Deposition of Kappa and Lambda Light Chains in Amyloid Filaments of Dialysis-Related Amyloidosis1,2

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ABSTRACT

β2-Microglobulin (β2m) is considered to be the amyloidogenic precursor in dialysis-related amyloidosis, although the implication of other relevant cofactors in the pathogenesis of this disease has also been hypothesized. It is conceivable that substances found in amyloid deposits might represent something more than simple codeposition, possibly playing a pathogenic role in amyloidogenesis. Along these lines, a detailed analysis of the protein composition of amyloid fibrils purified from synovial material surgically obtained from nine patients on long-term dialysis was carried out. By the use of sodium dodecyl sulfate-polyacrylamide gel electrophoresis, several other protein components, in addition to β2m, were found. These were characterized by NH2 amino-terminal sequencing and immunoblotting. In fibrils obtained by water extraction, which fulfill the electron microscopy criteria of highly pure amyloid material, polyclonal kappa and lambda light chains were detected with a concentration of 15 μg/mL. In the water extraction material, the β2m concentration was 200 μg/mL. Light microscopy immunohistochemistry was performed on samples from five patients. Amyloid deposits reacted with anti-β2m, and anti-light (kappa, lambda), chain antibodies. The Immunoreaction of amyloid filaments to anti-β2m, anti-lambda, and anti-kappa light chain antibodies was also tested by electron microscopy by use of the immunogold staining procedure. Amyloid filaments were labeled by the three antibodies and showed a different intensity of immunostaining apparently related to their different aggregation pattern. These observations demonstrate that polyclonal immunoglobulin light chains (kappa and lambda) are not contaminants but, together with β2m, represent a major constituent of amyloid deposits in dialysis-related osteoarticular amyloidosis, thus indicating their possible role in amyloidogenesis.

Key Words: β2-Microglobulin, biochemistry, immunohistochemistry, immunoelectronmicroscopy, carpal tunnel syndrome

Amyloid deposition in bones and synovia is a frequent complication in patients on long-term regular dialysis treatment: it is also accepted that its prevalence increases steeply with time spent on dialysis, being present in almost 100% of the patients after 20 yr of treatment (1). Typical osteoarticular lesions include the carpal tunnel amyloidosis and arthropathies frequently associated with bone cystic accumulation. The syndrome may lead to invalidating complications when bone fractures or nerve compression occur. β2-Microglobulin (β2m) is the recognized precursor of dialysis amyloidosis (2,3) and its precipitation takes place mainly as an intact molecule (4), although there is also evidence that β2m proteolytic fragments may be produced locally by a specific lysine cleavage (5), thus enhancing amyloidogenesis.

However, several clinical and experimental observations argue against a simple precipitation of β2m as the unique mechanism of amyloidogenesis. A main point in favor of a multifactorial origin of dialysis amyloidosis is the predilection for the synovia of osteoarticular tissues and tendon sheaths, suggesting the presence of local cofactors such as heparan sulfate-glycosaminoglycan and collagen, which might favor the formation of fibrils within these structures (6,7). Another issue is the presence, in the fibril preparation, of α-2-macroglobulin, a well-recognized antiproteolytic protein. Along with a whole family of antiproteases, α-2-macroglobulin could favor intact β2m precipitation and may also inhibit possible amyloid removal (8,9). In addition, levels of serum β2m do not correlate with the extension and severity of the osteoarticular lesions or with the prevalence of carpal tunnel syndrome (10). Taken together, these data suggest it to be rather unlikely that β2m can produce amyloid deposits by itself.

Considering the basic hypothesis of the possible
existence of local cofactors, we undertook a detailed analysis of amyloid fibrils obtained from synovial material by means of biochemical, histochemical, and immunocytochemical methods. This investigation describes the colocalization of β2m with immunoglobulin light chains in hemodialysis-associated amyloid deposits, the presence of which has already been hypothesized in our laboratories (11).

EXPERIMENTAL DESIGN

Synovial and tendon sheath samples from patients with dialysis-related amyloidosis were processed for the biochemical and immunocytochemical evaluation of amyloid deposits and for the immunohistostructural study of amyloid filaments. Biochemical studies included purification of amyloid fibrils, electrophoresis (sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE)) to characterize the protein composition of amyloid deposits, protein sequencing to identify proteins, and immunoblotting to validate the identification. The protein content of amyloid deposits was determined by immunoenzymatic methods. Light microscopy immunohistochemistry was carried out to assess the immunoreactivity of amyloid deposits. The immunoreaction of amyloid filaments to β2m and kappa and lambda light chain antibodies was tested in electron microscopy by use of the immunogold staining procedure in order to better define the distribution of the antigens in amyloid deposits.

METHODS

Materials

Acrylamide, N,N-methylenebisacrylamide (BIS), ammonium persulfate, alkaline phosphatase–goat anti-rabbit immunoglobulin G (IgG), and premixed substrate reagents (KIT No. 170-6432) were obtained from Bio-Rad (Richmond, CA); hexyl-D glucopyranoside and Type IV collagenase from Clostridium histolyticum were from Sigma (St. Louis, MO); polyvinylidene difluoride membranes (Problot) were from Applied Biosystems (Foster City, CA); standard proteins for molecular weight determinations were from Pharmacia (Uppsala, Sweden); rabbit anti-human light chains, anti IgG, anti-IgA, and anti-IgM antibodies were from Serva (Heidelberg, FRG); Hybond C super membranes were from Amersham (Little Chalfont, United Kingdom); anti-kappa and anti-lambda light chains and anti-IgG (gamma chains) antibodies used for immunocytochemistry and immunoelectron microscopy were obtained from Dako-Dakopatts (Copenhagen, Denmark); anti-β2m monoclonal antibodies were provided by Dr. Vittorio Bellotti (Biochemistry Department, University of Pavia).

Patients

Nine patients on regular dialysis treatment for more than 10 yr were considered for this study. Eight suffered from carpal tunnel syndrome and underwent surgery for the removal of tendon amyloid plaques; one had synovial amyloidosis of the main joints and hip fracture and underwent hip joint replacement. All patients were clinically stable, and the initial course of their renal disease was in all cases unrelated to amyloidosis as evaluated by available clinical data (Table 1). Patients were informed of the experimental use of tissue samples obtained during surgery.

Purification of Amyloid Fibrils

Large amyloid fragments from the carpal tissues of four patients were used for extracting amyloid fibrils, which were purified following a combination of the methods proposed by Gejyo et al. (2) and Gorevich et al. (3). Surgical specimens of carpal tunnel amyloid tissue were carefully dissected from other material, kept on ice, and immediately processed for fibril purification. This material, homogenized in the presence of phosphate-buffered saline (PBS), pH 7.4 (2 mL of PBS/100 mg of tissue), was centrifuged at 50,000 rpm (Beckman L8–704; Beckman Instruments Inc., Fullerton, CA) for 30 minutes, and the supernatant was discarded. This step was repeated nine times; at this point, the optical density measurement at 280 nm was less than 0.050. The pellet material was homogenized in water following the procedure suggested by Gorevich et al. (2), and four aqueous fractions were obtained.

At the end of the water extraction procedure, the residual pellet was submitted to the more denaturing extractive procedure suggested by Gorevich et al. (3) with first a 6 M guanidine buffer and then a 2 M guanidine–4 M acetic acid buffer. The material extracted by the denaturing buffer was then dialyzed in 6- to 8-kg cut-off membranes against 200 vol of distilled water, and the resulting precipitate was frozen. The material extracted was analyzed by electron microscopy, demonstrating a fibrillar pattern in the water extraction sample, whereas a more amorphous pattern was detectable.

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Age (yr)</th>
<th>Time on Dialysis (months)</th>
<th>Cause of Renal Insufficiency</th>
<th>Clinical Signs of Amyloid Involvement</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>51</td>
<td>200</td>
<td>Pyelonephritis</td>
<td>Bilateral CTS, trigger finger, bone cysts</td>
</tr>
<tr>
<td>2</td>
<td>34</td>
<td>168</td>
<td>Pyelonephritis</td>
<td>CTS</td>
</tr>
<tr>
<td>3</td>
<td>76</td>
<td>152</td>
<td>Hypertension</td>
<td>CTS, bone cysts, spondyloarthropathy, hip fracture</td>
</tr>
<tr>
<td>4</td>
<td>69</td>
<td>184</td>
<td>Pyelonephritis</td>
<td>CTS, bone cysts</td>
</tr>
<tr>
<td>5</td>
<td>57</td>
<td>180</td>
<td>APKD</td>
<td>CTS</td>
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<td>CTS, bone cysts</td>
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<tr>
<td>7</td>
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<td>172</td>
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<td>CTS</td>
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<tr>
<td>8</td>
<td>56</td>
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</tr>
<tr>
<td>9</td>
<td>60</td>
<td>154</td>
<td>Unknown</td>
<td>CTS, bone cysts</td>
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a CTS, carpal tunnel syndrome; APKD, adult polycystic kidney disease.

Table 1. Patient characteristics
in the material obtained with the guanidine CH$_3$COOH buffer in reducing conditions.

**SDS-PAGE and Immunoblotting**

For electrophoresis, samples were boiled for 2 min in 250 mM Tris HCl (pH 6.8) containing 2.5% SDS and 5% B-mercaptoethanol. SDS-PAGE (total monomer (T) = 10 to 20%, cross-linkage (C) = 2.4%) was performed according to Laemmli (12), and proteins were alternatively stained with silver stain, Coomassie R 250, and colloidal gold (Bio-Rad Kit). Gels (0.7 mm thick) were cast by mixing two polyacrylamide solutions (10 and 20%) containing 2.4% BIS in 0.33% tetramethylethylenediamine. The staining gel was constant in acrylamide (5%) and BIS (0.13%) concentrations. Electrophoresis was performed overnight. For immunoblotting, proteins were transblotted electrophoretically to Hybond membranes with a Bio-Rad dry transblot cell. Transfer was carried out for 3 h with 300 mA in 20 mM Tris-glycine (pH 8). Polyclonal monospecific rabbit anti-human kappa and lambda light chains, anti-IgG, anti-IgA, and anti-IgM antibodies were used for immunoblotting, and the complex linked to nitrocellulose was visualized with alkaline phosphatase linked to goat anti-rabbit IgG in the presence of nitroblue tetrazolium and 5-bromo-4-chloro-3-indolyl phosphate. Washing steps were done according to the manufacturer’s instructions (Bio-Rad).

**Protein Content**

The total protein content was determined by the method of Lowry et al. (13). The quantitation of the kappa and lambda light chains was performed by an immunoenzymatic method with a pool of Bence-Jones proteins of different subclasses as standard and compared with the $\beta_{2m}$ content assayed by ELISA. Similar ELISA determinations were used for gamma chains and albumin.

**Protein Sequencing**

Proteins were transblotted electrophoretically to PVDF membranes in a semidry apparatus TE 70 Semiphore (Hoefer, San Francisco, CA). Transfer was continued for 1.5 h at 200 mA with 10 mM (cycloexylamino)1-propane sulfonic acid buffer (pH 11) with 6% methanol. Sequence analysis was recorded with a Millipore Pro-Sequencer 6625 (Bedford, MA), and the resulting phenylthiohydantoin amino acid derivatives were identified with the on-line Waters 600E 120A PTH analyzer (Bedford, MA). Protein homology was searched for in the EMBL Database with a FASTA program (Intelligenetics, Mountain View, CA).

**Tissue Preparation for Morphologic Examination**

Synovial tissue from the hip joint of one patient and tendon sheath samples surgically removed from four patients with dialysis-related amyloidosis were formalin fixed and paraffin embedded for light microscopy study; other small samples from two of these patients were fixed in 2.5% glutaraldehyde in 0.13 M phosphate buffer for 2 h. After being rinsed in phosphate buffer, part of the samples was postfixed in 1% osmium tetroxyde ($\text{OsO}_4$) in phosphate buffer and routinely processed for electron microscopy, whereas the remaining samples were dehydrated avoiding $\text{OsO}_4$ postfixation and embedded in London Resin White for the performance of immunoelectron microscopy.

**Immunohistochemistry**

Histology sections from paraffin-embedded samples were freed from paraffin, rehydrated, and when required, pretreated with pepsin. After endogenous peroxidase inhibition and preincubation with normal suine serum or normal rabbit serum, the sections were incubated with different primary antibodies: rabbit anti-human kappa and lambda light chains, anti-mouse kappa and lambda light chains, anti-mouse IgG, anti-mouse IgA, and anti-mouse IgM antibodies. The complex linked to nitrocellulose was visualized with alkaline phosphatase linked to goat anti-rabbit IgG in the presence of nitroblue tetrazolium and 5-bromo-4-chloro-3-indolyl phosphate. Washing steps were done according to the manufacturer’s instructions (Bio-Rad).

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**Figure 1.** Protein composition of amyloid material in the extracts of the purification procedure, following a combination of the techniques described by Gejyo et al. (2) and Gorevich et al. (3). In the final water extract, 10 to 15 $\mu$g/mL of $\kappa$ and $\lambda$ chains and 200 $\mu$g/mL of $\beta_{2m}$ were detectable, whereas no $\kappa$ or $\lambda$ chains were present in the last PBS washing. This finding points to the presence of light chains in the amyloid fibrils, whereas $\gamma$ chains and albumin are probably contaminants or loosely bound to the fibrils.
chains polyclonal antibody diluted 1:1,000, 1:2,000, and 1:4,000 in Tris-buffered saline, respectively, or mouse anti-human β2m monoclonal antibody undiluted. After being washed in Tris-buffered saline the sections were incubated with second biotinylated antibodies (suine anti rabbit Ig or rabbit anti mouse Ig) and the immunoreaction was shown with diaminobenzidine by the avidin biotin complex (ABC) method.

For the simultaneous labeling of kappa light chains and β2m, an indirect immunostaining method combining two different enzymatic reactions revealed by differently colored substances was used (14). In our experiment, the sections were first incubated with a mixture of normal suine serum and normal rabbit serum, than with a mixture of primary antibodies at the same dilution used in each single detection, and subsequently with a mixture of second antibodies. The immunoreactivity of kappa light chains was revealed by diaminobenzidine by a peroxidase antiperoxidase method, and the immunoreaction with β2m was revealed by alkaline phosphatase with nitroblue tetrazolium by the ABC method.

Immunoelectron Microscopy

Immunostaining was performed on ultrathin sections of London Resin White-embedded samples by the immunogold staining procedure. Sections collected on 300-mesh nickel grids were incubated in 1:30 normal goat serum in 0.015 M PBS with 0.2% bovine serum albumin and 0.02% NaN₃ and then in different primary antibodies: grid No. 1, rabbit anti-human kappa light chains polyclonal antibody diluted 1:1,000; grid No. 2, rabbit anti-human β2m polyclonal antibody diluted 1:200 (Dakoc); grid No. 3, mouse anti-human β2m monoclonal antibody undiluted; grid No. 4 (by a double-labeling technique), a mixture of rabbit anti-human kappa light chains polyclonal antibody diluted 1:1,000 in mouse anti-human β2m monoclonal antibody. Grids No. 5 and No. 6 were not incubated with primary antibodies, remaining in normal goat serum as negative controls. Incubation with primary antibodies was carried out overnight at 4°C. After several changes in washing buffers, grids No. 1, 2, and 5 were incubated with goat anti-rabbit second antibody conjugated with 10-nm colloidal gold particles (GAR-G10 Bio Cell, Cardiff, U.K.) diluted 1:10 in 0.02 M Tris HCI buffer with 1% BSA (pH 8.2); grids No. 3 and 6 were incubated with goat anti-mouse second antibody conjugated with 20-nm gold particles (GAM-G20 Bio Cell) diluted 1:10 in the same buffer; grid No. 4 was incubated with a mixture of GAR-G10 and GAM-G20 diluted 1:10. Incubation with second antibodies was continued for 1 h at room temperature. After being washed in buffer solutions, grids were counterstained with uranyl acetate and lead citrate and then observed in a Jeol JEM 1010 (Tokyo, Japan) electron microscope operating at 80 KV. In a subsequent experiment, the immunoreactivity to lambda light chains was also tested by the same methodology.

RESULTS

Kappa and lambda light chains could be detected in amyloid deposits by biochemical and light and electron microscopy immunocytochemical methods.

Biochemistry

The protein composition of amyloid fibrils was determined by SDS-PAGE, western blot, and ELISA during the sequential steps of preparation. As men-
TABLE 2. Amino acid sequence of 25-kd proteins extracted from amyloid fibrils of synovial deposits of 25 patients who underwent surgery for carpal tunnel syndrome

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<tr>
<td>Normal Light Chain</td>
<td>D</td>
<td>I</td>
<td>Q</td>
<td>M</td>
<td>T</td>
<td>Q</td>
<td>S</td>
<td>P</td>
<td>S</td>
<td>S</td>
<td>L</td>
<td>S</td>
<td>A</td>
<td>S</td>
<td>V</td>
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<tr>
<td>Patient 1</td>
<td>I/Q</td>
<td>Q</td>
<td>M</td>
<td>T/P</td>
<td>Q</td>
<td>S</td>
<td>P/Q</td>
<td>S/Y</td>
<td>L</td>
<td>S</td>
<td>A</td>
<td>S</td>
<td>V</td>
<td>G</td>
<td>D</td>
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<tr>
<td>Patient 2</td>
<td>I/Q</td>
<td>Q/D</td>
<td>M/L</td>
<td>T/P</td>
<td>Q</td>
<td>S</td>
<td>Q</td>
<td>S</td>
<td></td>
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<td></td>
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<tr>
<td>Patient 3</td>
<td>I</td>
<td>Q</td>
<td>L</td>
<td>T</td>
<td>Q</td>
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<tr>
<td>Patient 4</td>
<td>I</td>
<td>Q</td>
<td>M/L</td>
<td>T</td>
<td>Q</td>
<td>S</td>
<td>Q</td>
<td>S</td>
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*No value listed indicates undetermined amino acid.

Immunoelectron Microscopy

In ultrathin sections of samples processed for immunoelectron microscopy, amyloid filaments were detected. They were significantly packed in short bundles or aggregates, being only occasionally loosely arranged surrounding larger areas of tightly packed amyloid filaments.

DISCUSSION

Although the pathogenesis of β2m amyloidosis remains unclear, it has been suggested that elevated plasma levels of this protein play an important role in conditioning amyloidogenesis (15). This hypothesis is supported by the finding of a beta-pleated sheet structure (16) and the observation that spontaneous birefringent aggregates are produced by β2m when incubated in an in vitro system at a concentration higher than 0.3 mg/mL (17). Initially, it was suspected that the length of time spent on dialysis could be correlated with increased β2m plasma levels. However, a comparison of levels before and after 10 yr

Immunohistochemistry

In all samples, amyloid deposits displayed a strong Congo red positivity with the characteristic dichromia under polarized light (Figure 3a and b). In serial sections, they appeared to be immunoreactive both with anti-β2m and anti-kappa light chain antibodies (Figure 3c); the two colocalized antigens showed a distinct pattern of immunoreactivity in some areas (Figure 3c, arrows and arrowheads). Light microscopy studies also demonstrated reactivity to lambda light chains and gamma heavy chains. Control sections processed avoiding the primary antibody incubation were unstained. A control section with an antialbumin antibody was also unstained.

Electron Microscopy and Immunoelectron Microscopy

Amyloid filaments were detected in ultrathin sections of all samples. They were slightly curvilinear and haphazardly arranged, showing the characteristic ultrastructural configuration of β2m amyloid filaments. Most frequently, they appeared tightly packed in short bundles or aggregates, being only occasionally loosely arranged surrounding larger areas of tightly packed amyloid filaments.

In ultrathin sections of samples processed for immunoelectron microscopy, amyloid filaments with different aggregation patterns, as described above, also showed a different immunoreactivity pattern: tightly packed amyloid filaments were strongly immunolabeled by the anti-β2m monoclonal antibody (Figure 4) and we were weakly reactive to the anti-kappa light chain antibody (Figure 5). On the contrary, in a few areas of loosely arranged amyloid filaments their immunoreactivity to anti-kappa light chain antibody was very intense (Figure 6). When the two antigens β2m and kappa light chains were colocalized by a double-staining technique, the immunoreactivity of tightly packed (Figure 7) and loosely arranged (Figure 8) amyloid filaments to both antibodies was comparable with that obtained in each single localization. Immunoreactivity to lambda light chains was also found in the same specimens, showing a spatial arrangement similar to that of the kappa light chains (not shown).
of dialysis argues against this hypothesis (10). In addition, the plasma levels of β2m in patients with carpal tunnel syndrome undergoing regular dialysis do not differ from those found in patients not affected by any form of dialysis amyloidosis (10). Taken together, these data indicate that besides the simple accumulation of β2m, other factors may play a role in the process of fibrillogenesis.

This study shows that polyclonal kappa and lambda light chains are also a component of this peculiar form of amyloid. Our preliminary studies showed that in addition to β2m, uremic patients on regular dialytic treatment have high concentrations of a protein macromolecule (85 kd) in carpal synovia and also in plasma (18). This protein was defined as an aggregate of smaller molecular weight components, all reactant with thioflavin T; one of these was recognized as kappa light chain (25 kd). In this immunocytochemical study, we demonstrate that kappa and lambda light chains colocalize with β2m in amyloid deposits and that in some areas the two antigens maintain a distinct pattern of immunoreactivity both in light and electron microscopy. The latter allowed us to observe a loose arrangement of amyloid filaments in areas intensely labeled with the anti-kappa light chain antibody and a pattern of tightly packed amyloid filaments strongly immunoreactive with the anti-β2m antibody.

We failed to show complete immunoglobulins in the water extraction of β2m fibrils, but the free polyclonal kappa and lambda light chains can be detected in a ratio κ+λ/β2m of approximately 5/100 to 7.5/100. These light chains can be specifically extracted from β2m amyloid and do not represent a contamination because they are no longer present in the supernatant after nine PBS washings.

Although we cannot define the mechanisms of light chain deposition in dialysis-related amyloid tissue, we hypothesize that light chains might precipitate owing to their substantial instability in an aqueous environment. In this respect, it is known from other models of amyloidosis, where light chains do represent the pathogenic precursor, that these proteins assume an insoluble structure owing to both the high rate of glycosylation and a few possible puntiform amino acid substitutions (19,20): one of the most recurrent substitutions (L → M in position 4) was also found in this study. Protein sequencing (Table 2) allowed us to determine the polyclonal characteristics of amyloid-related immunoglobulin light chains.

It is difficult to explain why polyclonal light chains are present in amyloid fibrils of patients with dialysis amyloidosis. Although we were unable to demonstrate that gamma chains are also constituents of the amyloid fibrils, we did detect gamma chains by immunohistologic techniques in amyloid deposits of the same patients; moreover, immunoblots for gamma chains were positive when amyloid fibrils were treated with the guanidine/CH₃COOH extraction. Gamma chains could therefore be considered as contaminants or they might be loosely bound to the amyloid material. Al-

Figure 3. (a and b) A large amyloid deposit in subsynovial connective tissue stained by Congo red (a) showing green birefringence under polarized light (b); original magnification, x100. (c) Colocalization of β2m (blue, arrowheads) and kappa light chain (brown, arrows). Anti-β2m antibody undiluted, ABC method; anti-kappa light chain antibody 1:1,000 peroxidase antiperoxidase method; original magnification x100.
Figure 4. Tightly packed amyloid filaments intensely reactive with the anti-β2m antibody. Immunogold staining method, 20-nm gold particles; original magnification, × 56,700.

Figure 5. Tightly packed amyloid filaments weakly labeled by anti-kappa light chain antibody. Immunogold staining method, 10-nm gold particles; original magnification, × 73,300.

Figure 6. Loosely arranged amyloid filaments strongly immunoreactive with anti-kappa light chain antibody. Immunogold staining method, 10-nm gold particles; original magnification, × 73,300.

though it cannot be stated on the basis of our data, we can speculate that the presence of both light chains and intact immunoglobulins might be the expression of immune activity against a specific target or possibly β2m.

In this respect, it should be emphasized that low-affinity autoantibodies to β2m of the IgG class have been detected in sera from patients with a heterogeneous range of diseases such as lupus erythematosus (21,22) and rheumatoid arthritis (23,24). The finding of immunoglobulins in rheumatoid articular collateralous tissue (25) is also of interest, because immunoglobulins could represent an expression of immune activity of synovial lining cells both in rheumatoid arthritis and in dialysis amyloidosis. Therefore, it is theoretically possible that a similar process could take place in dialysis patients, leading to the formation of anti-β2m antibodies; this point is particularly relevant, considering the recent observation that β2m in patients on regular long-term dialysis undergoes a series of posttranslational chemical processes known as nonenzymatic glycosylation and browning (26), which could give more antigenic properties to the molecule.
In particular, Miyata et al. (26,27) found that most of the \( \beta_2m \)-forming amyloid fibrils have the characteristics of advanced glycation end-products (AGE) of the Maillard reaction and react with anti-AGE antibody as well as with an antibody against an Amadori product, an early product of the Maillard reaction, but normal \( \beta_2m \) does not react with either antibody. Moreover, AGE-\( \beta_2m \) enhances the direct migration (chemotaxis) and random cell migration (chemokinesis) of human monocytes in a dose-dependent manner and increases the secretion of tumor necrosis factor-alpha and interleukin (IL)-1\( \beta \) from macrophages. Those authors postulate that AGE-\( \beta_2m \) participates in the pathogenesis of dialysis-related amyloidosis as foci where monocytes/macrophages accumulate and initiate an inflammatory response that leads to bone and joint destruction: the induction of cytokine release would lead to local bone resorption and, at the same time, to the production of collagenase, causing matrix protein destruction. If such an inflammatory response with macrophage activation does occur, the discovery of the presence of Ig light chains and possibly complete Ig would not be surprising.

The finding of a pathogenic role of IL-1\( \beta \) was confirmed by Moe et al. (28), who found that the \( \beta_2m \)-induced calcium efflux from cultured neonatal mouse calvariae was blocked by an IL-1\( \beta \)-neutralizing antibody and by an IL-1\( \beta \) receptor antagonist. Tenosynovial tissues in patients with dialysis amyloidosis have also been shown to produce IL-1 and IL-6 (29), pointing to a similar pathogenic mechanism for the development of carpal tunnel syndrome. Little is known about the mechanism of amyloid fibril formation, and we cannot prove that light chains or immunoglobulins favor or delay this process, but the problem of \( \beta_2m \) solubility in the presence of interacting molecules, such as antibodies, is a central question in the pathogenesis of \( \beta_2m \) amyloidosis and might open new therapeutic perspectives.

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