AMYLOID IS AN eosinophilic substance which under the light microscope has a hyaline appearance and which is deposited in the walls of small blood vessels and extracellularly in various organs. These deposits, when extensive, interfere with normal function. Many controversies arose about the cause and composition of amyloid for a century after it was named by Rudolph Virchow in 1854. The first major breakthrough regarding the nature of amyloid occurred in 1959 when electron microscopy showed that this apparently structureless material was actually fibrillar. The ability to solubilize the fibrils enabled subsequent characterization of their major protein constituents. Analytical protein chemistry techniques have elucidated several distinct chemical forms of amyloid although all of them share the common physical properties of polarization birefringence after Congo red staining, nonbranching fibrils with a diameter of 70 to 100 Å by electron microscopy, and a twisted β-pleated sheet conformation by x-ray diffraction. Except for intracerebral amyloid plaques, all deposits of amyloid also contain a nonfibrillar glycoprotein moiety, the P component. This amyloid P (AP) component is derived from a normal serum precursor (SAP) structurally related to one of the acute-phase reactants, C-reactive protein (CRP). Both SAP and CRP belong to the pentraxin family of plasma proteins.

In the past decade, monographs resulting from four international symposia have reflected the accelerated activity and progress in amyloid research. Several chemical types of amyloid have been identified, most of which circulate in the blood before being deposited in extracellular sites. Except for their common morphologic and physical properties, the various amyloid diseases are widely disparate and occur in diverse clinical settings. A classification of amyloidosis based on the major protein subunits present in the fibrils is shown in Table 1.

Historically, amyloidosis was classified according to whether it occurred de novo ("primary") or was "secondary" to a recognizable preexisting or coexisting chronic infectious or inflammatory disease. In the past 60 years, rare hereditary amyloid syndromes have been well documented. Most primary, secondary, and hereditary syndromes are systemic, ie, they involve more than one organ system. Isolated or tumor-like collections of amyloid in various organs also have been described. Recently, new amyloid disorders were recognized.

This review summarizes recent advances in understanding of the amyloid diseases. They are of interest to hematologists for at least three reasons: (a) Many of the precursor proteins circulate in the blood, (b) one of the more common types of amyloidosis is a plasma cell dyscrasia, and (c) many patients with amyloid disorders require hematological evaluation at some time during the course of their disease.

**DIAGNOSIS AND CLINICAL FINDINGS**

The diagnosis of amyloidosis is based on biopsy of involved tissue. Apple-green birefringence under polarized light after Congo red staining and/or the typical fibrillar structure evident by electron microscopy constitute the most reliable methods. Biopsy of any involved site may yield the diagnosis. Because of the propensity for amyloid to involve the walls of small blood vessels, biopsies of internal organs such as liver, kidney, or gastrointestinal tract may lead to hemorrhage. Rectal biopsy provides a positive diagnosis in most patients with primary systemic amyloidosis, but the specimen must include submucosa. Recent experience with abdominal fat aspiration suggests that this simple low-risk method is the diagnostic procedure of choice in evaluation for systemic amyloidosis with a 60% to 85% positive yield. Patients presenting with carpal tunnel syndrome have a lower incidence (0% to 17%) of positive abdominal fat aspirates. Tenosynovial amyloid is often unrecognized on routine histology, emphasizing the need to request the Congo red stain if amyloid is suspected.

Clinical manifestations of amyloidosis vary widely and depend on the organ system predominantly involved.

Renal involvement with proteinuria associated with the nephrotic syndrome and/or Bence Jones proteinuria is common; the latter finding is restricted to the AL type of...
Amyloidosis has been described in a number of connective tissue syndromes, including adult and juvenile rheumatoid arthritis, systemic lupus erythematosus, dermatomyositis, Behcet’s disease, Reiter’s syndrome, osteoarthritis, ankylosing spondylitis, and Sjögren’s syndrome.66-71 Amyloid arthropathy occasionally may be prominent in multiple myeloma.52-64 The carpal tunnel syndrome occurs in systemic amyloidosis due to immunoglobulin light chain deposition (AL), hemodialysis amyloidosis associated with deposition of β₂-microglobulin (β₂M), and in certain hereditary forms of amyloidosis.17-26,22,23,26,72 Destructive bone lesions are rare in amyloidosis but have been reported in association with plasma cell dyscrasias and hemodialysis-related amyloid.73-75

Cutaneous amyloid may be observed as a manifestation of systemic involvement or may be localized.76-77 Waxy skin plaques or nodules and purpura are the most common manifestations. Because of infiltration in the walls of small blood vessels which become fragile, ‘scratch’ and spontaneous palpebral purpura are especially suggestive of the diagnosis.

Amyloid deposition in the pancreatic islets is common in type II diabetes mellitus and islet cell tumors.78,79 Amyloid has been observed in serosal membranes,80 lymph nodes,26,81,82 and breast tissue.83 Deposition in the thyroid may result in a goiter.84 Amyloid stroma is often present histologically in patients with medullary thyroid carcinoma.85 Other neoplasms occasionally associated with amyloid deposition are Hodgkin’s disease,86 hairy cell leukemia,87 and renal cell carcinoma.88 Amyloidosis has been observed in cystic fibrosis with chronic pulmonary infections,89 Gaucher’s disease associated with monoclonal gammapathy,90 and cyclic neutropenia.91 Amyloid nephrotic syndrome has been identified in heroin addicts.92

## Table 1. Classification of Amyloid Diseases According to Major Protein Constituent in Fibrils

<table>
<thead>
<tr>
<th>Clinical Type</th>
<th>Protein Component</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Primary systemic (immunocytic)</td>
<td>AL (BJP)</td>
</tr>
<tr>
<td>2. Myeloma-associated (immunocytic)</td>
<td>AL (BJP)</td>
</tr>
<tr>
<td>3. Secondary systemic (reactive)</td>
<td>AA</td>
</tr>
<tr>
<td>4. Familial: autosomal recessive</td>
<td>AA</td>
</tr>
<tr>
<td>5. Reactive/induced in animals</td>
<td>AA</td>
</tr>
<tr>
<td>6. Familial: autosomal dominant</td>
<td>Prealbumin (TTR), Alp protein A-I</td>
</tr>
<tr>
<td>7. Senile cardiac</td>
<td>Prealbumin (TTR), α-ANP</td>
</tr>
<tr>
<td>8. Hemodialysis-associated</td>
<td>β2M</td>
</tr>
<tr>
<td>9. Central nervous system</td>
<td>β-Protein (A4)</td>
</tr>
<tr>
<td>10. Endocrine tumor-associated</td>
<td>β-Protein (A4)</td>
</tr>
<tr>
<td>11. Curative</td>
<td>β-Protein (A4)</td>
</tr>
<tr>
<td>12. Islet cell tumor</td>
<td>Cystatin C</td>
</tr>
<tr>
<td>13. Localized</td>
<td>Cystatin C</td>
</tr>
<tr>
<td>14. With systemic amyloidosis</td>
<td>Cystatin C</td>
</tr>
<tr>
<td>15. Keratin</td>
<td>Cystatin C</td>
</tr>
</tbody>
</table>

Abbreviations: AL, amyloid light chain; BJ, Bence Jones protein; AA, amyloid A; TTR, transthyretin; α-ANP, α-atrial natriuretic peptide; β2M, β₂-microglobulin.

Amyloid. Occasional resolution of renal amyloidosis has been observed;25-28 such spontaneous improvement in other organs involved with amyloid has been reported rarely.25 Cardiac involvement is especially likely to occur in primary systemic and some forms of hereditary and senile amyloidosis.30-37 Congestive cardiomyopathy with conduction defects and arrhythmias are frequently observed. Pericardial tamponade and valve involvement also have been described.32,33 Electrocardiography, echocardiography, angiography, technetium scanning, and myocardial biopsy are useful in diagnosis.36,38-41 Echocardiography provides the most sensitive noninvasive means for detection of cardiac amyloid infiltration.38,39 Amyloid deposition in arteries and arterioles may lead to severe vascular ischemic symptoms in the heart, extremities, or jaw.42-44 Respiratory involvement may occur as isolated involvement of the tracheobronchial tree or as nodular or diffuse amyloid deposition in the lungs.45-47 Gastrointestinal tract manifestations include macroglossia, dysphagia, motility disturbances, diarrhea, malabsorption syndrome, bleeding, infarction, and perforation.50,51 Liver findings include disproportionate hepatomegaly, nonspecific liver function studies, jaundice, and portal hypertension with ascites.55-57 Patients with neurological involvement may have peripheral or autonomic neuropathy,56-60 dementia, or cerebral hemorrhage.61

Amyloid infiltration of joints leads to marked periarticular thickening, most prominently manifest as the shoulder-pad sign; infiltration of muscles results in pseudohypertrophy.62-65

## HEMATOLOGIC FINDINGS

A variety of hematologic abnormalities may be observed in amyloid patients. Purpura is common on physical examination, especially scratch purpura or spontaneous periorbital purpura after a Valsalva maneuver.20,22,23,84 Lymphadenopathy is unusual but may be the presenting manifestation in an occasional patient.20,24,25 Splenomegaly is initially present in approximately 10% of patients, and splenic rupture has been reported rarely.20,22,23,56,56 Functional hyposplenism has been reported in 20% to 25% of patients with systemic amyloidosis26,77-79 and is present in 62% of those with primary hepatic amyloid.76 In such cases, Howell-Jolly bodies are evident on peripheral smear, and splenic uptake is decreased or absent on radionuclide scans.26,77,78 Hyposplenism does not occur with splenomegaly, probably because of massive replacement of splenic tissue by amyloid.98 A monoclonal immunoglobulin component in serum or urine is detectable by immunoelectrophoresis or immunofixation in approximately 80% of patients with primary systemic amyloidosis (AL).22,100,101 The most frequent abnormalities are Bence Jones proteinemia and proteinuria. In contrast to the usual distribution in myeloma, X Bence Jones proteins (BJPs) are more frequent than κ22,23,98,100-102 Polyclonal hypergammaglobulinemia may occur in patients with secondary amyloidosis. Most patients with AL have marrow plasmacytosis, and
some have sheets or clusters of plasma cells. Amyloid deposits are occasionally found in the walls of small blood vessels on marrow biopsy or rarely in the aspirate; 30% of patients with AL amyloidosis have a positive bone marrow. Rouleau is commonly observed in patients with monoclonal or polyclonal hypergamma globulinemia. Ten to 15% of patients have anemia; it is normocytic in those with coexisting myeloma or secondary amyloid, and microcytic in patients with hemorrhage and associated iron deficiency. Leukopenia and thrombocytopenia are unusual at presentation. Coagulation abnormalities are often present in patients with hepatic amyloidosis. Acquired factor X deficiency is an unusual but well-documented complication of amyloidosis; the clotting factor appears to be rapidly cleared from the circulation and bound by amyloid deposits. Factor X deficiency has been reported to resolve after splenectomy or chemotherapy. Rarely, increased fibrinolytic activity may account for a hemorrhagic diathesis; in such a circumstance α amino caproic acid may be of benefit. Lytic bone lesions of the type characteristic of myeloma rarely occur in amyloidosis.

**IMMUNOGLOBULIN LIGHT CHAIN-DERIVED AMYLOID (AL)**

An interesting historical parallel exists between amyloid and BJPs. Amyloid was named and popularized by Virchow in the mid-nineteenth century. Its structural features were not elucidated for more than 100 years, however, and its chemical nature remained controversial until the early 1970s. Similarly, BJP was described in 1848 and was used by clinicians as a diagnostic test for multiple myeloma for more than a century before its structure was clarified. Not until 1962 was it shown that BJP was the monoclonal immunoglobulin (Ig) light (L) chain portion of the serum M-component, which was often present in excess of heavy (H) chains and thus appeared commonly in the urine of patients with myeloma. A short time later, it was demonstrated that both H and L chains could be split by proteolytic enzymes into common (C) and variable (V) regions. The association between amyloid, BJP, and mar row plasmacytosis had been stressed by Apitz and Magnus-Levy for more than 40 years. In the 1960s, studies by Oserson et al and the Mayo Clinic group provided further evidence of this association and called attention to the relationship between myeloma and "primary" systemic amyloidosis. More recently, it has become clear that most patients presenting with the clinical manifestations of de novo systemic deposits of amyloid of the nonhereditary type (ie, primary) have an identifiable monoclonal Ig abnormality in serum, urine, or both. Amyloid deposition in these individuals tends to be distributed in the heart, tongue, gastrointestinal tract, skin, ligaments, and peripheral nerves. Involvement of liver, kidneys, spleen, and adrenals, a distribution more characteristic of "secondary" systemic amyloid, also may occur, however. Because of the overlap in amyloid deposition, differentiation into primary and secondary types on the basis of organ involvement or anatomic location is often unreliable in the individual patient.

The role of Ig components in the pathogenesis of nonhereditary primary systemic amyloidosis was clearly demonstrated by Glenner et al, who established by amino acid sequence studies the identity of monoclonal Ig L chains (BJPs) and certain amyloid fibril proteins. In patients with nonfamilial primary systemic amyloidosis or plasma cell dyscrasias associated with amyloidosis, the major protein constituent of the isolated amyloid fibril protein usually consists of intact monoclonal Ig L chains (BJPs), fragments from their amino-terminal (V) region, or both. Therefore, the amyloid in these patients (AL) consists of extracellular deposits of intact molecules or fragments of BJP. Amyloid has been identified in vitro even in patients without evidence of an M-protein in serum or urine. Tetramers or larger polymers of BJPs are selectively retained in the circulation and may predispose to amyloid deposition. Amyloid has been rarely observed in patients with γ heavy chain disease, a disorder typically unaccompanied by evidence of monoclonal L chain production. Because immunoglobulin heavy chains are not known to form amyloid, this finding has been unexplained. The recent demonstration of abnormal L chain genes in both γ and α heavy-chain disease may be pertinent in this regard. Low-level production of L chain fragments that are difficult to detect could, nevertheless, lead to amyloid deposition. Patients with primary systemic amyloidosis occasionally have been shown to have chemical types of amyloid other than AL. Approximately 80% of patients with nonhereditary primary (ie, without evidence of coexisting chronic infections or inflammatory disease) systemic amyloidosis have an identifiable M-protein in serum, urine, or both. Exclusive identification of BJPs, especially λ, has been especially common. Thus, accurate characterization of urinary protein by immunological techniques is critically important in this group of patients. Approximately 15% to 20% of BJPs appear to be amyloidogenic in that they have the property of precipitating as fibrillar material resembling amyloid after in vitro proteolytic digestion. This amyloidogenic property is associated with the V segment and is more commonly observed with λ than with κ monoclonal L chains, a finding in accord with the L-chain distribution noted in amyloid patients. The V region of the λ subclass VI appears to be especially amyloidogenic, although all λ subclasses have been identified. AL amyloid is composed of intact molecules or fragments of monoclonal L chains (ie, BJPs), and the amino-acid sequence of each BJP [molecular weight (mol wt) 23,000 daltons] is unique. No specific amino-acid sequence common to all amyloidogenic BJPs or AL proteins has yet been identified. BGP dimers may function as a primitive antibody; if so, such antigen-binding activity may be important in etiology and pathogenesis. The experimental studies relating BJPs to amyloid explain the rather high incidence of amyloidosis in patients with "light chain disease." Although it certainly occurs, amyloidosis has been infrequent in patients with the typical symptoms and signs of overt myeloma. These data are consistent with the hypothesis that patients with plasma cell dyscrasias who secrete intact BJPs or fragments thereof that possess amyloidogenic properties
have a clinical picture dominated by the features of primary systemic amyloidosis instead of the usual manifestations observed in typical myeloma. The resulting clinical illness, therefore, would be more dependent on the molecular structure of the individual BJPs synthesized than on any intrinsic difference between primary systemic amyloidosis and multiple myeloma.5,26 Such a hypothesis does not dictate that every patient producing amyloidogenic L chains necessarily need develop clinical amyloidosis; some clearly do not, suggesting that additional factors play an important role in tissue deposition of amyloid fibrils.2,5,26,13,15,16 Whether the BJP is transformed into amyloid fibrils intra- or extracellularly is not clear.147,149 Monoclonal antibodies (MoAbs) directed to non-light-chain antigens on plasma cells react with certain tissue amyloid deposits from patients with plasma cell dyscrasia-related amyloidosis.190 The tendency for some L-chain monomers or dimers to associate into high-mol wt polymers as well as biosynthetic studies demonstrating secreted L-chain fragments may have a bearing on whether incorporation into amyloid fibrils occurs.26,13,15,16 Both clinical types of AL, nonhereditary primary systemic and plasma cell dyscrasia-associated, have been termed immunocyte-derived amyloidosis by Glenner.5

The relationship of systemic light-chain deposition and light-chain nephropathy to amyloidosis is unclear.123,13,15,155 About 150 cases of light-chain deposition disease have been reported, most of which have had evidence of myeloma or an asymptomatic plasma cell dyscrasia.123,155 Nearly all cases have been associated with κ L chains.152,15,155,160 The deposits may occur in virtually any organ, especially the kidney. The monoclonal L chains in the deposits have been identified by immunohistochemical methods but do not show polarization birefringence after Congo red staining or a fibrillar structure by electron microscopy. Although systemic light-chain deposition has been observed with myeloma and Bence Jones proteinuria, serum M-components are frequently absent and the concentration of monoclonal L chains in urine is low. Marrow plasmacytosis usually is mild, but its monoclonal nature has been demonstrated.155,157 The most prominent findings usually are in the kidneys, in which glomerular and tubular deposits have been observed. A nodular type of glomerular involvement, resembling diabetic glomerulosclerosis, has been a striking finding in some patients.156,157,15,160 Most patients develop renal failure. An amyloid-like glomerulopathy without monoclonal L-chain deposits also has been described.151 There is some evidence indicating that the monoclonal L chains in light-chain deposition disease are either abnormally large or small and perhaps are glycosylated and synthesized as such.123,155 κ BJPs have a lesser tendency to form amyloid fibrils than their λ counterparts. Monoclonal κ chains in L-chain deposition disease may not contain an amino-acid sequence that permits a β-pleated sheet conformation in tissues and thus may not form amyloid.

**AMYLOID A (AA)**

In contrast to primary amyloidosis, patients with systemic amyloidosis secondary or reactive to chronic infections or inflammatory disorders and certain tumors have as the major protein constituent in their amyloid fibrils a protein (AA) that is not derived from immunoglobulin L chains.162,163 Recurrent infections in patients with tuberculosis, leprosy, chronic osteomyelitis, bronchiectasis, decubitus ulcers, paraplegia, chronically infected burns, chronic skin infections associated with parenteral drug abuse, hypogammaglobulinemia, and Whipple's disease are associated with AA.164 In addition, chronic inflammatory diseases, especially rheumatoid arthritis and juvenile chronic arthritis, are occasionally associated with amyloidosis of this type. Patients with other connective tissue disorders and Crohn's disease rarely develop AA.164 Neoplasms associated with AA include Hodgkin's disease, renal cell carcinoma and, very rarely, other tumors. AA appears to be derived by proteolytic cleavage of a heterogeneous minor component of normal plasma (SAA), which has a mol wt of ~12,500 daltons, is transported in association with high-density lipoprotein, and behaves as an acute-phase reactant.165,166 An increased concentration of SAA appears to be necessary for formation of AA; the apoprotein precursor protein is elevated in inflammatory and neoplastic diseases.167 The function of SAA is obscure; however, recent evidence indicates that it may play a role in modulating synthesis of collagenase.168 AA is a single polypeptide chain usually consisting of 76 amino acids and having a mol wt of approximately 8,500 daltons.169,170 Larger and smaller sizes of the protein have been identified as well.169 AA has been found in fibroblasts, plasma cells, Kupffer cells, and polymorphonuclear leukocytes, but appears to be synthesized by hepatocytes. Loss of affinity for Congo red after treatment with potassium permanganate is characteristic of AA; this histochemical property has proved useful in distinguishing AA from AL.21 The amyloid related to β₂-microglobulin deposition in chronic hemodialysis patients also is permanganate-sensitive, however. Immunohistochemistry using MoAbs specific for AA protein appears to be the method of choice for diagnosis of this type of amyloidosis.171

In addition to chronic infectious or inflammatory diseases and occasional tumors, AA also is the fibrillar protein in the only type of hereditary amyloidosis inherited as an autosomal recessive, familial Mediterranean fever (FMF). The amyloidosis of FMF occurs primarily in Sephardic Jews and persons of Turkish descent and may be the presenting manifestation of the disorder.172,173 Gertz et al described an FMF-like syndrome in a kindred in which the disorder appeared to be transmitted in an autosomal dominant pattern.174 The AA protein has been identified in amyloid fibrils from a patient with the Muckle-Wells syndrome, a type of hereditary amyloidosis characterized by nerve deafness, fever, urticaria, and the nephrotic syndrome.175 AA also is the chemical type that occurs in a variety of animal species with reactive or experimentally induced amyloidosis.163,176 Lavi et al showed that AA may form from faulty processing of SAA by peripheral blood monocytes.177 However, defective macrophage (Kupffer cell) function precedes development of amyloidosis in mice.178 Reduced SAA-degrading activity has been described in patients with secondary amyloidosis; the decrease in such activity parallels the serum albumin level.179 In mice, one of three SAA...
AMYLOIDOSIS

isotopes appears to be the precursor of amyloid fibril protein AA; this isotype (SAA) is selectively removed from the circulation. Mice resistant to amyloidosis have defective expression of the gene for SAA. A genetic marker for susceptibility to reactive systemic amyloidosis in patients with juvenile arthritis has been described; its applicability to other inflammatory disorders associated with amyloidosis is unknown.

PREALBUMIN (TRANSTHYRETIN [TTR]-DERIVED AMYLOID

A number of heredofamilial amyloid syndromes have been described; an excellent review of these disorders appeared recently. Unlike FMD, all other hereditary amyloidoses show an autosomal dominant mode of inheritance. Most are late-onset disorders with slow progression over a number of years; they are manifest as polyneuropathies, cardiomyopathies, or nephropathies. In most of these syndromes as well as in some types of senile cardiac amyloid, the major protein in the fibrils has been thyroxine-binding prealbumin, also known as TTR. This transport protein, which also binds retinol binding protein, has a normal plasma concentration of 20 to 40 mg/dL and is synthesized by the liver as a single polypeptide chain of 127 amino acids. Prealbumin is a negative acute-phase reactant (ie, its concentration decreases with inflammation); serum levels of the protein are also low in many patients with prealbumin amyloidosis. The normal circulating molecule has an extensive β-structure and is composed of a tetramer of noncovalently linked subunits, each of which has a mol wt of 14,000 daltons. A variant protein has been found in most types of familial amyloid polyneuropathy; both the normal and the abnormal forms of prealbumin are found in the deposits, but the latter appears to predominate. Single amino-acid substitutions have been identified at positions 30, 33, 60, 77, 84, 111, and 122 of the prealbumin molecule in the different types of hereditary amyloidoses. Six of these seven substitutions have been shown to result from single nucleotide changes in the prealbumin gene. In Portuguese, Japanese, United States, and Swedish families with familial amyloidotic polyneuropathy type I, the variant protein differs from normal plasma prealbumin in having a substitution of methionine for valine at position 30. Immunological, chemical, and direct DNA tests have been developed for detection of the specific mutations in the prealbumin variants; these assays are useful in identification of family members at risk while still in the preclinical phase of the disease. Such methods also are valuable for genetic counseling purposes. The ability to identify affected family members before clinical onset of disease will allow assessment of potentially valuable therapeutic regimens for these unusual disorders.

Not all of the familial amyloidotic polyneuropathy syndromes are associated with prealbumin deposits. In the Iowa type characterized by lower limb neuropathy, peptic ulcers and nephrotic syndrome, a variant form of apolipoprotein A-I has been demonstrated in the amyloid fibrils. Apolipoproteins occur in other types of amyloid. The precursor (SAA) of reactive systemic amyloidosis is an apolipoprotein. In addition, apolipoprotein A-II is present in the amyloid of mice with early senescence.

AMYLOID SECONDARY TO DEPOSITION OF β2M IN CHRONIC HEMODIALYSIS PATIENTS

During the past decade, a number of groups have reported that some patients on long-term hemodialysis develop the carpal tunnel syndrome. This finding occurs primarily in patients who have been on dialysis for 7 to 10 years. The carpal tunnel syndrome usually is bilateral. Cystic bone lesions or pathological fractures occur in some patients, and such lesions may be observed in the carpal bones. These symptoms and findings are the result of amyloid deposition in synovium and bone. Systemic involvement has been observed in some patients. The fibrillar protein in this circumstance has the usual tinctorial properties of amyloid. The electron microscopic appearance of the fibrils tends to be curvilinear rather than straight. The major protein constituent in this type of hemodialysis-associated amyloid consists of monomers and dimers of β2M. Like AA, β2M-derived amyloid is permanganate sensitive. This protein is normally present on all cell membranes other than erythrocytes and trophoblastic cells and is a component of the HLA class I molecule. The primary structure of β2M is homologous to that of the constant domains of immunoglobulin H and L chains. β2M binds preferentially to collagen; this property may be important in the pathogenesis of amyloidosis in patients on long-term hemodialysis. Like SAA, β2M may function in regulating collagen breakdown. β2M is a low-mol-wt protein (11,800 daltons) and, like immunoglobulin L chains and other small proteins, is filtered at the glomerulus and reabsorbed in the proximal tubule. β2M is too large to pass through most dialysis membranes; thus, it accumulates in the plasma of patients with chronic renal failure. The serum β2M concentration in chronic hemodialysis patients is 40 to 50 times higher than normal, but the elevated levels alone do not correlate well with the risk for developing amyloid. Thus, other systemic or local tissue factors may be important in determining whether amyloid deposition occurs. The use of polysulfone dialysers or extracorporeal adsorbents may increase the clearance of β2M and thus reduce the likelihood of amyloid deposition in this patient group.

AMYLOIDOSIS INVOLVING THE CENTRAL NERVOUS SYSTEM: ALZHEIMER'S DISEASE, DOWN'S SYNDROME, AND HEREDITARY CEREBRAL AMYLOID ANGIOPATHY

Alzheimer's disease, formerly believed to be a rare cause of dementia, is now recognized as the fourth leading cause of death in the United States and may be the most common type of amyloid-associated disease. Amyloid is present in three types of lesions in the brain of patients with Alzheimer's disease: neurofibrillary tangles, senile (neuritic) plaques, and blood vessels. As is usual, amyloid deposition in the plaques and vessels is extracellular but the neurofibrillary tangle with its paired helical filaments is a unique form of intracellular amyloid. Similar though less frequent amyloid lesions have been found in brains of normal elderly persons, patients with other neurodegenerative disorders, and several species of aged mammals. The major component of the fibrils in all three sites is β-protein (also
known as A4), a unique 28- to 43-amino acid polypeptide with a mol wt of approximately 4,200 daltons. In virtually all individuals with Down's syndrome aged more than 35 years, the same lesions occur in the brain and are also composed of β-protein. The gene for β-protein and that for familial Alzheimer's disease have been localized to chromosome 21 but these two genes do not appear to be linked. Because Down's syndrome is characterized by trisomy of chromosome 21, a third copy of the β-protein gene exists in these patients and may account for an overproduction of the protein which, after many years, leads to amyloid deposition in the brain. As with other chemical types of amyloid, the β-protein originates from a larger precursor (βAPP or PreA4); in this case, the precursor molecule appears to be a 695-amino acid glycoprotein of about 79,000 daltons. The β-protein sequence is near the C-terminal region of the precursor sequence; the precursor protein has been suggested to be a glycosylated cell receptor and related to the core protein of a heparan sulfate proteoglycan. The mRNA for β-protein has been identified in multiple cell types in the brain as well as other sites, including heart, kidney, and spleen. Immunoactivity for βAPP has been identified in the same nonneural sites as well as in adrenal gland and liver. Native βAPP occurs as a heterogeneous group of membrane-associated proteins in brain and other tissues. The cellular source of βAPP and its function remain to be determined. The widespread distribution of the precursor protein as well as the elevated ratio of serum βAPP (PreA4) to cerebrospinal fluid βAPP (PreA4) suggest that it circulates in the blood before it is incorporated into vessels, plaques, and tangles in the brain. Serum levels of βAPP (PreA4) are increased in patients with Down's syndrome but are normal in Alzheimer's patients. Although the role of β-protein in Alzheimer's disease is still unclear, the finding that a prionmyloid Congo red-negative type of amorphous nonfibrillar β-protein appears to precede development of neuritic plaques and neurofibrillary tangles in both Alzheimer's disease and Down's syndrome argues strongly for its importance in pathogenesis of the brain lesions. No evidence exists for production of an abnormal protein precursor in either Alzheimer's disease or Down's syndrome; nevertheless, this remains a possibility. A processing abnormality or catabolic defect constitutes another potential mechanism for amyloid deposition. A serine protease inhibitor, α-antichymotrypsin, is a component of amyloid deposits in neuritic plaques and blood vessel walls from brains of Alzheimer's disease patients. Alterations in the local concentration of inhibitor could promote deposition of β-protein into amyloid by inhibiting its clearance (excess activity) or by permitting increased proteolytic cleavage of βAPP and formation of β-protein (decreased activity).

Amyloid has been identified in Creutzfeldt-Jakob disease and other transmissible spongiform encephalopathies. The amino acid sequence of the "prion" fibrillar protein in these disorders differs from that of β-protein found in Alzheimer's disease and Down's syndrome. Cerebral amyloid angiopathy occurs in nearly all patients with Alzheimer's disease and aged Down's syndrome. In addition, cerebral amyloid angiopathy is a significant cause of spontaneous cerebral hemorrhage and has been described in kindreds from Iceland and the Netherlands in which it is transmitted as an autosomal dominant trait. Amyloid from the Dutch patients is biochemically identical to the β-protein seen in Alzheimer's disease and Down's syndrome. The Icelandic form of cerebral amyloid angiopathy is characterized by a protein closely related to the cysteine protease inhibitor cystatin C (previously known as γ-trace) which is found deposited in blood vessels. This protein is a 120-amino acid polypeptide with a mol wt of 13,000 daltons and is present in body fluids, especially the cerebrospinal fluid. It is produced by neuroendocrine cells within and outside the central nervous system. A variant protein has been identified in the cerebrovascular amyloid deposits, which is 10 residues shorter than the native protein and has an amino-acid substitution of glutamine for leucine at residue 58. As with other chemical types of amyloid, monomers and polymers of the protein are present in the deposits. Variant proteins reported in human amyloid diseases are shown in Table 2.

**AMYLOID OF OTHER TYPES: ENDOCRINE AND CUTANEOUS**

Certain tumors of endocrine glands (eg, medullary thyroid carcinoma and pancreatic islet cell tumors) have an amyloid stroma on histological examination. The localized amyloid of medullary thyroid carcinoma is composed of a precursor form of the hormone secreted by the tumor; i.e., procalcitonin. Amyloid also may be found in other types of calcitonin-producing tumors. The amyloid present in patients with islet cell tumors appears to be a protein distinct from insulin or proinsulin and has been termed islet amyloid polypeptide (IAPP). This molecule also may be involved in the pancreatic islet amyloid observed in elderly persons and persons with type II diabetes. A form of senile cardiac amyloid in which the deposits are restricted to the atria (isolated atrial amyloid or IAA) is composed of α atrial natriuretic peptide (αANP). This peptide is the C-terminal 28-amino acid fragment derived from a larger prohormone.

### Table 2. Variant Proteins in Human Amyloidosis

<table>
<thead>
<tr>
<th>Protein</th>
<th>Variant*</th>
<th>Clinical Syndrome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prealbumin (tranzthryatin)</td>
<td>Methionine 30</td>
<td>FAP I</td>
</tr>
<tr>
<td></td>
<td>Isoleucine 33</td>
<td>Jewish hereditary</td>
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<td></td>
<td>Alanine 60</td>
<td>Appalachian hereditary</td>
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<td></td>
<td>Tyrosine 77</td>
<td>Illinois/German hereditary</td>
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<td>Serine 84</td>
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<td>Danish hereditary</td>
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<td></td>
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<td>Senile cardiac</td>
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<td>Apolipoprotein A-I</td>
<td>Arginine 26</td>
<td>FAP III Iowa</td>
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<tr>
<td>Cystatin C</td>
<td>Glutamme 58</td>
<td>Hereditary cerebral amyloid angiopathy (Iceland)</td>
</tr>
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* Amino acid substitutions.
molecule (126 amino acid residues) and has potent natriuretic and diuretic effects.

Cutaneous involvement with amyloid occurs commonly as part of systemic deposition or may be localized to the skin.75,77 The lesions may be macular, papular, or nodular. Studies to date indicate that Ig L chains or AA protein are present in the skin of patients with systemic amyloidosis.72 Immunoreactivity to keratin has been reported in some patients with localized cutaneous amyloidosis.228

THERAPEUTIC CONSIDERATIONS

Therapy of amyloidosis is unsatisfactory, and evaluation of various approaches has been hindered by the lack of ability to determine accurately its extent in an individual patient.229,230 Despite the inert nature of amyloid fibrils and their presence in a relatively inaccessible extracellular location, amyloidosis occasionally is reversible.25,28,29,145,231 Thus, the fibrils can be mobilized and apparently dissolved under some circumstances. Potential therapeutic approaches consist of those directed to prevention of amyloid precursor protein synthesis, prevention of amyloid fibril deposition, and removal or dissolution of amyloid deposits from tissues.232,233

Colchicine has been shown to block production of secondary AA amyloid in mice.234 For more than a decade, colchicine has been known to prevent or abort febrile attacks in most patients with FMF.235-237 Israeli investigators subsequently showed that this agent can prevent amyloidosis in FMF patients.238 Moreover, colchicine can prevent additional deterioration of renal function in FMF patients with amyloidosis who have proteinuria but who have not progressed to the overt nephrotic syndrome. The effect of colchicine in prevention of amyloidosis in FMF patients appears to be independent of the drug's action on amelioration or prevention of the febrile attacks.239 The potential salutary effect of colchicine in other AA disorders associated with secondary (reactive) amyloidosis is unclear, but certainly needs to be examined.

Results of treatment in AL, usually with a myeloma-type chemotherapy regimen, have been unimpressive, although occasional successes have been reported.239-250 This approach is predicated on the rationale that treatment of such patients with alkylating agents and corticosteroids has as its objective reduction of the circulating monoclonal L-chain precursor by reducing the number of plasma cells synthesizing that protein in the bone marrow or elsewhere.22,26,242 Such an approach is aimed at retarding further deposition of amyloid in tissues rather than reversing amyloid that is already present. The median survival of patients with this plasma cell dyscrasia is 12 months, although long survival has been reported rarely.22,26,242,247 Congestive heart failure, urinary light chain excretion, hepatomegaly, and the presence of overt multiple myeloma are adverse prognostic factors within the first year.247 Elevated serum creatinine, overt multiple myeloma, orthostatic hypotension, and monoclonal serum component are adverse variables for those patients surviving more than 1 year.247

Chemotherapy has been of benefit in occasional patients with AL and the nephrotic syndrome.176,229,239,241,244,245 Improvement was evident within 1 year after institution of treatment and consisted of reduction in proteinuria and disappearance of edema. Hepatomegaly resolved in the four patients in whom it was initially present. Remission of the nephrotic syndrome, disappearance of serum and urine M-protein, stable renal function, and resolution of hepatomegaly may occur after chemotherapy despite advancing renal amyloidosis on subsequent biopsy.250,251 Thus, repeat biopsy of previously involved tissue is important in assessing the efficacy of therapy for AL. This point is further emphasized by reports of spontaneous remission in amyloid nephrotic syndrome.25,28,29,145 Occasional patients with histologically proven amyloidosis but without nephrotic syndrome have improved after chemotherapy, including one in whom histological regression of hepatic amyloid was demonstrated after melphalan-prednisone treatment.98,241,249,250

In a randomized trial, 55 patients with primary systemic amyloidosis received either melphalan-prednisone or placebo.242 Although laboratory parameters improved in 10 patients receiving chemotherapy, survival was not significantly different in the two groups. Because of its efficacy in FMF, colchicine also has been used in AL patients.73,246,250,253,234 Two groups of investigators have demonstrated improved survival in colchicine-treated patients as compared with historical controls.246,251 Kyle et al compared melphalan-prednisone with colchicine in a prospectively randomized cross-over study.246 No significant difference in survival was demonstrated when the two groups were analyzed in aggregate. Melphalan-prednisone–treated patients fared significantly better, however, when patients receiving only one regimen were analyzed or when survival was determined from the time of entry into the study until death or disease progression. These results suggested that melphalan-prednisone was superior to colchicine for treatment of AL amyloidosis. Use of melphalan-prednisone plus or minus colchicine should be compared in a randomized trial in patients with AL.

Dimethylsulfoxide (DMSO) administered either orally or transdermally has been reported to benefit occasional amyloid patients, but the agent has been difficult to tolerate orally and the results, in general, have been disappointing.255,256 Several other pharmacologic agents have been reported to have toxic or beneficial effects in experimental and clinical amyloid disorders. Various immunosuppressive drugs, including several cytotoxic chemotherapeutic agents in clinical use, accelerate casein-induced amyloidosis in mice.257 Such findings sound a note of caution regarding use of these drugs in non-AL types of amyloid, especially reactive (AA) systemic amyloidosis. Patients with amyloid cardiomyopathy have long been considered to be sensitive to digitalis. Isolated amyloid fibrils bind digoxin; such binding may be related to the enhanced toxicity of digitalis in patients with amyloid heart disease.258 Calcium channel-blocking agents can aggravate congestive heart failure in patients with cardiac amyloidosis.259 Gertz et al reported selective binding of nifedipine to isolated amyloid fibrils; the binding was largely irreversible and not calcium dependent.260 Autonomic neuropathy may result in incapacitating symptoms in amyloid patients. A norepinephrine precursor, L-threo-3,4-dihydroxyphenylserine, appears to be effective in treatment of
orthostatic hypotension. A long-acting somatostatin analogue may be helpful in controlling diarrhea in patients with autonomic dysfunction.

Use of polysulfone dialysers or extracorporeal adsorbers may reduce the risk of β₂M amyloidosis in chronic hemodialysis patients. Plasmapheresis has been used to lower the level of the circulating amyloid precursor in patients with familial amyloidotic polyneuropathy and light-chain-associated renal failure. Immunoabsorbents specific for each amyloid precursor constitute a potential approach that may be more efficient in removal of circulating proteins from plasma. Use of DNA testing to identify persons at risk of development of familial prealbumin amyloidosis while still in the preclinical phase of their disease will be useful in assessing the efficacy of various therapeutic regimens for these disorders.

Renal transplantation has been performed in selected patients with amyloidosis, most of whom have had the AA type (FMF or reactive systemic). Graft survival has been reported in six patients, four of whom had evidence of BJP. Five of the six patients were alive and fully rehabilitated 12 months posttransplant. One patient had evidence of recurrent cardiac amyloid by electron microscopy at the time of the report. No information was given regarding use of cytotoxic chemotherapy. Additional data will be necessary before the role of cardiac transplantation for amyloidosis can be adequately assessed.

Effective therapy for Alzheimer's disease and other forms of amyloidosis awaits elucidation of further insights into the origin and pathogenesis of fibrillar deposition in patients with these disorders.

REFERENCES


CONCLUSION

Although amyloid deposits in all clinical conditions share common physical properties relating to the presence of a β-pleated sheet conformation, it is now clear that many different chemical types exist and additional ones are likely to be described in the future. Immunohistochemical techniques using antisera specific for each of the known amyloid fibril proteins will aid greatly in subclassifying these disorders. Figure 1 is a schematic of the pathogenesis of amyloidosis. In most circumstances, a circulating precursor protein may result from overproduction of either intact or aberrant molecules (plasma cell dyscrasias), reduced degradation or excretion (SAA in some secondary amyloid syndromes and β₂M in patients on long-term hemodialysis), or genetic abnormalities associated with variant proteins (familial autosomal dominant polynuropathies). Proteolysis of a larger protein precursor molecule with production of low-mol-wt fragments that polymerize and assume a β-conformation as tissue deposits in an extracellular location characterizes amyloidogenesis. Knowledge acquired during the past decade has confirmed and extended the concept that amyloidosis is a generic term referring to a final common pathphysiologic pathway for tissue protein deposition in a wide variety of diseases.

NOTE ADDED IN PROOF

The β-amyloid precursor protein (PreA4) has been reported to be a coagulation factor XI, inhibitor (Broze GJ, Higuchi DA, Smith RP. Blood 74:56a, 1989 [abstr, suppl])

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