

# Matrix metalloproteinases and tissue inhibitors of metalloproteinases in synovial fluids from patients with rheumatoid arthritis or osteoarthritis

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## Abstract

**Objective**—Matrix metalloproteinases (MMPs) are expressed in joint tissues of patients with rheumatoid arthritis (RA) and osteoarthritis (OA). The objective of this study was to define the steady state levels of seven different MMPs and two tissue inhibitors of metalloproteinases (TIMPs) as well as the potential metalloproteinase activity in the synovial fluid (SF) to provide more insight into the role of MMPs in cartilage destruction in RA and OA.

**Methods**—Levels of MMP-1, MMP-2, MMP-3, MMP-7, MMP-8, MMP-9, MMP-13, TIMP-1, and TIMP-2 in SF aspirated from knee joints of 97 patients with RA and 103 patients with OA were measured by the corresponding one step sandwich enzyme immunoassays. Proteolytic activity of MMPs in these SFs was examined in an assay using [<sup>3</sup>H]carboxymethylated transferrin substrate in the presence of inhibitors of serine and cysteine proteinases after activation with *p*-amino-phenylmercuric acetate (APMA). Destruction of RA knee joints was radiographically evaluated.

**Results**—Levels of MMP-1, MMP-2, MMP-3, MMP-8, and MMP-9 were significantly higher in RA SF than in OA SF. MMP-7 and MMP-13 were detectable in more than 45% of RA SFs and in less than 20% of OA SFs, respectively. Among the MMPs examined, MMP-3 levels were extremely high compared with those of other MMPs. Direct correlations were seen between the levels of MMP-1 and MMP-3 and between those of MMP-8 and MMP-9 in RA SF. Although the levels of MMP-1 and MMP-3 increased even in the early stage of RA, those of MMP-8 and MMP-9 were low in the early stage and increased with the progression of RA. Molar ratios of the total amounts of the MMPs to those of the TIMPs were 5.2-fold higher in patients with RA than in OA, which was significant. APMA-activated metalloproteinase activity in SF showed a similar result, and a direct correlation was seen between the molar ratios and the activity in RA SF.

**Conclusions**—Our results show that high levels of MMP-1, MMP-2, MMP-3, MMP-8, MMP-9, and TIMP-1 are present in RA SF and suggest that once these

**MMPs are fully activated, they have an imbalance against TIMPs, which may contribute to the cartilage destruction in RA.**

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Destruction of cartilage is a common pathological feature in various arthritides, including rheumatoid arthritis (RA) and osteoarthritis (OA), and is a major cause of joint dysfunction, which is followed by impairment of the “quality of life” in those patients. Two pathways are known for the destruction of the cartilage. Firstly, an intrinsic pathway by which chondrocytes themselves degrade cartilage extracellular matrix (ECM) and, secondly, an extrinsic pathway by which tissues or cells other than chondrocytes, such as inflamed synovium, pannus tissue, and infiltrated inflammatory cells, break down the ECM of cartilage mostly through synovial fluid (SF). In both pathways, enzymic digestion of the ECM is ascribed to cartilage destruction.

Many proteinases belonging to all classes of proteinases are expressed in joint tissues of patients with RA and OA. Among them, however, matrix metalloproteinases (MMPs) are believed to have a key role in the joint destruction in the arthritides.<sup>1–3</sup> MMPs, a gene family of neutral Zn<sup>2+</sup> metalloproteinases, are composed of at least 18 members, which are classified into five subgroups of structurally related MMPs: (a) collagenases, including tissue collagenase (MMP-1), neutrophil collagenase (MMP-8), and collagenase 3 (MMP-13); (b) gelatinases such as gelatinase A (MMP-2) and gelatinase B (MMP-9); (c) stromelysins, including stromelysin 1 (MMP-3) and stromelysin 2 (MMP-10); (d) membrane-type MMPs (MT-MMPs),<sup>4–8</sup> including MT1-MMP (MMP-14), MT2-MMP (MMP-15), MT3-MMP (MMP-16), MT4-MMP (MMP-17), and MT5-MMP (MMP-24); and (e) other MMPs such as matrilysin (MMP-7), stromelysin 3 (MMP-11), metalloelastase (MMP-12), MMP-19,<sup>9</sup> enamelysin (MMP-20),<sup>10</sup> and MMP-23.<sup>11</sup> The enzymic activities of these MMPs are strictly controlled by inhibition with specific inhibitors—that is, tissue inhibitors of metalloproteinases (TIMP-1, TIMP-2, TIMP-3, and TIMP-4).<sup>2, 12, 13</sup> Therefore, the balance between the amounts of MMPs and TIMPs in SF and local tissue may be a determinant of whether MMPs attack the cartilage ECM.

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MMP-1 and MMP-3 are produced by synovial lining cells in RA,<sup>14-16</sup> whereas MMP-2 is produced by stromal cells in the sublining synovial layer.<sup>14</sup> MMP-8 and MMP-9 are secreted by neutrophils,<sup>2</sup> and MMP-9 is also produced by macrophages<sup>17</sup> and synovial cells.<sup>18</sup> In addition, the expression of these MMPs in chondrocytes has also been confirmed.<sup>19-22</sup> On the other hand, various MMPs are present in arthritic SF, which are produced by the joint tissues and infiltrated inflammatory cells. Several groups have independently reported that the levels of MMP-1, MMP-3, MMP-8, MMP-9, and TIMP-1 in SF from patients with RA are raised compared with those from patients with OA or with post-traumatic knee injury.<sup>18 23-28</sup> However, little or no information is available for the levels of other MMPs, such as MMP-2, MMP-7, MMP-13, and TIMP-2 in RA and OA SF. In addition, there are few reports that describe the actual levels of several MMPs in SF measured simultaneously in the same patients, and the relations between these levels and their potential enzymic activity or the disease progression of RA.

In this study we determined the steady state levels of MMP-1, MMP-2, MMP-3, MMP-7, MMP-8, MMP-9, MMP-13, TIMP-1, and TIMP-2 in the SF from patients with RA or OA, and examined the correlation between the levels and potential proteolytic activity in the same samples.

## Methods

### PATIENTS

Ninety seven outpatients with RA and 103 outpatients with knee OA attending at the Department of Orthopaedic Surgery, National Defence Medical College Hospital, Tokorozawa were studied. Table 1 summarises the general and clinical characteristics of the patients and their knees.

Diagnosis of the patients with RA was based on the American College of Rheumatology 1987 revised criteria.<sup>29</sup> They were further subdivided into three groups according to the method of Larsen *et al*,<sup>30</sup> based on the radiographic findings of femorotibial joints from which the SFs were aspirated; 17 patients

were in the early stage, which corresponds to the Larsen grade 0-I, 57 patients in the middle stage corresponding to the Larsen grade II-III, and 23 patients in the advanced stage corresponding to the Larsen grade IV-V. All the patients with RA were treated with non-steroidal anti-inflammatory drugs (NSAIDs). Fifty of these patients also received low dose steroid treatment (prednisolone, maximum 10 mg/day). Some patients with RA were treated with disease modifying antirheumatic drugs—that is, sodium aurothiomalate (17 patients), auranofin (10), sulfasalazine (5), D-penicillamine (11), bucillamine (18), or mizoribine (4), and nine patients received methotrexate, either alone or in combination with steroid.

Knee OA was diagnosed by clinical and radiological evaluations based on the American College of Rheumatology criteria.<sup>31</sup> Patients who presented with obvious joint injury or with generalised OA were excluded from the study. Fifty two patients with OA received various NSAIDs for knee pain.

Patients with RA or OA were not treated with intra-articular injection of steroids, chondroitin polysulphate or hyaluronic acid for at least one month before this study. SF was aspirated from the knee joints of patients with RA or OA under aseptic conditions as part of a therapeutic procedure, and stored at  $-80^{\circ}\text{C}$  before being used as described previously.<sup>25</sup>

### MEASUREMENT OF MMP-1, MMP-2, MMP-3, MMP-7, MMP-8, MMP-9, MMP-13, TIMP-1, AND TIMP-2

Levels of MMP-1, MMP-2, MMP-3, MMP-7, MMP-8, MMP-9, MMP-13, TIMP-1, and TIMP-2 in SF were measured by the corresponding one step sandwich enzyme immunoassay (EIA) systems as described previously.<sup>27 32-39</sup> Briefly, the assay systems used two simultaneous immunoreactions of a solid phase monoclonal antibody and a horseradish peroxidase labelled Fab' fragment of another monoclonal antibody. The antibodies were raised against the zymogens (proMMPs) of human MMP-1, MMP-2, MMP-3, MMP-7, MMP-8, MMP-9, MMP-13, and bovine TIMP-1, or oligopeptides prepared from the amino acid sequence of human proMMP-7 and TIMP-2. The EIA systems for MMP-1, MMP-3, MMP-8, and MMP-13 measure both precursor and active forms of the MMPs, and the systems for these MMPs, except for MMP-8, detect the complex with TIMPs.<sup>27 32 34 37</sup> However, those for MMP-2, MMP-7, and MMP-9 measure only their precursor forms.<sup>33 35 36</sup> The EIA system for TIMP-1 determines the whole amount of TIMP-1, including free TIMP-1 and the complexed forms with active MMPs and proMMP-9,<sup>36 38</sup> but the EIA system for TIMP-2 detects free TIMP-2 and TIMP-2 complexed with active MMPs but not the complex with proMMP-2.<sup>39</sup> Detection limits of these systems for MMP-1, MMP-2, MMP-3, MMP-7, MMP-8, MMP-9, MMP-13, TIMP-1, and TIMP-2 are 0.12, 1.0, 0.63, 0.16, 0.50, 0.24, 0.63, 1.24, and 6.30 ng/ml, respectively. Values are calculated as mol/l for

Table 1 Characteristics of the patients and their knees. Results are shown as mean (SD), (median), and range

Group (n, F/M*)	Age (years)	Disease duration (years)	Aspirated SF* volume (ml)
<b>RA</b>			
All (97, 74/23)	57.6 (12.1) (58)	13.0 (9.3) (11)	17.0 (13.5) (14.0)
Early stage (17, 14/3)	46.0 (15.4) (51)	5.0 (3.9) (4.5)	19.4 (12.4) (17.0)
Middle stage (57, 41/16)	59.0 (10.2) (59)	13.1 (8.7) (12)	18.6 (15.1) (18.0)
Advanced stage (23, 19/4)	62.2 (9.0) (62)	21.5 (10.0) (21.5)	11.7 (7.5) (10.0)
	44-82	3.5-42	2-30
<b>OA</b>			
All (103, 83/20)	64.0 (10.3) (64)	7.4 (6.8) (5)	14.7 (8.2) (14.0)
	44-86	0.25-30	3-45

\*F = female; M = male; SF = synovial fluid.

Table 2 Levels of MMP-1, MMP-2, MMP-3, MMP-8, and MMP-9 in SF from knee joints of patients with rheumatoid arthritis and osteoarthritis. Results are shown as means (SD), (median), and range (nmol/l)

Group	MMP-1	MMP-2	MMP-3	MMP-8	MMP-9
RA					
All	35.1 (24.8)*** (36.1)	28.7 (8.3)*** (28.2)	1980 (1360)*** (1853)	13.2 (18.6)*** (6.36)	10.6 (14.8)*** (5.77)
Early stage	0.2–90.6 38.5 (20.3)* (37.6)	14.8–52.2 30.2 (9.4) (28.2)	27–5384 2150 (1110)* (2012)	0.01–121.34 6.08 (8.83)† (2.25)	0.02–65.54 3.91 (5.94)† (1.04)
Middle stage	1.7–74.2 39.8 (24.1)** (40.5)	16.2–52.2 28.3 (7.8) (26.8)	100–4254 2180 (1340)** (2031)	0.01–31.31 14.9 (17.2) (8.61)	0.02–24.25 12.7 (16.2) (7.60)
Advanced stage	0.4–90.6 20.9 (23.4) (16.6)	14.8–47.9 28.7 (8.4) (28.9)	121–5384 1350 (1350) (801)	0.01–86.11 14.1 (24.7) (6.26)	0.02–62.92 10.4 (14.0) (8.39)
	0.2–71.3	14.8–49.3	27–4024	0.01–121.34	0.02–65.54
OA					
All	5.74 (5.17) (3.95)	20.3 (7.7) (19.7)	282 (53) (180)	ND	ND
	0.1–28.4	6.2–52.2	8–1192		

\*p<0.05 versus corresponding values in advanced stage of RA; \*\*p<0.01 versus corresponding values in advanced stage of RA; \*\*\*p<0.001 versus corresponding values in OA all.

†p<0.05 versus corresponding values in middle and advanced stages of RA.

ND = not detected (see "Results").

the comparison using the following molecular weights: 51 929 for proMMP-1, 70 952 for proMMP-2, 52 220 for proMMP-3, 27 916 for proMMP-7, 51 098 for proMMP-8, 78 426 for proMMP-9, 51 647 for proMMP-13, 20 685 for TIMP-1, and 21 755 for TIMP-2, which were calculated from their amino acid sequences.<sup>40</sup>

#### MEASUREMENT OF CARBOXYMETHYLATED TRANSFERRIN-DEGRADING ACTIVITY IN SF

Carboxymethylated transferrin (Cm-Tf)-degrading activity in SF was measured using [<sup>3</sup>H]Cm-Tf.<sup>41</sup> SF samples (52 RA and 60 OA) were incubated with 1 mM *p*-aminophenylmercuric acetate (APMA) at 37°C for 18 hours to activate proMMPs. Ten microlitres of the activated samples was incubated with the same amount of [<sup>3</sup>H]Cm-Tf and assayed at 37°C for six hours in the presence of 2 mM phenylmethane sulphonyl fluoride and 5 mM *N*-ethylmaleimide to inhibit serine and cysteine proteinases. After the reaction, undegraded [<sup>3</sup>H]Cm-Tf was precipitated with trichloroacetic acid and centrifuged. The radioactivity in the supernatants was measured in a liquid scintillation counter. One unit of activity is defined as the amount of enzyme that degrades 1 µg Cm-Tf/min at 37°C.

#### STATISTICAL ANALYSIS

Differences between the two diagnostic groups and among the radiographical stages of RA were analysed by the Mann-Whitney U test for unpaired variables and the Kruskal-Wallis test, respectively. Correlations were sought using Spearman's rank correlation coefficient ( $r_s$ ). *p* Values less than 0.05 were considered significant.

#### Results

##### LEVELS OF MMP-1, MMP-2, MMP-3, MMP-7, MMP-8, MMP-9, MMP-13, TIMP-1, AND TIMP-2 IN KNEE SF FROM PATIENTS WITH RA OR OA

MMP-1, MMP-2, MMP-3, MMP-8, and MMP-9 were measurable by the EIA systems in more than 90% of RA SFs, and the mean (SD) concentrations were 35.1 (24.8), 28.7 (8.3), 1980 (1360), 13.2 (18.6), and 10.6

(14.8) nmol/l, respectively (table 2). Although MMP-1, MMP-2, and MMP-3 were also detectable in all the SF from patients with OA, MMP-8 and MMP-9 were detected only in fewer than 2% of the samples. The levels of MMP-1, MMP-2, MMP-3, MMP-8, and MMP-9 were significantly higher in patients with RA than in those with OA (table 2). On the other hand, MMP-7 and MMP-13 were measurable in 61% and 46% of SF from patients with RA, respectively, and in only 15% and 18% of the OA SF, respectively. The levels of MMP-7 (1.95 (3.61) nmol/l *v* 0.77 (1.52) nmol/l, RA *v* OA) and MMP-13 (0.05 (0.04) nmol/l *v* 0.02 (0.07) nmol/l, RA *v* OA) in SF were extremely low compared with those of MMP-1, MMP-2, MMP-3, MMP-8, and MMP-9.

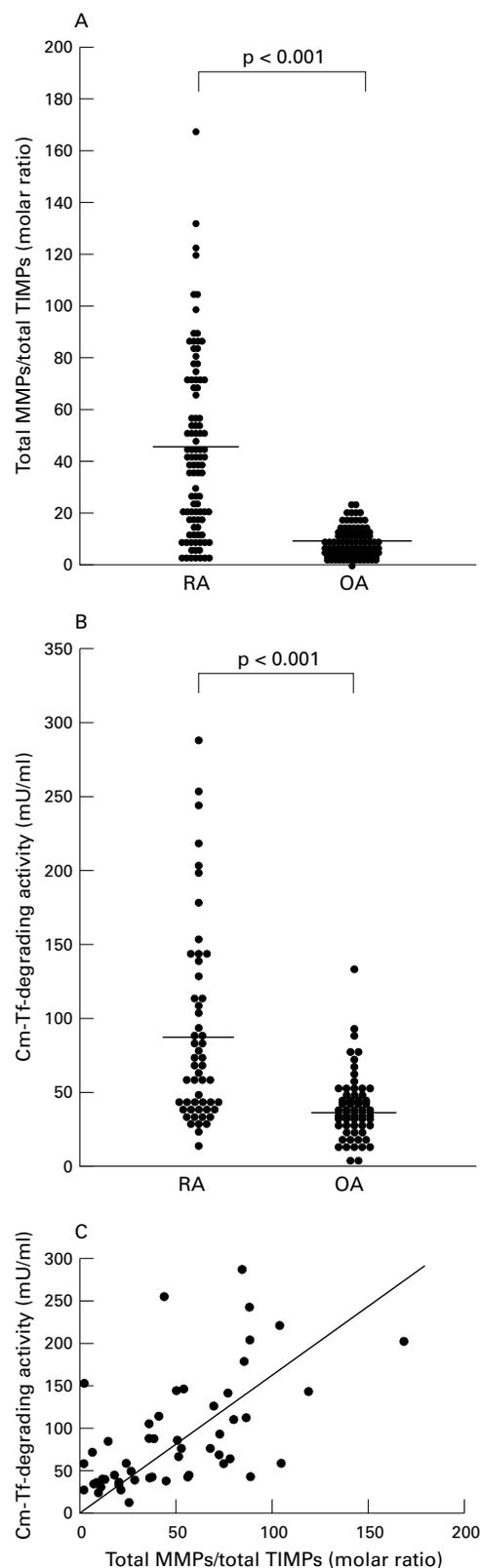
When the levels of MMP-1, MMP-2, MMP-3, MMP-8, and MMP-9 in SF in the different RA stages were compared, MMP-1 and MMP-3 were high even in the early stage and decreased significantly in the advanced stage (table 2). On the other hand, the levels of MMP-8 and MMP-9 were not high in the early stage, and appeared to increase with the progression of the RA stage (table 2). The levels of MMP-2 were not obviously changed.

Table 3 Levels of TIMP-1 and TIMP-2 in SF from knee joints of patients with rheumatoid arthritis and osteoarthritis. Results are shown as mean (SD), (median), and range (nmol/l)

Group	TIMP-1	TIMP-2
RA		
All	47.4 (24.9)*** (40.5)	7.22 (1.76) (7.31)
Early stage	14.2–121.3 36.9 (12.3) (37.1)	2.62–12.42 7.39 (1.29) (7.31)
Middle stage	17.5–67.0 49.5 (25.9) (41.4)	4.88–9.75 7.26 (1.91) (7.41)
Advanced stage	14.2–121.3 50.1 (27.0) (43.9)	2.62–12.42 6.97 (1.62) (7.31)
	15.2–114.7	2.67–10.07
OA		
All	28.9 (15.4) (27.1)	6.93 (1.75) (6.81)
	8.7–90.3	3.50–12.97

\*\*\*p<0.001 versus corresponding values in OA all.

**Figure 1** Molar ratios of matrix metalloproteinases (MMPs) to tissue inhibitors of metalloproteinases (TIMPs), carboxymethylated transferrin (Cm-Tf)-degrading activity, and correlation between the molar ratios and the activity. (A) Molar ratios of total MMPs to total TIMPs were calculated as described in "Materials and methods". Bars indicate mean values in RA and OA synovial fluids (SFs). (B) Cm-Tf-degrading activity in SF was measured after the *p*-aminophenylmercuric acetate (APMA) activation in the presence of serine and cysteine proteinase inhibitors as described in "Materials and methods". Bars indicate mean values in RA and OA SFs. (C) Correlation between the molar ratios and the Cm-Tf-degrading activity. Note a significant direct correlation ( $r_s=0.580$ ,  $p<0.001$ ).



Both TIMP-1 and TIMP-2 were detectable in all RA and OA SFs. Although TIMP-2 levels did not differ between RA and OA SFs, the levels of TIMP-1 were significantly higher (1.5-fold) in RA SF than in the OA samples (table 3). The TIMP-1 levels also appeared to increase with progression of the RA stages

(table 3), though they were not significantly different.

#### MMPs/TIMPs RATIO AND ENZYMIC ACTIVITY IN KNEE SF FROM PATIENTS WITH RA OR OA

Since the ratio of enzymes to their inhibitors is one of the critical factors for their proteolytic activities, the molar ratios of MMPs to TIMPs in SF from patients with RA and patients with OA were calculated. Figure 1A shows that the ratios in RA (44.3 (33.9), mean (SD)) were significantly higher (5.2-fold) than those in OA (8.53 (5.53)). We further determined Cm-Tf-degrading activity in the presence of serine and cysteine proteinase inhibitors after the APMA activation, and found that the levels of activity in SF are significantly higher (2.2-fold) in patients with RA than those in patients with OA (fig 1B). Moreover, a significant correlation ( $r_s=0.580$ ,  $p<0.001$ ) was observed between the molar ratio and the activity in SF from patients with RA (fig 1C), but such a correlation was not obtained with OA SF ( $r_s=0.287$ ,  $p=0.141$ ) (data not shown).

#### RELATIONS BETWEEN THE LEVELS OF MMPs AND TIMPs IN SF FROM PATIENTS WITH RA OR OA

Table 4 summarises the data on the relations between the levels of two MMPs in SF from patients with RA and patients with OA. Significant correlations were obtained between the levels of MMP-1 and MMP-3 in both RA ( $r_s=0.698$ ,  $p<0.001$ ) and OA ( $r_s=0.749$ ,  $p<0.001$ ). In addition, a highly significant correlation was observed between the levels of MMP-8 and MMP-9 in RA SF ( $r_s=0.896$ ,  $p<0.001$ ). There were also weak correlations between the levels of MMP-1 and MMP-2 in both RA ( $r_s=0.227$ ,  $p<0.05$ ) and OA ( $r_s=0.202$ ,  $p<0.05$ ), and between the levels of MMP-2 and MMP-3 in OA SF ( $r_s=0.253$ ,  $p<0.05$ ).

The levels of TIMP-1 correlated with those of MMP-8 and MMP-9 in RA SF ( $p<0.001$ ) (table 5). Significant correlations of the levels of TIMP-1 with those of MMP-1, MMP-2, and MMP-3 were also observed in OA SF, whereas such correlations were not present in patients with RA (table 5). On the other hand, levels of TIMP-2 in SF showed no significant correlation in either RA or OA.

#### Discussion

These studies have shown that among the seven different MMPs (MMP-1, MMP-2, MMP-3, MMP-7, MMP-8, MMP-9, and MMP-13) the steady state levels of MMP-1, MMP-2, MMP-3, MMP-8, and MMP-9 are significantly enhanced in SF from patients with RA compared with OA SF. The levels of MMP-7 and MMP-13 also seem to be higher in RA than in OA, as they were detectable in more than 45% of the RA SF samples but measurable only in fewer than 20% of the OA samples. Of these MMPs, the levels of MMP-3 were extremely high in both RA and OA SF. MMP-3 is capable of not only degrading many cartilage ECM components such as aggrecan but also of activating other proMMPs, including proMMP-1, proMMP-7, proMMP-8, proMMP-9, and proMMP-13.<sup>42</sup> It is difficult

Table 4 Correlation (Spearman's rank correlation coefficient ( $r_s$ )) between the levels of two MMPs in SF from patients with rheumatoid arthritis and osteoarthritis

RA	$r_s$	OA	$r_s$
MMP-1 v MMP-2	0.227*	MMP-1 v MMP-2	0.202*
MMP-1 v MMP-3	0.698***	MMP-1 v MMP-3	0.749***
MMP-1 v MMP-8	-0.131	MMP-2 v MMP-3	0.253*
MMP-1 v MMP-9	-0.143		
MMP-2 v MMP-3	0.112		
MMP-2 v MMP-8	0.039		
MMP-2 v MMP-9	0.094		
MMP-3 v MMP-8	0.043		
MMP-3 v MMP-9	-0.011		
MMP-8 v MMP-9	0.896***		

\* $p < 0.05$ ; \*\*\* $p < 0.001$ .

Table 5 Correlation (Spearman's rank correlation coefficient ( $r_s$ )) of the levels of TIMP-1 and TIMP-2 with the levels of MMPs in SF from patients with rheumatoid arthritis and osteoarthritis

	TIMP-1		TIMP-2	
	RA	OA	RA	OA
MMP-1	0.016	0.555***	0.111	-0.111
MMP-2	0.038	0.194*	0.148	0.087
MMP-3	-0.021	0.533***	-0.010	-0.090
MMP-8	0.413***	NE	-0.054	NE
MMP-9	0.385***	NE	-0.092	NE

\* $p < 0.05$ ; \*\* $p < 0.01$ ; \*\*\* $p < 0.001$ .  
NE = not examined.

to define the functional role of the high levels of MMP-3 in RA SF as it may exist mainly as a proenzyme.<sup>43</sup> However, once proMMP-3 in RA SF can be activated on the surface of the articular cartilage, or within the cartilage after permeation from SF, this MMP may act as one of the most powerful MMPs for the loss of cartilage matrix through direct cleavage of cartilage ECM and activation of other proMMPs. The data of an early study on the collagen-induced arthritis in MMP-3 deficient mice suggested that joint destruction is not altered by the expression levels of MMP-3.<sup>44</sup> However, recent studies have shown that generation of an MMP cleavage site in aggrecan—that is, the FVDIPEN epitope, and degradation of cartilage collagen fibrils are strongly reduced in the MMP-3 deficient arthritic mice.<sup>45 46</sup> In our study the MMP-3 levels were enhanced in the early and middle stages of RA and decreased in the advanced stage. Thus, these data, in addition to the findings from MMP-3 deficient mice, suggest that MMP-3 may have a central role in the initiation and progression of the cartilage destruction.

When soluble proteinases act in local tissues, ratios of the proteinases to their inhibitors are considered to be an important determinant. In our study the calculated values for molar ratios of MMPs to TIMPs in RA SF were ~44 on average, suggesting the possibility of imbalance in favour of MMPs. However, the ratios do not simply indicate the generation of the degradative activity based on the MMP/TIMP imbalance, even if MMPs are fully active, as TIMP-3 and TIMP-4, which may be expressed in RA joint tissues, were not measured. In addition,  $\alpha_2$  macroglobulin, a general inhibitor to proteinases, including MMPs, is present in RA SF.<sup>47</sup> Thus we examined the total potential MMP activity in an assay using Cm-Tf substrate by blocking the proteinase activities of other classes with inhibitors and activation of proM-

MPs in SF by the APMA treatment, and showed that the activity is detectable in all the RA samples. Furthermore, the molar ratios of MMPs to TIMPs in RA SF directly correlated with the levels of Cm-Tf-degrading activity. These data suggest the possibility that MMPs (that is, active MMPs plus proMMPs) in RA SF could become proteolytically active by overcoming the activity of TIMPs and other inhibitors, if proMMPs are fully activated. In OA SF, only MMP-1, MMP-2, and MMP-3 were mainly detected and their levels correlated well with that of TIMP-1, suggesting that the activities of these MMPs are well balanced by TIMP-1. However, the molar ratios of MMPs to TIMPs were ~9 on average in OA SF. Although definite direct correlation could not be obtained between the ratios and the activity, it is conceivable that the potential imbalance between MMPs and TIMPs is also present in OA SF, as low levels of APMA activated, Cm-Tf-degrading activity were detectable in all the OA samples.

It is notable that a highly significant correlation was present between the levels of MMP-1 and MMP-3 in both RA and OA SF. Coordinate mRNA expression of MMP-1 and MMP-3 has been shown in the synovium and articular cartilage by northern blotting<sup>48-50</sup> and in situ hybridisation studies.<sup>51-53</sup> Wolfe *et al* insisted that cartilage is a source of MMP-1 and MMP-3 in SF from patients with RA or OA according to their data of the relative levels of MMP-3 mRNA expression to those of MMP-1 in chondrocytes.<sup>54</sup> However, as articular chondrocytes are embedded in a large amount of ECM components, and thus the number of chondrocytes exposed to joint cavity is small, MMPs produced by chondrocytes may be a minor source in RA SF, if any. On the other hand, hyperplastic lining cells of RA synovium are faced to the joint cavity and produce a large amount of MMP-1 and MMP-3.<sup>14-16</sup> Therefore, it is reasonable to think that the lining cells are a major cellular source of MMP-1 and MMP-3 in RA SF. This is supported by the finding that the production levels of MMP-1 and MMP-3 in RA SF are decreased in the advanced stage of RA, in which inactive fibrotic synovium is often found. There were weak but significant correlations between the levels of MMP-2 and those of MMP-1 and MMP-3 in RA or OA SF. This suggests that MMP-2 is also derived mainly from the synovial tissue. Compared with the levels of MMP-1, MMP-2, MMP-3, MMP-8 and MMP-9, MMP-7 and MMP-13 were present at extremely low levels in both RA and OA SF. MMP-7 expression has been shown in OA cartilage,<sup>55</sup> but its production in RA or OA synovium is rare.<sup>14 56</sup> MMP-13 is also highly expressed in OA cartilage, but its expression in RA synovium is negligible,<sup>57</sup> though a previous study showed low expression.<sup>58</sup> Thus these data suggest that the levels of MMPs and TIMPs in SF reflect the production mainly by synovial tissue, but not by articular cartilage.

MMP-8 and MMP-9 in SF are almost selective to RA, as they were measurable in more than 90% of RA SFs but detected in only 2% of

OA SF. It is well known that the major cellular source of MMP-8 is neutrophils.<sup>2 59</sup> Although a recent study has claimed that it is also expressed by synovial fibroblasts,<sup>60</sup> our separate study showed that MMP-8 is undetectable in the culture media from RA synovial fibroblasts stimulated with interleukin 1.<sup>56</sup> Thus production of MMP-8 by synovial cells, if any, is considered to be negligible. On the other hand, MMP-9 has been immunolocalised to various cells, including macrophages, giant cells, chondrocytes, and synovial cells as well as neutrophils in chronic synovitis and RA.<sup>14 17 18</sup> However, a strong direct correlation between MMP-8 and MMP-9 in RA SF suggests the possibility that both MMPs are derived mainly from neutrophils infiltrated into synovial cavity. The levels of MMP-8 and MMP-9 in RA SF also correlated with those of TIMP-1. TIMP-1 is produced by the hyperplastic lining cells of RA synovium.<sup>14 16</sup> However, platelets contain a large amount of TIMP-1,<sup>61</sup> and the platelet count in RA SF is high, with a correlation to the white blood cell count in the SF.<sup>62</sup> Thus the source of raised levels of TIMP-1 in RA SF may be a mixture of platelets and synovial lining cells.

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## Matrix metalloproteinases and tissue inhibitors of metalloproteinases in synovial fluids from patients with rheumatoid arthritis or osteoarthritis

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