

Targeted Treatment for Amyloidosis

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Amyloid is a pathological extracellular deposit with a fibrillar ultrastructure (reviewed in [1]). In addition to the amyloid fibrils that constitute the bulk of the deposits, abundant glycosaminoglycans and proteoglycans are always present, as is the non-fibrillar, normal globular plasma glycoprotein, serum amyloid P component. There are about 30 different types of amyloid in humans, characterized by the particular specific protein that forms the fibrils. Each type of amyloid contains only a single amyloid fibril protein, and the precursor proteins from which the fibrils are derived are very diverse with little or no similarity or relationship between them.

Amyloid deposits are discrete focal structures rather than diffuse infiltrates, although they may be massive. Amyloid deposits can be either local, confined to a single anatomic site or tissue/organ system, or systemic, when the deposits are widely distributed in the parenchyma of organs and the extracellular tissue matrix throughout the body including blood vessel walls. In systemic amyloidosis the fibrils are always derived from globular precursor proteins by misfolding of the native structure followed by aggregation of partly misfolded intermediates in the characteristic amyloid fibril conformation. Regardless of the identity of the precursor protein, all amyloid fibrils are rigid, non-branching, straight or curvilinear, composed of two or more protofibrils of indeterminate length and about 100 Å in diameter. The core structure of all amyloid fibrils consists of antiparallel β -pleated sheets arranged with their long axes perpendicular to the long axis of the fibril. This structure specifically binds the histochemical dye, Congo-red, from alkaline alcoholic solutions, in an ordered molecular array which gives pathognomonic red-green birefringence when viewed in strong cross-polarized light. This is the gold standard for histological diagnosis of amyloid.

Systemic amyloidosis, fatal within 6 months of diagnosis in up to 20% of patients, causes about one per thousand deaths in developed countries and remains an important unmet medical need

Amyloid deposition usually disrupts the structure and function of affected tissues and organs, leading to disease that is known as amyloidosis and can be local or systemic depending on the distribution of the deposits. Local amyloidosis, either a single nodule or mass, or multiple deposits in the respiratory or urogenital tract or around the eyes, can be very troublesome but is rarely fatal. In contrast, cerebral amyloid angiopathy, local amyloid caused by A β deposition confined to the cerebral vasculature, is the cause of a substantial proportion of cerebral hemorrhages [2]. Systemic amyloidosis is usually fatal and is the cause of about one per thousand deaths in developed countries. The clinical manifestations of systemic amyloidosis are protean and non-specific and thus the diagnosis is often made late when there is already major organ damage. Despite major advances in diagnosis, monitoring and treatment, and much improved outcomes for many patients, systemic amyloidosis remains a major unmet medical need [1].

Amyloid fibrillogenesis can result from the inherent instability of a native wild-type protein, from overproduction of a normal protein with reduced stability and increased propensity to misfold, or from production of an abnormal unstable protein. There are therefore both acquired and hereditary forms of amyloidosis. The most common form of systemic amyloidosis is the AL type. The international nomenclature comprises A for amyloidosis and the second and other letters identify the amyloid fibril protein, in this case L for monoclonal immunoglobulin light chains [3]. AL amyloidosis, formerly known as primary amyloidosis, is thus a complication of monoclonal gammopathy of any type ranging from myeloma through monoclonal gammopathy of uncertain significance, to the whole variety of B/plasma cell dyscrasias. It accounts for about 60% of all cases referred to the UK

National Amyloidosis Centre. AA amyloidosis, formerly known as secondary or reactive systemic amyloidosis, is a complication of chronic inflammatory and infective diseases in which there is a sustained acute-phase response with overproduction of serum amyloid A protein, a very sensitive and dynamic major acute-phase protein. Although becoming rare in the developed world due to greatly improved treatments for inflammatory arthritides, Crohn's disease, chronic infection, etc., AA amyloidosis is

of considerable importance in Israel because of the prevalence of familial Mediterranean fever of which it is a major complication causing nephrotic syndrome, renal failure and death. A β 2m amyloidosis, so-called dialysis-related amyloidosis, is a serious complication of long-term dialysis for end-stage renal failure in which β 2-microglobulin, normally catabolized by the kidneys, is not adequately cleared and accumulates in the plasma, rising in concentration from its normal value of 1–2 mg/L to up to 70 mg/L. After 5–7 years it is then deposited as amyloid fibrils in and around bones and joints, causing pain, bone cysts and pathological fractures. ATTR amyloidosis is caused by the plasma protein, transthyretin. Normal wild-type TTR is inherently amyloidogenic and commonly forms amyloid fibrils in the elderly, sometimes producing clinically significant senile cardiac amyloidosis. Mutations in the TTR gene which encode destabilized TTR variant proteins are the most common cause of hereditary systemic amyloidosis, usually presenting as familial amyloid polyneuropathy, but this is very rare with only about 10,000 affected patients worldwide. There are several other types of hereditary amyloidosis, caused by mutations in the genes for other plasma proteins encoding destabilizing amino acid substitutions. Although these diseases are all extremely rare, they have provided important insights into molecular mechanisms of fibrillogenesis and the pathogenesis of amyloidosis.

AMYLOID-ASSOCIATED DISEASES

In contrast to the rarity of amyloidosis, there are two extremely common and very important diseases in which amyloid deposits are always present: Alzheimer's disease with cerebral and cerebrovascular A β deposits and neurofibrillary tangles with structure very closely related to amyloid fibrils, and type II diabetes with amyloid deposits in the pancreatic islets of Langerhans composed of islet amyloid polypeptide. Unlike the globular precursors of systemic amyloid fibrils, A β and islet amyloid polypeptide are intensely fibrillogenic natively unstructured polypeptides, cleaved from larger precursor proteins. Furthermore, also unlike the situation in systemic amyloidosis, it is not known whether and how much these local amyloid deposits contribute to the pathogenesis of dementia and diabetes respectively. Treatments that eliminate the deposits would be very informative about this point.

TREATMENT FOR SYSTEMIC AMYLOIDOSIS

The natural history of amyloid is persistence of the deposits, which usually continue to accumulate inexorably. A notable

TTR = transthyretin

feature of all amyloid deposits is that they are largely ignored by the usually very efficient physiological mechanisms by which abnormal protein debris is cleared from the interstitial space in the tissues. Dead cells, effete matrix and structural proteins, and blood cells and plasma proteins extravasated in injury, are normally rapidly cleared with no local or systemic clinical consequences. In contrast, although macrophages and giant cells are occasionally seen, especially around local rather than systemic amyloid deposits, amyloid deposition usually elicits no cellular or inflammatory reactions. Nevertheless, amyloid deposits can regress when the abundance of the respective fibril precursor protein is sufficiently reduced. Amyloid must

therefore be degraded *in vivo* but usually not at a sufficient rate to overcome new deposition.

The current therapeutic strategy in amyloidosis is therefore to provide vigorous supportive management, including organ replacement/transplantation if necessary, for as long as needed for production of the fibril precursor protein to be controlled and ideally abolished. The latter is not always possible, especially in the hereditary forms of amyloidosis, but the increasingly powerful and effective new medications for myeloma produce a good or complete clonal response in a substantial proportion of AL amyloidosis patients, associated with improved organ function, regression of amyloid, and greatly prolonged survival. It is essential to closely monitor serum free light chain concentrations to ensure adequate therapy for the best possible clonal response [4]. Similarly, effective anti-inflammatory treatment, or whatever is needed to control the acute-phase response and maintain circulating SAA concentrations in the normal range, is life saving in AA amyloidosis. Rigorous compliance with colchicine therapy for FMF prevents and ameliorates AA amyloidosis even in patients who do not experience complete relief of symptoms. The key is to control SAA production, closely monitoring SAA values in all patients with AA amyloidosis, and tailoring their treatment to keep these as low as possible [5]. However, many patients are already in severe or end-stage organ failure when diagnosed with amyloidosis and new approaches are desperately needed to save them.

SERUM AMYLOID P COMPONENT

SAP is a constitutive, normal, homopentameric, plasma glycoprotein, closely related to C-reactive protein – the classical acute-phase protein [6]. SAP is always present in amyloid deposits of all types because it binds avidly, albeit reversibly, to all different types of amyloid fibrils. We used this property

SAA = serum amyloid A protein
FMF = familial Mediterranean fever
SAP = serum amyloid P component

to develop radiolabelled human SAP as a highly specific, safe, non-invasive, quantitative tracer for diagnosis and monitoring of systemic amyloid deposits of all types [7]. SAP scintigraphy has provided unique information on the natural history of amyloidosis and its response to treatment, leading to the establishment of the UK National Health Service National Amyloidosis Centre. Our Centre, with a staff of over 50 funded directly by the UK Department of Health, now sees over 3000 amyloidosis patients annually, comprising the whole UK national caseload and many patients from elsewhere, and follows the largest and most diverse cohort of such patients in the world.

Binding of SAP to amyloid fibrils stabilizes them and promotes the formation and persistence of amyloid [8]. It is therefore a valid therapeutic target and we have developed a drug, (R)-1-[6-[(R)-2-carboxy-pyrrolidin-1-yl]-6-oxo-hexanoyl]pyrrolidine-2-carboxylic acid (CPHPC), which depletes almost all SAP from the circulation for as long as it is given [9,10]. CPHPC was intended to remove all SAP from amyloid deposits and thereby promote their regression. However, although amyloidosis patients treated long term with CPHPC remained well and apparently had more stable organ function, there was no amyloid regression. Furthermore, CPHPC removed much but never all the SAP from the amyloid deposits [10]. We therefore devised an additional novel approach.

Transthyretin amyloidosis is being targeted by several companies with novel small molecule drugs and with gene suppression

ELIMINATION OF AMYLOID DEPOSITS

The unique capacity of CPHPC to remove essentially all circulating SAP while leaving some SAP in the tissues as a specific marker for the amyloid deposits enables us to target the deposits with anti-SAP antibodies. Such antibodies obviously cannot be given to patients with normal circulating concentrations of SAP because immune complex formation in the circulation would cause major life-threatening complications. The antibody would also be consumed and would not be available to bind to the SAP in the amyloid deposits. In human SAP transgenic mice with massive AA visceral amyloid deposits, treatment with CPHPC to deplete their circulating SAP and yet leave SAP in the amyloid, followed by a single dose of anti-SAP antibody, is well tolerated with no detectable clinical or biochemical effects [11]. The antibodies localize to the residual SAP in the amyloid deposits, activate complement, and trigger rapid massive local accumulation of macrophages. The macrophages swiftly fuse into multinucleate giant cells which surround and engulf all the amyloid and promptly destroy it. After about 10–14 days almost all the amyloid has disappeared, along with the macrophages, and normal tissue architecture is restored [11].

Our patents covering the invention of this new therapeutic approach were licensed to GlaxoSmithKline in 2009 and the

first clinical trial in patients with systemic amyloidosis is now in progress, using CPHPC followed by fully humanized anti-SAP antibodies. So far there have been no adverse effects. If in due course there is evidence for elimination of amyloid deposits and no side effects or adverse reactions are observed, the new treatment should eventually become available for treatment of all types of systemic and local amyloidosis.

TRANSTHYRETIN AMYLOIDOSIS

TTR is a normal, non-glycosylated, homotetrameric plasma protein that transports retinol-binding protein, and therefore also retinol (vitamin A), and thyroxine in the blood; hence its name. TTR is inherently amyloidogenic and causes clinically silent microscopic amyloid deposition in a substantial proportion of individuals over the age of 80 years [12]. More massive TTR amyloid deposition in the myocardium causes senile cardiac amyloidosis, a restrictive cardiomyopathy leading to

fatal diastolic heart failure [13]. This condition is much more common in carriers of the Val122Ile TTR polymorphism which is present in ~4% of black Americans of West African

origin, comprising about 1.3 million people among whom about 13,000 are homozygous for the trait [14]. Until recently senile cardiac amyloidosis was rarely diagnosed because elderly subjects with heart failure were assumed to have had coronary artery disease and/or hypertension and were not investigated appropriately. Recently it has emerged that the bone-scanning agent ^{99m}Tc-DPD is a specific quantitative tracer for cardiac TTR amyloid. The mechanism underlying this specificity is not known, but the results are impressive and it is now clear that cardiac TTR amyloidosis is much more common than previously recognized [15]. Work is in progress to document the prevalence and natural history, and to ascertain the scope for potential therapeutic intervention.

Hereditary systemic TTR amyloidosis can be caused by over 90 different, variably penetrant mutations in the *TTR* gene [16]. The Val30Met variant is by far the most common, occurring mostly in specific locations in northern Portugal, northern Sweden and Japan, but cases have been reported worldwide. About 10,000 individuals are affected in total, usually presenting with autosomal dominant familial amyloid polyneuropathy, a severe progressive peripheral and autonomic neuropathy. The amyloid is always systemic with variable cardiac, renal, ocular, splenic and, rarely, also leptomeningeal involvement. Onset can be from the twenties to the seventies, always after potential child-bearing age so the gene is transmitted before the illness starts, and inexorably fatal progression over 10–15 years represents a grave unmet medical need.

We did some work with GlaxoSmithKline, seeking to develop a small molecule compound to stabilize TTR and prevent its

misfolding and amyloid aggregation [17]. Pfizer has already marketed such a compound, Vyndaquel (tafamidis), but its efficacy is not yet impressive. Diflunisal, the generic non-steroidal anti-inflammatory drug, has also been investigated in a clinical trial as a TTR stabilizer. Other companies are active in the field, with Isis having an antisense oligonucleotide and Alnylam a small interfering RNA, both of which substantially reduce the circulating TTR concentration. There are thus grounds for optimism that one or more of these approaches may be efficacious and become registered drugs. Importantly, if our CPHPC + anti-SAP antibody treatment to eliminate existing amyloid deposits is shown to be safe and effective, it will complement all these other approaches.

CONCLUSIONS

Amyloidosis is a complex, serious and very diverse condition. Patient management required for optimal outcomes is very challenging, involving many specialties, close monitoring and extreme attention to detail. It should be conducted and/or directed only by expert physicians in specialist centers. Despite major advances in diagnosis and management, leading to much improved outcomes in such centers, the condition is still almost always fatal. Based on increasing knowledge of the pathobiology and innovative scientific and technical advances in biomedicine, new therapeutic approaches are now in development. There are grounds for cautious optimism that these may lead to substantially better treatment and results in the next few years.

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“Whenever anyone has offended me, I try to raise my soul so high that the offense cannot reach it”

Rene Descartes (1596-1650), French philosopher and mathematician who has been dubbed The Father of Modern Philosophy, and is perhaps best known for the philosophical statement “*Cogito ergo sum*” – “I think therefore I am”

“One day work is hard, and another day it is easy; but if I had waited for inspiration I am afraid I should have done nothing. The miner does not sit at the top of the shaft waiting for the coal to come bubbling up to the surface. One must go deep down, and work out every vein carefully”

Arthur Sullivan (1842-1900), English composer, best known for his series of 14 operatic collaborations with the dramatist W.S. Gilbert, including such enduring works as *H.M.S. Pinafore*, *The Pirates of Penzance* and *The Mikado*. Sullivan composed 23 operas, 13 major orchestral works, eight choral works and oratorios, two ballets, incidental music to several plays, and numerous hymns and other church pieces, songs, and piano and chamber pieces