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Commentary

The Pathogenesis of Amyloidosis

Understanding General Principles

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Until the beginning of the 1970s, the deposition of amyloid was a puzzling phenomenon. Amyloid was known to occur as life-threatening diseases of highly variable clinical manifestations or as deposits localized to one tissue or organ. When it was understood that the amyloid fibril is the main component of amyloid[1] and when methods to extract these fibrils were established, it was also possible to apply protein chemical methods to purify protein components from the fibrils and analyze them. With this approach, Benditt and Eriksen[2] showed that the amyloid substances differ in protein composition according to clinical type, and they were able to demonstrate that in secondary systemic amyloidosis, a specific protein is present that is not found in primary amyloid material. N-terminal amino acid sequence analysis of this protein, termed protein A (later protein AA) revealed a previously unknown protein.[3] Benditt and Eriksen also postulated that several different amyloid fibril proteins may occur. At about the same time, Glenner et al[4] showed, again by establishing protein chemical methods, that the amyloid fibril in primary amyloidosis consists of N-terminal fragments of monoclonal immunoglobulin light chains. Since that time, many additional amyloid fibril proteins, characteristic of specific amyloid diseases, have been characterized by amino acid sequence analysis. To date, at least 18 different human amyloid fibril proteins are known and of these, 10 occur in systemic forms of amyloid and 8 only in localized types of amyloid.[5] Among these latter is the main component in Alzheimer's plaques and vascular amyloid, the Aβ protein.[6,7]

Consequently, amyloid is a biochemically, very heterogeneous material in spite of its similarities in properties and staining characteristics. Obviously the similarities do not reside in the composition of the main component. Instead, amyloidosis is today regarded as a group of "protein folding diseases"[8,9] in which it is in the three-dimensional arrangement of the composite protein molecules that most properties of amyloid arise. The amyloid is characterized by cross β-pleated sheet fibrils, and this unifying structure, proposed by Glenner and coworkers,[10] holds true for all types of amyloid. The unique β-sheet fibril is very resistant to physical agents and also gives the amyloid substance many of its characteristic properties, including affinity to the dye Congo red and green birefringence after such staining. Fibrils with these characteristics can easily be created *in vitro* from amyloid proteins or short peptide segments thereof, proving that no other components are necessary for these properties. Such fibrils have affinity for Congo red and exhibit green birefringence.

The exact mechanism that converts proteins into amyloid fibrils *in vivo* are not known. The proteins in amyloid fibrils are sometimes mutant as in some familial amyloidosis but are more often of wild-type. Many of the proteins such as transthyretin and immunoglobulin light chains have normally a high degree of β-sheet structure. An α-helix to β-sheet transition may occur in the pathogenesis of some amyloids. Concerning transthyretin-amyloidosis, it has been suggested that amyloid fibrils are formed from near-native molecule forms on the folding-unfolding pathway.[8,11] Because many of the amyloid fibril proteins are truncated at a rather regular fashion either N-terminally or C-terminally, it is possible that a proteolytic step is important in many forms of amyloidosis.

Although the protein fibril is the main component of the amyloid substance, there are other components present as well in which importance in the amyloid pathogenesis is yet not well established. Thus, all forms of amyloid contain the pentraxin glycoprotein amyloid P-component (AP) that most probably is bound to the protein fibrils directly. AP may be important in the pathogenesis of AA-amyloidosis,[12] perhaps by increasing the resistance of the fibril to proteolysis. Glycosaminoglycans, mainly in proteoglycan form, are ubiquitous ingredients of the amyloid substance. Basement membrane heparan sulphate proteoglycan may be most important and may promote protein aggregation into amyloid fibrils.[13] Also other basement membrane components may be present in amyloid and of importance in amyloidogenesis.

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The additional components of amyloid include several apolipoproteins, notably apolipoprotein E (apoE), apoJ, and apoA-I.[14] One in particular, apoE, has attracted much interest. ApoE is a constituent of certain plasma lipoprotein fractions and is important in the transport of cholesterol and triglycerides. It is synthesized mainly in the liver and the brain. By immunohistochemistry and immune electron microscopy, apoE was found to be present in amyloid plaques in Alzheimer's disease.[15] ApoE exists in three closely related allelic forms, apoE2, apoE3, and apoE4, and it was found that compared with the other forms, apoE4 occurs significantly more often in individuals with late onset sporadic or familial Alzheimer's disease,[16,17] and apoE4 is now regarded as a risk factor for Alzheimer's disease. It has been shown that Alzheimer patients with apoE4 alleles have a larger Aβ load compared with individuals without apoE4. Presence of apoE4, particularly at homozygocity is also significantly correlated to the occurrence and the degree of cerebral amyloid angiopathy.[18]

The mechanistic role of apoE4 in Aβ-amyloidogenesis and cell destruction is not known. ApoE binds to Aβ[17,19] to form extremely resistant complexes.[20] Most probably, these apoE-Aβ complexes contain truncated apoE molecules consisting of C-terminal fragments.[20] However, apoE4 is a less efficient Aβ binding molecule compared with the other apoE isoforms, and an impaired clearance of Aβ in individuals with apoE4 may be important in the amyloidogenesis.[21] Furthermore, apoE4 but not apoE3 is neurotoxic *in vitro*.[22] ApoE and particularly apoE4 enhances fibril formation *in vitro*[23] and has been proposed to act as a "pathological chaperone" by direct effects in the amyloid fibrillogenesis by induction of β-pleated sheet conformation.[15] This *in vitro* effect is seen not only with Aβ but also in other amyloid fibril proteins including protein AA.[24] This is a mechanism similar to that proposed for the tentative protein X in prion-associated diseases.[25]

Given the importance of apoE4 as a risk factor in Alzheimer's disease and the constant finding of apoE complexed with Aβ protein in amyloid plaques and in vascular amyloid, there is no wonder that the possible role of apoE in the pathogenesis of other types of amyloidosis is being explored. There are several reports that apoE is present in a variety of biochemically different amyloids,[26–28] including AA- (secondary) amyloidosis. Protein AA is an N-terminal fragment of about 50% larger precursor, serum AA, which is an acute phase apolipoprotein of high density lipoprotein (HDL) and of putative function in cholesterol metabolism at acute inflammation.[29] However, apoE4 genotype is no risk factor for AA-amyloidosis.[30,31] Similar to the situation in Aβ amyloid, apoE forms soluble complexes with protein AA.[26] AA-amyloidosis is the only type of systemic amyloidosis for which there exists a readily available mouse model. AA-amyloidosis is easily induced in many strains of mouse by a persistent inflammatory challenge. This model is therefore particularly suitable for testing of modifying mechanisms in amyloid fibrillogenesis.

In this issue, Kindy et al[32] using apoE null mice show quite convincingly that apoE is important for AA-amyloid formation. Kindy et al showed earlier that murine AA-amyloid deposits like human AA-amyloid is associated with apoE.[33] In the study by Kindy et al, two protocols for amyloid induction were used, one rapid with silver nitrate subcutaneously combined with an intravenous injection of amyloid enhancing factor (AEF) and another more slow with casein subcutaneously. The earliest studied time point was after 5 days. They found that apoE deficient mice can develop AA-amyloidosis but do so at a reduced rate. Both apoE knockout and wild-type animals increased their amyloid load during the observation time but the amount of amyloid in apoE deficient mice never reached that of wild-type animals. Most importantly, reconstitution of apoE production in the knockout animals by introduction of adenovirus expression of human apoE3 gene restored the degree amyloid formation to be comparable with that of wild-type mice. In recent studies two other research groups, using the same strains of animals from the same breeder as the study by Kindy et al, reported that experimental AA-amyloidosis readily was induced in apoE deficient mice.[34,35] In the first study, AA-amyloidosis was induced by an intravenous injection of AEF combined with a subcutaneous injection of silver nitrate, and the occurrence of amyloid was estimated after 72 hours. In the second study, amyloidosis was induced by AEF intraperitoneally and silver nitrate subcutaneously and the effects studied at 48 and 72 hours. Another induction modality was with complete Freund's adjuvant and *Mycobacterium butyricum* intraperitoneally and the effects were studied after two weeks. In all of these experiments, AA-amyloidosis developed in apoE-deficient as well as wild-type mice and there was no definite quantitative difference between the groups.

So, here we have three basically similar studies but with somewhat contradictory results. It is difficult to point out which differences in the studies have influenced to give these somewhat divergent results. The most obvious question is the quality of the AEF that was used in order to speed up the induction of amyloidosis. AEF is a poorly defined cell-free extract of amyloid that when given at induction of experimental AA-amyloidosis most remarkably shortens the time for the first amyloid deposits to occur.[36] Recent experiments indicate that presence of amyloid fibril proteins in fibrillar conformation may act as nidi similar to what is implicated for prion-diseases.[37] It is possible that different extraction procedures give rise to AEF preparations with different activities and that a very efficient preparation may override a partial resistance to amyloid induction by the lack of apoE. In addition to this putative variation, there is the possibility that some unknown environmental factors may play a role; development of another kind of amyloid (islet amyloid polypeptide-derived amyloid in the islets of Langerhans) developed only after that transgenic mice expressing human islet amyloid polypeptide were involuntarily put on a somewhat more fat-containing diet.[38] Finding these unknown factors may be of great importance as it may uncover factors that are mechanistic in the development of human AA-amyloidosis. That may help us to understand which individuals are in the risk zone to develop AA-amyloidosis.

Much of the amyloid research over the last few decades have been focused on the great biochemical variability in amyloid diseases. Several recent studies, including the study by Kindy et al,[32] show that although this variability exists, there may be certain uniform factors in the development of amyloid fibrils. These factors include apoE, heparan sulfate proteoglycan, and AP. It is conceivable that uncovering the additional participation of these factors in amyloidogenesis may point towards possible general therapeutic principles and may provide hope for the future for patients with different amyloid diseases.

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