EXTENDED REPORT

Characterisation of the cell type-specificity of collagenase 3 mRNA expression in comparison with membrane type 1 matrix metalloproteinase and gelatinase A in the synovial membrane in rheumatoid arthritis

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Objective: To study the pattern and cell type-specificity of collagenase 3, membrane-type 1 matrix metalloproteinase (MT1-MMP), and gelatinase A mRNA expression in the synovial membrane in rheumatoid arthritis (RA).

Methods: The mRNA expression of collagenase 3, MT1-MMP, and gelatinase A was characterised by northern blot analysis, reverse transcriptase-polymerase chain reaction, and in situ hybridisation. In situ hybridisation was performed in combination with the immunohistochemical detection of cell type-specific antigens.

Results: Synovial membrane specimens from 19 of 21 patients with RA expressing collagenase 3 mRNA were positive for MT1-MMP and gelatinase A mRNA. In control samples from patients without destructive inflammatory joint diseases collagenase 3 mRNA was not expressed and only in two of seven cases was a coexpression of MT1-MMP and gelatinase A mRNA detected. Fibroblast-like cells of the synovial membrane were found to be the predominant source of collagenase 3, MT1-MMP, and gelatinase A mRNA expression in lining and sublining layers as well as at the synovial membrane-cartilage interface. Additionally, the expression of MT1-MMP mRNA was detected in endothelial cells. Collagenase 3 mRNA expression was found in about 5% of CD68 positive macrophages.

Conclusions: Collagenase 3 mRNA is expressed simultaneously with MT1-MMP and gelatinase A mRNA in fibroblast-like cells of the synovial membrane in RA. These results suggest (*a*) a broad extracellular proteolytic potential of fibroblast-like cells and (*b*) an important role of cell surface associated procollagenase 3 activation by MT1-MMP and gelatinase A for cartilage degradation by invading fibroblast-like cells.

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The process of progressive cartilage and bone destruction in chronic inflammatory and degenerative joint diseases involves distinct matrix metalloproteinases (MMPs) known to cleave extracellular matrix components with high efficiency. In addition, MMPs can activate each other by cleaving propeptides from zymogens.[1 2] As a result, the simultaneous expression of different MMPs might substantially increase the local proteolytic potential of activated invading cells.[3]

Collagenase 3 (MMP-13) is of special interest for the pathogenesis of cartilage degradation because it cleaves type II collagen of hyaline cartilage more efficiently than two other human collagenases, interstitial collagenase (MMP-1) and neutrophil collagenase (MMP-8).[4] Accordingly, collagenase 3 was detected in osteoarthritic cartilage[5 6] and in the synovial membrane in rheumatoid arthritis (RA).[7–9] In vitro it has been shown that procollagenase 3 can be efficiently activated by membrane-type 1 MMP (MT1-MMP) directly or by progelatinase A (MMP-2) activation, suggesting a membrane associated activation mechanism of procollagenase 3.[10–12] The hypothesis of a proteolytic cascade involving MT1-MMP, gelatinase A, and collagenase 3 is supported by the coordinate expression of these three MMPs in different malignant tumours.[13] In osteoarthritic cartilage the expression of MT1-MMP correlates with the activation of progelatinase A.[14] In RA, collagenase 3, MT1-MMP, and gelatinase A were detected in synovial membrane samples of patients by immunohistochemistry.[15] Activated forms of MT1-MMP and gelatinase A were found in synovial membrane specimens of

patients with RA, but not in normal synovial membrane samples.[16 17]

In the present work the mRNA expression pattern of collagenase 3, MT1-MMP, and gelatinase A was studied in synovial membrane samples derived from a cohort of patients with RA who were characterised clinically, as previously reported.[18] The cell type-specificity of the expression of collagenase 3 mRNA was analysed in comparison with that of MT1-MMP and gelatinase A mRNA using combined detection of the mRNA expression of these three MMPs by in situ hybridisation with immunohistochemical characterisation of cell type-specific antigens.

METHODS
Tissue preparation
For the histological characterisation of the cell type-specific expression of collagenase 3 mRNA in the synovial membrane in RA, synovial membrane specimens were obtained from 10 patients with RA who were undergoing wrist or knee

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Table 1 Evaluation of the collagenase 3 mRNA expression in various areas of the synovial membrane of patients with RA

Tissue sample	Synovial lining	Subsynovial interstitium	CD68+ macrophages	Lymphoid infiltrates	Endothelial cells
1	++	+/–	+/–	–	–
2	++	++	+/–	–	–
3	++	++	+/–	–	–
4	–	–	–	–	–
5	+/–	–	–	–	–
6	++	++	+/–	–	+/–
7	+/–	+/–	–	–	–
8	+++	++	+/–	–	+/–
9	++	++	+/–	–	–
10	+++	+	+/–	–	–

–, No positive cells; +/–, <5% positive cells; +, 5–30% positive cells; ++, 30–60% positive cells; +++, >60% positive cells.
The histopathological examination of various structures of the synovial membrane was combined with the detection of collagenase 3 mRNA expression by in situ hybridisation. In addition, macrophages were labelled immunohistochemically with an anti-CD68 mAb and endothelial cells were identified using an mAb against type IV collagen of the basal membrane. Synovial membrane samples of 10 different patients with RA were examined

synovectomy (three and one patient, respectively) and metacarpophalangeal arthroplasty (six patients) at the Schulthess Clinic, Zürich, Switzerland. For the characterisation of the mRNA expression pattern of collagenase 3, MT1-MMP, and gelatinase A in the synovial membrane in RA, synovial membrane specimens were obtained from 36 patients with RA who were undergoing wrist synovectomy at the Clinic of Orthopaedics, Berlin-Buch, Germany, as previously reported.[18] Samples from the synovial membrane-cartilage interface were obtained from six of these 36 patients with RA who, in addition to synovectomy, underwent dissection of the distal articular surface of the ulna. Synovial fluid samples obtained from the knees of a separate group of patients with RA examined and treated at the Clinic of Rheumatology, Berlin-Buch, were used as a source for generating primary synovial fibroblast cell cultures, as previously described.[18] All of the patients involved in the present work met the American College of Rheumatology 1987 revised criteria for RA.[19] No patient received intra-articular steroids just before the intervention. Control synovial membrane samples were obtained from seven patients without destructive inflammatory joint diseases who were undergoing plastic operation because of rupture of the anterior or posterior cruciate ligament. The synovial membrane samples were snap frozen in liquid nitrogen for northern blot analysis and in situ hybridisation, and embedded in paraffin for histopathologic evaluation. Synovial membrane-cartilage specimens were fixed in 4% phosphate buffered paraformaldehyde for four hours, subsequently decalcified in 10% EDTA, and embedded in paraffin.

Reagents
Mouse monoclonal antibodies (mAb) against leucocyte common antigen CD45 (clones 2B11 and PD7/26) and macrophage antigen CD68 (clone PG-M1) were obtained from Dako (Hamburg, Germany). Mouse antifibroblast mAb (clone D7-Fib) was from Serotec Ltd (Kidlington, Oxford, United Kingdom). The mAb against basement membrane type IV collagen have been produced and characterised by Gay and Fine.[20] Alkaline phosphatase conjugated streptavidin was obtained from Dako (Hamburg, Germany). T3 and T7 RNA polymerases, digoxigenin uridine triphosphate, and other reagents for RNA synthesis were from Boehringer (Mannheim, Germany). The nucleic acid detection system from Boehringer was used for determining the efficiency of RNA labelling. Biotin-SP conjugated goat antimouse IgG (Fcγ fragment-specific) was obtained from Dianova (Hamburg, Germany). TRIzol reagent and Super Script II RNase H reverse

transcriptase were from Gibco BRL (Eggenstein, Germany). Restriction enzymes were from New England BioLabs (Schwalbach, Germany), and [α-^{32}P]dATP was purchased from DuPont de Nemours (Bad Homburg, Germany). For reverse transcriptase-polymerase chain reaction (RT-PCR), 20-mer primers were generated for the amplification of a 631 bp complementary DNA (cDNA) fragment of collagenase 3 (Nos 787–1417, Genebank accession No X75308), an 818 bp cDNA fragment of MT1-MMP (Nos 170–987, Genebank accession No D26512), and an 808 bp cDNA fragment of gelatinase A (Nos 928–1735, Genebank accession No J03210).

Northern blot analysis
For northern blot analysis, total RNA was isolated from synovial membrane specimens using the TRIzol reagent according to the manufacturer's instructions. Total RNA (25 µg) was analysed on denatured agarose gels. The procedure was performed as previously described.[7] For generating probes for northern blot analysis, a 631 bp cDNA fragment of collagenase 3 (Nos 787–1417, Genebank accession No X75308), an 818 bp cDNA fragment of MT1-MMP (Nos 170–987, Genebank accession No D26512), and an 808 bp cDNA fragment of gelatinase A (Nos 928–1735, Genebank accession No J03210) were amplified. The entire glyceraldehyde-3-phosphate dehydrogenase (GAPDH) cDNA was used as a control for RNA loading. The northern blots were analysed with a Fujix Bio-Imaging Analyzer BAS 2000 (Fuji, Tokyo, Japan). For quantitative evaluation of collagenase 3 mRNA expression the signals of both transcripts were summed.

Reverse transcriptase-polymerase chain reaction (RT-PCR)
Primary synovial fibroblast cultures were established as described previously.[18] Cells were lysed in TRIzol reagent and RNA was prepared according to the manufacturer's protocol. Synthesis of complementary DNA was performed with oligo $(dT)_{12-18}$ primer and M-MuLV reverse transcriptase (Boehringer). DNA was amplified by PCR under standard conditions using a three step protocol with 27 cycles (22 for collagenase 3): denaturation at 94°C/25 seconds, annealing at 62°C/60 seconds (58°C for collagenase 3), and extension at 72°C/120 seconds (90 seconds for MT1-MMP and gelatinase A). Reaction products were loaded onto 1.5% agarose gels, electrophoresed, and visualised by ethidium bromide staining.

In situ hybridisation and immunohistochemical analysis
A combined approach of non-radioactive in situ hybridisation of collagenase 3, MT1-MMP, or gelatinase A mRNA and
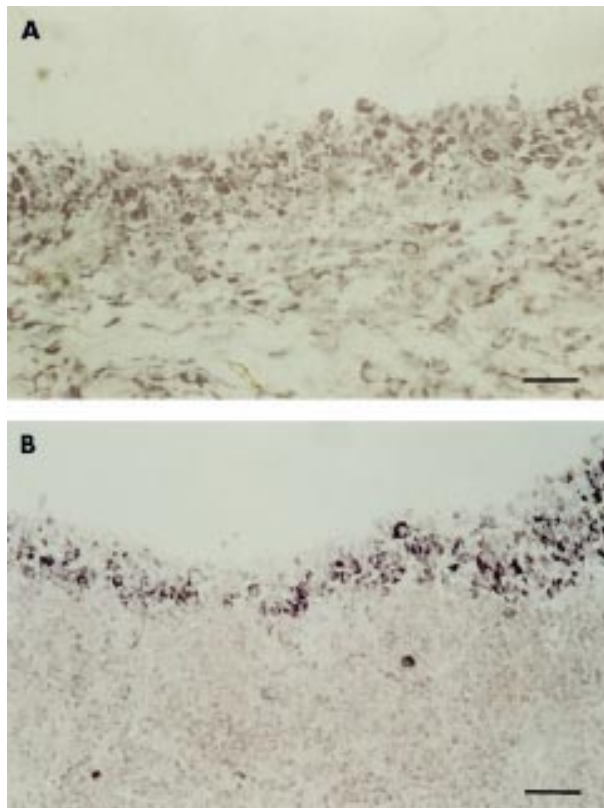
Figure 1 In situ hybridisation of collagenase 3 and interstitial collagenase mRNA in the synovial membrane from patients with RA (NBT/BCIP colour reagent). Collagenase 3 mRNA was detected in lining and sublining layers (A), whereas the mRNA expression of interstitial collagenase was largely restricted to the synovial lining (B). These data were confirmed by analysing synovial membrane samples of six patients with RA. Scale bar 100 μm in A; 130 μm in B.

Figure 2 Cell type-specific mRNA expression of collagenase 3 in the synovial membrane in RA. Collagenase 3 mRNA was detected by in situ hybridisation (NBT/BCIP colour reagent; black) and expressed in fibroblast-like cells (arrowheads) of the synovial membrane which were immunohistochemically labelled with mAb D7-Fib (APAAP method; red) known to recognise fibroblasts (A). Lymphocytic infiltrates (L) in the synovial membrane were negative for collagenase 3 mRNA expression (B). Scale bar 80 μm in A; 130 μm in B.

immunohistochemical detection of cell surface antigens or type IV collagen using the alkaline phosphatase-anti-alkaline phosphatase (APAAP) method was used as described.[21] In situ hybridisation was performed using digoxigenin labelled RNA probes and applying the nitroblue tetrazolium chloride/5-bromo-4-chloro-3-indolylphosphate (NBT/BCIP) signal detection method. For generating RNA probes, a 271 bp cDNA fragment of collagenase 3 (Nos 905–1176, Genebank accession No X75308), an 818 bp cDNA fragment of MT1-MMP (Nos 170–987, Genebank accession No D26512), and an 808 bp cDNA fragment of gelatinase A (Nos 928–1735, Genebank accession No J03210) were amplified. The probes were subcloned into pBluescript II KS (+/−) (Stratagene, Heidelberg, Germany) and linearised either with *Xba*I or *BamH*I for RNA polymerase reaction of the antisense or sense probe, respectively. The experiments were performed on frozen sections of synovial membrane specimens (7 μm) and sections from the synovial membrane-cartilage junction embedded in paraffin (4 μm).

RESULTS

Characterisation of the cell type-specific mRNA expression of collagenase 3 in the synovial membrane in RA

The localisation of collagenase 3 mRNA in synovial membranes was analysed by in situ hybridisation investigation of synovial membrane specimens of 10 patients with RA (table 1). A strong and diffuse mRNA expression of collagenase 3 localised in the synovial lining as well as in sublining layers was detected in 7/10 samples (table 1). In two other specimens
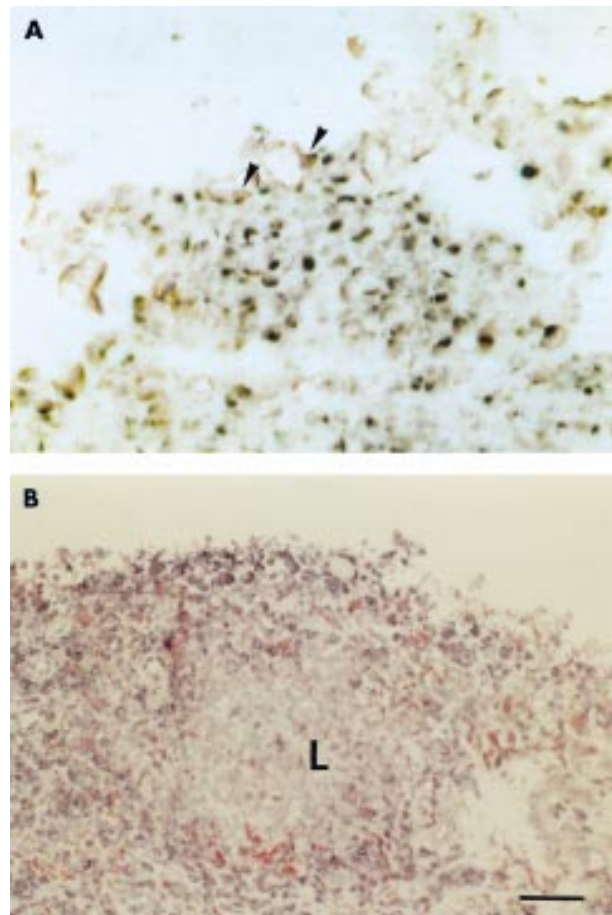
only a few single cells expressed collagenase 3 mRNA in the lining and sublining layers. One sample was completely negative for collagenase 3 mRNA expression. In contrast with collagenase 3, the mRNA expression of interstitial collagenase was detected in all 10 synovial membrane samples (data not shown), confirming our previous data obtained by northern blot analysis.[18] In addition, the mRNA expression of interstitial collagenase was largely restricted to the synovial lining, whereas collagenase 3 mRNA was detected in lining and sublining layers (fig 1).

To investigate the cellular origin of collagenase 3 expression in the synovial membrane in RA, in situ hybridisation of collagenase 3 mRNA was combined with immunohistochemical detection of cell type-specific antigens. As shown in fig 2A, collagenase 3 mRNA is expressed in fibroblast-like cells which were marked with the antifibroblast mAb D7-Fib. In parallel, a quantitative evaluation of collagenase 3 mRNA expression in non-fibroblast cells was performed. The analysis of 10 synovial membrane specimens by counting 500 CD68 positive macrophages for each sample showed that <5% of these cells were positive for collagenase 3 mRNA expression (table 1). A semiquantitative analysis of collagenase 3 mRNA expression in lymphoid infiltrates of the synovial membrane showed that this tissue was entirely negative for collagenase 3 mRNA expression (table 1, fig 2B). Collagenase 3 mRNA was detected in only a few endothelial cells in 2/10 synovial membrane

Table 2 mRNA expression pattern of collagenase 3, MT1-MMP, and gelatinase A in synovial membrane specimens of patients with RA

mRNA expression			
Collagenase 3	MT1-MMP	Gelatinase A	n
Patients with RA			
+	+	+	19
+	−	+	2
−	+	+	8
−	−	−	7
Non-arthritic controls			
−	−	−	5
−	+	+	2

Synovial membrane specimens of 36 patients with RA and seven control patients without destructive inflammatory joint diseases were investigated by RT-PCR analysis for the expression of collagenase 3, MT1-MMP, and gelatinase A mRNA.
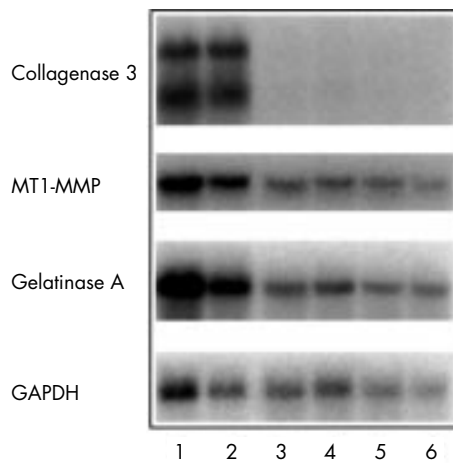

Figure 3 mRNA expression of collagenase 3, MT1-MMP, and gelatinase A in the synovial membrane in RA. Northern blot analysis was performed using total RNA from six separate synovial membrane specimens of patients with RA by successive hybridisation with radioactively labelled cDNA probes corresponding to collagenase 3, MT1-MMP, and gelatinase A, as indicated on the left. The mRNA expression levels of MT1-MMP and gelatinase A were only slightly raised in patients with RA expressing collagenase 3 mRNA in the synovial membrane (lanes 1 and 2) compared with patients without collagenase 3 mRNA expression (lane 3–6). GAPDH was used to check the loading of RNA.

samples (table 1). Therefore, fibroblast-like cells represent the predominant source of collagenase 3 mRNA expression in the synovial membrane in RA.

Simultaneous mRNA expression of collagenase 3, MT1-MMP, and gelatinase A in the synovial membrane and at the synovial membrane-cartilage interface in RA
MT1-MMP and gelatinase A contribute to membrane associated procollagenase 3 activation.[10 11] To analyse the potential role of these procollagenase 3 activation mechanisms for cartilage and bone degradation in RA, the mRNA expression of collagenase 3, MT1-MMP, and gelatinase A was investigated in synovial membrane specimens from 36 patients with RA by RT-PCR analysis (table 2). In a previous study of the same patients, collagenase 3 mRNA was detected only in 21 of these 36 synovial membrane samples.[18] As shown in table 2, 19 synovial membrane samples with collagenase 3 mRNA expression were also positive for MT1-MMP and gelatinase A mRNA. In two other cases a simultaneous mRNA expression was found only for collagenase 3 and gelatinase A. An analy-
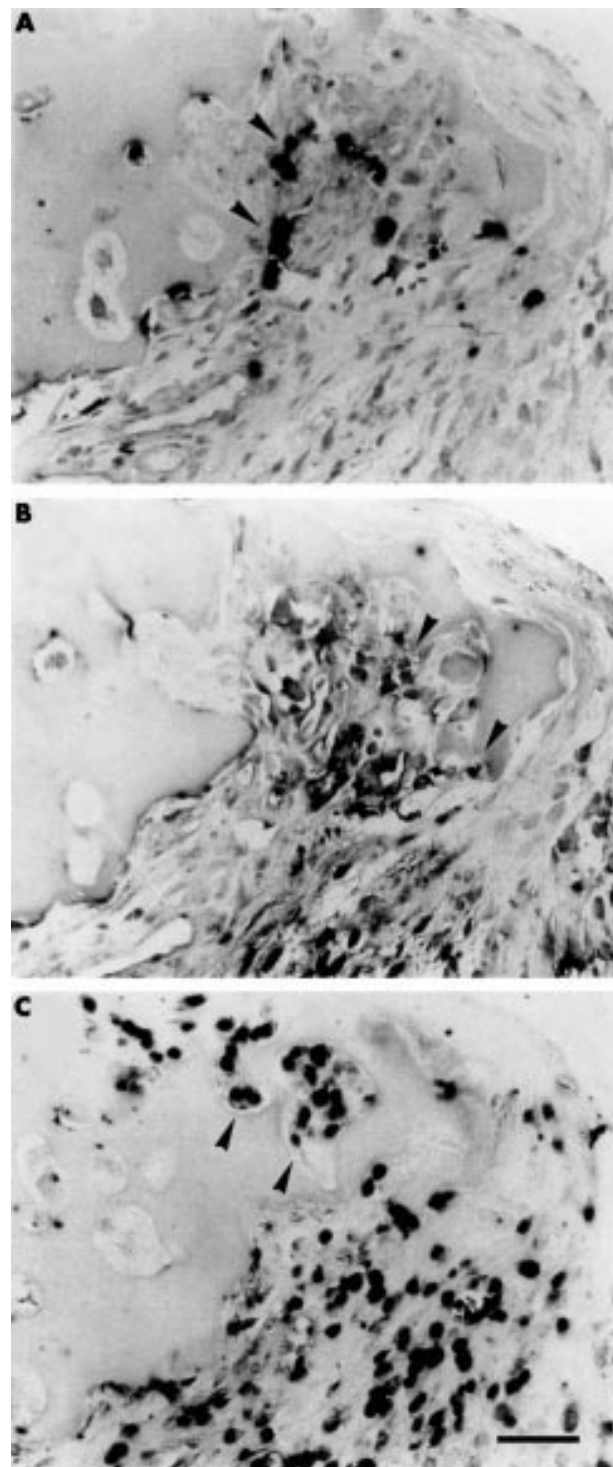

Figure 4 Localisation of collagenase 3, MT1-MMP, and gelatinase A mRNA expression at the synovial membrane-cartilage interface. Collagenase 3 (A), MT1-MMP (B), and gelatinase A mRNA (C) are colocalised in the synovial membrane adjacent to the cartilage (arrowheads), as shown for serial sections of the synovial membrane-cartilage interface of a patient with RA by in situ hybridisation (NBT/BCIP colour reagent; black). This observation was confirmed by analysing synovial membrane-cartilage samples of six patients with RA. Scale bar 100 μm.

sis of synovial membrane specimens without collagenase 3 mRNA expression for the expression of MT1-MMP and gelatinase A mRNA showed that MT1-MMP and gelatinase A mRNA were coexpressed in 8/15 cases, whereas in seven synovial membrane specimens the mRNA expression of both
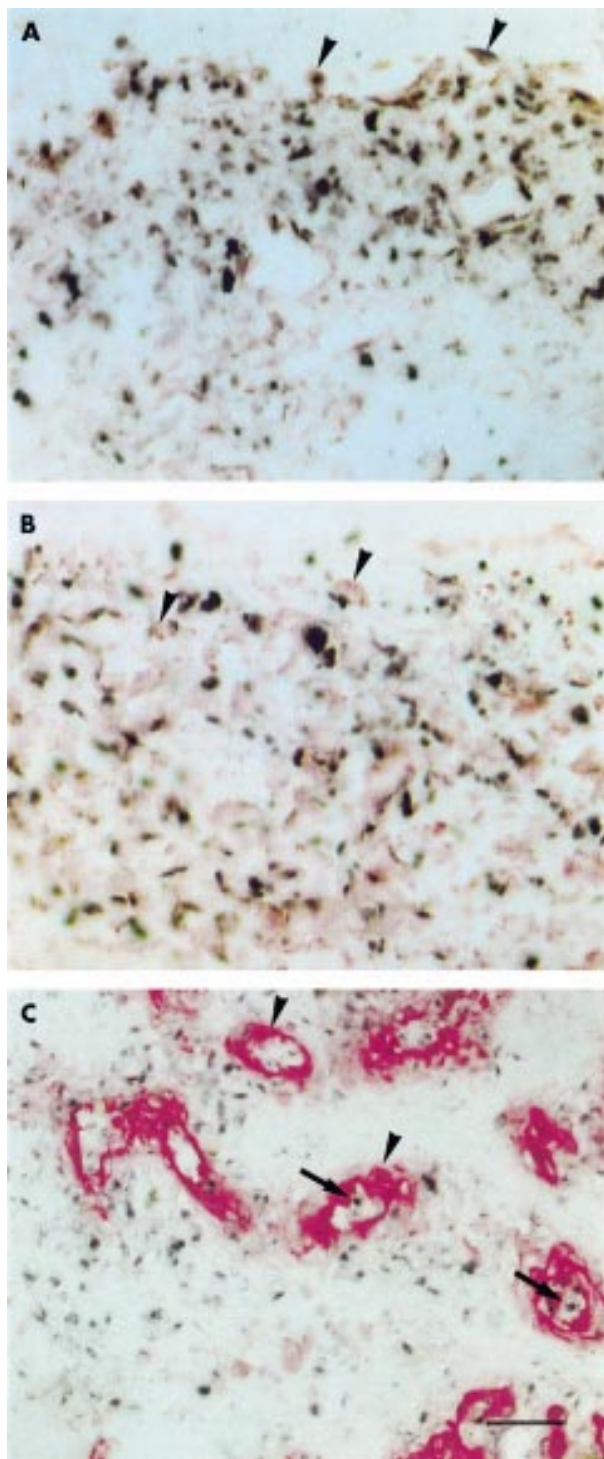
Figure 5 Cell type-specific mRNA expression of MT1-MMP and gelatinase A in the synovial membrane in RA. MT1-MMP and gelatinase A mRNA were detected by in situ hybridisation (NBT/BCIP colour reagent; black). Both MMPs are expressed in fibroblast-like cells (arrowheads) of the synovial membrane, which were immunohistochemically labelled with mAb D7-Fib (APAAP method; red) as shown in (A) and (B), respectively. MT1-MMP mRNA expression (black) was detected in endothelial cells (arrows) marked with an mAb against type IV collagen of the basal membrane (red, arrowheads) (C). Scale bar 80 µm in A and B; 200 µm C.

Figure 6 mRNA expression of collagenase 3, MT1-MMP, and gelatinase A in the synovial membrane in RA. RT-PCR was performed on total RNA from primary synovial fibroblast cultures of patients with RA using specific primers for collagenase 3, MT1-MMP, gelatinase A, and GAPDH as indicated on the left. All 10 fibroblast cell cultures expressed basal levels of MT1-MMP and gelatinase A mRNA, whereas basal collagenase 3 mRNA expression was detected only in 4/10 cultures.

MMPs was lacking. In synovial membrane preparations of seven patients without destructive inflammatory joint diseases collagenase 3 mRNA could not be detected. At the same time, only in two of these specimens was a coexpression of
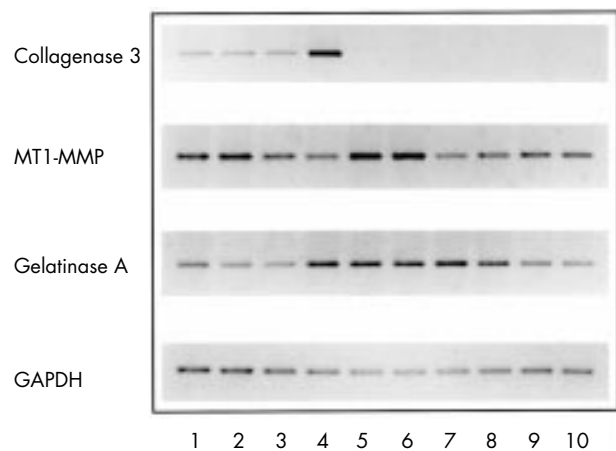
MT1-MMP and gelatinase A mRNA found. Therefore, MT1-MMP and gelatinase A mRNA are expressed (*a*) in most of the synovial membrane samples in RA and (*b*) simultaneously in nearly all cases.

Northern blot analysis was performed to compare the mRNA expression level of MT1-MMP and gelatinase A in the synovial membrane between patients with RA with and without collagenase 3 mRNA expression. In 7/21 synovial membrane specimens expressing collagenase 3 mRNA the mRNA expression levels of MT1-MMP and gelatinase A were slightly raised, as shown in comparison with synovial membrane samples without collagenase 3 mRNA expression in a representative northern blot from six separate synovial specimens (fig 3). These data suggest that the regulation of transcription of collagenase 3, MT1-MMP, and gelatinase A mRNA in the synovial membrane in RA is different, particularly the transcriptional regulation of collagenase 3 in comparison with MT1-MMP and gelatinase A.

The localisation of MT1-MMP and gelatinase A mRNA in the synovial membrane was investigated by in situ hybridisation. A diffuse mRNA expression pattern of both MMPs was detected in lining and sublining layers (data not shown). Furthermore, MT1-MMP and gelatinase A mRNA were colocalised with collagenase 3 mRNA in the synovial membrane adjacent to the cartilage, as shown for serial sections of the synovial membrane-cartilage interface in fig 4.

Characterisation of the cell type-specific mRNA expression of MT1-MMP and gelatinase A in the synovial membrane in RA

To elucidate the cellular origin of MT1-MMP and gelatinase A mRNA expression in the synovial membrane, in situ hybridisation of both MMPs was combined with immunohistochemical detection of cell type-specific antigens. MT1-MMP and gelatinase A mRNA were detected in fibroblast-like cells of the synovial membrane using the antifibroblast mAb D7-Fib, as shown in figs 5A and B. At the same time, endothelial cells labelled with a mAb against basement membrane type IV collagen showed a pronounced mRNA expression of MT1-MMP (fig 5C). However, endothelial cells were negative for gelatinase A mRNA expression (data not shown). At the same time, CD45 positive leucocytes did not express MT1-MMP mRNA (data not shown), whereas CD68 positive

macrophages were found to express MT1-MMP mRNA, as previously reported.[22] Only a few CD68 positive macrophages and CD45 positive leucocytes were positive for gelatinase A mRNA (data not shown). Therefore, fibroblast-like cells of the synovial membrane, being the predominant source of collagenase 3 mRNA expression, also express MT1-MMP and gelatinase A mRNA.

Investigation of MT1-MMP and gelatinase A mRNA expression in primary synovial fibroblast cell cultures

The mRNA expression of MT1-MMP and gelatinase A was analysed in primary synovial fibroblast cell cultures which were previously characterised for collagenase 3 mRNA expression.[18] A basal collagenase 3 mRNA expression was detected only in 4/10 primary synovial fibroblast cell cultures. However, all these 10 fibroblast cell cultures showed comparable basal levels of MT1-MMP and gelatinase A mRNA expression by northern blot and RT-PCR analyses (fig 6). Therefore, the restricted mRNA expression of collagenase 3 and the constitutive MT1-MMP and gelatinase A mRNA expression in primary synovial fibroblast cell cultures are similar to their mRNA expression pattern in synovial membrane samples in RA.

DISCUSSION

Distinct MMPs participate in cartilage and bone degradation in RA by cleavage of different extracellular matrix components and by processing each other through zymogen activation.[1] Because in RA the chronically inflamed and invasive growing synovial membrane consists of different cell types, the identification of cells producing MMP is important (*a*) to understand the cellular origin of cartilage and bone degradation and (*b*) to improve our knowledge about the role of cell-cell interactions for MMP zymogen activation and, therefore, the generation of a high extracellular proteolytic potential.

The analysis of the expression pattern of collagenase 3 suggests an important role of this MMP in several pathological conditions, especially in malignancies and chronic inflammatory processes.[5 7 13 23] The expression of collagenase 3 in aggressively growing malignant tumours reflects its ability to cleave efficiently a wide range of extracellular matrix components.[4 24] In parallel, collagenase 3 mRNA expression in the synovial membrane of patients with RA is correlated with severe clinical forms of the disease in these patients.[18]

In this study we showed for a cohort of patients with RA[18] that collagenase 3, MT1-MMP, and gelatinase A mRNA are simultaneously expressed in the synovial membrane, particularly at the synovial membrane-cartilage interface. Fibroblast-like cells of the synovial membrane marked with mAb D7-Fib were identified to express collagenase 3, MT1-MMP, and gelatinase A mRNA. As a result, these three MMPs potentially combine their proteolytic activity towards distinct extracellular matrix components. In addition, experiments in vitro showed that MT1-MMP directly or indirectly through progelatinase A activation can activate procollagenase 3, suggesting a membrane associated activation mechanism of collagenase 3.[10–12] Tissue inhibitor of matrix metalloproteinases-2, supposed to play a part in the regulation of procollagenase 3 activation within this proteolytic cascade,[25] has been shown to be coexpressed with MT1-MMP and gelatinase A in the synovial membrane in RA.[16] Therefore, the simultaneous mRNA expression of collagenase 3, MT1-MMP, and gelatinase A in fibroblast-like cells of the synovial membrane suggests that (*a*) these three MMPs form a complex proteolytic potential towards distinct extracellular matrix components and (*b*) that high collagenolytic activity through procollagenase 3 activation by MT1-MMP and gelatinase A is generated at the surface of fibroblast-like cells. As a result, fibroblast-like cells might have a high potential to invade adjacent cartilage and bone structures.

Although in adult human tissues the expression of collagenase 3 is strongly restricted to pathological conditions, MT1-MMP and gelatinase A seem to be more broadly expressed.[26 27] The present work shows a simultaneous expression of MT1-MMP and gelatinase A mRNA not only in most of the synovial membrane samples in RA but also in some of the control specimens (table 2). Interestingly, the mRNA expression level of MT1-MMP and gelatinase A did not substantially differ between all RA samples and was only slightly increased in some of the synovial membrane specimens with collagenase 3 mRNA expression compared with samples without collagenase 3 mRNA expression or control samples (fig 3). These data implicate a rather constitutive mRNA expression pattern of MT1-MMP and gelatinase A in the synovial membrane, which is supported by the analysis of MT1-MMP and gelatinase A mRNA expression in the synovial membrane after traumatic joint injury,[28] and by in situ hybridisation studies of MT1-MMP in rheumatoid and normal synovial membrane samples.[22] Accordingly, all primary synovial fibroblast cell cultures analysed in the present work showed basal mRNA expression levels of MT1-MMP and gelatinase A, whereas collagenase 3 mRNA could be detected only in some of these cell cultures.[18]

To confirm that mainly fibroblast-like cells of the synovial membrane in RA express collagenase 3 as well as MT1-MMP and gelatinase A, in situ hybridisation of these three MMPs was combined with the immunohistochemical detection of antigens specific for distinct non-fibroblast cell populations. Quantitative analysis showed that <5% of CD68 positive macrophages were positive for collagenase 3 mRNA expression (table 1). Only in 2/10 synovial membrane samples did a few endothelial cells express collagenase 3 mRNA (table 1). The evaluation of lymphoid infiltrates in synovial membrane preparations showed that collagenase 3 mRNA is not expressed in this tissue (fig 2B), suggesting that T cells in the rheumatoid synovial membrane do not express collagenase 3 mRNA. Accordingly, CD45 positive leucocytes were negative for collagenase 3 mRNA expression, except for a few single cells, as previously described.[18] These data do not completely confirm a rather broad cellular expression pattern of collagenase 3 in synovial membrane samples in RA observed by combined immunohistochemical detection of collagenase 3 and cell type-specific antigens.[8 15] However, immunohistochemical detection of collagenase 3 seems to be less suitable for the characterisation of the cell type-specific expression pattern of collagenase 3 than the detection of collagenase 3 mRNA by in situ hybridisation, because collagenase 3, like other MMPs, represents a secretory proteinase that is mainly located in the extracellular space.

In contrast with the restricted cellular mRNA expression pattern of collagenase 3, MT1-MMP mRNA was detected in different cell types of the synovial membrane in RA. In this study a marked MT1-MMP mRNA expression was found not only in fibroblast-like cells but also in endothelial cells. The latter finding is supported by immunohistochemical detection of MT1-MMP in synovial blood vessels.[16] Because MT1-MMP has been described as playing a part in blood vessel formation,[29 30] it can be expected that MT1-MMP also participates in angiogenesis in RA. Furthermore, CD68 positive macrophages were identified as expressing MT1-MMP mRNA in the synovial membrane in RA.[22] In contrast with MT1-MMP, gelatinase A is expressed in the synovial membrane in RA only in a few CD68 positive macrophages and CD45 positive leucocytes and not in endothelial cells. Therefore, it might be presumed that the cooperation between fibroblast-like cells and macrophages could further increase their invasive potential due to membrane associated activation of procollagenase 3 and progelatinase A by MT1-MMP.

Of note, collagenase 3 mRNA predominantly expressed in fibroblast-like cells of the synovial membrane in RA was detected mainly in stromal cells of malignant tumours.[31 32]

These observations suggest that soluble factors released by non-fibroblasts and/or tumour cells as well as direct cell-cell contacts might be responsible for the induction of collagenase 3 expression in fibroblasts known to be differently regulated compared with other MMPs.[33–35]

Based on the data of the present study, the relevance of pro-collagenase 3 activation by MT1-MMP and gelatinase A for cartilage degradation should be analysed in vivo. In addition, further efforts are needed to clarify the regulation of collagenase 3 mRNA expression in response to soluble factors and cell-cell interactions in fibroblast-like cells of the synovial membrane in RA.

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