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Senile Aortic Amyloid

Evidence for Two Distinct Forms of Localized Deposits

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Aortic tissues obtained at autopsy were examined from 84 patients (age, 18–96 years). Amyloid deposits were present in the media in 61 of 63 (97%) of the patients above the age of 50. In addition, intimal amyloid deposits were present in 35% of this group. Intimal amyloid differed from medial amyloid both in its morphologic characteristics and its association with atherosclerosis. An antiserum raised to a low molecular weight protein extracted from amyloid fibrils of the aortic media reacted specifically with medial amyloid but did not react with intimal deposits. Neither type of amyloid reacted with anti-ATTR (senile systemic amyloid), anti-AANF (isolated atrial amyloid), or antisera to other known forms of amyloid. These findings are consistent with the presence of two separate forms of localized amyloid in the aging aorta. (Am J Pathol 1992, 140:871–877)

Several forms of localized senile peripheral amyloid angiopathy have been described (in the aorta, aortic branches, pulmonary and temporal arteries). It has been suggested, but not confirmed that they represent deposition of different amyloid fibril proteins.[1] It has also been suggested that there are three different forms of senile aortic amyloidosis, namely intimal, medial, and adventitial amyloid.[2–10]

The aortic media is a common site for senile amyloid deposition. The highest prevalence has been found by Schwartz,[3] who found it in all autopsy cases above the age of 55. Intimal amyloid is less common, and frequencies up to 13.3% have been found.[6] Although adventitial amyloidosis is a manifestation of senile systemic amyloidosis,[11] the nature of medial and intimal aortic amyloid is unknown. Both the intimal and the medial amyloid deposits are histochemically positive for tryptophan[1,5–9] and are permanganate resistant,[1,5,8,9] but there have been indications that intimal and medial amyloidosis represent two separate entities. Atheromatous lesions are associated with intimal amyloidosis but have been reported to be negatively correlated with the medial form.[6]

In this study, we attempt to further characterize the different forms of localized aortic amyloid using immunohistochemistry and immune electron microscopy. We also show that the amyloid deposits often seen in the temporal arteries are related to the aortic medial amyloid.

Material and Methods

Aortic tissues from 84 autopsy cases (18–96 years, 54 males and 30 females) were studied. Age and sex distribution are given in Table 1. In 44 of the 84 autopsy cases, tissue from the common carotid artery close to its origin was also studied.

Light Microscopy

Two samples of tissue from the thoracic and two from the abdominal aorta of each patient were removed, formalin-fixed and paraffin-embedded (Figure 1); sections (6 μm thick) were stained with alkaline Congo red[12] and studied in polarized light for deposits of amyloid in the intima, media, and adventitia. Tissue from the common carotid artery was treated identically.

The quantity of medial amyloid in each of the four pieces of tissue was evaluated by the following scoring system: 0 = no amyloid; 1 = very small scattered deposits; 2 = small deposits uniformly distributed or patchy extensive deposits; 3 = extensive deposits uniformly dis-

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Table 1. *Age and Sex Distribution and Total and Average Score of Medial Amyloid in the Aorta in 84 Patients Studied for Aortic Amyloid*

Age (yr)	Men	Women	Total	Total score*	Average score
11–20	1	0	1	0	0
21–30	4	1	5	0	0
31–40	5	1	6	0	0
41–50	7	2	9	6	0.7
51–60	4	1	5	14	2.8
61–70	7	5	12	48	4.0
71–80	14	11	25	108	4.3
81–90	11	7	18	102	5.6
91–100	1	2	3	21	7.0
Total	54	30	84		

* Range of total score for each set of tissues = 0–12.

tributed. Each patient was thus assigned a score from 0 to 12.

Surgical specimens of the temporal artery from 28 patients (10 men and 18 women between 54 and 86 years of age) with suspected giant-cell arteritis were studied for amyloid content and the presence of giant-cell arteritis. Several sections of each specimen were made and stained with Congo red and hematoxylin-eosin. Giant-cell arteritis was diagnosed when the artery wall was infiltrated by inflammatory cells. All sections were studied blindly on two different occasions.

Purification of Medial Amyloid

Pieces of thoracic aorta were taken at autopsy from old persons (≥70 years) and stored at −20°C. Aortic tissue (from nine patients) that was shown by light microscopy to be rich in medial amyloid was used. After trimming off the adventitia and intima, the tissue (about 20 g) was homogenized in 0.15 M NaCl and centrifuged at 5,000 × g. To remove soluble proteins, this step was repeated five times. The amyloid containing pellet was then suspended in 50 ml 0.05 M Tris HCl buffer, pH 8.0, and incubated with collagenase (10%, w/v) from Clostridium histolyticum (Boehringer, Mannheim, Germany) at 37°C overnight and finally centrifuged. Before and after the collagenase treatment, the presence of amyloid in the pellet was confirmed in the polarization microscope after Congo red staining. After five cycles of homogenization in distilled water and centrifugation, the remaining amyloid pellet was lyophilized. Medial aortic tissue without amyloid from two patients was treated in the same way. For further purification, pellet material was defatted in chloroform-methanol (2:1) and dissolved in 6M guanidine HCl in 0.1 M Tris HCl buffer, pH 8.0 containing 0.1 M EDTA (ethylenediaminetetraacetic acid) and 0.1 dithiothreitol. After centrifugation, the solution was chromatographed on a Sepharose CL 6B (Pharmacia, Uppsala, Sweden) column which was equilibrated with 5 M guanidine HCl in distilled water. Fractions corresponding to peaks registered at 280 nm were pooled, precipitated with saturated ammonium sulphate, dialyzed against deionized water, and lyophilized.

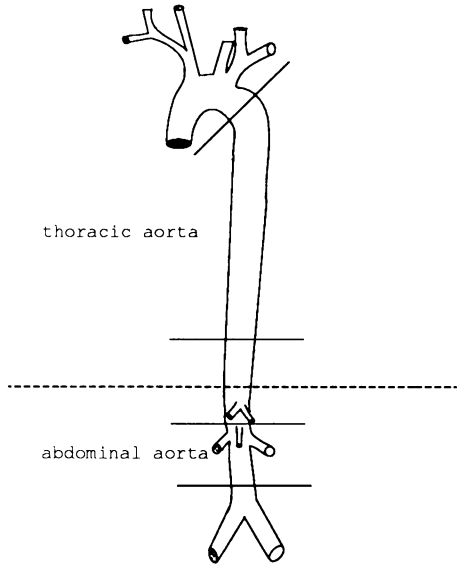

Figure 1. *Outline of the aortic tree showing sites from which samples were taken for analysis.*

Immune Electron Microscopy and Immunohistochemistry

Antisera

The materials corresponding to the two retarded protein peaks on the gel-filtration elution profile (see below) of the medial aortic amyloid were used for raising antisera in two guinea pigs (Ma 55 and Ma 58) by standard techniques. Rabbit antisera to protein AA,[13] ATTR,[11] AANF,[14] AIAPP[15] and seminal vesicle amyloid protein[16] have been characterized previously. Rabbit antiserum to cystatin C was a gift from Dr. A. Grubb (Lund).

Immunogold Electron Microscopy

The two antisera to medial aortic amyloid materials were tested by immunogold electron microscopy[17] on amyloid containing aortic medial tissue after fixation in 2.5% glutaraldehyde and Epon embedding. Sections were mounted on formvar-coated nickel grids and etched with saturated $NaIO_4$. The antisera were absorbed with normal human serum (1:10, v:v) and human amyloid P component (10 mg/ml undiluted antiserum) and diluted 1:25–1:50 with 1% bovine serum albumin in 0.05 M Tris HCl

buffer, pH 7.4, before incubation with the sections for 2 hours. After treatment with protein A-gold conjugate (Auroprobe, 20 nm particles Janssen, Beerse, Belgium), sections were contrasted with uranyl acetate and lead citrate. The electron microscope used was a JEOL 100 SX at 80 kV.

Immunohistochemistry

The antisera to the medial aortic amyloid materials were absorbed with amyloid P-component and normal human serum.[14] The peroxidase-antiperoxidase (PAP) method[18] was used on sections of various known and unknown amyloid types including intimal, medial, and adventitial aortic amyloid as well as amyloid in the temporal and carotid arteries. Formalin-fixed and paraffin-embedded tissues were taken from the laboratory files. After deparaffination, the sections were incubated with antiserum in a dilution of 1:100–1:200. The PAP method was also used when tissues containing intimal and medial amyloid were incubated with antisera to other amyloid proteins. Adventitial amyloid containing tissues were incubated with anti-ATTR. All these antisera were absorbed to specificity[14] before incubation.

Electrophoresis and Western Blot Analysis

Since only the antiserum to the last retarded peak (peak 2) material (antiserum Ma 58) was specific for the amyloid in immunohistochemistry studies, this was used for Western blot analysis. Protein material corresponding to peak 2 was studied by sodium dodecyl sulfate-polyacrylamide 10–20% gradient gel electrophoresis (SDS-PAGE).[19] For Western blot,[20] the proteins were then transferred to a Immobilon PVDF Transfer Membrane (Millipore, Bedford, MA) that was incubated with the absorbed antiserum (1:200). The antigen-antibody complexes were labelled with 20 nm Biocell A-gold conjugates and enhanced with a silver enhancing kit (BioCell, Cardiff, England).

Results

Light Microscopy

The frequency of amyloid in the aortic intima, media and adventitia is shown in Tables 1 and 2.

Intimal Amyloid

Intimal amyloid was consistently present in association with atheromatous lesions and was in the form of irregular lumps that showed a bright green birefringence after staining with Congo red (Figure 2a). Above the age of 50, the frequency of intimal amyloid was 35%, but there was no amyloid in the intima below this age (Table 2).

Medial Amyloid

Medial amyloid was much more frequent (97%) than the intimal form above the age of 50. Below 50 years of age, there was a single case of medial amyloid in a 42-year-old male with juvenile diabetes mellitus. Amyloid was most often present in the inner half of the media and was in the form of nodules and thin streaks often in close relation to elastic fibers (Figure 2d). The amount of medial amyloid was increased with increasing age (Table 1). There was no difference between the sexes. The thoracic aorta contained more medial amyloid than the abdominal aorta (total score, 171 vs. 127).

Adventitial Amyloid

Adventitial amyloid (Figure 2c) was only found in two cases, both from elderly patients. The amyloid was found in the connective tissue and in the walls of vasa vasorum.

Amyloid in the Common Carotid Artery

In 15 of the 44 cases studied, amyloid was present in the media. All patients were above the age of 50. In 39% of the cases that had aortic medial amyloid, concomitant amyloid was present in the media of the common carotid artery. The morphology of the medial amyloid in these two locations was identical.

Amyloid in the Temporal Artery

Twelve of the 28 cases studied had amyloid in the vessel wall. It was located on both sides of the internal elastic lamina (Figure 2f) and sometimes extended into the inner half of the media. Streaks of amyloid were parallel to the elastic lamina. Nine cases showed giant-cell arteritis, but only three of them had concomitant amyloid deposition in the vessels. The findings suggest that occurrence of giant-cell arteritis and amyloid deposits is coincidental.[21]

Immunohistochemical Findings

Both antisera (Ma 55 and Ma 58) raised to the two different fractions of the separated medial aortic amyloid material labelled the medial aortic amyloid deposits. However, while antiserum to peak 2 material (Ma 58) reacted only with the amyloid, antiserum to peak 1 material (Ma 55) also reacted diffusely with other tissue components. Consequently, antiserum Ma 58 was used for all subse-

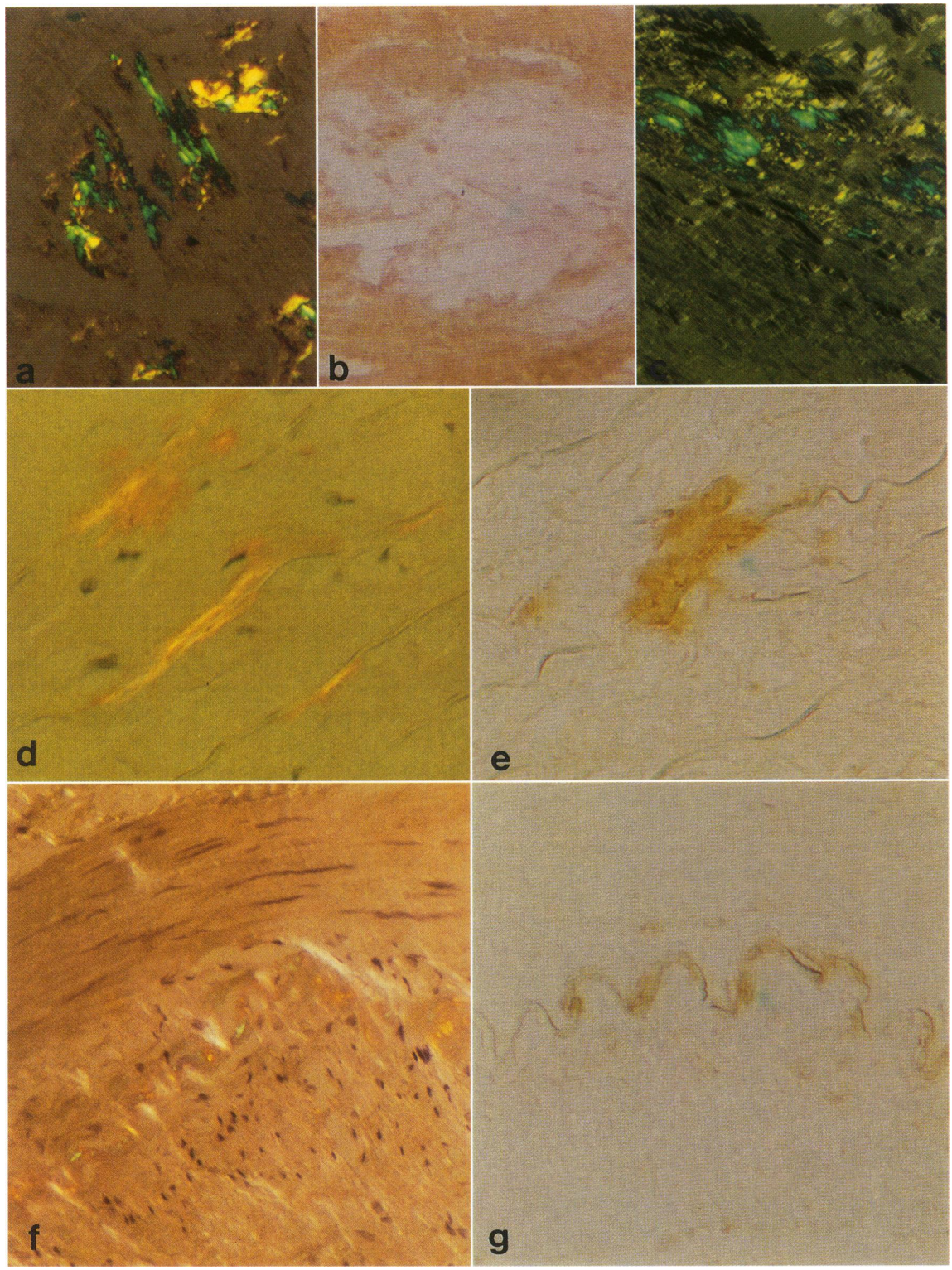

Figure 2. *Aortic intimal* (a, b), *adventitial* (c) *and medial* (d, e) *amyloid, studied in polarized light after staining with Congo red* (a, c, d). *Intimal and adventitial amyloid exhibit a brighter green birefringence than the medial amyloid. Antiserum to medial amyloid reacts specifically (PAP method) with this type of amyloid* (e) *but not with intimal deposits* (b). *In temporal arteries, small amyloid deposits occur along the internal elastic lamina* (f, *Congo red, polarized light). This amyloid reacts with antiserum to aortic medial amyloid* (g); ×*425* (a, b, d, g, e); ×*265* (c); ×*340* (f).

Table 2. *Frequency of Aortic Amyloid in the Intima, Media, and Adventitia of at Least One of Four Pieces of Tissue from Each Patient*

Age (yr)	No.	Location of amyloid deposits		
		Intima	Media	Adventitia
11–50	21	0	5%	0
51–96	63	35%	97%	3%

quent studies. Ma 58 labelled medial (Figure 2e) but not intimal (Figure 2b) or the adventitial amyloid deposits. The antiserum also reacted with amyloid in the temporal and carotid arteries (Figure 2g). It did not react with amyloid deposits containing fibril proteins AA, AL, ATTR, AANF, Aβ, AIAPP, AIns, ACys and undetermined fibril proteins in the localized amyloids of adrenal gland and seminal vesicle. Neither intimal nor medial amyloid reacted with anti-ATTR, anti-AANF, anti-AL, anti-AIAPP, anti-Cystatin C, or antiserum to seminal vesicle amyloid protein. Anti-ATTR antiserum reacted with amyloid in only one of the two cases with adventitial deposits. The nature of the amyloid in the second case was not determined.

Electron Microscopic Findings

Electron microscopy of aortic media showed extracellular amyloid fibril deposits. The fine fibrils were disorderly arranged in compact bundles. No definite relation to any type of cell could be demonstrated. The antiserum Ma 55 reacted diffusely, whereas antiserum Ma 58 labelled amyloid fibrils specifically (Figure 3).

Partial Characterization of Medial Aortic Amyloid

Treatment of the pellet with collagenase resulted in a much higher concentration of amyloid, consistent with the resistance of amyloid fibrils to this enzyme. Light microscopic analysis of supernatant and pellet materials after homogenization in water showed that virtually all amyloid remained in the pellet (which was used for further analysis).

Gel filtration of dissolved medial aortic amyloid gave rise to two retarded peaks (peak 1 and peak 2) (Figure 4). When control material was gel filtrated in the same way, a different elution pattern lacking peak 2 was seen (Figure 4). Several bands were seen on SDS-PAGE when the medial aortic protein material from peak 2 was analyzed (Figure 5). However, Western blot revealed that the material in only two of these bands reacted with the antiserum Ma 58 to aortic medial amyloid. A strong reaction was seen corresponding to a molecular mass slightly less than 6 kDa, while a weak band appeared at about 30 kDa (Figure 5).

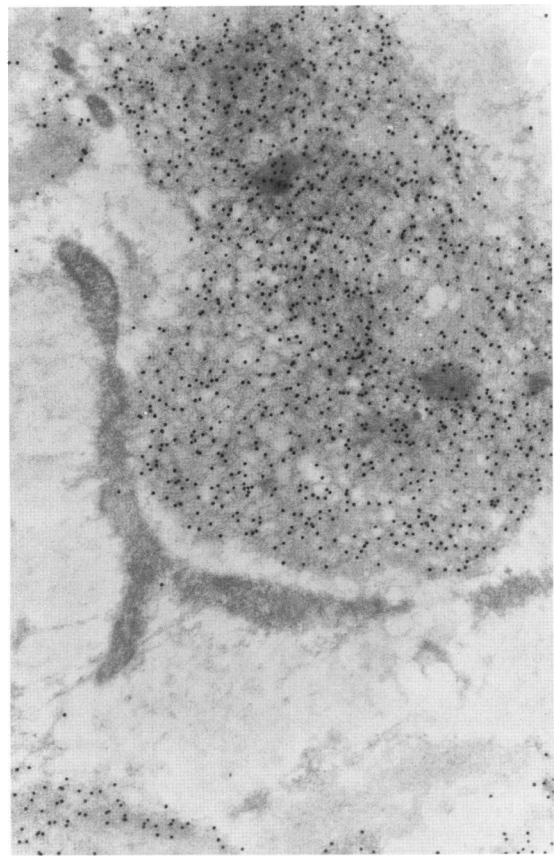

Figure 3. *Immunogold labelling of aortic medial amyloid with antiserum to this amyloid. No reaction is seen outside the amyloid deposits; 20 nm gold particle, ×25,000.*

Discussion

This study confirms the previously described high frequency of aortic medial amyloid in aged patients. [1–10]

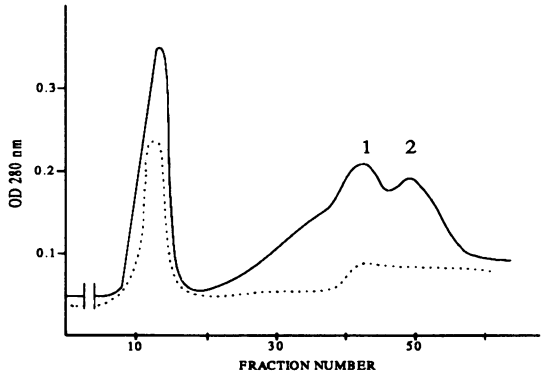

Figure 4. *Gel-filtration elution patterns of aortic medial amyloid (—) and control aortic material (····). The amyloid material gives rise to two retarded protein peaks, the smaller of which is lacking in chromatography of control material.*

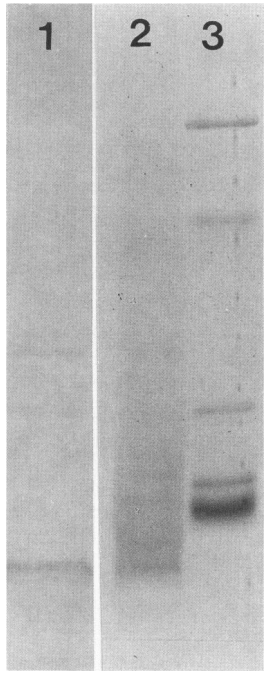

Figure 5. *SDS-PAGE (lane 2) of peak 2 material from gel filtration of aortic medial amyloid (Figure 4). When a guinea pig antiserum raised to this protein fraction is used in Western blot analysis of SDS-PAGE separated dissolved aortic medial amyloid (lane 1), a strong reaction with a protein corresponding to less than 6 kDa and a weak reaction with a second protein material of higher molecular mass (about 30 kDa) is seen. Molecular weight markers (lane 3) are from above bovine serum albumin (68 kDa), ovalbumin (43 kDa), soybean trypsin inhibitor (20.4 kDa), lysozyme (14.4 kDa), and aprotinin (6 kDa).*

Our data also confirm that the prevalence of amyloid is higher in the thoracic than in the abdominal portion of the vessel.[5] This pattern of distribution is in contradistinction to that observed for atherosclerosis. Thus, there does not appear to be any relationship between medial amyloid and atherosclerotic lesions, although both tend to increase with increasing age. By contrast, the intimal amyloid deposits occur in topographic association with atherosclerotic lesions. Although we do not know if these two lesions are related, the finding is of great potential interest and motivates further investigation. In our study, intimal amyloid occurred in 35% of patients over 50 years, almost three times the frequency which has been reported previously. [6] The reason for this difference is unclear.

The antiserum to a partially purified protein fraction from extracted aortic medial amyloid reacted strongly with the medial amyloid but not with other structures in the aortic wall. Furthermore, the antiserum did not react with any other type of amyloid that we studied. This finding supports our previous observation[7] that the medial amyloid in the aorta is chemically different from other known amyloid forms. Its strict localization to the media favors a local origin for the fibril precursor protein similar to the situation in other localized amyloids such as the β-protein in the brain and islet amyloid polypeptide (IAPP) in the islets of Langerhans.

The immunohistochemical data indicate that the amyloid in the aorta, common carotid arteries and temporal arteries is of identical nature. This finding raises the possibility that medial aortic amyloid represents a form of more generalized vascular amyloid, perhaps derived from locally produced precursor proteins rather than plasma proteins. Although a generalized degenerative process associated with aging in larger vessels seems probable, a systematic evaluation of additional parts of the vascular tree will be required to further understand the extent and nature of this type of amyloid.

Since studies of other amyloid proteins have given important knowledge of certain diseases,[22] purification and characterization of the protein in aortic medial amyloid is of interest. The amount of material obtained in this study was not sufficient for chemical analysis, but studies are currently in progress in our laboratory.

Interestingly, no reaction was obtained with the amyloid deposits in the intima when the antiserum to aortic medial amyloid was used, strongly suggesting that there are two different and common forms of localized amyloid deposits in the aorta. Since the intimal deposits are associated with atherosclerotic plaques, it is possible that the fibril protein in this form of amyloid is derived from the plasma.

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