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A Histochemical Study of Hemodialysis Related Amyloidosis

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Summary. **It is well known that β2-microglobulin (MG) amyloidosis shows characteristic morphological features including bundle or nodule formation of aggregated fibrils and engulfment by various cells. We studied the histochemical properties of β2-MG amyloid fibrils using glycoprotein, glycosaminoglycan (GAG) and/or proteoglycan detection methods. A large amount of glycoprotein was noticed in β2-MG amyloid fibrils. GAGs and/or proteoglycans were detected only around amyloid bundles or nodules, but not on amyloid fibrils themselves. These results suggest that the above histochemical characteristics may be related to amyloid formation.**

INTRODUCTION

The osteoarthropathy associated with hemodialysis (HD) related amyloidosis has been noted to be one of the major complications of long term HD patients.[1,2] Since Gejyo et al.[3] identified the constitutional protein of these amyloid fibrils as β2-microglobulin (MG), subsequent histochemical and biochemical studies have been reported. However, the mechanism of the deposition remains unclear. Additional constituents of the β2-MG amyloid deposits other than β2-MG have been reported such as glycoproteins, glycosaminoglycans (GAGs) and proteoglycans.[4] In order to clarify the relationship between these constituents and β2-MG amyloid fibrils we investigated the histochemical properties of the amyloid fibrils by electron microscopy.

MATERIALS AND METHODS

The subjects were 6 long term HD patients with carpal tunnel syndrome or carpal bone cysts. The mean HD duration was 9.8 years. The tissues were obtained from synovial sheaths and bone cyst contents at the time of the release or curretage operation. For light microscopy the tissues were fixed with 10% formalin and stained with hematoxylin eosin and Congo red. PAP method with anti human β2-MG antibody (DAKO, Denmark) was performed for identifying the constitutional protein of amyloid fibrils. For electron microscopy the tissues were fixed with 2.5% glutaraldehyde and 1% osmium tetroxide, dehydrated by ethanol and embedded in epoxy resin by the conventional method. Ultrathin sections were stained with uranyl acetate and lead citrate. For histochemical studies they were also treated with periodic acid methenamine (PAM) satin according to the modified method of Kobayashi.[5] Polyethylene imine (PEI) (molecular weight 1,800) was used for the detection of negative charge by Okada's method.[6] Toluidine blue O[7] and ruthenium red[8] block stains were also applied for detection of glycosaminoglycans (GAGs) and/or proteoglycans. For immunoelectron microscopy the tissues were fixed with 4% paraformaldehyde and embedded in glycolmethacrylate resin.[9] Immuno-gold labeling method was used with anti human β2-MG antibody (DAKO Denmark).

RESULTS

Many eosin and Congo-red positive small nodules were aggregated in all examined tissues. These nodules showed birefringence under polarized light. The constitutional protein was confirmed as β2-MG by PAP method. Electron microscopic study revealed that these nodules consisted of aggregated bundles which were composed of several tightly packed fibrils. The longitudinal axial directions of the bundles were straight or curvilinear. The fibrils were frequently engulfed by macrophages or interstitial cells (Fig. 1a). Some fibrils were degenerated in the

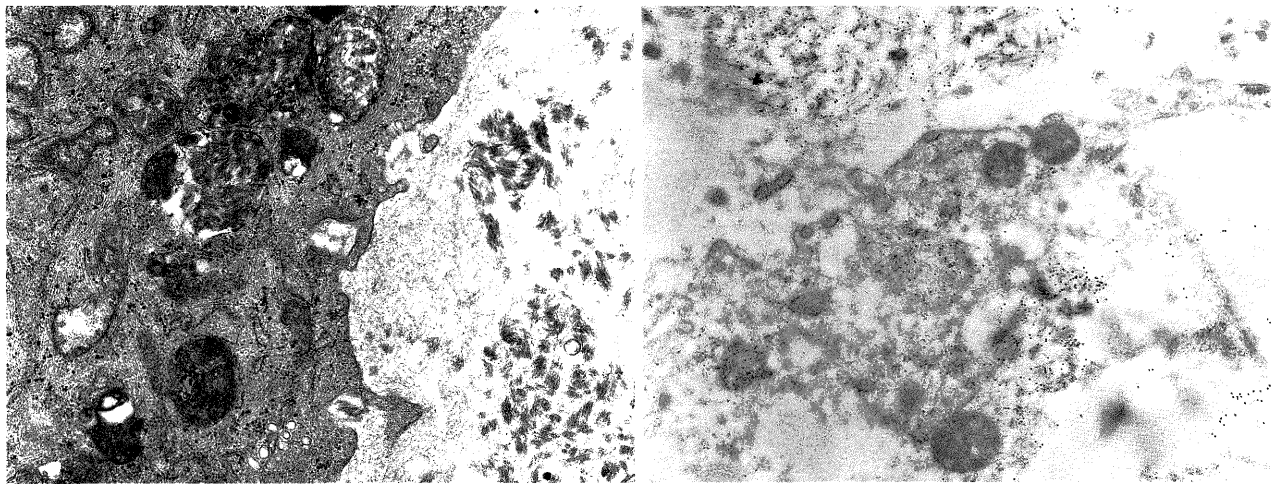

Fig. 1a. Engulfment of β2-MG amyloid fibrils by an interstitial cell. ×14,000
Fig. 1b. Immuno-gold labeling method. Both extra and intracellular amyloid fibrils are labeled with gold particles. ×18,000

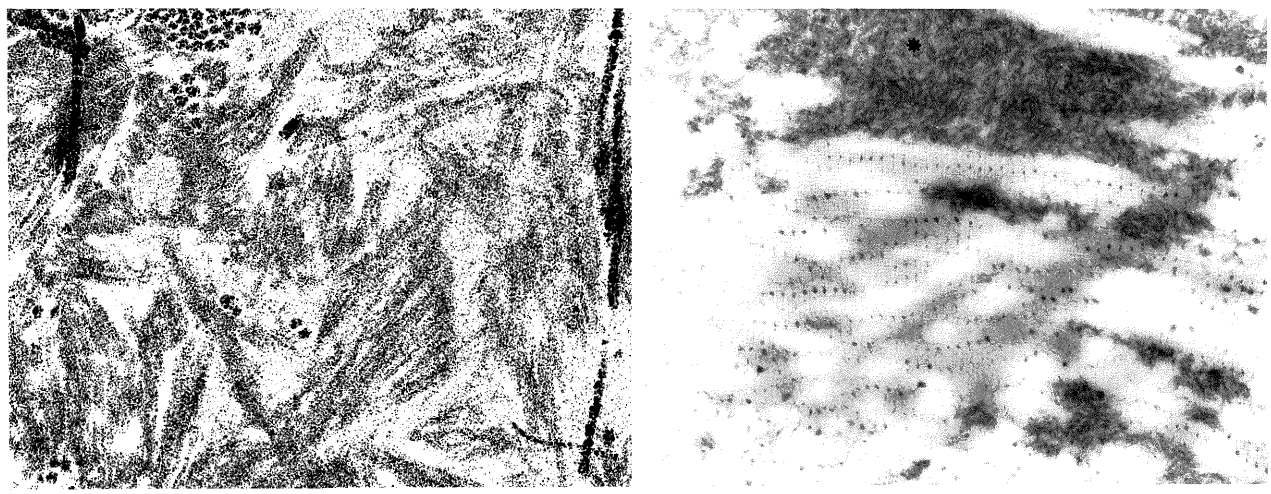

Fig. 2a. Amyloid fibrils are strongly stained with PAM stain. ×4,200
Fig. 2b. PEI reactive dots are observed only on the surface of collagen fibers, and not on amyloid fibrils (*). ×14,000

phagosome-like vacuoles. Immuno-gold labeling method revealed that both extracellular and intracellular engulfed fibrils were labeled by gold particles (Fig. 1b). However, gold particles were not recognized in the intracellular productive systems such as the rough endoplasmic reticulum and Golgi apparatuses.

β2-MG amyloid deposits were stained with electron microscopic PAM stain as intensely as collagen fibers located near amyloid fibrils. Higher magnification demonstrated that amyloid fibrils themselves were strongly stained (Fig. 2a). In the examination with PEI positive reaction was observed on collagen fibers, but not on amyloid fibrils (Fig. 2b). Toluidine blue O block stain revealed fibrilar structures surrounding the amyloid nodules or bundles, which were considered to be GAGs or proteoglycans (Fig. 3). Chondrocyte-like cells were seen in amyloid deposits. These zones did not contain amyloid fibrils, although many amyloid fibrils were recognized adjacent to them (Fig. 4a). The zones showed metachromasia with toluidine blue O stain on semi-thin sections of epoxy resin. Ruthenium red block stain demonstrated many positive dots in this zone (Fig. 4b).

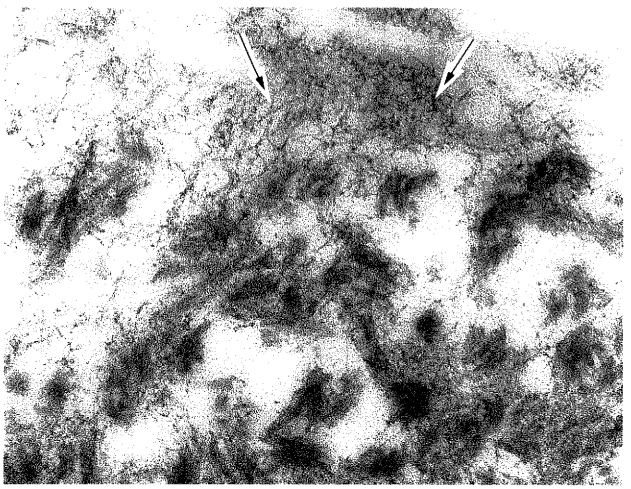

Fig. 3. Toluidine blue block stain. GAGs and/or proteoglycans like structures are detected surrounding amyloid bundles or nodules (arrows). ×56,000

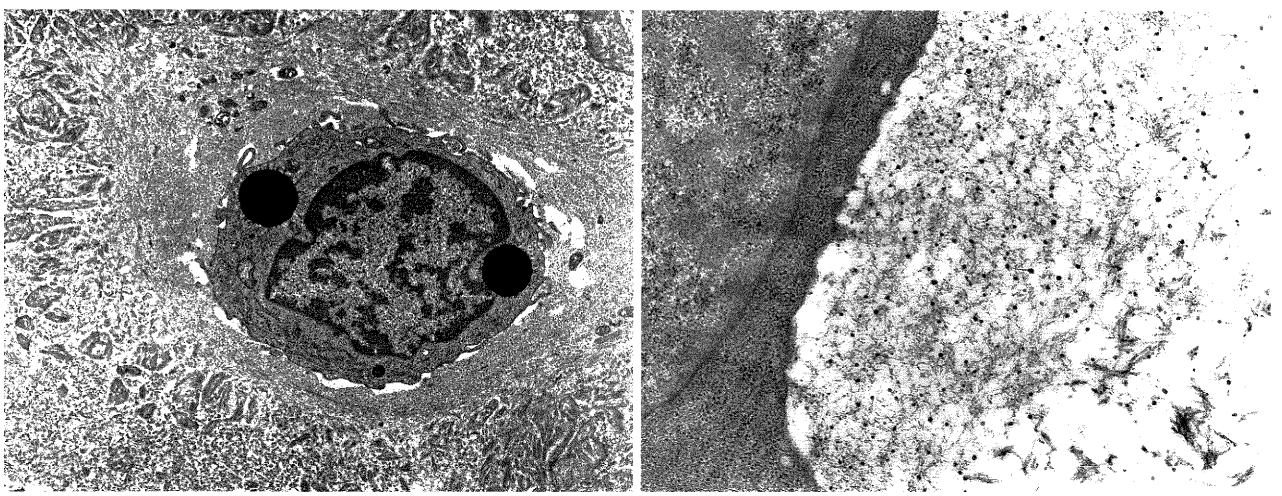

Fig. 4a. A chondrocyte like cell surrounded by amorphous zones in amyloid deposits. ×4,500
Fig. 4b. The amorphous zone show many reactive dots to ruthenium red. ×20,000

DISCUSSION

In primary or secondary amyloid deposits the arrangement of amyloid fibrils shows random cross patterns of single amyloid fibrils. The new type amyloid fibrils derived from β2-MG revealed the specific morphological feature of bundle or nodule formation of aggregated fibrils. Additionally in primary or secondary amyloidosis engulfment of amyloid fibrils by macrophages and other cells are seldom observed.[10] However, in β2-MG amyloidosis amyloid fibrils were frequently engulfed by both macrophages and interstitial cells. These morphological findings were seemed to be specific for β2-MG amyloid fibrils. It is suspected that these characteristic features may relate to the property of the precursor protein itself or compound substances in the amyloid deposits. However, the mechanisms have not been completely clarified.

β2-MG amyloid fibrils showed strong argyrophilic character with PAM staining. This fact indicates that they contain much glycoprotein or saccharides.[5] Since β2-MG is a non-glycated protein in its native form,[11] it may be glycated in the process of amyloid formation. The glycated β2-MG may produce new antigenicity and generate the tendency for active

engulfment. In primary or secondary renal amyloidosis spiculae, bundles of amyloid fibrils are strongly stained with PAM stain.[12,13] The glycation of β2-MG may also be related to the bundle or nodule formation.

Using PEI it was noted that β2-MG amyloid fibrils did not demonstrate negative charged characteristics compared with collagen fibers. The surface negative charge of collagen fibers is thought to be derived from the tight attachment of GAGs and/or proteoglycans to collagen fibers.[7,8] The glycoproteins or saccharides contained in β2-MG amyloid fibrils may not be GAGs and/or proteoglycans. We investigated the relationship between amyloid fibrils and GAGs and/or proteoglycans by histochemical technique. According to our results GAGs and/or proteoglycans existed only surrounding the amyloid bundles or nodules. Ruthenium red stain demonstrated that amyloid fibrils were not observed in GAG and proteoglycan rich areas. These results suggest that GAGs and/or proteoglycans may regulate the deposition and formation of β2-MG amyloid fibrils. However, GAGs and proteoglycans may not tightly attach to β2-MG amyloid fibrils themselves. It can be suspected that in long term HD patients noxious substances such as uremic toxins, superoxides[14,15] and cytokines[16] which accelerate inflammation may deteriorate or diminish GAGs and/or proteoglycans. Consequently, amyloid fibrils may be apt to be formed in the damaged tissues in which GAGs and/or proteoglycans are reduced.

It is also well known that β2-MG amyloidosis is complicated more frequently in higher aged and longer term HD patients.[1,2] It can be assumed that molecular changes or degeneration of β2-MG and GAGs and/or proteoglycans in the tissues may progress gradually during long term HD. We suppose that this progression may be one of the necessary conditions for amyloid formation.

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