Studies with radiolabelled serum amyloid P component provide evidence for turnover and regression of amyloid deposits \textit{in vivo}

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1. Quantitative scintigraphic and turnover studies, utilizing the specific binding affinity of serum amyloid P component for amyloid fibrils, have been developed as a tool for evaluating amyloid deposits \textit{in vivo}.

2. Serial studies in over 300 patients have shown characteristic, diagnostic tissue distributions of amyloid in different types of amyloidosis. There is generally a poor correlation between quantity of amyloid and associated organ dysfunction.

3. Contrary to previous expectations, regression of amyloid has been demonstrated systematically for the first time: AA, AL and variant transthyretin-associated amyloid deposits often regress rapidly, and sometimes completely, if the supply of fibril protein precursors is substantially reduced.

INTRODUCTION

Amyloidosis is a generic term for a heterogeneous group of disorders caused by the extracellular deposition of protein in a characteristic abnormal fibrillar form. There are acquired and hereditary types, and amyloid deposits may be focal, localized or systemic in distribution. When focal or small in amount, amyloid may be only an incidental finding at post mortem, and, indeed, such deposition in the brain, heart, seminal vesicles and joints is an almost universal accompaniment of ageing. In contrast, systemic amyloidosis or significant local deposition disrupts the normal structure and function of the affected tissues and is usually progressive. The systemic forms are frequently fatal.

Amyloid deposits are composed largely of protein fibrils, the peptide subunits of which differ in different forms of the disease and form the basis for the present nomenclature and classification of clinical amyloidosis syndromes. Examples include the acute-phase reactant serum amyloid A (SAA) in AA amyloidosis (formerly known as secondary amyloidosis), monoclonal immunoglobulin light chains in AL type (formerly known as primary amyloidosis) and genetic variants of transthyretin (TTR) which are associated with familial amyloid polyneuropathy (FAP). Glycosaminoglycans, predominantly of heparan sulphate and dermatan sulphate type, are always tightly, although non-covalently, associated with the fibrils. A non-fibrillar glycoprotein, amyloid P component (AP), is present as a minor constituent in all forms of amyloid. It is derived from and identical with serum amyloid P component (SAP), a member of the pentaxin family of plasma proteins, which includes C-reactive protein (CRP). SAP undergoes calcium-dependent binding to amyloid fibrils and is the same in all cases regardless of the fibril type. Although there are some correlations between fibril protein type and clinical manifestations, there are also many forms of acquired and hereditary amyloidosis in which there is little or no concordance between the fibril protein, or the genotype of its precursor, and the clinical phenotype. There are evidently other unknown genetic and/or environmental factors that determine whether, when and where clinically significant amyloid deposits form.

Clinically significant amyloidosis is not rare. Amyloid deposits in the brain and cerebral blood vessels are a central part of the pathology of Alzheimer’s disease, which is the fourth most common cause of death in the Western World, and amyloid is present in the islets of Langerhans of the
pancreas in most patients with Type II diabetes mellitus. Amyloid deposition in the bones, joints and periarticular structures eventually affects most patients who are on long-term haemodialysis for end-stage renal failure and is a frequent cause of serious morbidity among the approximately 500000 such individuals worldwide. Acquired systemic amyloidosis complicating myeloma and other B cell dyscrasias, or chronic infections and inflammatory diseases, is very important because of the difficulty which is often still experienced in making the diagnosis, its poor prognosis and the increasing availability of effective treatments. Hereditary amyloidosis is very rare, except in a few geographic foci, but is of importance as a model for understanding the pathogenesis of amyloid deposition.

Confirmation of the diagnosis of amyloidosis rests upon demonstration of the tissue amyloid deposits, which has traditionally necessitated histopathological examination of affected tissues. Material containing amyloid gives green birefringence when it is stained with Congo Red and viewed under crossed polarized light, and this characteristic universal property remains the gold standard by which other diagnostic techniques are judged in amyloidosis. Immunohistochemical staining of amyloidotic tissues remains the simplest method for identifying the amyloid fibril type. However, biopsies provide small samples of tissue which can only provide limited information on the anatomical distribution, extent and natural history of amyloid deposits. A major advance in clinical amyloidosis has been the development of radiolabelled SAP as an agent which specifically targets amyloid deposits in vivo. Combined scintigraphic imaging and metabolic analyses using labelled SAP have provided substantial new information on the natural history of many different forms of amyloid and their response to treatment [1-8]. For the first time the status of amyloid deposits throughout the body has been evaluated directly, rather than just the effects of these deposits on organ function. Most encouragingly, it has been clearly demonstrated in several different forms of amyloid that reduction of the supply of the protein precursors of amyloid fibrils can be followed by major regression of the deposits. Although many anecdotal accounts of such regression in AA amyloid and a few in AL amyloid have been reported, the unavoidable limitations of biopsies meant that direct confirmation of such regression was rarely available.

AP and SAP

Amyloid deposits in all different forms of the disease, both in man and in animals, contain the non-fibrillar glycoprotein AP. AP is identical with and derived from the normal circulating plasma protein, SAP. As a member of the pentraxin protein family it consists of ten identical non-covalently associated subunits, each of mass 25462 Da [9], which are non-covalently associated in two pentameric disc-like rings interacting face-to-face. SAP is a calcium-dependent ligand-binding protein, the best-defined specificity of which is for the 4,6-cyclic pyruvate acetal of D-galactose. It is therefore a lectin, but it also binds avidly and specifically to DNA [10], to chromatin [11] and to glycosaminoglycans [12], particularly heparan and dermatan sulphates, and this latter interaction may well underlie the deposition of SAP as AP in amyloid. Aggregated, but not native, SAP also binds specifically to C4-binding protein and fibronectin from the plasma, although SAP is not complexed with any other protein in the circulation. In addition to being a plasma protein, SAP is also a normal constituent of certain extracellular matrix structures. It is covalently associated with collagen and/or other matrix components in the lamina rara interna of the human glomerular basement membrane [13] and it is also present on the microfibrillar mantle of elastin fibres throughout the body [14].

The normal function of SAP is not known, nor is its role, if any, in the pathogenesis of amyloidosis. However, no deficiency of SAP has been described and it has been extremely stably conserved in evolution. There is a single copy of its gene on chromosome 1, and although there are reports of variation in a single amino acid residue, Ser- or Pro-82, mass spectrometric analyses of an extensive series of SAP preparations revealed only Ser-82 [9]. There is thus no significant polymorphism of the amino acid sequence, and furthermore the single biotennary oligosaccharide chain attached to Asn-32 is the most invariant glycan of any known glycoprotein [9]. This all suggests that SAP is likely to have important physiological function(s). The interaction with DNA, to which SAP is the single serum protein to undergo specific calcium-dependent bindings, and with chromatin are of particular interest in this regard. In binding to native long chromatin, SAP selectively and completely displaces histone H1, thereby solubilizing the chromatin [11]. Coupled with the fact that SAP from whole serum also binds specifically to chromatin within normal nuclei, and that SAP binds in vivo to extracellular deposits of chromatin, these observations suggest that SAP may participate in the handling of chromatin released by dead cells in vivo.

SAP is only produced by hepatocytes and its plasma concentration is tightly regulated: women, mean 24 (SD 8) mg/l, range 8-55 mg/l (n=274); men, mean 32 (SD 7) mg/l, range 12-50 mg/l (n=226) [15]. The value remains normal even during massive deposition of SAP in amyloid, indicating that even though SAP is not a major acute-phase protein, unlike its close homologue CRP, the rate of synthesis and secretion can be remarkably increased [3]. The normal plasma half-life of SAP is 24 h and in the absence of amyloid it is taken up and catabolized by hepatocytes. Persistence of SAP in the circulation is absolutely dependent on intact-
ness of its oligosaccharide, loss of the terminal sialic acid residues of which is associated with extremely rapid uptake and catabolism in the liver [9].

The three-dimensional structure of SAP has lately been solved to atomic resolution [16]. The tertiary fold of the subunit is dominated by antiparallel β-sheets forming a flattened β-barrel with jellyroll topology and a core of hydrophobic side chains. On one side of the jellyroll there is a short helix sitting above the Cys-36–Cys-95 disulphide bridge and adjacent to the Asn-32 glycosylation site. The calcium-binding site on the other side of the subunit consists of two loops coordinating two calcium ions 0.4 nm apart. Remarkably, the arrangement of β-strands in the SAP subunit is very similar to the subunit fold of concanavalin A and pea lectin, despite the absence of sequence similarity with these plant proteins. The SAP structure, with relatively short and tightly hydrogen-bonded loops joining the β-strands, also fits with the known resistance of SAP to digestion by proteinase. Despite some claims to the contrary, SAP is probably not itself a proteinase inhibitor; however, its own inherent resistance to degradation could well be of importance in amyloid deposits in vitro and it is clear that once associated with fibrils in the tissues SAP/AP is not susceptible to catabolism [3].

**SAP AS A SPECIFIC TRACER IN AMYLOIDOSIS**

The universal presence in amyloid deposits of AP, shown to be derived from circulating SAP [17–19], suggested the use of radioisotope-labelled SAP as a diagnostic tracer in clinical amyloidosis.

For clinical studies SAP was isolated in sterile, pure form from the plasma of single accredited donors to the British National Blood Transfusion Service. Donors in this scheme are prospectively and rigorously screened for transmissible conditions and the plasma was heat-treated before isolation of the SAP [1, 4]. The pure protein is oxidatively labelled with radiiodine using N-bromosuccinimide under conditions which preserve its function intact: $^{123}$I (half-life 13.2 h) is used for whole body scintigraphic imaging and $^{125}$I (half-life 60 days) for metabolic studies. In each $^{123}$I-imaging study patients receive an intravenous bolus of 100 μg of SAP carrying about 180 MBq of radioactivity, resulting in an effective dose equivalent of less than 4 mSv after thyroid blockade. More than 800 patient studies have now been performed in our unit and no adverse effects have been encountered.

In healthy individuals SAP is largely confined to the plasma compartment, from which it is cleared with a half-time of about 24 h [3]. SAP is metabolized exclusively in the liver [20] and in radioiodine tracer studies the associated radioactivity is released rapidly back into the circulation, mainly in the form of iodotyrosine; there is no retention or extravascular accumulation of radioactivity, all of which can be recovered in the urine within 14 days. The metabolism of SAP is unaltered in patients undergoing an acute-phase response or with other diseases. In patients with amyloidosis, labelled SAP is initially cleared from the plasma more rapidly, reflecting extravascular sequestration of the protein in the amyloid deposits. SAP is not significantly metabolized once it has localized to amyloid, and labelled SAP molecules have been shown to persist in this site unaltered for many months. The turnover studies indicate that plasma SAP is in a dynamic equilibrium with the AP within amyloid deposits; in most patients with clinically significant systemic amyloidosis the pool of SAP/AP within the amyloid is substantially larger than the normal plasma pool, by a factor of 200-fold in some cases. The localization of up to 95% or more injected labelled SAP in patients with advanced systemic amyloidosis thus represents a dilution phenomenon: extremely small numbers of radioactive SAP molecules are distributed among a vast excess of unlabelled native molecules within the plasma and the amyloid deposits alike. Since the latter compartment is much larger, most of the labelled SAP appears 'trapped' within the amyloid. However, there is free exchange of SAP between the two compartments during all phases of amyloid deposition and mobilization, as well as during the steady state.

$^{123}$I is a medium-energy, short half-life isotope with pure γ-emission, ideal characteristics for a scintigraphic imaging agent. High-resolution scans are obtained 24 h after $^{123}$I-SAP administration [1, 2] and show that, in healthy subjects and patients with diseases other than amyloidosis, the tracer is confined to the circulating blood pool. Whole body images thus show the major blood vessels and organs which contain a large blood volume; degraded radioactive material can be seen in the urinary bladder. In patients with amyloidosis uptake of $^{123}$I-SAP, diagnostic of amyloid, can be seen in one or more sites in the vast majority of cases (Fig. 1). Deposits can be readily identified in the kidneys, liver, spleen, adrenal glands, bone marrow and periarticular structures.

The characteristic organ distribution of amyloid differs in the different forms of the disease, which in some cases, for example bone marrow deposits in patients with AL type, is completely specific [2, 8]. Uptake of tracer into separate organs can be measured using computed region of interest analyses [6], and whole body retention of radioactivity, an index of the total quantity of amyloid within a patient, can also be determined scintigraphically [1, 3].

**SPECIFICITY, QUANTITATIVE ASPECTS AND CLINICAL APPLICATIONS OF LABELLED SAP STUDIES**

The binding properties of SAP to purified amyloid fibrils in vitro are characteristic of a protein-
ligand interaction; Scatchard analysis suggests that the binding constant is in the order of $10^{-10}/\text{mol}$ [4]. The mouse model of experimentally induced AA amyloid is analogous to reactive systemic amyloidosis in man, and has been used to confirm that radioiodinated pure human SAP behaves (and localizes to amyloid deposits) in vivo in a manner identical with the native unlabelled protein in fresh whole normal human serum [19]. Clinical scintigraphic studies in over 300 amyloid patients and 200 patients with other diseases have demonstrated that labelled SAP only localizes to amyloidotic organs and not unaffected tissues or to those involved in other disease processes. When labelled SAP scans and turnover studies are repeated in amyloid patients after a short interval, identical results are obtained [3]. There is no evidence of any interaction between labelled SAP and normal "tissue" AP, and nor is there any interaction between labelled control proteins, including albumin and CRP, and amyloid fibrils in vitro or in animal or clinical studies [21, 22].

SAP can be extracted from amyloidotic tissues in quantities which correlate with the amount of amyloid present in the tissue sample [19, 23]. No SAP can be extracted from normal tissues under similar conditions. Data obtained at post mortem from 15 patients with amyloidosis who, shortly beforehand, had undergone quantitative radioiodinated SAP studies, indicate that the uptake of labelled protein into tissue is proportional to both the amount of native SAP in these tissues and also to the quantity of amyloid estimated independently [23].

Labelled SAP imaging and limited turnover studies can be conveniently performed together and provide diagnostic results in more than 90% of patients with systemic amyloidosis. As a noninvasive technique with radiation dosimetry well within routine limits, SAP scintigraphy is evidently suitable as a method for screening patients known to be at risk of developing amyloidosis, such as those with chronic inflammatory diseases, monoclonal gammopathies and amyloidogenic gene mutations. Scans yield information on the organ distribution and quantity of amyloid, whereas turnover studies provide a measurement of the whole body amyloid burden, including the diffuse small (e.g. vascular) deposits which are beyond the resolution of scintigraphy. Serial studies have provided an effective method for elucidating the natural history of the amyloidoses, and to directly monitor the effects of treatment on the amyloid deposits themselves.

**RADIOLABELLED SAP STUDIES DEMONSTRATE TURNOVER AND REGRESSION OF SYSTEMIC AMYLOID DEPOSITS**

**AA amyloidosis**

SAP scintigraphy is completely specific and extremely sensitive for the diagnosis and evaluation of the tissue distribution of AA amyloid deposits [2, 7]. The spleen is always involved and is probably the first organ in which significant amyloid deposition occurs. Amyloid is demonstrable in the kidneys in at least 80% of cases, the adrenal glands in up to one-half and the liver about one-quarter (Fig. 1). Extensive liver and spleen uptake obscures visualization of the kidneys and adrenal glands in some patients. The scintigraphic appearance of AA amyloid deposits in patients with differing underlying inflammatory conditions is similar, although in all cases there is very limited correlation between the quantity of amyloid in any organ and the resulting degree of functional impairment, for example, some 5% of patients with AA amyloidosis have extensive renal deposits but do not have significant proteinuria. Moderate impairment of splenic and adrenal function is common, although liver function is rarely disturbed until very large quantities of amyloid are present. The majority of visceral organs affected by AA amyloid are not clinically enlarged. The early and universal involvement of the spleen renders the SAP scan useful for screening patients with suspected AA amyloidosis.
Sub-clinical deposits were identified in two out of 40 patients with rheumatoid arthritis attending our own rheumatology clinic.

Prospective serial SAP isotope studies have shown that AA amyloid is progressive in most patients in whom the underlying inflammatory disorder remains active. The rate of accumulation, however, varies considerably, not only among different individuals but also between different organs within the same individual. In some patients with rheumatoid arthritis complicated by AA amyloidosis, the whole body load of amyloid may double over a 2 year period in the presence of a median plasma SAA level of 100 mg/l, whereas occasionally in others there may be no appreciable change at all. Indeed, in a few patients the quantity of amyloid may progressively increase for a period and then reach a plateau level despite there having been no change in clinical inflammatory activity or the acute-phase response. This phenomenon has also been documented in one individual with subclinical amyloidosis [7].

Among patients with AA amyloid in whom the underlying acute-phase response had subsided, either spontaneously or after treatment, serial scans over a 2–3 year period have shown an absence of progression of amyloid in about one-half of cases, and regression in the remainder. The rate of regression varied considerably from patient to patient, but some cases with very extensive deposits at presentation gave near normal scans within 3 years. In most individuals regression of AA amyloid is accompanied by clinical benefits, for example, reduction of proteinuria.

AL amyloidosis

In a series of 100 patients with systemic AL amyloidosis [8], labelled SAP studies demonstrated that the deposits were considerably more heterogeneous with respect to their anatomical distribution, quantity, rate of progression and response to treatment than in AA type. Positive scans were specific for amyloidosis, but were diagnostic in only 85% of cases with histologically proven AL amyloid. Deposits were identified in the spleen in 64% of cases, the liver 49%, kidneys 25%, carpal tunnel region 23% and adrenal glands in 5%. Bone and bone marrow images were obtained in one-third of patients and have not been observed in patients with any other type of amyloidosis. Disappointingly, cardiac deposits were identified only occasionally, probably as a result of motility of the heart, ventricular blood pool activity, chest wall attenuation of signal and possibly delayed passage of labelled SAP across the non-fenestrated myocardial capillary endothelium [23]. Most of the AL patients in whom negative scans were obtained had clinical evidence of amyloid limited to a single organ system, such as the heart, peripheral and autonomic nerves, lungs, muscle, skin or lymph nodes. However, the majority of patients with cardiac amyloid had substantial deposits in other organs which were imaged readily. There have been no studies as yet in which asymptomatic individuals with monoclonal gammopathy have been screened for AL amyloid using these techniques.

Serial studies, performed at 6 monthly intervals, have been obtained in 27 patients, of whom 18 underwent cytotoxic chemotherapy. Regression of amyloid was observed in one-third of treated cases (Fig. 2) [6, 8], but in no untreated case. An absence of progression was noted in one-third of both treated and untreated individuals. The rate of accumulation of amyloid in patients with progressive disease was often slower than expected given the speed with which clinical deterioration typically occurs in systemic AL amyloidosis. Preliminary observations suggest that a favourable labelled SAP study result, that is, a small whole body amyloid load, lack of progression or regression of deposits, is an indicator of a better prognosis.

Hereditary systemic amyloidosis

In most patients with familial amyloid polyneuropathy due to mutations in the gene for plasma TTR,
there are major visceral amyloid deposits which can be imaged and quantified. Renal deposits are demonstrable by SAP scanning in most cases, the spleen in two-thirds and the adrenal glands in one-third. Cardiac amyloid deposits cannot be readily identified (although they are frequently present), and the small peripheral and autonomic nerve deposits are also beyond the limits of detection of this method.

A much rarer clinical syndrome is hereditary non-neuropathic systemic amyloidosis, a form of which was first described by Ostertag [24] in 1932. This disorder is now known to be caused by various point mutations in the genes of at least three different plasma proteins, namely apolipoprotein A1 [25-27], lysozyme [28] and the α-chain of fibrinogen [29]. Major visceral amyloid deposits, variably involving the liver, spleen, kidneys and adrenal glands, have been shown by SAP scintigraphy in all affected individuals. As in every other type of amyloidosis, the correlation between the amount of amyloid and resulting organ dysfunction in hereditary amyloidosis is poor. Extensive amyloid deposits have been demonstrated by SAP scintigraphy in many apparently healthy carriers of amyloidogenic gene mutations, some individuals having now been followed up for 5 years without clinical manifestations of disease (Fig. 1). Longer term studies of affected patients and gene carriers should shed considerable light on the penetrance and natural history of these disorders.

Hereditary amyloidosis due to variants of TTR are potentially amenable to treatment by orthotopic liver transplantation, since, although transthyretin is also produced in the choroid plexus, the liver is the only source of plasma TTR [30]. So far more than 70 liver transplants have been performed for FAP-associated with variant TTR [30a] and SAP scintigraphy has provided objective evidence of regression of visceral amyloidosis in several such cases [31].

DISCUSSION

Scintigraphic and turnover studies with radiiodinated SAP, based on the affinity of SAP for all types of amyloid fibril, are new specific methods for confirming the presence of amyloid in tissues. Labelled SAP scans survey the whole body macroscopically for the presence and anatomical distribution of amyloid in a quantitative manner, and SAP turnover studies provide information on the whole body amyloid load. The technique has been used prospectively in over 400 patients with amyloid and has already provided a number of new insights into the natural history of amyloidosis. These include the observation that there is a consistently poor correlation between the quantity of amyloid in an organ and the resulting degree of functional impairment. Amyloid deposits accumulate at rates which vary substantially between different organs in a single subject and between individuals with similar types of amyloidosis, even when the rates of amyloid fibril precursor protein supply are apparently similar. In some patients amyloid accumulation may plateau without any measurable alteration in the precursor supply. In patients with amyloidosis in whom the supply of fibril precursors is reduced, either as a result of therapy directed towards the underlying process or through a natural remission, substantial regression of amyloid frequently occurs. This has been observed over periods of 6 months to 3 years in patients with AA, AL and variant TTR-associated amyloidosis, and is usually associated with clinical benefits. In some such cases, however, the function of affected organs may continue to deteriorate despite halting the accumulation of amyloid, presumably because irreversible structural damage has already occurred.

These findings indicate that amyloid deposits may be mobilized from tissues and, therefore, that they may always be in a dynamic state of turnover. The natural history of most forms of amyloidosis is that deposition far exceeds the capacity for mobilization, an observation which has previously fuelled the widespread belief that amyloid deposition is irreversible.

Encouragingly, it has lately been possible to label SAP with 99mTc, an inexpensive and universally available γ-emitting isotope, to produce a tracer which gives images of quality comparable with those obtained with radiiodine [32]. It is also anticipated that knowledge of the three-dimensional structure of SAP may permit the development of designer low-molecular-mass analogues of the protein.

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