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INVITED REVIEW

Amyloidosis

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Amyloidosis

Amyloidosis is a heterogeneous group of disorders characterized by extracellular deposition of abnormal protein fibrils which are derived from different proteins in different forms of the disease. Asymptomatic amyloid deposition in a variety of tissues is a universal accompaniment of ageing, and clinical amyloidosis is not rare. Intracerebral and cerebrovascular β-protein amyloid deposits are a hallmark of the pathology of both sporadic and familial Alzheimer's disease, β_2-microglobulin-derived amyloid is a common complication of long term haemodialysis, and islet amyloid polypeptide is the fibril protein in the universal islet amyloidosis of type II diabetes mellitus. New fibril proteins have lately been identified in hereditary amyloidosis, including variants of gelsolin, apolipoprotein AI, lysozyme and fibrinogen. The development of radiolabelled serum amyloid P component (SAP) scintigraphy has allowed amyloid to be diagnosed non-invasively *in vivo* for the first time, provided unique insight into the distribution and size of amyloid deposits, and yielded novel information on the natural history and the effects of treatment. Amyloid deposits are in a state of dynamic turnover and can regress if new fibril formation is halted. The recent elucidation of the three dimensional structure of human SAP may enable the design of specific therapeutic agents.

Keywords: amyloid, amyloidosis

Introduction

Amyloidosis is a disorder of protein metabolism characterized by the extracellular deposition of abnormal protein fibrils[1–3]. It may either be localized, or systemically distributed throughout the body (Tables 1 and 2), causing organ damage and serious morbidity. Major visceral involvement, especially of the kidneys and heart, is usually fatal.

The fibrils which form the bulk of amyloid deposits are derived from a variety of precursor proteins in different forms of the disease but are always intimately associated with sulphated glycosaminoglycans. In addition, the normal circulating glycoprotein, serum amyloid P component (SAP), specifically binds to the fibrils and is a universal constituent of amyloid deposits.

Amyloid is defined by its pathognomonic tinctorial properties with the dye Congo red[4], and by its characteristic ultrastructural morphology. The development of a method[5] for isolating and purifying amyloid fibrils from tissue permitted the precise identification and characterization of the fibril proteins, and amyloidosis is now classified on this basis[6] rather than according to clinicopathological features, as in the past.

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Clinical amyloidosis syndromes

AA (REACTIVE) AMYLOIDOSIS

Diseases complicated by AA amyloidosis are characterized by their ability to provoke a sustained acute phase response and include chronic inflammatory disorders, chronic local or systemic microbial infections, and malignant neoplasms (Table 3). Amongst the connective tissue diseases, SLE is unique in that it is only very exceptionally complicated by AA amyloidosis and a

Table 1. Acquired amyloidosis syndromes

Clinical syndrome	Fibril protein
Systemic AL amyloidosis, associated with immunocyte dyscrasia, myeloma, monoclonal gammopathy, occult dyscrasia	AL fibrils derived from monoclonal immunoglobulin light chains
Local nodular AL amyloidosis (skin, respiratory tract, urogenital tract, etc) associated with focal immunocyte dyscrasia	AL fibrils derived from monoclonal immunoglobulin light chains
Reactive systemic AA amyloidosis, associated with chronic inflammatory diseases	AA fibrils derived from serum amyloid A protein (SAA)
Senile systemic amyloidosis	Transthyretin (TTR) derived from plasma TTR
Focal senile amyloidosis:	
atria of the heart	Atrial natriuretic peptide
brain	β-protein
joints	Not known
seminal vesicles	Seminal vesicle exocrine protein
prostate	β_2-microglobulin
Non-familial Alzheimer's disease, Down's syndrome	β-protein derived from β-amyloid protein precursor (APP)
Sporadic cerebral amyloid angiopathy	β-protein derived from β-amyloid protein precursor (APP)
Inclusion body myositis	β-protein derived from β-amyloid protein precursor (APP)
Sporadic Creutzfeldt-Jakob disease, kuru (transmissible spongiform encephalopathies, prion diseases)	Prion protein (PrP) derived from prion protein precursor
Type II diabetes mellitus	Islet amyloid polypeptide (IAPP), amylin, derived from its precursor protein
Endocrine amyloidosis, associated with APUDomas	Peptide hormones or fragments thereof (e.g. precalcitonin in medullary carcinoma of thyroid)
Haemodialysis-associated amyloidosis; localized to osteoarticular tissues or systemic	β_2-microglobulin derived from high plasma levels
Primary localized cutaneous amyloid (macular, papular)	?Keratin-derived
Ocular amyloid (cornea, conjunctiva)	Not known
Orbital amyloid	Not known

similar contrast exists between Crohn's disease in which amyloid occurs in 1 to 8% of cases and ulcerative colitis in which it is extremely rare.

AA amyloid deposition has a predilection for parenchymal organs and may be widely distributed without causing symptoms[7]. The spleen is always affected but the common involvement of the kidneys is most closely associated with an adverse prognosis and usually presents with non-selective proteinuria due to amyloid deposition in the renal glomeruli. This may lead to a nephrotic syndrome before terminating in end stage renal failure. The second most common presentation is organomegaly. Involvement of the heart and gastrointestinal tract is frequent though it rarely causes functional impairment[1].

AL (MONOCLONAL IMMUNOGLOBULIN) AMYLOIDOSIS

AL amyloid may be associated with almost any dyscrasia of the B lymphocyte lineage ranging from frank malignancy of plasma cells (multiple myeloma) to 'benign' monoclonal gammopathy in which the only demonstrable abnormality may be the overproduction of monoclonal light chains. In some cases AL amyloid may be the only evidence of an underlying plasma cell dyscrasia. AL amyloid occurs in up to 15% of cases of myeloma and in a lower proportion of other plasma cell disorders[8].

AL amyloid affects principally the mesenchymal tissues causing peripheral and autonomic neuropathy, carpal tunnel syndrome, macroglossia, restrictive cardiomyopathy and arthropathy of the large joints, although visceral organs or indeed any tissue may be involved. It has a poor prognosis especially when the heart is involved (survival for 6–18 months) and usually presents as a restrictive cardiomyopathy[9].

HEREDITARY SYSTEMIC AMYLOIDOSIS

There are many forms of hereditary systemic amyloidosis

Table 2. Hereditary amyloidosis syndromes

Clinical syndrome	Fibril protein
Predominant peripheral nerve involvement, familial amyloid polyneuropathy (FAP). Autosomal dominant	Transthyretin (TTR) genetic variants (most commonly Met30, but over 40 others described)
Predominant peripheral nerve involvement, familial amyloid polyneuropathy (FAP). Autosomal dominant	Apolipoprotein AI (apoAI) N-terminal fragment of genetic variant Arg26
Predominant cranial nerve involvement with lattice corneal dystrophy. Autosomal dominant	Gelsolin, fragment of genetic variant Asn187 or Tyr187
Non-neuropathic, prominent visceral involvement (Ostertag-type). Autosomal dominant	ApoAI, N-terminal fragment of genetic variants Arg26, Arg 50, Arg60, etc.
Non-neuropathic, prominent visceral involvement (Ostertag-type). Autosomal dominant	Lysozyme genetic variant Thr56 or His67
Non-neuropathic, prominent visceral involvement (Ostertag-type). Autosomal dominant	Fibrinogen α-chain, fragment of genetic variants, Leu554 or Val526
Predominant cardiac involvement, no clinical neuropathy. Autosomal dominant	TTR genetic variants Thr45, Ala60, Ser84, Met111, Ile 122
Hereditary cerebral haemorrhage with amyloidosis (cerebral amyloid angiopathy). Autosomal dominant	
Icelandic type (major asymptomatic systemic amyloid also present)	Cystatin C, fragment of genetic variant Glu68
Dutch type	β-protein derived from genetic variant APP Gln693
Familial Alzheimer's disease	β-protein derived from genetic variant APP Ile717, Phe717 or Gly717
Familial dementia—probable Alzheimer's disease	β-protein derived from genetic variant APP Asn670, Leu671
Familial Creutzfeldt-Jakob disease, Gerstmann-Sträussler-Scheinker syndrome (hereditary spongiform encephalopathies, prion diseases)	Prion protein (PrP) derived from genetic variants of PrP precursor protein 51-91 insert, Leu 102, Val117, Asn178, Lys200
Familial Mediterranean fever, prominent renal involvement. Autosomal recessive	AA derived from SAA
Muckle-Well's syndrome, nephropathy, deafness, urticaria, limb pain	AA derived from SAA
Cardiomyopathy with persistent atrial standstill	Not known
Cutaneous deposits (bullous, papular, pustulodermal)	Not known

and most of them are rare. However, autosomal dominant inheritance and the adult onset of symptoms have ensured persistence of the traits which cause this devastating disorder. All the various syndromes are characterized by point mutations leading to the production of variant amyloidogenic proteins. Variant transthyretin, causing familial amyloid polyneuropathy, was the first of these proteins to be identified[10] and more than 40 mutations in the transthyretin gene have since been described[1]. Mutations in the apolipoprotein AI[11,12] and gelsolin[13] genes also cause neuropathic systemic amyloid. Recently, mutations have been discovered in the lysozyme[14] and α-fibrinogen[15] genes, leading to expression of amyloidogenic variant proteins in patients with Ostertag-type, non-neuropathic amyloidosis.

Familial Mediterranean fever is an autosomal recessive inflammatory disease that may be complicated by AA amyloidosis especially in some ethnic groups.

SENILE SYSTEMIC AMYLOIDOSIS

Some amyloid is present in all aged individuals. It may be systemic, with deposits of fibrils composed of normal wild type transthyretin, but it is most abundant in the heart. These may cause cardiac failure but usually they are clinically silent. Asymptomatic focal deposits in the brain (β-protein), corpora amylacea of the prostate (β_2-microglobulin), joints and seminal vesicles (precursor protein(s) unknown) are also common[1].

Table 3. Conditions associated with reactive systemic (AA) amyloidosis

Chronic imflammatory disorders
Rheumatoid arthritis
Juvenile chronic arthritis
Ankylosing spondylitis
Psoriasis and psoriatic arthropathy
Reiter's syndrome
Adult Still's disease
Behçet's syndrome
Crohn's disease
Chronic microbial infections
Leprosy
Tuberculosis
Bronchiectasis
Decubitus ulcers
Chronic pyelonephritis in paraplegics
Osteomyelitis
Whipple's disease
Malignant neoplasms
Hodgkin's disease
Renal carcinoma
Carcinomas of gut, lung, urogenital tract
Basal cell carcinoma
Hairy cell leukaemia

DIALYSIS RELATED AMYLOIDOSIS

Amyloid in which the fibrils consist of β_2-microglobulin[16] affects a large proportion of patients receiving long term haemodialysis and it has also been reported in patients on continuous ambulatory peritoneal dialysis[17] and even in a patient with chronic renal failure but not yet on dialysis[18]. Initially thought to be confined to joints, bones and periarticular tissues, deposits of β_2-microglobulin in many other tissues and several cases of fatal extensive disease have been reported. It causes carpal tunnel syndrome, arthralgias of the large joints and bone cysts often leading to pathological fractures. Renal transplantation is the only effective treatment with rapid normalization of blood β_2-microglobulin levels and resolution of symptoms.

LOCALIZED AMYLOIDOSIS

Deposits of amyloid localized to particular organs or tissues occur in a wide variety of different forms and are presumably consequent on either the local production of fibril precursors (nodular AL amyloid, cutaneous amyloid, endocrine amyloid) or the properties of a particular microenvironment that predisposes to localization and fibril formation of systemically distributed circulating precursor proteins. For example, the bony/periarticular β_2-microglobulin deposits in dialysis related amyloid. Many local forms of amyloid are common accompaniments of ageing and are rarely of clinical significance. Protein precursors of tumour related amyloid remain unidentified[19] but are probably derived from tumour related or locally produced proteins such as keratin.

CEREBRAL AMYLOIDOSIS

The brain and intracerebral blood vessels are rarely affected in the systemic amyloidoses but are common and important sites for the local deposition of amyloid (Table 4) in the absence of amyloid elsewhere in the body. The most frequent and important type of amyloid in the brain is that related to Alzheimer's disease, which is the commonest cause of dementia. The vast majority of cases of Alzheimer's disease are sporadic but there are also families with an autosomal dominant pattern of inheritance[20,21]. The triad of cerebral amyloid angiopathy, neurofibrillary tangles and neuritic plaques seen in Alzheimer's disease is also seen in the dementia that occurs in all Down's syndrome patients over the age of forty

Table 4. Cerebral amyloidosis

Age-related amyloid angiopathy with or without intracerebral deposits
Hereditary amyloid angiopathy of meningeal and cortical vessels associated with cerebral haemorrhage:
a Icelandic type; **b** Dutch type
Hereditary amyloid angiopathy affecting the entire CNS
Alzheimer's disease: sporadic, familial or associated with Down's syndrome
Cerebral amyloid associated with prion disease
Sporadic spongiform encephalopathy: Creutzfeldt-Jakob disease (CJD)
Familial prion disease: familial CJD, Gerstmann-Sträussler-Scheinker (GSS) disease and atypical familial prion disease
Prion disease in animals
Familial oculoleptomeningeal amyloidosis

Table 5. Amyloidogenic and non-amyloidogenic variants of transthyretin

10	20	30	40
GPTGTGESKC	PLMVKVLDAV	RGSPAINVAV	HVFRKAADDT
R	***E***	***M***	***I P***
		A	***L***
		L	
S			
50	60	70	80
WEPFASGKTS	ESGELHGLTT	EEEFVEGIYK	VEIDTKSYWK
G T R AR	***P GP HKA***	***K L LHN***	***A Y***
A I	***R***		
V			
90	100	110	120
ALGISPFHEH	AEVVFTANDS	GPRRYTIAAL	LSPYSYSTTA
S QN	***G***	***V***	***M C***
N			
		R T	M
		V	
127			
VVTNPKE			
I			

The wild type sequence of transthyretin is shown above. Amino acid substitutions shown in bold italics below are associated with transthyretin amyloidosis; others shown in normal type are not.

years[22]. These intracerebral and cerebrovascular amyloid deposits are hallmarks of the neuropathological diagnosis and are derived from the amyloidogenic β-protein fragment of the so-called amyloid precursor protein (APP).

Fibril proteins and their precursors

AA

Amyloid A protein (AA) which has a molecular weight of around 8000 daltons is derived from the circulating precursor serum amyloid A (SAA) by proteolytic cleavage. SAA, an apolipoprotein of HDL particles, is an acute phase reactant. Synthesis of SAA by hepatocytes is regulated by cytokines especially IL-1, IL-6 and TNF and its concentration may increase by up to several thousand fold during the acute phase response but its function is not known. SAA is polymorphic but there is no evidence that any particular form is either more or exclusively amyloidogenic.

AL

AL proteins consist of either the whole molecule or fragments of monoclonal immunoglobulin light chains, and in any individual case there may be a mixture of these. The fragments are derived from the N-terminal region and consist of all, or part, of the variable (VL) domain. The L chain of the circulating or urinary monoclonal paraprotein is either identical to, or clearly the precursor of, AL isolated from the amyloid deposits. AL is more commonly derived from λ than from κ chains despite the fact that the latter predominate among normal immunoglobulins and the paraprotein products of immunocyte dyscrasias.

TRANSTHYRETIN

Transthyretin (TTR), formerly known as prealbumin, is produced by the liver and choroid plexus and is involved in the transport of thyroid hormones and vitamin A. Genetic variants of the TTR molecule, all involving a single amino acid substitution, are the most common cause of hereditary amyloidosis (Table 5). TTR is composed almost entirely of β-sheets, is an inherently amyloidogenic molecule and little change is apparently required for this property to be greatly enhanced.

β-PROTEIN

The fibril protein in the intracerebral and cerebrovascular amyloid of Alzheimer's disease, Down's syndrome

and hereditary amyloid angiopathy of Dutch-type, is β-protein, a 39–43 residue sequence derived by proteolysis from a high molecular weight precursor protein (APP) encoded on the long arm of chromosome 21. There is controversy over whether or how the β-protein fragment *per se*, or the amyloid fibrils which it forms, contribute to the neuronal dysfunction and damage which underlie the dementia. However, the fact that APP mutations may cause Alzheimer's disease[20,21] and produce the same neuropathology as sporadic cases, including tangles, argues strongly that the APP and β-protein pathway is of primary pathogenetic significance. Further support for this concept comes from the fact that all individuals with Down's syndrome (trisomy 21) over the age of forty years develop typical Alzheimer's disease[22]. Since APP is coded by a gene on chromosome 21 it seems that excessive gene dose with wild-type APP can have the same effect as the APP variants in familial Alzheimer's disease.

β_2-MICROGLOBULIN

β_2-microglobulin is the amyloid fibril protein of dialysis-related amyloidosis[16]. It is a non-polymorphic single chain polypeptide with a molecular weight of 11 800 daltons, and is the light chain of Class I MHC antigens present on all nucleated cells. It is continually shed from cell membranes and is normally filtered by the kidney and catabolised in the proximal tubule. The failure of haemodialysis membranes to clear β_2-microglobulin efficiently leads to persistently raised plasma levels and this presumably predisposes to its deposition as amyloid fibrils. The strong affinity of β_2-microglobulin for collagen *in vitro* may explain the striking predisposition for β_2-microglobulin amyloid to deposit in the collagen rich tissues of joints[23].

POLYPEPTIDE HORMONES

Amyloid often occurs in endocrine organs in connection with ageing and in some polypeptide hormone secreting tumours. The localized amyloid deposits occurring in certain polypeptide hormone secreting tissues consist mainly of hormone or pro-hormone. Examples are calcitonin related amyloid in medullary carcinoma of the thyroid, islet amyloid polypeptide derived amyloid which occurs in 95% of patients with Type II diabetes[24] and atrial natriuretic peptide in isolated atrial amyloidosis.

Nature of amyloid deposits

FIBRILLAR COMPONENTS

Amyloid is defined histologically by its binding of the dye Congo red and the subsequent green birefringence when viewed under polarized light[4]. This unique property resides in the fibrils themselves and is retained in isolated fibril preparations. Most, though not all, proteins which are precursors of amyloid fibrils are rich in β-sheet secondary structure. This, together with the apparent resistance of amyloid fibrils to proteolysis, led to the concept that amyloid fibrils consist exclusively of a stack of anti-parallel β-pleated sheets arranged with their long axis perpendicular to the long axis of the fibril, resembling the structure proposed for silk, which is also highly proteinase resistant. However, fibrillar morphology *per se* does not require β-pleated sheet structure, and X-ray fibril diffraction studies suggest that a shared repeating structure in different amyloid fibrils may derive from similar intermolecular packing motifs rather than a shared secondary structure.

Another feature of all amyloid deposits is the universal presence of sulphated glycosaminoglycans (GAGs) which are probably laid down simultaneously with the fibrils. These fibril-associated GAGs are heparan sulphate and dermatan sulphate in all forms of amyloid which have been investigated[25]. Fibrils isolated by water extraction contain 1–2% by weight of GAG carbohydrate, none of which is covalently associated with the protein. The significance of GAGs remains unclear but it is possible that they may be the ligands to which SAP, another universal constituent of amyloid deposits, binds.

NON-FIBRILLAR COMPONENTS

Human serum amyloid P component (SAP), a decameric plasma glycoprotein, binds in a calcium dependent manner to all forms of amyloid fibril and is universally present in amyloid deposits[26], including the cerebral amyloid of Alzheimer's disease. SAP which is produced only by the liver is also a normal constituent of glomerular basement membrane and of elastic microfibrils.

SAP is remarkably resistant to proteolytic degradation in the presence of calcium. This resistance to proteinase digestion is likely to be an important aspect of the normal function of SAP, and may also contribute to the persistence of amyloid deposits by protecting them against digestion. Protection could result simply from coating of the abnormal amyloid fibrils by SAP, which is completely unaltered with respect to its normal circulating form, and which would, therefore, not be

expected to trigger macrophage activation and phagocytosis. However, the proteinase resistance of SAP itself may be a significant factor.

The three dimensional structure of SAP has recently been solved by atomic resolution[27]. Availability of the complete structure to 2Å resolution of SAP and determination of its ligand binding sites now offer the opportunity of direct modelling of competitive inhibitors of SAP binding and for producing binding site homologues, either of which could be used as drugs to displace SAP from amyloid deposits *in vivo*. This would open up new avenues for the treatment of amyloidosis, enabling the body to mobilize and degrade the fibrils which may otherwise be inappropriately protected by SAP.

Fibril formation

A necessary condition for the formation and deposition of amyloid fibrils is the presence of an autologous protein precursor, which is either circulating or produced locally, and which is abnormal either in structure, concentration or both. Until recently, it was generally accepted that the pathogenesis of amyloid fibril deposits involved the partial proteolytic cleavage of qualitatively or quantitatively abnormal precursor proteins into fragments with a propensity for aggregating into anti-parallel β-pleated sheets[2,3]. However, there is now evidence that intact undegraded variant transthyretin[28] and β_2-microglobulin[29] molecules can aggregate to form fibrils in familial amyloid polyneuropathy and dialysis related amyloidosis respectively.

Although the pathogenesis of amyloid fibril formation is not known, the primary structure of precursor proteins is crucial in determining their amyloidogenicity. This is clearly shown by the hereditary amyloidoses involving genetic variants of proteins in which single amino acid substitutions convert the non-amyloidogenic wild type molecules into highly amyloidogenic variants.

The presence of precursor proteins is obviously necessary for fibril formation but the factors that determine their deposition as amyloid fibrils in some individuals but not others, and the variation in the time and anatomical distribution of deposits, are not known. Mice given repeated inflammatory stimuli over a period of three weeks or more eventually develop AA amyloidosis but the latent period can be reduced to 1–2 days in mice which have previously received a single intravenous injection of an extract of amyloidotic tissue. The nature and mode of action of this so called amyloid enhancing factor[30] is not known, but it may be responsible for the marked individual variation in susceptibility to fibril formation and elucidation of the processes involved will clearly be of considerable clinical relevance.

Fibril degradation

The natural history of amyloid deposits is to persist and accumulate. This apparent resistance to proteolytic degradation has been ascribed to the silk-like antiparallel β-pleated sheet structure of the fibril proteins, and it is also possible that the universal presence of SAP, in an apparently native unaltered state, may be involved in some way. However, amyloid deposits can regress once supply of the precursor proteins is reduced. Evidence for this was previously available only from histological studies in isolated case reports[31]. However, there are now prospective data from serial studies with ^{123}I-SAP scans demonstrating regression of amyloid deposits following treatment to reduce precursor protein levels in patients with AA, AL, β_2-microglobulin and hereditary transthyretin amyloidosis[7,32–35].

Diagnosis

HISTOLOGICAL

Congo red staining

Since the original method of Congo red staining was first described[36], it has undergone several modifications to improve its sensitivity, specificity, and reliability[4]. It has been suggested that apple green birefringence in polarized light, the unique optical property of Congo red stained amyloid, depends on the β-pleated sheet configuration of the peptide chains in the fibrils[37], allowing planar dye molecules to fit edgewise into the face of the pleated sheets with their long axes in the axis of the filament. However, the structure of amyloid fibril-Congo red complexes has not yet been solved. Other methods of detection of amyloid include fluorescent stains, such as thioflavin T or S, and metachromatic stains such as crystal violet. The fluorescent dyes, because of their high sensitivity, may be helpful in screening tissues for amyloid deposits but positive results should be confirmed by more specific methods.

Sensitivity and specificity. The optical properties of Congo red stained amyloid may be affected in very thin or very thick sections, with loss of green birefringence. A thickness of 5–10 μm is optimal. Congo red staining of amyloid is more intense in tissues fixed in alcohol or Carnoy's solution than those fixed in formalin but this may lead to a higher incidence of false positive results. Staining of non-amyloid components may be removed by alcohol and this effect is enhanced by the addition of sodium hydroxide. False positive staining may occur with collagen and this may be avoided by saturation of the staining solution with NaCl. Congo red solutions are

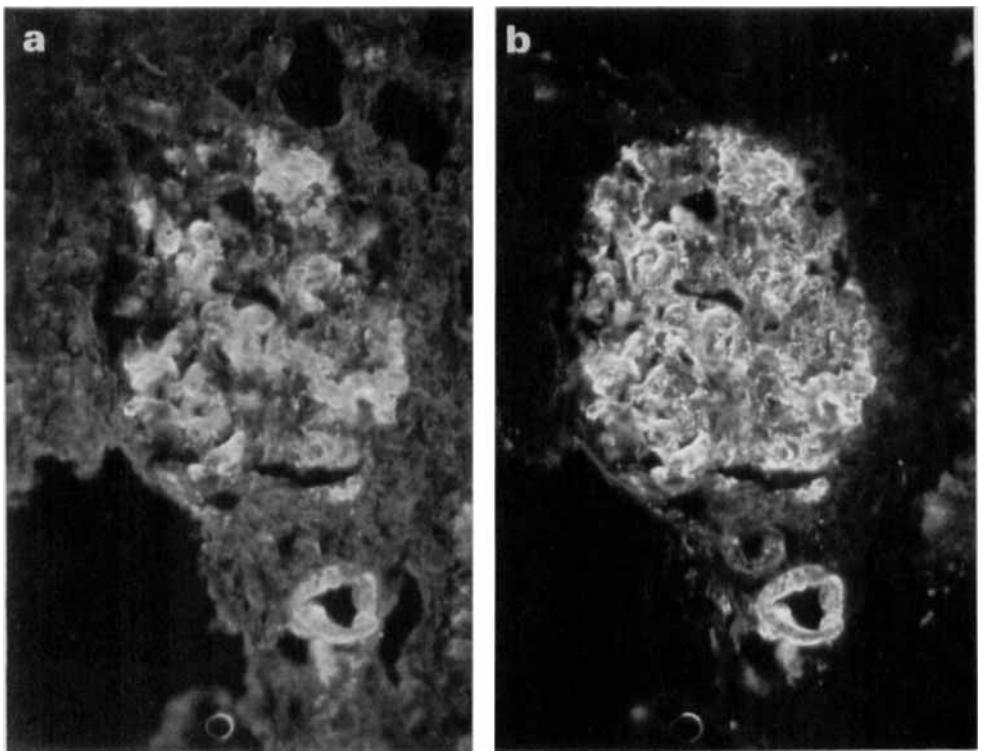

Figure 1. Renal glomerular amyloid deposits in a case of AA amyloidosis complicating renal adenocarcinoma. **a** immunofluorescence staining with rhodamine-labelled rabbit anti-human AA antibodies. **b** same field stained with fluorescein-labelled sheep anti-human SAP antibodies. ×400.

unstable and should be freshly prepared every 2 months. Good microscopic technique, knowledge of the variables involved in Congo red staining and inclusion of positive and negative controls in every staining run is, therefore, critical to the sensitivity and specificity of the technique.

Classification of amyloid proteins on the basis of Congo red staining. Congo red staining after autoclaving or treating the tissue with potassium permanganate or alkaline guanidine has been used to subclassify amyloidosis[38] but these non-specific techniques are unreliable and are no longer appropriate. They have been rendered obsolete by the general availability of immunochemical methods which can precisely identify and classify amyloid proteins in tissues.

Immunohistochemical methods for the detection and classification of amyloid

Antibodies against all known amyloid fibril proteins are available and can be used in both immunofluorescence (Figure 1) and immunoperoxidase techniques. The immunoflorescence method requires the use of frozen sections. The immunoperoxidase technique is more sensitive and has the advantage of being able to detect amyloid in formalin fixed paraffin embedded tissues. However, formalin fixation may mask the epitopes of amyloid proteins especially in AL and transthyretin-related amyloid. Pre-treatment of sections with trypsin may be necessary to break down the cross-linking bridges and 'unmask' the epitopes although the process itself may damage and alter the antigenic determinants of the proteins[39]. Antigenicity is much better preserved with frozen sections. In AL amyloidosis, only about half of the cases are stainable with standard antisera to κ or λ light chains probably because the light chain fragment is usually derived from the variable domain and may contain unique epitopes that are not recognized by antibodies mainly directed against constant domain determinants. Although fibril proteins can be identified immunohistochemically and enable the amyloid to be classified, the demonstration of amyloidogenic proteins in tissues does not on its own establish the presence of amyloid. Congo red staining with green birefringence is always required.

Electron microscopy

Under the EM, fibrils are rigid, non-branching and 10–15 nm in diameter. Each fibril is in turn composed of aggregates of 2 to 5 filaments, arranged in a twisted ribbon pattern. However, amyloid fibrils cannot always be convincingly identified ultrastructurally, and EM alone is not sufficient for the diagnosis of amyloidosis.

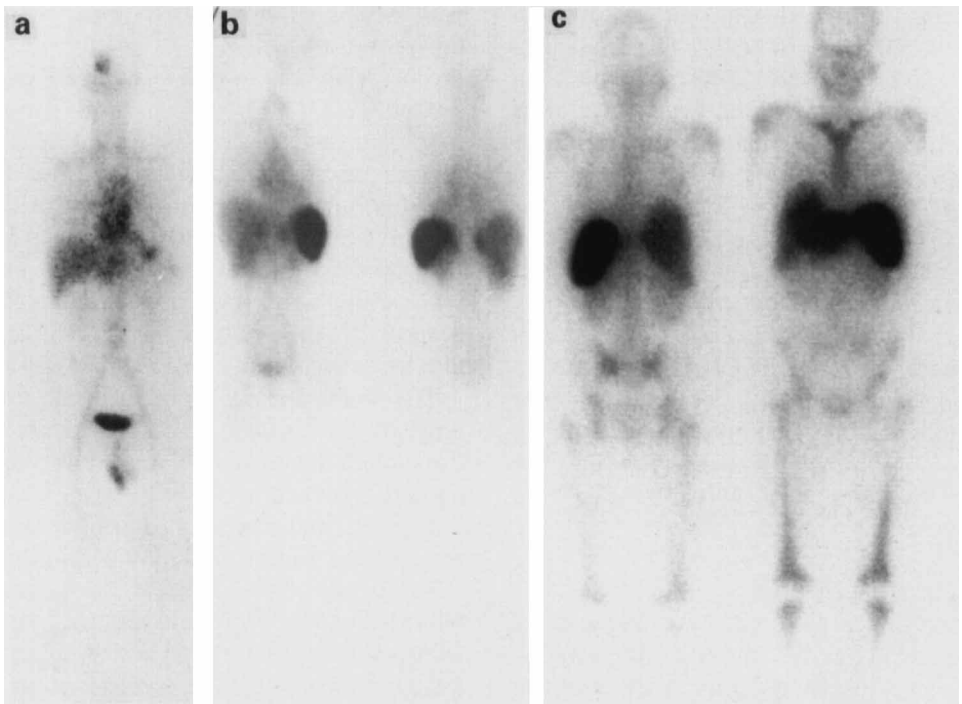

Figure 2. Whole body scintigraphs 24 h after intravenous injection of ^{123}I-labelled human SAP. **a** anterior view of normal control subject showing distribution of residual tracer in the blood pool and radioactive breakdown products in urine in the bladder, note the absence of localization or retention of tracer anywhere in the body. **b** anterior (left) and posterior (right) views of patient with juvenile chronic arthritis complicated by AA amyloidosis. There is uptake of tracer in the spleen, kidneys and adrenal glands, a typical distribution of AA amyloid in which the spleen is involved in 100% of cases, kidneys in 75% and adrenals in 40%. **c** posterior (left) and anterior (right) views of patient with monoclonal gammopathy complicated by extensive AL amyloidosis. There is uptake and retention of tracer in the liver, spleen, kidneys, bone marrow and soft tissues around the shoulder. Note the complete absence of blood pool or bladder signal compared to **a** indicating a heavy whole body amyloid load. This pattern of amyloid distribution revealed by scintigraphy is pathognomonic for AL amyloidosis, bone marrow uptake never having been seen in any other type.

NON-HISTOLOGICAL

In vivo scintigraphy with ^{123}I-labelled human serum amyloid P component

When SAP labelled with ^{123}I (a pure medium energy gamma emitter) is injected into the circulation in patients with amyloidosis, it rapidly and specifically localizes to the deposits in proportion to the quantity of amyloid present, allowing the deposits to be visualized and quantified by scintigraphy (Figure 2)[40–43]. This has enabled important observations regarding amyloid to be made for the first time *in vivo* including: the different patterns of distribution of amyloid in different forms of the disease, the demonstration of amyloid in sites not normally available for biopsy and the demonstration of a poor correlation between the quantity of amyloid present in a given organ and the level of organ dysfunction. Scintigraphy also allows the study of the natural history of the disease and the effects of treatment[7,32–35].

Echocardiography, radiology and gene studies

Two-dimensional echocardiography showing small, concentrically hypertrophied ventricles, generally impaired contraction, dilated atria, homogeneously thickened valves, and 'sparkling' echodensity of ventricular walls can be diagnostic of cardiac amyloidosis whilst ECG classically shows low voltage complexes.

Radiology generally contributes little to the diagnosis of amyloid. However, in β_2-microglobulin amyloid associated with haemodialysis there is almost always some skeletal involvement producing multiple periarticular cystic bone lesions which grow in size and number with increasing duration of dialysis. Commonly affected sites include the carpal bones, femoral and humoral heads, acetabulum, tibial plateau and distal radius, and amyloid deposition may result in pathological fractures especially in weight bearing bones[44]. Extraosseous radiological findings include swelling of articular and periarticular soft tissue mainly involving joint capsules, synovia and tendons and these, can be visualized and quantified by ultrasonography[45].

In some patients, AL amyloid is the only feature of an underlying plasma cell dyscrasia with no demonstrable morphological evidence of the clonal population on bone marrow aspiration or trephine biopsy, nor of its products such as a paraprotein band in the serum or Bence Jones proteins in the urine. However, provided they represent at least 1% of the cells in the marrow or among peripheral leukocytes, these clonal B or plasma cells can be identified using Southern blotting to detect immunoglobulin gene rearrangements[46,47]

In hereditary systemic amyloidosis and familial Alzheimer's disease, DNA sequencing has identified the causative gene mutations. It is also used together with other methods, such as allele specific oligonucleotide hybridization, to screen relatives who may be carriers of the mutation and therefore at risk of developing disease.

Fibril protein characterization

The ultimate biochemical description of any case of amyloidosis requires extraction of fibrils from amyloidotic organs or biopsy tissue, isolation of the subunits and complete characterization by protein sequencing and mass spectrometry.

Effects of amyloid deposition

Amyloid deposits exert their pathological effects largely through their physical presence, which distorts tissue architecture and hence disrupts normal function, leading ultimately to organ failure. Organ function may also decline, without any demonstrable changes in amyloid load, suggesting secondary effects due to the presence of amyloid, such as renal interstitial fibrosis.

Clotting factor deficiency is a rare but serious complication of amyloidosis. Acquired deficiency of factor X and other factors is probably due to adsorption of the clotting proteins to the fibrils, and occurs only in AL amyloidosis. The devastating haemorrhagic complications that result are often unresponsive to treatment even with replacement of clotting factors but may improve with resection of amyloid laden spleen. However, bleeding complications are more commonly due to direct amyloid infiltration of the blood vessels.

Clinical management

Although there is no specific therapy that can prevent the development of amyloidosis or promote fibril resorption, effective measures aimed at reducing the supply of precursor proteins can reduce or halt disease progression, and in some cases lead to regression of amyloid deposits. Ideally, patients with conditions predisposing to amyloidosis should, whenever possible, be treated aggressively to reduce the load of fibril precursor protein, in order to prevent deposition in the first place.

In AA anyloidosis, suppression of the underlying disease process by alkylating agents in rheumatoid arthritis[48] and juvenile chronic arthritis[7], and colchicine in familial Mediterranean fever[49] have been shown to preserve renal function and improve survival together with scintigraphic evidence of regression of amyloid deposits[7,35]. Tumour resection in Castleman's disease has also led to regression of AA amyloid deposits[50].

The results are more variable and less dramatic in AL amyloid, the therapy of which is aimed at suppressing the underlying clone of plasma cells so as to reduce or abolish production of precursor monoclonal light chains[9]. Cytotoxic drug regimes are identical to those used for the treatment of multiple myeloma.

In dialysis-related amyloid, β_2-microglobulin levels fall rapidly to normal limits following a successful renal transplant. Indirect evidence that this is accompanied by regression of amyloid deposits in some patients[43] has recently been confirmed on follow-up scintigraphic studies.

An exciting recent development has been the introduction of liver transplantation as treatment for familial amyloid polyneuropathy caused by transthyretin gene mutations[33]. The liver is the main source of transthyretin and transplantation leads to rapid and complete disappearance of variant transthyretin from the plasma. Most of the liver transplant recipients report an improvement, especially in symptoms related to autonomic neuropathy, and there is also scintigraphic evidence of regression of amyloid deposits[33]. This surgical form of gene therapy holds much promise for patients with hereditary transthyretin related amyloidosis.

The mainstay of management of patients with amyloidosis remains supportive therapy. Failure of vital organs should be managed actively and aggressively, including allogeneic transplantation whenever possible. The prognosis of renal failure caused by amyloidosis has been improved by maintenance dialysis and renal transplantation. The presence of cardiac involvement in AL amyloid is associated with a poor prognosis but there is now evidence that these patients may benefit from cardiac transplantation and chemotherapy[32]. Amyloidosis is thus no longer mainly the concern of the histopathologist and the morbid anatomist, but is increasingly recognized as a common, clinically important dynamic process with diverse aetiology. It is amenable to non-invasive diagnosis *in vivo* and is often susceptible to active and effective management.

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