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Kenichi Ohashi · Ryuko Kawai · Mitsuru Hara
Yasunori Okada · Shintaro Tachibana
Yosuke Ogura

Increased matrix metalloproteinases as possible cause of osseoarticular tissue destruction in long-term haemodialysis and β_2-microglobulin amyloidosis

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Abstract Immunolocalization of matrix metalloproteinases (MMPs) and tissue inhibitors of matrix metalloproteinases (TIMPs) in periarticular tissues of β_2-microglobulin amyloidosis patients was investigated. MMP-1 (interstitial collagenase) the most strongly expressed of the MMPs, was localized in the synovial lining cells, mesenchymal cells in granulation tissue and nodular amyloid deposits, and chondrocytes within areas of cartilage erosion. Expression of MMP-1 was correlated with the degree of macrophage infiltration and synovial cell hyperplasia, but it was not correlated with the degree of amyloid deposition or haemodialysis period. Expression of MMP-1 appeared more intense than that of TIMP-1 and TIMP-2 in highly inflammatory cases. MMP-2 was mildly expressed in the interstitial fibroblasts and MMP-3 was faintly stained in the extracellular matrix of the synovial membrane. MMP-9 (gelatinase B) was found to be strongly positive in the osteoclasts which increased in the progressing osteolytic lesion from the destructive arthropathy. These results suggest involvement of MMPs in inflammation with an imbalance between expression of MMPs and TIMPs being closely related to pathogenesis of the destructive arthropathy.

Key words β_2-Microglobulin amyloidosis · Matrix metalloproteinases · Tissue inhibitors of metalloproteinases · Destructive arthropathy · Bone cyst

K. Ohashi (✉)
Department of Pathology, Faculty of Medicine, Tokyo Medical and Dental University, 1–5–45, Yushima, Bunkyo-ku, Tokyo, 113 Japan
Fax: (81) 3–5803–0123

K. Ohashi · R. Kawai · M. Hara
Department of Pathology, Toranomon Hospital, 2–2–2, Toranomon, Minato-ku, Tokyo, 105 Japan

K. Ohashi · M. Hara
Okinaka Memorial Institute of Medical Research, 2–2–2, Toranomon, Minato-ku, Tokyo, 105 Japan

Y. Okada
Department of Molecular Immunology and Pathology, Cancer Research Institute, Kanazawa University, 13–1, Takara-machi, Kanazawa, Ishikawa, 920 Japan

S. Tachibana
Department of Orthopaedic Surgery, Toranomon Hospital, 2-2-2, Toranomon, Minato-ku, Tokyo, 105 Japan

Y. Ogura
Kidney Center, Toranomon Hospital, 2-2-2, Toranomon, Minato-ku, Tokyo, 105 Japan

Introduction

The systemic amyloidosis which is frequently seen in patients with long-term haemodialysis, namely β_2-microglobulin amyloidosis, has attracted a good deal of attention [16, 40]. The constituent amyloid protein was identified as β_2-microglobulin (β2M), with a calculated molecular weight of 11,000 daltons [8], and previous pathological studies have revealed this type of amyloidosis to be characterized by two features: amyloid deposition showing a preferential tissue distribution (in the periarticular tissue and intervertebral discs) and an inflammatory reaction, featuring macrophage infiltration and synovial cell hyperplasia, usually observed around the amyloid deposits [14]. Destructive changes in joints (destructive arthropathy) occur in long-term haemodialysis patients, but the mechanisms underlying the destruction of cartilaginous and osseous tissues and relationship between such changes and β2M amyloid deposits themselves are not clear.

A number of classes of proteinases, including serine proteinases, cathepsins and matrix metalloproteinases (MMPs), have been implicated in the degradation of extracellular matrix components in various diseases. Among them, the MMPs such as interstitial collagenase (MMP-1), gelatinases (MMP-2 and MMP-9) and stromelysin-1 (MMP-3), and their specific inhibitors, tissue inhibitors of metalloproteinases (TIMPs), have been the subject of much study [4, 17, 22, 23, 27, 33–36]. An imbalance between the amounts of MMPs and TIMPs is considered to be a determinant of the joint destruction during the course of rheumatoid arthritis (RA) and in the

mechanism of tumour invasion and metastasis [18, 21, 22]. However, no information is available on the expression and localization of MMPs and TIMPs in the tissue of haemodialysis-related amyloidosis.

In the present study, we investigated immunolocalization of MMP-1, -2, -3 and -9, and TIMP-1 and -2 in the periarticular tissues of haemodialysis-related amyloidosis patients to assess their possible role in destructive changes. In addition, expression of MMPs and TIMPs in RA and non-specific synovitis was examined for comparison.

Materials and methods

This study was performed using surgical operation specimens from 34 haemodialysis-related amyloidosis patients (33 with carpal tunnel syndrome and 1 with destructive arthropathy at the hip joint), 2 RA patients (carpal tunnel syndrome) and 2 non-specific synovitis patients (carpal tunnel syndrome). Synovium and transverse ligament samples were resected from the carpal tunnel syndrome patients. In 22 of the amyloidosis patients with carpal tunnel syndrome, amyloid deposits were mainly observed in the synovium, with band-like deposits in the synovial membrane and/or nodular deposits in the subsynovial connective tissue. Resected synovium from these 22, the 2 RA patients and the 2 non-specific synovitis patients was used for the present immunohistochemical study. In the remaining 11 amyloidosis patients with carpal tunnel syndrome, nodular amyloid deposits were observed only in the transverse ligament, and samples of the latter were therefore investigated. From the destructive arthropathy patient, the femoral head, including the synovium, articular capsule and bone, was resected and used for the study. All the cases of amyloidosis, RA and non-specific synovitis examined were pathologically diagnosed at the Department of Pathology, Toranomon Hospital, Tokyo. The period of haemodialysis, mainly performed using cuprophane membranes, ranged from 8 to 25 years. Synovium, ligament and femoral head specimens were fixed in buffered formalin solution and routinely processed to paraffin blocks. Bone tissue from the destructive arthropathy patient was decalcified with EDTA before paraffin embedding. From 9 amyloidosis patients, 2 RA patients and 2 non-specific synovitis patients, fresh frozen specimens of synovial tissue were also obtained, snap-frozen in dry ice-acetone and stored at –80°C until use for the immunohistochemical analysis.

Paraffin-embedded specimens were cut serially at 4 μm thickness and deparaffinized. Frozen specimens were cut serially at 8 μm thickness. In all amyloidosis cases, Congo red staining was performed according to the method of Puchtler et al. [38]. Amyloid deposits were confirmed histologically by positive staining for Congo red and characteristic green birefringence under polarized light. To check the present cases for amyloid heterogeneity, immunostaining for β2M, amyloid A protein, κ-light chain, λ-light chain and prealbumin (DAKOpatts) was also conducted. Foci of positive staining in each case were compared with the site of amyloid deposits. In the present series, all amyloid deposits were positively stained only for β2M and were negative for amyloid A protein, κ-light chain, λ-light chain and prealbumin.

MMP-1 (41–1E5), MMP-2 (75–7F7), MMP-3 (55–2A4), MMP-9 (56–2A4), TIMP-1 (50–3D2) and TIMP-2 (67–4H11) were immunostained using monoclonal antibodies provided by Dr. K. Iwata, Fuji Chemical Industries, Ltd., Takaoka, Japan. These antibodies have been characterized and reported previously [5–7, 29, 42]. All the antibodies for MMPs recognize both zymogen and active forms. Concentration of the primary antibodies used for immunostaining were 1 μg/ml for MMP-1, 4 μg/ml for MMP-2, 8.3 μg for MMP-3, 1 μg for MMP-9, 10 μg for TIMP-1 and 1 μg for TIMP-2. In earlier studies, proteoglycans, such as chondroitin sulphate and heparan sulphate, were found to be consistently increased in amyloid deposits [31, 32]. A monoclonal antibody against chondroitin sulphate (CS56, Seikagaku-kogyo) was therefore applied to investigate the characteristics of MMP-positive cells. The relationship between the degree of MMP expression and that of macrophage infiltration was also investigated using a monoclonal antibody against CD68, a membrane antigen of macrophages (Kp-1, DAKOpatts). Monoclonal antibodies against MMP-2, 3, TIMP-1 and TIMP-2 reacted only with frozen sections. Monoclonal antibodies against MMP-1, MMP-9, chondroitin sulphate and CD68 proved useful for both frozen and paraffin sections, with immunoreactivity in the two cases being essentially the same.

Frozen sections were fixed in acetone for 5 min and subsequently immersed in methanol containing 0.3% (v/v) H_2O_2 for 20 min, washed with phosphate-buffered saline (PBS), incubated in either normal horse or goat serum (DAKOpatts) for 10 min depending on the species in which the biotinylated antibody had been raised, and the primary antibody incubation was then per-

Table 1 Immunohistochemical data for matrix metalloproteinases (*MMPs*) and their tissue inhibitors (*TIMPs*) in frozen sections of synovial tissue. Cases 1, 2: non-specific synovitis patients; cases 3, 4: rheumatoid arthritis patients; cases 5–13: haemodialysis-related amyloidosis patients. Amyloid deposition and cell infiltration: –, none; 1+, mild; 2+, moderate; 3+, severe. Synovial cell proliferation: –, flat; 1+, focal faint proliferation; 2+, mild villous change; 3+, moderate villous change; 4+, marked villlous change with piling up of synovial lining cells. Immunoreactivity: –, negative; 1+, focal faint positive staining; 2+, moderately positive staining; 3+, diffuse strong positive staining

Case no.	Haemodialysis period (years)	Amount of amyloid deposition	Synovial cell proliferation	Cell infiltration	Immunoreactivity					
					MMP1	MMP2	MMP3	MMP9	TIMP1	TIMP2
1	–	–	2+	1+	1+	–	–	1+	1+	–
2	–	–	2+	1+	1+	–	–	1+	1+	–
3	–	–	3+	2+	3+	1+	–	1+	1+	–
4	–	–	4+	3+	2+	1+	1+	3+	1+	2+
5	11	2+	1+	1+	1+	–	–	1+	–	–
6	11	1+	3+	1+	2+	–	–	1+	1+	–
7	12	2+	2+	1+	1+	–	–	1+	1+	2+
8	13	2+	2+	1+	–	–	–	–	1+	1+
9	14	1+	3+	2+	2+	1+	–	2+	1+	1+
10	15	1+	2+	1+	1+	1+	–	–	1+	1+
11	22	2+	2+	2+	2+	–	–	1+	1+	–
12	23	3+	2+	1+	1+	–	–	1+	1+	2+
13	25	3+	3+	1+	2+	–	–	–	1+	1+

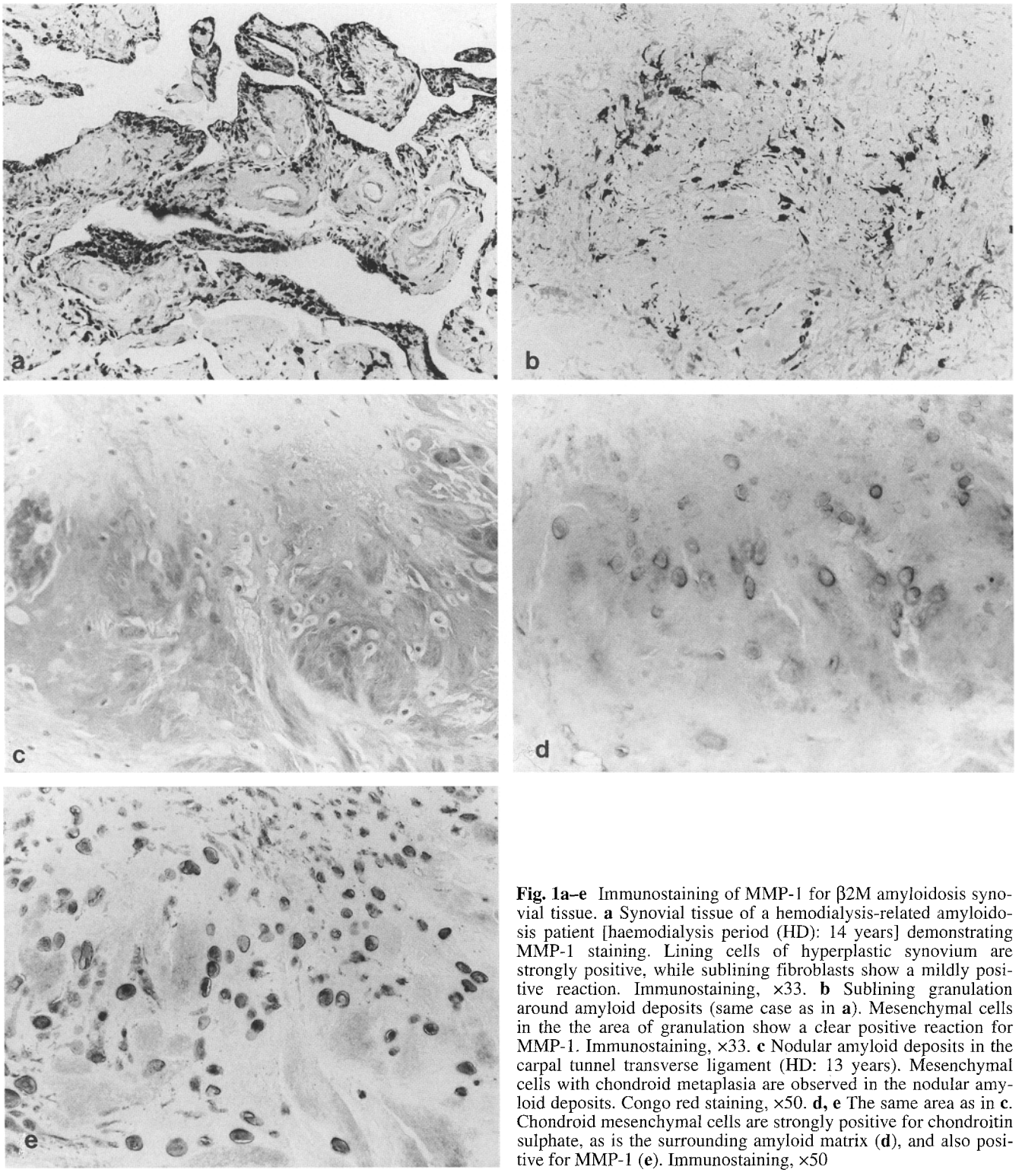

Fig. 1a–e Immunostaining of MMP-1 for β2M amyloidosis synovial tissue. **a** Synovial tissue of a hemodialysis-related amyloidosis patient [haemodialysis period (HD): 14 years] demonstrating MMP-1 staining. Lining cells of hyperplastic synovium are strongly positive, while sublining fibroblasts show a mildly positive reaction. Immunostaining, ×33. **b** Sublining granulation around amyloid deposits (same case as in **a**). Mesenchymal cells in the the area of granulation show a clear positive reaction for MMP-1. Immunostaining, ×33. **c** Nodular amyloid deposits in the carpal tunnel transverse ligament (HD: 13 years). Mesenchymal cells with chondroid metaplasia are observed in the nodular amyloid deposits. Congo red staining, ×50. **d, e** The same area as in **c**. Chondroid mesenchymal cells are strongly positive for chondroitin sulphate, as is the surrounding amyloid matrix (**d**), and also positive for MMP-1 (**e**). Immunostaining, ×50

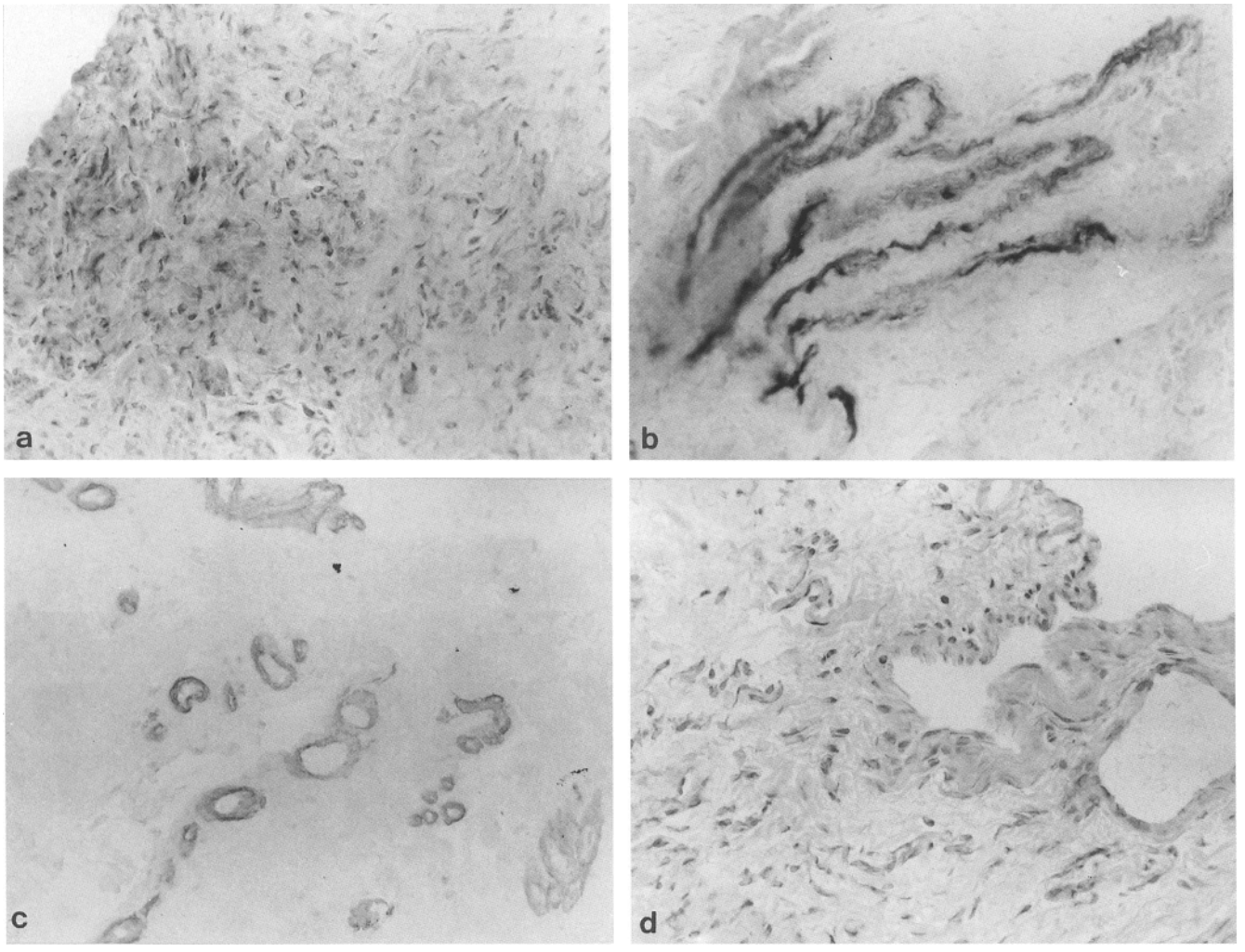

Fig. 2 **a** Synovial tissue of a haemodialysis-related amyloidosis patient (HD: 15 years). Sublining fibroblasts show a mildly positive reaction for MMP-2. Immunostaining, ×40. **b** Synovial tissue of a haemodialysis-related amyloidosis patient (HD: 14 years). Endothelial cells of increased small vessels show a positive reaction for MMP-9. Immunostaining, ×40. **c** Synovial tissue of a haemodialysis-related amyloidosis patient (HD: 15 years). Endothelial cells of small vessels show a positive reaction for TIMP-1. Immunostaining, ×33. **d** Synovial tissue of a haemodialysis-related amyloidosis patient (HD: 12 years). Sublining fibroblasts show a positive reaction for TIMP-2. Immunostaining, ×40

formed. Paraffin-embedded sections were laid on poly-L-lysine-coated slides, and deparaffinized. Digestion by 0.1% trypsin for 20 min at 37°C was required when antibody against CD68 (Kp-1) was applied. Sections were incubated in methanol containing 0.3% (v/v) H_2O_2, washed in PBS, and incubated in 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 immunoglobulin and streptavidin (Nichirei). After three additional washes, bound peroxidase was developed with 0.02% diaminobenzidine (Sigma) at pH7.6 in 0.05 M TRIS buffer plus 0.015% H_2O_2. The slides were counterstained with haematoxylin or methyl green. Sections incubated with normal mouse serum in place of the primary antibody served as negative controls. Immunoreactivity was classified into four grades: (–), no reaction; (1+), focal and/or faint reaction; (2+), moderately positive reaction; and (3+), diffuse and/or strong positive reaction. Degree of amyloid deposition was classified into three grades: (1+), mild; (2+), moderate; and (3+), severe. Inflammatory cell infiltration, which was mainly composed of lymphocytes in RA and non-specific synovitis, and of macrophages in amyloidosis, was classified into four grades: (–), none; (1+), mild; (2+), moderate; and (3+), severe. Degree of synovial cell proliferation was classified as follows: (–), flat synovium; (1+), focal and/or faint proliferation; (2+), mild villous proliferation; (3+), moderate villous proliferation; and (4+), severe villous proliferation with multilayering of lining cells. In the present series, (4+) synovial proliferation was only observed in RA patients.

Results

Immunoreactivity findings for MMP-1, -2, -3 and -9, and TIMP-1 and TIMP-2 in frozen sections of carpal tunnel synovium from the haemodialysis-related amyloidosis, non-specific synovitis and rheumatoid arthritis patients are summarized in Table 1. In the haemodialysis-related amyloidosis cases, MMP-1 was most strongly expressed of the MMPs, being observed in 8 of 9 cases. Expression of MMP-1 appeared to correlate with the grade of macrophage infiltration and synovial cell hyperplasia. It was

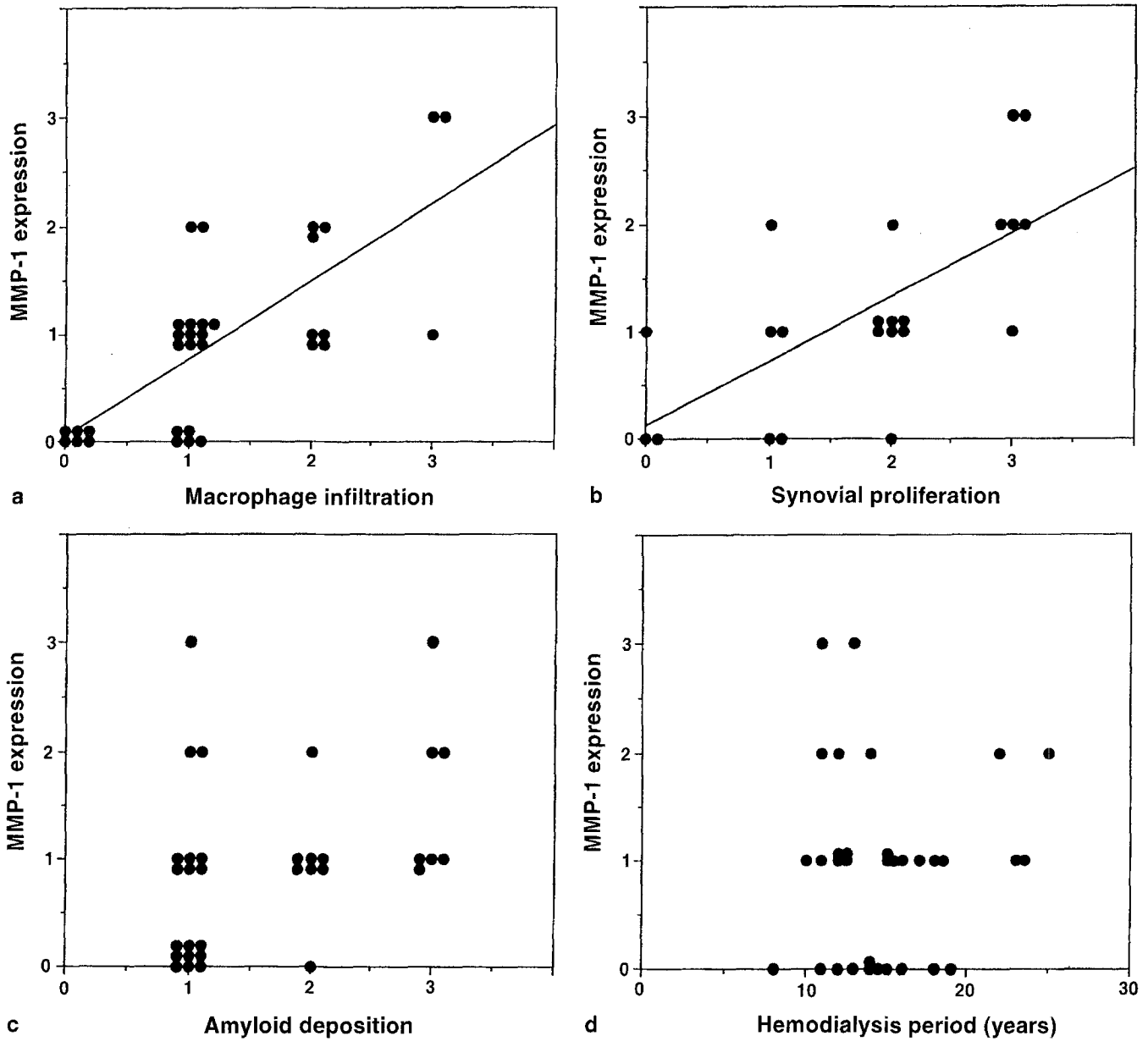

Fig. 3 Correlation of degree of MMP-1 immunostaining with macrophage infiltration (**a**), synovial cell hyperplasia (**b**), amyloid deposition (**c**), and haemodialysis period (**d**). A positive direct correlation was found for **a** and **b**. Lines were determined by regression analysis

immunolocalized in the hyperplastic synovial lining cells, in the mesenchymal cells in the nodular amyloid deposits and in the accompanying granulation tissue in highly inflammatory cases (Fig. 1a,b,e). In cases with (2+) synovial cell proliferation, sublining fibroblasts also showed a mildly positive reaction (Fig. 1a). In the large nodular amyloid deposits, mesenchymal cells stained positively for MMP-1 usually showed chondroid metaplasia with abundant and clear cytoplasm, surrounded by basophilic mucoid substance (Fig. 1c). Chondroid mesenchymal cells in nodular amyloid deposits were also clearly positive for chondroitin sulphate proteoglycans (Fig. 1d). CD68-positive macrophages infiltrated mainly around the nodular amyloid deposits, accompanied by proliferation of small blood vessels. Chondroid cells in the nodular amyloid deposits were negative for CD68. In the haemodialysis-related amyloidosis cases, expression of MMP-2, -3 and -9 was generally weaker than that of MMP-1. MMP-2 was expressed in 2 cases and localized in the sublining fibroblasts (Fig. 2a). Intracellular expression of MMP-3 was not observed in any of the amyloidosis cases and only the extracellular matrix of the synovial surface was faintly stained. MMP-9 was mildly positive in cells of the small vessels, regarded as evidence of neovascularization (Fig. 2b). In 2 of 9 cases, mesenchymal cells in granulation tissue and nodular amyloid deposits were also positively stained for MMP-9. However, the reactivity was much weaker than that for MMP-1. In highly inflammatory cases, expression of TIMP-1 and TIMP-2 appeared less intense than that of MMP-1. TIMP-1 was mildly expressed in 8 cases and lo-

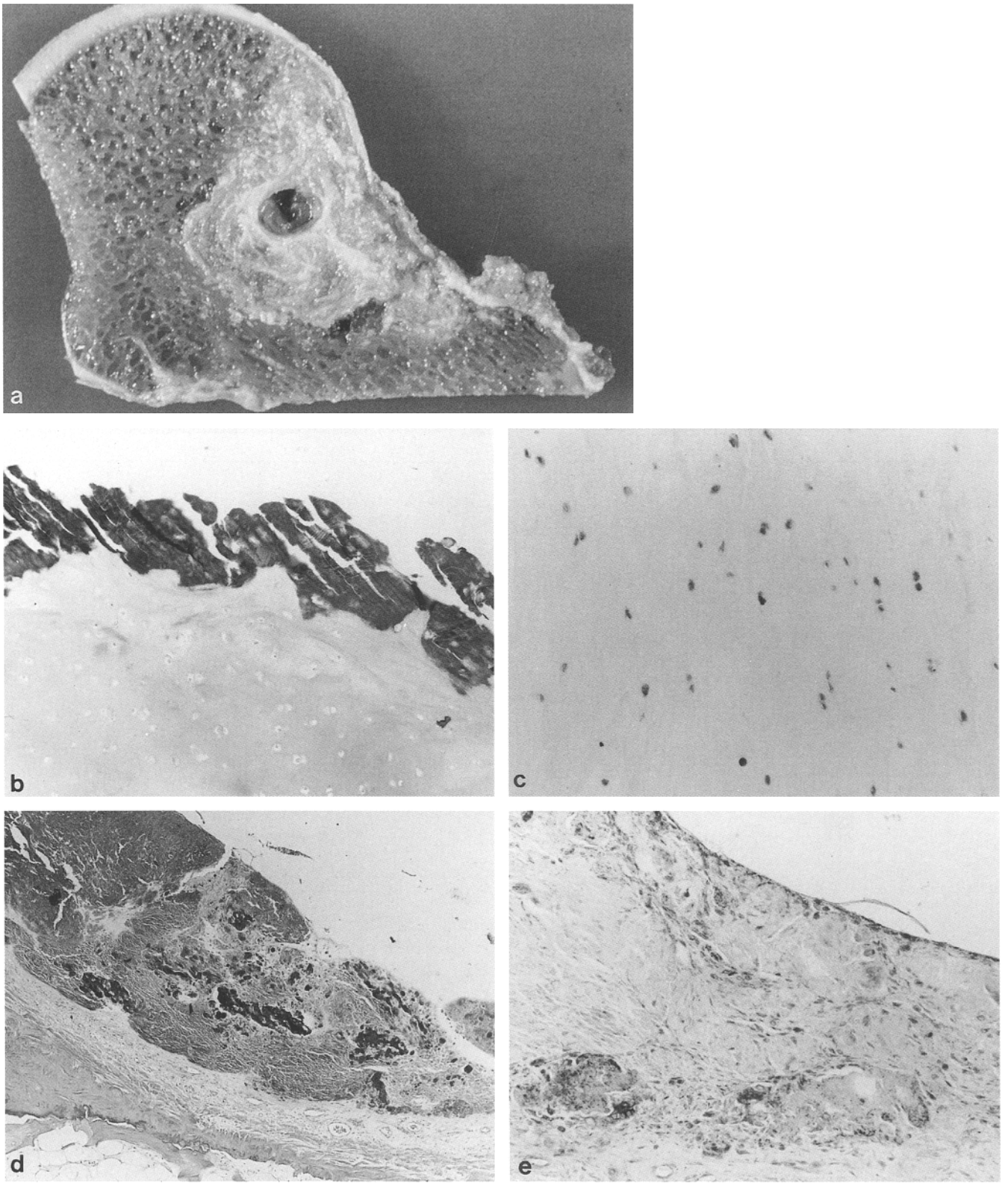

Fig. 4a–e

calized mainly in cells of the small neovascularization vessels, as with MMP-9 (Fig. 2c). In 3 cases, synovial lining cells were also mildly positive for TIMP-1. TIMP-2 was expressed in 6 cases, localized in the sublining fibroblasts (Fig. 2d).

In the 2 cases of RA, synovial villous proliferation with multilayering of synovial lining cells and marked lymphocyte infiltration were observed. Localization of MMPs and TIMPs was similar to the amyloidosis cases, although the grades of synovial proliferation and inflammatory cell infiltration were higher, and the expression of MMPs and TIMPs, especially that of MMP-2, -3 and -

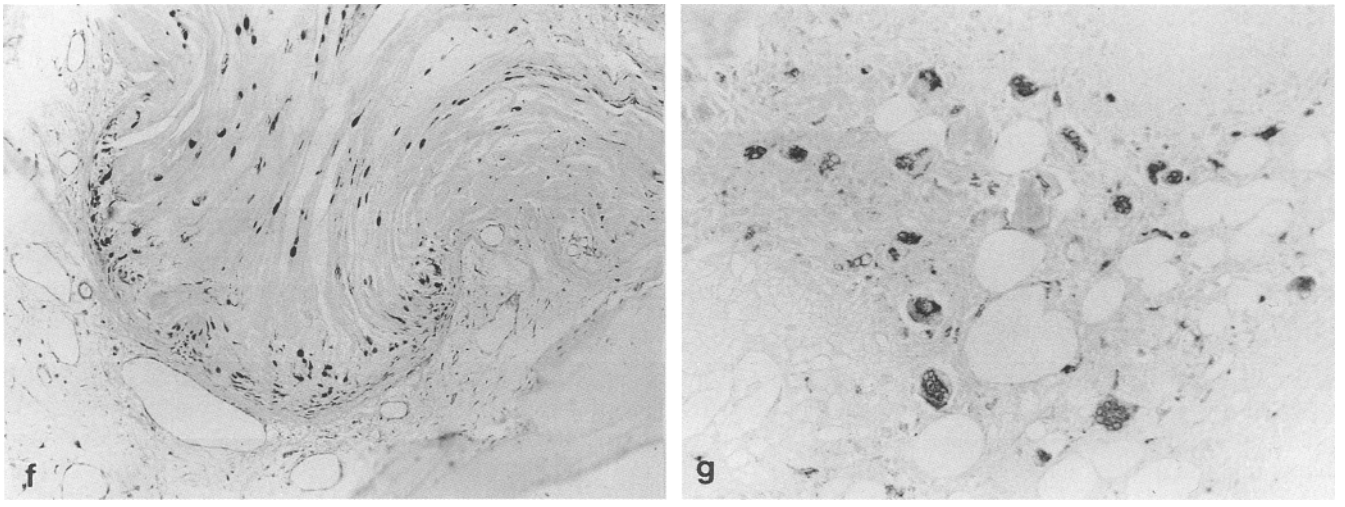

Fig. 4a–g Femoral head demonstrating destructive arthropathy due to haemodialysis-related amyloidosis (HD: 25 years). **a** Macroscopic appearance. The articular cartilage distant from the cystic lesion is well conserved, but that adjacent to the amyloid deposits and surrounding inflammatory reaction is eroded. **b** Articular cartilage distant from the cystic lesion. Band-like amyloid deposits are observed on the articular surface. Congo red staining, ×40. **c** Chondrocytes beneath the band-like amyloid deposits show a positive reaction for MMP-1. Immunostaining, ×40. **d** Articular capsule adjacent to the cystic lesion. The band-like amyloid deposits are markedly thicker than in **b** and articular cartilage has almost disappeared. Congo red staining, ×25. **e** The same area as in **d**. Mesenchymal cells in contact with the thick band-like amyloid deposits show a strong positive reaction for MMP-1. Immunostaining, ×40. **f** Amyloid deposits in the articular cartilage adjacent to the cystic lesion have destroyed the articular capsule and penetrated into the bone tissue. Mesenchymal cells in contact with the invading amyloid show a positive reaction for MMP-1. Immunostaining, ×25. **g** Bone tissue at the limits of the cystic lesion. Increased osteoclasts show a distinct positive reaction for MMP-9. Immunostaining, ×40

9, was more distinct. MMP-3 was clearly expressed in 1 case, localized in the synovial lining cells. In the mildly non-specific synovitis cases, expression of MMPs was weak. MMP-1 and MMP-9 were faintly stained in the synovial lining cells and in the small vessels, respectively. TIMP-1 was mildly positive as in the RA and amyloidosis cases, localized in the synovial lining cells and small vessels.

Using paraffin and frozen sections of amyloidosis patients, correlation of MMP-1 expression with macrophage infiltration, synovial cell proliferation, amyloid deposition and haemodialysis period was examined. The results are summarized in Fig. 3. A good correlation was observed with macrophage infiltration and synovial cell proliferation but not with amyloid deposition and haemodialysis period.

The immunolocalization of MMP-1 and MMP-9 in the resected hip joint tissues including synovium, articular capsule and femoral bone was studied. Macroscopically, the articular cartilage of the femoral head showed irregularities of the surface and erosion, leading to destruction of the subchondral bone and cyst formation (Fig. 4a). Histologically, amyloid deposition in the articular cartilage was prominent. At sites distant from the cystic lesion where the normal thickness of the articular cartilage was still well conserved, amyloid deposits formed a thin dense band along the cartilaginous surface (Fig. 4b). Chondrocytes under the band-like amyloid deposits showed a positive reaction for MMP-1 (Fig. 4c). Articular cartilage became progressively thinner as the thickness of the band-like amyloid deposits increased (Fig. 4d). Mesenchymal cells in and around the area of amyloid deposits showed distinct expression of MMP-1 (Fig. 4e). In the area adjacent to the cystic lesion, amyloid deposits replaced the entire thickness of the articular cartilage, and penetrated into the bone tissue. Granulation and macrophage infiltration were found around invading amyloid deposits with MMP-1 expressed in the mesenchymal cells (Fig. 4f). Within the cystic lesion, amyloid deposits, necrotic bone trabeculae and granulation were noted. Bone trabeculae at the limits of the lytic change were eroded with increased osteoclastic activity. MMP-9 was clearly expressed in the increased osteoclasts (Fig. 4g) and MMP-1 was also faintly positive in a small number of these cells. Periosseous fibroblasts showed a positive reaction for MMP-1.

Discussion

Matrix metalloproteinases and their inhibitors have been reported to play an important role in degrading articular extracellular matrix materials. Immunolocalization of MMPs and TIMPs in the synovial and cartilaginous tissues of RA or osteoarthritis (OA) patients has revealed MMP-1, MMP-3 and TIMP-1 in synovial lining cells, MMP-2 in sublining fibroblasts and MMP-9 in inflammatory cells and osteoclasts [10, 33–36]. In situ hybridization technique demonstrated the presence of MMP-1, MMP-3 and TIMP-1 mRNAs in the rheumatoid synovial lining cells [4, 22].

In the present cases with β2M amyloid deposits, MMP-1, which can degrade type I, II, III and X collagens, was most strongly expressed of the MMPs examined. MMP-1 is considered to be a major factor in the

destructive changes occurring in β2M-amyloidosis-related synovitis and its expression in the synovial lining cells was common to both RA and amyloidosis cases in the present study. However, the finding of MMP-1 in mesenchymal cells in granulation tissue and nodular amyloid deposits was specific for the β2M amyloidosis and provides evidence of a direct relationship between amyloid deposits and destructive changes. In highly inflammatory cases, expression of TIMPs appeared less intense than that of MMP-1 and an imbalance between the two might be implicated in the destructive process.

Endothelial cells produce MMPs and TIMPs. In the previous reports, it has been suggested that the activity of MMPs and TIMPs played an important part in neovascularization, especially in tumour tissues [11, 13]. It is likely that the induction and arrest of capillary endothelial cell migration during neovascularization require precise regulation of extracellular matrix synthesis and degradation. Therefore the presence of MMP-9 and TIMP-1 in the increased capillary endothelial cells of amyloidosis synovium is possibly related to the neovascularization with a role in degradation of basement membranes surrounding intact capillaries.

In both RA and β2M amyloidosis cases, hyperplastic changes of the synovial lining cells are commonly observed, but the two conditions differ with regard to sublining inflammatory reactions. RA synovitis is initiated by an immune response to as yet unknown stimuli, and is characterized by lymphocyte infiltration with follicle formation. Macrophages are intermingled in the inflammation to some extent. However, β2M-amyloidosis-related synovitis is characterized by exclusive infiltration of macrophages, which are frequently accompanied by multi-nucleated giant cells and small vessel proliferation. In previous papers, expression of MMP-1 and MMP-3 was reported to correlate with the degree of synovial cell hyperplasia and lymphocyte infiltration in RA and OA tissues [22, 35]. In the present study, the expression of MMP-1 correlated with the degree of synovial hyperplasia and macrophage infiltration, but not with the degree of amyloid deposition or dialysis period. Although the types of inflammatory cells are quite different in RA and amyloidosis, the present results suggest that common mediators produced by synovial cells and/or macrophages regulate the production of MMPs. Circulating levels and local production of cytokines, such as interleukin 1(IL-1) and tumour necrosis factor alpha (TNFα), are elevated in both RA and β2M amyloidosis patients [1, 3, 9, 37]. Immunohistochemically, hyperplastic synovium and infiltrating macrophages in tissues with amyloid deposits were positively stained for IL-1 and TNFα [24, 30]. Production of MMPs is induced in synovial cells by treatment with IL-1 and TNFα in vitro [19, 35]. Cytokines, such as IL-1 and TNFα, are thus considered to be possible factors which regulate the MMP production in both RA and amyloidosis synovium in vivo. Another possible factor related to MMP-1 production in amyloidosis synovium is β2M itself, since it has been reported to induce MMP-1 in rabbit synovial fibroblasts [2]. However, our study did not show correlation of MMP-1 expression with the degree of amyloid deposition or dialysis period.

In AL amyloidosis, where the constituent protein is immunoglobulin light chain, amyloid deposits are also observed in the periarticular tissue. Despite the similarity in deposition pattern, destructive arthropathy rarely occurs in AL cases. In tissues with AL-type amyloid deposits, synovial cell hyperplasia and inflammatory cell infiltration are rarely found and the presence of the latter in β2M amyloidosis patients therefore provides an important clue to the underlying mechanism. Miyata et al. [25] recently demonstrated that β2M amyloid deposits are modified by advanced glycation end products (AGEs) and the AGEs induce monocyte chemotaxis and macrophage secretion of IL-1 and TNFα in vitro. However, the reasons why other proteins modified with AGEs, such as collagen in diabetes mellitus patients, do not induce macrophage infiltration are unclear. Other factors which might be responsible for the macrophage infiltration should be excluded.

Mesenchymal cells demonstrating the chondroid metaplasia frequently observed in the nodular amyloid deposits did not show a macrophage phenotype, suggesting a fibroblast origin. Interestingly these cells were positively stained for both MMP-1 and chondroitin sulphate. In β2M and other systemic amyloidosis, the presence of increased proteoglycans at sites of deposits is considered to be related to a microenvironment suitable for amyloidogenesis [15, 20, 31]. It has also been reported that β2M exhibits high collagen-binding affinity in vitro, and in vivo amyloid deposits were frequently observed along collagen bundles under the electron microscope [12, 28]. We can thus hypothesize that fibroblasts which secrete both MMPs and proteoglycan promote remodelling of the extracellular matrix, facilitate the β2M-binding affinity of collagens and alter the microenvironment, making it suitable for amyloidogenesis.

In haemodialysed patients who develop arthropathy, destructive changes of bone with formation of cystic lesions are frequently observed [26, 39]. β2M amyloid deposits are considered to be the main pathogenetic factor, but exactly how the process proceeds is still a matter of controversy. Solé et al. [39] reported consistent the histological finding of amyloid deposits in cartilage leading to fissures and irregularities in the articular surface in 23 cases of dialysis-related destructive arthropathy. In our present case, band-like amyloid deposits were observed along the cartilaginous surface, and MMP-1 was expressed in chondrocytes under the amyloid deposits and in mesenchymal cells in sites of amyloid deposits and granulation tissue. Irregular surface and erosion of the articular cartilage may be due to damage by secreted MMPs. In addition, the distinct expression of MMP-9 in the increased osteoclasts around lytic changes of bone is significant, given its important role in abnormal bone resorption in RA cases and in carcinoma metastasis [36, 41]. Abnormal expression of MMP-1 and MMP-9 by mesenchymal cells in the invading amyloid deposits,

granulation and increased osteoclasts is probably closely related to the development of bone cysts.

In conclusion, investigation of MMPs and TIMPs in tissues with β_2-microglobulin amyloid deposits demonstrated strong expression of MMP-1, correlated with the grade of inflammatory reaction. The results indicate that MMP-1 produced by synovial cells, mesenchymal cells and chondrocytes, and MMP-9 by osteoclasts are directly involved in the destructive process of amyloid arthropathy, with cytokines secreted by synovial lining cells and macrophages being possible mediators. The possibility clearly warrants further attention.

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