Prealbumin: its association with amyloid

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SUMMARY In recent years prealbumin has been shown to be a major component of two forms of systemic amyloid, senile systemic amyloid (SSA), and familial amyloidotic polyneuropathy (FAP). Despite the fact that the amyloid fibril proteins associated with these two forms of amyloid, designated ASc₁ and AF, respectively, share many similarities the clinical features of the two diseases are remarkably different. To understand better this paradox the clinical, histochemical, immunological and biochemical features of SSA and FAP were reviewed.

A prealbumin like protein (AF_P) associated with amyloid was first described in 1978 by Costa *et al* in Portuguese familial amyloidotic polyneuropathy (FAP).¹ Shortly afterwards protein ASc₁, which had previously been isolated from senile cardiac amyloid,² was also reported to be related to prealbumin.³ Senile cardiac amyloid has subsequently been designated senile systemic amyloid (SSA).^{4,5} Since these initial findings, other forms of FAP (Japanese, Jewish, Swedish-American, German-English, Swedish) and familial amyloidotic cardiomyopathy (Danish) have been shown to contain proteins related to prealbumin.⁶⁻¹¹ Despite the apparent similarity in the biochemical nature of SSA and FAP the clinical manifestations of these two forms of amyloidosis are strikingly different. On the one hand, SSA occurs in late life and is generally associated with minor clinical manifestations; by contrast, FAP is generally a disease of early and middle age and is always fatal.

Prealbumin

Human prealbumin is a product of chromosome 18.¹² It is a tetrameric protein of 55 kD molecular weight, composed of four identical non-covalently bound monomers of 127 amino acid residues arranged with tetrahedral symmetry.¹³ A narrow cylindrical channel runs through the centre of the protein molecule and contains two binding sites for thyroxine.¹⁴ Independent binding sites for retinol binding protein, the specific plasma carrier of vitamin A, are probably

located on the outside of each subunit.¹⁵ Each dimer contains two β sheets, composed of eight strands,¹⁶ a structure which may account for its unusual stability and resistance to dissociation. The β structure makes human prealbumin an ideal candidate for the deposition of twisted β pleated sheet fibrils into amyloid deposits. This conclusion is based on x-ray and infrared analysis of systemic and localised amyloid fibrils, which show that they have a β pleated sheet configuration.¹⁷

Senile systemic amyloid (SSA)

CLINICAL FEATURES

Senile systemic amyloid is a disease related to age, which has a predisposition for the heart. The incidence and distribution of SSA was determined in a prospective immunohistochemical study of atrium, ventricle, aorta, lung, renal cortex and rectum removed at necropsy from 85 consecutive elderly patients (80 years or older). Twenty one patients (25%) had SSA (ASc₁) type amyloid. Of this group, 17 had cardiac disease and 19 had small extracardiac deposits.⁴ Although the mean heart weight, percentage with heart failure, and incidence of myocardial infarction were higher for the group of patients with cardiac amyloid, none of these differences was significant in this small group of patients.

The widespread nature of ASc₁ type amyloid was subsequently confirmed in 13 patients who died from extensive ASc₁ type amyloid infiltration of the heart (mean heart weight 598 g; range 370-950 g). The

Table 1 Comparison of clinical features of senile systemic amyloidosis (SSA) and familial amyloidotic polyneuropathy (FAP)

	SSA	FAP
Age	Elderly (> 70 years)	Young (25–50 years)
Incidence	Common (> 25% above 80 years)	Rare
Transmission	Unknown	Autosomal dominant
Predominant organ disease	Heart	Nerves (autonomic, sensory, motor)
Other	Many others (all tissue studied except brain; very rare in spleen; observed only in renal medulla and vessels)	Many others (vitreous, heart, renal cortex and medulla, intestines, skin, muscles, spleen)
Clinical course	Generally benign	Uniformly fatal in about 10 years

patients ranged in age from 74 to 102 years. Twenty seven different tissues were studied in this group. All tissues except brain showed ASC₁ type amyloid deposits.⁵ Unlike other forms of renal amyloid, SSA deposits occurred only in the renal medulla and vessels, with complete sparing of the glomeruli. The spleen was rarely affected, and disease was limited to very small vascular deposits (table 1).

SSA does not produce symptoms in most patients. In a small percentage of patients with extensive heart disease severe cardiac symptoms can occur. Careful examination of the conduction system shows widespread deposits in the sinus node and the atrio-ventricular node in the heart of such patients (Johansson B, Westermark P, unpublished observations). Moderately large amounts of amyloid may be seen in the alveolar walls.⁵ The lung function has not been evaluated in SSA, but it is probably impaired in some patients.

HISTOCHEMICAL, IMMUNOLOGICAL, AND BIOCHEMICAL FEATURES

SSA shows the typical red-green birefringence of amyloid when stained with alkaline Congo red and viewed with polarised light. Like most other types of amyloid, it is resistant to treatment with permanganate and contains both tryptophan and P component (table 2).¹⁸

SSA deposits in tissue sections react strongly by immunofluorescence with antihuman prealbumin and

anti-ASC₁. Both of these reactions can be blocked by purified human prealbumin or ACC₁.¹⁹ These antisera fail to react with amyloid of the amyloid fibril protein of immunoglobulin light chain type and amyloid fibril protein A types (table 2).¹⁹ Despite the similarity of antihuman prealbumin and anti-ASC₁ in binding to amyloid tissue deposits the antisera react differently in the immunoprecipitation reaction (table 3). Antihuman prealbumin forms lines of identity with human prealbumin and pooled human serum but fails to react with purified whole ASC₁. Unabsorbed anti-ASC₁, on the other hand, reacts with ASC₁ but not with human prealbumin.¹⁹

The biochemical structure of ASC₁ is not completely known. Studies by Sletten *et al* have shown that peptic digests of ASC₁ have shown peptides with complete homology, with residues 70–90, 96–107, 109–115, and 121–127 in human prealbumin.³ In all studies of ASC₁ it has been noted that ASC₁ is relatively insoluble in water, whereas human prealbumin is completely soluble. The study of ASC₁ by electrophoretic and immunoblot techniques has indicated that it is composed of at least three components.²⁰

A subunit similar to human prealbumin: This protein (i) has a molecular weight identical with that of the normal prealbumin monomer on SDS-PAGE; (ii) reacts strongly with antihuman prealbumin both in immunoblot and immunodiffusion reactions; (iii) contains at least one cysteinyl residue (the normal prealbumin monomer contains a single cysteine at position 10); and (iv) is characterised by weaker than normal non-covalent forces between subunits and the presence of an abnormal disulfide bond when compared with that of normal prealbumin.

A somewhat smaller 13 kD protein This protein (i) reacts strongly with anti-ASC₁ and poorly with antihuman prealbumin; (ii) seems to lack a cysteinyl residue; (iii) represents the major component of ASC₁.

Smaller proteins (i) Some of these proteins contain cysteine; (ii) some react strongly with anti-ASC₁ and weakly with antihuman prealbumin. Thus these proteins seem to be fragmentation products of the prealbumin like protein or the 13 kD protein, or both.

The prealbumin like protein of SSA has recently

Table 2 Comparison of histochemical and immunological features of SSA and FAP deposits in tissue sections

	SSA	FAP
Congo red reaction	+	+
Permanganate treatment	Resistant	Resistant
Tryptophan	+	+
P-component	+	+
Binding reaction (fluorescence or peroxidase)		
Anti-prealbumin	+	+
Anti-ASC ₁	+	+
Anti-amyloid fibril protein of immunoglobulin light chain type,		
Anti-amyloid fibril protein A	–	–

Table 3 Comparison of immunological and biochemical features of normal prealbumin monomer (PA) with prealbumin variants ASc_1 and AF associated with senile systemic amyloidosis (SSA) and familial amyloidosis polyneuropathy (FAP), respectively

	Prealbumin	ASc_1	Amyloid fibril
Size (SDS-PAGE analysis)	~ 14 kD	Three components Prealbumin like component (~ 14 kD) 13 kD fragment Smaller fragments	Three components (AF_s) Prealbumin like component (~ 14 kD) 13 kD fragment Smaller fragments
Primary structure	127aa	Homology with prealbumin at residues 70–90, 96–107, 109–115, 121–127; no methionine substitution in position 30	AF_{Ja} , AP_P , AF_P , AF_{SA} : 127aa with varying N-terminal deletion: amino acid substitution at position 30 (Val → Met) in some molecules AF_{Je} : 127aa with cleavage at position 48 in some molecules; amino acid substitution in position 49 (Thr → Gly) or position 33 (Phe → Ile) in some molecules
Disulfide bonds	No	Prealbumin like protein only	Prealbumin like protein only
Immunological reactivity Western blot with anti prealbumin	+	+ Prealbumin like protein – Smaller components	AF_s : + with prealbumin like protein – with smaller components
Immunoprecipitation with anti-prealbumin	+	– Whole ASc_1 + Purified PA like proteins – Smaller proteins	AF_{Ja} , AF_J , AF_P , AF_{SA} + AF_s : + or – with whole amyloid fibril (individual variations) + purified prealbumin like protein – smaller proteins
Immunoprecipitation with anti- ASc_1	–	+ Whole ASc_1	AF_s : + Whole amyloid fibril
Circulating prealbumin variants		Unknown	AF_{Ja} , AF_P , AF_{SA} : Variant PA (Val → Met) and normal prealbumin AF_s : Variant PA (Val → Met) and probably normal PA

been isolated and, unlike protein AF, has been found to lack a methionine substitution at position 30 (B Johansson, K Sletten, P Westermarck, unpublished observations) (table 3).

Familial amyloidotic polyneuropathy (FAP)

CLINICAL FEATURES

The forms of FAP related to human prealbumin fall predominantly into the type I (Andrades) group of disorders. This group is described in Portugal, Japan, and Sweden and in single families of different or mixed ethnic origin. Attempts to find a common ancestry have not been successful. The inheritance is autosomal dominant, and the usual age range at which symptoms begin is 25–50 years. Insidious disease of the autonomic nervous system usually appears early. Peripheral neuropathy characteristically affects the legs most severely, with paresthesias and sensory loss preceding motor loss.²¹ Gastrointestinal disease (motility dysfunction and malabsorption)²² and car-

diac disease (conduction defects and restrictive cardiomyopathy)^{23,24} are prominent features. Abnormal glomerular function may occur,²⁵ and, unlike SSA, amyloid deposits in the glomeruli have been described.²⁶ The duration from onset of clinical symptoms until death ranges from 3 to 26 years (mean 10.8 years).²⁷

HISTOCHEMICAL, IMMUNOLOGICAL, AND BIOCHEMICAL FEATURES

Amyloid associated with FAP is nearly indistinguishable from SSA in its tinctorial and immunological properties (tables 2, 3). Like protein ASc_1 , protein AF_s (in tissue sections) binds antihuman prealbumin and anti- ASc_1 with equal avidity. In addition, anti- ASc_1 forms a line of identity between protein ASc_1 and protein AF_s .²⁸ Antihuman prealbumin has been shown to precipitate with AF_P ,¹ AF_{SA} ,⁸ AF_{Ja} ,⁶ AF_{Je} ,²⁹ and, in some patients, with AF_s (P Westermarck, K Sletten, B O Olofsson, unpublished observations). Immunoblot studies of AF_s have shown a pattern very similar to that described above for ASc_1 .²⁰

Amino acid sequencing studies have shown that a prealbumin variant with a single amino acid substitution at position 30 (Val→Met) is present in the FAP amyloid of the Japanese,^{30,31} Swedish-American,³² Portuguese³³ and Swedish types. Both the Swedish-American and Swedish amyloid fibrils also contain normal prealbumin. The AF_{1a} and AF_S have heterogeneous N-terminal sequences, resulting from the presence of complete human prealbumin variants.^{30,34} AF_{SA} and AF_P have been reported to start at position 1.^{32,33} Patients with these four types of FAP have the Val→Met prealbumin variant (as well as normal prealbumin) in the serum or plasma.^{33,35,36} The Val→Met substitution does not affect the ability of monomers to form stable tetramers, nor the binding affinity for retinol-binding protein or thyroxine.³³ The prealbumin variant in the Jewish type, by contrast, has a substitution at position 49 (Thr→Gly)²⁹ or position 33 (Phe→Ile).³⁷

A nonapeptide containing the substituted methionine at position 30 has been synthesised, and an antibody to this peptide has been used to identify circulating human prealbumin variant by radioimmunoassay.³⁸ This assay seems to have identified Japanese family members at risk before clinical disease of the nervous system occurred. The variant prealbumin molecule has also been identified as a preclinical marker in the serum or plasma of Swedish-American and Portuguese family members. Human prealbumin has been isolated from these subjects and has been digested with cyanogen bromide (which cleaves at the methionine residue). The additional peptides derived from the mutant prealbumin have been identified by purification and sequencing³⁹ or by immunoblotting techniques.⁴⁰

In addition, DNA isolated from peripheral blood leucocytes of three Japanese patients with FAP has been shown to contain new restriction sites for endonuclease NsiI or BallI in the prealbumin locus.⁴¹ These mutations were consistent with the substitution of adenine (A) for guanine (G). The resultant change in the nucleotide sequence from GTG to ATG explains the substitution of methionine for valine at position 30 in prealbumin. Similar findings have been reported in the offspring of patients with Portuguese FAP.⁴² The absence of additional variant nucleotide sequences in these subjects supports the hypothesis that the prealbumin variant associated with SSA entails one or more post-translational events.

Familial cardiac amyloid was first discovered in a Danish family⁴ and has recently been shown to contain prealbumin.¹ The disease has its onset in early to middle age and leads to progressive cardiac failure and death over two to six years. Several other organs (skin, spleen, kidney) and fat have been affected in some patients.

Comparison of SSA and FAP

Although SSA is the most common form of systemic amyloidosis, it is also the most benign. The heart (atria and ventricles) is most often and abundantly affected, but only a small fraction of patients with SSA develop massive cardiomegaly with fatal outcome. Although SSA is a widespread disorder, organ failure at extracardiac sites is virtually unknown. FAP, by contrast, affects young and middle age patients who inherit the disease. A variable pattern of progressive autonomic, sensory, and motor neuropathy develops, leading to death. In some forms of FAP extraneural tissue disease may predominate.

Despite the contrasting clinical course of these two forms of systemic amyloid they share many biochemical characteristics. Table 2 shows that SSA and FAP cannot be distinguished by standard histochemical or immunological methods. It is clear that both ASc₁ and AF proteins differ from normal prealbumin. Specific structural abnormalities of the AF proteins have been determined, but many unanswered questions remain about the exact structure of protein ASc₁ (table 3).

Conclusions

The amyloid fibril proteins ASc₁ and AF, associated with SSA and FAP, respectively, contain prealbumin variants. These variants seem to contain several protein components, one of which may be very similar to normal prealbumin. In most types of human prealbumin related FAP studied to date this protein has at least one amino acid substitution. A smaller component appears to lack cysteine and reacts poorly with antiprealbumin. This is the major component of ASc₁ and of AF_S in some patients and can be detected with anti-ASc₁. The finding of a similar smaller protein in ASc₁ and AF_S is consistent with the observation that anti-ASc₁ reacts both with ASc₁ and AF_S in double diffusion but fails to react with native prealbumin. As sera or plasma from four different types of FAP have shown a prealbumin variant with an amino acid substitution at position 30, it is speculated that this abnormality leads to important conformational changes of the prealbumin molecule, which change its precipitability and contribute to the amyloidogenic potential of these proteins.

Unlike the prealbumin variants found in amyloid fibrils and blood of FAP patients and family members, no direct evidence of amino acid substitution has yet been shown for protein ASc₁. The deletion of cysteine from the N-terminus in the 13kD protein of ASc₁ may be caused by post-translational cleavage of the prealbumin monomer. Alternatively, it is possible that the cysteine residue is modified and not detectable

by the ^{14}C -iodoacetamide reaction, which was used to identify this amino acid. To date no structural studies of putative circulating prealbumin variants have yet been performed in patients with SSA.

The clinical diversity of the prealbumin related amyloidoses represents an important challenge to our better understanding of the associated prealbumin variants and their role in the mechanisms and sites of amyloid deposition.

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