Variation with Age and Disease of an Amyloid A Protein-Related Serum Component

C. Julian Rosenthal and Edward C. Franklin

From the Irvington House Institute, Rheumatic Disease Study Group, Department of Medicine, New York University Medical Center, New York 10016

Abstract Using the radioactively-labeled alkaline-degraded acid-soluble fraction of amyloid ([^{125}I]DAA), we developed a radioimmunoassay for the previously described amyloid-related component of the human serum (SAA). Screening the sera of 228 normal individuals and of 297 patients with a variety of illnesses, we found that SAA is a component of all human sera, including cord blood (mean 94±57 ng/ml). The concentration of this component increases significantly with the aging process, reaching very high levels in the eighth and nine decades. It is also elevated in all cases of amyloidosis (except for those associated with nephrotic syndrome) as well as in many patients with myeloma, macroglobulinemia, lymphoma, carcinoma, rheumatoid arthritis, and tuberculosis. A marked increase was noted in the early stages of a variety of acute inflammatory and infectious states with a return to normal levels paralleling clinical improvement and faster than the erythrocyte sedimentation rate. The possible implications of this component in the genesis of amyloid and in the immune process are discussed.

INTRODUCTION

Recent studies from a number of laboratories have demonstrated the existence of at least two major types of amyloid fibril proteins. One, found mainly in patients with primary amyloidosis and amyloidosis associated with multiple myeloma, consists primarily of fragments of immunoglobulin light chains (1, 2). The other, present in the fibrils of patients with secondary amyloidosis and some familial forms of amyloidosis, as well as in the amyloid in three experimental animals—monkey (3), duck (4), and guinea pig (5)—has as a major component a unique protein known as amyloid A (AA) [1] protein (6). This material was previously called A component by Benditt and Eriksen (7), amyloid of unknown origin by Ein, Kimura, Terry, Magnotta, and Glenner (8), acid-soluble fraction by Pras and Reshef (9), Franklin, Pras, Levin, and Frangione (10), and Levin, Franklin, Frangione, and Pras (11), and amyloid subunit by Husby, Sletten, Michaelson, and Natvig (12).

As described by Pras and Reshef (8) and Levin et al. (11), AA is a polypeptide with a mol wt of 8,500 daltons, which is insoluble in water, but soluble in solutions with low pH. Amino acid sequence studies (3, 8, 10, 11) of human and primate AA showed heterogeneity at the amino terminus (11) and in some instances also at the carboxy terminus (8) and have raised the possibility that it is the product of enzymatic digestion of a precursor molecule. Chemical (8, 10, 12) and immunologic studies (11, 12) showed AA extracted from different patients to be virtually identical and provided clear evidence that this is a unique protein with no homology to any known protein.

The finding (13, 15), with relatively insensitive techniques, of an antigenically related α1 globulin component having a mol wt of about 100,000 in the sera of few normal individuals and in a majority of patients suffering from chronic diseases such as tuberculosis, rheumatoid arthritis, Hodgkins' disease, etc., has raised the possibility that this may be the putative precursor molecule and that more detailed investigation of its

Presented in part at the meeting of the Association of American Physicians, Atlantic City, N. J., 7 May 1974.

Dr. Rosenthal's present address is the Department of Medicine, Downstate Medical Center, State University of New York, Brooklyn, N. Y. 11203.

Received for publication 16 August 1974 and in revised form 16 December 1974.

[1] *Abbreviations used in this paper:* AA, amyloid A; BGG, bovine γ-globulin; BSA, bovine serum albumin; DAA, degraded amyloid A; SAA, Amyloid A-related serum component.

incidence, nature, and possible origin may shed some light on the pathogenesis of amyloidosis. This component will henceforth be called serum amyloid A-related protein (SAA).

To study in greater detail the distribution of this component in the normal and diseased population and its possible relation to the aging process, which is frequently accompanied by amyloid deposits in the brain and other tissues (16, 17), and to obtain additional information on the physicochemical properties of this SAA, we have developed a radioimmunoassay to determine its concentration. This report describes the immunoassay and the results of studies on sera from a large group of normal individuals of varying ages and patients with a variety of pathologic states.

METHODS

Antisera against AA. AA was extracted from the water-soluble amyloid of a patient with familial Mediterranean fever by the technique of Pras and Reshef (9).

To solubilize the protein in physiologic solvents, AA was degraded (DAA) with 0.1 N NaOH for 1 h at 37°C and neutralized. Immunization of five rabbits was carried out with a 1:1 mixture of complete Freund's adjuvant and antigen, in the footpads (1 mg/animal). Several animals produced detectable amounts of antibody but only one yielded an antiserum suitable for the development of an immunoassay after 3 mo of bimonthly boosting. During part of the immunization period, AA conjugated to horse γ globulin was used for boosting. However, since this did not increase the immunogenicity of the material, antibody levels were maintained for over a year by monthly injections of 0.5 mg DAA (in 0.5 ml saline mixed 1:1 with complete Freund's adjuvant subcutaneously). The antisera were screened by Ouchterlony analysis (18) and immunoelectrophoresis (19). To determine the amount of antibody, quantitative precipitin analyses (20) were performed with sera from several individuals known to have relatively large amounts of SAA. In one of the early studies, a trace amount of radioactive AA (50,000 cpm/tube) was added to the antigen in the event that the amount of antibody was not sufficient to be detectable by the Folin-Ciocalteu method (21), but subsequent, experiments were done without the radioactive antigen.

Labeling of AA with Iodine-125. DAA, neutralized with sulfuric rather than hydrochloric acid, was used for iodination. Initially iodination at pH 7 gave erratic results with yields of iodination less than 50% and [^{125}I]DAA binding to excess antibody less than 70%. For this reason experiments were performed to improve the solubility of DAA and the iodination conditions. The solubility of DAA at pH's ranging from 6 to 10 in 0.1 M phosphate or barbital buffer was first established, and then the optimal pH for the iodination was determined. Iodination was performed by the procedure of Greenwood, Hunter, and Glover (22). 2 mCi iodine-125 as NaI in 0.1 NaOH (New England Nuclear, Boston, Mass.) was mixed with 10 μg of DAA dissolved in 0.2 M barbital buffer, pH 8.6; 50 μg chloramine T in 0.1 M barbital buffer, pH 8.6, (2 mg/ml) was added. Oxidation was stopped after 30 sec with 60 μg sodium metabisulfite in 0.1 M barbital buffer (1.2 mg/ml). Subsequently the labeled DAA was separated from the free iodine-125 by filtration through a Sephadex G-25 column (0.7 × 30 cm), with 0.1 M barbital buffer as eluant. 0.5-ml fractions were collected and counted in a gamma counter (Nuclear-Chicago Corp., Des Plaines, Ill.). The most radioactive fraction collected in the first eluted peak was used for the radioimmunoassay. To minimize adsorption of the ^{125}I-labeled AA, the Sephadex column was pretreated with 30 ml of 1% bovine serum albumin (BSA) in 0.1 M barbital buffer, pH 8.6. 0.5 ml of BSA was also present in the tubes used to collect the eluates. The [^{125}I]AA was stored at −20°C after adding 0.1 ml of 1% Thiomerosal (Sigma Chemical Co., St. Louis, Mo.) solution. Trasylol, an inhibitor of proteolytic digestion, was added in early experiments to minimize possible enzymatic digestion of [^{125}I]-AA, but was later omitted. Since the [^{125}I]AA prepared by this procedure was rapidly inactivated as a result of "iodination damage", enzymatic iodination by the procedure of Marchalonis (23), modified by Thorell and Johansson (24), was also attempted. 5 μg DAA in 25 μl 0.2 M barbital buffer, pH 8.6 was added to 2 mCi Na [^{125}I]. Oxidation by 1 μl 0.88 mM peroxide (Fisher Scientific Co., Pittsburgh, Pa.) in the presence of 4 μg lactoperoxidase (Calbiochem, San Diego, Calif.) in 4 μl 0.1 M barbital buffer was stopped after 2–5 s by adding 0.5 ml 0.1 M barbital buffer, pH 8.6. The subsequent steps were identical to those in the technique previously described.

Radioimmunoassay for SAA was carried out according to the general principles described by Yalow and Berson (25), with polyethylene glycol (26) for the precipitation of the antigen-antibody complexes in triplicate tubes for each sample. To 0.5 ml of specific rabbit antiserum against AA, diluted 1:1000 (50% of the concentration required for maximal binding) in 0.5% bovine gamma globulin (BGG-fraction II-Sigma Chemical Co.) in 0.1 M barbital-buffered saline, pH 8.2, was added 0.2 ml of the sera to be tested, followed by 0.3 ml of a solution of [^{125}I]DAA in 0.5% BGG having 50,000 cpm. Each series was accompanied by 10 serial dilutions of pure DAA in 0.5% BGG to establish a standard curve. After thorough mixing and incubation for 2 h at 37°C, 0.5 ml of cold 30% polyethylene glycol (Matheson, Coleman & Bell, East Rutherford, N. J.) in water was added to each tube to precipitate antibody-antigen complexes. After centrifugation at 3,000 rpm for 30 min at 4°C, the supernate was discarded, and the precipitate counted in a gamma counter (Nuclear Chicago). The mean of the triplicate samples for each serum was used to calculate the percent of the total radioactivity in the precipitate. The SAA concentration was calculated from a standard curve obtained by plotting on a semilogarithmic graph, the percentage of counts per minute precipitated against the quantity of "cold" DAA added. This curve was linear from 7 to 180 ng of DAA in the reaction mixture. The results were expressed as DAA equivalents in nanograms per milliliter. The SD of duplicate determinations in different assays performed 48 h after iodination of DAA was 4.6%. In view of the possibility that the binding affinities of DAA and SAA may differ and the fact that the molecular weight of the serum component is ten times that of AA, these values do not represent the precise concentration of SAA.

Subjects. The SAA concentration was first determined in the sera of 228 normal subjects including 117 randomly selected blood donors, aged 15–65 yr, from the New York Central Blood Bank; 71 individuals, aged 60–95 yr, from two old-age homes who did not have any of the chronic disorders known to be associated with amyloidosis, nor any acute inflammatory or infectious disease during the 2 mo preceding the study; 18 children, aged 1–15 yr, with congenital cardiac or neurologic disorders, but without any acute or chronic acquired disease, and 22 cord blood samples

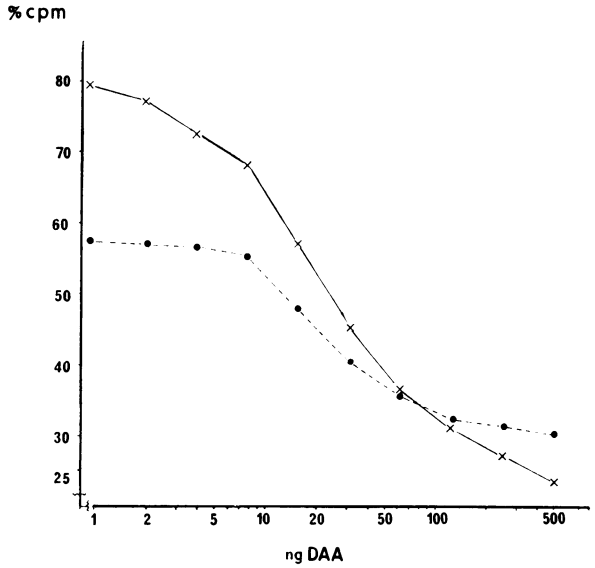

FIGURE 1 Curves comparing the displacement by cold DAA of [^{125}I]DAA iodinated by two different methods: chloramine T oxidation (×——×) and enzymatic iodination with lactoperoxidase (●- - -●). The data are plotted on a semilogarithmic scale.

from normally delivered newborns. All assays were done on serum remaining after the performance of routine tests.

Subsequently the sera of 157 consecutive patients admitted to the Medical Service of the University Hospital of New York University over a 6-wk period and 27 pregnant women at the time of delivery were examined. When analysis of the results of this survey revealed a marked elevation of SAA levels in certain disorders, 107 additional patients with these diseases seen in the clinics or hospitalized on wards for chronic patients at Bellevue and Goldwater hospitals were studied.

Statistical analysis. The statistical significance of the variations of SAA concentration in different groups of subjects tested was calculated by applying the Student *t* test (27). The values found in various age groups were compared with the mean SAA concentration in the first three decades of life, while those from different groups of patients were referred to the mean of SAA levels in the sera of the normal subjects aged 31–70. Different groups were also compared by the analysis of variance method (28).

RESULTS

Radioimmunoassay. To develop optimal conditions for the radioimmunoassay, it was first necessary to study the solubility of DAA and to establish optimal conditions for radioiodination and precipitation. Studies of the solubility of DAA indicated that it was maximal at alkaline pH's greater than 10, and that it decreased significantly below a pH of 8.2. Because of this, iodination was performed at a pH of 8.6 rather than 7.5, as is usually recommended (29), and a pH of 8.2 was chosen for the performance of the radioimmunoassay. Two techniques of radioiodination were tried. Use of chloramine T (22) resulted in an iodination yield of 85%. The iodinated DAA was 95% precipitable by excess antibody. The lactoperoxidase method (23, 24) was only 55% effective in iodination and resulted in a labeled protein that was 85% precipitable by excess antibody.

As shown in Fig. 1, the slope of the curve measuring the displacement of labeled DAA by cold antigen was more suitable when chloramine T-labeled antigen was used than with lactoperoxidase DAA. Because of these advantages, chloramine T oxidation was chosen even though the decrease in binding of [^{125}I]DAA, probably a result of radiation damage during storage, was 50% greater than that of material labeled by the lactoperoxidase method. Because of these difficulties, which were only partially alleviated when the use of Trasylol, an inhibitor of proteolysis, was discontinued, each batch of iodinated material was used for no longer than 1 wk.

Assessment of the rabbit antisera to AA. The specificity of the antisera collected at various times after immunization or boosting was tested by double diffusion. After absorption with horse γ-globulin, used as a carrier in some of the booster injections, only a single precipitin line was noted when the antisera were reacted with whole serum or tissue extracts. The precipitin curves with radioactive DAA by rabbit antisera obtained at selected intervals from 1 to 12 mo after immunization indicated that the antibody titer remained relatively constant over a 1-yr period. The precipitin curve in Fig. 2, carried out with increasing amounts of serum to which a constant trace amount of labeled AA was added, indicates a relatively narrow zone of equivalence. The decrease in precipitated radioactivity beyond the equivalence point is consistent with

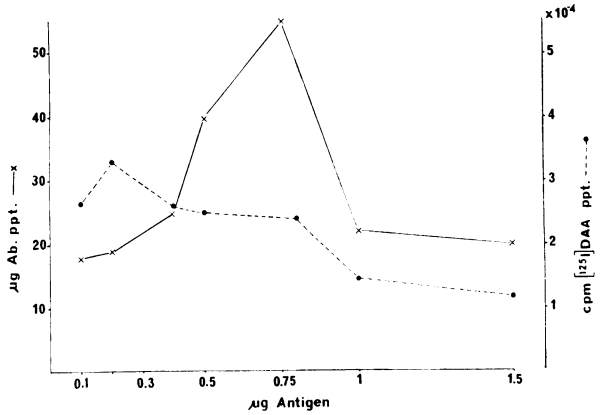

FIGURE 2 Precipitin curve obtained with a rabbit antiserum against degraded amyloid A fraction and increasing amounts of a serum whose content of SAA was determined previously. The amount of antigen is expressed in micrograms of DAA. The interrupted line indicates the precipitate from a constant amount of radioactive iodinated degraded amyloid A fraction added to each tube.

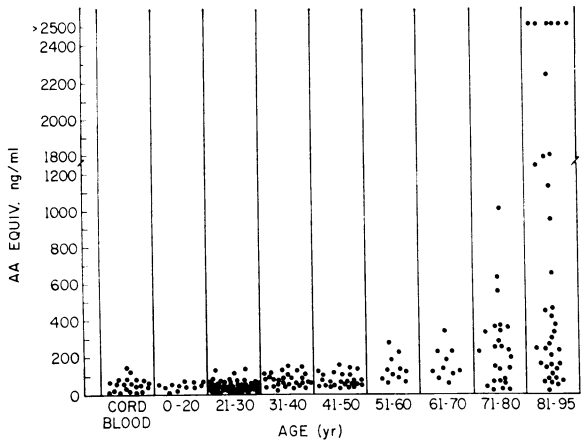

FIGURE 3 Concentration of SAA in the normal population in relation to age.

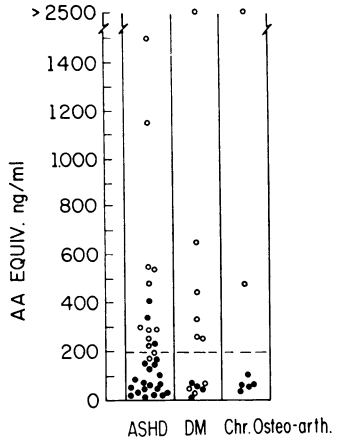

FIGURE 4 Concentration of SAA in certain common diseases of the elderly. ASHD, atherosclerotic heart disease; DM, diabetes mellitus; Chr. Osteo-arth., chronic osteoarthritis. The interrupted line indicates the upper level of SAA in the normal population under 70 yr of age. ● ≤ 70 yr; ○ ≥ 71 yr.

but does not necessarily prove that AA and SAA react with the antiserum in an identical manner.

Concentration of SAA in normal and patient sera. SAA was found in the sera of all normal subjects regardless of age (Fig. 3). In the cord blood and during the first three decades of life, its concentration was 50 ±40 ng/ml. The level increased slowly but steadily with age and reached the highest values over the age of 70. This increase was statistically significant ($P < 0.01$) in all age groups over the age of 30 yr. While some individuals over the age of 70 had a serum concentration of this component equal to that found in the first three decades of life, the majority had a moderately or markedly increased concentration of SAA ($P < 0.001$). That this rise of the SAA in the elderly was not due to some age-associated chronic disorders such as arteriosclerosis, diabetes mellitus, or chronic osteoarthritis is shown by the results depicted in Fig. 4, which clearly demonstrate that among patients suffering from these disorders, only those over the age of 70 had an increased concentration of SAA, while those younger than 70 had normal levels, with the exception of three patients with myocardial infarction of less than 1 wk duration.

Among the patients with various pathologic states (Fig. 5) included in the initial survey of hospitalized patients, the SAA concentration in patients under the age of 70 was significantly elevated in patients with pulmonary tuberculosis, lymphoma of various histologic types, rheumatoid and psoriatic arthritis, carcinoma, and leukemia. The results of patients over 70 yr were not included in the calculation of the means of each group, since it was not possible to differentiate an age-related elevation of SAA concentration from that associated with these diseases. When the number of patients suffering from these disorders was enlarged by including patients, most of whom appeared to be clinically in a quiescent stage, seen in the clinics or hospitalized in the wards for the chronically ill, some

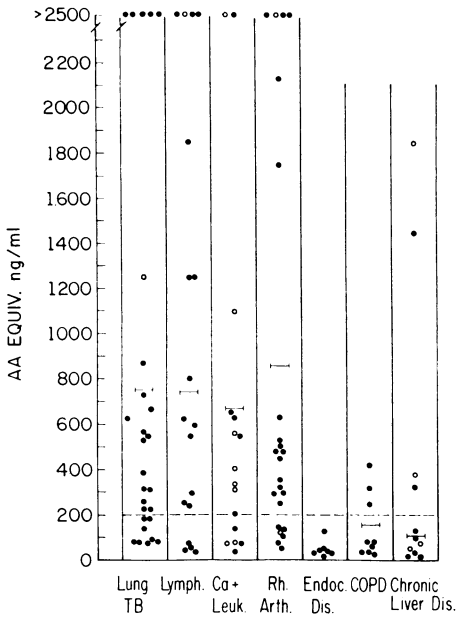

FIGURE 5 Concentration of SAA in various diseases states. Lung TB, lung tuberculosis; Lymph, lymphomas; Ca + Leuk., carcinoma and leukemia; Rh. Arth., rheumatoid arthritis; Endoc. Dis., endocrine diseases; COPD, chronic obstructive lung disease. The bars (|—|) represent the means of SAA values of patients under 70 in each group. Since values higher than 2,500 ng/ml were not estimated, many of the means are higher than those indicated. The interrupted line indicates the upper level of SAA in the normal population under 70 yr of age. ● ≤ 70 yr old; ○ ≥ 71 yr old.

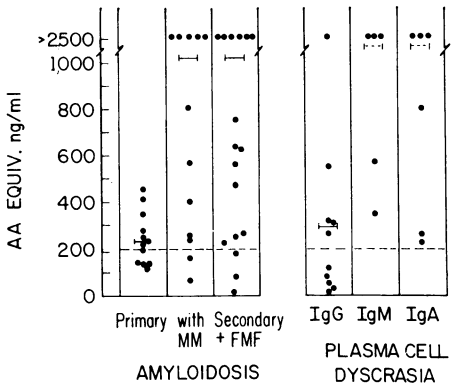

FIGURE 6 Concentration of SAA in patients with amyloidosis and certain plasma cell dyscrasias. Amyloidosis, primary; with myeloma (MM); secondary and familial Mediterranean fever (FMF). All values were from patients younger than 70 yr of age. Legend otherwise identical to that of Fig. 5.

were found to have normal SAA levels. However, the overall mean concentration of SAA in patients with these diseases, remained elevated and was approximately four to six times the mean of 95±57 ng/ml of the 67 normal subjects aged 31–70 yr.

In contrast, patients with chronic liver diseases, with the exception of two patients with biliary cirrhosis, and those with chronic obstructive lung disease, various endocrine disorders (Fig. 5), including diabetes mellitus, as well as those with artherosclerotic heart disease (except for three patients with recent myocardial infarction) and chronic osteoarthritis (Fig. 4) had levels of the SAA within the normal limits.

The concentration of the SAA was also elevated ($P < 0.01$) in 33 patients with amyloidosis (Fig. 6). This

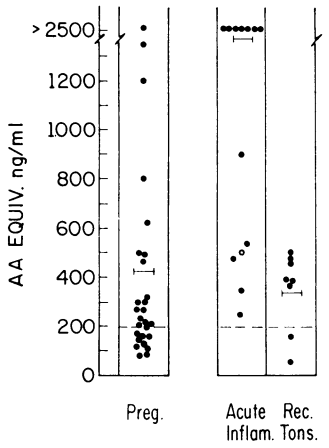

FIGURE 7 Concentration of SAA in pregnancy (Preg.) acute inflammatory states (Acute Inflam.), and recurrent tonsillitis (Rec. Tons.). Remainder of legend identical to that of Fig. 5. ● ≤ 70 yr old; ○ ≥ 71 yr old.

elevation was only moderate (mean, 235 ng/ml) but statistically significant ($P < 0.001$) in patients with primary amyloidosis. In those with secondary amyloidosis and with amyloidosis associated with multiple myeloma, the mean SAA level was markedly elevated (1,151 ng/ml and 1,130 ng/ml, respectively). This was statistically significant ($P < 0.01$), despite the large range of values in each of these two groups (SD, 1,089 and 1,102). An increase of the SAA concentration was also found in the sera of patients with monoclonal gammopathies (Fig. 6) especially those with IgA myeloma and macroglobulinemia (mean, 1,464 ng/ml and 1,431 ng/ml, respectively). All three patients with secondary amyloidosis whose SAA level was normal, as well as many of those with monoclonal gammopathies without a rise of SAA, had nephrotic syndrome.

Among the 27 pregnant women whose SAA level was determined at the time of delivery (Fig. 7), 70% had values above the upper limit of the normal (208 ng/ml). The mean of 431 ng/ml of the whole group was significantly elevated, a finding in agreement with previous qualitative studies (30) by Husby and Natvig. Somewhat unexpected was the finding that all 13 patients with acute infectious and inflammatory disorders included in the initial survey of hospitalized patients (Fig. 7) had a marked elevation of the SAA level (mean, 1,668 ng/ml), statistically highly significant ($P < 0.001$). This group included cases of pneumonia, meningitis, diverticulitis, cholecystitis, subacute bacterial endocarditis, hepatitis, and acute rheumatic fever. Similarly, a group of children 4–15 yr old with recurrent tonsillitis (Fig. 7) had a mean concentration of 342 ng/ml, six times that of normal children of this age. In followup studies of the SAA level in nine patients with acute inflammatory diseases after recovery (Table I), it was noted that the level of this serum component appeared to return to normal as soon as the clinical status improved, faster than the return to normal of the sedimentation rate.

In diseases where it was applicable, there was no close correlation between the SAA level and the serum levels of C'3, immunoglobulins, C-reactive protein, nor with the sedimentation rate (Table II). While both the SAA level and the other tests generally appear to parallel activity, the results would seem to preclude identity between the SAA and the factors involved in these other tests.

DISCUSSION

The presence of a serum component antigenically related to the acid-soluble fraction extracted from the amyloid fibrils was first noted by Levin et al. (13) and by Husby et al. (14), using the double immuno-

diffusion. With this rather insensitive technique, SAA was found in the sera of most patients with primary and secondary amyloidosis and in a large number of patients suffering from myeloma, macroglobulinemia, hypogammaglobulinemia, as well as of some chronic diseases such as rheumatoid arthritis, tuberculosis, and chronic liver diseases. Amyloid-related serum component was also found in a small percentage of normal subjects, but could not be detected in cord blood. Using the somewhat more sensitive technique of immunoelectro-osmophoresis, Husby and Natvig (30) found the amyloid A-related serum component in 10% of the normal individuals and reported that the frequency increased to 50% in sera from apparently normal subjects between the ages of 80 and 95 yr as well as in the sera from pregnant women. A higher incidence of positive sera was also noted in a few patients with various lymphoproliferative disorders, some other malignant diseases, and primary biliary cirrhosis, but not in acute hepatitis. Some of these results were recently confirmed by Benson, Skinner, Lian, Cohen, and Shirahana (15).

The physicochemical properties and the composition of this serum component are only partially known. It was found to have the electrophoretic mobility of an $\alpha 1$ globulin (13, 30), to be resistant to boiling (30), and to have an approximate mol wt of 100,000 in physiologic solvents (13, 30).

Recent studies have indicated that its mol wt is slightly lower [2] and amino acid analyses of partially purified amyloid-related protein from the sera of two subjects appeared to be different from the acid-soluble fraction of amyloid fibrils (15).

Because of its much greater sensitivity, the radioimmunoassay for the determination of the SAA reported in this study has allowed more precise quantitation of this protein, thus permitting a more detailed analysis of its distribution in the normal and diseased populations. Previous studies of assays for ACTH and other hormones have indicated the possibility of detecting in the serum a presumably larger precursor molecule, with the help of a radioimmunoassay in which the standard displacement curves were obtained with a purified protein of smaller mol wt, with an antibody that reacted with some antigenic sites common to both the small and the large molecule (29). The use of such a system introduces several difficulties which, while they may preclude an accurate estimate of the precise concentration of the larger component, do not negate its use for comparative estimates of the type reported here, in view of the antigenic identity of the two proteins (13). There are two major limitations to precise quantitation. One is that the amino acid sequence of the serum related component is not known. If AA is a piece of a larger protein that consists primarily of an unrelated protein, it seems likely that the actual concentration of the SAA would be about 10 times greater than that calculated. If, on the other hand, the serum component consists of two or more repeating units of AA, the actual concentration would be less and would lie somewhere between the one listed

[2] Rosenthal, C. J., and E. C. Franklin. Unpublished observations.

TABLE I

Followup Study of Patients with Acute Infectious and Inflammatory Processes

Patient	Age	Diagnosis	Date	ESR	SAA
				mm/h	*ng/ml*
C. J.	17 mo	Ac. meningitis (*Hemophilus influenzae*)	2/28	65	1,075
			4/2	34	10
W. L.	52 yr	Ac. cholecystitis	2/12	88	>2,500
			2/20	77	280
P. H.	54 yr	Pneumococcal pneumonia	4/7	38	475
			4/17	35	100
L. I.	27 yr	Viral pneumonia	4/7	39	90
			4/12	35	20
S. T.	9 yr	Acute rheumatic fever	4/23	58	1,250
			5/10	45	125
T. W.	8½ yr	Acute rheumatic fever	4/23	60	950
			5/15	50	130
L. W.	14 yr	Acute rheumatic fever (recurrent)	5/1	60	>2,500
			6/5	59	1,050
P. A.	24 yr	Erythema nodosum	4/15	130	950
			5/13	145	170
A. J.	72 yr	Pneumonia	5/21	82	305
			5/28	70	260

ESR, erythrocyte sedimentation rate.

TABLE II

Comparison between the SAA Level and Other Tests Usually Abnormal in Inflammatory States

	ESR			CRP			C'3			Total Ig's		
	No. patients	Range	Mean	No. patients	Range	Mean	No. patients	Range	Mean	No. patients	Range	Mean
		mm/1 h			*mm*			*U*			*mg/100 ml*	
SAA < 200 ng/ml	20	16–80	25.9	11	0–8	1.0	6	45–130	87	11	720–1,991	1,093
SAA > 200 ng/ml	40	14–160	44.4	15	0–8	1.53	3	35–122	68	15	535–2,430	1,250

CRP, C-reactive protein; ESR, erythrocyte sedimentation rate.

and the upper limit expressed above. The other limitation is lack of information concerning the avidities of the antiserum for AA and SAA. If they are not the same, the effect of the two antigens on the precipitation of the labeled antigen might differ, and thus add further problems in accurate quantitation. Indeed, as shown in Fig. 2, such differences in avidity could not be excluded with certainty when quantitative precipitin analyses were done with a mixture of radioactive DAA and serum in the hope of making the precipitin analysis more sensitive. For these reasons we have expressed the SAA concentrations in terms of nanograms of DAA per milliliter, thus avoiding any errors that could be introduced by the above-mentioned unknown factors.

The results of the determination of the SAA in the normal population (Fig. 3) have demonstrated for the first time that this component is a physiologic constituent of all normal sera, present at a mean concentration of 94±57 ng/ml, and that its concentration in serum increases slowly but steadily in parallel with the aging process. The increase was most striking in individuals over the age of 70 and appeared to be unrelated to the common age-associated diseases. This finding appears to be consistent with pathological and electron microscopic observations by Divry (31), Marinesco (32), and Terry (33), indicating the presence of amyloid in the senile plaques of brains from old individuals, as well as with more recent studies by Schwartz (16), suggesting that the aging process in man, ducks, mice, and dogs is associated with the deposition of amyloid in the brain, heart, and Langerhans' islets of the pancreas.

Our studies confirmed and quantitated the previously reported increase of the SAA in pregnancy (30), in some chronic inflammatory diseases such as tuberculosis, rheumatoid and psoriatic arthritis (13, 15, 30), and biliary cirrhosis (30) as well as in the sera of patients with lymphoproliferative disorders, myeloma, macroglobulinemia, and some other malignancies, including a few cases of leukemia. The mean SAA concentration in amyloidosis was more elevated in patients with secondary amyloidosis than in patients with primary amyloidosis or myeloma-associated amyloid. However, in several patients with amyloidosis and in some patients with monoclonal gammopathies complicated by the nephrotic syndrome, the SAA level was normal or low. It is of interest that in patients with amyloidosis associated with myeloma and those with primary amyloidosis, the increase of the SAA level occurs in the face of tissue deposits of amyloid, which in our experience often contain little or no acid-soluble fraction (34), though some of these do contain this protein (35). This discrepancy suggests the possibility of a blockade in the transfer of the nonimmunoglobulin amyloid precursor from the blood vessels to the tissues, or such a marked excess of light chain production and deposition that the amyloid fibrils consist primarily of light chain fragments.

In contrast to the results in these diseases, the SAA level remains normal in a number of illnesses with no apparent active inflammatory, proliferative, or immunologic features, such as chronic obstructive lung diseases, diabetes mellitus, chronic osteoarthritis, arteriosclerosis (except for patients with recent myocardial infarctions), and chronic liver diseases (with the exception of biliary cirrhosis, known to be associated with certain peculiar immunologic phenomena). Because of the elevated levels of SAA seen in a variety of diseases other than amyloidosis, it is important to emphasize that the SAA level is of no value as a possible diagnostic test for the presence of amyloid.

Of particular interest in relation to possible factors involved in the pathogenesis of amyloidosis was the significant rise of SAA seen in acute inflammatory and infectious disorders, and in preliminary studies, its rapid return to normal in parallel with the improvement of the clinical state. This observation, which must be confirmed by further investigations, is consistent with the increase of SAA concentration during the early stages of acute myocardial infarction, in patients with biliary cirrhosis and with chronic inflammatory and proliferative disorders (lymphoma and other malignancies), and with the relatively normal levels during the quiescent stages of these disorders. These findings suggest that the SAA may be released much like an acute phase reactant in many common inflammatory and immune disorders. It is conceivable that if these processes are short-lived, and the release of SAA only transient, it can be rapidly cleared from the circulation or removed from the tissues. In contrast, if the liberation of SAA persists for longer periods, or if the liberation is massive, it may lead to the formation of amyloid by mechanisms yet to be uncovered. It seems possible that during this process, the nonimmunoglobulin amyloid precursor may interact with fragments of immunoglobulin light chains, thus accounting for the presence of both components in the deposits.

In view of the depressed state of cellular and humoral immunity often seen in patients with amyloidosis and in experimental animals with the disease (36) and during the aging process (16), it would be tempting to implicate this material in these phenomena. The precise physiologic role of the SAA remains to be defined. It is hoped that the radioimmunoassay herein reported will be a useful tool in future studies to investigate many of the unknown aspects of this SAA,

to uncover its site of origin, and to establish its possible role in the formation of the amyloid fibrils.

ACKNOWLEDGMENTS

We would like to thank Dr. Gideon Goldstein for helpful advice in setting up the radioimmunoassay technique, Drs. Isadore Rossman, Martin Meltzer, I. Karten, and S. Leslie for their help in the clinical studies, and Ms. Marlene Chavis and Ms. Linda Earle for expert secretarial work.

This work was supported in part by U. S. Public Health Service Grants AM 01431, AM 05064, and ND 07719, The Michael and Helen Schaffer Fund, and the New York Chapter of the Arthritis Foundation, Inc.

REFERENCES

1. Glenner, G. G., W. D. Terry, M. Harada, C. Isersky, and D. Page. 1971. Amyloid proteins: proof of homology with immunoglobulin light chains by sequence analysis. *Science (Wash. D. C.).* **172**: 1150–1151.
2. Isersky, C., D. Ein, D. L. Page, M. Harada, and G. G. Glenner. 1972. Immunochemical cross reactions of human amyloid proteins with immunoglobulin light chains. *J. Immunol.* **108**: 486–493.
3. Hermodson, M. A., R. W. Kuhn, K. A. Walsh, H. Neurath, N. Ericksen, and E. P. Benditt. 1972. Amino acid sequence of monkey amyloid protein A. *Biochemistry.* **11**: 2934–2944.
4. Eriksen, N., S. Fowler, and L. H. Ericsson. 1974. Origin of amyloid protein A. *Fed. Proc.* **33**: 1562. (Abstr.)
5. Skinner, M., M. D. Benson, A. S. Cohen, E. J. Cathcart, and J. B. Lian. 1974. N-terminal amino acid sequence of experimentally induced guinea pig amyloid. *Fed. Proc.* **33**: 618. (Abstr.)
6. Wegelius, O., editor. 1975. Amyloid Symposium. Academic Press, Inc., New York. In press.
7. Benditt, E. P., and N. Eriksen. 1972. Chemical classes of amyloid substances. *Am. J. Pathol.*, **65**: 231–249.
8. Ein, D., S. Kimura, W. D. Terry, J. Magnotta, and G. G. Glenner. 1972. Amino acid sequence of an amyloid protein of unknown origin. *J. Biol. Chem.* **247**: 5653–5655.
9. Pras, M., and T. Reshef. 1972. The acid soluble fraction of amyloid—A fibril forming protein. *Biochem. Biophys. Acta.* **271**: 193–203.
10. Franklin, E. C., M. Pras, M. Levin, and B. Frangione. 1972. The partial amino acid sequence of the major low molecular weight component of two human amyloid fibrils. *FEBS (Fed. Eur. Biochem. Soc.) Lett.* **22**: 121–123.
11. Levin, M., E. C. Franklin, B. Frangione and M. Pras. 1972. The amino-acid sequence of the major non-immunoglobulin component of some amyloid fibrils. *J. Clin. Invest.* **51**: 2773–2776.
12. Husby, G., K. Sletten, T. E. Michaelson, and J. Natvig. 1972. Antigenic and chemical characterization of non-immunoglobulin amyloid proteins. *Scand. J. Immunol.* **1**: 393–400.
13. Levin, M., M. Pras, and E. C. Franklin. 1973. Immunologic studies of the major non-immunoglobulin amyloid protein of amyloid. *J. Exp. Med.* **138**: 373–380.
14. Husby, G., T. E. Michaelsen, K. Sletten, and J. B. Natvig. 1973. Immunochemical characterization of a non-immunoglobulin amyloid fibril subunit and a structurally related serum component. *Scand. J. Immunol.* **2**: 319. (Abstr.)
15. Benson, M. D., M. Skinner, J. Lian, A. S. Cohen, and T. Shirahama. 1974. Amyloid serum component: relationship to aging. *Fed. Proc.* **33**: 619. (Abstr.)
16. Schwartz, P. 1970. Amyloidosis—cause and manifestation of senile deterioration. Charles C Thomas, Publisher, Springfield, Ill. 395 pp.
17. Ehrlich, J. C., and I. M. Ratner. 1961. Amyloidosis of the islets of Langerhans. A restudy of islet hyalin in diabetic and nondiabetic individuals. *Am. J. Pathol.* **38**: 49–59.
18. Ouchterlony, O. 1958. Diffusion in gels. Methods for immunological analyses. *Prog. Allergy.* **5**: 1–78.
19. Scheidegger, J. J. 1955. Une micro-methode de l'immuno-éléctrophorèse. *Int. Arch. Allergy Appl. Immunol.* **7**: 103–110.
20. Kabat, E. A. 1961. Precipitin reaction. *In* Experimental immunochemistry. E. A. Kabat and M. M. Mayer, editors. Charles C Thomas, Springfield, Ill. 2nd edition. 22–96.
21. Lowry, O. H., N. J. Rosebrough, L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193**: 265–276.
22. Greenwood, F. C., W. M. Hunter, and J. S. Glover. 1963. The preparation of ^{131}I-labeled human growth hormone of high specific radioactivity. *Biochem. J.* **89**: 114–123.
23. Marchalonis, J. J. 1969. An enzymic method for the trace iodination of immunoglobulins and other proteins. *Biochem. J.* **113**: 299–305.
24. Thorell, J. I., and B. G. Johansson. 1971. Enzymatic iodination of polypeptide with ^{125}I to high specific activity. *Biochem. Biophys. Acta.* **251**: 363-369.
25. Yalow, R. S., and S. A. Berson. 1960. Immunoassay of endogenous plasma insulin in man. *J. Clin. Invest.* **39**: 1157–1175.
26. Desbuquois, B., and G. D. Aurbach. 1971. Use of the polyethylene glycol to separate free and antibody-ground peptide hormones in radioimmunoassay. *J. Clin. Endocrinol. Metab.* **33**: 732–738.
27. Bulmer, M. G. 1967. Principles of Statistics. The M.I.T. Press, Cambridge, Mass. 145–154.
28. Bahn, A. K. 1972. Basic Medical Statistics. Grune & Stratton, Inc., New York. 178–182.
29. Hunter, W. M. 1974. Preparation and assessment of radioactive tracers. *Br. Med. Bull.* **30**: 18–23.
30. Husby, G., and J. B. Natvig. 1974. A serum component related to nonimmunoglobulin amyloid protein AS, a possible precursor of the fibrils. *J. Clin. Invest.* **3**: 1054–1061.
31. Divry, P. 1927. Étude histochimique des plaques séniles. *J. Neurol. Neuropathol. Psychiatr.* **27**: 643–657.
32. Marinesco, G. 1928. Nouvelles réchèrches sur les plaques séniles. *Bull. Sect. Sci. Acad. Roum.* **11**: 1–25.
33. Terry, R. D. 1963. The fine structure of neurofibrillary tangles in Alzheimer's disease. *J. Neuropathol. Exp. Neurol.* **22**: 629–642.
34. Franklin, E. C., and C. J. Rosenthal. 1974. Chemical heterogenicity of human amyloid. *Fed. Proc.* **33**: 758. (Abstr.)
35. Husby, G., J. B. Natvig, T. E. Michaelsen, K. Sletten, and H. Host. 1972. Unique amyloid protein subunit common to different types of amyloid fibril. *Nature (Lond.).* **244**: 362–364.
36. Cathcart, E. S., M. A. Scheinberg, and M. Bennett. 1974. Amyloidosis and age-related phenomena in casein-treated mice. *Fed. Proc.* **33**: 758. (Abstr.)