Alterations of synovial tissue and their potential role in the deposition of $\beta_2$-microglobulin-associated amyloid

Goetz Ehlerding¹, Juergen Schaeffer¹, Werner Drommer², Toshio Miyata³, Karl Martin Koch¹ and Juergen Floege²

¹Division of Nephrology, Hannover Medical School, ²Department of Pathology, Hannover Veterinary School, Germany, and ³Institute of Medical Sciences and Department of Medicine, Tokai University School of Medicine, Isehara, Japan

Abstract

**Background.** Beta-2-microglobulin-associated amyloidosis (AB2M) is a frequent complication of long-term dialysis treatment. Uraemic retention of beta-2-microglobulin ($\beta_2$M) apparently constitutes the basis for AB2M. However, it is unclear why clinical manifestations are largely confined to osteoarticular tissues. It has been speculated that synovial inflammatory changes, induced by uraemia and/or dialysis therapy could predispose this tissue to amyloid deposition.

**Methods.** We investigated which local synovial alterations preceded or paralleled amyloid deposition. Using immunohistology we evaluated synovial leukocyte infiltration (B and T lymphocytes, monocytes/macrophages), cell proliferation, fibroblast activation (de novo expression of $\alpha$-smooth-muscle actin), the expression of extracellular matrix components (heparan sulphate proteoglycan, collagen types I, III, IV), and advanced glycation end-products (AGEs).

**Results.** Synovial AB2M was detected in 20 of 36 chronic peritoneal and haemodialysis patients and none of eight non-uraemic controls. Notably, non-AB2M synovial amyloid was present in six additional dialysis and three control patients. Cellular infiltration was largely restricted to patients with advanced AB2M deposits. The infiltrates consisted mainly of macrophages and progressed with increasing degrees of AB2M deposition. In advanced cases they exhibited characteristics of a foreign-body reaction. Other infiltrating leukocyte types, altered cell proliferation, or fibroblast activation were absent or uncommon in periarticular tissue of dialysis patients with and without AB2M. Neither dialysis treatment nor the presence of AB2M deposits appreciably altered the qualitative matrix composition in periarticular tissue. AGEs were present in AB2M deposits, the extracellular synovial matrix of dialysis patients (of both, patients with and without AB2M) and, to a lesser degree, in synovia of controls.

**Conclusions.** These data suggest that, except for AGE formation, alterations of none of the parameters assessed, and in particular no inflammatory tissue alterations, precede periarticular AB2M. Rather synovial tissue, possibly modified by AGES, seems to have an intrinsic propensity for amyloid deposition and inflammatory changes appear to only arise secondary to amyloid deposition.

**Key words:** advanced glycation end-products; amyloidosis; $\beta_2$-microglobulin; dialysis; immunohistology; lymphocytes; macrophages

Introduction

Since 1980, when Assenat first described amyloid material in synovial tissue of patients on long-term dialysis treatment [1], this new type of amyloidosis has emerged as a frequent and often debilitating complication in patients with end-stage renal disease. In 1985 $\beta_2$-microglobulin ($\beta_2$M) was identified as the precursor protein and major constituent of the amyloid deposits [2], resulting in the term AB2M for this type of amyloidosis.

It is widely accepted that the retention of $\beta_2$M, based on impaired renal clearance in uraemic individuals, is the main pathogenic process underlying AB2M formation [3,4]. Additional systemic factors may also contribute to the pathogenesis of AB2M by increasing the ‘amyloidogenicity’ of the precursor protein $\beta_2$M, including limited proteolysis [5], amino-acid replacement [6] or the formation of advanced glycation end products (AGEs) [7] of $\beta_2$M.

Even if the above mechanisms contribute to AB2M formation, they cannot explain why AB2M deposits are largely confined to osteoarticular structures, such as synovial membranes, the carpal tunnel, tendons, ligaments, cartilage and bone, while clinically relevant visceral AB2M deposition is exceptional [3,4]. Presumably, either constitutive properties of the osteoarticular tissues or properties acquired during
uraemia are responsible for the striking tissue predilection of AB2M. In this regard collagens and proteoglycans, in particular heparan sulphates, have been suspected to be important factors in the pathogenesis of AB2M deposition [8–10]. In addition, inflammatory cells and/or inflammatory lesions have repeatedly been described in the vicinity of AB2M deposits [11–18]. Since dialysis treatment is known to induce various inflammatory processes [19–25], it has also been speculated that local inflammation at the sites of AB2M predilection may induce or at least facilitate amyloid deposition [11–15, 26].

To clarify these later issues, we have investigated the tissue alterations that precede or accompany AB2M deposition in patients on haemodialysis for various lengths of time and have compared them to tissues obtained from non-uraemic control patients. Tissue parameters assessed included the degree of leukocyte infiltration, activation of resident synovial cells (as indicated by their increased or altered expression of intermediate filament cytoskeletal proteins), local cell proliferation as well as qualitative extracellular matrix protein alterations.

Subjects and methods

Patients and tissue samples

During routine joint surgery or autopsy specimens of periarticular tissue (joint capsule, cartilage and tendon) from shoulders, knees, hips, sternoclavicular joints as well as some specimens from the carpal tunnel were obtained from patients on regular dialysis treatment (n = 36, mean age 62 years, range 38–85 years) and controls without renal disease (n = 8, mean age 57 years, range 48–82 years).

Thirty-two of the dialysis patients were treated exclusively with haemodialysis (mean age 63 years, range 38–85 years), while two patients were treated exclusively with peritoneal dialysis (age 50 and 67 years, 8 and 16 months of peritoneal dialysis). The two further patients (age 57 and 61 years) switched after a period of 60 and 123 months on haemodialysis to peritoneal dialysis for 12 and 29 months. Only incomplete data about the history of dialysis regimes was available in several patients so that this issue could not be examined systematically. At the time of tissue sampling 12 patients were treated with standard Cuprophane® membranes, 12 patients were on low-flux “biocompatible” membranes (polymide or polysulphone) while six patients were treated with high-flux dialysis membranes. All haemodialysis patients were treated 4–5 h three times per week, while all four peritoneal dialysis patients were on daily automatic peritoneal dialysis (APD).

Further details of the patients, controls and specimens obtained are shown in Table 1.

Histology and immunohistology

After fixation (buffered 4% formaldehyde at pH 7.2 or methacarn solution: 60% methanol, 30% chloroform and 10% acetic acid) and embedding in paraffin, sections were stained with H & E using standard methodology and with Puchtel’s Congo-red as described [27,28]. If Congo red staining was positive, the presence of amyloid was confirmed using green birefringence under polarized light and additionally by immunoreactivity for serum amyloid P component.

For immunostaining, methacarn-fixed tissue was used. Sections of 6 µm were deparaffinized in xylene and stepwise descending ethanol concentrations. Endogenous peroxidase activity was blocked (0.75% H₂O₂) and normal serum (species of the secondary antibody, 20%) was used to block non-specific antibody binding.

Incubation with the various primary antibodies was carried out overnight at 4°C, the optimal working dilution for primary antibodies (see below) was determined with different reference tissues fixed in the same way (tonsils for B and T lymphocytes; subcutaneous calcification with foreign-body reaction for macrophages; tendons, cartilage and liver for the collagens and heparan sulphate proteoglycan; tissues with known types of amyloidosis). DAB (3,3′-diaminobenzidine-tetrahydrochloride) with 0.03% H₂O₂ was used as a chromogen after incubation with an appropriate biotinylated secondary antibody followed by incubation with streptavidin-labelled horseradish peroxidase. Mayer’s Haemalaun and Congo red was used for counterstaining.

In control specimens the primary antibodies were replaced by either irrelevant mouse monoclonal antibody or normal rabbit immunoglobulins. In these cases no tissue staining was observed (data not shown).

Immunohistochemistry—qualitative studies

Typing of amyloid. In every patient positive for amyloid according to the criteria mentioned above (Congo-red positive, green birefringence under polarized light and immunoreactivity for serum amyloid P component), immunostaining was performed to characterize the type of amyloid. The following antibodies directed against different types of amyloid precursor proteins were used:

| Table 1. Characteristics of the patients and tissue specimens investigated |
|-----------------------------|-----------------------------|
|                            | Dialysis patients | Controls |
|-----------------------------|-----------------------------|
| Individuals (n)             | 36                          | 8         |
| Age years (mean ± SD)       | 62±13                      | 57±12     |
| Source of tissue (n)        | autopsy  | 19 | 7 |
| surgery                     | 17 | 1 |
| Time on dialysis months     | 89; 5–221                   |
| Renal diseases (n)          | tubulointerstitial disease, | 2 |
| nephroclerosis               | 3 |
| glomerulonephritis          | 9 |
| diabetes mellitus           | 3 |
| secondary amyloidosis       | 2 |
| (unknown origin)            | 5 |
| algiesic nephropathy         | 4 |
| polycystic kidney disease   | 1 |
| chronic pyelonephritis      | 7 |
| others/unknown              | |
| Tissues obtained from (n)   | knees | 33 | 10 |
|                            | hips | 38 | 10 |
|                            | shoulders | 38 | 12 |
|                            | sternoclavicular joints | 6 | 1 |
|                            | carpal tunnel | 3 |
| Total                       | 118 | 33 |
Synovial alterations in dialysis amyloidosis

(a) monoclonal mouse IgG 2a-kappa antibody against human serum amyloid A (Dakopatts, Copenhagen, Denmark);
(b) polyclonal rabbit immunoglobulin fraction against human kappa-light chains (Dakopatts);
(c) polyclonal rabbit immunoglobulin fraction against human lambda light chains (Dakopatts);
(d) polyclonal rabbit immunoglobulin fraction against human prealbumin/transhylaurin (Dakopatts);
(e) polyclonal rabbit immunoglobulin fraction against human β2-M (Dakopatts);
(f) monoclonal mouse IgG antibody against human β2M (ICN Pharmaceuticals, Costa Mesa USA).

Cell infiltration, activation, and proliferation

Cells in the specimens were characterized by immunostaining using antibodies against several cellular antigens:
(a) monoclonal mouse IgG1 kappa antibody against the human leukocyte common antigen (Dakopatts);
(b) polyclonal rabbit immunoglobulin fraction against the CD3 antigen, specific for T lymphocytes (Dakopatts);
(c) monoclonal mouse IgG2a kappa antibody against the CD20 antigen, specific for B lymphocytes (Dakopatts);
(d) monoclonal mouse IgG1 kappa antibody against the CD68 antigen, specific for macrophages and macrophage derived synovial intimal type A cells [29] (Dakopatts);
(e) monoclonal mouse IgG2a kappa antibody against x-smooth muscle actin, which is expressed by a subset of activated fibroblasts [30] (Dakopatts);
(f) monoclonal mouse IgM kappa antibody against the proliferating cell nuclear antigen, an auxiliary protein to DNA polymerase d, present mainly in the S-phase of the cell cycle [31,32] (Dakopatts).

Extracellular matrix proteins

Synovial tissue specimens from hips and shoulders of six dialysis-patients with and without AB2M as well as from three controls were stained with antibodies against several matrix components:
(a) polyclonal rabbit IgG fraction against human collagen I (Institute Pasteur de Lyon, Lyon, France);
(b) polyclonal rabbit IgG fraction against human collagen III (Institute Pasteur de Lyon);
(c) monoclonal mouse IgG1 antibody against human collagen IV (Dakopatts);
(d) monoclonal mouse IgM kappa antibody against heparan sulphate proteoglycan (Seikagaku Tokyo Co, Tokyo, Japan).

AGEs

Synovial tissue specimens from 12 dialysis patients with and six patients without AB2M as well as from four controls were stained with:
(a) Rabbit anti-AGE IgG, that was raised by the immunization of rabbit with AGE-modified keyhole limpet haemocyanin (KLH), which was prepared by incubating KLH with 0.1 M D-glucose at 37 °C for 90 days in 0.4 M phosphate buffer (pH 7.4). IgG from immune serum was purified on protein A agarose. The IgG fraction was affinity purified further by adsorbing it to a CNBr-activated Sepharose 4B column containing immobilized AGE-modified BSA. IgG was then dialyzed against 20 mM sodium phosphate buffer (pH 7.4).
(b) Murine anti-AGE monoclonal IgG, reactive against N-carboxymethyl) lysine [33].

Both rabbit anti-AGE polyclonal and mouse anti-AGE monoclonal antibodies reacted with in vitro-prepared AGE-proteins such as AGE modified KLH and BSA, but not with their normal counterparts nor the early Maillard Amadori compounds [7].

Competition experiments to confirm the specificity of immunostaining were done with the rabbit anti-AGE IgG or mouse anti-AGE monoclonal IgG, which were preincubated for 30 min at 37 °C with an excess of AGE-modified BSA. AGE-modified BSA had been prepared by incubating BSA (essentially fatty free acid-free grade) with 0.1 M D-glucose at 37°C for 60 days in 0.1 M phosphate buffer (pH 7.4). Counterstaining with Congo-red was performed in all sections.

Immunohistochemistry—quantitative studies

Specimens of the joint capsule of hips, knees, shoulders and sternoclavicular joints with sufficient cross-sectional area for 20 different high power fields were used for morphometry (62 specimens from dialysed patients, 19 from controls). All smaller specimens (especially the transverse carpal ligament) or specimens with other than AB2M type of amyloidosis were excluded.

Sections fulfilling the above criteria were immunostained for the CD68 antigen and counterstained with Congo-red. For morphometry we used an image analysis system (Leitz, Wetzlar, Germany). The relative amount of amyloid in one section was estimated with a point counting method in 20 high-power fields in every section and categorized as:

negative: no amyloid;
minute: >0 but <1% of cross-sectional area positive;
marked: >1% of cross-sectional area positive.

Simultaneously, infiltrating CD68 positive monocytes/macrophages were quantified on a semi-quantitative scale in the same 20 fields of view (counting of individual cells was not possible because of the presence of large, confluent monocyte/macrophage infiltrates in several specimens):
grade 0 = no CD68 positive cells;
grade 1 = diffuse distribution of maximally 20 CD68 positive cells per high-power field;
grade 2 = 20–100 CD68 positive cells per high-power field with focal loose accumulation of cells;
grade 3 = more than 100 CD68 positive cells per high-power field, in part with confluent infiltrates, in which individual cells could no longer be delineated;
grade 4 = homogenous, confluent infiltrates of CD68 positive cells.

On 10 intersections of gridlines with the surface of the synovial membrane the number of synovial type A cells were determined semiquantitavely:
grade 0 = no CD68 positive cells;
grade 1 = a single layer of CD68 positive cells;
grade 2 = 2–3 layers of CD68 positive cells on the surface of the synovial membrane;
grade 3 = more than 3 layers of CD68 positive cells on the surface of the synovial membrane.

The mean score for synovial type A cells per section was calculated using the 10 individual scores.
Statistical evaluation

Data are given as the mean ± SD. Statistical significance was evaluated using ANOVA and Fisher’s PLSD test with statistical significance defined as \( P < 0.05 \).

Results

Frequency and type of amyloidosis

Amyloid was found in at least one of the specimens obtained from 26 of 36 patients on dialysis. Notably, only 20 of the 26 amyloid-positive dialysis patients had AB2M amyloid, while other types were detected in the six remaining patients (Table 2). A mixed synovial AB2M and AA type amyloidosis was detected after 146 months of haemodialysis in one 69-year-old patient, who suffered from chronic renal failure due to secondary amyloidosis of unknown origin (Table 2).

Time on dialysis was 106 (range 14–210) months in AB2M negative vs 160 (range 8–221) months in AB2M-positive patients. The mean age was 54 years (45–73 years) in AB2M-negative patients vs 61 years (38–85) in AB2M-positive patients (difference not significant).

In the specimens obtained from non-uraemic patients, amyloid was detected in three of eight controls. One 82-year-old patient with traumatic femoral fracture had massive, transthyretin immunoreactive amyloid deposits of the hip-joint capsule. Two others (55 and 63 years) had minute amyloid deposits that could not be classified with the antisera employed in this study (Table 2).

Localization of amyloid deposits

Joints in dialysis patients with AB2M were affected with variable frequency. In all patients from whom a sternoclavicular specimen had been obtained (5/20 AB2M-positive patients) this tissue contained AB2M. AB2M was detected with decreasing frequency in hips, shoulders and knees (Table 3). In the carpal ligament, AB2M was never detected in the few specimens examined, which is consistent with a previous report [34] and which may indicate that in the carpal tunnel the tendon sheats rather than ligaments are preferentially affected by AB2M deposition.

AB2M was found in the synovial membrane in diffuse or sometimes focal deposits (Figure 1a,b). The extent of amyloid deposition in synovial tissue ranged from less than 0.05 to about 70% of the cross-sectional area. Cartilage from joints with synovial amyloid deposits was always involved as well and showed superficial, ‘band like’ deposition of AB2M (Figure 1c).

Leukocyte infiltration

Immunostaining for the leukocyte common antigen (CD45, a pan-leukocyte marker) demonstrated leucocytes or leukocyte-derived cells in all synovial specimens investigated. Further characterization of these cells showed that synovial lymphocyte infiltration was very rare in both dialysis patients and controls. Their occurrence was largely confined to specimens obtained during hip replacement surgery or specimens with soft-tissue calcification. If lymphocytic infiltration was detected, it was mostly composed of T-cells (Figure 2a). The demonstration of B cells was exceptional in all specimens investigated.

The predominant infiltrating cells in synovial specimens with or without AB2M from dialysis patients or controls were CD68 positive. These cells are either infiltrating monocytes/macrophages or resident, monocyte-derived synovial intimal type A cells. The number of resident synovial type A cells was variable in all groups, and the scores were not different between the groups.

Synovial infiltration by monocyte/macrophages was either diffuse or focal (Figure 2b,c). Predominantly in the vicinity of large amyloid deposits multinucleated, CD68-positive giant cells were frequently encountered (Figure 2d). Similar infiltrations of macrophages with giant-cell formation occurred near soft-tissue calcifications in patients with and without AB2M.

Morphometry was performed on 62 specimens from dialysis patients: 10 specimens were obtained from dialysis patients with no AB2M in any location examined; 12 specimens were AB2M negative but AB2M had been detected in other specimens in these patients; 16 specimens presented minute AB2M deposits, and 24 had marked amyloid deposits. As controls 19 specimens from non-uraemic patients were available.

Table 2. Presence and type of amyloid detected in the individuals studied

<table>
<thead>
<tr>
<th>Type of amyloid</th>
<th>Dialysis patients (n=36)</th>
<th>Controls (n=8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AB2M</td>
<td>19</td>
<td>–</td>
</tr>
<tr>
<td>AB2M/AA</td>
<td>1</td>
<td>–</td>
</tr>
<tr>
<td>Transthyretin (senile amyloid)</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Transthyretin (senile)/AA</td>
<td>1</td>
<td>–</td>
</tr>
<tr>
<td>AA</td>
<td>1</td>
<td>–</td>
</tr>
<tr>
<td>κ/λ light chain</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Unknown</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Total positive</td>
<td>26</td>
<td>3</td>
</tr>
</tbody>
</table>

Table 3. Presence of amyloid in different joints of AB2M-positive haemodialysis patients

<table>
<thead>
<tr>
<th>Joints</th>
<th>AB2M positive specimens (n)</th>
<th>AB2M negative specimens (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Knees</td>
<td>11</td>
<td>9</td>
</tr>
<tr>
<td>Shoulders</td>
<td>19</td>
<td>6</td>
</tr>
<tr>
<td>Hips</td>
<td>25</td>
<td>3</td>
</tr>
<tr>
<td>Sternoclavicular joints</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>Transverse carpal ligament</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Total</td>
<td>60</td>
<td>21</td>
</tr>
</tbody>
</table>
Grading of CD68-positive cells showed no difference between controls and amyloid-negative specimens from dialysis patients without AB2M in any location (Table 4). The CD68 scores in AB2M positive dialysis patient specimens without AB2M in the section investigated or with minute AB2M deposits (<1% of cross-sectional area) were slightly elevated, but not significantly different from controls (Table 4). In specimens with marked AB2M deposition, CD68-positive cell scores were significantly increased as compared to AB2M-negative dialysis patients and controls (Table 4).

In specimens with marked deposition of AB2M, infiltration of CD68-positive cells was not a constant finding: In some specimens with very large AB2M deposits, a normal CD68 score was obtained. On the other hand several specimens showed marked CD68-cell positive cell infiltration without concurrent AB2M deposition. However, in these cases a close spatial relationship of AB2M deposits and CD68-positive cell infiltration cannot be ruled out, since no serial sections were analysed.

In the cases in which specimens of cartilage were obtained in addition to the synovial membrane specimens from the same joint, AB2M deposition in hyaline cartilage was never accompanied by monocyte/macrophage infiltration.

**Synovial cell proliferation and fibroblast-activation**

Positive immunostaining for PCNA was exceptional and could only be demonstrated in some sections inside the vessel wall or synovial layer (Figure 3a), indicating that no pathological cell proliferation, occurred in the tissues investigated. No difference of the staining pattern was noted between the patient groups or between specimens with and without AB2M.

Alpha-smooth muscle actin was constitutively expressed in smooth muscle cells of vessel walls in all specimens and was normally absent in the interstitium of the synovial tissue (Figure 3b). De novo expression of α-smooth-muscle actin in fibroblasts, indicating the conversion to a myofibroblast phenotype [30], was never detected in specimens obtained from either dialysis patients or controls.

**Matrix components**

In all specimens from AB2M-positive and -negative joints as well as from controls spatial distribution and intensity of collagen types I, III and IV and heparan sulphate proteoglycan were not different (Figure 4a–d).

Immunostaining for heparan sulphate proteoglycan was generally weak and the staining pattern was not different in patients with and without AB2M and in controls. The strongest immunostaining was found in the walls of small vessels (Figure 4d) while the interstitium only sometimes was diffusely or focally positive for heparan sulphate proteoglycan. In the vicinity of and inside single AB2M amyloid deposits a mild
enhancement of immunoreactivity for heparan sulphate proteoglycan could be demonstrated (Figure 4e), but most amyloid deposits were negative for heparan sulphate proteoglycan (Figure 4d). Heparan sulphate proteoglycan positive and negative amyloid deposits could be found in the same section.

Advanced glycation end-products

Staining of variable intensity for AGEs was detected in the interstitium of all synovial specimens (Figure 5a). In general, the intensity of staining was stronger in specimens from dialysis patients than in control specimens. Preincubation with AGE-modified bovine serum albumin completely abolished the staining (Figure 5b), suggesting that the staining was specific. AGEs were also detected in some, albeit not all AB2M deposits (Figure 5c) preferentially those of advanced cases. Interstitial AGE staining was not appreciably altered in the vicinity of the AB2M.

Discussion

In the present study we have attempted to unravel reasons for the striking predilection of AB2M deposits for synovial tissues [3,4]. Several important findings have emerged from this study. First, unless osteoarticular amyloid was classified by immunohistochemistry, we would have mistaken a significant percentage of amyloid deposits in dialysis patients as AB2M. It is also noteworthy, that we detected clinically asymptomatic synovial amyloid deposits (of non-AB2M origin) in three of eight control patients. Similar to this later observation, others have detected non-AB2M amyloid in 29–58% of synovial tissue samples from non-uraemic, routine surgery or autopsy patients [35–37]. Taken together, these findings suggest that synovial tissue may have an intrinsic propensity for amyloid deposition. Nevertheless, the frequency of clinical manifestations of AB2M in synovial or osteoarticular structures is much higher than in other types of amylo-
Vimentin, this finding cannot be related to di
numbers (Table 4). Since patients with and without showed that these cells are capable of producing amyl-
tissues, we were unable to detect either qualitative or with peripheral monocytes.
main finding of the present study was that in 10 chronic synovia [12,13,17,26,43], i.e. an attempted phagocyt-
resemble observations published previously [12,13,15– findings to the uraemic synovial tissue di
Y
these findings in the advanced AB2M amyloid cases is indicative of a reactive foreign-body reaction. All fibrils was not a consistent finding [44]. All these
cases with advanced amyloid deposition. Frequently in formed in very few cases only [44,45], that the capacity
appearance of AB2M in the synovia and a significantly the macrophages. However, in this respect it has to be
therapy. Rather, increased synovial monocyte
/
oidoses. Consequently there has been considerable speculation about the potential role of uremia and/or synovial inflammatory processes triggered by dialysis in the pathogenesis of AB2M [38,39]
To assess synovial inflammatory processes and if so, their temporal relationship with AB2M, we first investiga
ted whether cellular infiltration could be demonstra
in the synovial tissues of chronic dialysis patients. As reported previously [29,40,41] and con
firmed in the present study, most leukocytes in normal synovial tissue are monocyte/macrophages, while other types of leukocytes are only rarely encountered. The main finding of the present study was that in 10 chronic dialysis patients with no evidence of AB2M in synovial tissues, we were unable to detect either qualitative or more subtle quantitative changes of synovial leucocyte numbers (Table 4). Since patients with and without AB2M had similar durations of regular dialysis treat
ment, this finding cannot be related to different lengths of exposure to either the uraemic milieu or dialysis therapy. Rather, increased synovial monocyte/macro
phage counts appeared to be closely linked to the appearance of AB2M in the synovia and a significantly increased infiltration of the cells was only observed in cases with advanced amyloid deposition. Frequently in these later cases, we noted giant-cell formation, which is indicative of a reactive foreign-body reaction. All these findings in the advanced AB2M amyloid cases resemble observations published previously [12,13,15–17,26,42,43]. Since neither we nor others have access to longitudinal samples from chronic dialysis patients, we cannot exclude with certainty that transient micro-inflammation may precede and form a nidus for AB2M formation. However, the fact that we did not observe histological findings corresponding to this hypothesis, either in amyloid free dialysis patients, or in early AB2M cases, argues strongly against this potential mechanism. Our observation, that synovial monocyte/macro
phage infiltration and giant-cell formation occurs predominantly in advanced cases and is absent in non-AB2M specimens, suggests, that synovial monocyte/macrophage infiltration and giant-cell formation have to be regarded as responses to rather than causes of amyloid formation.
Our hypothesis is also consistent with the observa
tion of anti-β2-microglobulin-positive fibrils inside ves
icles of macrophages or multinucleated cells of uraemic synovia [12,13,17,26,43], i.e. an attempted phagocyt
osis of the fibrils. On the other hand, in vitro studies with peripheral monocytes/macrophages [44] or syn
ovial cells [45] obtained from uraemic individuals showed that these cells are capable of producing amyloid like fibrils or Congo-red material respectively.
Consequently it might be argued that the presence of AB2M fibrils in uraemic macrophages in vivo may indicate amyloid formation by these cells, which would challenge our hypothesis of a purely reactive role of the macrophages. However, in this respect it has to be noted that the in vitro studies cited above were performed in very few cases only [44,45], that the capacity of monocytes/macrophages to produce amyloid like fibrils was not a consistent finding [44]. All these caveats render an extrapolation from the in vitro findings to the uraemic synovial tissue difficult.
Besides leukocytic infiltration, increases in local cell proliferation and activation of resident cells, in particu
lar fibroblasts, also characterize synovial inflammation and have been demonstrated, for example, in rheumat
oid arthritis, osteoarthritis or following trauma [46]. To screen for cell proliferation and activation in our

<table>
<thead>
<tr>
<th>Table 4. Results of morphometry</th>
</tr>
</thead>
<tbody>
<tr>
<td>Relative amount of amyloid</td>
</tr>
<tr>
<td>(% of cross-sectional area per specimen)</td>
</tr>
<tr>
<td>None</td>
</tr>
<tr>
<td>Age (years)</td>
</tr>
<tr>
<td>Time on dialysis (months)</td>
</tr>
<tr>
<td>Minute (&gt;0% but &lt;1%)</td>
</tr>
<tr>
<td>Age (years)</td>
</tr>
<tr>
<td>Time on dialysis (months)</td>
</tr>
</tbody>
</table>

Relationship between the amount of amyloid and infiltration by CD68-positive cells in synovial tissue of 29 dialysis patients and five controls. All individuals with other than AB2M types of amyloid were excluded from this analysis. Data are means ± SD and (number of specimens examined).

Also mean age and time on dialysis are shown. Since more than one specimen from certain patients may be analysed in some groups, a statistical evaluation is not possible.

*Significant difference vs controls; **significant difference vs dialysed patients without AB2M.
AB2M deposits induce little activation of local synovial cells.

Apart from inflammatory changes, the composition of the extracellular matrix and/or its alterations in uraemia could also favour AB2M deposition. In an in vitro assay, Homma et al. have detected a high affinity of collagen for β2M [10]. This phenomenon might contribute to the pathogenesis of AB2M, since the synovial tissues we examined, although not showing any qualitative alterations from the normal staining pattern, were rich in collagens. Nevertheless the role of collagen in the amyloidogenesis has to remain speculative, since the above study [10] used a collagen preparation from skin, i.e. a site not normally involved in AB2M deposition [47]. Apart from the collagens, proteoglycans are presumed to play a role in amyloid fibril formation [8,9,48,49]. In this respect it is noteworthy that we were able to detect heparan sulphate proteoglycan, a normal constituent of the synovial membrane [50], in some AB2M deposits. However, whether heparan sulphate proteoglycans have a role in the onset of AB2M cannot be deduced from our study since they were not a constant feature in the amyloid deposits and showed no apparent relation to the severity of the amyloidosis.

In addition to the composition of the extracellular matrix, chemical modifications of physiologically expressed matrix proteins could also contribute to AB2M deposition. Recently it has been noted that sugar cross-linking of proteins, i.e. AGE formation, is markedly enhanced in uraemic patients and AGE plasma levels in uraemia increase by an order of magnitude [51]. AGE-modified β2M has also been detected in AB2M and has been suggested as playing an important role in the amyloidogenesis [33,52]. The current study confirms these data and extends them by showing that AGE formation is not confined to the tissue. Since the latter finding was also noted in uraemic patients without amyloid deposits, an attractive hypothesis to be tested in future studies is whether AGE-modified extracellular matrix promotes the formation of AB2M.

In conclusion, three major findings have evolved from this study. (i) The synovial membrane is frequently affected by amyloid deposition of various origins, in both uraemic and non-uraemic patients. This indicates that the synovial tissue per se may promote amyloid formation. (ii) Inflammatory changes, which sometimes exhibit characteristics of a foreign-body reaction, appear to arise in response to AB2M deposition and are unlikely to contribute to its initiation. (iii) AGE formation in the synovial extracellular matrix but no qualitative matrix protein alterations preceded amyloid formation. Whether AGE formation indeed renders the synovial extracellular matrix more amyloidogenic remains to be elucidated.

Acknowledgements. The technical support of K. Franke and K. Rohn is gratefully acknowledged. This study was supported by a grant from the Deutsche Forschungsgemeinschaft (SFB 244/C6).
Fig. 4 a–d. Immunostaining for different matrix components. (a) Positive immunostaining for collagen I is abundant in the synovial membrane obtained during autopsy from a patient after 96 months of regular dialysis treatment; (b) immunostaining for collagen III is nearly absent. (c) Positive immunostaining for collagen IV is confined to the basement membrane of the vessel wall (arrows). (d) Heparan sulphate proteoglycan is constitutively expressed in the vessel wall in the synovial membrane of the same patient as Figure 3a (arrows). (e) Amyloid deposits demonstrated either a mild positive immunoreactivity (arrows) or (d) were negative for heparan sulphate proteoglycan immunostaining (arrowheads). Original magnifications (a–d) × 100, (e) × 400.
References

Synovial alterations in dialysis amyloidosis


Received for publication: 28.8.97
Accepted in revised form: 11.1.98