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CURRENT CONCEPTS OF THE STRUCTURE AND PATHOGENESIS OF THE AMYLOID DISEASES

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Amyloidosis is a generic name for a heterogeneous group of diseases of man and a variety of animal species marked by the deposition of characteristic fibrillar extracellular deposits that ultimately impair the function of the involved organs. Though the incidence of the well characterized forms of amyloidosis is not great, study of the amyloid diseases has attracted a great deal of attention in recent years because of the complexity of the mechanisms involved in their pathogenesis [5, 6, 17].

A better understanding of the amyloid diseases requires firstly a clearer knowledge of the proteins that constitute the fibrils and secondly, a delineation of the pathogenetic mechanisms involved in their deposition. As regards the former, a great deal is now known about the common types of amyloid in which the protein subunits of the fibrils have been well characterized. Unfortunately, little knowledge is available concerning the rarer or localized types. Table 1 summarizes the nature of the major amyloid subunits of the common types and lists also their probable precursors. It is apparent that many questions remain to be resolved (tab. 1).

In addition to the major protein constituents of the fibrils, all amyloid deposits contain small amounts of the P component, a molecule structurally and perhaps functionally closely related to C-reactive protein (CRP), which in man and mice has some features of an acute phase reactant. So far, only the first 24 residues of the tissue P component and the related serum component have been reported and the sequence as well as the subunit composition and tertiary structure indicate a close relationship to the CRP family of molecules [13, 15, 16, 19].

Though our knowledge of the chemical nature of the amyloid substances is rather limited, it appears enormous compared to our understanding of pathogenesis which is exceedingly complex for a number of reasons. Firstly, since amyloidosis is

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type	major component	precursor	current knowledge
primary and myeloma	'L' chain fragment [7]	L chain	well characterized
secondary	AA [2, 12]	SAA	well characterized
endocrine			
medullary thyroid carcinoma	thyrocalcitonin [21] precursor	?	little
pancreas	?	?	?
cutaneous	?	?	?
senile < CNS	?	?	?
senile < cardiac	ASC [22]	?	little
familial			
FMF	AA	SAA	well characterized
Portuguese	? prealbumin [3]	?	poorly characterized

Tab. 1 - Correlation of the major types of amyloidosis with their protein subunits and precursor proteins.

a generic term encompassing a variety of different disorders, perhaps different types of amyloid are caused by different factors, a possibility that appears quite likely. Secondly, the common secondary (AA) type of amyloid which occurs in most experimental animals can be induced and influenced by an infinite number of often mutually contradictory factors, ranging from aggression and fighting to imbalance of hormone production; from immunodeficiency states to conditions marked by excessive antibody formation. Thus, with a plethora of factors influencing the induction of amyloidosis, it is difficult to define a simple common pathogenetic mechanism. Thirdly, most types of amyloid, with the exception of the AA type, are rare and difficult to induce experimentally. Because of these complexities, it is not surprising that many theories of pathogenesis have come into and often gone out of vogue. Nevertheless, several features appear common to all types. Firstly, there appears to be excessive production, locally or systemically, of a protein or protein precursor destined to be deposited as fibrils. Secondly, of those individuals exposed to a given stimulus only a fraction (rarely more than 20%) develop the disease. Thirdly, in the two well studied types, i.e. AA and L chain related amyloidosis and perhaps in the others as well, there appears to be produced a large precursor which is degraded or processed to give the amyloid subunit. Though this appears to occur at sites distant from the place of synthesis, it cannot be excluded with certainty that both in the L chain and AA related types processing may occur at or near the site of production. Consequently, it seems likely that in the localized forms, processing of a locally produced precursor characteristic of a particular tissue (skin, pancreas, etc.) may occur in or near the cells involved in synthesis and result in the formation of localized deposits [5].

1. *Structural studies*

During the past six years, largely through the work of Glenner, it has been recognized that primary and myeloma amyloid consists of L chain fragments or

entire L chains [7]. Concurrently, we [12] and others [2, 20] have worked out the structure of the AA protein from human as well as a host of experimental secondary amyloids and know most of the sequence of the 12,000 dalton SAA precursor [1, 18]. As shown in tab. 1, the information on the rarer or localized forms is sketchy or completely absent.

2. *Pathogenesis*

The complexity of the pathogenesis of amyloidosis is indicated by the abundance of often contradictory data extant in the literature. Boiled down to its simplest form, in those types that have been studied in some detail, amyloid development is associated invariably with the excessive production of the precursor protein. This has been amply demonstrated in L chain and AA amyloid, appears probable in the endocrine types, and may be applicable to all forms of the disease. Yet overproduction alone appears not to be sufficient, since in general only a fraction (usually less than 20 %) of those at risk appears to develop the disease. In the case of L chain related amyloid, due to the enormous structural variability of the variable regions of the L chains and the known predominance of λ related proteins, one could consider the possibility that there are structural differences which make some proteins 'amyloidogenic' and others not [9]. In the case of AA related amyloid, though minor structural differences have been noted between different AA proteins, and also between SAA and AA deposits in mice [8], these differences are so subtle that it seems less likely that they are responsible for differences in amyloidogenesis. Consequently, it seems more probable that differences in processing may predispose some individuals to form amyloid fibrils and protect others in the face of an appropriate stimulus. Such a difference in degradative patterns may be inborn, or develop as a result of the underlying disease or perhaps accompany the aging process.

Based on some of our recent studies, it seems possible in the AA type of amyloidosis in man and inbred strains of mice said to vary in their susceptibility to develop amyloidosis, that differences in the processing of SAA and AA proteins by peripheral blood monocytes and perhaps other cells may play a role in the development and susceptibility to amyloidosis [10, 11, 23]. In tissue culture system with human peripheral blood monocytes, we have demonstrated that these cells bear on their surface a number of proteases which degrade SAA in two readily distinguishable patterns. One of these results in complete degradation of SAA without the appearance of any identifiable intermediate. This pattern was observed in 15/30 normal subjects but in none of 13 patients with amyloidosis. When tested, cells of such individuals degraded AA protein as well [10].

The second pattern of degradation yielded an intermediate product similar to AA in size and immunologic properties. In some donors this intermediate persisted, while in others it was further degraded after more prolonged incubation. Individuals with a persistent AA intermediate failed to degrade AA protein whereas those with a transient intermediate did. This pattern of degradation was seen in 1/2 of normal subjects and in each of 10 patients with amyloidosis.

Evidence that the enzymes reside on the surface of the cells and that degradation does not require interiorization or release of enzyme is provided by the finding that the supernatants were not active enzymatically, that an intermediate digestion product was found outside the cell (a phenomenon not seen following

intracellular degradation) and by the observation that degradation was not abolished by a variety of maneuvers which inhibited interiorization including fixation with 0.1% glutaraldehyde [10].

More recently, by a functional enzymatic assay of fractionated cells and by surface labelling with H_3DFP and H_3 Ac-Ala-Ala-Pro-Val-CH_2Cl, we have identified a series of elastase-like enzymes ranging in molecular weight from 25×10^3 to 68×10^3. Most of these degraded SAA to an AA intermediate. However, enzyme species ranging from 40 to 58×10^3 daltons degraded AA and SAA completely [11, 23].

While it would be attractive to postulate a single processing phenomenon applicable to all types of amyloidosis, this hope was soon shattered when we studied the effect of monocytes on Bence Jones proteins from patients with and without amyloidosis. The effect of most cells on these Bence Jones proteins was insignificant; in fact, it was striking to observe the selective degradation of SAA and the minimal effect on Bence Jones proteins, RNase, HSA and IgG [14]. In contrast, lysosomes from human kidneys were very effective in degrading all Bence Jones proteins [4, 14]. Therefore, if proteolytic processing plays a role in amyloid formation, it must involve different proteases in different organs in the various types of amyloidosis; presumably processing takes place by cells and proteases in close proximity to the sites of deposition and appears to be distinctive for the different forms of the disease.

Thus, if one were permitted to speculate, it seems not unlikely that in some of the localized forms of amyloid, as for example the pancreatic and cutaneous types, the disease occurs in individuals who produce too much, or perhaps an abnormal form of a protein unique to that organ (perhaps insulin in the case of the pancreas, or certain cutaneous proteins) and whose cells can process it to a fibrillar form. It is also conceivable that the processing error is the basic problem and that certain individuals with faulty processing might develop the disease even without overproduction of the precursor. Also, it should be obvious that the approach is still in its infancy and that tissue macrophages, various components of the RES and other tissue deserve study.

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