Annu. Rev. Med. 2006. 57:223–41
doi: 10.1146/annurev.med.57.121304.131243

First published online as a Review in Advance on Aug. 24, 2005

AMYLOIDOSIS

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Key Words amyloid fibril, diagnosis, imaging, treatment

Abstract Amyloidosis is a clinical disorder caused by extracellular deposition of insoluble abnormal fibrils, derived from aggregation of misfolded, normally soluble, protein. About 23 different unrelated proteins are known to form amyloid fibrils in vivo, which share a pathognomonic structure although they are associated with clinically distinct conditions. Systemic amyloidosis, with amyloid deposits in the viscera, blood vessel walls, and connective tissue, is usually fatal and is the cause of about one per thousand deaths in developed countries. This rarity and the variable involvement of different organs and tissues are often responsible for missed or delayed diagnosis, and amyloidosis remains a considerable clinical challenge. However, recent elucidation of important aspects of pathogenesis, as well as developments in diagnosis, monitoring, and treatment, have greatly improved outcomes, especially when patients are managed in specialist centers.

INTRODUCTION

Amyloidosis has been known for more than 150 years, but recently protein misfolding has been increasingly recognized in the pathogenesis of many other diseases. Unfortunately, those without experience in pathobiology have tended to confuse and conflate amyloid with all types of protein aggregation present in disease. In 1984 Glenner, a pioneer of amyloid studies, first isolated the previously undescribed protein that forms amyloid fibrils in Alzheimer's disease, expanding interest in amyloid by linking it to one of the commonest and most important human diseases. He named this protein β-protein, reflecting his earlier original discovery of the cross β core structure of amyloid fibrils. The Alzheimer's deposits soon came to be called β-amyloid, the protein itself amyloid-β, and its precursor the amyloid precursor protein, as if this were the only type of amyloid! Amyloid is a precise term with critical implications for patients with life-threatening disease, who receive life-threatening as well as life-saving therapy (1, 2). However, in much work on Alzheimer's disease, the term amyloid is used indiscriminately for β-protein regardless of whether it is soluble, variously aggregated, or in the pathognomonic amyloid fibril form. Increasing recognition of protein misfolding and abnormal aggregation in other important diseases has unfortunately led to these clinically

and pathologically unrelated conditions also being widely, and incorrectly, cited as examples of amyloid and amyloidosis.

WHAT IS, AND WHAT IS NOT, AMYLOIDOSIS

Amyloidosis

Amyloidosis is disease caused by extracellular amyloid deposits. There is no clinical or pathological evidence of disease in the absence of such deposits. In local amyloidosis, the amyloid is restricted to a particular organ or tissue. In systemic amyloidosis, deposits can be present in any or all of the viscera, connective tissue, and blood vessel walls, although intracerebral amyloid deposits are never found. Acquired amyloidosis is a complication of preexisting primary disease that produces either an inherently amyloidogenic abnormal protein or greatly increased amounts of potentially amyloidogenic normal protein. Hereditary amyloidosis is caused by mutant genes encoding variant proteins whose structure makes them amyloidogenic.

Other Diseases in Which Amyloid Occurs

Amyloid is a histopathological feature of Alzheimer's disease and type 2 diabetes mellitus, but unlike amyloidosis, as defined above, it is not established that amyloid causes these diseases. Amyloid, composed of the islet amyloid polypeptide, is frequently present in the Islets of Langerhans in diabetic patients, but it is not universal. In Alzheimer's disease and in its transgenic mouse models, there is a poor correlation between cognitive impairment and abundance of the intracerebral amyloid deposits composed of β-protein. However, trisomy 21 (Down's syndrome), which is characterized by early-onset Alzheimer's disease, and all the mutations responsible for familial Alzheimer's disease cause increased production of β-protein. Furthermore, soluble small oligomers of β-protein have recently been shown to mediate cognitive dysfunction under physiological conditions in vivo, implicating this early aggregated form of β-protein rather than amyloid fibrils in pathogenesis (3). Amyloid clearly causes damage and disease in other parts of the body, but treatments that specifically prevent and/or remove the cerebral amyloid plaques of Alzheimer's and the pancreatic islet amyloid deposits will be required to determine whether these particular forms of amyloid contribute significantly to the pathogenesis of dementia and diabetes, respectively.

Transmissible spongiform encephalopathy (TSE) is also frequently cited as an example of "amyloidosis," even though full-blown TSE disease can develop in the absence of amyloid. No amyloid is detectable histopathologically in the brains of mice with some strains of scrapie, nor in the brains of patients with fatal familial insomnia (a form of TSE), nor of cows with bovine spongiform encephalopathy. Furthermore, abundant cerebral amyloid deposits of PrP^{Sc} (the disease-associated form of PrP^{C}, the cellular prion protein) do not produce disease

in brains of transgenic mice lacking PrP^{C} (4). Thus, although PrP^{Sc} is central to the pathogenesis of TSE and can aggregate to form typical amyloid deposits in vivo, such deposits are not a necessary condition for the disease.

Protein Misfolding Diseases That Are Not Amyloid or Amyloidosis

In Huntington's and the related neurodegenerative diseases caused by polyglutamine repeats, and in Parkinson's disease, abnormal protein aggregates are present within the nucleus and the cytoplasm of neurons, respectively. Although these aggregates may share some biophysical properties with amyloid fibrils, they are definitely not amyloid deposits by any of the established clinical and pathological criteria. The conflation of these diseases with amyloidosis is seriously misleading. In genuine amyloidosis, the deposits are unequivocally harmful, and measures to prevent their formation and/or promote their removal are clinically beneficial. In contrast, among rat neurons transfected with huntingtin, those cells with abundant aggregates survive, whereas those with fewer or no such aggregates die prematurely (5). This compellingly illustrates the fact that abnormal protein aggregation of vastly different scale and radically different location—intranuclear, intracytoplasmic, and extracellular—is likely to have widely different effects on cell, tissue, and organ function and structure, and hence on development of disease, and will require appropriately different approaches to therapy.

AMYLOID

The identification of amyloid, either histologically or in fibrils formed in vitro, depends on the pathognomonic red-green dichroism observed when preparations correctly stained with alkaline alcoholic Congo red are viewed in intense unidirectional polarized light (6). This optical effect is produced by alignment of the dye molecules along the fibrils. Binding of thioflavin T usually corresponds with Congo red birefringence but is less specific. Congo red staining for amyloid is not a very sensitive test and requires an adequate amount of amyloid, sufficiently thick tissue sections, technically correct staining and visualization procedures, and adequate observer experience. In the British National Health Service National Amyloidosis Center, which annually sees >1100 patients with known or suspected amyloidosis and has reviewed thousands of biopsies in the past 25 years, we continue to receive both false positive and false negative Congo red diagnoses of amyloid from other hospitals all over the world.

All amyloid fibrils share a common cross β core structure with the polypeptide chains running perpendicular to the fibril long axis, regardless of the particular protein from which they are formed (7). In negatively stained electron microscopy, amyloid fibrils are usually about 10 nm in diameter, straight, rigid, nonbranching, of indeterminate length, and composed of twisted protofibrils. Transmission electron

microscopy reveals the typical fibrillar ultrastructure of tissue amyloid deposits, but this is not clinically diagnostic, and morphological appearances are also not sufficient for definite identification of synthetic in vitro fibrils as amyloid. Ex vivo fibrils cannot be crystallized, and there is no high-resolution structure of natural amyloid fibrils, but biophysical methods are now yielding much more detailed molecular structures of amyloid fibrils created in vitro (8).

All amyloid deposits contain abundant heparan sulfate and dermatan sulfate proteoglycans and glycosaminoglycan chains, some of which are tightly bound to the fibrils (9). These glycan molecules may contribute to amyloid fibrillogenesis as well as stabilization of the fibril structure (10). All amyloid deposits also contain amyloid P component, which is identical to and derived from the normal circulating plasma protein of the pentraxin family, serum amyloid P component (SAP) (11). SAP undergoes avid (Kd ~1 μmol/l), specific, calcium-dependent, reversible binding to amyloid fibrils of all types. This causes a remarkably high specific concentration of SAP in amyloid deposits, of which it may comprise up to ~15% by mass. SAP binds both to glycosaminoglycans and to protein ligands specifically present on all types of amyloid fibril. Binding of SAP, which is itself highly resistant to protease digestion, stabilizes amyloid fibrils and protects them from degradation by proteases and phagocytic cells in vitro (12), and presumably has similar effects in vivo. Mice with targeted deletion of the SAP gene show delayed and reduced deposition of amyloid (13), and there is some in vitro evidence that SAP may promote amyloid fibrillogenesis. Human SAP is also a normal constituent of extracellular matrix in the microfibrillar mantle of elastic fibers and in the glomerular basement membrane, both sites at which early amyloid fibril deposition is often observed. Nevertheless, amyloid formation does not require SAP, since with a sufficiently powerful stimulus the SAP knockout mice develop amyloid deposits exactly like wild-type SAP-sufficient controls (13). However, human SAP binds much more avidly than mouse SAP to amyloid fibrils and is about 30 times more abundant in human amyloid deposits than mouse SAP is in mouse amyloid. Human SAP may therefore be involved more significantly in pathogenesis and/or persistence of human amyloid. Other plasma proteins, such as apolipoprotein E, are sometimes detectable in amyloid deposits, but none with the universality and abundance of SAP.

CLINICAL AMYLOIDOSIS

Local Amyloidosis

The commonest form of local amyloidosis is caused by foci of otherwise benign monoclonal B cells or plasma cells producing monoclonal immunoglobulin light chains (L) that are deposited as AL amyloid, most frequently in the respiratory tract, urogenital tract, or skin (14). (Amyloidosis nomenclature uses the letter A to designate amyloid, followed by an abbreviation of the name of the fibril protein.) Local amyloid composed of β-protein within the walls of cerebral blood

vessels can be responsible for Congophilic angiopathy, which can cause cerebral hemorrhage and stroke. Peptide hormones forming amyloid deposits in benign or malignant tumors of endocrine tissue, and microscopic senile amyloid deposits composed of various proteins in the arterial wall, the heart, the seminal vesicles, and the prostate, are incidental histological findings; there is little evidence that these amyloid deposits cause disease.

Acquired Systemic Amyloidosis

Systemic AL amyloidosis, previously known as primary amyloidosis, is the most common form of clinical amyloid disease in developed countries. It is usually secondary to an otherwise benign low-grade monoclonal gammopathy and may also complicate multiple myeloma or other clonal B cell diseases. The fibrils are usually formed from part or all of the N-terminal variable domain of monoclonal immunoglobulin light chains. The deposits can affect any part of the body except the brain and lead to a wide range of tissue and organ dysfunction with extremely varied clinical presentation. The diagnosis is frequently made incidentally when a biopsy is taken. A high index of suspicion of amyloidosis is mandatory in any patient with unexplained nephropathy, cardiac failure, peripheral or autonomic neuropathy, or hepatic or splenic enlargement and/or dysfunction. Macroglossia, especially associated with any of the above, is virtually diagnostic of AL amyloidosis.

Reactive systemic AA amyloidosis, formerly known as secondary amyloidosis, is a complication of chronic infections and inflammatory conditions. It is characterized by a sustained acute phase response in which there is persistently increased production of serum amyloid A protein (SAA). SAA is an apolipoprotein of high-density lipoprotein particles, produced predominantly in the liver, and is, together with C-reactive protein, the most dynamic acute phase protein. SAA concentration rises from <5 mg/liter in healthy subjects to as much as 2000 mg/liter at the peak of a severe acute phase response. In rheumatoid arthritis, juvenile rheumatoid arthritis, other types of inflammatory arthritis, Crohn's disease, familial Mediterranean fever and the other hereditary periodic fever syndromes, the SAA concentration can remain at tens or hundreds of milligrams per liter for months or years unless the inflammatory activity remits or is suppressed by therapy. Up to 10% of individuals with sustained high SAA values may eventually develop AA amyloidosis. This always involves the spleen but typically presents with proteinuria and/or hepatosplenomegaly; cardiac involvement is very rare. In some hereditary fever syndromes, the genetic bases of which are being elucidated (15), the incidence of AA amyloidosis can be much higher. A small number of patients with AA amyloidosis have no clinically overt inflammatory disease, although some are carriers of inherited fever syndrome genes.

Dialysis-associated amyloidosis is a complication of long-term dialysis for end-stage renal failure. β_2-microglobulin (β_2M) is cleared and catabolized only by the kidney and is very poorly cleared by peritoneal dialysis or hemodialysis.

In end-stage renal failure its circulating concentration therefore rises from the normal 1–2 mg/liter to ~50–70 mg/liter. After 7–10 years of dialysis, patients may present with painful and disabling $A\beta_2M$ amyloid deposition in and around bones and joints. This intractable complication of long-term dialysis is effectively treated only by renal transplantation. However, the incidence of $A\beta_2M$ amyloidosis is apparently now falling, possibly owing to use of new dialysis membranes, cleaner dialysis fluids, and higher-flux dialysis.

In patients over the age of 80 years, wild-type transthyretin (TTR) amyloid deposits in the heart, kidneys, and respiratory tract are an almost universal incidental autopsy finding, but some elderly patients with more extensive TTR amyloid deposits in the heart develop restrictive cardiomyopathy or symptoms that mimic coronary artery disease. This senile cardiac amyloidosis is untreatable except by heart transplantation, and it is seldom diagnosed because most elderly subjects with cardiac failure are automatically assumed to have coronary artery disease. The correct diagnosis is suggested by the cardiac echo and possible low-voltage electrocardiogram, and it is confirmed by cardiac biopsy. In most patients the amyloid consists of wild-type TTR, but sometimes amyloidogenic TTR variants are present.

Hereditary Systemic Amyloidosis

This rare disorder is very difficult to treat and is usually fatal, but its study has provided invaluable information on amyloid fibrillogenesis and pathogenetic mechanisms, which has enabled development of new potentially therapeutic drugs for amyloidosis. The most common cause of hereditary amyloidosis is mutation in the gene for TTR, which affects perhaps ~10,000 individuals worldwide. The other amyloid fibril proteins that cause hereditary amyloidosis are apolipoproteins AI and AII, fibrinogen A α-chain, gelsolin, and lysozyme in systemic amyloidosis; cystatin C in the Icelandic form of hereditary cerebral hemorrhage with amyloidosis; and β-protein in the Dutch form of this disease. Over 80 mutations, most of which are amyloidogenic, are known in TTR, and new amyloidogenic mutations in the other proteins listed continue to be discovered.

Severe and ultimately fatal peripheral and/or autonomic neuropathy are major features of hereditary TTR amyloidosis (familial amyloid polyneuropathy), but fatal cardiac and significant renal involvement are also common. ApoAI amyloidosis sometimes causes neuropathy, but this is not a feature of the other hereditary types, which typically involve the viscera. Age of onset, distribution of amyloid deposits, and clinical presentation can vary widely both within and between families, even with the same mutation. All the amyloidogenic mutations are dominant, but they are variably penetrant and there may be no family history. AL amyloidosis is sometimes diagnosed by exclusion because rigorous positive immunohistochemical identification of AL-type fibrils is not possible in some cases, and without use of the new sensitive and reliable test for free immunoglobulin light chains in serum (16, 17), the amyloid deposits may be the only sign of monoclonal

gammopathy. Thus, there is scope for misdiagnosis of hereditary amyloidosis as AL-type (18), and the inappropriate use of dangerous cytotoxic regimens aimed at ablation of clonal B cell disease. It is therefore absolutely mandatory that the amyloid fibril type is positively identified in all systemic amyloidosis patients and/or that there is comprehensive gene screening for all known amyloidogenic mutations.

AMYLOID FIBRILLOGENESIS

In Vivo

Amyloid formation in vivo occurs with both normal wild-type proteins and with genetically variant amyloidogenic proteins. The fibrils may contain the intact amyloidogenic protein or be composed of proteolytic cleavage fragments. Wild-type TTR is inherently amyloidogenic, and even at normal concentrations it forms amyloid fibrils in almost all individuals over 80 years of age, sometimes causing senile systemic amyloidosis. The other amyloidogenic wild-type proteins, SAA and β_2M, form amyloid only when persistently present at grossly supraphysiological concentration. Abnormal proteins with enhanced amyloidogenicity can be acquired, as are the monoclonal immunoglobulin light chains responsible for AL amyloidosis, or inherited, as in the familial amyloidoses. However, amyloid does not appear immediately in any of these situations. There is always a lag period, often of many years, between the first appearance of the potentially amyloidogenic protein and the deposition of clinically significant amyloid. In mice, this lag period can be dramatically shortened by intravenous injection of a crude extract of amyloidotic tissue, known as amyloid enhancing factor (19). The same effect can be achieved using isolated amyloid fibrils and probably reflects a seeding process. Amyloidosis is extremely rare in children and even young adults, and increasing age may thus favor amyloid deposition, although the underlying mechanisms are not known.

In Vitro

Typical amyloid fibrils can be formed in vitro from isolated purified proteins, including (*a*) amyloidogenic variant proteins that are associated with amyloid deposition in vivo, (*b*) their wild-type counterparts that do not form amyloid in vivo, and (*c*) wild-type proteins that can form amyloid in vivo. No additional biological molecules are required. Amyloid fibrillogenesis involves partial unfolding of the native precursors and the population of relatively unstable partly folded states, which then stabilize themselves by intermolecular binding and aggregation to form fibrils containing cross β structure. Seeding with natural or synthetic preformed fibrils greatly accelerates fibrillogenesis. Amyloidogenic variant proteins from acquired or inherited types of amyloidosis are inherently less stable than their wild-type counterparts and misfold under relatively physiological conditions.

Transformation of wild-type proteins into amyloid fibrils requires a significantly harsher denaturing environment. However, it has recently been demonstrated that almost any natural protein or synthetic polypeptide, even those exclusively composed of α-helices in their native state, can be induced by denaturation to refold and aggregate in vitro as amyloid fibrils with typical cross β core structure (20). The amyloid fold is evidently a very stable, low-energy state for all polypeptide chains and apparently depends predominantly on main-chain interactions independent of the specific amino acid side chains.

Mechanisms of Amyloid Fibril Formation and Persistence In Vivo: Unanswered Questions

Why do only 23 or so natural proteins form amyloid fibrils in vivo? Do these proteins have intrinsic properties that make them more amyloidogenic in vivo than all others, or does their amyloidogenicity depend on interactions with other biological molecules such as glycosaminoglycans and SAP? Or do they evade normal protective mechanisms, such as chaperones, that prevent other misfolding proteins from forming and persisting as amyloid fibrils? Because any protein can form amyloid fibrils in vitro, understanding why this does not happen, or at least does not become detectable, in vivo is critical to understanding the pathogenesis of amyloid. There are no answers yet to these questions, nor do we know what determines the precise time, location, or effect of amyloid deposition in any form of the disease.

Amyloid fibrils are intrinsically very stable but can be digested in vitro by proteases and by phagocytic cells, and amyloid deposits regress in vivo when the abundance of the amyloid fibril precursor protein is sufficiently reduced (21, 22). The persistence and accumulation of amyloid deposits, which are the usual natural history observed in clinical practice, thus reflect greater amyloid deposition than amyloid removal over time. However, in some patients whose supply of amyloid fibril precursor is completely removed, amyloid deposits can remain completely unchanged for many years. Nothing is known about the mechanisms responsible for amyloid clearance in vivo, but it presumably requires degradation of the fibrils and associated materials by adjacent cells. The stability of amyloid fibrils, enhanced by tightly bound glycosaminoglycans and SAP, suggests that dissolution by physical processes alone is extremely unlikely under physiological conditions. It is unusual to see any inflammatory or foreign-body reaction in or around amyloid deposits, and they do not elicit a systemic inflammatory reaction or acute phase response. We have speculated that this reflects masking of the abnormal structure of the fibrils by bound SAP, which is identical to the normal circulating SAP (11). There are no receptors for SAP on phagocytic cells, and coating of bacteria with human SAP has a powerful antiopsonic effect (23). SAP may thus protect amyloid fibrils from degradation and shield their abnormal structure from recognition and elicitation of an inflammatory response.

PATHOGENICITY OF AMYLOID

Regardless of the duration of their exposure to acquired or hereditary amyloidogenic precursor proteins, patients with amyloidosis present with clinical signs and symptoms only when amyloid deposits become demonstrable. The relationship of total-body amyloid load to severity of tissue damage varies between patients, but increasing amyloid load increases severity of disease in each individual, and stabilization or regression of amyloid burden is associated with clinical stability or improvement. The presence of typical amyloid deposits is thus a necessary condition for expression of the clinical manifestations of amyloidosis and is very closely related to their severity.

Variously aggregated amyloidogenic proteins are toxic for susceptible parenchymal cells cultured in vitro. This observation has prompted pervasive speculation that cytotoxicity is an important pathogenetic mechanism in diseases associated with protein misfolding. However, it is not surprising that misfolded proteins with exposed hydrophobic surfaces, which can interact avidly with cellular membranes and other hydrophobic ligands, can be cytotoxic in vitro. An artificial cell culture is an unphysiological environment, lacking the high in vivo protein concentration of plasma and the extracellular fluid, as well as the complex and highly organized in vivo microenvironment of diverse cell types and extracellular matrix. Extrapolation from artificial cell culture model systems to in vivo mechanisms of disease is thus questionable.

Reversible neuronal dysfunction in vivo caused by physiologically formed soluble small oligomers of β-protein may reflect an aspect of the pathogenesis of Alzheimer's disease (3), but there is no such physiologically robust evidence in other situations. In particular, the increasingly popular extrapolation of the "toxicity" concept to local and systemic amyloidosis is not supported by compelling evidence. On the contrary, clinicopathological observations indicate that the physical presence of extracellular amyloid deposits, disrupting the structure and thereby the function of the tissue, is sufficient to explain their pathogenicity, as illustrated by the following examples:

1. In cardiac amyloidosis, vigorous myocardial contractility is sustained despite massive amyloid infiltration; heart failure occurs because the inelastic amyloid restricts ventricular filling.
2. Deposition of TTR amyloid fibrils in the vitreous humor of the eye, in immediate contact with the retina, causes blindness. However, when the opaque amyloid-laden vitreous is replaced with artificial clear medium, even after many years of blindness, perfect vision is immediately restored. The exquisitely sensitive retina is evidently not damaged by adjacent long-standing TTR misfolding, oligomerization, aggregation, and amyloid fibril formation.
3. Amyloid deposition usually starts early in organs transplanted for end-stage kidney, heart, or liver failure in hereditary systemic amyloidosis. However,

there is no evidence of graft dysfunction, despite continuous exposure to the amyloidogenic precursor, until sufficient deposits accumulate to cause tissue damage, and this may take many years.

These and many similar observations do not support the idea that misfolded amyloidogenic proteins or prefibrillar aggregates cause disease by toxic effects on cells.

IMAGING AMYLOID IN VIVO

SAP Scintigraphy

SAP is a highly conserved, invariant plasma glycoprotein of the pentraxin family that becomes specifically and highly concentrated in amyloid deposits of all types as a result of its calcium-dependent binding to amyloid fibrils. It circulates in the plasma at a concentration of ~20–30 mg/liter, so in the absence of amyloid there is ~100 mg of SAP in the body. However, in patients with amyloidosis there may be as much as 20,000 mg of SAP in the deposits, in equilibrium with the free SAP in the circulation and the extracellular fluid. We exploited this phenomenon to develop the powerful technique of radiolabeled SAP scintigraphy for diagnosis and quantitative monitoring of amyloid deposits (24). Following intravenous injection, the tracer distributes between the free and the amyloid-bound SAP pools in proportion to their size and can then be imaged and quantified. This safe, noninvasive method uniquely provides invaluable information on the presence, distribution, and extent of amyloid deposits throughout the body, and serial scans monitor progress and response to therapy. With the short-half-life ^{123}I isotope routinely used, the method is not informative about cardiac amyloidosis; nor is it useful for imaging intracerebral amyloid, owing to the relatively slow penetration of SAP into the brain. Nevertheless, SAP scintigraphy has revolutionized our knowledge of the natural history of systemic amyloidosis and its response to treatment (Figures 1–4) (25). Key findings have been (*a*) the unequivocal demonstration that amyloid deposits of all types can regress when the abundance of the amyloid fibril precursor protein is sufficiently reduced; (*b*) the distinctly different anatomical patterns of amyloid deposition in different types of amyloidosis, some of which (e.g., bone marrow deposits in AL) are pathognomonic; (*c*) detection of amyloid deposition in the spleen, kidneys, and adrenals before these organs have shown any clinical abnormality but when they are nonetheless at high risk of failure; and (*d*) detection of explosive late onset of AA amyloid deposition in patients with predisposing conditions such as rheumatoid arthritis. Determination of the actual amyloid load is also critical, especially in AL disease, where clinical and pathological findings may be identical in patients who have greatly different amounts of amyloid and correspondingly different risk/benefit balances when they receive cytotoxic chemotherapy. Unfortunately, owing to its cost and technical complexity, SAP scintigraphy is routinely available only in the UK National Amyloidosis Center.

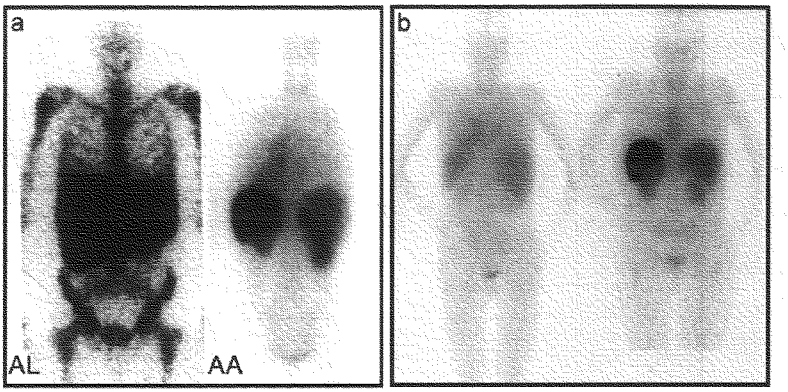

Figure 1 Whole-body scintigraphy with ^{123}I-labeled serum amyloid P component in systemic amyloidosis. (*a*) *Left:* Anterior view of a typical patient with AL amyloidosis showing massive liver and spleen amyloid and the pathognomonic deposits throughout the bone marrow that are not seen in any other type of amyloidosis. *Right:* Posterior view of a typical patient with AA amyloidosis showing amyloid in the spleen, kidneys, and adrenal. The left adrenal is obscured by the overlying spleen, but the right is clearly visible above the kidney. (*b*) Posterior scans taken a year apart in a patient with longstanding rheumatoid arthritis who suddenly developed AA amyloidosis. The earlier scan (*left*) is normal; the later one (*right*) shows heavy splenic and significant renal amyloidosis.

Echocardiography, including new techniques of strain rate tissue Doppler imaging, provides essential information on the extent and progression of cardiac amyloid deposits (26). Recent developments in magnetic resonance imaging can also contribute to assessment of the severity of cardiac amyloidosis (27), as can serum assays of specific cardiac markers, N-terminal pro-BNP (28), and troponin T (29).

TREATMENT OF AMYLOIDOSIS

Local amyloid masses can only be treated surgically. The twin aims of management in systemic amyloidosis are (*a*) reduction of the supply of amyloid fibril precursor proteins so that amyloid deposition ceases and regression of existing deposits can occur, and (*b*) scrupulous general care, including dialysis and organ transplantation if necessary, to keep patients alive long enough for the benefits of reduced fibril precursor abundance to be realized. The prognosis of systemic amyloidosis remains grave for most patients, especially those with AL disease, in whom the diagnosis is often made after substantial visceral and/or neural involvement has occurred. However, recent advances have greatly extended median survival. Awareness of the compromised functional reserve of amyloidotic organs and extreme care to protect

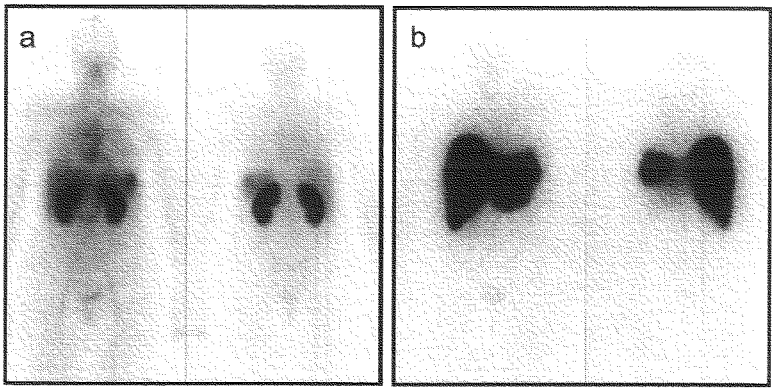

Figure 2 (*a*) Anterior (*left*) and posterior (*right*) views of a patient with AL amyloid who presented with minor proteinuria and no other clinical or investigational evidence of disease. There is substantial renal amyloid but no scintigraphically detectable deposits elsewhere. (*b*) Anterior (*left*) and posterior (*right*) views of a different patient with AL amyloid who also presented with minor proteinuria and no other clinical or investigational evidence of disease. There is massive amyloid deposition in the liver and spleen. The kidneys are not visualized, probably because the tracer, which distributes according to the amount of amyloid, is all taken up elsewhere. Note that, in contrast to (*a*), there is no residual tracer in the circulation, indicating a heavy whole-body amyloid load. This patient did not tolerate intensive chemotherapy and developed liver failure.

renal function are critical. Outcomes are much better in centers with specialist expertise, and all systemic amyloidosis patients should ideally be managed in, or with advice from, such centers.

Rational management has been facilitated by the recent availability of routine assays for circulating SAA in AA amyloidosis (30) and free immunoglobulin light chains in AL amyloidosis (16, 17). Treatment of the primary inflammatory conditions responsible for AA amyloidosis, to reduce SAA values (ideally to normal), dramatically improves survival and is associated with arrest of amyloid deposition and frequently regression of deposits (21). New biological drugs (such as antibodies to, and inhibitors of, key cytokine mediators of inflammation, including tumor necrosis factor and IL-1) potently suppress the acute phase response in many patients with rheumatoid arthritis, seronegative spondyloarthropathies, Crohn's disease, and some hereditary periodic fevers, and thus prevent AA amyloidosis or treat it if it is already present. Treatment with colchicine is mandatory to prevent AA amyloidosis in familial Mediterranean fever, even when it does not completely control symptoms (31). Excision of the lymphoid masses producing IL-6 is essential in Castleman's disease complicated by AA amyloid, relieving symptoms and allowing regression of amyloid (32).

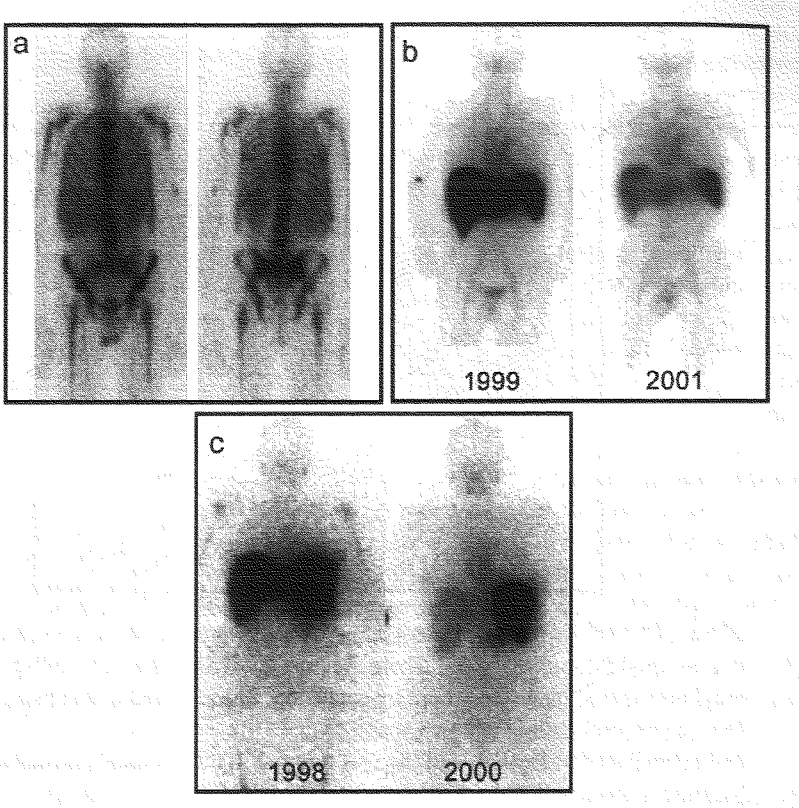

Figure 3 (*a*) Anterior (*left*) and posterior (*right*) views of a patient with AL amyloid who presented with multiple fractures over four years. X-ray and bone scan were normal but bone biopsy unexpectedly revealed amyloid. No monoclonal gammopathy was identifiable at that time, but bone amyloid is frequent in AL and may be the main clinical feature. (*b*) Serial anterior views showing regression of AA amyloidosis in a juvenile rheumatoid arthritis patient treated with chlorambucil, in whom the SAA concentration was suppressed to <10 mg/l. (*c*) Serial anterior views showing regression of AL amyloidosis in a patient treated with high-dose melphalan and stem cell rescue.

Ablation of the B cells that produce amyloidogenic monoclonal immunoglobulin light chains is associated with arrest of amyloid deposition, regression of deposits in many cases, preservation of organ function, and enhanced survival in AL amyloidosis. Availability of the robust, sensitive Freelite™ immunoassay for free immunoglobulin light chains in serum (16, 17) has been one of the most important advances in management of AL amyloidosis (22, 33). The amyloid fibril precursor protein can now be monitored prospectively and chemotherapy tailored accordingly. Sustained reduction of the serum concentration of free monoclonal light chains reduces amyloid deposition, and suppression by 50% or more is

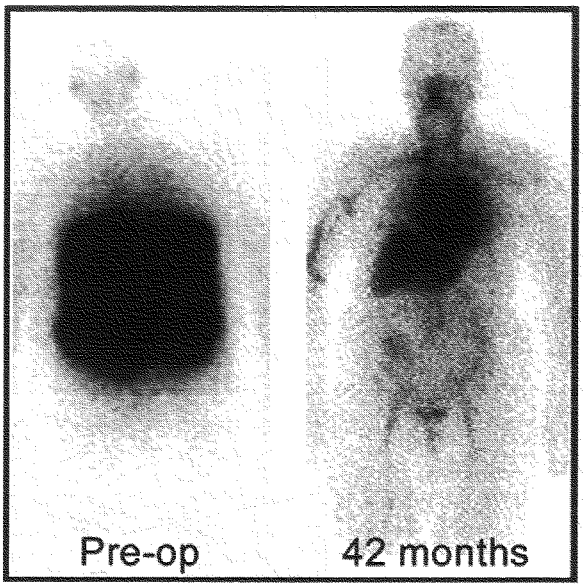

Figure 4 Serial anterior views of a 56-year-old woman who developed renal failure due to amyloidosis at age 33. There was no family history, and a diagnosis of AL amyloidosis was made elsewhere by exclusion. She received two consecutive kidney transplants, each failing within 5 years. At age 51 massive hepatic amyloidosis caused end-stage liver failure (*left*) and she was referred to the National Amyloidosis Center. The mutation encoding the amyloidogenic Glu526Val variant of fibrinogen A α-chain was then identified and her amyloid shown by immunohistochemistry to be composed of fibrinogen. She received liver and kidney transplants and remains perfectly well with no sign of amyloidosis seven years later. In the 42-months-postoperative scan (*right*), tracer is distributed only in the blood pool, showing up the heart, liver, major blood vessels, and the transplanted kidney in the right iliac fossa.

associated with enhanced survival (22). There is wide variation between patients in their capacity to tolerate the adverse side effects of chemotherapy, and also in the efficacy of different chemotherapy regimens, which reflects the monoclonal nature of AL amyloidosis; some clones resist all treatment. Oral melphalan and prednisolone are often better tolerated than more aggressive treatment and may induce a response, but substantial toxicity can still occur and clonal suppression may be delayed or absent. Intermediate-dose infusional chemotherapy regimens, such as vincristine, adriamycin and dexamethasone, or melphalan and dexamethasone, are often well tolerated and may induce swifter responses (34, 35). High-dose chemotherapy with rescue by peripheral stem cell transplantation can rapidly suppress the clone but has a high case fatality rate in patients with large whole-body amyloid load, with heart and kidney damage, and with advancing age (36–38). Results outside specialist amyloidosis centers are extremely poor. Other current

approaches include thalidomide, alone or in combination chemotherapy (39, 40), and Rituximab™ in patients with CD20 positive clones. The satisfactory response to less intense chemotherapy indicates that high-dose regimens are excessive in some cases, but there is presently no way to identify which individuals will tolerate and respond best to which treatment. The key point is to sufficiently suppress production of the amyloidogenic free light chain without unacceptable toxicity, and this requires careful individual monitoring.

At present, apart from transplantation to replace failed organs, and liver transplantation to remove the source of amyloidogenic proteins of hepatic origin, only symptomatic treatment is available for hereditary systemic amyloidosis. The liver is the source of plasma TTR, and over 700 liver transplants have been performed for treatment of hereditary TTR amyloidosis since this "surgical gene therapy" approach was introduced in 1991 (41, 42). In younger patients carrying the common Met30Val amyloidogenic mutation, the outcome is generally good, with arrest of neuropathy and regression of visceral amyloid (43); however, vitreous amyloid progresses, perhaps because TTR is also produced within the central nervous system. Cardiac amyloid may also progress, and there have been some combined heart and liver transplants. In older Met30Val patients and in patients with other amyloidogenic TTR mutations, liver transplantation has arrested neither amyloid deposition nor progression of clinical disease (44). The livers of patients with hereditary TTR amyloidosis contain only microscopic amyloid deposits in the blood vessels and interstitial tissues, and they retain normal liver function. This has led to use of these organs, explanted during transplantation of normal livers into TTR amyloidosis recipients, for salvage transplantation in recipients with terminal liver disease for whom normal livers were not available (45). The "domino" procedure has greatly prolonged the lives of terminal liver failure patients, but unfortunately the first such recipient has lately developed symptomatic systemic TTR amyloidosis eight years after transplantation (46).

In a patient with hereditary fibrinogen amyloidosis (AFib) who had received two consecutive renal transplants and then developed amyloidotic liver failure, combined liver and kidney transplantation was dramatically effective (47), and several more AFib patients have now received liver transplants. These operations have demonstrated that the liver is the sole site of synthesis of plasma fibrinogen, but the appropriate roles of liver and/or renal transplantation in management of this disease have yet to be determined. Patients with apoAI amyloidosis can develop kidney, liver, and cardiac amyloidosis, and several organ transplants have been performed, so far with excellent results. In one apoAI amyloidosis patient, aged 44, who underwent combined heart and kidney transplantation for end-stage failure 12 years ago, there are minor amyloid deposits in the transplanted heart and kidney but no organ dysfunction despite no intervention to reduce production of the amyloidogenic protein. In contrast, in a patient with lysozyme amyloidosis and a marked familial phenotype of hepatic amyloidosis leading to hepatic rupture, liver transplantation was eventually followed by fatal reaccumulation of liver amyloid.

Elucidation of aspects of the molecular pathogenesis of amyloid and amyloidosis has triggered a variety of novel approaches to therapy. We have developed a drug that targets SAP with the goal of eliminating it from amyloid deposits, in the hope that this may reduce amyloid deposition and/or accelerate amyloid clearance (48). Preliminary open-label studies are in progress in patients with systemic amyloidosis to optimize dosing and SAP depletion, and an initial short-term study in Alzheimer's disease has just been completed. There have been no adverse drug effects during >30 patient-years of exposure. Neurochem, Inc. have just completed an international double-blind controlled clinical study in AA amyloidosis of Fibrillex™, a small-molecule glycosaminoglycan analogue aimed at blocking the proamyloidogenic interaction between SAA and proteoglycans (49). The drug was well tolerated, but the primary clinical endpoints were not achieved; detailed analysis of outcomes is awaited. Small-molecule ligands that stabilize the native tetrameric structure of TTR and prevent its fibrillogenesis are being actively investigated for prophylaxis and therapy in TTR amyloidosis (50). Other strategies include stabilizing native structures of amyloidogenic proteins and preventing and reversing fibrillogenesis, as well as disrupting established deposits, by using antibodies, synthetic peptides, and small-molecule drugs. Some of these potential new therapies may enter clinical trials within the next few years and offer exciting prospects for improvements in treatment.

ACKNOWLEDGMENTS

The work of the Center for Amyloidosis and Acute Phase Proteins is supported by Medical Research Council Program Grant G79/00051, a grant from the Wolfson Foundation, and National Health Service R&D funds. The National Health Service National Amyloidosis Center is funded entirely by the UK Department of Health. I thank Philip Hawkins for critical and constructive discussions and his outstanding performance as Clinical Director of the National Amyloidosis Center, Beth Jones for skillful preparation of the manuscript, and David Bishop for expert production of the figures.

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