Virchows Arch (1997) 430:479–487

ORIGINAL ARTICLE

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Visceral organ involvement and extracellular matrix changes in β_2-microglobulin amyloidosis – a comparative study with systemic AA and AL amyloidosis

Received: 23 August 1996 / Accepted: 9 January 1997

Abstract Patterns of amyloid distribution and extracellular matrix changes in the heart and gastrointestinal tract were compared among β_2-microglobulin (B2M), AA (secondary), and AL (primary and multiple myeloma-associated) amyloidosis cases. B2M amyloid was found to be mainly distributed in the small arterioles, venules, endocardium and muscularis propria of these organs, the deposits characteristically forming subendothelial nodular lesions in the vessels. A marked increase of chondroitin sulfate (CS) was consistently detected in B2M amyloid. Heparan sulfate (HS) also showed an increase in amyloid deposits, but with less reactivity than CS in the small arterioles or venules. Basement membrane structures stained positively for laminin and collagen type IV were replaced by negative amyloid deposits. In the AL cases, the muscularis propria of the gastrointestinal tract was involved in amyloid deposits, as seen for the B2M type, but the vascular amyloid deposits were localized in the media and adventitia of larger vessels. Immunoreactivity for HS was more intense than that for CS, and no increase in laminin or collagen type IV was observed. In the AA cases, amyloid deposits were distributed in the capillaries, small arterioles, interstitium of the myocardium and mucosa. Immunoreactivity for laminin and collagen type IV was marked, and more intense than that for HS and CS. Although the existence of a direct relationship between increase in extracellular matrix material and amyloidogenesis remains to be proven, the observed variation in extracellular matrix changes in the background of each type of amyloidosis may indicate different binding sites of the amyloid precursor proteins, resulting in the specific histological features and distribution.

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Key words Haemodialysis · β_2-Microglobulin · Amyloidosis · Chondroitin sulfate · Heparan sulfate

Introduction

A newly established form of systemic amyloidosis, seen frequently in patients on long-term haemodialysis, has attracted increasing attention [14, 31]. The major constituent protein in so-called haemodialysis-related amyloid has been identified as β_2-microglobulin (B2M) [7], with a molecular weight calculated to be about 11,800 Da, which is similar to that of native B2M. It is well known that B2M amyloidosis has a preferential tissue distribution, involving the synovium, ligament, articular cartilage and intervertebral discs. Arthropathy caused by this type of amyloid is characterized by carpal tunnel syndrome, persistent oligoarticular swelling and effusions, lytic bone lesions, and destructive spondyloarthropathy [14, 31]. Recent case reports with examination of autopsy materials have revealed that it may also affect the various visceral organs, especially in long-term haemodialysis patients [3].

In our previous study, we established that amyloid deposition occurs much earlier in the cervical intervertebral discs than in the visceral organs, and that this early deposition is closely associated with tissue degeneration attributable to mechanical stress [21]. An increase of glycosaminoglycans, such as chondroitin sulfate (CS), is a constant finding shown not only in the amyloid deposits but also in the surrounding degenerative tissue changes [22]. Co-deposition of basement membrane components, such as heparan sulfate (HS) proteoglycan, has been reported in experimentally induced models of AA (secondary) amyloidosis and many kinds of human amyloidosis [12, 16, 26, 27]. Extracellular matrix changes of visceral organ deposits in B2M type have not been sufficiently detailed to determine whether their extracellular matrix changes are different from those of periarticular deposits or not or whether extracellular matrix changes of B2M type are different from those of other types.

To define and compare the pattern of amyloid distribution and extracellular matrix changes in B2M, AA (secondary) and AL (primary or multiple myeloma-associated) amyloidosis, we collected 11 B2M, 8 AA and 8 AL cases featuring visceral organ deposition. To rule out organ-specific changes in extracellular matrix, we performed a comparative study using the same organs, the heart and gastrointestinal tract. B2M amyloid deposits in visceral organs are generally scanty, and it is almost impossible to extract and purify the extracellular matrix associated with amyloid fibrils from these tissues. We investigated extracellular matrix changes, such as HS, CS, dernatan sulfate (DS) proteoglycan (decorin), collagen type IV, laminin and fibronectin immunohistochemically, using various monoclonal antibodies or antisera. The role of extracellular matrix changes in amyloidogenesis is discussed.

Materials and methods

This study was performed using autopsy specimens from 11 B2M, 8 AL, and 8 AA amyloidosis patients. In all 11 B2M amyloidosis cases, amyloid deposits were observed in both visceral organs and periarticular tissues, such as synovium, ligament and intervertebral discs. Seven of the B2M amyloidosis cases and all of the individuals suffering from AL and AA amyloidosis had been diagnosed at the Department of Pathology, Toranomon Hospital, Tokyo. Three other B2M amyloidosis patients had been diagnosed at the Department of Pathology, Japan Red Cross Medical Center, Tokyo, and one at the Department of Pathology, Nakano General Hospital, Tokyo. Clinical profiles of patients undergoing haemodialysis are summarized in Table 1, cuprophane membrane being used in almost all cases. Of the 8 AL-type patients, 4 presented with multiple myeloma and 4 were diagnosed as having primary amyloidosis. Of the 8 AA-type patients, 5 had been associated with rheumatoid arthritis, 1 had a bladder cancer, 1 had pulmonary tuberculosis, and 1 had polycystic kidneys.

Organs taken at autopsy were fixed in 10% formalin solution and routinely processed to paraffin blocks, which were cut serially at 3 or 6 μm and deparaffinized. Sections cut at 6 μm were stained for Congo red according to the method of Puchtner et al. [24]. Amyloid deposition was confirmed histologically by positive staining and characteristic green birefringence under polarized light. Using sections cut at 3 μm, immunohistochemical staining of B2M, AA protein, κ-light chain, λ-light chain (Dakopatts, Copenhagen) and various extracellular matrix components was performed. Foci of positive staining in each case were compared with the sites of amyloid deposition. In the present B2M amyloidosis cases, amyloid deposits showed positive staining only for B2M and never showed a positive reaction for AA protein, or the κ- and λ-light chains.

The monoclonal antibodies and antisera against extracellular matrix components used in the present study are listed in Table 2. For CS staining, four kinds of monoclonal antibodies were applied. CS56 recognizes the native glycosaminoglycans, and MO225 the long chains of d-unit type, while 2B6 and 3B3 detect the disaccharides, ΔDi-4S and ΔDi-6S, remaining after digestion by chondroitinase ABC. To detect heparan sulfate we used the

Table 1 Visceral organ involvement of haemodialysis-related amyloidosis (*HD* haemodialysis, *CGN* chronic glomerulonephritis, *Y* years, *G-I tract* gastrointestinal tract, *U bladder* urinary bladder; *italics* indicate samples examined immunohistochemically)

No. of cases	Age	Sex	Cause	HD period	Amyloid deposition
1	71	M	Gout	12Y	*Heart*, *G-I tract*, adrenal gland
2	82	M	CGN	16Y	*Heart*, *G-I tract*, prostate
3	66	F	CGN	16Y	*Heart*, *G-I tract*, pancreas, liver
4	66	F	Unknown	17Y	*Heart*, *G-I tract*, liver, kidney, ovary, uterus, U bladder
5	36	M	CGN	18Y	*Heart*, *G-I tract*, lung, pancreas, liver, kidney, adrenal gland, tongue, prostate, U bladder, epididymis
6	59	M	CGN	18Y	*Heart*, *G-I tract*, salivary gland
7	53	M	Unknown	18Y	*Heart*, *G-I tract*, peritoneum
8	53	M	CGN	19Y	*G-I tract*
9	72	M	CGN	19Y	*Heart*, *G-I tract*, lung, liver, kidney, salivary gland, tongue, prostate, epididymis
10	54	F	Unknown	21Y	*Heart*, *G-I tract*, salivary gland, U bladder, uterus
11	68	F	Unknown	21Y	*Heart*, *G-I tract*, liver, kidney, lung, pancreas, thyroid adrenal gland, ovary, tongue

Table 2 Monoclonal and polyclonal antibodies used in the present study

Extracellular matrix	Clone	Dilution	Pretreatment	Source	Reference
β2-Microglobulin	(poly)	100	Trypsin	DAKO	
Amyloid A protein	mc1	50	Trypsin	DAKO	[15]
κ Light chain	(poly)	500	–	DAKO	
λ Light chain	(poly)	500	–	DAKO	
Type IV collagen	JK199	200	Pronase E	Shiseido	[11]
Laminin	(poly)	50	Pepsin	Sanbio	
Fibronectin	MF1B	10	Microwave	Cappel	
Heparan sulfate	10E4	100	–	Seikagaku Kogyo	[6]
Chondroitin sulfate	CS56	200	–	Seikagaku Kogyo	[2]
Chondroitin sulfate	MO225	100	–	Seikagaku Kogyo	[33]
Chondroitin sulfate, ΔDi-4S	2B6	100	Chondroitinase ABC	Seikagaku Kogyo	[4]
Chondroitin sulfate, ΔDi-6S	3B3	100	Chondroitinase ABC	Seikagaku Kogyo	[4]
Dermatan sulfate proteoglycan (decorin), core protein	6B6	1000	Chondroitinase ABC	Seikagaku Kogyo	[29]

10E4 monoclonal antibody, which recognizes glycosaminoglycans, not core proteins. In the present study, since all materials were taken at autopsy, the preservation of tissues was inferior to that usual with experimental models. This is presumably the reason why the monoclonal antibody for detecting core protein of heparan sulfate proteoglycan (perlecan) did not react with basement membrane structures of the present tissues. The 6B6 antibody detects a core protein of DS proteoglycan, decorin. Pretreatments with pronase E, pepsin, chondroitinase ABC, or microwave heating were necessary for collagen type IV, laminin, ΔDi-4S and ΔDi-6S disaccharide, DS and fibronectin staining, as shown in Table 2. For HS, formic acid pretreatment was also performed, but it did not change the immunoreactivity of positive control sections. Basement membrane structures in kidney tissue were stained as a positive control for HS, laminin, collagen type IV, and fibronectin. Matrix material and chondrocytes in cartilaginous tissue were stained as a positive control for CS. Dermal interstitium of the skin was utilized for DS proteoglycan. As negative controls, sections were incubated with normal serum.

For immunohistochemistry, paraffin-embedded sections were laid on poly-L-lysine-coated slides, and deparaffinized. Digestion by 0.1% pronase E for 60 min at room temperature, by 0.1% pepsin for 120 min at 37°C, or by 5 U/ml chondroitinase ABC for 60 min at 37°C, and heating by microwave oven for 10 min at 95°C were performed where required. Subsequently, sections were incubated in methanol containing 0.3% (v/v) H_2O_2, washed in PBS, and exposed to normal serum, followed by reaction with primary antibodies for 2 h at room temperature in humidified chambers. Excess antibody was removed by washing with PBS, and the bound antibodies were labelled with biotinylated anti-mouse or anti-rabbit immunoglobulin and streptavidin–peroxidase (Nichirei). After three additional washes, bound peroxidase was visualized with 0.02% diaminobenzidine (Sigma) at pH 7.6 in 0.05 M Tris buffer plus 0.015% H_2O_2. Immunoreactivity was classified into four grades: (–) no reaction, (±) questionable or slightly positive reaction, (+) mildly positive reaction, (++) strongly positive reaction.

Results

Table 1 summarizes data for the organ distribution of B2M amyloid. The heart and gastrointestinal tract most frequently demonstrated amyloid deposition, 10 and all of 11 cases being affected, respectively. In the gastrointestinal tract, the stomach and small intestine were most often involved. Histologically, the amyloid deposits were found to be distributed mainly in the small vessels, and the amounts were generally small. With regard to parenchymal deposition, amyloid was localized in the endocardium and valves of the heart and the muscularis mucosae and muscularis propria of the gastrointestinal tract. Interstitial deposition in the prostate, tongue, salivary gland, ovary or peritoneum was also observed in numbers of cases.

AA- and AL-type amyloid deposits were also commonly observed in the heart and gastrointestinal tract. Table 3 summarizes findings for the localization of the three types of amyloid in the heart and gastrointestinal tract. B2M-type amyloid deposits in the heart were localized in the intramyocardial small vessels and endocardium. Interstitial deposition surrounding individual myocytes was not observed in any of the cases, in contrast to the frequent occurrence of AA amyloid deposits in this site, forming nodular lesions and sometimes replacing the myocytes. AL-type amyloid deposits in the intramyocardial interstitium were noted at a frequency intermediate between those for the AA and B2M types.

In the gastrointestinal tract, both B2M and AL types showed parenchymal deposition in the muscularis mucosae and muscularis propria layers of smooth muscle. A total of 4 B2M cases showed amyloid deposits in the muscularis propria and 1 in muscularis mucosae, the corresponding figures for AL being 3 and 4 cases. Amyloid deposits of both types often involved the muscularis propria massively with replacement of muscle fibres. However, no AA cases showed amyloid deposits in the muscularis mucosae and muscularis propria, although 7 of the 8 showed parenchymal deposition of fine granules in the mucosal interstitium around the basement membranes of glands, associated with erosion or haemorrhage. This was not the case for B2M amyloid.

Deposits of all three types were commonly observed in the vascular wall. However, the histological features differed. In the B2M cases, small to medium-sized arterioles or venules located in the myocardium or submucosa were frequently involved by amyloid deposits localized mainly in the subendothelium, forming nodules with luminal stenosis or occlusion (Figs. 1a, f, 2a). Such characteristic subendothelial nodular lesions were found in all cases and in every organ with amyloid deposits. Outer layers of the media and adventitia were rarely affected. With the AL type, a wider range of vessel sizes demonstrated deposits, including medium to large arterioles and small arteries. Amyloid deposits were localized in the media and adventitia (Fig. 2g), and the walls usually showed marked thickening. Ischaemic changes in the myocardium, such as interstitial fibrosis and degeneration of myocytes, were observed around these vessels. Subendothelial nodular lesions were rarely observed in

Table 3 Amyloid deposition in the heart and gastrointestinal tract (*AA* secondary amyloidosis, *AL* primary and multiple myeloma-associated amyloidosis, *Aβ$_2$M* haemodialysis-related amyloidosis)

Type of amyloid	No. of cases	Heart			Gastrointestinal tract[a]			
		Vessels	Endocardium	Interstitium	Vessels	Mucosa	Muscularis mucosae	Muscularis propria
AA	8	8	2	7	8	8	0	0
AL	8	5	3	4	6	4	4	3
Aβ$_2$M	11	10	3	0	11	0	1	4

[a] If amyloid was found in a layer in any part of the digestive tract, the layer was considered to be affected for the purpose of this table

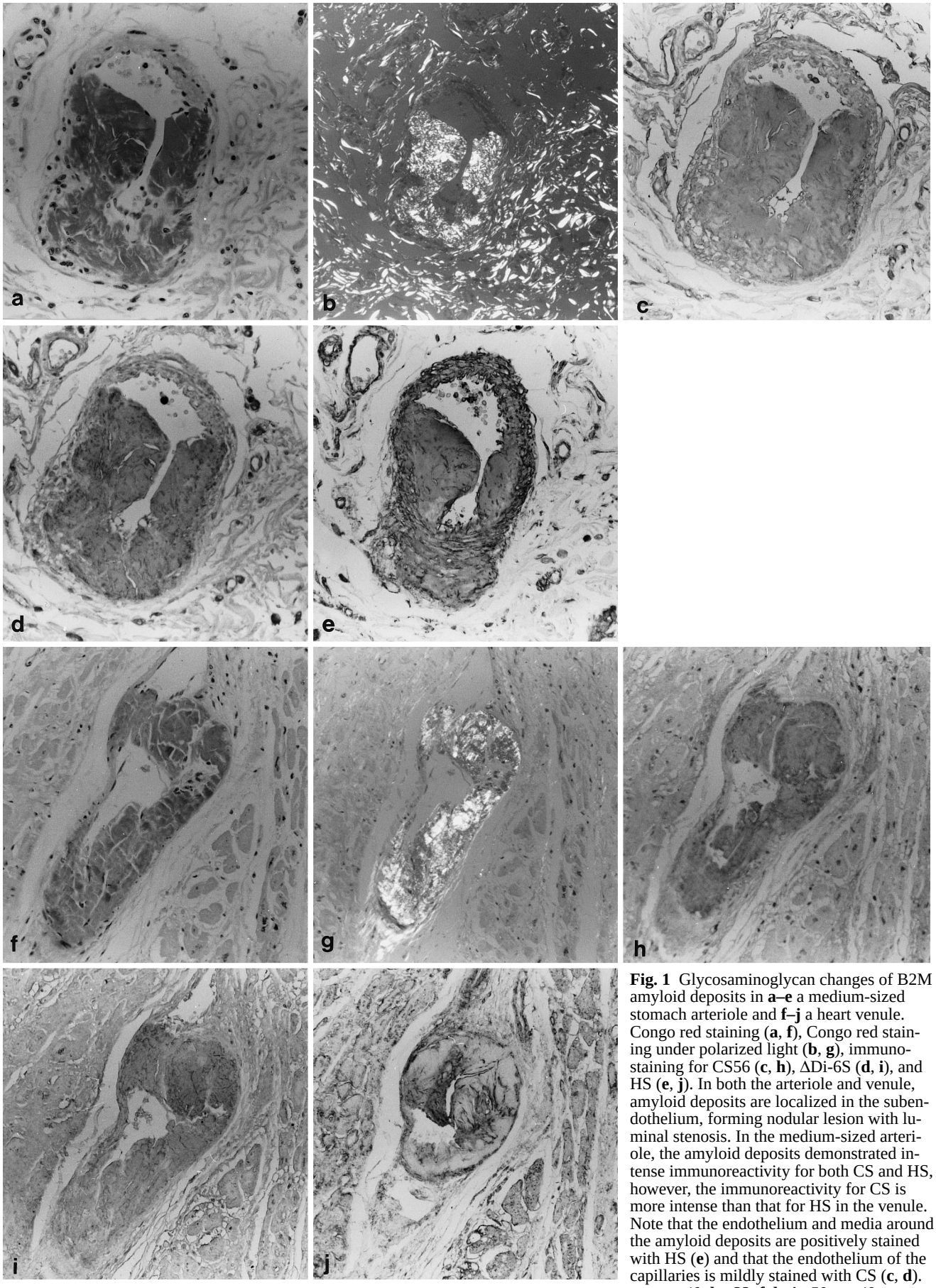


Fig. 1 Glycosaminoglycan changes of B2M amyloid deposits in **a–e** a medium-sized stomach arteriole and **f–j** a heart venule. Congo red staining (**a**, **f**), Congo red staining under polarized light (**b**, **g**), immunostaining for CS56 (**c**, **h**), ΔDi-6S (**d**, **i**), and HS (**e**, **j**). In both the arteriole and venule, amyloid deposits are localized in the subendothelium, forming nodular lesion with luminal stenosis. In the medium-sized arteriole, the amyloid deposits demonstrated intense immunoreactivity for both CS and HS, however, the immunoreactivity for CS is more intense than that for HS in the venule. Note that the endothelium and media around the amyloid deposits are positively stained with HS (**e**) and that the endothelium of the capillaries is mildly stained with CS (**c**, **d**). **a**, **c–e** ×40, **b** ×33, **f**, **h–j** ×50, **g** ×40

AL-type cases. AA amyloid was frequently apparent in capillaries and small arterioles (Fig. 3a), which showed circumferential thickening with the deposits tending to extend toward the interstitium around the basement membrane. Subendothelial nodular lesions were not observed in any AA-type case.

The results of immunohistochemical studies of extracellular matrix changes in the heart and gastrointestinal tract are summarized in Table 4. With B2M-type amyloid deposits, a marked increase of CS was observed consistently in both the vascular wall and the muscularis propria, along with binding of antibodies recognizing native glycosaminoglycan chains and disaccharides after chondroitinase ABC digestion (Figs. 1c, d, h, i, 2c). The intima of the normal vessels and myocytes of smooth muscle layers also showed mildly positive reactions for CS. Amyloid deposits in the muscularis propria and medium-sized arterioles showed positive for HS, immunoreactivity for HS being almost as same as that for CS (Fig. 1e). In contrast, those in the small arterioles or venules with thin smooth muscle layers of the media were much weaker for HS than that for CS (Figs. 1j, 2d). The intima and media of small vessels, especially around the amyloid deposits, showed a positive reaction for HS. Laminin and collagen type IV were localized to the intima and media of vessels, and myocytes of the muscularis propria. In vessels with amyloid deposits, basement membrane structures stained with laminin and collagen type IV were displaced or replaced by amyloid, and their immunoreactivity in the amyloid was very weak or negative (Fig. 2e, f). Fibronectin was also observed in the intima of normal vessels and the myocytes. A slight increase in fibronectin was occasionally observed in the amyloid deposits. The DS proteoglycan, decorin, was positively stained in the fibrous interstitium around but not within the amyloid deposits.

Both HS and CS showed an increase in the AL amyloid deposits as seen in B2M type, the reactivity for HS being generally stronger (Fig. 2i, j). In the large arterioles and small arteries, whole layers of media including amyloid deposits showed an intense reaction for HS. Immunoreactivity for laminin and collagen type IV was much weaker or negative (Fig. 2k, l). Fibronectin showed questionable to mild reactivity in the amyloid deposits, and DS proteoglycan was shown only in the fibrous interstitium around the amyloid deposits.

In the AA-type cases, amyloid deposits localized in capillaries, arterioles and interstitium showed a positive reaction for HS and CS, but the immunoreactivity was generally weak compared with that in the B2M and AL amyloid (Fig. 3d). In contrast, the immunoreactivity for laminin and collagen type IV was marked with intense and homogeneous staining in the amyloid deposits, demonstrating a clear difference from the B2M and AL cases (Fig. 3b, c). Fibronectin was positively stained in intima and myocytes, and not in the amyloid deposits (Fig. 3e). DS proteoglycan was found to be present in the fibrous interstitium around the amyloid deposits.

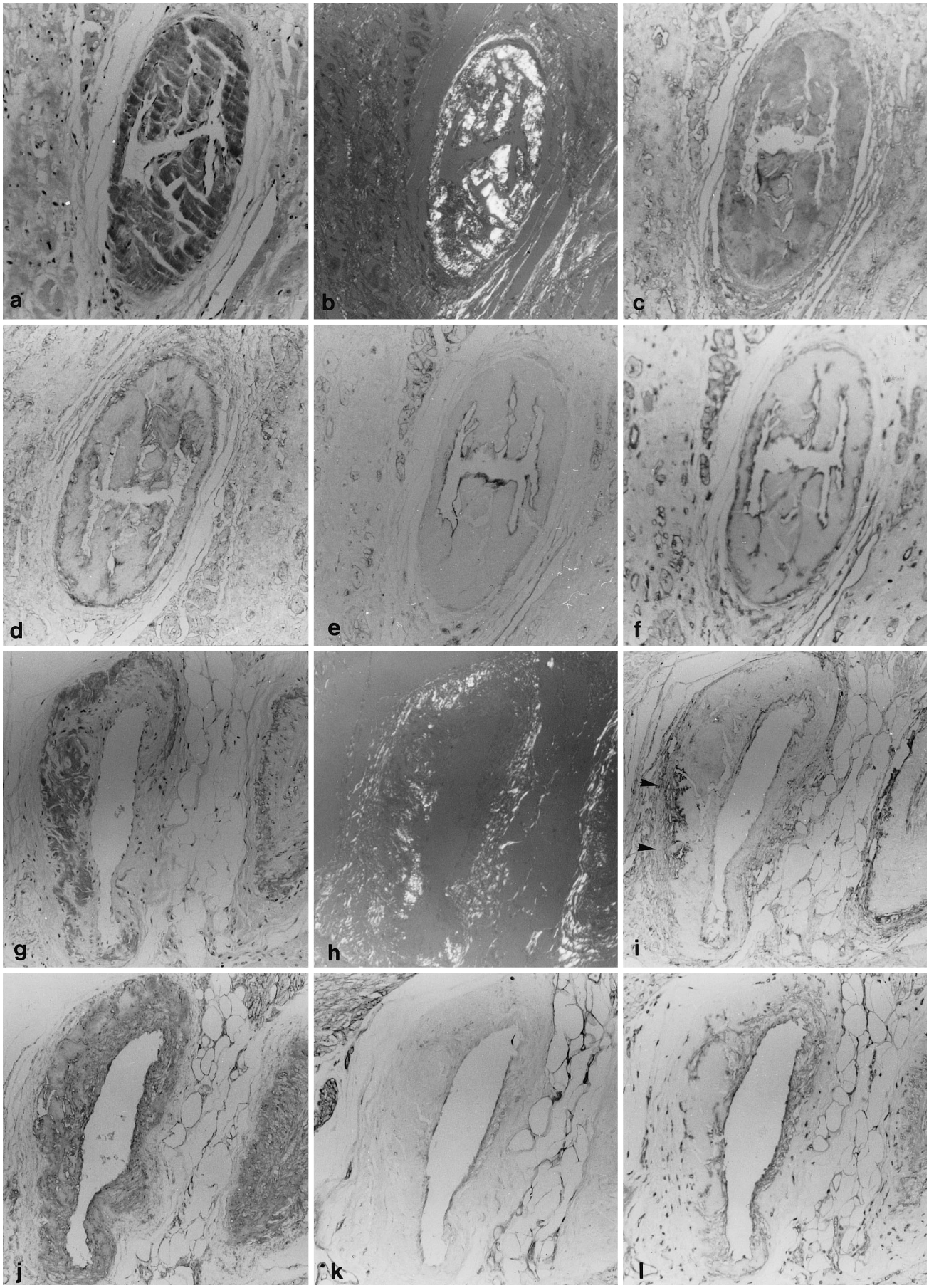
Discussion

Type-specific distribution of amyloid deposits, especially in the gastrointestinal tract, has long been the subject of research, and early investigators such as Gilat et al and Yamada et al. reported the AA type to be mainly distributed in the mucosal interstitium and small vessels of the submucosa, while AL cases demonstrated involvement of the muscularis propria and vessels of all layers [8, 32]. Our results on the distribution of AA and AL amyloid are therefore in line with previous reports. In B2M type, parenchymal deposits were shown in the muscularis propria of the gastrointestinal tract and not in the mucosal interstitium, which is a similar distribution to that in the AL type. However, with respect to the histological features of vascular wall deposits, the B2M type differed from the AL type. Campistol et al. reported three B2M amyloidosis cases with visceral organ involvement and also pointed out subendothelial nodular lesions in the small arterioles [3]. In the present study, we confirmed that such subendothelial nodules in small arterioles and venules are specific findings for B2M amyloidosis.

Extracellular matrix changes, especially of the acidic glycosaminoglycans, have been investigated in various amyloid deposits [12, 26, 27, 34]. In experimentally induced-mouse AA amyloidosis, an increase of the basement membrane form of HS proteoglycans, perlecan, has been reported [1, 16]. An increase of HS or CS/DS in amyloid fibrils can also be detected by biochemical methods in the human AA and AL amyloidosis, Alzheimer's disease and prion amyloidosis [17, 20, 23]. In B2M amyloidosis, glycosaminoglycan changes in periar-

Table 4 Summary of immunohistochemical findings. Extracellular matrix changes in amyloid deposits *CollV* type IV collagen, *Lam* laminin, *Fib* fibronectin, *HS* heparan sulfate, *CS* chondroitin sulfate, *DS* dermatan sulfate, – no reaction, ± slight reaction, + mild reaction, ++ strong reaction)

Type of amyloid	Site of deposition	ColV	Lam	Fib	HS	CS				DS
						CS56	MO225	2B6	3B3	
AA	Vessel	+/++	+/++	±/+	±/+	±	±	-/±	±/+	–
AL	Vessel	–/±	–/±	±/+	+/++	±/+	+	±	±/+	–
	Muscular layer	–/±	–/±	+	++	+	+	±/+	+	–
$A\beta_2m$	Vessel	–	–	±	+/++	+	++	+/++	++	–
	Muscular layer	±	–/±	±/+	++	+/++	++	+/++	++	–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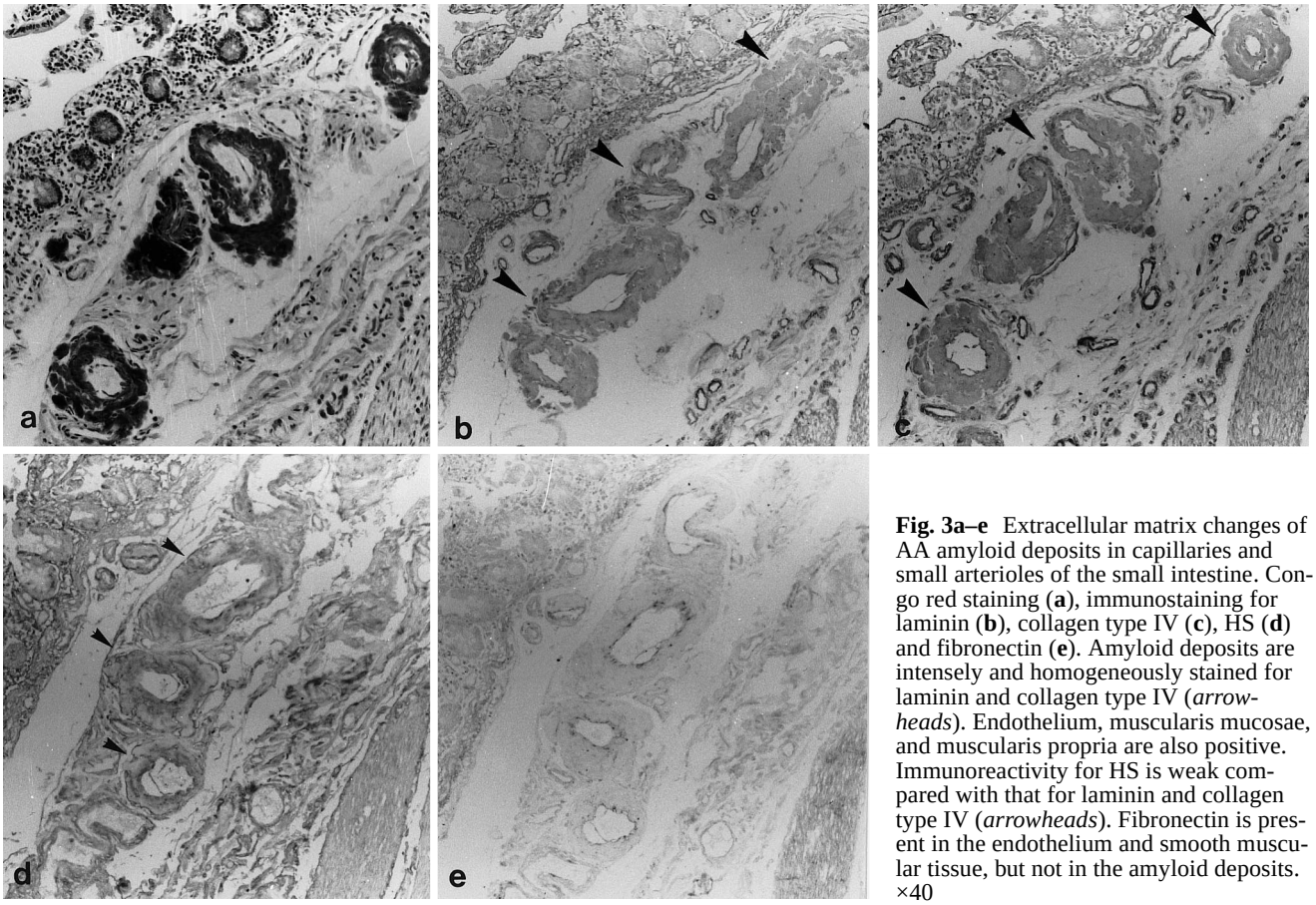

Fig. 3a–e Extracellular matrix changes of AA amyloid deposits in capillaries and small arterioles of the small intestine. Congo red staining (**a**), immunostaining for laminin (**b**), collagen type IV (**c**), HS (**d**) and fibronectin (**e**). Amyloid deposits are intensely and homogeneously stained for laminin and collagen type IV (*arrowheads*). Endothelium, muscularis mucosae, and muscularis propria are also positive. Immunoreactivity for HS is weak compared with that for laminin and collagen type IV (*arrowheads*). Fibronectin is present in the endothelium and smooth muscular tissue, but not in the amyloid deposits. ×40

ticular deposits include increments of both HS and CS as demonstrated immunohistochemically, with the increase in CS being more marked than that of HS in the amyloid associated with ligaments and intervertebral discs [22]. Biochemical studies have also confirmed CS to be a major component in the amyloid-rich periarticular tissues [22, 23].

In the present study, we have confirmed that type-specific changes of extracellular matrix occur in association with amyloid deposits in the heart and gastrointestinal tract. Lyon et al. demonstrated co-deposition of laminin, collagen type IV in amyloid deposits of mouse AA amyloidosis model [16]. We have confirmed that an increase in laminin and collagen type IV also occurs in human AA amyloidosis, the immunoreactivity for laminin and collagen type IV being much stronger than that for HS or CS. Previous biochemical studies demonstrated total amounts of glycosaminoglycans from 3 to 15 times those extracted from AL as opposed to AA amyloid fibrils [17, 20, 23]. The present finding of relatively weak immunoreactivity for glycosaminoglycans in AA-type deposits is thus consistent with reports in the literature.

The amino acid sequence of B2M is homologous with the sequences of the constant regions of both classes of light chains (κ and λ) [5]. B2M and AL types also have similar features with regard to their distribution and associated extracellular matrix changes, as shown by parenchymal deposition in the muscularis propria of the gastrointestinal tract and increased glycosaminoglycans, but factors other than primary structure of protein appear to be associated with differences in protein binding to the vessel wall. Kisilevsky hypothesized a close association between amyloidogenesis and abnormal metabolism of basement membrane components [12], and thus differences in the extracellular matrix could be the background to the variation in binding of different amyloid precursor protein, resulting in specific histological features and distribution of amyloid deposits. A CS-dominated increase of glycosaminoglycans is probably associated with the subendothelial B2M-type nodular lesion, and an HS-dominant increase might be responsible for

Fig. 2 Extracellular matrix changes of **a–f** B2M and **g–l** AL amyloid deposits in a small heart arteriole and artery. Congo red staining (**a**, **g**), Congo red staining under polarized light (**b**, **h**), immunostaining for CS56 (**c**, **i**), HS (**d**, **j**), laminin (**e**, **k**), and collagen type IV (**f**, **l**). In B2M type, subendothelial nodular deposits are positively stained with CS. The endothelium and thin media are positively stained with HS, but the immunoreactivity in the amyloid deposits is weak. Laminin staining is only present in the endothelium, and collagen type IV in the endothelium and thin media. In AL type, amyloid deposits localized in the outer media and adventitia are intensely positive for HS, but the immunoreactivity for CS is comparatively weak (*arrowheads*). The endothelium and media around the amyloid deposits are also positive for HS. Laminin is present only in the endothelium. Collagen type IV is stained in the endothelium and media, but not in the amyloid deposits. **a–f** ×50; **g–l** ×25

the circumferential AL-type deposits in the media and adventitia.

In previous studies, we demonstrated that an increase in CS, which is probably induced by mechanical stress, is closely related to the B2M amyloidogenesis in periarticular tissue or intervertebral discs [22]. The "mechanical stress theory" cannot be applied directly to the vascular wall deposition; however, various pathological states, such as hyperglycaemia, high levels of angiotensin II and atherosclerosis, have been reported to induce CS/DS synthesis in endothelium [13, 25, 30]. Endothelium and vascular smooth muscle express different kinds of CS/DS proteoglycans, biglycan being present in the former and biglycan and decorin predominating in the latter [10], and it is possible that some unknown damage to endothelium causes abnormal overproduction of CS proteoglycan, possibly biglycan, before amyloid deposition. This might provide a particularly suitable microenvironment for B2M amyloidogenesis, resulting in formation of characteristic subendothelial nodular lesions in the vessel walls. Further characterization of CS proteoglycans associated with B2M amyloid and determination of factors that induce overproduction of CS proteoglycans in vessel walls appear warranted.

The role of extracellular matrix components in amyloidogenesis has been analysed by a number of authors in vitro, with increase in formation of β-sheet structure of SAA protein being associated with binding to HS [18]. HS proteoglycans had been reported to show a high affinity for the Aβ protein in Alzheimer's disease [19, 28], but the question as to whether this is also the case for other amyloid precursor proteins is unclear. Various collagens, including type IV, bind to B2M strongly in vitro [9]. With regard to CS, there is no available information on a possible role in amyloidogenesis, but the results of the present investigation indicate that its affinity for different amyloid types warrants attention. Affinity for the glycosaminoglycans and alteration of the secondary structure of B2M as a result of binding should also be examined as a next step.

In summary, the present study revealed specific histological features and extracellular matrix changes associated with B2M amyloidosis, subendothelial nodular lesions in small vessels and a CS-dominated increase of glycosaminoglycans being characteristic. A close relationship between amyloidogenesis and specific binding sites in the extracellular matrix were suggested by our findings. However, further studies of the interaction between amyloid precursor proteins and glycosaminoglycans, and particularly between B2M and CS, are necessary to prove this hypothesis.

Acknowledgements We thank Tamiko Takemura, MD, Department of Pathology, Japan Red Cross Medical Centre, and Takumi Akashi, MD, Department of Pathology, Tokyo Medical and Dental University, for providing us with B2M amyloidosis cases. This study was supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science, Sports and Culture of Japan.

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