A COMPARATIVE APPROACH TO SECONDARY AMYLOIDOSIS

MINIREVIEW

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ABSTRACT   In various species including phylogenetically lower animals, the
pathological protein amyloid has been found. The characteristics of
amyloid depend upon the β pleated structure of its fibrils. Various
groups of amyloid can be distinguished based on the chemical proper-
ties of the fibrils which reveal a different major protein in each
group: secondary amyloid, amyloid of immunoglobulin origin, APUD-
amyloid, senile cardiac amyloid, and amyloid-like substances.
In secondary amyloid, the major protein is protein AA which is
related to the serum protein SAA. There is a relationship between
induced secondary amyloid and the immune system, especially impair-
ment of T cells and dysfunction of macrophages. The formation of
protein AA containing amyloid will depend upon prolonged elevation of
SAA levels and the extrusion of lysosomal enzymes from RE cells.
The physiological relationship between SAA and the immune system,
which it depresses, is unclear.

INTRODUCTION

Amyloidosis is a well known pathological change in vertebrates,
characterized by extracellular deposits of variable amounts of a histo-
logically homogeneous and biologically inert, proteinaceous material. As a
consequence of these deposits, cell function is compromised or fails and
clinical disease results. The name "amyloid" was given to the material,
after Virchow (113) described its chemical composition as cellulose-like
and comparable to the amyloid encountered in botany (4). Amyloid is com-
posed of rigid nonbranching fibrils (the amyloid fibrils), which are
randomly arranged and occur in extracellular groundsubstances. Amyloid
fibrils may also occur as parallel strands. The fibrils are of undetermined
length and have a diameter of about 75 - 150 Å (Table I).

TABLE I

The diameter of purified and negatively stained amyloid fibrils.

species	diameter in Å	references
man	40 - 80	38
	70 - 100	21
	80	42
cattle	129-157	42
hamster	146	42

The fibrils are composed of two intertwined filaments (45,78). Despite a variable chemical composition in different amyloid groups, the fibrils have a similar molecular structure when examined by X-ray diffraction and infrared spectroscopy: the β pleated sheet structure (38).

The reason for presenting this review of amyloid to comparative immunologists is the link between amyloid and the immune system in the two major amyloid groups: secondary amyloid and amyloid of immunoglobulin origin. A comparative study in phylogenetically diverse animals, especially of secondary amyloid, can reveal unknown mechanisms which are basic to its pathogenesis.

CHARACTERISTICS

Characteristics of amyloid, such as congophilia with typical green birefringence in polarized light and its resistance to proteolysis (38) and autolysis (31), result from the β pleated molecular structure. In this arrangement, the (poly)peptide layers are more closely (4.75 Å) linked to each other by hydrogen bonds, than in the α helix or triple helix configuration of other fibrillar proteins. Neutral and acidic mucopolysaccharides occur between the amyloid fibrils and are adsorbed to them (119). Moreover, substances from connective tissue e.g. collagen and basement membrane materials, lipids and serum proteins (e.g. fibrinogen., immunoglobulin proteins, complement factors) occur in varying quantities (67). For human amyloid, many studies have been performed to elucidate the role of a 95 Å pentagonal structure, the plasma (P) component or protein AP (73,102), extractable from amyloid-containing tissues. This protein is identical to the protein $C_1 t$ (84,60,69), thought to be a part of the complement system, although there is still controversy (81). Its Ca-dependent affinity for polyanions might clarify its presence in amyloid (83).

When subjected to various histochemical procedures, most staining methods reveal variable results. Only reactions like Congo red for the β pleated fibrillar proteins will be specific. Wolman's standardized toluidine blue method (with red birefringence) will also react with these fibrillar proteins (22). Iodine binding appears to form a complex with a component in a pepsin-labile glycoprotein of the interfilamentous matrix of amyloid fibrils (22). Other widely used staining methods e.g. thioflavin with fluorescence and toluidine blue with metachromasia and yellow birefringence, react for acidic mucopolysaccharides and are less specific (119, 107,108,22). To distinguish between protein AA-containing and non-containing amyloid (see below), both staining methods of Wright et al (120) using primary oxydation with potassium permanganate prior to the Congo red stain are recommended for several species (40).

CLASSIFICATION

Amyloid can be classified into various groups, which in most instances depend upon clinical parameters or its deposition pattern. The basic division is: I. Secondary amyloid, accompanies disease such as chronic infections or rheumatic disease, and can be induced in laboratory mammals by repeated injections of various substances e.g. casein or endotoxin; it shows a "typical" distribution pattern (kidney, liver, spleen); II. Primary amyloid, is not associated in most cases with a primary disease and is characterized by an "atypical" distribution pattern (tongue, heart, cerebral vessels).

After purification of amyloid fibrils, best performed according the method of Pras et al (86), four groups have been found each of which contains a different major protein component. The protein of each group has its own precursor. a. In secondary amyloid, also called amyloid of unknown origin (36) the major protein, protein AA, is a unique component showing no homology with any known immunoglobulin nor with any protein so far identified. It is similar in different patients and is homologous in various species (man, monkey, mink, mouse, guinea pig, duck (102,49,32,33,10) rabbit (2) and cattle (40)). Human and monkey protein AA are most nearly identical and consist of 76 amino acid residues and have a molecular weight of about 8500 (68,48). Murine protein AA (103) and our bovine protein AA (40) which have a molecular weight of about 10,000, after hydrolysis, showed an amino acid analysis (92,40) similar to human protein AA and that of other species. Protein AA shows immunological cross reactivity with a serum α globulin, SAA, which is associated with high density lipoprotein (10) and whose serum levels fluctuate in a manner analogous to acute-phase reactants (90,74,75,1,100). SAA has a molecular weight of 100,000 - 200,000 Daltons. Under acidic conditions a low molecular weight protein of 12,500 - 15,000 Daltons can be obtained from SAA, the SAAL (51,104). SAAL has a NH_2-terminal amino acid sequence identical with that of protein AA (100,101,91) and is believed to be the precursor of protein AA. Other constituents of amyloid fibrils not containing protein AA will be components derived from normal tissue (50).

b. A large proportion of the "primary" amyloid group and the amyloid of myeloma patients is derived from immunoglobulin proteins and is called amyloid of immunoglobulin origin (36). From the amyloid fibrils in these cases, a major protein has been isolated which differed in each patient and was immunologically related to the paraprotein of the same patient. Moreover chemical homology has been found (amino acid composition, NH_2-terminal sequence studies) with light chain proteins (κ and λ) from the monoclonal paraprotein, especially its variable part (38). The immunoglobulin origin of these fibrils was favoured since the amyloid-like β pleated fibrils were created in vitro in several laboratories by mild proteolytic digestion of Bence-Jones proteins (38). In vivo formation of fibrils will occur by lysosomal cleavage of the paraprotein in RE-cells (36,64), but lysosomes may also form in plasma cells of myeloma patients (66).

c. APUD-amyloid. In some cases of the primary amyloid group localized amyloid deposits occur in endocrine glands of the APUD-series (82) and tumours of these glands. Studies on insular pancreatic amyloid and amyloid in medullary thyroid carcinomas indicate that this type of amyloid originates from polypeptide hormone-related proteins of these glands (82, 116,23,109). Using acidification or proteolytic enzymes, it has been possible to create amyloid-like β pleated fibrils from insulin, glucagon and other APUD-hormones (37,72,62). Differing from other types of amyloid, in the APUD-amyloid, tryptophan is lacking (82,23,40) and tyrosine is present in only small quantities (115). By means of electron microscopy the fibrils

may be taller than in other types of amyloid (117).

    d. <u>Senile amyloid</u>. Most cases of senile amyloid which occur as primary amyloid during old age, are categorized in one of the former three groups. Senile cerebral amyloid in LM- and EM-immunochemical studies contains immunoglobulins near the area of amyloid fibrils (53,54). This type of amyloid has much in common histochemically with APUD-amyloid (85). From senile cardiac amyloid using isolated amyloid fibrils a unique protein: protein A_{sca} has been found (118).

    e. In addition to these four groups of amyloid, amyloid-like or related fibrils can be found in the "calcifying odontogenic tumour" (80,15,35) and in the corpora amylacea of several epithelial organs (but not in the corpora amylacea of the brain, 106,96). Human prostatic corpora amylacea (46) and corpora amylacea of the bovine mammary gland (87) like those of other species (rat,9) are well known examples. Whether these proteins are categorized in one of the former groups, still must be determined. In the mammary corpora amylacea, positive findings for tryptophan (9) excludes the APUD-group.

THE RELATIONSHIP OF INDUCED SECONDARY AMYLOIDOSIS TO THE IMMUNE SYSTEM

    Several groups of investigators have made comprehensive studies of immunological phenomena associated with induced amyloidosis, mostly using mice and guinea pigs, not rats, since in this species amyloidosis is rare and unreproducable (39); in most cases splenic amyloidosis has been studied. Regarding <u>humoral immunity</u> (B cells), between amyloid deposits and the localization of plasma cells, no relation has been found (47). Amyloidosis occurs in human patients and in mice with hypogammaglobulinaemia (3). Antibody production against various antigens is unchanged (20,6), but increase of B cell activity has been found in studies of antibody dependent cytotoxity (121). In <u>cell-mediated immunity</u> (T cells), there is a correlation between impaired T cell function and amyloid (19). In nude mice and thymectomized ones, amyloid can be induced by injecting casein (47). During the first phase after beginning casein injections, T cells increase in the spleen (the first or pyroninophilic phase of Teilum, 110). During the predeposit phase and the deposit phase of amyloid, the number of pyroninophilic cells decreases (47). Daily injections of thymosin restored T cell function and reduced the incidence of casein-induced amyloidosis (93), suggesting thymic involvement. In casein induced amyloidosis, mice and guinea pigs became tolerant for casein (14,16,17,18) and BCG-injections exert a protective influence against amyloidosis (122,47), further evidence associating it with cell-mediated immunity.

    Regarding <u>macrophages</u>, during amyloid induction, after the pyroninophilic phase, a second phase, the predeposit phase is characterized by an increase of PAS-positive macrophages in perifollicular areas of the spleen (110). During this phase, carbon clearance (47) and mitogen induced cellular cytotoxicity was elevated (121). At the onset of amyloid deposition, the macrophage activity decreased (47, 121). In the predeposit phase in mice, the first amyloid occurred in macrophages, and if spleens were explanted <u>in vitro</u>, there was an increase of intracellular amyloid masses. Moreover, in the supernatant, amyloid-related low molecular weight proteins were observed (6,7,8). The formation of amyloid fibrils in lysosomes of macrophages by cleavage of precursors has been proposed (36). In this context the finding of parallel aligned intralysosomal amyloid fibrils in macrophages in induced murine amyloidosis was explained as formation (99).

Phagocytosis should result in a random pattern of intravacuolar amyloid fibrils (98,52). In induced phagocytosis of amyloid, however, in an autogenous in vivo model using murine amyloid (111) and in xenogenous models with human (76) and bovine amyloid (44), vacuoles were found containing parallel fibrils. Moreover, in induced amyloidosis of hamster liver, during the predeposit phase, no intracellular amyloid fibrils occurred (41). After enzyme-histochemical LM and EM analyses however, activity of lysosomal enzymes of sinusoidal lining cells (acid phosphatase, β glucuronidase, β glucosaminidase) has been found in extracellular deposits of amyloid (41). After recovery from the induction system, regression of the amyloid masses has resulted from phagocytosis (30,34). In some cases, glomerular amyloidosis of the kidney, however, should increase (30).

AMYLOID ENHANCING FACTOR (AEF), AMYLOID INDUCING FACTOR (AIF) AND SAA

Induced amyloidosis can be accelerated by injecting extracts from amyloid containing tissue, splenic cultures from mice with amyloid or their supernatants (63, 58,59). Splenic tissue of pretreated mice implanted in dialysis bags will also enhance amyloid induction (61). The enhancing influence of splenic cells from pretreated donors might depend upon the transfer of antigen-RNA complexes (AEF) (47). Moreover, injecting splenic cells from normal donors enhanced amyloidosis (5). Splenic suspensions and splenic nuclear fractions from pretreated donors induced amyloidosis in recipients without amyloid inducing treatment, especially after subsequent immunosuppressive treatment. In these cases, amyloid induction should result from an antigen-DNA complex (AIF) which influences protein synthesis by macrophages (47).

The significance and relation of substances AEF and AIF to SAA are still unknown. They may influence the production or release of SAA(L) and/or the release of lysosomal enzymes from RE-cells, which both are required for the formation of secondary amyloid. Because: a. elevation of SAA levels alone does not result in amyloidosis (12,51), b. substances from leucocytes stimulate amyloid formation (65), c. vascular permeability is increased prior to amyloid deposition (94,95,97), d. the major non-protein AA fraction of secondary amyloid fibrils contains normal tissue proteins (50) and e. the activity of lysosomal enzymes has been found in newly formed amyloid without the finding of intralysosomal amyloid prior to amyloidosis in our hamsters (41), the proposed pathogenesis of secondary amyloid involves extracellular conversion of SAA after leakage from lysosomal enzymes. Except for precursor of amyloid, SAA probably is a normal protein regulating immunereactivity, because it contains immunosuppressive potencies (11). References concerning the origin of SAA are controversial: formation in macrophages has been proposed (8), and antigenic cross reactivity with fibroblasts (70,71,79) and plasma cells (114) has been described. Moreover, the liver in which relatively large quantities of SAA are found (75) might be its origin, just as it is the site of origin of many other acute-phase reactants.

THE INCIDENCE OF AMYLOIDOSIS IN VARIOUS SPECIES

Spontaneous amyloidosis is a common finding in man and many other mammalian species. In some species (e.g. sheep, goat and rat) amyloid is rare (57), whilst in others, it has a high incidence (e.g. cattle, 43).

Since the review of Jakob (57) several incidental findings also concerning
other species, have been published. The second group in which amyloidosis
commonly occurs is birds, especially in the group of ducks, geese and swan,
Anatidae (88,89,26,27,29,55,56,77,105). In phylogenetically lower groups,
reports of amyloidosis are rare. In tortoises an "epizooty" has been des-
cribed (112) and in snakes it has been found (28). Moreover in the earth-
worm, *Lumbricus terrestris*, congophilic degeneration has been found after
prolonged captivity (24), and in the honey bee, *Apis mellifera*, amyloidosis
of the queens receptaculum seminis has been described (13).

   In domestic species, evident strain differences exist between the
incidence of spontaneous amyloidosis and the resistance to inducing agents.
In nearly all cases, the type of amyloid in the sense of classification,
given above, is unknown and many have been described as primary. The
finding of protein AA antigen in the different types of bovine renal
amyloidosis (40), may indicate that protein AA - (secondary) amyloid can
have a higher incidence than expected. Studies of canine cases with the
staining methods after Wright et. al. (120) showed that also in this
species secondary amyloid occurs most frequently (unpublished results).

CONCLUSION

   The characteristics of amyloid depend upon the β pleated structure of
the amyloid fibrils. In spite of this structure, the chemical composition
varies in different groups of amyloid: secondary amyloid containing
protein AA, amyloid of immunoglobulin origin, APUD-amyloid, senile cardiac
amyloid, and amyloid-like substances. In amyloid of immunoglobulin origin,
the relationship to the immune system is clear. In secondary amyloid, con-
taining protein AA, a relationship to the acute-phase reactant SAA does
exist, although the physiologic role of this protein and its relationship to
the immune system is still unclear. However, many investigations have shown
comprehensive changes in the immune system, especially of T cells and
macrophages. The formation of amyloid fibrils will depend upon: a. prolonged
elevation of SAA levels (the amyloidogenic protein) b. extrusion of
lysosomal enzymes. The finding of parallel intralysosomal amyloid fibrils
can be explained by phagocytosis. The pathogenesis of APUD-amyloid, senile
amyloid and amyloid-like substances, is less clear than the former groups.
Amyloidosis is a common pathological finding in mammals and birds and it
has been described in reptiles. Amyloid may also occur in the earthworm,
Lumbricus, and in certain insects. Since invertebrates and fishes,
amphibians and reptiles possess immune capabilities (25), a comparative
study of amyloidosis in phylogenetically lower animals may contribute to
elucidating the evolutionary role of the immune system in secondary
amyloidosis.

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