AMYLOID AND AMYLOIDOSIS OF THE SKIN

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Amyloidosis is widely distributed in the animal kingdom. The clinical features of this disorder are a consequence of the destruction and replacement of vital organs by the extracellular deposition of a proteinaceous substance known as amyloid. This material has been recognized by its homogeneous eosinophilic appearance when viewed by light microscopy, and by its staining properties with alkaline Congo red and certain metachromatic dyes. Hence, it is not surprising that recent biochemical and immunologic studies of amyloid substance have provided strong support for the clinical impression that amyloidosis may be a term that encompasses a variety of different disorders. Before discussing the nature of the cutaneous types of amyloidosis and to discuss some of the possible pathogenetic mechanisms.

Amyloidosis is relatively rare as a clinically significant disease. Nevertheless, because of its varied manifestations, its frequent association with a number of diseases involving the immune system, and the deposition of amyloid as part of the aging process, amyloidosis has attracted the interest of clinicians, chemists, immunologists, and pathologists since its recognition 125 years ago. Progress has been hampered by difficulties in isolating the amyloid substance and by the fact that an almost infinite number of apparently dissimilar stimuli can induce its production. Two major observations during the last 15 years have advanced our understanding of the amyloid substance and have given us significant insights into some factors that may play a role in the pathogenesis of the disease. The first was the discovery, through the use of the electron microscope, that the apparently homogeneous amyloid material consists of characteristic long fibrils. These have a diameter of 100 to 150 Å and are made up of two longitudinal subunits or filaments 40-60 Å in diameter separated by a clear space of 25 to 50 Å [1-4]. The second was the isolation of the fibrils in a state of high purity, thereby allowing sophisticated study of the constituent proteins [3,5], and the consequent demonstration of the existence of several different types of amyloid substance.

Since it appears likely that the different clinical forms of amyloidosis may ultimately be shown to be associated with characteristic types of amyloid fibrils, it seems appropriate to describe briefly the more important clinical forms of amyloidosis before discussing the amyloid fibrils.

CLASSIFICATION

The following major forms of amyloidosis are recognized: (1) primary amyloidosis and the type associated with plasma cell and lymphoid neoplasms, (2) secondary amyloidosis, (3) a variety of heredofamilial forms, (4) amyloidosis associated with aging, and (5) amyloidosis of certain endocrine organs. The diagnosis in all types can be suspected on the basis of the clinical and laboratory features but requires histologic documentation to be certain. In the event that an involved organ is not suitable for biopsy, a rectal or gingival biopsy is likely to yield positive results in over 90% of the patients [6,7]. For optimal results, the tissue must be carefully stained with alkaline Congo red or certain metachromatic dyes, and should be properly studied by polarization microscopy when Congo red is used. The diagnostic value of specific fluorescent antisera remains to be determined [8]. The quantitation of certain serum proteins related to amyloid (see below), while theoretically and potentially rewarding, has not proved useful due to a lack of specificity. It is generally possible to classify a particular patient on the basis of the presence or absence of other associated diseases and the tissue distribution of the amyloid deposits, together with careful immunochemical analyses of serum and urine. However, it is now widely appreciated that clinical manifestations and organ localization are often insufficient by themselves to achieve this goal in view of the frequent occurrence of deposits in atypical sites [9-11]. It seems likely that in time a precise classification will be based on the chemical nature of the amyloid proteins [12-14].

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Abbreviations:

AA: amyloid A protein
AL: amyloid L-chain protein
FMF: familial Mediterranean fever
SAA: serum A-related protein
Secondary Amyloidosis

The most common and perhaps the most “typical” form of amyloidosis is that which accompanies many chronic illnesses and is therefore called the “secondary form” of amyloidosis. In the past, suppurative conditions such as osteomyelitis, tuberculosis, and bronchiectasis headed the list of associated diseases; presently, this type of amyloidosis is more often encountered in patients with paraplegia and other chronic neurologic disorders, Hodgkin’s disease, leprosy, and rheumatoid arthritis. It is of interest that amyloidosis is rarely if ever encountered in systemic lupus erythematosus. In secondary amyloidosis, deposits are most prominent in the kidneys, spleen, liver, and adrenals, and only rarely involve the heart, gastrointestinal tract, or musculoskeletal system.

Primary Amyloidosis

Although known of for many years, the primary type of amyloidosis and the type associated with plasma cell and lymphocyte neoplasms are now being recognized with an increased frequency because of the wider use of tissue biopsies and the routine performance of immunochemical analyses of serum and urine. These two types of amyloidosis can be considered together on the basis of biochemical and clinical evidence. The amyloid substances deposited and the organ distribution in these forms appear to be identical [11-14]. In addition, it is now widely appreciated that an ever-increasing number of patients with primary amyloidosis, if carefully studied, are found to have homogeneous immunoglobulins in the serum and/or urine and ultimately develop morphologic and clinical evidence of a plasma cell dyscrasias [11].

It seems possible that such an evolution may take place in all individuals with the primary form of amyloidosis, if they are followed long enough. Generally this type of amyloid infiltrates predominantly the tongue, heart, skeletal muscle, skin ligaments, and gastrointestinal tract but it can also involve the same organs as the secondary type. Such patients often come to the attention of physicians because of the presence of a carpal tunnel syndrome, and occasionally because of the appearance of a neuropathy, periartricular thickening, or synovial infiltration. Some of the localized varieties of amyloidosis may well belong to the primary form.

Familial Forms

A variety of familial forms, many with characteristic geographic localizations and tissue distributions, have been described. The most prevalent of these appear to be the type associated with familial Mediterranean fever (FMF) and the Portuguese type. Their large number and clinical heterogeneity preclude a detailed description of these disorders [15].

Amyloid Associated with Aging

Although clinically often inapparent, small deposits with many of the features of amyloid can often be found, if carefully looked for, in the senile plaques of the brain and in tissues such as the aorta, pancreas, testes, and other endocrine organs. Their incidence increases with age, and it may be that these deposits will ultimately be shown to be an invariable accompaniment of aging. At this time, they appear to be the best single indicator of the aging process [16].

Amyloidosis of Endocrine Organs

For reasons that will become apparent later, the “amyloid” often found in certain endocrine glands in diseases such as diabetes mellitus and medullary carcinoma of the thyroid should be considered separately. These types of amyloidosis which are usually localized to a single gland are clinically asymptomatic and are discovered either at autopsy or on pathologic examination of a surgically removed endocrine tumor.

NATURE OF AMYLOID FIBRILS

More than 95% of the amyloid substance consists of the characteristic fibrils described above [1-4,17]. In addition, a minor component, the P-component, has been noted in most amyloid deposits [18,19]. In general, amyloid fibrils are composed of two major types of proteins which exist either singly or in combination. One of these, present as the major and often the sole constituent in amyloids of the primary and myeloma-associated types, consists of fragments of immunoglobulin light chains. This group of proteins is now called AL (amyloid L chain)* protein and is usually, but not always, associated with a similar L chain containing immunoglobulin light chains. This group of proteins is now called AL (amyloid L chain)* protein and is usually, but not always, associated with a similar L chain containing immunoglobulin light chains. This group of proteins is now called AL (amyloid L chain)* protein and is usually, but not always, associated with a similar L chain containing immunoglobulin light chains. This group of proteins is now called AL (amyloid L chain)* protein and is usually, but not always, associated with a similar L chain containing immunoglobulin light chains. This group of proteins is now called AL (amyloid L chain)* protein and is usually, but not always, associated with a similar L chain containing immunoglobulin light chains.
nigators were able to produce fibrils with the appearance of amyloid fibrils by proteolytic digestion of some, but not all, Bence Jones proteins, especially those belonging to the Vα subclass [22,23], and by their finding that antisera to these amyloid subunits cross-reacted with κ or ι light chains [24]. While there can be little doubt that these immunoglobulin-related proteins constitute most if not all of the protein in this type of amyloid fibril, it is not yet known whether the fragments are synthesized as such, or if they are the result of degradation of an intact L chain. Although the latter appears more likely, the former possibility can be excluded only by in vitro biosynthetic studies.

Amyloid fibrils from patients with the secondary type of amyloidosis and certain familial forms [13,14,25,26] as well as amyloid isolated from monkeys [27], ducks [27], and guinea pigs [18] with experimentally induced amyloidosis, consist primarily of another protein that is unrelated to any known immunoglobulin. This protein, known as the AA (amyloid A) protein, generally has a molecular weight of 8,500 daltons, although a fragment half this size has been isolated from the fibrils of one patient with rheumatoid arthritis [28]. Complete amino acid sequence studies of two such proteins from man [25,26] and one from the monkey [27], and partial amino acid sequence studies of several other proteins from man, duck, guinea pig and other species, have been reported. These studies have revealed striking similarities, but have also disclosed some differences, especially in the carboxy terminal half of different human proteins, and have clearly documented the expected homologies between the human and primate and subprimate molecules. It is of interest that the human protein is often heterogeneous at the amino terminus and that several additional residues precede the first residue in guinea pig amyloid, a finding which suggests that it, too, may be derived from a large precursor by proteolysis [13,18,25]. In addition to the AA protein, varying quantities of immunoglobulin L chain-related proteins, often polyclonal in nature, are also present in these amyloid fibrils [25].

Using antisera to this component, it has been possible to detect in serum an antigenically-related larger component known as SAA (serum A-related protein)* which has been purified and which appears to be the precursor of the tissue component [29–31]. Radioimmunoassay has permitted accurate quantitation of this serum component and has revealed a number of interesting and clinically useful features which can be summarized as follows [31]: (1) The concentration of SAA is very low (less than 200 ng/ml) and remains in that range during the first five decades of life but increases significantly in the older age groups, often reaching levels more than 10 times greater in the eighth and ninth decades. (2) The concentration of SAA increases in all types of amyloidosis, cancer, infections, rheumatoid arthritis, multiple myeloma, macroglobulinemia, lymphomas and a variety of other chronic illnesses, but less so in a small number of patients with systemic lupus erythematosus. As a consequence, the almost invariably increases noted in the sera of patients with amyloidosis cannot be used as a simple diagnostic test. (3) In acute infections, and in exacerbations of certain chronic diseases, the protein often behaves like an acute-phase reactant, quickly returning to normal levels as the disease is brought under control. (4) The concentration of SAA increases in pregnancy. In serum, the SAA protein exists as complexes with a molecular weight of 85,000 and about 170,000, apparently bound to serum albumin. After acid treatment, a fragment antigenically related to the AA protein with a molecular weight of 12,000 can be isolated by chromatography on Sephadex. Preliminary studies of this protein suggest that it shares part of its amino acid sequence with the AA protein [32–34], and that AA constitutes the aminoterminal portion of SAA from which it may be derived by proteolysis [33,35].

Lest readers be left with the impression that all amyloid consists of one or the other of these molecules, they should be warned that little is known about the nature of some other forms of amyloid, such as the senile type. Furthermore, based on older histochemical studies [36] coupled with the recent demonstration that β-sheet fibrils can be formed from insulin and glucagon [37], and the tentative identification of thyroglobulin in the amyloid of medullary carcinomas of the thyroid [38], one must seriously consider the possibility that other peptides may yield fibrils with the properties of amyloid. The significance of the minor component found in most amyloid deposits and known as the P-component or doughnut, because of its characteristic morphologic appearance, remains unknown. This protein has a molecular weight of 180,000 daltons, is composed of 5 identical non-covalently linked subunits, and is antigenically related to an α1 serum glycoprotein [18,19].

Although few of these advances have found immediate clinical application, it seems likely that they will soon be of value in practice. In particular, antisera specific for the conformational antigens of the fibrils may prove useful as sensitive diagnostic reagents in examining biopsies. The availability of antisera to specific amyloid proteins may allow us to classify patients more accurately and perhaps to characterize the nature of some of the as yet ill-defined types of amyloids which, because of their diffuse distribution in small deposits, do not lend themselves readily to chemical analyses. For example, in the case of senile amyloid, preliminary histochemical results by Wright (1974, personal communication), which suggest that this type of amyloid is related to L chains, could receive definitive support by immunochemical studies.
PATHOGENESIS

While of utmost interest in terms of understanding and, hopefully, eventually controlling the disease successfully, studies of possible etiologic and pathogenetic mechanisms are so complex that they cannot be discussed in detail. Hence, only a few salient points will be mentioned. The existence of a plethora of models and factors which can induce amyloid precludes precise conclusions concerning pathogenesis, especially since in many instances apparently contradictory mechanisms have been implicated [14]. While the biochemical studies of the amyloid substance have yielded potentially important clues, they have fallen short of providing us with a clear insight into pathogenesis. Hopefully the application of advances in the fields of cellular and humoral immunity to studies of the disease will aid us in the next few years in unravelling the many questions that remain. In general, amyloidosis is associated with exposure to a large antigenic load (as is the case in the casein model, hyperimmununized horses, and chronic infections in man). Recent studies indicate that the deposition of amyloid may be accompanied by depressed T-cell function [39], can be accelerated by immunosuppressive agents, and often accompanies naturally occurring immunodeficiency states [14]. Of particular interest in this regard is the recent observation that the SAA protein from mouse serum seems to diminish the amount of antibody produced in vitro to sheep red blood cells [40]. This finding, taken together with the existence of naturally occurring and experimental amyloid in situations where the immune system is overwhelmed by an antigenic load or where it undergoes neoplastic transformation, raises the possibility that the AA protein may be a substance with immunoregulatory properties, produced most probably by cells belonging to the immune system [41]. These findings suggest the possibility that amyloid can be stopped or is reversible if the stimulus is removed. Although some instances of regression of the disease after cure of the underlying condition have been reported, the resistance of amyloid to phagocytosis and proteolysis hampers the ready removal of the material by the host’s defense mechanisms [42-44].

One promising note has been sounded recently. Stimulated by the successful use of colchicine in aborting the febrile episodes of FMF, a disease often accompanied by amyloidosis, Kedar et al [45] have shown that colchicine can prevent the appearance of amyloidosis in mice given casein, a finding that has also been reported by Shirahama and Cohen [46]. Although the precise mechanisms of action of colchicine in this regard are unknown, the drug is sufficiently nontoxic to warrant a large-scale controlled trial in this otherwise hopeless disease.

CUTANEOUS AMYLOIDOSES

Amyloidosis is of interest to dermatologists for three reasons. Firstly, although the skin is rarely, if ever, involved in the secondary form of the disease, amyloid deposits occur frequently in the skin in the primary and myeloma-related types of the disease. Secondly, and probably more importantly, there exist several localized types of amyloidosis which involve only the skin and are virtually never associated with systemic disease. These cutaneous forms of amyloidosis, while often annoying to the patient, are quite benign. Lastly, amyloidosis is on occasion a late complication of certain chronic dermatologic conditions such as hidradenitis, purpurative stasis ulcers, psoriasis, lupus erythematosus, dermatomyositis, basal cell carcinoma, epidermolysis bullosa, and lepromatous leprosy. Since this type of amyloidosis is identical to the secondary form associated with many chronic diseases, it will not be discussed further. All these types of amyloidosis are reviewed in detail in three papers by Brownstein and Helwig [47-49].

In the primary and myeloma-associated types, papules are the most common cutaneous lesions. They are usually nontender and nonpruritic. Most often they are skin colored or yellow and translucent or waxy in appearance. They are rarely ulcerated, but often have a hemorrhagic component. The face, especially the eyelids, scalp, and neck are most frequently involved, although on occasion lesions can occur also on the trunk and extremities. Nodules or tumors, often very large in size with a hemorrhagic component due to involvement of the blood vessels, are sometimes seen. Cutaneous plaques which may impair the motion of the fingers, toes, and facial muscles, and may result in ulcerations of the skin may be present. Blood vessel involvement may result in purpura, commonly involving the face and eyelids, which is often brought on by minor trauma such as rubbing. Of lesser importance are alopecia, waxy discoloration of the skin, and abnormalities of the nails such as crumbling, brittleness, and anonychia. In this type of systemic amyloidosis involving the skin, the amyloid deposits are seen in the walls of the blood vessels, especially in the periadventitial zone and in the papillary corium and are associated with an attenuated epidermis, but a normal keratin and granular layer. The epidermis is usually unininvolved while the connective tissue of the corium and the dermis are often heavily infiltrated by amyloid deposits. The larger deposits are sometimes infiltrated with foreign-body giant cells and foci of plasma cells, but generally the amyloid deposits are quite acellular.

Of greater concern to the dermatologist are the primary localized benign cutaneous forms of amyloidosis, which are never associated with systemic involvement. The most common variant is lichen amyloidosis where amyloid deposits of the papillary corium give rise to pruritic papules, most commonly on the lower extremities. The lesions are most frequently seen in the pretilial area but also on the calves, thighs, ankles, and dorsa of the feet. The papules are usually firm, nontender, hyperkeratotic, and discrete. On occasion, individual
lesions occur in close proximity and may sometimes fuse to give verrucoid plaques. The papules range in size from 1 to 10 mm in diameter and vary in color from that of normal skin to gray or yellowish brown. They occur in individuals of all ages and may last for many years. Occasionally the upper extremities, shoulders, sacrum, and abdominal and chest wall may also be involved.

In this form of localized amyloidosis, the deposits are limited to the dermal papillae and do not involve the epidermis from which they are usually separated by a thin layer of collagen. The epidermis may be thinned and the keratin layer is usually compact and focally thickened. The deeper cutis and subcutaneum are uninvolved, and, as is true of all types of amyloidosis, there are few if any inflammatory cells in the lesions. Unlike the systemic types of amyloidosis where the capillary walls are involved, the deposits in lichen amyloidosis, but chemical and immunochemical elucidation of their precise composition remains to be undertaken.

The macular form of amyloidosis is less common and consists of oval, poorly delineated, hyperpigmented patches of aggregated, grayish brown macules. Its distribution and pathologic features resemble the papular type and it may progress to the papular form in time.

The third type of cutaneous amyloidosis, the tumefactive form, is less clearly benign since, in a significant number of cases, it may progress to a systemic form of the disease. The lesions are seen on the extremities, trunk, or face, involve the corium and subcutaneous tissue, and may affect the large blood vessels and cause thinning of the epidermis.

Lastly, asymptomatic deposits with the staining characteristics of amyloid are seen in a variety of dermatologic conditions such as seborrhoeic kerasoses, basal cell carcinomas, Bowen's disease, cylindromas, and pilomatrixomas.

In considering the pathogenesis of these localized forms of the disease, it is imperative to first establish the nature of the amyloid and the cells producing them. So far, there is no information as to whether they are associated with the AA protein, L chains, or if they represent a yet different type of protein. This association could best be determined with fluoresceinated antisera or by biochemical analyses. Then, one would have to establish that the deposits are locally produced and search for agents or stimuli which may cause a local disturbance in immune regulation that can set off the synthesis of the amyloid precursor. It seems possible that some as yet undefined local infectious agent or toxic products may initiate such a process, perhaps in genetically predisposed individuals. With currently available tools, answers to these questions will be soon forthcoming.

REFERENCES

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DISCUSSION

Epstein: What is the doughnut molecule?

Franklin: The doughnut, which is not a constituent of the amyloid fibril and generally makes up less than 5% of the amyloid substance, appears to be a pentamer existing as a 170,000 dalton serum globulin whose nature and function remain unknown. Partial amino acid sequence studies by Skinner have not shed significant light on this question. This component has been identified in all types of amyloidosis and may perhaps interact in a more or less specific manner with the major amyloid constituents.

Windhorst: Children with chronic granulomatous disease, other patients with chronic infections, and aged people have prominent intracellular deposits of a substance called lipofuscin. Do you have any information about a possible relationship between amyloid and this material?

Franklin: I am not aware of any relation to lipofuscin. The intracellular identification of amyloid has proved to be a difficult task since many cells develop intracellular fibrils resembling amyloid in situations of stress. Thus, we assume intracellular fibrils in cells in the vicinity of amyloid to be amyloid, but we can’t be sure. A few years ago we used some of our antisera to conformational antigens on the fibrils to stain cells and were able to get fluorescence with some cells surrounding amyloid deposits. Obviously these studies should be pursued further.

Tan: Do you think that problems encountered in determining the cell making AA protein might be related to the fact that you have an antiserum to denatured AA protein which cross-reacts poorly with native AA protein in the cell of origin? This problem is seen with antiserum raised against solubilized histones which reacts very well with solubilized histones in vitro but not with native histones in cell smears or tissue sections.

Franklin: This is one possibility but not a likely one since these antisera react well with the native serum component which gives a reaction of complete identity with the AA protein. It seems more likely to us that either the protein is rapidly turned over and does not accumulate in the cell, or that we have not yet looked at the correct cell. We are currently looking at the question in somewhat greater detail.

Kirkpatrick: Do nude mice have unusual susceptibility to amyloid? I ask because of your reference to prevention of amyloidosis with thymus hormone. Also, do NZB mice develop amyloidosis and is it possible to delay or prevent it with thymus cells?

Franklin: Both types of animals are likely candidates. The results in nude mice are somewhat contradictory and not very striking. Amyloid has been seen in NZB mice.

Kantor: Have you looked for homology of amyloid with other acute phase reactants such as C-reactive protein, fibrinogen, and complement?

Franklin: The AA protein is different from C-reactive protein, fibrinogen, and a host of minor, known and unknown serum globulins. With Peter Lachmann we have ruled out a relation to a few complement components but we are continuing to look at this question further since it is obviously an attractive idea to link the AA protein to complement.

Gigli: Is it possible that the doughnut and the fibrils seen in amyloidosis are part of the same molecule resembling Clq?

Franklin: On the basis of the morphology of these doughnuts and their size, this possibility seems unlikely since the doughnuts are very large. However, as I mentioned before it would be attractive to implicate complement in these amyloid deposits and we will continue to look for a relation to complement in the future.