

12.12.3 Amyloidosis 2218

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section 12 Metabolic disorders 2218 Levy R, et al. (2015). Phenotypic and genotypic characteristics of cryopyrin-associated periodic syndrome: a series of 136 patients from the Eurofever Registry. *Ann Rheum Dis*, 74(11), 2043–9. Liu Y, et al. (2012). Mutations in proteasome subunit β type 8 cause chronic atypical neutrophilic dermatosis with lipodystrophy and elevated temperature with evidence of genetic and phenotypic heterogeneity. *Arthritis Rheum*, 64, 895–907. Niel E, Scherrmann JM (2006). Colchicine today. *Joint Bone Spine*, 73, 672–8. Schneiders MS, et al. (2005). Manipulation of isoprenoid biosynthesis as a possible therapeutic option in mevalonate kinase deficiency. *Arthritis Rheum*, 54, 2306–13. Sfriso P, et al. (2012). Blau syndrome, clinical and genetic aspects. *Autoimmun Rev*, 12, 44–51. Simon A, et al. (2013). Schnitzler's syndrome: diagnosis, treatment, and follow-up. *Allergy*, 68, 562–8. Smith EJ, et al. (2010). Clinical, molecular, and genetic characteristics of PAPA syndrome: a review. *Curr Genomics*, 11, 519–27. Sönmez HE, et al. (2016). Familial Mediterranean fever: current perspectives. *J Inflamm Res*, 17, 13–20. Twig G, et al. (2014). Mortality risk factors associated with familial Mediterranean fever among a cohort of 1.25 million adolescents. *Ann Rheum Dis*, 73, 704–9. Zhao Y, Shao F (2016). Diverse mechanisms for inflammasome sensing of cytosolic bacteria and bacterial virulence. *Curr Opin Microbiol*, 29, 37–42.

12.12.3 Amyloidosis Mark B. Pepys and Philip N. Hawkins

ESSENTIALS Amyloidosis is the clinical condition caused by extracellular deposition of amyloid in the tissues. Amyloid deposits are composed of amyloid fibrils, abnormal insoluble protein fibres formed by misfolding of their normally soluble precursors. About 30 different proteins can form clinically or pathologically significant amyloid fibrils in vivo as a result of either acquired or hereditary abnormalities. Small, focal, clinically silent amyloid deposits in the brain, heart, seminal vesicles, and joints are a universal accompaniment of ageing. Clinically important amyloid deposits usually accumulate progressively, disrupting the structure and function of affected tissues and lead inexorably to organ failure and death. There is no licensed treatment which can specifically clear amyloid deposits, but intervention which reduces the availability of the amyloid fibril precursor proteins can arrest amyloid accumulation and may lead to amyloid regression with clinical benefit. Pathology—amyloid fibrils of all types are similar: straight, rigid, and nonbranching; of indeterminate length and 10–15 nm in diameter; and with their subunit proteins arranged in a stack of twisted anti-parallel β -pleated sheets. The fibrils bind Congo red dye producing pathognomonic green birefringence when viewed in high-intensity cross-polarized light, and the protein type can be identified by immunostaining or proteomic analysis. Amyloid deposits always contain a nonfibrillar plasma glycoprotein, serum amyloid P component, the universal presence of which is the basis for use of radio-isotope-labelled serum amyloid P component as a diagnostic tracer. Clinicopathological correlation—amyloid may be deposited in any tissue of the body,

including blood vessels walls and connective tissue matrix; clinical manifestations are correspondingly diverse. Although there are some typical clinical presentations related to fibril type, there are many forms of amyloidosis in which there is little or no concordance between the fibril protein, or the geno- type of its precursor, and the clinical phenotype. Identification of the amyloid fibril protein is always essential for appropriate clinical management. Specific types of amyloidosis

Monoclonal immunoglobulin light chain (AL) amyloidosis—the most prevalent type of systemic amyloidosis currently diagnosed. The fi- brils consists of all or part of the variable (VL) domain of monoclonal immunoglobulin light chains. May complicate any B-cell dyscrasia but most cases are associated with otherwise ‘benign’ ‘monoclonal gammopathy of undetermined significance’ (MGUS). Highly variable idiopathic disease but characteristic presentations include involve- ment of the heart (restrictive cardiomyopathy), kidneys (protein- uria, renal failure), gut (motility disorders, malabsorption), tongue (macroglossia), and nerves (painful sensory polyneuropathy; auto- nomic neuropathy). Treatment is with cytotoxic chemotherapy, similar to that used in myeloma, aimed at suppression of the causa- tive B-cell clone.

Transthyretin (ATTR) amyloidosis—caused by wild type transthyretin (TTR) forming amyloid predominantly in the heart, mostly in eld- erly men. Previously diagnosed extremely rarely but shown by new imaging methods to be much more common with prevalence lately approaching that of AL amyloidosis, and potentially exceeding it in future.

Hereditary systemic amyloidoses—include hereditary ATTR amyloidosis caused by mutations in the TTR gene. Presents as progres- sive peripheral and autonomic neuropathy and varying degrees of visceral involvement (formerly known as familial amyloidotic poly- neuropathy), and sometimes with predominant cardiomyopathy.

Reactive systemic (AA) amyloidosis—fibrils composed of AA pro- tein derived from the acute phase protein, serum amyloid A protein (SAA). It can be a complication of any chronic inflammatory disorder (e.g. rheumatoid arthritis, Crohn’s disease) or chronic infections in which SAA concentrations are persistently increased. Has become very rare in recent years due to improved management of chronic inflammation and infection. Usually presents with proteinuria and/or organomegaly (e.g. hepatosplenomegaly); nephrotic syndrome may develop before progression to end-stage renal failure.

Introduction Amyloidosis and amyloid deposits Amyloid deposits can be systemic, that is present anywhere in the body. The predominant locations and most damaging clinical

12.12.3 Amyloidosis 2219 effects can vary widely in different types of amyloidosis and in dif- ferent individuals. In systemic amyloidosis the deposits can be in the extracellular space of any tissue and any organ except the brain, because the amyloid fibrils are derived from circulating, normally soluble, globular plasma proteins. There are also localised forms of amyloidosis in which the deposits are confined to a single tissue or organ system, such as the cerebral blood vessels walls in cerebral amyloid angiopathy or localised amyloid of the respiratory system or urogenital tract. Crucially, in all forms of amyloidosis, tissue damage and thus disease are directly caused by the amyloid de- posits that disrupt normal tissue architecture and thus function. In contrast, the histopathology in some other diseases includes local amyloid deposits, for example, the A β amyloid plaques in the brain in Alzheimer’s disease and islet amyloid in the islets of Langerhans in the pancreas of patients with type 2 diabetes, but there is no direct evidence that these deposits cause disease. They should therefore not be included as types of amyloidosis. Similarly, microscopic amyloid deposits are frequently present in the elderly, in the seminal vesicles and the walls of large arteries, without evidence that they necessarily cause disease. Systemic amyloidosis causes about 1 in 1500 of all deaths in developed countries but this figure will likely rise as the true prevalence of wild type ATTR amyloidosis is recognized and is a serious and important disease because it is

often difficult to diagnose, it is usually fatal, and its management is complex and costly. Most systemic amyloidosis is a complication of other underlying primary conditions, which include monoclonal gammopathies of all types, chronic inflammatory disorders, and dialysis for end-stage renal failure. Hereditary amyloidosis is very rare, except in a few geographic foci, but its diversity is remarkable. It is important because of its poor prognosis, the complexity of clinical management, the difficult genetic issues involved, and its considerable value as a model for understanding the pathogenesis of amyloid deposition. Clinicopathological correlation Although there are some correlations between fibril protein type and clinical manifestations, there are also many forms of acquired and hereditary amyloidosis in which there is little or no concordance between the fibril protein, or the genotype of its precursor, and the clinical phenotype (Tables 12.12.3.1 and 12.12.3.2). There are evidently genetic and/or environmental factors, distinct from the amyloid fibril protein itself, that determine whether, when, and where clinically significant amyloid deposits form. The nature of these important determinants of amyloidogenesis is obscure. Furthermore, the mechanisms by which amyloid deposition causes disease are poorly understood. While a heavy amyloid load is invariably a bad sign, there may be a poor correlation between the local amount of amyloid and the level of organ dysfunction. Active deposition of new amyloid is often associated with accelerated deterioration compared with stable, long-standing deposits. Nascent or newly formed amyloid fibrils generated in vitro may also be cytotoxic to cultured cells, whereas aged or ex vivo fibrils are generally inert, but it is not known if or how this relates to effects in vivo. In most forms of systemic amyloidosis there is overwhelming evidence that tissue damage and resultant disease are caused by the physical presence and accumulation of amyloid deposits, and not by cytotoxicity of the amyloidogenic proteins or their prefibrillar aggregates. Clinical types of amyloidosis

Reactive systemic (AA) amyloidosis	Associated conditions
AA amyloidosis occurs in association with chronic inflammatory disorders, chronic local or systemic microbial infections, and, occasionally, malignant neoplasms. In western Europe and the United States	Table 12.12.3.1 Acquired amyloidosis syndromes
Clinical syndrome	Fibril protein
Systemic AL amyloidosis, associated with immunocyte dyscrasia, myeloma, monoclonal gammopathy, occult dyscrasia	AL derived from monoclonal immunoglobulin light chains
Local nodular AL amyloidosis (skin, respiratory tract, urogenital tract, etc.) associated with focal immunocyte dyscrasia	AL derived from monoclonal immunoglobulin light chains
Reactive systemic AA amyloidosis, associated with chronic active diseases	AA derived from SAA
Non-hereditary wild-type transthyretin amyloidosis	Transthyretin derived from plasma transthyretin
Sporadic cerebral amyloid angiopathy	A β derived from APP
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
Primary localized cutaneous amyloid (macular, papular) ?	Keratin-derived
Systemic ALECT2 amyloidosis causing renal dysfunction	Leucocyte chemotactic factor 2
Ocular amyloid (cornea, conjunctiva)	Not known
Orbital amyloid	AL or AH derived from monoclonal Ig

Ig AA, amyloid A; AH, monoclonal immunoglobulin heavy chain fragment; AL, monoclonal immunoglobulin light chain; ALECT2, leucocyte chemotactic factor 2 amyloid; APP, amyloid precursor protein; Ig, immunoglobulin; SAA, serum amyloid A protein.

section 12 Metabolic disorders 2220 States of America, the most frequent predisposing conditions are idiopathic rheumatic diseases, notably including rheumatoid arthritis and juvenile idiopathic arthritis (Box 12.12.3.1). AA amyloidosis has become increasingly rare, reflecting improved treatment of chronic inflammatory disorders, and for reasons that are not clear, the incidence is lower in the United States of America than in Europe. Amyloidosis is exceptionally rare in systemic

lupus erythematosus, related connective tissue diseases, and in ulcerative colitis in which there is a blunted acute phase response of serum amyloid A protein, the precursor of AA amyloid fibrils. Tuberculosis and leprosy are important causes of AA amyloidosis where these infections remain endemic. Chronic osteomyelitis, bronchiectasis, chronically infected burns, and decubitus ulcers, as well as the chronic pyelonephritis of paraplegic patients, are other well-recognized associations (Box 12.12.3.1). Hodgkin's disease and renal carcinoma, which often cause fever, other systemic symptoms, and a major acute phase response, are the malignancies most commonly associated with systemic AA amyloidosis. Intriguingly, about 10% of patients with AA amyloidosis do not have a clinically obvious chronic inflammatory disease, and may erroneously be assumed to have AL amyloidosis. The commonest identifiable pathologies in such cases in our own experience have been previously undiagnosed inherited periodic fever syndromes and cytokine-secreting Castleman's disease tumours of the solitary plasma cell type, located in either the mediastinum or the gut mesentery. Clinical features AA amyloid involves the viscera, but may be widely distributed without causing clinical symptoms. More than 90% of patients present with nonselective proteinuria resulting from glomerular deposition, and nephrotic syndrome may develop before progression to end-stage renal failure. Haematuria, isolated tubular defects, nephrogenic diabetes insipidus, and diffuse renal calcification rarely occur. Kidney size is usually normal, but may be enlarged, or, in advanced cases, reduced. End-stage chronic renal failure is the cause of death in 40 to 60% of cases, but acute kidney injury may be precipitated by hypotension and/or salt and water depletion following surgery, excessive use of diuretics, or intercurrent infection. The second most common presentation is with organ enlargement, such as hepatosplenomegaly or thyroid goitre, with or without overt renal abnormality, but in any case, amyloid deposits are almost always widespread at the time of presentation. Involvement of the heart and gastrointestinal tract is frequent, although the former rarely causes functional impairment.

Table 12.12.3.2 Hereditary amyloidosis syndromes

Clinical syndrome	Fibril protein	Predominant peripheral nerve involvement
hereditary transthyretin amyloidosis associated polyneuropathy (familial amyloid polyneuropathy).	Transthyretin variants (commonly Met30, >120 others described)	
Autosomal dominant	Apolipoprotein A-I N-terminal fragment of Arg26 variant	Predominant cranial nerve involvement with lattice corneal dystrophy.
Autosomal dominant	Gelsolin, fragment of variants Asn187 or Tyr187	Oculoleptomeningeal amyloidosis.
Autosomal dominant	Transthyretin variants	Non-neuropathic, prominent visceral involvement.
Autosomal dominant	Apolipoprotein A-I N-terminal fragment of variants	Lysozyme variants
Fibrinogen α -chain variants	β 2-microglobulin variant Asn76	Predominant cardiac involvement, no clinical neuropathy, hereditary transthyretin amyloidosis associated cardiomyopathy.
Autosomal dominant	Transthyretin variants	Hereditary cerebral haemorrhage with amyloidosis (cerebral amyloid angiopathy).
Autosomal dominant: Icelandic type (major asymptomatic systemic amyloid also present)	Cystatin C, fragment of variant Glu68	Dutch type A β derived from APP variant Gln693
Cutaneous deposits (bullous, papular, pustulodermal)	Not known	Amino acids: Arg, arginine; Asn, asparagine; Gln, glutamine; Glu, glutamic acid; Met, methionine; Tyr, tyrosine. A β , amyloid β ; APP, amyloid precursor protein; SAA, serum amyloid A protein.

Box 12.12.3.1 Conditions associated with reactive systemic amyloid A amyloidosis

Chronic inflammatory disorders • Rheumatoid arthritis • Juvenile idiopathic arthritis • Ankylosing spondylitis • Psoriasis and psoriatic arthropathy • Reiter's syndrome • Adult Still's disease • Behçet's syndrome • Crohn's disease • Hereditary periodic fever syndromes

Chronic microbial infections • Leprosy • Tuberculosis • Bronchiectasis • Decubitus ulcers • Chronic pyelonephritis in paraplegics • Osteomyelitis • Whipple's disease

Neoplasms • Castleman's disease • Hodgkin's disease • Renal carcinoma • Carcinomas of gut, lung, and urogenital tract • Basal cell

12.12.3 Amyloidosis 2221 AA amyloidosis may become clinically evident early in the course of associated disease, but the incidence increases with the duration of the primary condition. The mean duration of chronic rheumatic diseases, such as rheumatoid arthritis, ankylosing spondylitis, or juvenile rheumatoid arthritis, before amyloid is diagnosed is 12 to 14 years, although they can present much sooner. For most patients, the prognosis is closely related to the degree of renal involvement and the effectiveness of treatment for the underlying inflammatory condition. In the presence of persistent uncontrolled inflammation, 50% of patients with AA amyloidosis die within 5 years of diagnosis; however, if the acute phase response can be consistently suppressed, proteinuria can cease, renal function can be retained, and the prognosis is much better. The availability of chronic haemodialysis and transplantation prevents early death from uraemia *per se*, but amyloid deposition in extrarenal tissues may be responsible for a less favourable prognosis than for some other causes of end-stage renal failure. Amyloidosis associated with immunocyte

dyscrasia: monoclonal immunoglobulin

light chain (AL) amyloidosis Associated conditions AL amyloidosis may complicate almost any dyscrasia of cells of the B-lymphocyte lineage, including multiple myeloma, malignant lymphomas, and macroglobulinaemia, but most cases develop on a background of monoclonal gammopathy of undetermined significance. Amyloidosis occurs in up to 10% of cases of myeloma, in a lower proportion of other malignant B-cell disorders, and about 2% of patients with monoclonal gammopathy of undetermined significance, which are, of course, much more common than myeloma. A monoclonal paraprotein or abnormal serum free light chain concentration can be detected in the serum of most patients with AL amyloidosis, confirming a monoclonal gammopathy. Subnormal levels of some or all serum immunoglobulins, or increased numbers of marrow plasma cells may provide less direct clues to the underlying aetiology. Until recently it has been the practice to diagnose apparently primary cases of amyloidosis, with no previous predisposing inflammatory condition or family history of amyloidosis, as AL type by exclusion. However, it has now been recognized that autosomal dominant, hereditary non-neuropathic amyloidosis, particularly that caused by variant fibrinogen α -chain, may be poorly penetrant and of late onset, so there may be no family history. The coincident occurrence of a monoclonal gammopathy, which occurs in more than 10% of the healthy older population, may then be gravely misleading, and it is essential to exclude by genotyping all known amyloidogenic mutations, and to seek positive immunohistochemical or proteomic identification of the amyloid fibril protein in all cases. Clinical features AL amyloidosis occurs equally in men and women, usually over the age of 50 years, but occasionally in young adults. It has a lifetime incidence (and is the cause of death) of between 0.5 and 1 in 1000 individuals in the United Kingdom. The clinical manifestations are protean, as virtually any tissue other than the brain may be directly involved. Uraemia, heart failure, or other effects of amyloidosis usually cause death within 2 years of diagnosis, unless the underlying B-cell clone is effectively suppressed. The heart is affected in 90% of patients with AL amyloidosis. In 30% of these, restrictive cardiomyopathy is the presenting feature and in up to 50% of these patients it is fatal. Other cardiac presentations include arrhythmias and angina. Measurement of circulating brain natriuretic peptide (BNP) or its precursor N-terminal-proBNP provide a sensitive index of cardiac dysfunction in cardiac AL amyloidosis, and often show rapid improvement when production of the aberrant clonal light chain is substantially suppressed by cytotoxic chemotherapy. This suggests that amyloidogenic light chains, perhaps in an aggregated

prefibrillary form, may themselves have intrinsic cardiotoxicity, in addition to their deposition as amyloid fibrils. Renal AL amyloidosis has the same manifestations as renal AA amyloidosis, but the prognosis is worse. Gut involvement may cause disturbances of motility (often secondary to autonomic neuropathy), malabsorption, perforation, haemorrhage, or obstruction. Macroglossia occurs rarely, but is almost pathognomonic. Hyposplenism sometimes occurs in both AA and AL amyloidosis. Painful sensory polyneuropathy, with early loss of pain and temperature sensation, followed later by motor deficits, is seen in 10 to 20% of cases, and carpal tunnel syndrome in 20%. Autonomic neuropathy leading to orthostatic hypotension, impotence, and gastrointestinal disturbances may occur alone or together with the peripheral neuropathy, and has a very poor prognosis. Skin involvement takes the form of papules, nodules, and plaques, usually on the face and upper trunk, and involvement of dermal blood vessels results in purpura, occurring either spontaneously or after minimal trauma, and is very common. Articular amyloid is rare, but may mimic acute polyarticular rheumatoid arthritis, or it may present as asymmetrical arthritis affecting the hip or shoulder. Infiltration of the glenohumeral joint and surrounding soft tissues occasionally produces the characteristic 'shoulder pad' sign. A rare but serious manifestation of AL amyloidosis is an acquired bleeding diathesis that may be associated with deficiency of factor X, and sometimes also factor IX, or with increased fibrinolysis. It does not occur in AA amyloidosis, although in both AL and AA disease there may be serious bleeding in the absence of any identifiable coagulation factor deficiency due to widespread vascular amyloid deposits.

Senile amyloidosis Some amyloid is seen in all autopsies on individuals over 80 years of age, but it is not known whether this contributes to the ageing process or whether it is an epiphenomenon that becomes clinically important only when it is extensive. Wild-type transthyretin (cardiac) amyloidosis Up to 25% of older people have systemic deposits of wild-type transthyretin amyloid involving the walls of the heart and blood vessels, smooth and striated muscle, fat tissue, renal papillae, and alveolar walls. In contrast to most other forms of systemic amyloidosis (including hereditary transthyretin amyloid caused by point mutations in the transthyretin gene), clinical manifestations are usually restricted to the heart and carpal tunnel syndrome. The brain is not involved. The typical clinical picture of progressive restrictive cardiomyopathy is usually fatal within 5 years. The isoleucine 122 variant of transthyretin, which occurs in about 4% of black individuals of African ethnicity, is associated with a greatly increased risk of cardiac transthyretin amyloidosis, the phenotype of which is indistinguishable from the wild-type form. It is likely that senile cardiac amyloidosis is substantially underdiagnosed and may account for many cases of cardiac failure associated with preserved ejection fraction in the elderly.

section 12 Metabolic disorders 2222 **Senile focal amyloidosis** Microscopic and clinically silent amyloid deposits of different fibril types, localized to particular tissues, are very commonly found in older people. Deposits of β -protein ($A\beta$, see 'Alzheimer's disease') as amyloid in cerebral blood vessels and intracerebral plaques seen in normal older brains may or may not have been the harbinger of Alzheimer's disease had the patient survived long enough. Amyloid deposits composed of apolipoprotein A-I are present in most osteoarthritic joints at surgery or autopsy, usually in close association with calcium pyrophosphate deposits, and affect the articular cartilage and joint capsule. However, the significance of this age-associated articular amyloid, the amount of which is correlated with neither the presence nor clinical severity of osteoarthritis, is not known. The corpora amylacea of the prostate are composed of β 2-microglobulin amyloid fibrils. Amyloid in the seminal vesicles is derived from semenogelin I, an exocrine secretory product of the vesicle cells. Isolated deposits of cardiac atrial amyloid consist of atrial natriuretic peptide. The focal

amyloid deposits commonly present in atheromatous plaques of older subjects are of two types: containing fibrils either composed of medin, a fragment of lactadherin, or the N-terminal fragment of apolipoprotein A-I. Cerebral amyloid The brain is a very common and important site of amyloid deposition (Box 12.12.3.2), although, possibly because of the blood-brain barrier, there are never any deposits in the cerebral parenchyma itself in any form of acquired systemic visceral amyloidosis. However, cerebrovascular transthyretin amyloid may occur in familial amyloid polyneuropathy, although the serious clinical entity of oculoleptomeningeal amyloidosis is very rare and associated with certain uncommon transthyretin variants. The major forms of brain amyloid are confined to the brain and cerebral blood vessels, with the single exception of cystatin C amyloid in hereditary cerebral haemorrhage with amyloidosis, Icelandic type, in which there are major, though clinically silent, systemic deposits. Alzheimer's disease By far the most frequent and important type of amyloid in the brain is that related to Alzheimer's disease, which is the most common cause of dementia and affects more than 3 million individuals in the United States of America and a corresponding proportion of other Western populations. It is generally a disease of older people, and its prevalence is therefore increasing. The clinical differential diagnosis of senile dementia and the positive identification of Alzheimer's disease are difficult and often of limited precision in life. However, intracerebral and cerebrovascular amyloid deposits are hallmarks of the neuropathological diagnosis and are detectable by PET imaging with radionuclide-labelled ligands specific for amyloid fibrils composed of β -protein ($A\beta$). The Alzheimer's disease $A\beta$ protein is a 39- to 43-residue cleavage product of the large amyloid precursor protein. The vast majority of cases of Alzheimer's disease are sporadic, but there are also families with an autosomal dominant pattern of inheritance and usually early onset. In about 20 families there are causative mutations in the APP gene for amyloid precursor protein on chromosome 21, and most other kindreds have mutations in the genes for presenilin 1 (chromosome 14) and presenilin 2 (chromosome 1). All these mutations are associated with increased production from amyloid precursor protein of $A\beta_{1-42}$, the most amyloidogenic form of $A\beta$. Since all individuals with Down's syndrome (trisomy 21) develop Alzheimer's disease if they survive into their 40s, there is evidently a close link between amyloid precursor protein, $A\beta$ overproduction, $A\beta$ amyloidosis, and the pathogenesis of Alzheimer's disease. However, it remains unclear whether or how $A\beta$ per se, or the amyloid fibrils that it forms, contribute to the neuronal loss that underlies the dementia. Synthetic $A\beta$ fibrils formed in vitro are markedly cytotoxic, and cause the death of cultured cells by apoptosis and necrosis. Although it is not clear to what extent these findings reflect phenomena that may be responsible for neurodegeneration in vivo, there is increasing evidence, from both transgenic mouse models of Alzheimer's disease and in vivo intracerebral injection of different molecular conformations of $A\beta$, that small oligomeric prefibrillar aggregates of $A\beta$ are associated with and cause cognitive dysfunction. There is controversy about the correlation between the severity of dementia in Alzheimer's disease and the extent of amyloid angiopathy and plaques. Nevertheless, the fact that patients with Alzheimer's disease caused by amyloid precursor protein and presenilin mutations have exactly the same neuropathology as sporadic cases, including tangles, argues strongly that the amyloid precursor protein and $A\beta$ pathway can be of primary pathogenetic significance. In addition to the $A\beta$ deposits in the brains of patients with Alzheimer's disease and Down's syndrome, there are also extensive 'amorphous' deposits throughout the brain. These do not stain with Congo red, and are detectable only by immunohistochemical staining. Their significance is unknown. They apparently precede the appearance of histochemically identifiable amyloid, but are not necessarily the precursor of it because they are present in areas such as the cerebellum in which $A\beta$ is never seen. The nonfibrillar, nonamyloid protein apolipoprotein E is

demonstrable in many amyloid deposits, including those of Alzheimer's disease. The APOE4 gene (chromosome 19), encoding one of the three isoforms of this apolipoprotein, is strongly associated with a predisposition to develop Alzheimer's disease and with increased amounts of amyloid in the brain, but the underlying mechanisms are unknown. Another neuropathological feature of Alzheimer's disease, and some other neurodegenerative conditions, is the neurofibrillary tangle located intracellularly within neuronal cell bodies and processes. These tangles have a characteristic ultrastructural morphology of paired helical filaments, and are composed of an abnormally phosphorylated tau protein.

Box 12.12.3.2 Cerebral amyloidosis • Age-related amyloid angiopathy with or without intracerebral deposits (cerebral amyloid angiopathy) • Hereditary amyloid angiopathy of meningeal and cortical vessels associated with cerebral haemorrhage:

— Icelandic type (variant cystatin C)

— Dutch type (variant A β) • Hereditary amyloid angiopathy affecting the entire central nervous system • Alzheimer's disease: sporadic, familial, or associated with Down's syndrome • Cerebral amyloid associated with prion disease:

— Sporadic spongiform encephalopathy, Creutzfeldt-Jacob disease, variant Creutzfeldt-Jacob disease

— Familial prion disease, familial Creutzfeldt-Jacob disease, Gerstmann-Sträussler-Scheinker syndrome atypical familial prion disease • Familial oculoleptomeningeal amyloidosis

12.12.3 Amyloidosis 2223 phosphorylated form of the normal neurofilament protein, tau. They bind Congo red and then give the pathognomonic green birefringence of amyloid when viewed in polarized light. Although their electron microscopic ultrastructure is distinctly different from that of amyloid fibrils, the most recent review of amyloid nomenclature includes them as amyloid. All extracellular and most intracellular tangles are coated with serum amyloid P component. Sporadic cerebral A β amyloidosis and cerebral amyloid angiopathy At autopsy, up to 60% of the brains of nondemented older individuals contain A β amyloid in the cerebral blood vessels, and there may also be focal intracerebral A β plaques. These deposits may or may not have been harbingers of Alzheimer's disease, had the patients survived long enough, but are increasingly recognized as an important cause of cerebral haemorrhage and stroke, to be distinguished from atherosclerotic cerebrovascular disease. Progressive deposition of A β amyloid occurs in the walls of arteries of up to about 2 mm in diameter, arterioles, and capillaries in the cerebral cortex and leptomeninges. Deposits may less commonly occur in the cerebellum and brainstem. Cerebral amyloid angiopathy is a common cause of intracerebral haemorrhage beyond the age of 60 years, which has a very high mortality approaching 50%. Recognized consequences of the deposits also include progressive cognitive impairment, which may be rapid, and transient neurological symptoms. A β amyloid deposition interferes with vascular structure resulting in loss of smooth muscle cells, thickening of vessel walls, and luminal narrowing, endothelial dysfunction and fragility that renders vessels susceptible to microaneurysm formation and bleeding. Advancing age and APOE genotype are the only known risk factors for sporadic cerebral amyloid angiopathy. Polymorphisms in the APOE gene encoding the ϵ 2 and ϵ 4 isoforms are associated with greater risk of developing the disease and its severity, and individuals with both these alleles have the earliest onset of disease and greatest probability of recurrent stroke. The mechanism by which

apolipoprotein E contributes to disease are not clear, but may include promoting A β amyloid deposition and inducing structural changes in involved vessels. Hereditary cerebral haemorrhage with amyloidosis: hereditary cerebral amyloid angiopathy Icelandic type (OMIM 604312)

Cerebrovascular amyloid deposits composed of a fragment of a genetic variant of cystatin C are responsible for recurrent major cerebral haemorrhages starting in early adult life in members of families originating in western Iceland. There is autosomal dominant inheritance and appreciable, but clinically silent, amyloid deposits are present in the spleen, lymph nodes, and skin. There is no extra-vascular amyloid in the brain, and the neurological deficits, often including dementia, of surviving patients are compatible with their cerebrovascular pathology. Dutch type (OMIM 605714)

In families originating from a small region on the coast of the Netherlands the autosomal dominant inheritance of a genetic variant of A β , which is deposited as cerebrovascular amyloid, results in recurrent normotensive cerebral haemorrhages starting in middle age. There are also amorphous A β deposits in the brain and early senile plaques, without congophilic amyloid cores. Multi-infarct dementia occurs in survivors, but some patients become demented in the absence of stroke. Amyloid outside the brain has not been reported. Cerebral amyloid associated with prion disease

The neuropathology of a group of progressive, invariably fatal spongiform encephalopathies sometimes, but certainly not always, includes intracerebral amyloid plaques. These diseases are transmissible and in some cases hereditary. The sporadic and familial Creutzfeldt-Jacob disease, the familial Gerstmann-Sträussler-Scheinker syndrome, and kuru are caused by prions (PrP^{Sc}), conformational isoforms of the normal physiological cellular prion protein (PrP^C). The human diseases are closely related to the animal diseases scrapie of sheep and goats, transmissible encephalopathy of mink, elk, and male deer, and bovine spongiform encephalopathy. Variant Creutzfeldt-Jacob disease is apparently the result of transmission of bovine spongiform encephalopathy to humans. The significance of amyloid per se in these disorders is not clear because it is not always histologically detectable, and in some disorders is not seen, for example, fatal familial insomnia and bovine spongiform encephalopathy (which is apparently a result of the transmission of ovine scrapie to cattle). When scrapie or its human counterparts are transmitted to experimental animals by inoculation of affected brain tissue, the development of intracerebral amyloid depends on the strain of infectious agent and the genetic background of the recipient. Even when amyloid is present in the brain it is not seen elsewhere (e.g. in the spleen), although the latter is a rich source of the infective agent. However, when the infective agent is exhaustively and highly purified from brain or spleen it forms typical congophilic amyloid fibrils composed of the proteinase-resistant subunit PrP^{Sc}, and when amyloid deposits are present in affected brains they immunostain with antiprion antibodies. The amyloid fibril protein is thus directly related to the cause of the encephalopathy, but histologically demonstrable amyloid deposition is evidently not necessary for the expression of disease. Indeed, recent work in transgenic and knockout mouse strains clearly demonstrates both that prion amyloid deposition is not a necessary condition for the development of transmissible spongiform encephalopathy, and that expression of the normal cellular isoform, PrP^C, is absolutely required. Neuronal damage may perhaps be caused by a cytotoxic interaction between prefibrillar PrP^{Sc} aggregates and the normal PrP^C, or indeed by other mechanisms entirely. This is a different situation from the extracerebral amyloidosis, and from cystatin C and nonhereditary cerebral amyloid angiopathies, in which amyloid deposition is invariably present when there is clinical disease, and is unequivocally the cause of tissue damage.

Hereditary systemic amyloidosis Hereditary transthyretin amyloidosis (OMIM 176300) Hereditary transthyretin amyloidosis is an autosomal dominant syndrome with onset at any time from the second decade onwards. It is characterized by progressive peripheral and autonomic neuropathy

and varying degrees of visceral involvement especially affecting the heart, the vitreous of the eye, kidneys, thyroid, and adrenals. There are usually amyloid deposits throughout the body involving the walls of blood vessels and the connective tissue matrix; the pathology is due to these deposits. Major foci exist in Portugal,

section 12 Metabolic disorders 2224 Japan, and Sweden but familial amyloid polyneuropathy has been reported in most populations throughout the world. There is considerable variation in the age of onset, rate of progression, and involvement of different systems, although within families the pattern tends to be more consistent. There is remorseless progression over 5 to 15 years and the disorder is invariably fatal. Death results from the effects and complications of peripheral and/or autonomic neuropathy, or from cardiac or renal failure. Hereditary transthyretin amyloidosis is caused by mutations in the gene for the plasma protein transthyretin (formerly known as prealbumin). The most frequent of these worldwide causes a methionine for valine substitution at position 30 in the mature protein, but more than 100 amyloidogenic mutations have been described; substitution of alanine for threonine at position 60 is the most frequent cause in the United Kingdom. There is often little correlation between the underlying mutation and the clinical phenotype, which is evidently determined by other genetic and possibly also environmental factors, although in a few cases certain mutations are uniquely associated with particularly aggressive or relatively organ-limited disease. The amyloidogenic transthyretin mutations are not always penetrant, and asymptomatic methionine-30 homozygotes over the age of 60 years have been reported. Rare kindreds with the apolipoprotein A-I arginine 26 variant, which usually causes non-neuropathic amyloidosis, may present with prominent peripheral neuropathy resembling transthyretin familial amyloid polyneuropathy (OMIM 107680). Familial amyloid polyneuropathy with predominant cranial neuropathy (OMIM 137350) Originally described in Finland, but now reported in other populations, this very rare, autosomal dominant, hereditary amyloidosis presents in adult life with cranial neuropathy, lattice corneal dystrophy, and distal peripheral neuropathy. There may be skin, renal, and cardiac manifestations and microscopic amyloid deposits are widely distributed in connective tissue and blood vessel walls; life expectancy approaches normal. The amyloid fibrils are derived from variants of the actin-modulating protein gelsolin, encoded by point mutations. Non-neuropathic systemic amyloidosis (OMIM 105200) In this rare autosomal dominant syndrome of major systemic amyloidosis without clinical evidence of neuropathy, the patterns of organ involvement and overall clinical phenotype vary between families. The kidneys are often the most severely affected organ, leading to hypertension and renal failure, but the heart, spleen, liver, bowel, connective tissue, and exocrine glands may all be involved. Following clinical presentation there is inexorable progression to death or organ failure requiring transplantation. Clinical presentation is typically around the sixth decade, but can occur in early adulthood. The amyloid proteins so far identified are genetic variants of apolipoprotein A-I and A-II, lysozyme, and least rarely the fibrinogen α -chain. A single kindred has lately been identified with a genetic variant of β 2-microglobulin. Familial amyloid cardiomyopathy Cardiac amyloidosis without overt involvement of other viscera or neuropathy, progressing inexorably to death, is associated with certain transthyretin gene mutations and is inherited in an autosomal dominant manner with variable penetrance (Table 12.12.3.2). By far the most common variant is transthyretin isoleucine 122, which occurs in 4% of African Americans and causes cardiac amyloidosis from the sixth decade onwards. Familial Mediterranean fever (OMIM 249100) Familial Mediterranean fever is an autosomal recessive autoinflammatory disorder caused by mutations in the gene MEFV on chromosome 16 that encodes a neutrophil-specific protein of unknown function

called pyrin (see Chapter 12.12.2). The disease is characterized by recurrent episodes of fever, abdominal pain, pleurisy, or arthritis, and predominantly occurs in non-Ashkenazi Jews, Armenians, Anatolian Turks, and Levantine Arabs. Among Sephardi Jews of North African origin, and in the other populations (except Armenians and, to a lesser extent, Ashkenazi Jews), untreated familial Mediterranean fever is eventually complicated in a high proportion of cases by typical systemic AA amyloidosis. Furthermore, some patients with familial Mediterranean fever present with AA amyloidosis before they have experienced any symptoms, and this is consistent with the recent finding that a substantial acute phase plasma protein response is frequently present, even in asymptomatic individuals. The variable incidence of amyloidosis in patients with familial Mediterranean fever from different populations is not wholly explained by their specific pyrin gene mutations, and is another illustration of the unknown genetic determinants of clinical amyloidosis. Heterozygosity for deletion of methionine at position 694 of pyrin is associated with dominantly inherited but otherwise typical familial Mediterranean fever in the British population.

Haemodialysis-associated amyloidosis In the past, almost all patients with end-stage renal failure who were maintained on haemodialysis for more than 5 years developed amyloid deposits composed of β_2 -microglobulin. These deposits were predominantly osteoarticular and were associated with carpal tunnel syndrome, large-joint pain and stiffness, soft tissue masses, bone cysts, and pathological fractures. Renal tubular amyloid concretions could also form. The serious clinical problems associated with such amyloidosis were a major cause of morbidity in patients on long-term dialysis. Furthermore, in some patients more extensive deposition occurred, most commonly in the spleen but also in other organs, and a few cases of death associated with systemic β_2 -microglobulin amyloid were reported. The β_2 -microglobulin was derived from the high plasma concentration that developed in renal insufficiency, which was not adequately cleared by dialysis. This condition also occurred (with lower incidence) in patients on continuous ambulatory peritoneal dialysis and has rarely been reported in patients with chronic kidney disease who have never been dialysed. Improved clearance of β_2 -microglobulin by current dialysis membranes and procedures has much reduced the incidence and severity of this form of amyloidosis.

Leucocyte chemotactic factor 2 amyloidosis A recently identified and still obscure form of systemic amyloidosis is associated with amyloid deposits derived from leucocyte chemotactic factor 2 (LECT2). The aetiology of this apparently sporadic disease is unknown. Most patients present in their sixth to seventh decades with slowly progressive renal impairment; proteinuria tends to be low grade and hypertension is a frequent accompaniment. Although splenic and adrenal amyloid deposits are frequently evident on serum

12.12.3 Amyloidosis ²²²⁵ amyloid P component scintigraphy, the clinical manifestations are predominantly renal. The disease is apparently most prevalent in Mexican Americans and South Asians, and renal biopsy data from the United States of America suggests that ALECT2 amyloidosis may account for up to 2.5% of amyloidotic renal biopsy specimens. The LECT2 gene, located on chromosome 5q31.1-q32, consists of four exons that encode 151 amino acids, of which 133 are present in the secreted product. In addition to having chemotactic activity for neutrophils, LECT2 may have other physiological functions related to cell growth and repair after damage. Although synthesized predominantly in the liver, it also is expressed in other tissues, including testis, vascular and endothelial smooth muscle cells, and kidney. The pathogenesis underlying ALECT2 amyloid formation is unknown, but the fibrils have been demonstrated to be derived from full-length, 133-residue LECT2 protein.

Endocrine amyloid Many tumours of APUD (amine precursor uptake and decarboxylation) cells that produce peptide hormones have amyloid deposits in their

stroma. These are probably composed of the hormone peptides; in the case of medullary carcinoma of the thyroid, the fibril subunits are derived from procalcitonin. In insulinomas, the amyloid fibril protein is a novel peptide first identified in that site and subsequently shown to be the fibril protein in the amyloid of the islets of Langerhans seen in type 2 (maturity-onset) diabetes. This peptide is called islet amyloid polypeptide, or amylin, and shows appreciable homology with calcitonin gene-related peptide. Amyloid of this type is an almost universal feature of the pancreatic islets in type 2 diabetes, and becomes more extensive with increasing duration and severity of the disease. Although the amyloid itself is probably not initially responsible for the metabolic defect in this form of diabetes, it is likely that progressive amyloid deposition, leading to islet destruction, subsequently does contribute to the pathogenesis. The possible hormonal or other role of islet amyloid polypeptide itself, which is produced by the islet β -cells, is also not yet clear. Rare localized amyloidosis syndromes Amyloid deposits localized to the skin occur in both acquired and hereditary forms of amyloidosis. Primary localized cutaneous amyloidosis presents in adult life as macular or papular lesions, the fibrils of which may be derived from keratin. Hereditary cutaneous amyloid lesions are rare, of unknown fibril type, and are sometimes associated with other, nonamyloid, multisystem disorders. Amyloid deposits in the eye cause local problems in the cornea (corneal lattice dystrophy) or conjunctiva, while orbital amyloid presents as mass lesions that can disrupt eye movement and the structure of the orbit. In one such case, the fibril protein has been identified as a fragment of IgG heavy chain. Lactoferrin and keratoepithelin have been identified as the amyloid fibril proteins in different cases of corneal amyloidosis. Localized foci of AL amyloid can occur anywhere in the body in the absence of systemic AL amyloidosis, the most common sites being the skin, upper airways and respiratory tract, and the urogenital tract. They may be associated with a local plasmacytoma or B-cell lymphoma producing a monoclonal immunoglobulin, but often the cells, which must be present to produce the amyloidogenic protein, are scattered inconspicuously in the affected tissue. The clinical problems caused by these space-occupying amyloidomas can often be ameliorated by surgical resection, but this is not always possible. Amyloid fibrils Regardless of their very diverse protein subunits, amyloid fibrils of different types are remarkably similar: straight, rigid, nonbranching, of indeterminate length, and 10 to 15 nm in diameter. They are insoluble in physiological solutions, relatively resistant to proteolysis, and bind Congo red dye, producing pathognomonic green birefringence when viewed in intense cross-polarized light. Electron microscopy reveals that each fibril consists of two or more protofilaments, the precise number varying with the fibril type. The X-ray diffraction patterns of all the different ex vivo amyloid fibrils, and of synthetic fibrils formed in vitro, that have been studied demonstrate the presence of a common core structure within the filaments: the subunit proteins are arranged in a stack of twisted antiparallel β -pleated sheets lying with their long axes perpendicular to the long axis of the fibril. Many different proteins, including molecules totally unrelated to amyloidosis in vivo, can be refolded after denaturation in vitro to form typical, stable, congophilic cross- β fibrils. Although it is not clear why only the 26 known amyloidogenic proteins adopt the amyloid fold and persist as fibrils in vivo, a major unifying theme is that, in all cases studied, the precursors are relatively destabilized. Even under physiological or other conditions they may encounter in vivo, they adopt partly unfolded states that involve the loss of tertiary or higher-order structure. These misfolded intermediates readily aggregate, with retention of β -sheet secondary structure, into protofilaments and fibrils. Once the process has started, seeding may also play an important facilitating role, so that amyloid deposition may progress exponentially as expansion of the amyloid template captures further precursor molecules. Amyloid fibril proteins and their precursors Immunoglobulin light chain AL proteins are derived from the N-

terminal region of monoclonal immunoglobulin light chains and consist of all or part of the variable domain. Intact light chains may occasionally be found, and the molecular weight therefore varies between about 8 and 30 kDa. The light chain of the monoclonal paraprotein is either identical to, or clearly the precursor of, AL isolated from the amyloid deposits. AL fibrils are more commonly derived from λ chains than from κ chains, despite the fact that κ chains predominate among both normal immunoglobulins and the paraprotein products of immunocyte dyscrasias. A particular λ -chain subgroup, λ VI, was initially identified as an AL protein in two cases of immunocyte dyscrasia-associated amyloidosis, before it had been recognized in any other form, and it is present in many more cases of AL amyloidosis. Furthermore, sequence analysis of Bence Jones proteins of both κ and λ types from patients with AL amyloidosis, and of AL proteins themselves, shows that these polypeptides contain unique amino acid replacements or insertions compared with nonamyloid monoclonal light chains. In some cases, the changes involve the replacement of hydrophilic framework residues by hydrophobic residues, changes likely to promote aggregation and insolubilization; in others, the monoclonal light chains from amyloid patients have been directly demonstrated to have decreased solubility and a greater propensity for precipitation than control nonamyloid proteins. The inherent amyloidogenicity of particular monoclonal light chains

section 12 Metabolic disorders 2226 has been elegantly confirmed in an in vivo model in which isolated Bence Jones proteins were injected into mice. Animals receiving light chains from AL amyloid patients developed typical amyloid deposits composed of the human protein, whereas animals receiving light chains from myeloma patients without amyloid did not. Amyloid A (AA) The AA protein is a single nonglycosylated polypeptide chain usually of mass 8000 Da and containing 76 residues corresponding to the N-terminal portion of the 104-residue serum amyloid A protein (SAA). Smaller and larger AA fragments, even some whole SAA molecules, have also been reported in AA fibrils. SAA is an apolipoprotein of high-density lipoprotein particles, and is the polymorphic product of a set of genes located on the short arm of chromosome 11. It is highly conserved in evolution and is a major acute phase reactant. Most of the SAA in plasma is produced by hepatocytes, in which the synthesis is under transcriptional regulation by cytokines (especially interleukin 1, interleukin 6, and TNF) acting via NF- κ B-like transcription factors, and possibly others. After secretion it rapidly associates with high-density lipoproteins, from which it displaces apolipoprotein A-I. The circulating concentration can rise from normal levels of up to 3 mg/litre to over 1000 mg/litre within 24 to 48 h of an acute stimulus, and with ongoing chronic inflammation the level may remain persistently high. Certain isoforms of SAA, the products of different genes, are predominantly synthesized elsewhere in the body by macrophages, adipocytes, and certain other cells. Although they also associate with high-density lipoproteins, their acute phase synthesis is stimulated differently and they presumably have different functions. There is also a closely related family of high-density lipoprotein trace apoproteins that are not acute phase reactants; they have been designated constitutive SAAs, although they do not form amyloid. The precursor of amyloid fibril AA protein is circulating SAA, from which amyloid fibril AA protein is derived by proteolytic cleavage. Such cleavage can be produced by macrophages and by a variety of proteinases, but since further cleavage of AA is readily demonstrable in vitro it is not clear why the AA peptide persists in amyloid. Furthermore, it is not known whether the process of AA fibril generation involves cleavage of SAA before and/or after aggregation of monomers. Persistent overproduction of SAA causing sustained high circulating levels is a necessary condition for deposition of AA amyloid, but it is not known why only some individuals in this state develop

amyloidosis. In mice, only one of the three major isoforms of SAA is the precursor of AA in amyloid fibrils. Human SAA isoforms are more complex, but homozygosity for particular types seems to favour amyloidogenesis, although there may also be ethnic differences. The normal functions of SAA are not known, although modulating effects on reverse cholesterol transport and on lipid function in the microenvironment of inflammatory foci have been proposed. A protein, homologous with SAA, produced by rabbit fibroblasts has been reported to act as an autocrine stimulator of collagenase production in vitro. Other reports of potent cell-regulatory functions of isolated denatured delipidated SAA have yet to be confirmed in physiological preparations of SAA-rich high-density lipoproteins. Regardless of its physiological role, the behaviour of SAA as an exquisitely sensitive acute phase protein with an enormous dynamic range makes it an extremely valuable empirical clinical marker. It can be used objectively to monitor the extent and activity of infective, inflammatory, necrotic, and neoplastic disease. Furthermore, routine monitoring of SAA should be an integral part of the management of all patients with AA amyloidosis or disorders predisposing to it, as control of the primary inflammatory process in order to reduce SAA production is essential if amyloidosis is to be halted, enabled to regress, or prevented. Automated immunoassay systems for SAA are available that are calibrated on the World Health Organization international reference standard.

Transthyretin, formerly known as prealbumin, is a normal nonglycosylated plasma protein with a relative molecular mass of 55 044 Da. It is composed of four identical noncovalently associated subunits, each of 127 amino acids. It is produced by hepatocytes and the choroid plexus, and is a significant negative acute phase protein. Each tetrameric molecule is able to bind a single thyroxine or triiodothyronine molecule and up to 15% of circulating thyroid hormone is transported in this way. Transthyretin also forms a 1:1 molecular complex with the vitamin A transporter, retinol-binding protein, and is essential for keeping it in the circulation. Transthyretin is encoded by a single-copy gene, but is appreciably polymorphic and more than 120 different point mutations encoding single residue substitutions have been identified. Normal wild-type transthyretin is an inherently amyloidogenic protein that forms the fibrils in wild-type transthyretin (also known as senile systemic) amyloidosis. Almost all variant forms of transthyretin have been associated with hereditary amyloidosis, and show decreased stability in vitro compared with the wild type. Individuals heterozygous for transthyretin mutations have a mixture of wild-type and variant transthyretin monomers in their circulating transthyretin, and if they develop amyloidosis both forms are often present, although the variant may predominate in the amyloid fibrils. In addition to intact protomers, ATTR amyloid fibrils also usually contain the C-terminal fragment, residue 49-127, of transthyretin. This is produced by a recently elucidated mechano-enzymatic mechanism, in which physiological scale shear forces critically facilitate cleavage of transthyretin at residue 48 by plasmin. This specific cleavage catalyses, and is necessary for, transthyretin amyloid fibril formation. Plasmin, the pivotal essential mediator of physiological fibrinolysis, is thus also a key pathogenic factor in transthyretin amyloidosis.

Amyloid beta (A β) The fibril protein in the intracerebral and cerebrovascular amyloid of Alzheimer's disease, Down's syndrome, and hereditary amyloid angiopathy of the Dutch type is a 39- to 43-residue sequence derived by proteolysis from a precursor protein of high molecular weight, the amyloid precursor protein (APP), encoded on the long arm of chromosome 21. Several isoforms of APP are generated by alternative splicing of transcripts from the 19-exon gene, yielding three major forms: APP695, APP751, and APP770. These are each single-chain, multidomain glycoproteins with the 47 residues of the C-terminal lying within the cytoplasm, a 25-residue membrane-spanning region, and the rest of the molecule lying extracellularly. APP751 and APP770 contain a 56-residue Kunitz-type serine protease inhibitor domain encoded by exon 7. Following glycosylation and

membrane insertion, APPs are cleaved extracellularly, close to the transmembrane sequence, by so-called APP secretase activity. This releases, in the case of APP751 and APP770, a molecule known as proteinase nexin II, which avidly

12.12.3 Amyloidosis 2227 binds factor XIa, trypsin, and chymotrypsin, as well as epidermal growth factor-binding protein and the γ subunit of nerve growth factor. The predominant species of mRNA found in the brain encodes APP695, which lacks the proteinase inhibitor domain, while mRNA for APP751 is the most abundant in other tissues. Despite this, 85% of secreted APP in the brain is proteinase nexin II. Interestingly, APP secreted by a glial cell line is substantially glycosylated with chondroitin sulphate glycosaminoglycan chains. APP also undergoes high-affinity interactions with heparan sulphate. These observations suggest that APP may have important functions in cell adhesion, cell migration, and modulation of growth-factor activities. APP proteinase nexin II is present in and released by platelets, and probably functions in the clotting cascade. The amyloidogenic peptide A β , encoded by parts of exons 16 and 17, corresponds to the part of the APP sequence that extends from within the cell membrane into the extracellular space. Secretase cleavage of APP to release the soluble form cannot therefore generate intact A β itself, or larger fragments containing it. However, there is an alternative processing pathway for APP, in which it is taken up whole by lysosomes and cleaved to yield fragments that do contain the whole A β sequence. Furthermore, APP cleaved at the N-terminus of A β , and soluble A β itself, are normally produced by cell lines and by mixed brain cells in culture, and are present in the cerebrospinal fluid. However, the source of the A β in the intracerebral amorphous deposits, and that which aggregates as amyloid fibrils in the brain and cerebral blood vessels, is still not known. The 42-residue form of A β (A β 1–42) is markedly the most amyloidogenic, and all the mutations in the APP and presenilin genes that are associated with hereditary Alzheimer's disease result in increased production of this fragment. Increased availability of the precursor is thus responsible for amyloidogenesis, but the pathogenesis of neuronal damage and dementia remain unclear.

Cystatin C Cystatin C (formerly called γ -trace) is an inhibitor of cysteine proteinases, including cathepsin B, H, and L. It is encoded by a gene on chromosome 20 and consists of a single nonglycosylated polypeptide chain of 120 residues. It is present in all major human biological fluids at concentrations compatible with a significant physiological role in proteinase inhibition. The normal concentration in cerebrospinal fluid is 6.5 mg/litre (range 2.7–13.7), but is much lower (2.7 mg/litre, range 1.0–4.7) in patients with the Icelandic type of hereditary cerebral amyloid angiopathy, in whom fragments of the glutamine-68 genetic variant of cystatin C form the amyloid fibrils. This reduced concentration is diagnostically useful, and is evident even in presymptomatic carriers of the cystatin C gene mutation. The point mutation that causes the disease encodes a glutamine for leucine substitution in the mature protein, and the amyloid fibril protein consists of the C-terminal 110 residues of the variant. This N-terminally truncated form is not detectable in the cerebrospinal fluid of affected patients, suggesting that cleavage takes place either in close proximity to fibril deposition or after the fibrils have formed. The variant cystatin C is less stable than the wild type and readily forms fibrils in vitro. It is not known whether cerebral haemorrhage in cystatin C amyloidosis is caused simply by the damaging effects of vascular amyloid deposition, or whether deficiency in inhibitory capacity for cysteine proteinases also plays a part.

Gelsolin Gelsolin is a widely distributed 90-kDa cytoplasmic protein that binds actin monomers, nucleates actin filament growth, and severs actin filaments. Alternative transcriptional initiation and message processing from a single gene on chromosome 9 are responsible for the synthesis of a secreted form of gelsolin (93 kDa), which circulates in the plasma at a concentration of about 200 mg/litre.

Its function in the blood is not known, but may be related to the clearance of actin filaments released by dying cells. In the Finnish type of hereditary amyloidosis, the amyloid fibril protein is a 71-residue fragment of variant gelsolin, with asparagine substituted for aspartic acid at position 15 (corresponding to residue 187 of the mature molecule), and the same mutation has been discovered in affected kindreds from different ethnic backgrounds. In one Danish family with the same phenotype there is a different mutation at the same nucleotide, predicting a tyrosine for aspartic acid substitution at residue 187. Synthetic and recombinant peptides that include the asparagine for aspartic acid substitution at residue 187 are less soluble than the wild-type sequence and readily form amyloid fibrils in vitro. Apolipoprotein A-I and A-II Apolipoprotein A-I is the most abundant apolipoprotein among the high-density lipoprotein particles, and participates in their central function of reverse cholesterol transport from the periphery to the liver. Apolipoprotein A-I variants are extremely rare, and may be phenotypically silent or may affect lipid metabolism. However, at least 15 different variants of apolipoprotein A-I, including single- and multiple-residue substitutions and deletions, have been associated with amyloidosis. These are inherited in an autosomal dominant manner and are usually highly penetrant, but there are marked variations in the age and manner of presentation, even within the same family and in different kindreds with the same mutation. The amyloid fibril protein consists, in all cases studied, of the first 90 or so N-terminal residues, even when the causative variant residue(s) are more distal. Wild-type apolipoprotein A-I is also amyloidogenic, forming the deposits associated with atheromatous plaques in older people. The various amyloidogenic mutations presumably encode sequence changes that render apolipoprotein A-I less stable and/or more liable to cleavage that yields the fibrillogenic N-terminal fragment. Predominantly renal amyloidosis has also been described in a handful of families in association with several different mutations in a normal stop codon in the gene for apolipoprotein A-II, which results in a peptide extension from residue 78. Lysozyme Lysozyme is the classic bacteriolytic enzyme of external secretions, discovered by Fleming in 1922. It is also present at high concentration within articular cartilage and in the granules of polymorphs, and is the major secreted product of macrophages. Lysozymes are present in most organisms in which they have been sought, although their physiological role is not always clear. The complete structures of hen egg-white and human lysozymes are known to atomic resolution, and their catalytic mechanism, epitopes, folding, and other aspects of their structure-function relationship have been analysed exhaustively. Wild-type lysozyme, unlike transthyretin and β 2-microglobulin, is not inherently amyloidogenic and, coupled with

section 12 Metabolic disorders 2228 the extensive knowledge of its structure and folding, it is therefore a valuable model for the investigation of amyloid fibrillogenesis. There is only one copy of the lysozyme gene in the human genome, and no disease is associated with lysozyme other than amyloidosis. The first lysozyme mutations identified to cause amyloidosis were the substitution of threonine for isoleucine at residue 56 in one family, and histidine for aspartic acid at residue 67 in another. These dramatic changes in residues that are extremely conserved throughout the lysozyme and related α -lactalbumin protein families destabilize the native fold, so that the variants readily adopt partly unfolded states, even under physiological conditions, and spontaneously aggregate in vitro, and evidently also in vivo, into amyloid fibrils. Several further amyloidogenic variants of lysozyme have subsequently been described. Islet amyloid polypeptide Islet amyloid polypeptide (IAPP; amylin) is a 37-residue molecule encoded by a gene on chromosome 12 and with 46% sequence homology to the neuropeptide calcitonin gene-related peptide. Islet amyloid polypeptide is produced in the β -cells of the pancreatic islets of Langerhans, and is stored in and

released from their secretory granules together with insulin. It has been reported to modulate insulin release and to induce peripheral insulin resistance, vasodilatation, and lowering of plasma calcium, but neither its physiological role nor its contribution to diabetes are known. The amyloidogenicity of islet amyloid polypeptide depends on the amino acid sequence between residues 20 and 29, as shown by in vitro fibrillogenesis with synthetic peptides. The synthetic decapeptide IAPP20–29, and even the hexapeptide IAPP25–29, form amyloid-like fibrils in vitro, whereas other fragments do not. There is also a correlation between conservation of this sequence and deposition of the amyloid in the islets of diabetic animals of different species. However, the role of the amyloid in diabetogenesis remains to be established. In the degu, a South American rodent, spontaneous diabetes is associated with islet amyloid composed of insulin, and xenogeneic insulin can also form amyloid in humans at the site of repeated therapeutic insulin injections.

β 2-microglobulin is a nonglycosylated, nonpolymorphic, single-chain protein of 99 residues, with a single intrachain disulphide bridge and a relative molecular mass of 11 815, encoded by a single gene on chromosome 15. It becomes noncovalently associated with the heavy chain of major histocompatibility class I antigens, and is required for transport and expression of the major histocompatibility complex (MHC) at the cell surface. Amino acid sequence homology places β 2-microglobulin in the superfamily that includes immunoglobulins, T-cell receptor α - and β -chains, Thy-1 (CD90), MHC class I and II molecules, secretory component, etc. Its three-dimensional structure is a typical β -barrel with two antiparallel pleated sheets comprising three and four strands, respectively, and closely resembles an immunoglobulin domain. β 2-microglobulin is produced by lymphoid and a variety of other cells, in which it stabilizes the structure and function of MHC class I antigens at the cell surface. When these complexes are shed by cleavage of the heavy chain at the cell surface, free β 2-microglobulin is released. The circulating concentration of β 2-microglobulin is 1 to 2 mg/litre and the protein is rapidly cleared by glomerular filtration and then catabolized in the proximal renal tubule. Impairment of renal function is associated with retention of β 2-microglobulin and increased circulating concentrations because there is no other site for its catabolism. Daily production of β 2-microglobulin is about 200 mg, and in patients in end-stage renal failure on haemodialysis, plasma β 2-microglobulin levels rise to and remain at levels of about 40 to 70 mg/litre. Isolated unaltered β 2-microglobulin can form amyloid-like fibrils itself in vitro, and most studies of ex vivo β 2-microglobulin fibrils show the whole intact molecule to be the major subunit, although fragments and altered forms of β 2-microglobulin have also been reported. Intriguingly, a highly amyloidogenic variant of β 2-microglobulin has been identified in a French kindred with hereditary systemic amyloidosis manifesting with slowly progressive gastrointestinal symptoms and autonomic neuropathy. Renal function and circulating concentration of β 2-microglobulin were normal. The Asp76Asn β 2-microglobulin variant was thermodynamically unstable and uniquely fibrillogenic in vitro under physiological conditions.

Glycosaminoglycans

Amyloidotic organs contain more glycosaminoglycans than normal tissues, and at least some of this is a tightly bound integral part of the amyloid fibrils. These fibril-associated glycosaminoglycans are heparan sulphate and dermatan sulphate in all forms of amyloid that have been investigated. Fibrils isolated by water extraction and separated from other tissue components contain 1 to 2% by weight glycosaminoglycan, none of which is covalently associated with the fibril protein. Interestingly, in systemic AA and AL amyloidosis, the only forms in which this has been studied so far, there is markedly restricted heterogeneity of the glycosaminoglycan chains, suggesting that particular subclasses of heparan and dermatan sulphates are involved. Immunohistochemical studies demonstrate the presence of proteoglycan core proteins in all amyloid deposits, and that these are closely related to fibrils at the ultrastructural level. However,

in isolated fibril preparations much of the glyco- saminoglycan material is free carbohydrate chains, and it is not yet clear whether this represents aberrant glycosaminoglycan metab- olism related to amyloidosis or is just an artefact of postmortem degradation of core protein. The significance of glycosaminoglycans in amyloid remains un- clear, but their universal presence, intimate relationship with the fibrils, and restricted heterogeneity all suggest that they may be im- portant. Glycosaminoglycans are known to participate in the organ- ization of some normal structural proteins into fibrils and they may have comparable fibrillogenic effects on certain amyloid fibril pre- cursor proteins. Furthermore, the glycosaminoglycans on amyloid fibrils may be ligands to which serum amyloid P component, an- other universal constituent of amyloid deposits, binds. Amyloid P component and serum amyloid P component Amyloid deposits in all different forms of the disease, both in hu- mans and in animals, contain the nonfibrillar glycoprotein, amyloid P component. Amyloid P component is identical to and derived from the normal circulating plasma protein, serum amyloid P com- ponent, a member of the pentraxin protein family that includes

12.12.3 Amyloidosis 2229 C-reactive protein. Human serum amyloid P component is secreted only by hepatocytes, is a trace constituent of plasma (women: mean 24 mg/litre, range 8–55, men: mean 32 mg/litre, range 12–50), and is not an acute phase reactant. Nevertheless, apart from the fibrils themselves, amyloid P component is always by far the most abun- dant protein in all amyloid deposits. Serum amyloid P component consists of five identical noncovalently associated subunits, each with a molecular mass of 25 462 Da, arranged in a pentameric disc-like ring. The tertiary fold of the subunit is dominated by antiparallel β -sheets, forming a flattened β -barrel with jellyroll topology and a core of hydro- phobic side chains. This is the lectin fold, shared with a variety of other animal, plant, and bacterial carbohydrate-binding proteins (lectins). Serum amyloid P component is a calcium-dependent ligand-binding protein; its best-defined specificity is for the 4,6- cyclic pyruvate acetal of β -d-galactose, but it also binds avidly and specifically to DNA, chromatin, glycosaminoglycans (particu- larly heparan and dermatan sulphates), and to all known types of amyloid fibrils. The latter interaction is responsible for the unique specific accumulation of serum amyloid P component in amyloid deposits. Aggregated, but not native, serum amyloid P compo- nent also binds specifically to C4-binding protein and fibronectin from plasma, although serum amyloid P component is not com- plexed with any other protein in the circulation. In addition to being a plasma protein, human serum amyloid P component is also a normal constituent of certain extracellular matrix structures. It is covalently associated with collagen and/or other matrix compo- nents in the lamina rara interna of the human glomerular basement membrane, and is present on the microfibrillar mantle of elastin fibres throughout the body. Although no deficiency of serum amyloid P component has been described, and it has been stably conserved in evolution, its physio- logical function remains unclear. There is a single copy of its gene on chromosome 1, no polymorphism of the amino acid sequence, and the single biantennary oligosaccharide chain attached to as- paragine at residue 32 is the most invariant glycan of any known glycoprotein. Studies of serum amyloid P component knockout mice have shown that serum amyloid P component is involved in host resistance to some infections, and contributes to the pathogen- esis of others, but these animals are otherwise healthy and have a normal lifespan. The serum amyloid P component molecule is highly resistant to proteolysis and, although not itself a proteinase inhibitor, its binding to amyloid fibrils in vitro protects them against proteolysis. Once bound to amyloid fibrils in vivo, serum amyloid P component per- sists for very prolonged periods and is not catabolized at all, by con- trast with its rapid clearance from the plasma (half-life 24 h) and prompt catabolism in the liver. These observations suggest that serum amyloid P component may

contribute to the persistence of amyloid deposits in vivo; serum amyloid P component knockout mice show retarded and reduced induction of experimental AA amyloidosis, confirming that serum amyloid P component is significantly involved in the pathogenesis of amyloidosis. Other proteins in amyloid deposits A number of plasma proteins, other than the fibril proteins themselves and the serum amyloid P component, have been detected immunohistochemically in some amyloid deposits. These include α 1-antichymotrypsin, some complement components, apolipoprotein E, and various proteins of the extracellular matrix and basement membrane. None of these is as universal, abundant, or selective as serum amyloid P component, and their role, if any, in the pathogenesis or effects of amyloid deposition is not known.

Diagnosis and monitoring of amyloidosis The gold standard for diagnosis of amyloid is Congo red staining of histological sections under strictly defined conditions, followed by viewing under intense cross-polarized light to detect the pathognomonic green birefringence. Although inherently not complex or challenging, the essential practical procedures are often not performed adequately, leading to a substantial proportion of false-negative and false-positive histopathological diagnoses which can be devastating for individual patients. The relative rarity of amyloidosis and its highly variable and protean clinical presentation also mean that clinicians often do not think of it and thus do not request its histological identification. The variable standard of histological assessment compounds the problem and together these factors explain why the diagnosis is very challenging in routine practice. The result is that amyloidosis is frequently diagnosed very late and patients already have advanced organ damage before they come to specialist attention. Even when properly stained and viewed, biopsies provide extremely small samples and therefore can never provide information on the extent, localization, progression, or regression of amyloid deposits, either locally or in the whole patient. The development of radiolabelled serum amyloid P component as a specific tracer for amyloid was therefore a major advance in clinical amyloidosis and provided a wealth of new information on the natural history of many different forms of amyloid and their response to treatment.

Histochemical diagnosis of amyloidosis Biopsy In many patients with systemic amyloidosis, amyloid is an unexpected finding on biopsy of the kidneys, liver, heart, bowel, peripheral nerves, lymph nodes, skin, thyroid, or bone marrow during investigation of undiagnosed clinical problems. When amyloidosis is suspected clinically, biopsy of the rectum or subcutaneous fat is the least invasive. Amyloid is present at these sites in 50 to 70% of cases of systemic AA or AL amyloidosis. Alternatively, a clinically affected tissue may be biopsied directly.

Congo red and other histochemical stains Many cotton dyes, fluorochromes, and metachromatic stains have been used to stain amyloid in tissue sections but Congo red staining, specifically using an alcoholic alkaline solution, and the resultant green birefringence when viewed with high-intensity cross-polarized light, is the pathognomonic histochemical test. The stain is unstable and must be freshly prepared every 2 months or less and it is essential that the batch of Congo red has been validated. It is critical to have a section thickness of 5 to 10 μ m and to include in every staining run a positive control tissue containing modest amounts of amyloid. Reliable reporting on the microscopic appearances

section 12 Metabolic disorders 2230 absolutely depends on the use of a microscope of suitable quality equipped to provide high-intensity cross-polarized light. It is impossible to rigorously recognize or exclude amyloid unless these conditions are all fulfilled. Immunohistochemistry Although many amyloid fibril proteins can be identified immunohistochemically, the demonstration of amyloidogenic proteins in tissues does not, on its own, establish the presence of amyloid. Congo red staining and green birefringence are always required, and immunostaining may then enable

the amyloid to be classified. Antibodies to serum amyloid A protein are commercially available and always stain AA deposits, similarly with anti- β_2 -microglobulin antisera and haemodialysis-associated amyloid. In AL amyloid, the deposits are stainable with standard antisera to κ or λ immunoglobulin light chains in only about one-half of cases, probably because the light-chain fragment in the fibrils is usually the N-terminal variable domain, which is largely unique for each monoclonal protein. Immunohistochemical staining of transthyretin, A β , and prion protein amyloid may require pretreatment of sections with formic acid or alkaline guanidine, or deglycosylation. Electron microscopy Amyloid fibrils cannot always be convincingly identified ultra structurally, and electron microscopy alone is not sufficient to confirm the diagnosis of amyloidosis. Problems of histological diagnosis The tissue sample must be adequate (e.g. the inclusion of sub-mucosal vessels in a rectal biopsy specimen), and failure to find amyloid does not exclude the diagnosis. The unavoidable sampling problem means that biopsy cannot reveal the extent or distribution of amyloid. Experience with Congo red staining and viewing is required if clinically important false-negative and false-positive results are to be avoided. Immunohistochemical staining requires positive and negative controls, including demonstration of the specificity of staining by absorption of positive antisera with isolated pure antigens. Proteomic analysis Laser capture microdissection of amyloid deposits from histological sections, followed by proteolytic digestion of the excised amyloid material and mass spectrometric identification of the fragments can also be used for typing. Suitable for use only in major centres of expertise, this highly specialized technique is an adjunct to immunohistochemistry for identification of amyloid fibril proteins, especially in the case of previously unknown fibrils, but appropriate interpretation of the results can be challenging. Nonhistological investigations Structural imaging Two-dimensional echocardiography showing small, concentrically hypertrophied ventricles, generally impaired contraction, dilated atria, homogeneously echogenic valves, and 'sparkling' echodensity of ventricular walls is strongly suggestive of cardiac amyloidosis. However, clinically significant restrictive diastolic impairment may be difficult to detect, even by comprehensive Doppler echocardiography and other functional studies. Myocardial strain imaging has emerged as a useful additional echocardiographic technique in cardiac amyloidosis, with the 'relative apical sparing' pattern of longitudinal strain being a strong pointer to the diagnosis. Cardiac MRI provides functional and morphological information on cardiac amyloid, and yields more accurate measurements of volume, mass, and wall thickness than echocardiography, which may be of particular benefit in sequential assessments. An advantage of cardiac MRI is myocardial tissue characterization, which provides information on the presence and extent of amyloid deposits. Characteristic patterns of global subendocardial and transmural late gadolinium enhancement have very high diagnostic sensitivity for cardiac amyloidosis and correlate with prognosis. T1 mapping studies performed after administration of gadolinium contrast can quantify the extracellular amyloid load and myocardial cell mass. Scintigraphy Scintigraphy following injection of technetium 99m-labelled 3,3'-diphosphono-1,2 propanodicarboxylic acid (99mTc-DPD), which was developed as a bone tracer, has lately been repurposed as a remarkably sensitive method for detecting the presence of cardiac transthyretin amyloid deposits. Localization of this tracer to the heart to a greater intensity than to the bones in the absence of a monoclonal gammopathy is pathognomonic for cardiac transthyretin amyloidosis. Cardiac localization of this tracer can also occur in some patients with AL, apolipoprotein A-I, and other types of amyloidosis, but usually to only a minor degree. Genetic and biochemical tests In cases of known or suspected hereditary amyloidosis, the gene defect must be characterized. If amyloidotic tissue is available, the fibril protein may be known and the corresponding gene can then be studied, but if no tissue containing amyloid is available, screening of the genes for known amyloidogenic proteins must be

undertaken. There are biochemical and immunochemical tests for screening the plasma for amyloidogenic variant protein products of mutant genes (e.g. for transthyretin and apolipoprotein A-I variants), but molecular genetic analysis of DNA is easier to perform and is the most direct approach. However, regardless of the DNA results, it is desirable, if possible, to directly identify the respective protein in the amyloid. Serum amyloid P component as a specific tracer in amyloidosis. The universal presence in amyloid deposits of amyloid P component, derived from circulating serum amyloid P component, is the basis for the use of radioisotope-labelled serum amyloid P component as a diagnostic tracer in amyloidosis. No localization or retention of labelled serum amyloid P component occurs in healthy subjects or in patients with diseases other than amyloidosis (Fig. 12.12.3.1a). Radioiodinated serum amyloid P component has a short half-life (24 h) in the plasma and is rapidly catabolized, with complete excretion of the iodinated breakdown products in the urine. However, in patients with systemic or localized extracerebral

12.12.3 Amyloidosis 2231 amyloidosis, the tracer rapidly and specifically localizes to the deposits, in proportion to the quantity of amyloid present, and persists there without breakdown or modification (Figs. 12.12.3.1b and 12.12.3c). Highly purified serum amyloid P component, isolated from donor plasma according to pharmaceutical current good manufacturing practice, is oxidatively iodinated under conditions that preserve its function intact. The medium-energy, short half-life, pure γ -emitter ^{123}I is used for scintigraphic imaging, and the long half-life isotope ^{125}I is used for metabolic studies. The dose of radioactivity administered (<4 mSv) is well within accepted safety limits and more than 30 000 studies have been completed without any adverse effects. In addition to high-resolution scintigraphs, the uptake of tracer into various organs can be precisely quantified and, together with highly reproducible metabolic data on the plasma clearance and whole-body retention of activity, the progression or regression of amyloid can be monitored serially and quantitatively. Dual modality three-dimensional single-photon emission computed tomography coupled with CT enables precise localization of ^{123}I -SAP to be determined when there is tracer uptake in unusual anatomical sites. Important observations regarding amyloid include the following: the different distribution of amyloid in different forms of the disease; amyloid in anatomical sites not available for biopsy (adrenals, spleen); major systemic deposits of forms of amyloid previously thought to be organ-limited; a poor correlation between the quantity of amyloid present in a given organ and the level of organ dysfunction; a nonhomogeneous distribution of amyloid within individual organs; and evidence for rapid progression and sometimes regression of amyloid deposits with different rates in different organs (Fig. 12.12.3.2). Examples of major regression of amyloidosis, when it has been possible to reduce or eliminate the supply of fibril precursor, are very encouraging. Studies with labelled serum amyloid P component thus make a valuable contribution to the diagnosis and management of patients with systemic amyloidosis, and in the United Kingdom these are routinely available for all known or suspected cases of amyloidosis in the National Health Service National Amyloidosis Centre at the Royal Free Hospital, London. Fig. 12.12.3.2 Serial posterior whole-body ^{123}I -serum amyloid P component scintigraphs of a man with AL amyloidosis complicating benign monoclonal gammopathy. At presentation (scan 1) there was uptake in the spleen, liver, and bone marrow, obscuring any possible renal signal. Chemotherapy was given before scan 2, which shows increased spleen uptake, reduced liver uptake, and some renal uptake, but no change in total amyloid load determined by measurements of the clearance and retention of the tracer (not shown). Subsequently, he had recurrent splenic infarctions and splenectomy was performed. Thereafter (scan 3) there was increased tracer uptake in the liver, although a notably lower total amyloid load. Six months later (scan 4) liver and kidney

uptake, plasma clearance, and whole-body retention of tracer were all reduced, indicating regression of amyloid. He was clinically much improved and remained well. (a) (b) (c) Fig. 12.12.3.1 Whole-body scintigraphs 24 h after intravenous injection of ¹²³I-labelled human serum amyloid P component. (a) Anterior view of a normal control subject showing the 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) Posterior (left) and anterior (right) views of a 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%. Note the reduced blood pool and bladder signal compared with (a). This patient, whose amyloid was diagnosed by renal biopsy 15 years before when nephrotic syndrome developed, and who was then treated with chlorambucil, had been in complete remission for 10 years during which there had been no acute phase response. At the time of this scan there was no biochemical abnormality in blood or urine, despite the very appreciable amyloid deposits, illustrating the discordance between the presence of amyloid and clinical effects. (c) Posterior (left) and anterior (right) views of a 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. This scintigraphic pattern of amyloid distribution is pathognomonic for AL amyloidosis; bone-marrow uptake has not been seen in any other type. Note the complete absence of blood pool or bladder signal resulting from complete uptake of the tracer dose into the substantial amyloid deposits.

section 12 Metabolic disorders 2232 Management of amyloidosis There have been substantial recent advances in the management of systemic amyloidosis, in particular active measures to support failing organ function while attempts are made to reduce the supply of the amyloid fibril precursor protein. Serial serum amyloid P component scintigraphy in more than 8000 patients with various forms of amyloidosis has shown that control of the primary disease process, or removal of the source of the amyloidogenic precursor, often results in regression of existing deposits and recovery or preservation of organ function. This strongly supports aggressive intervention, and relatively toxic drug regimens or other radical approaches can be justified by the poor prognosis. Such an approach, leading to reduced morbidity and improved survival, was the basis for the establishment of the National Health Service National Amyloidosis Centre. However, clinical improvement in amyloidosis is often delayed long after the underlying disorder has remitted, reflecting the very gradual regression of the deposits that is now recognized to occur in most patients. Continuing production of the amyloid precursor protein should be monitored as closely as possible in the long term, to determine the requirement for and intensity of treatment for the underlying primary condition. In AA amyloidosis this involves frequent estimation of the plasma SAA level, and in AL amyloidosis it requires monitoring of the serum free light-chain concentration or other markers of the underlying monoclonal plasma cell proliferation. Specific conventional therapies AA amyloidosis The treatment of AA amyloidosis ranges from potent anti-inflammatory and immunosuppressive biological drugs in patients with rheumatoid arthritis, to lifelong prophylactic colchicine in familial Mediterranean fever, and surgery in conditions such as refractory osteomyelitis and the tumours of Castleman's disease. Most patients with AA amyloidosis complicating inflammatory arthritis can now be treated effectively with one or other of the many biological agents now available (i.e. anticytokine (TNF, IL-1, IL-6) and anti-CD20 antibodies etc.); IL-1 inhibition with biological agents has revolutionized the management and prognosis of most patients with inherited periodic fever syndromes. Median SAA concentration has

been shown to be a strong predictor of both survival and renal outcome; sustained remission of inflammation in association with normal SAA values is associated with an almost 18-fold lower risk of death than median SAA levels of greater than 155 mg/L. Median survival of 79 to 137 months has been reported in a very large series. Approximately 40% of patients will eventually require renal replacement therapy, with a median time to dialysis from diagnosis of 78 months.

AL amyloidosis Treatment of AL amyloidosis is based on that for myeloma, although the plasma cell dyscrasias in AL amyloidosis are often very subtle and tolerance of chemotherapy is frequently limited by amyloidotic organ dysfunction. Chemotherapy for AL amyloidosis therefore needs to be tailored to individual patients based on age, performance status, organ involvement, cardiac staging, and the known toxicities of individual drugs, balancing the ideal for complete remission of the clonal disease with the need to minimize treatment-related morbidity and mortality.

Quantitative measurements of serum free light chains using the robust, sensitive, Freelite immunoassay are usually the most effective means for evaluating the early effects of chemotherapy as well as the requirement for ongoing treatment. Although partial haematological responses, that is, greater than 50% reduction in aberrant light chain concentration, can be beneficial, more complete clonal responses, that is, greater than 90% reduction, are strongly desirable and are associated with the best clinical outcomes. Serial measurements of the cardiac biomarker serum NT-proBNP are highly informative with respect to the response of cardiac amyloidosis to chemotherapy. Many different chemotherapy regimens are in current use, reflecting the paucity of clinical trials and international consensus, ranging from relatively low-intensity oral melphalan and dexamethasone through various intermediate-dose drug combinations to high-dose chemotherapy with autologous peripheral stem cell rescue in younger and fitter patients. In our current practice, most patients with AL amyloidosis receive a cyclic combination of the alkylating agent cyclophosphamide, the proteasome inhibitor bortezomib (Velcade) and dexamethasone, delivered 3- to 4-weekly over 3 to 6 months. More recently developed proteasome inhibitors including carfilzomib and ixazomib are undergoing evaluation in AL amyloidosis. The immunomodulatory drugs, thalidomide, lenalidomide, and pomalidomide, are also widely used in combination with dexamethasone and sometimes with proteasome inhibitor in addition. A multidisciplinary specialist approach is required to formulate and supervise optimal clinical management.

Specific innovative therapies

Transthyretin amyloidosis Several therapies have been undergoing advanced clinical testing in transthyretin amyloidosis and three have been approved by regulatory agencies. While hepatic transplantation has been shown to reduce neurological disease progression in patients with early-stage familial amyloid polyneuropathy associated with the methionine-30 substitution, in whom cardiac amyloid deposits are absent, this approach is limited by donor liver availability, associated morbidity and mortality, and continued deposition of wild-type transthyretin amyloid in the heart when cardiac amyloid is present at the time of surgery.

Small-molecule drugs New pharmacological therapies include the small-molecule drugs diflunisal and tafamidis, which are bound in the plasma by transthyretin and postulated to inhibit its conversion into amyloid fibrils. The pivotal trial of tafamidis involved 441 patients with wild-type as well as the hereditary form of disease. In 264 patients who received the active drug, all-cause mortality and cardiovascular-related hospitalizations were reduced as compared with placebo (30% reduction in death over a period of 2½

12.12.3 Amyloidosis 2233 years); decline in functional capacity and quality of life was also decreased. Tafamidis (dose 20 mg daily) is approved for familial amyloid polyneuropathy by the European Medicines Agency and authorized by NHS England for treatment of transthyretin familial

amyloid polyneuropathy in patients with stage 1 symptomatic polyneuropathy to delay peripheral neurological impairment and before liver transplantation. RNA interference therapy In 2018, two therapies—independently developed by competing biopharmaceutical companies (Alnylam and Akcea)—received marketing approval. These respectively use either an intravenously administered small interfering double-stranded RNA or a sub-cutaneous antisense oligonucleotide that complements the messenger transthyretin RNA. By selectively blocking the cognate sense mRNA transcript of the TTR gene, hepatic synthesis of the unstable transthyretin protein, which misfolds to give rise to amyloid deposits, is markedly inhibited. The Food and Drug Administration in the United States of America and the European Medicines Agency have approved both patisiran (Onpattro) and inotersen (Tegsedi), respectively, for treatment of the neuropathy caused by hereditary transthyretin amyloidosis. In the larger of the two phase III clinical trials, involving 255 patients, patisiran was shown to reverse neuropathy in most; it also had salutary effects on a composite measure of life quality, reducing autonomic manifestations and improving activities of daily living. These studies are encouraging for patients with transthyretin amyloidosis but also serve as a landmark in the field of diseases caused by the proteins misfolding. Patisiran and inotersen need to be repeatedly administered, and while this mandates an attractive business model for companies developing high-cost therapies for rare diseases, it is not clear that the high annual charges proposed and health gains achieved will be reimbursable worldwide. Access to the drugs may thus be limited for many affected patients. General measures The disabling arthralgia of β 2-microglobulin amyloidosis may partially respond to nonsteroidal anti-inflammatory drugs or corticosteroids, but even the most severe symptoms usually rapidly vanish following renal transplantation. The basis for this remarkable clinical response is unclear, since although transplantation rapidly restores normal β 2-microglobulin metabolism, regression of β 2-microglobulin amyloid may not be evident for many years. Supportive therapy remains critical in systemic amyloidosis, with the potential for delaying target organ failure, maintaining quality of life, and prolonging survival while the underlying process can be treated. Rigorous control of hypertension is vital in renal amyloidosis. Surgical resection of amyloidotic tissue is occasionally beneficial but, in general, a conservative approach to surgery, anaesthesia, and other invasive procedures is advisable. Should any such procedure be undertaken, meticulous attention to blood pressure and fluid balance is essential. Amyloidotic tissues may heal poorly and are liable to bleed. Diuretics and vaso-active drugs should be used cautiously in cardiac amyloidosis because they can reduce cardiac output substantially in the patient with stiff ventricles. Dysrhythmias may respond to conventional pharmacological therapy or to insertion of pacemakers and implantable defibrillators. Replacement of vital organ function, notably by dialysis, may be necessary, and cardiac, renal, and liver transplantation procedures have a role in selected cases. Excellent results of renal transplantation in end-stage renal failure have been obtained in patients with apolipoprotein A-I amyloidosis, and in selected patients with systemic AA and AL amyloidosis; it probably also remains the treatment of choice for patients with hereditary fibrinogen α -chain amyloidosis, although recurrent amyloid deposition of this type usually affects the transplant within 5 to 10 years. Future treatment possibilities As noted at the beginning of this chapter, most of the pathogenesis of disease in amyloidosis is caused by the accumulation of the extracellular amyloid deposits which physically disrupt tissue architecture and thus function. The deposition of more amyloid is invariably associated with clinical deterioration but sufficient reduction of fibril precursor protein abundance halts amyloid accumulation with slow regression of amyloid deposits in some patients, stabilization of clinical status and potential improvement. However, therapeutic interventions to directly target amyloid deposits and promote their clearance from the tissues

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