel filtration on a 0.9 × 52-cm Sephadex G-25 medium equilibrated with 10% formic acid was used (8). The P-component of amyloid was prepared according to Cathcart, Wollheim, and Cohen (3).

*Sera, serum proteins, and tissues.* Sera from 704 individuals were tested for the presence of protein ASC (see Tables II and III). The sera were obtained mostly from in-patients admitted to Rikshospitalet University Hospital, Oslo Sanitetsforenings Rheumatism Hospital, or the Norwegian Radium Hospital. Some sera were kindly supplied by Doctors H. G. Kunkel, A. I. Pick, and L. Å. Hansson. 55 sera were from patients with known amyloidosis (Table III). Some patients could be followed longitudinally for up to 4 yr, since sera from different bleedings had been stored at −20°C, and in six cases (all with juvenile rheumatoid arthritis), amyloidosis developed during the observation period.

Tissue specimens obtained at biopsy or autopsy from patients with established or suspected amyloidosis were examined for amyloid deposits by polarization microscopy after staining with Congo red (14, 15).

*Controls.* Controls were: cord sera from 18 new-born babies and 18 matching sera from their mothers, taken simultaneously at the time of delivery; sera from 34 healthy children matching in age and sex with 34 of the patients with juvenile rheumatoid arthritis, kindly supplied by Dr. H. M. Høyeraal; sera from 70 normal blood donors; and finally sera from 20 apparently healthy individuals between the ages of 80 and 95 yr (Table II).

*Serum proteins.* 120 isolated myeloma and macroglobulinemia proteins were included in addition to 30 κ- and λ-Bence-Jones proteins. Concentrated urine from 10 patients with myelomatosis, and 25 joint fluids from patients with rheumatoid arthritis were also tested.

*Antisera.* Antisera to amyloids I and XVIII were raised in rabbits as previously described (9, 13), using crude alkali-treated amyloid (DAM) as immunogen. In particular anti-DAM XVIII reacted strongly with purified protein AS and was chosen for the search of protein ASC in human sera. Before use, the antiserum was absorbed with three times its volume of pooled normal human sera. After absorption the antiserum was specific for protein AS in the test systems used. Antisera to human serum and to $F(ab')_2$ were also raised in rabbits.

*Immunological tests.* Double immunodiffusion in 1% agarose was used to test the sera, urines, joint fluids, and serum proteins for protein ASC. Crude DAM and purified protein AS from amyloids I and XVIII served as control antigens. The sera were screened undiluted, and in some cases twofold titration was performed. The plates were kept in a moist chamber at room temperature for 48 h before examination. Immunoelectrophoresis was performed using 1% agarose in barbital buffer pH 8.6 and ionic strength 0.025.

To increase the sensitivity of the immunodiffusion test system, immunoelectro-osmophoresis (16) in 1% agarose was performed using barbital buffer pH 8.6, ionic strength 0.05. The voltage was 2.5–2.8 V/cm and running time 55 min at room temperature. Sera and antisera were applied in wells 3 mm in diameter, the thickness of the gel being 1 mm and the center to center distance of the wells containing test serum and antiserum being 12 mm.

Single radial immunodiffusion (17) in 1% agarose with barbital buffer pH 8.6, ionic strength 0.025, was utilized to quantitate protein ASC. Since purified protein ASC was not available, a strongly positive serum, obtained from a patient (J. O.) with medullary carcinoma of the thyroid, was chosen for the standard reagent. The agarose gel contained absorbed anti-DAM XVIII at a final dilution of 1 in 10. The plates were photographed after incubation for 24 h at room temperature.

*Physical and chemical treatment of protein ASC-containing sera.* Selected sera reacting strongly with anti-protein AS (anti-DAM XVIII) were subjected to the following procedures before testing in double immunodiffusion: (*a*) Dialysis against various buffers with pH 2 and pH 3 (glysine-HCl), pH 4 and pH 5 (acetate buffer), and pH 11 (glysine-NaOH). (*b*) Treatment with sodium hydroxide or with hydrochloric acid at a final concentration of 0.1 N. (*c*) Boiling for 10 min, resulting in precipitation of most of the serum. A small supernate was tested as such, the sediment being treated with 0.1 N NaOH for 1 h before testing.

*Gel filtration.* Sera with high amounts of protein ASC were gel filtered on a column of Sephadex G-150 equilibrated with 0.1 M Tris-HCl buffer pH 7.6. The Kav values were estimated according to Laurent and Killander (18).

## RESULTS

*Protein ASC in human sera.* Many sera from patients with amyloidosis gave a single precipitation line when tested in double immunodiffusion against specifically absorbed antiserum to degraded amyloid (anti-DAM XVIII) which also reacted strongly with protein AS. The line fused with that obtained with autologous or homologous protein AS, demonstrating antigenic identity with the amyloid fibril subunit (Fig. 1). A precipitation line of identity was also obtained when crude DAM was applied in a neighboring well of a posi-

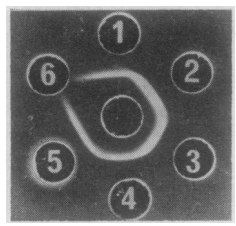


FIGURE 1 Detection of protein ASC in serum by double immunodiffusion. Anti-DAM XVIII in central well tested against (wells 1, 4) purified protein AS; (well 2) serum from patient J. O. with medullary carcinoma of the thyroid; (well 3) serum from patient J. B. with Waldenström's macroglobulinemia-associated amyloidosis; (well 5) serum from patient L. H. with amyloidosis secondary to rheumatoid arthritis; and (well 6) a negative control serum.

tive serum. Furthermore, the precipitation reaction between anti-DAM XVIII and the positive sera was totally blocked by adding purified protein AS or crude degraded amyloid (DAM) to the antiserum. Also absorption of anti-DAM XVIII with positive sera blocked the precipitation line corresponding to protein AS. Thus, both by direct precipitation and by absorption experiments there was antigenic identity between the serum component and protein AS. No reaction was seen when anti-DAM was tested against isolated immunoglobulins or immunoglobulin fragments, chains of different classes and subclasses, or with purified P-component derived from primary, macroglobulinemia-associated or secondary amyloidosis.

Preliminary titration experiments showed a considerable variability in the strength of the precipitation reactions in double immunodiffusion among the various sera, giving precipitation titers varying from 1 to 64. To further characterize protein ASC in individual sera, a quantitative assay with radial immunodiffusion was attempted. Linear curves with equal slope were obtained when the diameters of the precipitates were plotted against varying serum dilutions on a semilogarithmic paper (Fig. 2). A positive control serum (J. O.) was used as a standard, since protein ASC has not been isolated from serum in a pure form and since the purified antigenically identical protein AS has a much lower molecular weight. However, when other positive sera were correlated with this standard, reproducible results were obtained. Using this technique a pronounced quantitative variability was observed among the various sera tested (Table I), which correlated well with the precipitation titers of the same sera obtained by double immunodiffusion.

The presence of protein ASC was also tested for in various control sera and different age groups. Protein ASC was not detected in cord sera or in healthy children between 2 and 15 yr of age in quantities necessary to give positive results in double immunodiffusion. 2 out of the 70 normal adult blood donors were positive, and so were 50% of the aged control persons. 10 out of the 18 sera from pregnant women (56%) matching the cord sera were also positive (Table II).

However, when 50 of the negative sera from the normal blood donors and the 18 negative cord sera were tested using immunoelectro-osmophoresis, a precipitation line was obtained with 5 of the former and one of the latter sera (Fig. 3). Thus by this more sensitive immunologic technique, 10% of the normal sera were positive. A certain increase in the frequency of sera containing protein ASC was also observed among the various groups of patients when immuno-

TABLE I

*Quantitation of Protein ASC. Comparison of Radial Immunodiffusion Units* and Precipitation Titers‡*

| Patient | Radial immunodiffusion | Precipitation titers |
|---|---|---|
| J. O. | 100 | 64 |
| E. K. | 94 | 32 |
| U. O. | 89 | 16 |
| A. H. | 76 | 16 |
| K. C. | 73 | 16 |
| S. V. | 73 | 16 |
| A. G. | 71 | 4 |
| H. H. | 69 | 8 |
| M. N. | 65 | 8 |
| J. B. | 38 | 2 |
| J. N. | 26 | 1 |

* One unit is 1/100 of the concentration of ASC in the standard serum (J. O.).

‡ Reciprocal of the highest dilution of serum giving a visible precipitation line was used as titer.

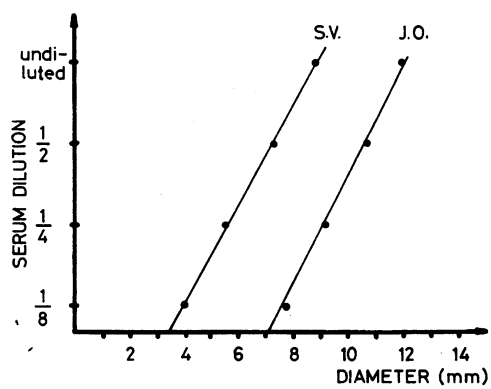


FIGURE 2 Quantitation of protein ASC in serum by radial immunodiffusion. The serum dilutions and the diameters of the precipitates are plotted on a semilogarithmic paper revealing linear quantitation curves with equal slope.

electro-osmophoresis was used for testing. The lower positivity of 3% in normal sera obtained with the somewhat less sensitive immunodiffusion test appeared to be preferable for the distinction between the actual groups of patients and the controls. Double immunodiffusion was therefore selected as a standard test for detection of protein ASC in serum in this study.

The positive sera came from various groups of patients, and were not only correlated with established amyloid disease, but also with diseases which are often complicated by amyloidosis. Many of these patients had been examined for amyloidosis by biopsies or at autopsy, but no evidence for established amyloidosis was found. In Table III, the frequencies of protein ASC in patients with and without amyloidosis are compared.

*Amyloidosis.* Protein ASC was found in the serum of 41 out of 45 patients with generalized amyloidosis including patients with primary and myeloma/macroglobulinemia-associated as well as secondary amyloidosis. Protein ASC was detected in 7 out of 10 patients with local amyloidosis, i.e., 1 patient with primary amyloidosis of the lung and 6 patients with amyloid-containing medullary carcinoma of the thyroid (Table III).

*Lymphoproliferative and other malignant disorders.* Protein ASC was found in the serum of 55% of the patients with myelomatosis and 80–90% of the patients with Waldenström's macroglobulinemia tested. The frequency of protein ASC was also high (66%) in the serum of Hodgkin's disease, and 5 of the 13 sera from patients with lymphomas were positive (38%). In serum from patients with other malignant tumors, protein ASC was also a rather frequent finding (Table III). In addition, 1 out of 10 urines from patients with myelomatosis and positive test for ASC in serum was positive. This urine was from a patient with myelomatosis and kidney damage with severe proteinuria, and the finding of protein ASC in the urine was probably due to leakage from serum.

*Rheumatic diseases.* Protein ASC was detected in more than half of the sera from patients with rheumatoid arthritis (Table III); 8 out of 17 joint fluids obtained from these patients were positive. Protein ASC was, however, found in higher concentrations in serum than in the joint fluid in all the eight cases. The protein ASC detected in joint fluids therefore appeared to be derived from the serum. The frequency of protein ASC in serum was lower among patients with disseminated lupus erythematosus (23%) than those with rheumatoid arthritis (Table III).

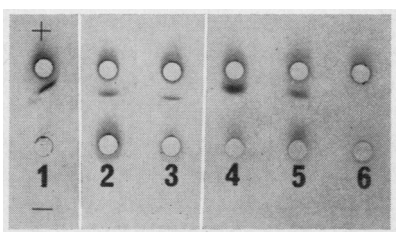


FIGURE 3 Immunoelectro-osmophoresis of (well 1) DAM I; (well 2) a control serum in which no protein ASC was detectable by double immunodiffusion showing a precipitation line; (wells 3–5) sera from three patients with amyloidosis; and (well 6) a negative control serum. Anti-DAM XVIII closer to the anode.

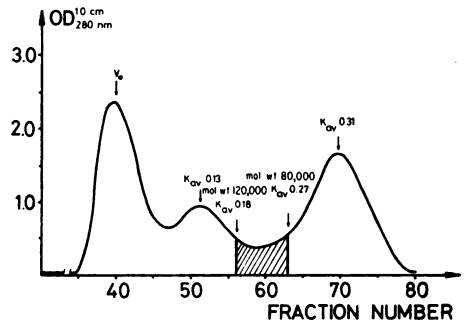


FIGURE 4 Gel filtration of serum from a patient (E. S.) with amyloidosis associated with Waldenström's macroglobulinemia on a 3.2 × 92.3-cm Sephadex G-150 column with $V_o$ = 236 ml. Elution rate 17.7 ml/h. Fraction volume 5.9 ml. Elution buffer 0.1 M Tris-HCl − 0.2 M NaCl − 2 mM EDTA containing 0.02% sodium azide pH 7.6 (temperature 23°C). Material reacting in double immunodiffusion with anti-DAM XVIII eluted at Kav 0.18–0.27, corresponding to mol wt 80,000–120,000.

*Liver diseases.* Protein ASC was not detected in the serum of any of the 18 cases with acute hepatitis. In chronic hepatitis and cirrhosis of the liver as well as in hepatoma, however, protein ASC was frequently found in the serum (30–40%, Table III).

*Immunodeficiencies.* The patients with immunodeficiencies were mostly children, and protein ASC was detected in the serum of about 50% of the patients,

TABLE II
*Protein ASC in Control Sera*

| Sera | No. positive/ no. tested | Positive |
|---|---|---|
| | | % |
| Aged, otherwise healthy individuals* | 10/20 | 50 |
| Pregnant women‡ | 10/18 | 56 |
| New-born babies (cord sera)‡ | 0/18 | 0 |
| Healthy children | 0/34 | 0 |
| Adults (normal blood donors) | 2/70 | 3 |

* Age: 80–95 yr.
‡ The cord sera matched the sera of the pregnant women.

TABLE III

*Protein ASC in Serum in Various Groups of Disease*

| Sera | Amyloidosis | | Patients with known amyloidosis excluded | | Total | |
|---|---|---|---|---|---|---|
| | No. positive/ no. tested | Positive | No. positive/ no. tested | Positive | No. positive/ no. tested | Positive |
| | | % | | % | | % |
| Primary amyloidosis | | | | | | |
| Generalized | 6/7 | —* | — | — | 6/7 | — |
| Local amyloidosis of the lung | 1/1 | — | — | — | 1/1 | — |
| Lymphoproliferative disorders | | | | | | |
| Myelomatosis | 3/4 | — | 66/121 | 55 | 69/125 | 55 |
| Waldenström's macroglobulinemia | 3/3 | — | 9/11 | 82 | 12/14 | 86 |
| Malignant lymphoma | — | — | 5/13 | 38 | 5/13 | 38 |
| Hodgkin's disease | — | — | 23/35 | 66 | 23/35 | 66 |
| Other malignant diseases | | | | | | |
| Medullary carcinoma of the thyroid | 6/9 | — | — | — | 6/9 | — |
| Hepatoma | — | — | 5/8 | — | 5/8 | — |
| Other malignant tumors | 2/2 | — | 8/22 | 36 | 10/24 | 42 |
| Rheumatic diseases | | | | | | |
| Rheumatoid arthritis‡ | 15/16 | 94 | 21/41 | 51 | 36/57 | 63 |
| Juvenile rheumatoid arthritis | 8/8 | — | 16/34 | 47 | 24/42 | 57 |
| Systemic lupus erythematosus | — | — | 6/26 | 23 | 6/26 | 23 |
| Liver diseases | | | | | | |
| Acute hepatitis | — | — | 0/18 | 0 | 0/18 | 0 |
| Chronic hepatitis | — | — | 12/41 | 29 | 12/41 | 29 |
| Primary biliary cirrhosis | — | — | 8/23 | 35 | 8/23 | 35 |
| Alcoholic and cryptogenetic cirrhosis | 1/1 | — | 15/41 | 37 | 16/42 | 39 |
| Other diseases | | | | | | |
| Ulcerative colitis | 1/2 | — | 3/3 | — | 4/5 | — |
| Cystic fibrosis of the pancreas | 1/1 | — | — | — | 1/1 | — |
| Familial Mediterranean Fever | 1/1 | — | — | — | 1/1 | — |
| Immunodeficiencies | | | | | | |
| Hypogammaglobulinemia | — | — | 17/33 | 52 | 17/33 | 52 |
| Other immunodeficiencies | — | — | 8/19 | 42 | 8/19 | 42 |
| Total | 48/55 | 87 | 222/489 | 45 | 270/544 | 50 |

* Percentage not given for groups less than 10.

‡ Including patients with ankylosing spondylitis.

including those with severe hypogammaglobulinemia (B-cell defects) as well as in other types of immunodeficiency (T-cell defects) (Table III). It was observed that ASC was present in the serum of patients where radial immunodiffusion showed extremely small amounts or absence of immunoglobulin.

*Additional studies on the relationship of protein ASC to amyloidosis.* 8 of 34 patients with juvenile rheumatoid arthritis and protein ASC in serum had established amyloidosis. In six of these patients the component was detected in serum from 1 to nearly 3 yr before amyloidosis was diagnosed by biopsies (Table IV). In four sera protein ASC was present continuously. However, in two cases the test for protein ASC became negative in a period during deposition of amyloid fibrils in the tissues (Table IV). In other patients the amyloidosis progressed in spite of a temporary disappearance of protein ASC, and in most cases ASC reappeared in serum after having been undetectable for shorter or longer periods.

*Physical and chemical characterization of protein ASC.* Gel filtration of positive sera showed that the molecular weight of protein ASC was approximately 100,000 (Fig. 4). The electrophoretic mobility revealed by immunoelectrophoresis corresponded to the $\alpha$–$\beta$-globulin region. As seen for the protein AS, the pro-

TABLE IV

*Relation of Protein ASC in Serum to Amyloidosis in Patients with Juvenile Rheumatoid Arthritis*

| Patient | Test specimen | Time of testing*/result | | | | | |
|---|---|---|---|---|---|---|---|
| A. H. | | 2/71 | 6/71 | 11/71 | 2/72 | 4/72 | 9/72 |
| | Serum | − | + | − | + | + | + |
| | Biopsy‡ | ND | ND | ND | − | − | + |
| M. L. | | 1/72 | 5/72 | 8/72 | 2/73 | | |
| | Serum | + | + | + | + | | |
| | Biopsy | ND | ND | + | ND | | |
| B. B. | | 6/69 | 10/69 | 2/71 | 3/72 | | |
| | Serum | − | + | + | + | | |
| | Biopsy | ND | ND | ND | + | | |
| K. N. | | 11/69 | 8/70 | 3/72 | 8/72 | 6/73 | |
| | Serum | + | + | + | − | + | |
| | Biopsy | ND | − | ND | + | ND | |
| A. B. | | 5/69 | 1/72 | | | | |
| | Serum | + | + | | | | |
| | Biopsy | ND | + | | | | |
| T. S. | | 4/69 | 12/70 | 12/71 | 2/72 | 3/72 | |
| | Serum | + | + | + | + | − | |
| | Biopsy | ND | ND | ND | + | ND | |

* Month/year.

‡ Examined for amyloid deposits by polarization microscopy after staining with Congo red.

tein ASC was very resistant. Sera containing ASC were still precipitated by anti-DAM XVIII after dialysis against buffers with pH values from 3 to 11, and also after adding NaOH to a final normality of 0.1. After boiling of the positive sera, full antigenic activity with anti-DAM XVIII was still observed in the supernate, and after treatment with 0.1 NaOH, the sediment was also precipitated by the antiserum. The supernate obtained by boiling showed two weak precipitation lines when tested against an antiserum to normal human serum. However, these lines showed a reaction of nonidentity with that obtained with protein ASC and anti-DAM XVIII. No precipitation was observed when the supernate was tested against a polyspecific antiserum to human immunoglobulins (anti-F(ab′)$_2$).

## DISCUSSION

The nonimmunoglobulin amyloid protein AS with the reported molecular weight of 7,000–9,000 daltons (10, 19, 20) corresponding to 76 amino acids (20) is shown to be an integral part of the amyloid substance in secondary amyloidosis (8, 10, 19). In addition, we have recently demonstrated the presence of protein AS in amyloid preparations derived from patients with primary amyloidosis as well as amyloidosis associated with myelomatosis or macroglobulinemia (9, 12). It has been shown that protein AS can form fibrils (8, 21, 22). Protein AS thus seems to be an essential component of the fibrillar elements of amyloid substances.

Another main component which has been found in amyloid fibrils consists of variable region fragments of monoclonal light chains (23), which under certain circumstances also can form fibrils in vitro (24). In addition, studies on a high molecular weight protein component eluted in the void volume on Sephadex G-100 (8) from degraded amyloid preparations have indicated that this component consists of light chains (8, 25).

The detection of a component antigenically related to protein AS in human sera from all the clinical types of amyloidosis investigated, and in diseases often complicated by amyloidosis, raises the question whether protein ASC is a soluble, circulating precursor of amyloid fibrils. The molecular weight of protein ASC (100,000) is far higher than that of protein AS (9, 145) as determined by the total amino acid sequence (20). Levin et al. (10) suggested that protein AS is a fragment of a larger protein, since the N-terminal part of the protein in their experiments seemed to be heterogenous. We have, however, found only one amino

acid, arginine, in the N-terminal position of protein AS of a secondary amyloid previously described (8, 20). However, this does not rule out the hypothesis that protein AS is a fragment, a degradation product or a subunit of protein ASC. Protein ASC was found in serum and protein AS itself was found in the amyloid of patients with primary as well as myeloma/macroglobulinemia-associated and secondary amyloidosis. This indicates an important relationship between protein ASC and protein AS in the pathogenesis of amyloidosis.

No antigenic relationship of protein ASC with the P-component of amyloid was found, thus confirming the recent observations of Levin et al. (10). This was not unexpected, since the degraded amyloid fibril (DAM) prepared according to Pras and co-workers Schubert, Zucker-Franklin, Rimon, and Franklin (4, 5) was used as the immunogen for the preparation of the anti-DAM antisera in the present study. DAM has previously been shown to have no antigenic relationship with the P-component (26). Furthermore, recent amino-terminal sequence studies of the P-component (27) revealed no similarities with protein AS in the primary structure. The anti-DAM antisera were heavily absorbed with pooled normal sera which have been shown to contain the P-component in equal amounts with sera from patients with amyloidosis (28). This absorption should further prevent reactivity of anti-DAM antisera with the P-component or related materials.

In addition to generalized amyloidosis, serum ASC was also found in the serum of patients with local types of amyloidosis, suggesting that protein AS-like material has a high tendency to localize to certain areas in these cases. Pearse, Ewen, and Polak (29) suggested that the amyloid fibrils occurring in medullary carcinoma of the thyroid had a special chemical composition significantly different from other types of amyloids. In spite of this, protein ASC was detected in the serum of many of these patients, indicating the presence of protein AS also in this type of amyloid substance. Further studies on the chemical composition of amyloid fibrils derived from endocrine tumors will throw more light on these questions.

Protein ASC was detected in only 3% of the normal blood donors by double immunodiffusion. However, immunoelectro-osmophoresis, which is a more sensitive technique, revealed a higher number of positive sera among these control persons (10%). This suggests that protein ASC is a serum constituent which is normally only present in minor quantities (10). However, under certain disease conditions, often prone to amyloidosis, the concentration in serum of protein ASC rises and may induce the formation of amyloid fibrils. Whether this is due to increased synthesis, reduced catabolism, or more local factors is not known, because the synthesis and biology of this protein is still obscure.

The high frequency of protein ASC in the serum of patients without established amyloid disease appears to be related to diseases which are often complicated by amyloidosis. This is so for the lymphoproliferative and other malignant disorders, and the rheumatic and allied diseases. It is therefore tempting to suggest that these patients with a high concentration of protein ASC in serum have amyloidosis at very early stages not detectable by conventional diagnostic procedures, or represent groups more likely to get amyloidosis. It was shown in this study that protein ASC was detected in serum years before the diagnosis of amyloidosis was established by biopsies. This finding supports the hypothesis that protein ASC is a circulating amyloid fibril precursor. Detection and quantitation of protein ASC in serum may therefore appear to be an important diagnostic test in amyloidosis. Since amyloidosis is also related to immunoglobulins or immunoglobulin fragments (23, 30), the demonstration of ASC in patients with hypogammaglobulinemia was of particular interest. Amyloidosis among such patients has, indeed, been reported (31, 32), showing that excess production of immunoglobulins or their fragments is not necessary for the development of amyloid substances.

The number of positive sera in chronic hepatitis being higher than that of acute hepatitis shows that protein ASC in serum increases when the condition becomes chronic.

The increase of protein ASC with age was not unexpected, and these findings are in accordance with the general increase with age of amyloidosis. However, there was an unexpectedly high number of sera containing ASC from pregnant women. This increase did not appear to be stable after delivery. The fact that all the matching cord sera were negative shows that protein ASC does not pass the placental barrier. The protein ASC detected in low concentration in one cord serum using immunoelectro-osmophoresis was possibly due to contamination with the strongly positive corresponding maternal serum.

There seemed to be a marked quantitative variability of the amount of protein ASC in serum among different individuals. Further studies are in progress in our laboratory to develop a more exact method for the quantitation of protein ASC, which may enable us to further correlate the serum concentration of protein ASC to the development of amyloidosis. It is hoped that this may lead to reliable methods for the diagnosis of manifest amyloidosis and possibly also of the precursor stage of this disease. This may open up the possibility for treatment or prophylaxis before manifest tissue damage has occurred.

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