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Expression of Matrix Metalloproteinase-9 (Gelatinase B) in Gouty Arthritis and Stimulation of MMP-9 by Urate Crystals in Macrophages

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Abstract To investigate the relevance of gelatinase-B (matrix metalloproteinase 9, MMP-9) in gouty arthritis (GA), we tested the occurrence of MMP-9 in GA patients and cell culture system. Gelatinolytic activity in the synovial fluid (SF) of patients with different kinds of arthritis was assessed by gelatin zymography. A predominant 92-kDa MMP-9 gelatinolytic activity was evident in rheumatoid arthritis (RA) and GA samples, but no activity was observed in osteoarthritis (OA) samples. Among the 53 SF samples (9 RA, 24 GA, and 20 OA) analyzed for MMP-9 and tissue inhibitor of metalloproteinase (TIMP-1) antigen levels by ELISA, MMP-9 antigen levels were elevated tenfold in GA SF compared with OA SF. In addition, GA synovial tissue extracts revealed elevated levels of MMP-9 expression as compared to OA tissue extracts by Western blot and RT-PCR analysis. Immunohistochemical studies demonstrated that MMP-9 immunoreactivity was more intense in GA than in OA synovial tissues. Furthermore, macrophages activation by gouty crystals in vitro was examined. Crystals stimulated MMP-9 gene expression in macrophage cell line and such stimulation was suppressed by PD98059. These findings suggest that the abnormal production of MMP-9 by macrophages is a reflection of the pathological conditions in joints of patients with GA, and that the activation of MMP-9 in the joint is known to play an important role in joint disease. J. Cell. Biochem. 89: 791–799, 2003.

Key words: gouty arthritis; MMP-9; MSU

Gout is a prevalent disease and typically has a characteristic clinical picture, such as excruciating pain and intense inflammation of a single lower extremity joint. A diagnosis is made with absolute certainty by identifying needle-shaped, negative birefringent crystals in synovial fluid (SF) or soft tissue aspirates [Wallace et al., 1967]. The main predisposing factors for gout in human are a family history, obesity, excessive consumption of alcohol, a diet high in purine, and raised triglyceride concentration [Nakanishi et al., 1999]. Disorder of nucleic acid metabolism leads to monosodium urate (MSU) crystals deposition in joints causing gouty arthritis (GA). Pioneering work in the early 1960s demonstrated that acute GA was a self-limiting inflammatory response to microcrystals of MSU that precipitate in tissues from supersaturated body fluids [Agudelo and Wise, 2001].

Matrix metalloproteinases (MMPs) are a family of structurally related endopeptidases that reabsorb macromolecules of the extracellular matrix. There are 18 known MMPs in humans, and more than 25 MMPs have been identified in the animal and plant kingdoms [Massova et al., 1998; Konttinen et al., 1999;

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Llano et al., 1999; Velasco et al., 1999]. They participate both in physiologic connective tissue remodeling and in pathological tissue destruction. MMPs are divided into three subclasses: the collagenases, the stromelysins, and the gelatinases. Gelatinases occur in two major forms, gelatinase-A (MMP-2) and gelatinase-B (MMP-9). These enzymes are capable of degrading type IV and V collagens, elastin, and gelatin [Johnson et al., 1998]. Recently, elevated levels of MMP-9 were demonstrated in the SF of patients with various inflammatory diseases. A correlation between the increased level of MMP-9 gelatinolytic activity in rheumatoid arthritis (RA) SF and the severity of the disease was also found [Hirose et al., 1992; Picarella-Stein et al., 1994; Koolwijk et al., 1995; Ahrens et al., 1996]. However, the relative expression of MMP-9 in human GA remains to be defined.

In order to understand the role of MMP-9 in GA, we undertook the present investigation to examine the expression of MMP-9 in GA patients and cell culture system. Synovial samples from the patients with osteoarthritis served as controls.

MATERIALS AND METHODS

Patient Populations

Samples were obtained from 54 patients with arthritis. Patients with RA and OA were selected according to the American College of Rheumatology criteria [Altman et al., 1986; Arnett et al., 1988]. Gout was diagnosed by the observation of urate crystals on SF analysis. SF was isolated, during therapeutic arthrocentesis, from 24 patients with GA, 9 patients with RA, 17 patients with OA, 2 patients with HA, and 2 patients with trauma. Synovial tissues were obtained during replacement surgery from patients with GA ($n = 3$) and OA ($n = 3$). Tissue samples were obtained from the knee and hip joints.

Sampling of Synovial Fluid

Samples of SF were aspirated from the knee joints of arthritis patients as part of their therapeutic program. The SF was then centrifuged at 3,000*g* for 10 min at 4°C. The cell-free supernatants were stored at −20°C until analysis.

Reagents and Antibodies

PD98059 was obtained from New England Biolabs (Beverly, MA). Endotoxin-free MSU crystals were prepared as previously described [Terkeltaub et al., 1991]. Anti-ERK1/2 phosphospecific and nonphosphospecific antibodies were obtained from SantaCruz Biotechnology (Santa Cruz, CA). Antibody to MMP-9 was purchased from Oncogene Science (Bayer Co., Morristown, NJ).

Cell Culture and MSU Treatment

Murine monocyte/macrophage cell line RAW 264.7 cells were cultured in RPMI-1640 supplement with 10% endotoxin-free, heat-inactivated fetal calf serum (GIBCO, Grand Island, NY), supplemented with 100 U/ml penicillin, and 100 μg/ml streptomycin. Before different treatments, cells were cultured in RPMI with 1% serum for 20 h. The potential inhibitors were added 30 min before the MSU treatment.

Enzyme-Linked Immunosorbent Assay (ELISA)

Concentrations of MMP-9 and TIMP-1 in SF were determined using commercially available ELISA kits purchased from Amersham (Amersham Biosciences, UK). In brief, SF (10 μl) was assayed for MMP9 and TIMP-1 which were then compared with a standard curve as suggested by the manufacturer.

Gelatin Zymograms

For the detection of gelatinase activity, 10 μl samples of unreduced SF (diluted 1:10) or cell culture supernatant (12 μl) were electrophoresed in 10% polyacrylamide gels impregnated with 0.2% (w/v) gelatin. After electrophoresis, gels were washed in 50 mM Tris-HCl, 5 mM $CaCl_2$, pH 8.0 for 1 h. Subsequently, gels were incubated in the same buffer at 37°C for 18 h. Gelatinase activities appeared as lysis zones, detected after staining the gels with Coomassie blue. Immunodepletion study of the gelatinolytic activity in GA SF was carried out by diluting samples in PBS (1:10) and incubation with 10 μg/ml MMP-9 (Oncogene Science) antibody for 2 h at room temperature. Subsequently, protein A/G plus-agarose (Santa Cruz Biotechnology) was added to the assay tubes, and incubation was continued for 1 h at room temperature. Supernatants were analyzed by gelatin zymography, and the precipitated fractions were accessed by Western blot analysis.

Western Blot Analysis of MMP9

Protein extracts and Western blot analysis were performed as previously described [Tsai

et al., 2002]. Precipitated SF fractions or total protein extracts (30 μg) were analyzed on blots and incubated overnight at 4°C with 1% bovine serum albumin in phosphate-buffered saline (PBS) and then with primary antibodies for 1 h. The proteins were detected by chemiluminescence (ECL, Amersham Life Science) or coloregenic substrates, nitro blue tetrazolium (NBT) and 5-bromo-4-chloro-3-indolyl-phosphate (BCIP), as suggested by the manufacturer (Sigma Chemical, St. Louis, MO).

RNA Preparation and Analysis

Total cellular RNA was isolated from tissues in patients with GA and OA by the ISOGEN kit according to the manufacturer's instructions (Nippon Gene, Japan). The primers used were: (A) human MMP9: GGTCCCCCACTG-CTGGCCCTTCTACGGCC and GCCCACCTC-CACTCCTCCCTTTCCTCCGGA; (B) human GAPDH: GGAGTCAACGGATTTGGT and GT-GATGGGATTTCCATTG; (C) mouse MMP-9: AGGCCTCTACAGAGTCTTTG and CAGTCC-AACAAGAAAGGACG; and (D) mouse G3PDH, TGAAGGTCGGTGTGAACGGATTTGGC and CATGTAGGCCATGAGGTCCACCAC. RT-PCR was performed by a one-step protocol of Pharmacia Biotech Ready-To-Go PCR Beads (Amersham Biosciences, Piscataway, NJ). Total RNA (2 μg), primers (10 pmol), and DEPC-treated water were added to RT-PCR Beads in a final volume of 50 μl. The amplification cycles of human MMP-9 were 94°C for 45 s, 60°C for 45 s, and 72°C for 2 min for 35 cycles. The amplification cycles of mouse MMP-9 were 95°C for 1.5 min, 55°C for 2 min, and 72°C for 3 min for 35 cycles. The amplification cycles of GAPDH were 94°C for 45 s, 55°C for 45 s, and 72°C for 1 min for 30 cycles. This program was preceded by 5 min at the given denaturation temperature followed by 10 min at 72°C. RT-PCR products were analyzed on a 1.2% agarose gel and visualized by ethidium bromide staining.

Immunostaining

Tissue specimens were preserved in 10% formalin and embedded in paraffin, serially sectioned onto microscope slides at a thickness of 4 μM, and then deparaffinized. Immunohistochemical staining was performed with a DAKO LSAB2 system kit as described by the manufacturer. Primary antibody against human MMP-9 (Santa Cruz Biotechnology) (1:50 dilution, in PBS) was used. Positive staining was indicated by red deposits for the alkaline-phosphatase method. Slides were counterstained with hematoxylin and evaluated with a brightfield microscope.

RESULTS

MMP-9 in Human SF

SF samples from patients with RA, GA, and OA were evaluated for the presence of gelatinase activity by gelatin zymography. With this method, several gelatinolytic species were identified in SF samples, which represent pro-MMP-2, pro-MMP-9, and the proteolytically active MMP-9–α_2-microglobulin complex, respectively (Fig. 1A,B). As shown in Figure 1A, all of the tested SF samples exhibited a 72-kDa form of MMP-2 without significant differences in intensities of the bands. In contrast, the

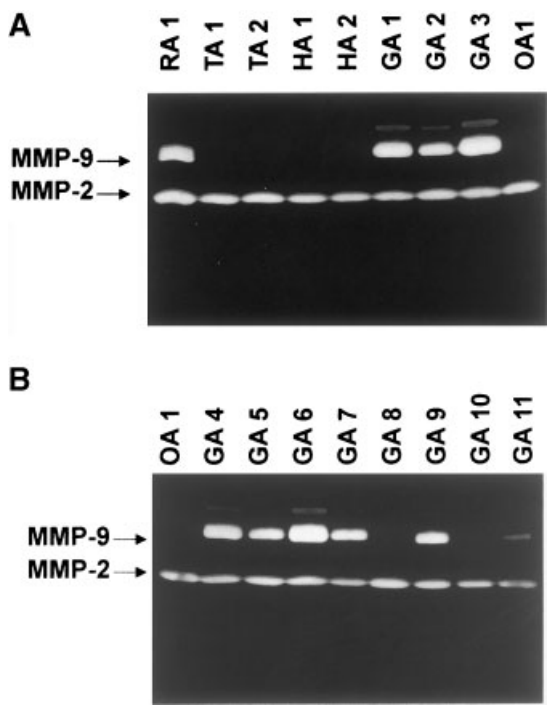

Fig. 1. Characterization of gelatinolytic activity in synovial fluid (SF) of patients with rheumatoid arthritis (RA), trauma arthritis (TA), hemoarthritis (HA), gouty arthritis (GA), and osteoarthritis (OA), using gelatin zymograms. **A**: SF from patients with RA (n = 1), TA (n = 2), HA (n = 2), GA (n = 3), and OA (n = 1). All SF samples demonstrated the presence of matrix metalloproteinase 2 (MMP-2; 72-kDa gelatinase A), while the RA and GA samples displayed MMP-9 (92-kDa gelatinase B). **B**: SF from patients with GA (n = 8) and OA (n = 1). All SF samples demonstrated the presence of MMP-2, while only GA samples displayed MMP-9. Similar results were obtained in three independent experiments.

levels of MMP-9-associated gelatinolytic activity including the proform and complex were elevated in RA and GA SF samples as compared with the OA samples tested. Heterogeneity of MMP-9-associated gelatinolytic activity was clearly shown among the samples derived from GA SF, except in 2 of 12 samples (samples 8 and 10) (Fig. 1A,B).

Verification of MMP-9 gelatinolytic activity was addressed by immunodepletion studies utilizing a monoclonal antibody specific for MMP-9 which effectively immunoprecipitates this enzyme. In this study, the GA SF samples previously identified to contain MMP-9-associated gelatinolytic activity were pretreated with MMP-9 antibody or control antibody, and immune complexes were immunoprecipitated. Supernatants were analyzed by gelatin zymography and immunopreciptated fractions were analyzed by Western blot. As shown in Figure 2A, gelatin zymography of MMP-9-immunoabsorbed samples demonstrated the preferential loss of MMP-9-associated gelatinolytic activities in GA SF samples. In contrast, immunoabsorption with MMP-9-specific antibody failed to affect the MMP-2-associated gelatinolytic activity found in GA SF samples. In addition, Western blot analysis confirmed the presence of MMP-9 in GF SF samples (Fig. 2B).

53 SF samples (24 GA, 9 RA, and 20 OA) were analyzed for MMP-9 antigen levels by enzyme-linked immunosorbent assay (ELISA) (Fig. 3A). MMP-9 antigen levels were significantly elevated ($P < 0.05$) in GA and RA SF samples as compared with OA SF samples. This result is consistent with the findings of gelatin zymography. Furthermore, the TIMP-1 antigen level in SF was also determined. Figure 3B shows that the TIMP-1 antigen level was lower in GA SF samples as compared with RA and OA SF samples. Nevertheless, there were no significant differences between GA and OA SF samples.

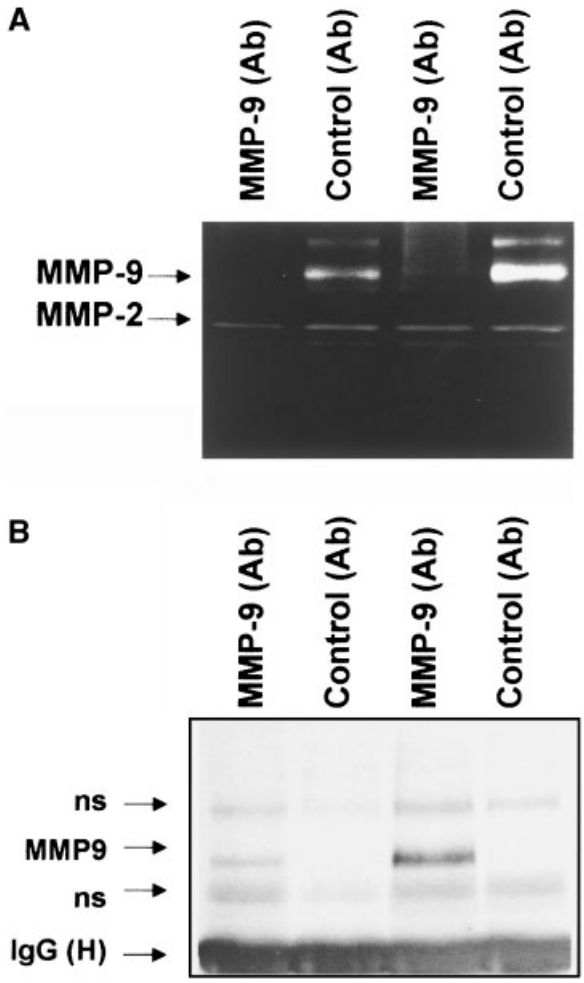

Fig. 2. Immunodepletion analysis of gelatinolytic activity in SF of patients with GA, using gelatin zymogram together with immunoprecipitation. **A**: SF from GA patients incubated with MMP-9 monoclonal antibody (Ab) or control Ab for 2 h at room temperature followed by the addition of protein A/G-plus agarose and further incubation for 1 h. Supernatants were analyzed by gelatin zymogram (A), and the immunoprecipitated fractions were analyzed by Western blot (B). A: All SF samples treated with control antibody or MMP-9 specific antibody demonstrating selective ablation of MMP-9-associated gelatinolytic activity with the continued presence of MMP-2 gelatinolytic activity. **B**: Immunoprecipitated fractions blotted and immunodetection performed with MMP-9 antibody. Similar results were obtained in three independent experiments (ns = nonspecific bands).

MMP-9 in Human Synovium Tissues

MMP-9 antigen levels were assessed in synovial tissue extracts from patients with GA and OA using Western blot (Fig. 4A). GA synovial tissue protein extracts demonstrated significantly the elevated protein levels of MMP-9 compared to those of OA samples. To further analyze for the eventual presence of MMP-9 mRNA in synovial tissues, GA and OA samples were studied using RT-PCR analysis (Fig. 4B). The result indicated this revealed the presence of MMP-9 (756 bp) in GA synovial tissue sample. In contrast, only a slight amount of MMP-9 was detected in OA synovial tissue sample. RNA extraction was controlled using primers for G3PDH (226 bp).

Studies on immunostaining with MMP-9 antibody were undertaken using paraffin-

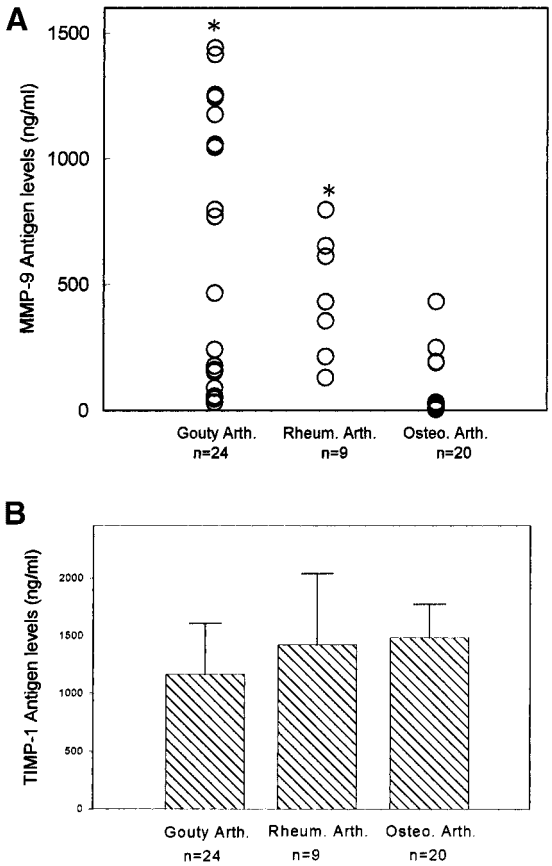

Fig. 3. MMP-9 and TIMP-1 antigen levels in SF from arthritis patients. SF samples obtained from patients with RA (n = 9), GA (n = 24), or OA (n = 20) were analyzed for MMP-9 (**A**) and TIMP-1 (**B**) expression by ELISA as described in "Materials and Methods." Values are the mean ± SD. $^*P < 0.05$ compared with OA samples, by Student's two-tailed *t*-test for unpaired values.

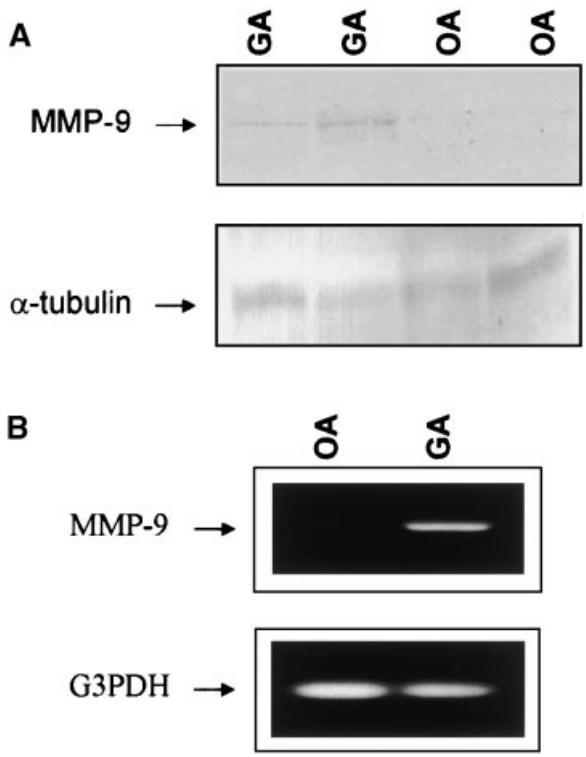

Fig. 4. Expression of MMP-9 in GA synovial tissues. **A**: Western blot analysis of MMP-9 protein in synovial tissues of patients with arthritis. Synovial tissue extracts (80 μg) from patients with GA (n = 2) or OA (n = 2) were prepared as described in "Materials and Methods" and were analyzed for MMP-9 protein expression. Similar results were obtained in three independent experiments. **B**: Characterization of MMP-9 mRNA in synovial tissues of patients with arthritis, using RT-PCR. Total RNA extracts (2 μg) from patients with GA or OA were prepared as described in "Materials and Methods" and were analyzed for MMP-9 mRNA (756 bp) and G3PDH mRNA (226 bp) expression. Similar results were obtained in three independent experiments.

embedded, paraformaldehyde-fixed GA and OA synovial tissues, to determine which cell types express MMP-9. As shown in Figure 5, MMP-9 immunoreactivity was more intense and widespread in GA synovium than in OA synovium. Representative sections of GA synovial tissue reacted with MMP-9 antibody and demonstrated significant immunostaining of tissue macrophages. In contrast, MMP-9 staining was nearly undetectable in control OA synovium.

MSU Crystal Induction of MMP-9 mRNA and Protein Synthesis

Since the potential of macrophages to synthesize MMP-9 was demonstrated in GA patients, we further evaluated the effect of MSU crystal on MMP-9 expression in RAW 264.7 monocyte/macrophage cells. Figure 6A showed that treatment of macrophages with MSU for 18 h induced MMP-9 gelatinolytic activity in a dose-dependent manner, whereas the getatinolytic activity of MMP-2 did not seem to be affected. In addition, a time course of MMP-9 getatinolytic activity induction by MSU was illustrated in Figure 6B. This result showed a significant increase of MMP-9 gelatinolytic activity within 24 h of stimulation. In order to investigate whether the increase of MMP-9 gelatinolytic activity was due to the induction of MMP-9 mRNA, RT-PCR analysis for total RNA extracted from macrophage cells were carried out. Figure 6C showed that MMP-9 mRNA expression was induced within 6 h in these cells.

PD98059 Blocks MSU-Induced MMP-9 Protein Synthesis

To gain insight into the mechanism of MSU signaling leading to MMP-9 up-regulation, cells

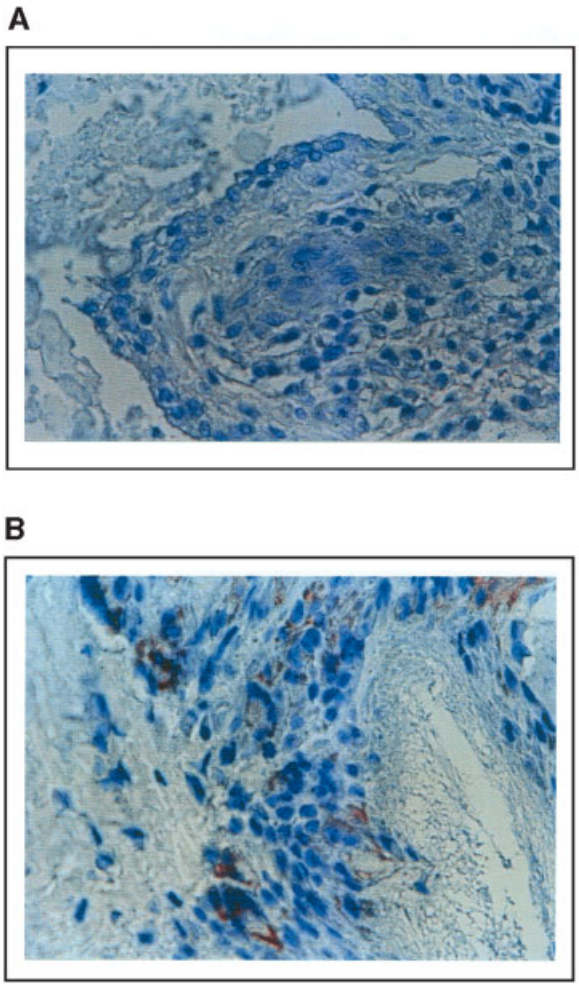

Fig. 5. Immunostaining for MMP-9 in synovial tissues from GA and OA patients. Paraffin-embedded synovial tissue sections obtained from a representative OA patient (**A**) and a representative GA patient (**B**) were prepared as described in ''Materials and Methods'' (×400).

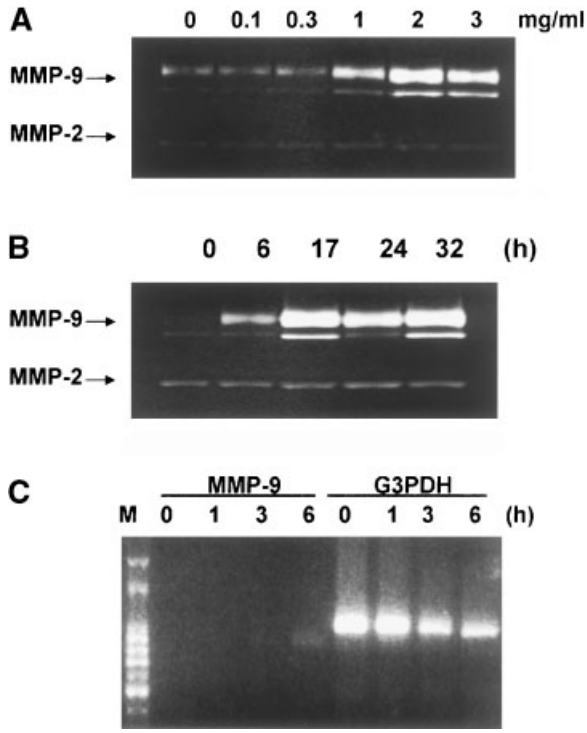

Fig. 6. Induction of MMP-9 mRNA and protein by MSU in mouse macrophages. **A**: Cells were incubated in the absence or presence of increasing concentration (0.1–3 mg/ml) of MSU for 24 h. **B**: Cells were cultured in the presence of MSU (2 mg/ml) for increasing period of time (0–24 h). MMP-9 levels in the culture medium were determined by gelatin zymography as described in ''Materials and Methods.'' **C**: Cells were incubated in the absence or presence of MSU (2 mg/ml) for increasing period of time (0–6 h). Total extracts (2 μg) were prepared as described in ''Materials and Methods'' and were analyzed for MMP-9 mRNA (825 bp) and G3PDH mRNA (983 bp) expression. Similar results were obtained in three independent experiments.

were exposed to MSU alone or in combination with PD98059, a specific inhibitor of mitogen-activated protein kinase (MAPK) kinase 1 (MEK-1) that prevents extracellular signal-regulated kinase (ERK1/2) phosphorylation. As shown by gelatin zymography, PD98059 suppressed the MSU-induced MMP-9 gelatinolytic activity but not the MMP-2 levels (Fig. 7A). For MEK1/2 activity analysis, total proteins were first extracted from cells treated with MSU alone or in combination with inhibitors for different time periods, and then detected by Western blot. As indicated in Figure 7B, PD98059 reduced the MSU-induced phosphorylation of ERK1/2. However, the levels of ERK1/2 detected by total antibody remained constant. This result is in accord with those observed in MMP-9 activation shown in Figure 6, which further suggests that ERK1/2 is as a key-signaling mediator for MMP-9 induction by MSU.

DISCUSSION

MMPs are a family of Zn^{2+} endopeptidases that possess the ability to break down extracellular matrix macromolecules associated with tissue destruction in various pathological conditions [Matrisian, 1992]. Previous studies have revealed that MMPs play a crucial role in tumor invasion [Westermarck and Kähäri, 1999]. For example, MMP-9 has been shown to be elevated in sera of patients with hepatocellular carcinoma and abundantly expressed in patients with various malignant tumors [Hosokawa et al., 1994; Duffy et al., 1995]. MMP-9 also

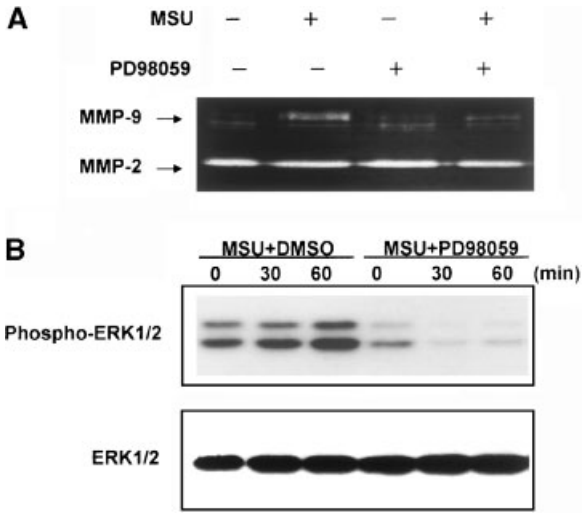

Fig. 7. Effects of protein kinase inhibitor PD98059 on MSU-induced MMP-9 production and effects of MSU and PD98059 on phosphorylation of ERK1/2. **A**: Cells were preincubated for 30 min with PD98059 (50 μM) followed by 24 h incubation in the presence of 1 mg/ml MSU. MMP-9 levels in the culture medium were determined by gelatin zymography as described in ''Materials and Methods.'' **B**: Cells were pretreated with DMSO (vehicle for inhibitor) or PD98059 (50 μm) for 30 min followed by MSU (2 mg/ml) treatment for increasing period of time (0–60 min). Cell lysates were analyzed by Western blotting with phosphospecific ERK1/2 antibody (**upper panels**). The membrane was stripped and reprobed with total antibody (**lower panels**). The resulting bands are shown.

plays a role in many other disease states including Alzheimer's disease [Fujimoto et al., 1996]. Recently, MMPs were found to play a key role in the destruction of joint structures, because increased plasma and tissue levels of MMP-9 have been noted in rat adjuvant arthritis and RA [Koolwijk et al., 1995; Ahrens et al., 1996]. Although some reports describing MMP expression in some types of arthritis such as RA and OA have been published [Hirose et al., 1992], this study is the first to show differential expression of MMP-9 between OA and GA. As a result, the abnormally elevated expression of MMP-9 was significantly demonstrated in the SF and synovium tissues of patients with GA.

MMP-9, like other members of MMPs, is secreted in the precursor latent form (92 kDa, pro-MMP-9). However, the activated form of this enzyme is discriminated from the latent form by its reduced molecular weight of 84 kDa. Furthermore, MMP-9 can occur in a SDS-stable complex with α 2-microglobulin-related protein that has a molecular weight of about 125 kDa [Triebel et al., 1992]. This study demonstrated an increased gelatinolytic activity in SF of patients with GA as compared to patients with OA by gelatin zymography. In particular, the gelatinolytic activity in SF samples was predominantly that of latent/proform MMP-9. Its binding to connective tissue substrates as well as irreversible binding to α 2-microglobulin may restrict the presence of activated soluble MMP-9 in SF. This result is consistent with the findings of earlier studies, which demonstrated increased levels of the latent form MMP-9-associated gelatinolytic activity in SF of patients with RA [Hirose et al., 1992; Koolwijk et al., 1995; Ahrens et al., 1996]. Nevertheless, all forms of MMP-9 gelatinolytic activity were found in the synovium tissues of patients with GA (data not shown). The results from Figures 1 and 3A showed that some patients with GA revealed low or undetectable MMP-9 activity and protein expression. This may due to the variability of crystal deposition in joints or different disease states.

Tissue inhibitors of metalloproteinases (TIMPs) are specific inhibitors for MMPs. TIMPs bind specifically with active MMPs to form 1:1 noncovalent, but tightly bound complexes that inactivate enzymes. For example, TIMP-1 is a glycoprotein and contains six disulfide bonds that constrain the molecule into two major domains. TIMP-1 can form a bimolecular complex with the latent form of 92-kDa MMP-9 [Cawston et al., 1981; Stetler-Stevenson et al., 1989; Williamson et al., 1990]. This study clearly showed an elevated expression of MMP-9 in SF with GA compared with OA. However, it is interesting to note that no significant difference in TIMP-1 expression appears between OA and GA patients (Fig. 3B). This result suggests that there is a relative decrease in TIMP-1 compared with the MMP-9 levels in GA. Similar results were obtained from in situ hybridization mRNA studies, which indicated that the overall level of TIMP gene expression was similar in the synovium of RA and OA patients [McCachren, 1991; Firestein and Paine, 1992]. Taken together, much of the connective tissue destruction seen in RA and GA may be due to a local imbalance between activated MMPs and TIMPs.

MMP-9 antigen levels in synovial tissue extracts were found to be elevated in GA compared with OA patients (Fig. 4). These results were consistent with those of previous tissue distribution studies in which the presence of MMPs within the synovium was demonstrated. Investigations by Abren et al. indicated that MMP-9

positive immunoreactivity associated with surface synovial lining cells, vascular endothelium, and synovial macrophages, along with weak staining of synovial fibroblasts in RA synovial tissues [Ahrens et al., 1996]. Increased tissue levels of MMP-9 have also been noted in rat adjuvant arthritis; tissue distribution of MMP-9 antigen in arthritic lesions associated with this model showed antigen expression in infiltrating leukocytes, osteoclases, articular chondrocytes, vascular endothelium, and synovial fibroblasts [Picarella-Stein et al., 1994; Singer et al., 1995]. In contrast, immunohistological analysis of GA synovial tissues indicated that MMP-9 is produced only by infiltrating cells of monocyte/macrophage lineage; positive immunoreactivity associated with lining cells and fibroblast cells was almost negligible (Fig. 5). However, the potential of macrophages to synthesize MMP-9 was clearly demonstrated in GA and RA patients. These findings are of interest since the inflamed synovium in GA and RA joints contains a large number of macrophages, implicating macrophages as possible mediators of the disease process.

Acute GA is a self-limiting inflammatory response to the intra-articular deposition of MSU microcrystals [Schumacher, 1989]. Many reports have indicated that crystals can directly stimulate production of proinflammatory cytokines such as tumor necrosis factor (TNF), interleukin-1 (IL-1), IL-6, and IL-8 [di Giovine et al., 1987, 1991; Guerne et al., 1989; Terkeltaub et al., 1991, Hachicha et al., 1995; Chapman et al., 1997]. In this study, we reported for the first time that the MSU crystals can up-regulate the expression of MMP-9 (Fig. 6). Previous studies have also demonstrated that MMP-9 can be induced by proinflammatory cytokines in monocytes/macrophages [Unemori et al., 1991; Saren et al., 1996]. Therefore, it is possible that crystals in a joint can directly stimulate the production of MMP-9 and the proinflammatory cytokines could amplify this response. Additionally, the induction of MMP-9 could be inhibited by pharmacologic blockade of ERK1/2 (Fig. 7). This result indicated that ERK1/2 is involved in MSU-stimulated macrophages signal transduction cascades, which is consistent with the reports of activation of MAPK cascade by MSU and CPPD in THP-1 cells [Liu et al., 2000].

In summary, the findings of our study clearly showed an abnormal MMP-9 expression pattern in the SF and synovium tissues of patients with GA. Furthermore, the potential of macrophages to synthesize MMP-9 was demonstrated in GA patients. It was also discovered that MSU crystals could induce MMP-9 expression in macrophages, and that the MMP-9 induction can be significantly abolished by PD98059 (MEK1 inhibitor). Taken together, the present findings indicated that the expression of MMP-9 by macrophages might play an important role in GA.

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