Expression of proteinases and inflammatory cytokines in subchondral bone regions in the destructive joint of rheumatoid arthritis


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Abstract

Objective. We previously described abnormalities in the bone marrow of patients with rheumatoid arthritis (RA), but were able to shed little light on the pathogenic roles of inflammatory cytokines and proteinases in joint destruction in the subchondral region in RA. This is the first report to describe the co-localization of cytokines and proteinases in this area.

Methods. Decalcified paraffin-embedded sections from 10 patients with RA and five patients with osteoarthritis (OA) were examined for the immunolocalization of cathepsins B, K and L and the localization of messenger RNAs for interleukin 1β (IL-1β), tumour necrosis factor α (TNF-α) and matrix metalloproteinase 9 (MMP-9). The cells were double-stained with anti-CD68 or anti-prolyl 4-hydroxylase (PH) antibody.

Results. An immunohistochemical study confirmed the expression of cathepsins B and L by CD68-positive mononuclear cells at the sites of significant cartilage and bone erosion from the subchondral region in all RA specimens. Osteoclast-like cells showed intense staining for cathepsin K and MMP-9. Osteoblast-like cells strongly expressed MMP-9. Analysis of serial sections revealed that expression of the IL-1β and TNF-α genes occurred near that of the cathepsins and MMP-9 in the subchondral region.

Conclusion. We conclude that inflammatory cytokines and tissue-damaging proteinases play important roles in joint destruction in the subchondral region in RA.

Key words: Rheumatoid arthritis, Cathepsins, Matrix metalloproteinase 9 (MMP-9), Inflammatory cytokines, Joint destruction, Immunohistochemistry, In situ hybridization.

Rheumatoid arthritis (RA) is an autoimmune disease characterized by chronic inflammation and progressive joint destruction. However, the mechanism of bone and cartilage destruction is still not completely understood. Inflammatory cytokines, such as interleukin 1β (IL-1β) and tumour necrosis factor α (TNF-α), appear to contribute directly to tissue damage in RA by inducing the release of tissue-damaging enzymes [1]. Histological studies [2, 3] have revealed that significant cartilage and bone erosion occurs primarily through the action of osteoclasts, but few analyses have localized tissue-damaging enzymes and inflammatory cytokines to the subchondral region.

Previously, we have investigated abnormalities of the bone marrow of patients with rheumatoid arthritis and demonstrated the pivotal role of bone marrow in the pathogenesis of RA [4–11]. In the present study, to analyse the possible role of inflammatory cytokines in joint destruction in the subchondral region and at the pannus–cartilage or pannus–bone junction, we investigated the distribution of tissue-damaging enzymes and inflammatory cytokines in these regions in RA patients.

It is generally recognized that osteoclasts are directly responsible for bone resorption. At the site of bone attachment, osteoclasts generate an acidic microenvironment within which bone demineralization and matrix degradation occur. We focused on proteinases functioning under low pH as candidate proteinases that degrade bone matrix in RA joints.

Cathepsins B, K and L are categorized as cysteine proteinases, and function at low pH. Cathepsins B and L are capable of degrading cartilage and bone matrix...
components and activating metalloproteinases (for review, see reference 12). Although cathepsins B and L are capable of depolymerizing collagen [13], they have been shown to be involved in the pathophysiology of rheumatoid joint destruction [14]. Cathepsin K [15] is capable of cleaving the triple helix of native collagens [16], and is involved in bone resorption. Matrix metalloproteinase 9 (MMP-9; also called 92 kDa gelatinase and gelatinase B) is a member of the MMP gene family, and the gelatinolytic activity of MMP-9 is optimal at pH 7.5, but 50–80% of its full activity is retained at pH 5.5–6.0 [17]. TNF-α contributes positively to the induction of MMP-9 by up-regulation of the binding sites for AP-1 and NF-κB [18].

In the present study, we investigated the co-localization of cytokines IL-1β and TNF-α and the enzymes cathepsins B, K and L and MMP-9 in the subchondral region of patients with RA to determine the direct contribution of inflammatory cytokines and the participation of these proteinases to the tissue-damaging process, not only at the pannus–cartilage junction but also in subchondral bone erosion in RA.

Materials and methods

Patients

Tissue specimens were obtained with informed consent from 10 patients with RA during total knee arthroplasty. All RA patients met the American College of Rheumatology revised criteria for RA [19]. Samples of thick articular cartilage tissue with subchondral bone, obtained from the lateral tibial plateau of five patients with the medial type of osteoarthritis (OA) who were undergoing total knee arthroplasty, served as the control. None of the OA patients had any relevant immunological background or history of systemic inflammation. The clinical backgrounds of these patients are summarized in Table 1.

Tissue preparation

Articular cartilage and adjacent subchondral bone samples, originating from the centre of the medial or lateral tibial plateau, and approximately 5 mm thick and 15 mm wide, were prepared for immunohistochemistry or in situ hybridization. After fixation in 4% paraformaldehyde (pH 7.4) at 4 °C for 24 h, the tissue samples were decalcified in 20% EDTA (pH 7.4) for 2 h in a microwave oven (H2800 Microwave Processor; Energy Beam Science Inc, Agawan, MA, USA) at 50 °C [20] and then for 22 h at 4 °C. The samples were then dehydrated through an ethanol series and embedded in paraffin. A 4-μm section of each sample was prepared using a microtome, mounted on 1-polylysine-coated slides and processed for further study. For histological evaluation, each slide was stained with haematoxylin and eosin.

Antibodies

Recombinant rat cathepsins B and L and the C-terminal polypeptide of mouse cathepsin K were produced in Escherichia coli. Polyclonal antisera raised against the purified enzymes and polypeptide were prepared in New Zealand White rabbits and purified by affinity chromatography as described previously [21]. Rabbit polyclonal antibodies against rat cathepsins B and L were tested for reactivity by immunoprecipitation with cultured human synovial cells from RA patients. A rabbit polyclonal antibody against the C-terminal polypeptide of mouse cathepsin K was purified by affinity chromatography and tested for reactivity by Western blotting with human cathepsin K. The primary antibodies to human CD68 and to human prolyl 4-hydroxylase (PH) were purchased from Dako (Santa Barbara, CA, USA) and Fuji Chemical (Toyama, Japan) respectively.

Immunohistochemical staining

Immunohistochemical staining was performed by the streptavidin–peroxidase technique, using Histofine SAB-PO kits (Nichirei, Tokyo, Japan) according to the manufacturer’s instructions [22]. Briefly, 4 μm-thick sections were rehydrated and placed in 0.03% hydrogen peroxide in methanol to block endogenous peroxidase. After washing, the sections were blocked with 10% normal goat non-immune serum to minimize background staining, followed by incubation with rabbit polyclonal antibody for 2 h at room temperature (RT). Normal rabbit serum was used as a control for the primary antibody against cathepsins and mouse IgG1 (Dako) for the primary antibody against CD68 and PH. After washing in phosphate-buffered saline (PBS; pH 7.2), the sections were incubated with secondary antibody for 20 min at RT in a humid chamber, and then incubated with peroxidase-conjugated streptavidin (Nichirei) for 20 min at RT in a humid chamber and washed in PBS. Finally, substrate reagent (3,3′-diaminobenzidine tetrahydrochloride; Dojindo, Tokyo, Japan) was added. Counterstaining was performed with haematoxylin, and sections were mounted.

<table>
<thead>
<tr>
<th>Table 1. Characteristics of the RA and OA patients at the time of surgery</th>
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<td><strong>Number of males, females</strong></td>
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<tr>
<td>1, 9</td>
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<td><strong>Age (yr): mean (range)</strong></td>
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<td><strong>CRP (mg/dl): mean (range)</strong></td>
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<td><strong>Disease duration (yr): mean (range)</strong></td>
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<td><strong>Number taking NSAIDs</strong></td>
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<td><strong>Treatment during previous 6 months</strong></td>
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<td><strong>Gold salts</strong></td>
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<td><strong>n-Penicillamine</strong></td>
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CRP = C-reactive protein; NA = not available; NSAIDs = non-steroidal anti-inflammatory drugs.
Preparation of probes for in situ hybridization

Digoxigenin-11-UTP-labelled single-strand cRNAs were prepared using the digoxigenin (DIG) RNA Labeling Kit (Boehringer Mannheim Biochemica, Mannheim, Germany) according to the manufacturer’s instructions. For the generation of probes, a human MMP-9 cDNA [23] was obtained by reverse transcription–polymerase chain reaction (RT-PCR), and subcloned into pGEM plasmid. The cDNA was either linearized with SacII and transcribed with SP6 RNA polymerase to generate a 540-base antisense (cRNA) probe, or linearized with SpeI and transcribed with T7 RNA polymerase to generate a sense probe. Similarly, a 840-base fragment of human IL-1β [24], a 546-base fragment of TNF-α [25] and a 360-base fragment of type I collagen [26] were obtained by RT-PCR and were subcloned into pGEM plasmids. They were then either linearized with SacII and transcribed with SP6 RNA polymerase to generate antisense (cRNA) probes, or linearized with SpeI and transcribed with T7 RNA polymerase to generate sense probes. Sequencing analysis confirmed the identity of each cDNA obtained by RT-PCR by comparison with published nucleotide sequences. The nucleotide sequence of the MMP-9 cDNA had less than 50% homology with other MMPs. Similarly, the type I collagen cDNA showed only 40% homology with other collagens.

In situ hybridization

In situ hybridization was performed as described previously [27]. Paraffin sections were dewaxed through an ethanol series and incubated with 1 mg of proteinase K (Boehringer Mannheim) in TE (0.1 M Tris, pH 8.0, 50 mM EDTA, pH 8.0) at 37°C for 10 min. Sections were then fixed with 4% paraformaldehyde in 0.1 M PBS (pH 7.4) for 20 min at RT and acetylated with 0.25% acetic anhydride (Katayama Chemical Co., Osaka, Japan) in 0.1 M triethanolamine (pH 8.0, Katayama Chemical Co.). This was followed by dehydration through an ethanol series and air-drying. A hybridization solution (50 ml) (50% deionized formamide, 10% dextran sulphate, 1 × Denhardt’s solution, 4 × SSC, 150 mg of E. coli tRNA) containing approximately 0.5 mg/ml of RNA probe was placed on each section, and hybridization was performed at 50°C for 16 h in a moist chamber. After hybridization, the slides were washed briefly in 5 × SSC (1 × SSC = 0.15 M NaCl, 0.015 M sodium citrate; Life Technologies, Grand Island, NY, USA) and for 30 min in 50% formamide (Nakalai Tesque, Kyoto, Japan), 2 × SSC at 50°C. After rinsing in 1 × TES (10 mM Tris–HCl pH 7.6, 1 mM EDTA, 0.5 M NaCl) for 15 min at 37°C, the slides were treated with RNase A (10 mg/ml in 1 × TES, Boehringer Mannheim) for 30 min at 37°C, then rinsed again in 1 × TES for 15 min at 37°C. The slides were then washed twice with 2 × SSC for 20 min at 50°C, followed by 0.2 × SSC in the same manner. Hybridized DIG-labelled probes were detected with the aid of a Nucleic Acid Detection Kit (Boehringer Mannheim) according to the manufacturer’s instructions. The slides were incubated with DIG buffer 1 (100 mM Tris–HCl, pH 7.5, 150 mM NaCl) for 2 min, and with 1.5% blocking reagent in DIG buffer 1 for 60 min at RT. Diluted Polyclonal sheep anti-DIG Fab fragment in DIG buffer 1 (100 ml/cm²) was mounted on the sections and incubated for 30 min at RT. After immunoreaction, the slides were rinsed twice in DIG buffer 1 for 15 min and equilibrated with DIG buffer 3 (100 mM Tris–HCl, pH 9.5, 100 mM NaCl, 50 mM MgCl₂) for 3 min. Colouring solution containing nitroblue tetrazolium and 5-bromo-4-chloro-3-indoyl phosphate in DIG buffer 3 was added to the slides, and the preparations were incubated at RT for 3 h.

Characterization of cells expressing cathepsins

To characterize cells observed in the subchondral region, immunohistochemical examination was performed using monoclonal antibodies against CD68 and PH. Sections were incubated with 10% normal goat non-immune serum in a humid chamber to minimize background staining, followed by incubation with marker antibodies for 2 h at RT. Sections were then incubated with a fluorescein isothiocyanate-conjugated goat anti-mouse Ig (G + M) antibody for 20 min at RT, then immunostained with rabbit polyclonal antibody against cathepsins using goat Texas red-conjugated anti-rabbit Ig (G + M) antibody as the secondary antibody. After mounting, sections were analysed using fluorescence microscopy (Nikon 9500; Nikon, Tokyo, Japan).

Analysis of sections

All slides were examined and graded according to the frequency of positively stained cells in the subchondral region, and were digitized with an HC2500 image analysis system (Fujifilm, Tokyo, Japan).

Results

Histological examination

In all RA patients examined, the subchondral bone region was characterized by cellular aggregation, which is compatible with our previous report of an increase in the number of mononuclear cells in bone marrow blood in RA [22]. The subchondral plate was thickened and composed of lamellar and woven bone containing many oval to round mononuclear cells. The surface of the subchondral plate was almost completely covered with multinucleated giant cells or flattened or cubic mononuclear cells. Fibroblastic and vascular proliferation was observed in the bone marrow space in all RA specimens (Fig. 1a–d). Extensive erosion of the subchondral plate and cartilage by cell-rich inflammatory tissue occupying the bone marrow space was also noted. In the control subjects, the layer structure of the articular cartilage, tidemark, calcified cartilage and subchondral plate were well defined (Fig. 1i and j), and there was clearly less cell infiltration across the tidemark than in RA patients.
Characterization of cells in the RA subchondral region

All multinucleated cells were immunopositive for CD68. Immunoreactivity for CD68 was detected in approximately 60% of mononuclear cells in subchondral cell aggregates and in all multinucleated cells (Fig. 1e). Approximately 30% of mononuclear cells stained positively for PH (Fig. 1f). The oval to round mononuclear cells in the woven bone and cubic mononuclear cells lining the trabecular bone surface expressed PH and the type I collagen gene (Fig. 1g and h).

Fig. 1. Pathohistological analysis of the subchondral bone region. In RA specimens (a–h), the region was characterized by cell aggregates in bone marrow spaces (BM) and extensive erosion of the cartilage (C) and subchondral plate (SB) by cell-rich inflammatory tissue occupying the bone marrow space (a, c). These tissues caused extensive erosion of the cartilage (b) and the subchondral bone (d). Immunohistochemical analysis revealed that mononuclear cells occupying RA subchondral bone marrow were composed of CD68-positive mononuclear cells (e) and PH-positive mononuclear cells (f). The subchondral plate was sometimes thickened and composed of lamellar bone and woven bone, and the surface of the subchondral plate was covered by flattened or cubic mononuclear cells (g). In situ hybridization revealed that the cubic mononuclear cells strongly expressed type I collagen mRNA (h). In control tissue (i, j), a normal layer structure was observed, composed of cartilage (C), tidemark (TM), calcified cartilage (CC), subchondral bone plate (SB) and fatty bone marrow. No significant erosion of cartilage or bone from the subchondral region was observed. Panels a–d, g and i–j are stained with haematoxylin and eosin. Original magnifications: a, c, ×40; i, ×100; d, e, g, ×200; b, h, j, ×400.
Distribution of proteinases and proinflammatory cytokine expression at the subchondral bone region

Joint destruction is due to an enzymatic reaction, and therefore we hypothesized that tissue-damaging proteinases would be observed in the subchondral region. To confirm the co-localization of these proteinases and cytokines, serial sections were analysed. An overview of the distribution of tissue-damaging proteinases and inflammatory cytokines in the RA subchondral region is shown in Table 2.

In control subjects, no notable immunoreactivity against cathepsin B and L was observed in the subchondral region (representative examples are shown in Fig. 2a). Multinucleated cells attached to the bone surface displayed immunoreactivity against cathepsin K, but few mononuclear cells had notable immunoreactivity against cathepsin K (Fig. 2b).

Multinucleated cells were intensely immunostained with cathepsin K (Fig. 2c) and were positively stained with the cRNA probe for MMP-9 (Fig. 2h) in all RA specimens and controls. However, the immunoreactivity of multinucleated cells for cathepsins B and L was weaker than that in mononuclear cells seen in the same section (Fig. 2d–f). The mRNA of TNF-α and IL-1β was expressed in all RA sections in fewer than 10% of multinucleated cells (Fig. 2i and j).

Mononuclear cells aggregated in the bone marrow of all RA specimens rather than OA specimens, and were assumed to play an important role in joint destruction in the subchondral region. Immunostaining indicated the presence of cathepsins B and L in 30–60% of the mononuclear cells in the bone marrow space. Their immunoreactivity was more intense than that in multinucleated cells in all RA samples. Mononuclear cells expressing both cathepsin K and MMP-9 mRNA accounted for approximately 10% of the mononuclear cells. TNF-α and IL-1β mRNA were found in less than 10% of the mononuclear cells invading the bone and cartilage and in the bone marrow space in all RA patients.

Cubic mononuclear cells lining the bone surface were morphologically similar to osteoblasts, and some reports have indicated osteoblastic osteolyis [28]. In the present study, immunohistochemical analysis indicated weaker immunoreactivity against cathepsin K in mononuclear cells compared with that in multinucleated cells in the same sections. The mRNA of MMP-9, IL-1β and TNF-α was expressed in more than 60% of all cubic mononuclear cells lining the bone surface (Fig. 2m–p).

In the negative controls, no notable immunoreactivity against non-immune rabbit serum was observed (a representative result is shown in Fig. 2g), and no notable signal was observed in situ hybridization using sense probes (representative results are shown in Fig. 2i and k).

Identification of the mononuclear cells expressing targeted proteinases and proinflammatory cytokines in the subchondral region

The number of synovial macrophages has been shown to correlate with the severity of joint destruction [29], and fibroblastic cells are thought to participate in joint destruction to some degree [30]. To characterize the cells expressing cathepsins, double immunostaining was performed in the present study. Immunoreactivity for cathepsin B was found in 30–60% of CD68-positive mononuclear cells and in fewer than 30% of PH-positive cells in all RA samples. Immunoreactivity for cathepsin L was found in more than 60% of CD68-positive mononuclear cells, and in fewer than 30% of PH-positive cells. Immunoreactivity for cathepsin K was found in fewer than 10% of CD68-positive mononuclear cells and in PH-positive cells (representative results for cathepsin K are shown in Fig. 3).

Discussion

RA is believed to be a chronic inflammatory disease characterized by synovial proliferation, and over the last decade several mechanisms of joint destruction at the pannus–cartilage/bone junction and at the articular cartilage have been suggested [for review, see reference 1]. Although substantial cartilage and bone erosion has been shown to occur by cellular infiltration from the subchondral bone region [3], the pathomechanism of joint destruction in the subchondral region remains to be clarified. In the present study, we analysed the RA subchondral region and determined the distribution of cathepsins and inflammatory cytokines using histological techniques.

In the inflamed RA synovium, proinflammatory cytokines, such as IL-1β and TNF-α, are produced mainly by macrophages and fibroblasts and contribute directly to tissue damage by induction of the release of tissue-damaging enzymes from synovial fibroblasts, macrophages and articular chondrocytes [1, 31, 32]. Sites at the pannus–cartilage junction show pronounced expression of IL-1β, TNF-α [33] and cathepsins B [34] and K [30], and RA synovium shows immunoreactivity against MMP-9 [35]. In the present study, we have demonstrated the expression of cathepsins, MMP-9 and inflammatory cytokines at the RA subchondral region.

| Table 2: Immunoreactivity and expression of mRNAs for lysosomal cysteine proteinases, MMP-9 and inflammatory cytokines in subchondral region of RA patients |
|------------------|------------------|------------------|
|                   | OCL              | aOB              | MNC              |
| Cathepsin B       | +                | ±                | +                |
| Cathepsin L       | + +              | -                | + +              |
| Cathepsin K       | + + + ±          | + + + ±          | + + + ±          |
| MMP-9             | + + + ±          | + + + ±          | + + + ±          |
| IL-1β             | ±                | + +              | +                |
| TNF-α             | ±                | + +              | +                |

OCL, CD68-positive multinucleated cells attached to the bone surface; aOB, cubic mononuclear cells lining bone surface, expressing mRNA of type I collagen (suggesting they are active osteoblasts); MNC, mononuclear cells in bone marrow spaces.

- 0%, ± 0–30%; +, 30–60%; ++, 60–90%; +++, 90–100%. 

- 0%, ± 0–30%; +, 30–60%; ++, 60–90%; +++, 90–100%.
Fig. 2. Immunolocalization and gene expression of tissue-damaging proteinases and proinflammatory cytokines in the subchondral region. In control tissue, no notable immunoreactivity against cathepsins B and L was observed at the subchondral region (a; representative of immunohistochemistry of cathepsin B), and immunoreactivity against cathepsin K was observed only in multinucleated cells. Immunohistochemical analysis showed that, in the subchondral region in RA patients, multinucleated osteoclast-like cells (arrows) stained positively for cathepsin K (c), L (d, f) and B (e). A considerable proportion of mononuclear cells filling the bone marrow (BM) spaces (asterisk) were positively immunostained for cathepsin B and L, but only a small proportion stained for cathepsin K. In negative controls, only faint immunoreactivity was observed using normal serum (g). Expression of matrix metalloproteinase-9 (MMP-9) mRNA was detected not only in osteoclasts but also in mononuclear cells at the bone surface and in the bone marrow space (h). Serial sections showed co-localization of the proinflammatory cytokines IL-1β (j) and TNF-α (l) with cathepsins and MMP-9. In negative controls only faint signals were observed using sense probes.
These findings suggest the contribution of these enzymes and cytokines to the pathogenesis of RA in the subchondral region, and the similarity between the pannus–cartilage junction and the subchondral region in the pathogenesis of RA.

We have previously reported abnormalities in the bone marrow of RA patients [4–8, 11, 36]. Increased bone resorption and enhanced osteoclastogenesis were observed specifically in the iliac bone marrow of patients with RA, especially those with severe RA [10]. Recently, we established stromal cell clones originating from both the synovium and the bone marrow of RA patients [37, 38]. The similar characteristics and functions of these cell clones, such as the production of inflammatory cytokines in RA synovium and bone marrow, may reflect the contribution of these stromal cell clones to the pathogenesis of RA. The origin of subchondral cell aggregation remains unknown. To answer this question, we employed a collagen-induced experimental arthritis model and found that bone marrow stromal cells migrated into the joint cavity and contributed to the proliferation of synovial cells [39]. In addition, other investigators have recently reported the invasion of osteoclastic cells and immunocytes into the subchondral bone [40].

In the present study, haematoxylin/eosin-stained sections from all patients showed severe erosion of cartilage by synovial cells, as well as cartilage and bone erosion in the subchondral region. Histochemical and immunohistochemical staining demonstrated the frequent presence at both the pannus–bone junction and in the subchondral region of CD68-positive mononuclear cells that were identified as macrophages, PH-positive mononuclear cells identified as fibroblasts, and CD68-positive multinucleated cells identified as osteoclasts [41]. A considerable proportion of macrophage-like cells possessed intense immunoreactivity for cathepsins B and L, and a small proportion had immunoreactivity for cathepsin K and MMP-9. Osteoclast-like cells expressed cathepsin K and MMP-9 mRNA diffusely, but cathepsins B and L were observed infrequently. Fibroblast-like and osteoblast-like cells showed weak immunoreactivity for cathepsins B and K compared with macrophage-like and osteoclast-like cells. Because cathepsin B and K display about half of their maximal proteolytic activity at neutral pH [14], these cathepsins may participate in degrading matrix protein when they are released from fibroblast-like and osteoblast-like cells.

In the present study, the subchondral plate was thickened in the loaded area, and cubic mononuclear cells, strongly expressing PH and type I collagen mRNAs and identified as active osteoblasts, lined the thick woven bone. Many osteoclasts were observed in all RA sections and expressed mainly MMP-9 and cathepsin K. Interestingly, we also found weak but distinct expression of the proteinases in active osteoclast-like cells. These cells also expressed MMP-9 mRNA and the proinflammatory cytokines IL-1β and TNF-α, which is indicative of a pathological condition. These findings are compatible with an in vitro study by Phnagakos and Kumar [42], who showed that TNF-α accelerates the expression of the MMP-9 gene in an osteoblast cell line, and with a histological study that demonstrated that inflammatory cytokines are produced by active osteoblasts in OA or Paget’s disease [43]. Proinflammatory cytokines were produced by macrophages or fibroblasts, as has been shown in the synovium [25], suggesting that similar pathological mechanisms of joint destruction occur in the subchondral bone region.

Fig. 3. Characterization of cells expressing cathepsin K in the subchondral region in RA patients. Multinucleated CD68-positive osteoclasts (a) stained intensely for cathepsin K (b), as did CD68-positive macrophages. Prolyl 4-hydroxylase (c), a marker for fibroblasts, was detected in a small proportion of mononuclear cells, and some of these were positively immunostained for cathepsin K (d). Original magnification ×400.

Fig. 2 (continued). [representative sections are shown in (i) for MMP-9 and in (k) for IL-1β]. Cubic mononuclear cells lining the bone surface had positive but weak immunoreactivity for cathepsin k (m) and expressed mRNAs for MMP-9 (n), TNF-α (o) and IL-1β (p). Original magnifications: b–f, m, p, ×100; a, g, ×200; h–l, n, o, ×400. B, bone.
Cathepsins B, K and L and TNF-α and IL-1β are localized at the pannus-cartilage junction; inflammatory cytokines enhance the production of the cysteine proteinases [31, 32]. In the present study, we showed the co-localization of the cathepsins, MMP-9 and the inflammatory cytokines in the subchondral region of RA patients. These findings suggest that cartilage and bone erosion in the subchondral region of RA patients is caused by a mechanism similar to joint destruction at the pannus-cartilage junction.

In conclusion, this histological study demonstrates that cathepsin K, MMP-9 and inflammatory cytokines are present in both osteoclast-like and osteoblast-like cells, and that cathepsin B and L occur primarily in macrophages appearing in the subchondral region. These results suggest that proteinases play a role in the occurrence of erosion in the subchondral bone and cartilage as well as at the pannus-cartilage junction. Moreover, the presence of the proinflammatory cytokines IL-1β and TNF-α, coexisting with these proteinases in the subchondral region, contributes directly to joint destruction in the subchondral region.

Acknowledgements

Rabbit polyclonal antibody against cathepsin K was kindly supplied by Dr Hideaki Sakai and Dr Yasuhiro Kobayashi (School of Dentistry, Nagasaki University). We wish to thank Kaori Izumi, Shouko Kuroda and Fumi Tamaki for excellent technical assistance. This research was supported by a grant from the Organization for Pharmaceutical Safety and Research and a grant-in-aid for Developmental Science Research from the Ministry of Education, Science, and Culture, Japan.

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