

Amyloidosis: a global problem common in Papua New Guinea

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SUMMARY

The increase in different precursor proteins that have been shown to form amyloid fibrils and the identification of common properties have not yet led to any unifying theory or mechanism for the pathogenesis of amyloidogenesis. Papua New Guinea holds a unique place in the story of amyloidosis and in this article we review the current status of amyloidosis research indicating how this relates to those forms relevant to Papua New Guinea. This review concentrates on secondary reactive amyloid (AA), which is found in the highest frequency in the world in parts of Papua New Guinea, and kuru, in which the amyloid protein itself is infectious. The history, pathogenesis and future prospects for these diseases are discussed in the light of what is known about other forms of amyloidosis.

Introduction

The designation 'amyloidosis' refers to a disease with deposition of an insoluble proteinaceous substance (amyloid) in different tissues and organs. Amyloid differs from other protein deposits by certain physicochemical properties, notably affinity for the dye Congo red and green birefringence when viewed under polarized light after this staining. The morphological appearance of amyloid has been known for a long time as has the great variability in tissue distribution, association with other diseases and clinical consequences. The explanation for this great diversity in distribution and clinical appearance seems to reside in the molecular nature of the amyloid. All types of amyloid consist of fibrils which vary only a little in morphology between the forms. However, there is much variation in the chemical composition of amyloid and, to date, 15 discrete proteins have been shown to form amyloid fibrils in humans (Table 1) (1). Each of these proteins occurs in one or a few clinical

syndromes (Table 1). Several amyloid syndromes, including different amyloid proteins (AA, AL, A-PrP), have already been described from Papua New Guinea (PNG) and one disease, kuru, now recognized as an amyloid disorder, has caused a unique epidemic in the country. There is, however, no doubt that other amyloid-associated diseases exist in Papua New Guinea, such as the most important form now recognized in Alzheimer's disease. With the progressive ageing of Papua New Guineans this globally prevalent amyloidosis will, undoubtedly, become more common here.

First descriptions of amyloidosis in PNG

In 1970 Cooke and Champness reported their findings from 1101 postmortem examinations carried out in Port Moresby, PNG, in which they found histological evidence of amyloidosis in 80 (7.3%), the highest prevalence of the condition in the world. A new juvenile primary amyloid syndrome of

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TABLE 1
HUMAN AMYLOID FIBRIL PROTEINS AND PRECURSORS*

Designation	Full-length Fragment (L) (F)	Precursor	Nature of precursor	Systemic or Localized	Clinical amyloid syndromes
AA	F	Serum AA	Apolipoprotein	S	Inflammation-associated amyloidosis
AApoA1	F	Apo A1	Apolipoprotein	L	Senile intimal aortic amyloidosis
AL	F	Light chain	Immunoglobulin	S	Plasma cell dyscrasia
AH	F	Heavy chain	Immunoglobulin	L	Plasmacytoma (?)
A β 2M	F, L	β 2-microglobulin	HLA class 1 light chain	S	Plasma cell dyscrasia
ATTR	F, L	Transthyretin	Carrier protein	S	Haemodialysis
ACys	F	Cystatin C	Protease inhibitor	S	Familial (cerebral haemorrhagic)
ALys	F	Lysozyme	Protease inhibitor	S	Familial (kidney)
AFibr	F	Fibrinogen	Clotting protein	S	Familial (kidney)
AGel	F	Gelsolin	Actin modulator	S	Familial (Finnish type)
A β PP	F	β -protein precursor	Transmembrane protein	L	Alzheimer's disease
APrP	F	PrP (prion protein)	Transmembrane protein	L	Kuru
ACalc	F, L	(Pro)calcitonin	Polypeptide hormone	L	Creutzfeldt-Jacob disease
AANF	L	Atrial natriuretic factor	Polypeptide hormone	L	Gerstmann-Sträussler-Scheinker syndrome
AIAPP	L	Islet amyloid polypeptide	Polypeptide hormone	L	Medullary thyroid carcinoma
					Senile atrial amyloid
					Non-insulin-dependent diabetes

* Based on a table in reference 1; for references to the clinical syndromes see reference 1

renal failure and goitre was recognized in eight children in whom no known predisposing cause for amyloidosis could be identified (2). Since Port Moresby General Hospital was the national centre for histopathology in PNG, specimens were referred from all over the country and the geographical distribution of amyloidosis described at that time merely reflected the referral patterns from the main medical centres. Anders et al. (3) examined the geographical distribution of amyloidosis cases presenting in Port Moresby. During an eleven-year period 73 cases were identified but none of the patients had originated from the highlands or north coast regions although these two areas accounted for 16% of medical admissions and made up a similar percentage of Port Moresby's population. A higher incidence area was noted in the inland, more sparsely populated region outside Port Moresby, but no predisposing chronic diseases or aetiological factors could be identified for the 32 cases of primary disease. Anders et al. (4) showed that amyloid fibril preparations from 8 representative patients contained the non-immunoglobulin protein AA, including one of the children with the renal failure and goitre syndrome.

Leprosy

All of the early descriptive amyloid series from PNG identified leprosy as a major cause of secondary amyloidosis. McAdam et al. (5) found amyloid in the rectal biopsies of 16 out of 190 patients with leprosy at Togoba Hospital in the Western Highlands of PNG and this represented 20% of the patients who had had lepromatous leprosy for more than two years. Recurrent erythema nodosum leprosum (ENL) reactions were the common feature of all multibacillary cases who developed amyloidosis. Serum amyloid A (SAA) concentrations were shown to rise during ENL episodes in parallel with the neutrophil leucocyte count, which is the earliest marker of these immune-complex-mediated reactions.

Leprosy and tuberculosis have long been known to be causes of secondary amyloidosis and in the early descriptions from PNG various other associations were also noted, including osteomyelitis, ankylosing spondylitis and chronic lung disease (6). In addition a few sporadic cases of myeloma-associated disease

have been seen in PNG; though uncommon here, in industrialized countries this may be the most frequently seen clinically important form of systemic amyloidosis. McAdam (7) reported the previously unrecognized association between amyloidosis and two chronic parasitic infections common in PNG: filariasis and malaria.

Filariasis

In a study from the Fly River basin of PNG, where both filariasis and malaria are hyperendemic, abdominal wall fat aspiration biopsies were performed on 27 adults found to have either elephantiasis or proteinuria of more than 30 mg/100ml. Six biopsies revealed amyloidosis and two of the six patients had a particularly striking history, describing multiple recurrent acute attacks of adenolymphangitis occurring every 2-4 weeks. During these attacks there was high fever, prostration and tender swelling of inguinal lymph nodes, accompanied by elevated concentrations of SAA.

Malaria

The first clue that malaria might be implicated as a cause of amyloidosis in PNG came from a closer examination of the villages of origin of the juvenile cases rather than the medical centre from which they were referred. An interesting cluster emerged in the Watut Valley area of the Morobe Province near Bulolo (8). This area had been the site of a gold rush in the 1920s and has been the focus of medical attention since the early 1960s (9), on account of the high incidence there of tropical splenomegaly syndrome (TSS) (10), also known as hyperreactive malarious splenomegaly (HMS) (11), a disorder of aberrant immune regulation in response to malaria (12-14). In most malarious areas splenomegaly occurs commonly in children under 10 years old but their spleens usually regress in size by puberty, whereas in HMS this reduction in size does not occur, leading to enormous splenomegaly in young and older adults. High concentrations of antimalarial IgM, with polyreactive autoantibodies and cryoglobulins (15), help differentiate the condition from other causes of splenomegaly (16).

McAdam (7) has reviewed the early studies

on amyloidosis in the Upper Watut Valley amongst peoples of the Anga linguistic group; they had originated in the Eastern Highlands above the altitude of malarial transmission but various clans had migrated down into adjacent valleys about 70-90 years ago and had met malaria for the first time (17). In the context of seasonal or mesoendemic malarial transmission, 80% of the inhabitants develop HMS and about 10% AA amyloidosis.

In 1974 an initial point prevalence study of amyloidosis used a simple field diagnostic technique of abdominal wall fat aspiration biopsy to provide histological evidence of amyloidosis (18). Two different clans of the Anga linguistic group were compared, one from the Watut Valley and the other living near Aseki above the altitude of malarial transmission. Nephrotic syndrome was so frequently observed in the Watut population that they had a locally well-accepted name for it in Melanesian Pidgin – ‘skin solap’ – whereas the condition was not known by their highland cousins. Of biopsies obtained in the Watut from 76 individuals who were more than 10 years old, 8 (11%) were positive for amyloid, which yielded a point prevalence of 6% in the total study population of 133. In contrast, none of the 72 biopsies in the highland study population of 129 people had histological evidence for amyloidosis (7). This is the closest estimate to a true point prevalence of amyloidosis ever undertaken and was only possible in this field setting because of the simplicity and local acceptability of the biopsy procedure (8). The major difference between these two groups was made particularly evident by an evaluation of malarial indices. The Watut people had high spleen rates and were more anaemic, with high concentrations of serum IgM, malarial antibodies and autoantibodies. Only a few young adult males in the highland population, who had almost certainly travelled to coastal areas for work, expressed any of these signs of malarial exposure. Concentrations of SAA protein in the serum were significantly higher in the Watut Valley peoples and rose during acute malarial attacks.

In 1983 two closely related and geographically similar adjacent Watut villages (one hour's walk between them) provided an opportunity to define the effectiveness of

malaria chemoprophylaxis, using chloroquine administered once a week, on the prevalence of amyloidosis. Villagers in Yokua had not been taking chloroquine prophylaxis (though offered it), whereas the people of Kaumanga had been taking chloroquine on a regular basis for about 5 years. A non-Anga village, Mirap, near the coastal town of Madang was selected as a control population, living in a hyperendemic malarial situation. The prevalence of amyloidosis in Yokua was twice as high as that in Kaumanga. The only case of amyloidosis in Mirap was in a 55-year-old man and there were no recollections by the inhabitants of medical histories consistent with nephrotic syndrome in this village. The differences between the mean spleen sizes of each village were significant: 47% of subjects in Yokua had Hackett grade 4 or 5 splenomegaly, but only 14% of subjects in Mirap and 13% of subjects in Kaumanga had such grossly enlarged spleens. On the basis of these preliminary data, antimalarial interventions would be extremely beneficial in this Watut Valley population, not only to reduce the splenomegaly (17) but also the associated anaemia and the high prevalence of AA amyloidosis. Whether insecticide-impregnated bednets or the various candidate malaria vaccines will be more effective than long-term chemoprophylaxis, now rendered less effective because of a general increase in drug resistance, remains to be seen.

Amyloidosis was found to be more prevalent in particular family groups. On the basis of family histories and amyloid biopsy results, pedigrees associated with amyloidosis were identified within each clan. Further genetic studies will investigate whether susceptibility to amyloidosis can be linked to various candidate genes encoding amyloid precursor proteins including SAA.

Kuru

Kuru is a degenerative disease of the central nervous system which occurred as an epidemic in the Okapa area of the Eastern Highlands. The disease runs a subacute course, with progressive cerebellar ataxia and, to a lesser extent, damage to the other motor systems of the brain, and is always fatal (19). The average duration is about 12 months. The degeneration which causes kuru and similar diseases is now known to be an amyloidosis. The remarkable

feature of this form of amyloid is that it is infectious. The purified amyloid protein can transmit the disease experimentally (20). The epidemic of kuru was caused by transmission of infected brain, principally to women and children, through the practice of endocannibalism, the mortuary consumption of dead relatives at funeral feasts.

The amyloid precursor protein is a transmembrane protein. In disease, the normal isoform of the protein undergoes a conformational change to an isoform which is largely beta-pleated, protease resistant and insoluble. This causes degeneration and vacuolation in neurones. The abnormal protein is deposited extracellularly, where it may form amyloid plaques. When plaques occur they are very common throughout the brain, especially in the cerebellum, but in many cases of kuru there are no plaques. However, there is always the abnormal amyloid protein; and with it, neuronal degeneration and loss, astrocytosis and widespread spongiform change.

Kuru and other cerebral spongiform encephalopathies, such as Creutzfeldt-Jacob disease (which causes presenile dementia), Gerstmann-Sträussler-Scheinker syndrome, fatal familial insomnia and scrapie (the equivalent of kuru in sheep), are all caused by an amyloid which is infectious. It is able to impose its abnormal conformation on other molecules of the precursor protein and induce them to adopt the abnormal pathogenic infectious isoform. The cell is stimulated to produce more of the amyloid precursor protein, which is host-coded, and the pathological process expands.

The probability of transmission to a new host depends on the conformational similarity between the host's amyloid precursor protein and the infecting amyloid; within the same species the probability is extremely high; as the distance between the species increases, the probability of transmission falls. However, if transmission does occur to a new species – as from humans to chimpanzees in the first such experiments with kuru (21) – the newly formed agent is host-coded and therefore transmits very easily within the new species. The route of inoculation also influences the probability of transmission; with the oral route transmission is less likely, even within the same species, and

the incubation period of the disease is very variable. This was the situation with the transmission of kuru by endocannibalism. Although transmission ceased about 40 years ago, a few cases of kuru are still occurring (the tail end of the epidemic) but only in people aged over 40 years and with an incubation period at least as long. The annual incidence today is about 5 compared to 200 in the late 1950s, and the epidemic will certainly die out: but it will probably take the lifespan of the cohort born before 1960 before it finally does so.

These infectious amyloid proteins and their precursors have been called prions or PrP (22). Genetic variation in the PrP gene may increase the probability of the development of the abnormal isoform, and thus the disease, and may determine the age at onset, the clinical form of the disease and the duration (23). Nevertheless, most cases of Creutzfeldt-Jacob disease are sporadic. Since kuru is an unusual variant of Creutzfeldt-Jacob disease it is presumed that the first case occurred sporadically (as happens in every human population at an annual incidence of about one per million), probably in the second decade of this century. Whether there are any genetic features in the PrP gene or other genes of the Fore people and their neighbours, to whom the disease was restricted, which increased their susceptibility and/or determined the clinical form of the disease as predominantly cerebellar ataxia rather than dementia, is not known but remains a strong possibility. The familial nature of the disease, however, can be explained entirely by the familial mourning rituals which determined its transmission.

Pathogenesis of amyloid

Amyloid proteins and amyloid fibril formation

All amyloid fibril proteins are small and can, for the most part, be divided into two main groups, one consisting of naturally occurring, full-length proteins and one containing small fragments of sometimes much bigger precursors. The nature of the fibril protein precursor is extremely variable. As seen from Table 1, the amyloid fibril precursor proteins include apolipoproteins, immunoglobulins, protease inhibitors and polypeptide hormones. A large number of familial forms of

amyloidosis have been described, many of which involve alterations in the protein sequence; thus far 70 variants in 8 human proteins have been described (Table 2) (24).

The basic unit of amyloid is the fibril and the molecular organization in the fibril is believed to be similar in all forms of amyloid, irrespective of chemical nature. A cross- β -structure has been characteristic of all amyloid fibril types that have been examined (25) and several of the protein precursors, for example immunoglobulin light chains, β_2 -microglobulin (26) and transthyretin (27), have a high degree of β -structure in their native forms. Formation of fibrils most probably also involves intermolecular β -sheet formation but the exact quaternary structure has not been resolved for any type of amyloid. Most likely, not only β -structure is present in the amyloid fibril but other secondary structures as well (28). The fibril may, however, have a sheet core, which theoretically can vary between different forms. Such a model allows for other structures to be present outside the core. Whatever the unifying molecular organization is, it should explain the important properties of the amyloid fibrils such as their insolubility, resistance to proteolysis, affinity for Congo red and green birefringence after this staining. It should also explain the binding of other substances *in vivo*, such as the amyloid P-component (AP).

The pathogenesis of all types of amyloid thus involves an altered three-dimensional structure of a protein leading to an ordered fibril formation (29). To what extent fibril formation also includes refolding of the subunit protein is not clear and this may well vary between the different proteins. In transthyretin, most antigenic sites present in transthyretin monomers are also present in the amyloid fibrils (30), certainly indicating that at least some normal folding is present (31,32). On the other hand, an amyloid-specific antigenic epitope was also demonstrated (30).

The reason why a protein starts forming an amyloid fibril is only partially understood. Many different factors may be involved and both abnormal or enhanced formation and deficient degradation have to be considered. In the pathogenesis of amyloid fibrils in general, the possibility of a 'nidus' on which fibril formation takes place has been given

increasing attention. Several *in vitro* studies with different amyloid fibril proteins have clearly shown that the presence of a small amount of preformed fibrils strongly enhances further fibril formation from a protein solution (33,34). It is believed that the preformed fibrils may act as the template on which elongation of fibrils takes place. This may mean that the limiting process is the formation of the first fibril (35). Therefore, the search for a putative nidus formation may be of greatest interest. In the transmissible cerebral amyloid forms (in humans: kuru, Creutzfeldt-Jakob disease and Gerstmann-Sträussler-Scheinker syndrome), as discussed earlier, there is strong evidence that the infective agent is a protein, which starts post-transcriptional changes in the homologous protein expressed by a gene in the recipient (36). These diseases are thus believed to result from a protein conformational change chain reaction. Similar mechanisms may well act in other amyloids. The amyloid enhancing factor (AEF), which basically is an amyloid extract, dramatically shortens the lag time between inflammatory stimulation and deposition of AA amyloid in the mouse model (37). The nature of AEF is not known but the presence of small fibril fragments (38) or even AA monomers may offer an explanation for this phenomenon. Interestingly, fibrils formed *in vitro*, made from short synthetic peptides, have AEF activity in the AA amyloid mouse model (39). The finding that synthetic fibrils, which were active as an amyloid enhancer, included those made from human TTR or IAPP peptides, i.e. heterologous structures, opens up the interesting possibility that a starting point for amyloid fibril formation may not necessarily be identical fibrils but may include other structures and components. Factors in ageing and even in the environment should be taken into consideration given the very high prevalence of certain amyloid forms in association with ageing and in some geographical areas. AA amyloidosis in PNG is a good example of the latter.

Other amyloid components

In addition to the main amyloid fibril proteins, other components are regularly found in the amyloid substance. Thus, within the extracellular amyloid deposits AP and glycosaminoglycans will always be present.

TABLE 2

INHERITED AMYLOID DISEASES WITH A MUTATION IN THE PRECURSOR PROTEIN*

Variant	Geographic kindred	Clinical manifestations ^a
TTR:		
Cys ₁₀ Arg	US	PN, eye, heart
Val ₃₀ Met	Portugal, Japan, Sweden, US	PN, AN, eye
Val ₃₀ Ala	US	AN, PN
Val ₃₀ Leu	Japan	PN, AN
Val ₃₀ Gly	US	Eye
Phe ₃₃ Ile	Israel	PN, eye
Phe ₃₃ Leu	US	PN, heart
Ala ₃₆ Pro	US	PN, eye, heart, kidney
Glu ₄₂ Gly	Japan, Italy	PN, AN, heart
Ala ₄₅ Thr	US	Heart, AN
Ala ₄₅ Asp	US	Heart, PN
Gly ₄₇ Arg	Japan	PN, AN
Gly ₄₇ Ala	Italy	PN, heart, kidney
Gly ₄₇ Val	Sri Lanka	CTS, PN
Thr ₄₉ Ala	Italy	Eye, PN, heart
Ser ₅₀ Arg	Japan	CTS, PN, AN
Ser ₅₀ Ile	Japan	Heart, PN, AN
Ser ₅₂ Pro	UK	PN, heart
Glu ₅₄ Gly	UK	PN
Leu ₅₅ Pro	US, Taiwan	PN, AN, heart, eye
Leu ₅₈ His	US	CTS, PN
Leu ₅₈ Arg	Japan	PN, AN, CTS, eye
Thr ₅₉ Lys	Italy	Heart
Thr ₆₀ Ala	US	Heart, PN, AN
Glu ₆₁ Lys	Japan	AN, PN
Phe ₆₄ Leu	US	PN, CTS, heart
Ile ₆₈ Leu	Germany	Heart
Tyr ₆₉ His	US	Eye
Lys ₇₀ Asn	US	CTS, eye, PN
Val ₇₁ Ala	France	PN, CTS, eye, AN
Ser ₇₇ Tyr	US, France	PN, AN, heart
Ile ₈₄ Ser	US	CTS, PN, eye, heart
Ile ₈₄ Asn	US	Eye, heart
Glu ₈₉ Gln	Italy	CTS, PN, heart
Ala ₉₇ Gly	Japan	PN, heart
Ile ₁₀₇ Val	US	CTS, PN, heart, AN
Leu ₁₁₁ Met	Denmark	Heart
Tyr ₁₁₄ Cys	Japan	PN, AN, eye, heart
Tyr ₁₁₄ His	Japan	CTS
Val ₁₂₂ Ile	US	Heart
Apo A1:		
Gly ₂₆ Arg	US	Kidney, PN, peptic ulcers
Leu ₆₀ Arg	UK	Kidney
Gelsolin:		
Asp ₁₈₇ Asn	Finland	PN, eye, kidney
Asp ₁₈₇ Tyr	Denmark	PN

Variant	Geographic kindred	Clinical manifestations ^a
Apo A2^b: Pro ₅ Gln		Spleen, liver
Lysozyme: Ile ₅₆ Thr Asp ₆₇ His	UK UK	Kidney, spleen Kidney
Fibrinogen α-chain: Glu ₅₂₆ Val Arg ₅₅₄ Leu	US Peru	Kidney Kidney
ABPP^c: Gln ₆₆₅ Asp Lys ₆₇₀ Asn & Met ₆₇₁ Leu Ala ₆₉₂ Gly Glu ₆₉₃ Gln Ala ₇₁₃ Thr Ala ₇₁₃ Val Val ₇₁₇ Ile Val ₇₁₇ Phe Val ₇₁₇ Gly	UK Sweden Netherlands Netherlands France UK UK, Japan, Italy US UK	Kidney Dementia Dementia or CH CH Dementia Schizophrenia (?) Dementia Dementia Dementia
Cystatin C: Leu ₆₈ Gln	Iceland	CH
PrP: (Insertion) ^d Pro ₁₀₂ Leu Pro ₁₀₅ Leu Ala ₁₁₇ Val Tyr ₁₄₅ (Stop) Asp ₁₇₈ Asn Val ₁₈₀ Ile Phe ₁₉₈ Ser Gln ₂₀₀ Lys Val ₂₁₀ Ile Gln ₂₁₇ Arg Met ₂₃₂ Arg	UK, US US, UK, Japan Japan France, UK Japan Finland, US, France Japan US US, Slovakia, Israel, France, Libya, Japan France, Italy Sweden Japan	Dementia (CJD) Ataxia, dementia (GSS) Dementia (GSS) Dementia (GSS) Dementia (GSS) Dementia, insomnia (CJD; FFI) Dementia (CJD) Ataxia, dementia (GSS) Dementia (CJD) Dementia (CJD) Dementia (GSS) Dementia (CJD)

* Based on a table in reference 24; for references to the diseases and variants see references 24 and 25

^a PN = peripheral neuropathy; AN = neuropathy of the autonomic nervous system; CTS = carpal tunnel syndrome; CH = cerebral haemorrhage; CJD = Creutzfeldt-Jakob disease; GSS = Gerstmann-Sträussler-Scheinker disease; FFI = fatal familial insomnia

^b Occurs only in mice

^c Isoform with 770 amino acids (AβPP770) has been used as the basis for numbering of the mutations

^d Insertion of octapeptide repeats between codons 51 and 91

Note - For protein abbreviations see Table 1

AP, identical to the plasma protein SAP, consists of five identical 24 kd subunits and belongs to the pentraxin family (also including C-reactive protein) (40). AP normally occurs in the basement membranes of the glomeruli and vessel walls and on elastic fibrils (41,42). It can in some amyloid make up 20% of the dry material (43). Among the suggestions for SAP function is the binding to exposed cellular DNA thereby preventing the formation of autoantibodies (44). Others have demonstrated that AP acts as an elastase inhibitor and, when it is bound to elastin fibres or AA fibrils, proteolytic cleavage is reduced, perhaps accounting for some of the notorious insolubility of amyloid (45,46). The impact of AP in the pathogenesis of amyloid is poorly understood.

The second component, glycosaminoglycan, occurs in different forms but heparan sulphate (HS) and dermatan sulphate (DS) predominate (47,48). At least HS is mainly present in a proteoglycan (PG) form. Thus, HSPG has been demonstrated in the human glomerular AA amyloid deposits. In the experimental mouse model of AA amyloidosis, HSPG has been shown to occur simultaneously with amyloid deposition in the spleen (49). HS has been shown to alter the secondary structure of synthetic polypeptides to form a β -structure rather than an α -helical conformation (50) and may therefore act as a 'pathological chaperone' for the amyloid protein. HSPG is a basement membrane component and it should be pointed out that other basement membrane structures have also been found in amyloid (51,52)

Recently, apolipoprotein E, found in different amyloids including β -amyloid from the brain, has attracted considerable interest (53). It was found that the apo E4 isoform was associated with Alzheimer's disease (54,55) and this protein has also been suggested as a pathological chaperone. It is perhaps noteworthy that the frequency of the apo E4 allele is amongst the highest in the world in PNG (56).

Inflammation as a cause of amyloidosis

The precursor protein of AA amyloidosis is serum amyloid A protein (SAA), an HDL-associated apolipoprotein (57), which increases in plasma concentration by up to 1000 fold

following severe inflammation (58). This massive increase in concentration is due to increases in synthesis of two gene products, SAA1 and SAA2, in response to the stimulatory activity of inflammatory cytokines. A normal healthy individual has low levels of SAA and this includes a high proportion of cSAA or constitutively synthesized SAA, which is coded for by a third member of the SAA gene family (59). There is a pseudogene located in the same area of chromosome 11p15.1. These forms of SAA are also expressed in extrahepatic sites, but these sites contribute little to the plasma concentration of SAA and there is site-specific differential expression of the isotypes compared to the liver (60). It has been clear for a long time that the requirements for AA amyloid formation are a high rate of synthesis of SAA as well as a second factor that promotes at least one of the stages between SAA and the fibrils of AA amyloid. A particular isoform in the mouse (SAA2) is preferentially deposited into fibrils but there is no similar preference in human AA amyloid (61). Although the human AA fibril protein is largely derived from SAA1, this may reflect the fact that SAA1 is present in higher concentrations than SAA2. In PNG there is a high frequency of expression of SAA2 isoforms (62) but this appears not to be associated with any predisposition to AA amyloidosis. Both SAA1 and SAA2 have been shown to be incorporated into AA amyloid fibrils. In familial mediterranean fever (FMF) neither SAA nor SAP appears to be linked to disease. Five alleles of SAA1 and three of SAA2 have been described but again no distinct association with amyloidogenesis demonstrated (63).

The most important cytokines inducing SAA synthesis are interleukin-1 and interleukin-6. Although interleukin-6 is regarded as the major cytokine that stimulates the acute phase response (64,65) there is considerable evidence that interleukin-1 might be a major inducing cytokine for SAA (66,67). In this instance, interleukin-1 receptor antagonist (IL-1RA, the third member of the interleukin-1 gene family) would be a major inhibitor of synthesis of SAA and it is the balance between cytokines, their soluble receptors and their antagonists that is the important factor in SAA synthesis. The regulatory regions for SAA genes reveal sites for induction by NF-kappaB and NF-IL6 and

these factors have been shown to act synergistically (68). Other cytokines that have minor stimulatory importance may be TNF and the IL-6 family members oncostatin M and leukaemia inhibitory factor (LIF).

In PNG many infectious diseases, such as malaria, tuberculosis and chronic respiratory tract disease, cause recurrent acute inflammatory episodes that induce the acute phase response. It is possible that the high incidence of AA-amyloidosis in PNG may result not from variants of fibril-forming proteins but from variation in inflammatory cytokine synthesis. TNF is a major inflammatory cytokine which, although not a strong inducer of SAA directly, is important in the synthesis of IL-1 and IL-6. It is known that inflammatory cytokines are beneficial in low amounts but under some conditions can be responsible for increased inflammatory disease or even death. Thus the TNF2 α polymorphism has been shown to be associated with autoimmune disease and more recently with an 8-fold increase in death from cerebral malaria (69). In the light of these recent discoveries of polymorphisms in cytokine genes that give rise to an increased disease severity or incidence, it is possible that such a polymorphism may occur in populations in which there is a high incidence of AA amyloidosis such as in the Watut Valley.

Another form of amyloid, the A β amyloid in Alzheimer's disease, is also strongly influenced by inflammation. The amyloid precursor protein (APP or BPP) is induced in response to IL-1 and IL-6 and the inflammation resulting from injury to the brain is capable of inducing amyloid plaque formation within a period of a few days – strong evidence that reinforced other observations (reviewed in 70). There is inflammation in affected brains, shown by using immunohistochemical methods that demonstrate a variety of inflammatory mediators and markers (71). Considerable debate now focuses on whether the inflammation is a consequence of toxicity of the amyloid peptide or amyloid plaque or whether the inflammation initiates and promotes the process. In prion type amyloid there is no clear role for inflammation in generation of the amyloid but it has been suggested that, once the amyloid seeding has

begun, inflammatory processes are induced, which may further accelerate disease progression.

Inflammation may also have a role in the localization and mechanisms of deposition of amyloid. Heparan sulphate has been implicated in amyloidogenesis because it is increased in expression before amyloid deposition (27) and binds to a variety of amyloid fibril components. Inflammation has been shown to increase both the rate of synthesis of heparan sulphate and its secretion (72,73). Other components of fibrils and amyloid-associated proteins may show altered expression during inflammation including various protease inhibitors.

Serum amyloid P component has been shown to be a sex-linked acute-phase protein in the Syrian hamster (74), in which species it may have a pro-amyloid function, but in humans it is not an impressive acute-phase protein. SAP concentrations do, however, show a modest increase in certain chronic disease states and the mean SAP concentrations in the Watut Valley populations were higher than normal, particularly after an inflammatory stimulus in those who had not been on malarial chemoprophylaxis. These individuals had higher indices of chronic malaria, including splenomegaly and elevated IgM concentrations. Since patients with macroglobulinaemia have been previously reported to have elevated SAP levels (75), the high serum IgM in the Watut group may be binding SAP after its enhanced acute-phase synthesis. Alternatively, the malarial polyclonally activated IgM contains germline-encoded IgM molecules (76) which resemble SAP in binding specificities (77) and compete for SAP binding sites, giving rise to higher serum levels of SAP. Whether SAP can act as a nidus for amyloid fibril formation, or can decrease fibril proteolysis, or is merely a bystander caught by a calcium-dependent affinity for amyloid fibrils, remains a highly controversial issue.

Conclusions

Amyloidosis remains the collective name for an increasing number of different conditions which are characterized by the pathological arrangement of fibrillar proteins in deposits of amyloid which stain characteristically with

cotton dyes, such as Congo Red, exhibiting green birefringence when viewed under polarization microscopy. There are at least 15 different proteins which have been identified as amyloid fibril proteins. There are also various bystander proteins, including AP and HSPG, which may be critical to define tissue tropism of amyloid and may be important for initiation or persistence of the insoluble amyloid fibrils.

In PNG there seems to be an unusually high prevalence of AA amyloidosis which complicates the clinical course of patients who have a variety of different predisposing inflammatory diseases. Amyloidosis in PNG can occur in families, and some kindreds have affected members in each generation. This suggests an autosomal dominant inheritance with variable penetrance but heterozygosity is so limited in PNG that one cannot rule out the possibility of a recessive trait for susceptibility to amyloidosis, expressed in those who are homozygous.

The principles of management of secondary amyloidosis are simple: prevent or treat the underlying causative disease, reduce the severity of inflammation and, on the basis of experimental studies in animals and humans in whom there is minimal toxicity, use colchicine to prevent amyloid fibril formation. There are several reports of reversal of secondary amyloid in patients whose predisposing disease has been controlled. There is thus a great advantage in making an early diagnosis before organ damage is too great. The diagnostic technique of abdominal wall biopsy is simple, without complication and easily used in field situations. In the future it would certainly be an advantage to screen high-risk families, kindreds and clans for a marker which might indicate susceptibility to amyloid so that individuals at risk can be identified, advised and treated appropriately, before it is too late to help them.

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