Reciprocal interaction between macrophage migration inhibitory factor and interleukin-8 in gout

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Abstract

Objective

Macrophage migration inhibitory factor (MIF) is a proinflammatory, chemotactic, and tissue destructive cytokine. This study determined monosodium urate crystal-induced MIF production and its interaction with interleukin (IL)-8 in gout.

Methods

Peripheral blood (PB), synovial fluid (SF), and clinical data were obtained from 98 patients with gout. SF and serum concentrations of MIF and IL-8 were measured using ELISA. SF monocytes and neutrophils were cultured with monosodium urate (MSU) crystals and the cytokine production was determined. The signalling pathways involved were determined using signal inhibitors. The interaction between MIF and IL-8 was investigated.

Results

SF MIF was higher in acute gout and that in serum was higher in patients with intercritical gout compared with controls. SF MIF was positively correlated with SF leukocyte and neutrophil counts and IL-8. The expression of MIF was similar in SF neutrophils and monocytes, while IL-8 was higher in monocytes. MSU crystals induced MIF production in monocytes and IL-8 production in neutrophils. This effect was decreased by inhibiting Fc-gamma receptor 1 and toll-like receptor 4. IL-8 increased MIF production in monocytes while MIF increased interleukin-8 production in neutrophils.

Conclusion

MIF and IL-8 are highly produced in acute gout. MSU crystals induced MIF production in monocytes and IL-8 production in neutrophils with a reciprocal interaction between the two cytokines.

Key words

gout, monosodium urate, macrophage migration inhibitory factor, interleukin-8

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Introduction

Gout is a crystal-induced inflammatory arthritis associated with hyperuricaemia that is characterised by the deposition of monosodium urate (MSU) in the tissues and joints (1, 2). The typical clinical feature of gout is periodic attacks of acute gout followed by asymptomatic intercritical periods (1, 3). In acute gout, the MSU crystals initiate and amplify acute attacks of autoinflammation (2, 4). Phagocytosis and activation of macrophages and neutrophils are key processes in this period (4). MSU crystals induce the formation of the intracellular NALP3/NLRP3 inflammasome, leading to activation of caspase-1 and promoting interleukin (IL)-1β secretion (4, 5). In addition to IL-1β, tumour necrosis factor alpha (TNF-α), IL-6, IL-8, and other chemokines also induce neutrophil recruitment and activation (4).

IL-8 is an essential chemokine for neutrophil recruitment and activation in acute gouty inflammation (6-8). Intra-articular injection of MSU crystals into rabbit knee joints induces severe acute arthritis following an increase in IL-8 production and subsequent neutrophil infiltration into synovial tissues (7, 9). The peak production of IL-8 in synovial fluid (SF) occurs 2 h after the intra-articular injection of MSU crystals (9). The result suggests that IL-8 play a part in the very early stages of acute gouty inflammation. MSU crystal strongly induces neutrophil influx and IL-8 receptor CXCR2 is essential for the neutrophil recruitment in experimental crystal-induced inflammation (10).

Macrophage migration inhibitory factor (MIF) is one of the inducers of IL-8 in inflammatory and neoplastic conditions (11-14). MIF is a proinflammatory cytokine, which promotes macrophages to produce TNF-α, IL-1β, IL-6, and IL-8, stimulates T cell activation, up-regulates tissue degrading matrix metalloproteinases, induces angiogenesis, and promotes osteoclastogenesis in rheumatoid arthritis (RA) (11, 15-20). These data suggest MIF is an upstream cytokine that regulates inflammation, angiogenesis and tissue destruction in inflammatory diseases. The concentration of MIF is increased in SF of acute gout and MSU crystal injection into the knee joint of mice increase the concentration of MIF. The injection of MIF promotes neutrophil influx and joint inflammation, while the intra-articular injection of MSU crystal dose not recruit neutrophils in the joint of *Mif*$^{-/-}$ mice. This study suggests MSU induces the production of MIF, and MIF induces neutrophil influx in gout joint (21). On the basis of its role in RA, we hypothesised that MIF and IL-8 could be key molecules in acute gout.

In this study, we measured the concentrations of MIF and IL-8 in the serum and SF of patients with acute and intercritical gout and analysed the relationship between their concentration and the clinical characteristics. SF monocytes and neutrophils were isolated and stimulated with MSU crystals to produce MIF and IL-8. The reciprocal interaction of MIF and IL-8 was studied through their stimulatory effect on each other.

Methods

Patients

Gout in the enrolled patients was confirmed by the presence of MSU crystals in the SF or uric acid deposition on dual-energy computed tomography. Thirty-one patients who had acute monoarthritis with typical gout symptoms and signs were defined as the acute gout group. Sixty-seven patients who did not have any joint symptoms between gout attacks and had a history of acute gout were defined as having intercritical gout. Written informed consent was obtained from patients and healthy volunteers. The protocol for this study was approved by Institutional Review Board for Human Research of Konkuk University Hospital.

Reagents

MSU crystals were purchased from Alexis (Lausen, Switzerland). Recombinant MIF, IL-1β, anti-Toll-like receptor 2 (TLR2), anti-TLR4, anti-CD64, anti-CD32, and anti-CD16 were purchased from R&D systems (Minneapolis, MN). LY294002, SB203580, SP600125, and AG490 were obtained from Calbiochem (Schwalbach, Germany). BAY11-7085 and SR11302 were obtained from Santacruz (Santa Cruz Biotechnology, Dallas, TX).

Cell isolation from peripheral blood and synovial fluid

Peripheral blood mononuclear cells (PBMCs) and SF mononuclear cells (SFMC) were isolated by density gradient centrifugation on Ficoll-paque-Plus (GE Healthcare, Amersham, UK), washed twice with RPMI-1640 medium (Sigma-Aldrich, St Louis, MO), counted using a Neubauer hemocytometer and resuspended at 1×10^6 cells/ml in fresh medium. CD14-positive monocytes were isolated from the PBMCs and SFMCs using monoclonal anti-human-CD14 antibody conjugated to microbeads (MicroBeads; Miltenyi Biotech, Bergisch Gladbach, Germany). Similarly, CD16-positive neutrophils were isolated from the SFMCs using monoclonal anti-human-CD16 antibody conjugated to microbeads (MicroBeads; Miltenyi Biotech, Bergisch Gladbach, Germany).

Enzyme-linked immunosorbent assay

In brief, a 96-well plate (Nunc, Roskilde, Denmark) was coated with 4 μg/ml monoclonal antibodies against MIF, IL-8, and IL-1β (R & D Systems) an incubated at 4°C overnight. After blocking with phosphate-buffered saline/1% bovine serum albumin/0.05% Tween 20 for 2 h at room temperature (22–25°C), test samples and the standard recombinant MIF, IL-8, and IL-1β (R & D Systems) were added to the 96-well plate and incubated at room temperature for 2 h. Plates were washed four times with phosphate-buffered saline/Tween 20, and then incubated with 500 ng/ml biotinylated mouse monoclonal antibodies against MIF, IL-8, and IL-1β (R & D Systems) for 2 h at room temperature. After washing, streptavidin-alkaline phosphate–horseradish peroxidase conjugate (Sigma-Aldrich) was incubated for 2 h, then washed again and incubated with 1 mg/ml *p*-nitrophenyl phosphate (Sigma-Aldrich) dissolved in diethanolamine (Sigma-Aldrich) to develop the color reaction. The reaction was stopped by the addition of 1 M NaOH and the optical density of each well was read at 405 nm. The lower limit of MIF, IL-8, and IL-1β detection was 10 pg/ml. Recombinant human MIF, IL-8, and IL-1β diluted in culture medium to concentrations of 10 to 2000 pg/ml were used as calibration standards. A standard curve was drawn by plotting the optical density against the log of the concentration of recombinant cytokines and used for determination of MIF, IL-8, and IL-1β concentrations in test samples.

MIF and IL-8 mRNA quantification by reverse transcription-polymerase chain reaction

Monocytes or neutrophils from PB and SF were incubated with various concentrations of MSU and cytokines. After 16 h of incubation, mRNA was extracted with RNAzol B (Biotex Laboratories, Houston, TX) in accordance with the manufacturer's instructions. Reverse transcription of 2 μg of total mRNA was performed at 42°C using the Superscript reverse transcription (RT) system (Takara, Shiga, Japan). Polymerase chain reaction (PCR) amplification of cDNA aliquots was performed by adding 2.5 mM dNTPs, 2.5 U of *Taq* DNA polymerase (Takara) and 0.25 μM of sense and antisense primers. The reaction was performed in PCR buffer (1.5 mM MgCl2, 50 mM KCl, and 10 mM Tris-HCl at pH 8.3) in a total volume of 25 μl. Reactions were processed in a DNA thermal cycler (Perkin-Elmer Cetus, Norwalk, CT) at cycles of 30 seconds of denaturation at 94°C, 1 min of annealing at 60°C *(*β*-actin*), 59°C (*MIF*), or 57°C (*IL-8*), followed by 1 min of elongation at 72 °C. PCR rounds were repeated for 25 cycles each for *MIF, IL-8* and β-*actin*, to correspond with the exponential phase of amplification of each transcript. The level of mRNA expression was presented as a ratio of target PCR product over β-*actin* product.

PCR assessment of MIF mRNA expression.

PBMC and SFMC were incubated with various concentrations of MSU crystals in the presence or absence of MIF. After 16 h of incubation, mRNA was extracted with RNAzol B (Biotex Laboratories, Houston, TX) in accordance with the manufacturer's instructions. RT of total mRNA (2 μg) was performed at 42°C using a SuperScript RT system (Takara). PCR was performed at a final volume of 20 μl in capillary tubes in a LightCycler (Roche Diagnostics, Basel, Switzerland). The following sense and antisense primers for each molecules were used: *MIF* sense, 5'- GTT CCT CTC CGA GCT CAC C -3'; *MIF* antisense, 5'- TGC TGT AGG AGC GGT TCT G -3'; *IL-8* sense, 5'- AAA CCA CCG GAA GGA ACC A-3'; *IL-8* antisense, 5'- TGTGTTGGCGCAGTGTGG -3'; β-*actin* antisense, 5'-TGT GTT GGC GAT CAG GTC TTT- G-3'. The reaction mixture contained 2 μl LightCycler FastStart DNA Master Mix for SYBR Green I (Roche Diagnostics), 0.5 μ*M* of each primer, 4 mM MgCl2, and 2 μl of template DNA. All of the capillaries were amplified in a LightCycler instrument with activation of polymerase (95°C for 10 minute), followed by 45 cycles of 10 seconds at 95°C, 10 seconds at 60°C *(*β*-actin*) or 59°C (*MIF*) or 57°C (*IL-8*), and 10 seconds at 72°C. The temperature transition rate was 20°C/second for all steps. The double stranded PCR product was measured during the 72°C extension step, by detection of fluorescence associated with the binding of SYBR Green I to the product. The fluorescence curves were analysed using LightCycler software, v. 3.0 (Roche Diagnostics), and the LightCycler was used to quantify *MIF* and *IL-8* mRNA. The relative expression level in each sample was calculated as the level of *MIF* and *IL-8* normalised to that of the endogenously expressed housekeeping gene (β-*actin*). Melting curve analysis was performed immediately after the amplification protocol, under the following conditions: 0 seconds (hold time) at 95°C, 15 seconds at 71°C, and 0 seconds (hold time) at 95°C. The rate of temperature change was 20°C/second except during the final step when it was 0.1°C/second. The melting peak generated represented the quantity of specific amplified product. The crossing point was defined as the maximum of the second derivative from the fluorescence curve. Negative controls that contained all elements of the reaction mixture except for the template DNA were also included. All samples were processed in duplicate.

Statistical analysis

The results are expressed as mean ±

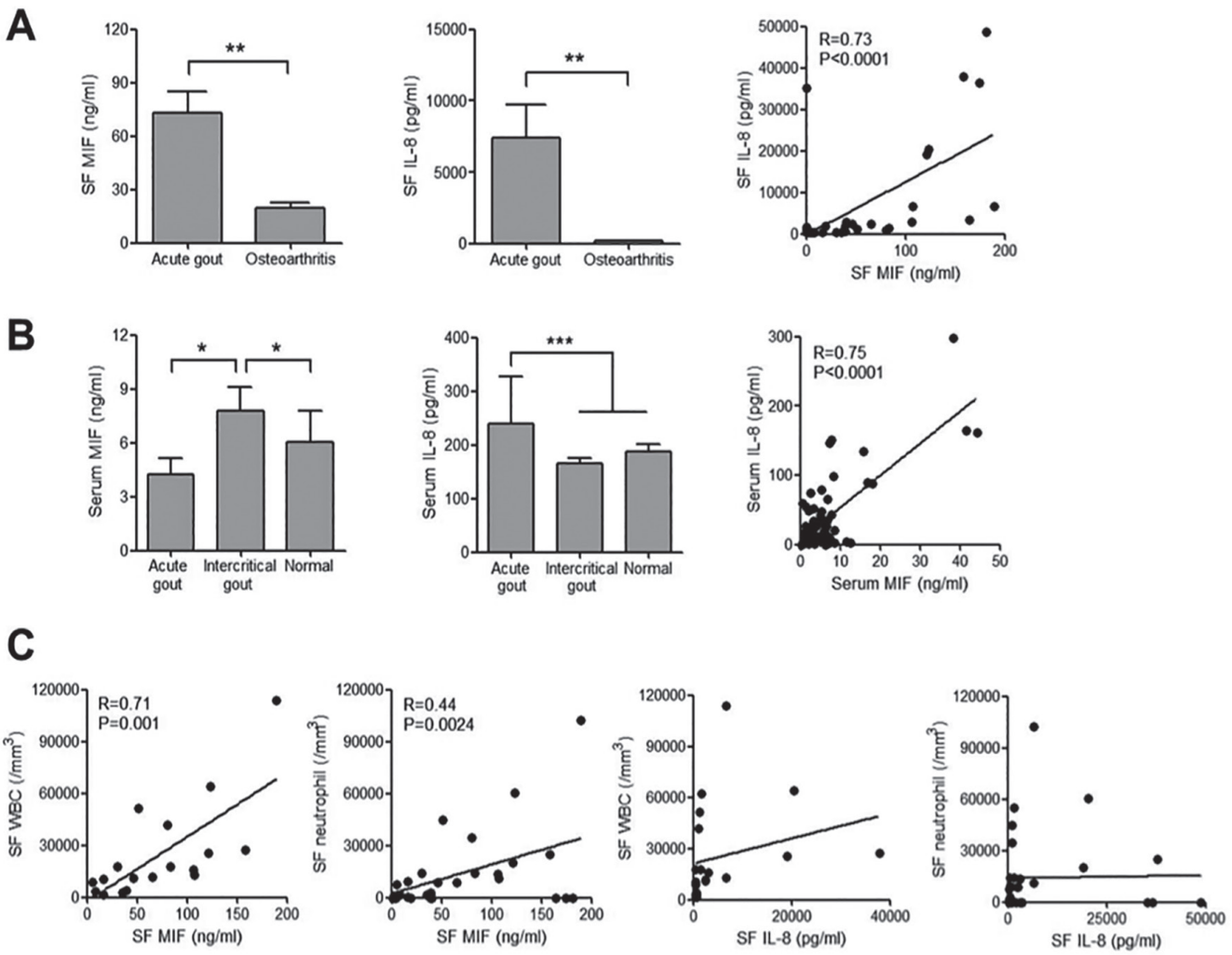

Fig. 1. The synovial fluid (SF) and serum concentration of macrophage migration inhibitory factor (MIF) and interleukin-8 (IL-8) in patients with gout.
A: The SF concentration of MIF and IL-8 in 26 patients with acute gout and 43 patients with osteoarthritis was measured using enzyme-linked immunosorbent assay (ELISA). The relationship between SF MIF and IL-8 in acute gout was analysed using Pearson's correlation coefficient (R).
B: Serum concentrations of MIF and IL-8 in 31 patients with acute gout, 67 patients with intercritical gout, and 36 healthy controls were measured using ELISA. The relationship of serum MIF and IL-8 in intercritical gout was analysed using Pearson's correlation coefficient. The data represent the mean ± standard error of the mean. *p<0.05, **p<0.01, and ***p<0.001. (C) The relationship between SF white blood cells and neutrophils and concentration of MIF and IL-8 in 26 patients with acute gout was analysed using Pearson's correlation coefficient.

standard error of the mean. Statistical differences were assessed using Mann-Whitney U-test or one-way analysis of variance with Bonferroni's multiple comparison post-hoc test. The correlation was analysed using Pearson's correlation coefficient. A p-value <0.05 was considered statistically significant.

Results

Clinical characteristics of patients with gout

Among the 98 patients with gout, 31 were diagnosed with acute gout with traditional symptoms and 67 patients were asymptomatic in a state of intercritical gout. The clinical characteristics of patients with acute gout are as follows; male 93.6%, age 54.5±2.9 years, serum erythrocyte sedimentation rate 41.5±6.5 mm/h, C-reactive protein 7.5±1.6 mg/dl, serum uric acid 6.5±0.6mg/dl, SF white blood cells 25288.8±6368.3/mm^3, SF neutrophils 14150.5±4171.0/mm^3 and presence of bone erosion 26.1%. In patients with intercritical gout, male 98.5%, age 55.1±1.8 years and serum uric acid 2.6±0.2 mg/dl.

SF and serum concentration of MIF and IL-8 in patients with acute and intercritical gout

SF MIF and IL-8 concentration was higher in patients with acute gout than in those with osteoarthritis (73.2±12.0 ng/ml *vs*. 19.8±2.9 ng/ml, p<0.001 for MIF; 7428.1±2281.4 pg/ml vs. 171.5±9.7 pg/ml, p<0.001 for IL-8). The SF concentration of MIF had a strong correlation with the SF concentration of IL-8 (R=0.73, p<0.0001, Fig. 1A). Serum MIF concentration was higher in patients with intercritical gout (7.8±1.4 ng/ml) than patients with acute gout (4.3±0.9 ng/ml, p=0.036) or healthy controls (6.1±1.8 ng/ml, p=0.02). Serum IL-8 concentration was higher in patients with acute gout (240.2±86.3 pg/ml), compared to that in patients with intercritical gout (164.8±10.1 pg/ml, p<0.0001) or healthy controls (187.7±14.5 pg/ml,

$p<0.0001$). In intercritical gout, serum MIF concentration were closely correlated with serum IL-8 concentration (R=0.75, $p<0.0001$, Fig. 1B).

There was a strongly positive correlation between SF MIF concentration and SF white blood cell count (WBC) in patients with acute gout (R=0.71, $p=0.001$). SF neutrophil count was significantly correlated with SF MIF concentration (R=0.44, $p=0.024$). However, SF IL-8 concentration was not associated with either SF WBC or neutrophil counts (Fig. 1C). Serum MIF and IL-8 concentration were not associated with clinical serologic measures such as ESR, CRP, WBC, and uric acid levels, or radiological results such as the presence of tophi and bone erosion (data not shown).

Basal expression of MIF and IL-8 in SFMC of acute gouty arthritis

The basal expression of *MIF* and *IL-8* mRNA was increased in SFMCs of patients with acute gout compared to those with osteoarthritis ($p<0.0001$, Fig. 2A-B). To determine the main cells producing *MIF* and *IL-8*, SFMCs were divided into CD16-positive neutrophils and CD14-positive monocytes, and their expression of the two cytokines was quantified using real time PCR. While the basal gene expression of *MIF* was similar in SF neutrophils and monocytes (Fig. 2C), the gene expression of *IL-8* was much higher in SF monocytes than in neutrophils in patients with acute gout ($p<0.0001$, Fig. 2D).

MSU crystal and IL-8-induced MIF production

Human PBMCs were isolated and cultured with various dosages of MSU crystals for 24 h. MSU crystal-induced *MIF* gene expression and production in PBMCs reached its maximal effect at 100 μg/ml of crystals. MSU crystals also increased the production of *MIF* from SFMCs. Subsequently, SFMCs were divided into neutrophils and monocytes. MSU crystals stimulated the production of *MIF* in SF monocytes, but not in SF neutrophils (Fig. 3A). There were no cytotoxic effects observed at the experimental dosages of MSU crystals used (data not shown).

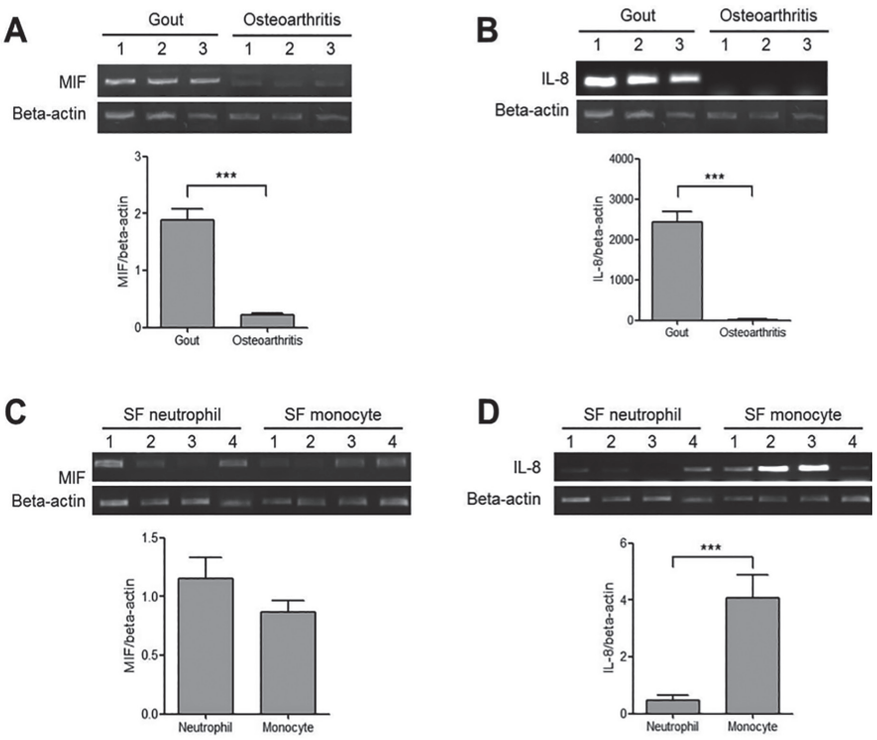

Fig. 2. The expression of macrophage migration inhibitory factor and interleukin-8 in synovial fluid mononuclear cells in patients with acute gout.

A-**B**: The basal expression of macrophage migration inhibitory factor (MIF) and interleukin-8 (IL-8) mRNA was determined using reverse transcription-polymerase chain reaction (RT-PCR) and real time PCR in synovial fluid mononuclear cells from 3 patients with acute gout and 3 patients with osteoarthritis.

C-**D**: The basal expression of MIF and IL-8 mRNA was determined using RT-PCR and real time PCR in SF CD16+ neutrophils and CD14+ monocytes from 4 patients with acute gout. Data were normalised to beta-actin and reported in relative expression units. The data represent the mean ± standard error of the mean of three independent experiments. ***$p<0.001$.

Since there was strong correlation between SF *MIF* and *IL-8* in acute gout, we hypothesised that *MIF* stimulates *IL-8* production or vice versa. When TPH-1 cells, a human monocyte cell line derived from acute monocytic leukaemia, were cultured with various doses of IL-8, IL-8 increased the mRNA expression and protein production of *MIF* in culture medium. The combination of IL-8 and MSU crystals further increased the production of MIF in the culture medium (Fig. 3B).

Signal pathways of MSU crystal-induced MIF expression

To define the signalling pathways of MSU crystal-induced *MIF* expression and production, PBMCs were cultured with MSU crystal in presence of the inhibitors for Fc-γ receptor (FcγR) and TLRs. After PBMCs were cultured with anti-FcγRI (CD64) and anti-TLR4 in the presence of MSU crystals, the gene expression and production of *MIF* in culture medium were significantly decreased (Fig. 4).

MSU crystal and MIF-induced IL-8 production

PBMCs were isolated and cultured with various dosages of MSU crystals for 24 h. MSU crystals did not increase the production of IL-8 in PBMCs. In SF neutrophils, MSU crystals increased IL-8 production in a dose-dependent manner, but did not affect its production in PB and SF monocytes (Fig. 5A). There were no cytotoxic effects observed at the experimental dosages of MSU crystals used (data not shown).

When SF neutrophils were cultured with various doses of MIF, MIF alone and combined with MSU crystals increased the gene expression of *IL-8*. While MIF alone tended to increase the production of MIF but there was no statistical significance ($p=0.07$), the combination of

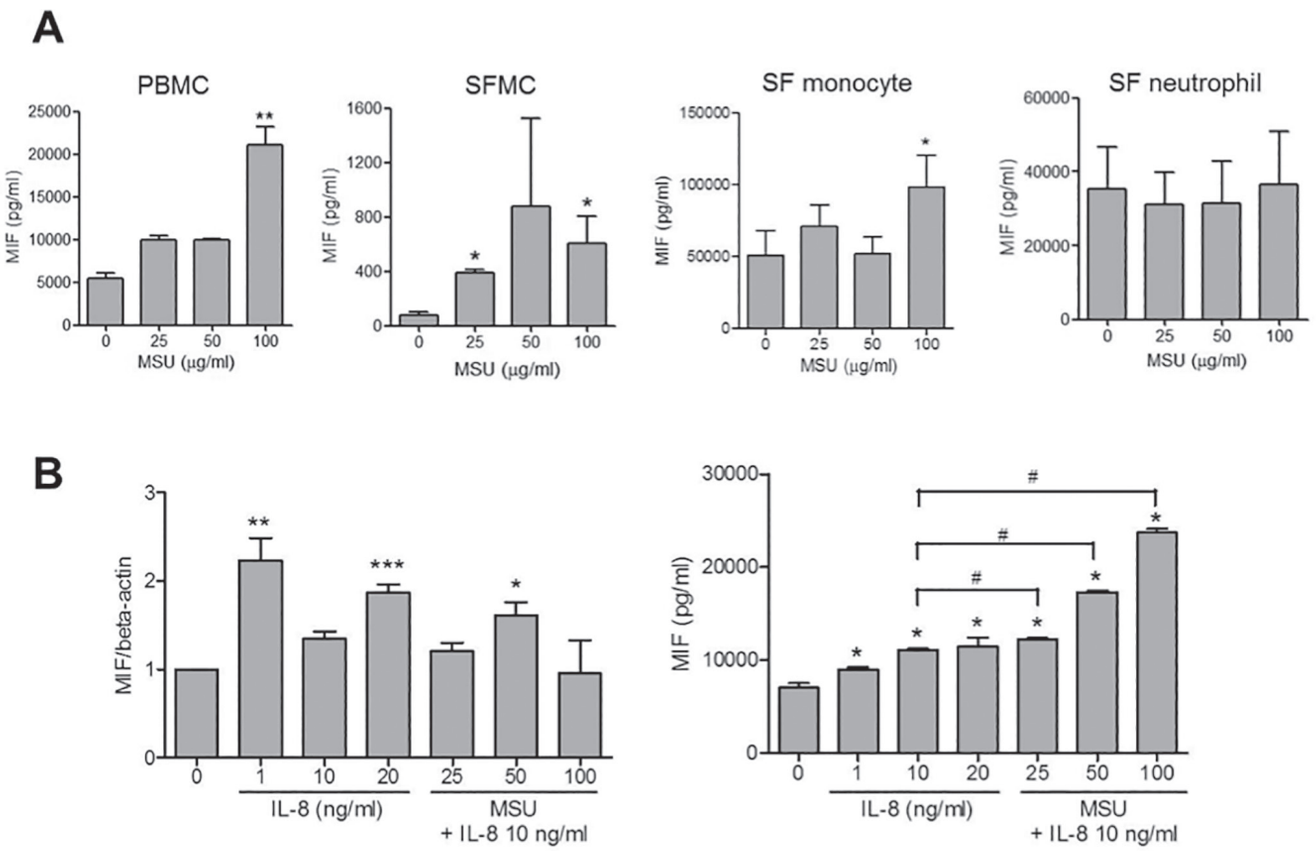

Fig. 3. The production of macrophage migration inhibitory factor induced by monosodium urate crystals and interleukin-8.
A: After peripheral blood mononuclear cells, synovial fluid (SF) CD14+ mononuclear cells, CD16+ SF monocytes and SF neutrophils were cultured with various doses of monosodium urate (MSU) crystals for 24 h, the production of macrophage migration inhibitory factor (MIF) was determined in the culture media using enzyme-linked immunosorbent assay (ELISA). The data represent the mean ± standard error of the mean. *p<0.05 and **p<0.01.
B: After THP-1 cells were cultured with either interleukin-8 (IL-8) or IL-8 and various doses of monosodium urate (MSU) crystals for 24 h, the gene expression of macrophage migration inhibitory factor (MIF) was determined using real time polymerase chain reaction, and the production of MIF in culture medium was determined using ELISA. Data were normalised to beta-actin and reported in relative expression units. The data represent the mean ± standard error of the mean. **p<0.01 and ***p<0.001.

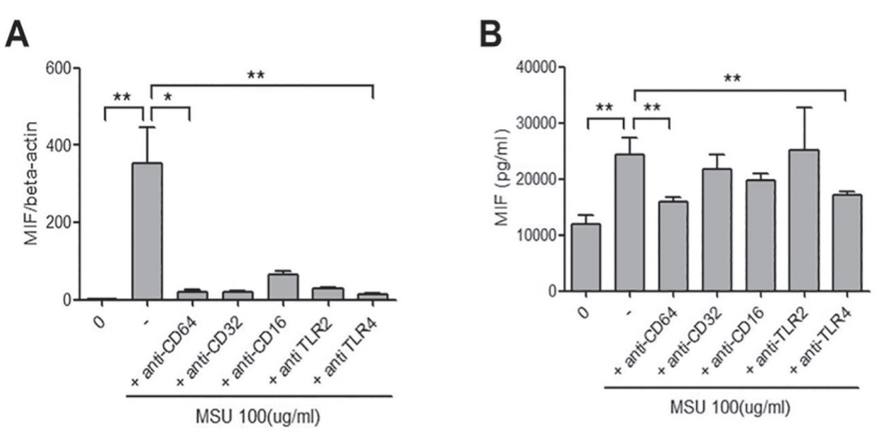

Fig. 4. Signalling pathways mediated monosodium urate crystal-induced macrophage migration inhibitory factor expression and production.
After peripheral blood mononuclear cells were cultured with 100 μg/ml of monosodium urate (MSU) crystals in the presence of signalling inhibitors such as anti-CD64, anti-CD32, anti-CD16, anti-toll-like receptor 2 (anti-TLR2) and anti-TLR4 for 24 h.
A: The gene expression of macrophage migration inhibitory factor (MIF) was determined using real time polymerase chain reaction.
B: The production of MIF in culture medium was determined using enzyme-linked immunosorbent assay. Data were normalised to beta-actin and reported in relative expression units. The data represent the mean ± standard error of the mean. *p<0.05 and **p<0.01.

MIF and MSU crystals increased the production of IL-8 in culture medium (Fig. 5B).

Discussion

This study analysed serum MIF and IL-8 concentrations in patients with acute gout. The initial results showed that SF MIF concentration was elevated in patients with acute gout, and that MIF concentration was correlated with that of IL-8. The pathogenic role of IL-8 in gout is well defined: IL-8 increases MSU-induced leukocytosis and induces other chemokines in the joints of patients with gout (9). Although the inflammatory role of MIF is well defined in RA and other inflammatory and neoplastic conditions, the pathogenic mechanism of MIF in acute gout has not been discovered yet. A previous study showed that the SF concentration of

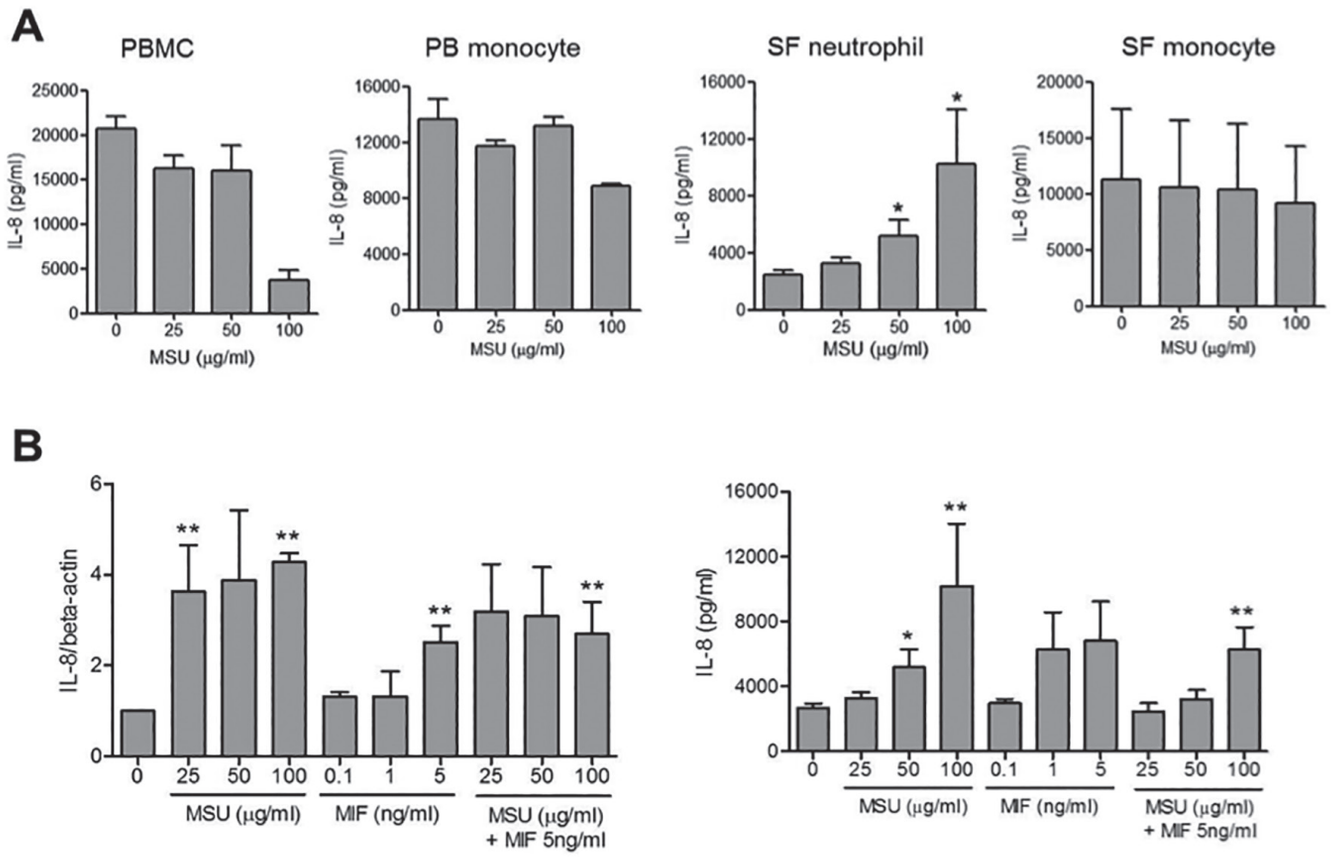

Fig. 5. The production of interleukin-8 is induced by monosodium urate crystals and macrophage migrating inhibitory factor.
A: After peripheral blood mononuclear cells, peripheral blood CD14+ monocytes, synovial fluid (SF) CD16+ neutrophils, and SF CD14+ monocytes were cultured with various doses of monosodium urate (MSU) crystals for 24 h, the production of interleukin-8 was determined in culture media using enzyme-linked immunosorbent assay (ELISA). The data represent the mean ± standard error of the mean. *p<0.05 and **p<0.01.
B: After SF neutrophils were culture with MIF, MSU crystals, and the combination of IL-8 and MSU crystals for 24 h, the gene expression of IL-8 was determined using real time PCR, and the production of IL-8 in culture medium was determined using ELISA. Data were normalised to beta-actin and reported in relative expression units. The data represent the mean±SEM. *p<0.05 and **p<0.01.

MIF is increased in acute gout. However, the study did not compare the MIF concentration in gout with that in other disease conditions or healthy subjects (21). Serum MIF concentration was elevated in patients with intercritical gout but not in those with acute gout. The serum MIF concentration was much lower than SF MIF level, indicating that the production of MIF is mainly localised in joint space rather than the systemic production. During the acute phase of gout, MIF is immediately produced and disappears immediately from the joint after resolution of the acute inflammation. Conversely, systemic MIF does not respond to inflammation as rapidly. However, the limitation of ELISA assays is the cytokine concentration does not reflect the bioactivity of the cytokines.

SF MIF concentration was strongly correlated with SF WBC count, which means SF WBCs are a major source of MIF in acute gout. In acute gout, high numbers of neutrophils infiltrate the joints. In this study, among SF WBCs, the percentage of neutrophils was 78.05% (data not shown). There was also a positive correlation between SF neutrophil count and MIF concentration; however, the degree of correlation was weaker than with that of total WBC count. This result suggests that the major cells producing MIF and IL-8 are not only the neutrophils, but also other more potent cells that cause acute inflammation. To define the cell source of MIF, SF WBCs were divided into CD14-positive monocytes and CD16-positive neutrophils. The expression of *MIF* was similar in both cells; however, monocytes expressed more *IL-8* than neutrophils. This result suggests that the most powerful player in acute gout inflammation is SF monocytes rather than neutrophils even with neutrophils being much more abundant than monocytes in the joints of patients with gout. Although IL-1β is known to be the most important cytokine in the pathogenesis of acute gout and anakinra, an IL-1 receptor antagonist, is used for intractable acute gout, MSU crystals alone do not directly stimulate IL-1β production. The combination of MSU crystal with lipopolysaccharide or free fatty acid induces IL-β production through toll-like receptors 4 or 2 (22, 23). However, the injection of pyogen-free MSU crystal alone induces acute inflammation of human joints (1, 24, 25). Taken together, a second stimulatory signal may be involved in triggering of acute gouty inflammation.

We tried to determine the direct effect of MSU crystals on the production of MIF and IL-8 in acute gout. After PBMCs and SFMCs were stimulated with MSU crystals, their production of MIF

was increased, while the production of IL-8 was not affected. As MIF and IL-8 levels were augmented in SF, SFMCs were divided into CD14+ monocytes and CD16+ neutrophils to determine which cells are more affected by MSU crystal stimulation of cytokine production. Interestingly, MSUs crystal affected the production of both cytokines in different cells. It directly induced MIF production in SF monocytes and IL-8 production in SF neutrophils. The result suggests that MSU crystals have different effects on different inflammatory cells in acute gouty inflammation.

Because there was a strong correlation between MIF and IL-8 and their production was directly induced by MSU crystals, we tried to determine their reciprocal interaction. In monocytes, IL-8 increased *MIF* gene expression and production. MSUs crystal augmented the IL-8-induced MIF production when monocytes were stimulated with both together. In our previous study we found that MIF increases IL-8 production and endothelial tube formation in RA synovial fibroblasts (11). Therefore, MIF was expected to affect IL-8 production of SF neutrophils in acute gout. MIF alone and combined with MSUs crystal increased IL-8 production in SF neutrophils. This result suggests that, in acute gout, not only are inflammatory cytokines produced from different cells after MSU crystal stimulation, but there is also a reciprocal interaction between the two cytokines. MSU crystals induced MIF in monocytes and IL-8 in neutrophils, with the IL-8 directly inducing MIF production from SF monocytes and MIF inducing IL-8 production in SF neutrophils. The cytokines' effects were augmented by MSU crystals. The interplay between MSU crystals, MIF, and IL-8 suggests a vicious cycle of acute inflammation in gout.

Since small molecular inhibitors of signalling pathway have become a therapeutic reality, further investigation of these pathways in inflammatory disorders has become more important (26, 27). The cell surface recognition receptors for MSU crystals suggested by previous studies are CD14, CD16, CD32, TLR2, and TLR4 (28, 29). We used inhibitors of Fcγ receptors, such as CD16, CD32, CD64, and inhibitors of TLR2 and TLR4. The inhibition of FcγI receptor and TLR4 reduced the MSU-induced MIF production. The genetic depletion of TLR2 and TLR4 impairs uptake of MSU crystal *in vitro*, production of IL-1β and TNF-α, and neutrophil influx and local induction of IL-1β in animal models (29). In the THP-1 cell line, MSU crystal-induced IL-8 expression is mediated through extracellular signal-regulated kinase (ERK)-1/ERK-2, c-Jun N-terminal kinase, and p38 mitogen-activated protein kinase (30). Further study into the signal pathways involved in MSU-induced MIF production is warranted.

In conclusion, the production of MIF and IL-8 was increased in the SF of patients with acute gout. MSU crystals induced MIF production in SF monocytes and IL-8 production in SF neutrophils. IL-8 induced MIF production from monocytes while MIF induced IL-8 production in neutrophils. The inhibition of MIF or IL-8 could block the reciprocal interaction and it will be a new therapeutic option in acute gout.

Key messages

- The expression and production of synovial MIF and IL-8 was increased in acute gout and there was positive correlation between MIF and IL-8.
- MSU crystals induced MIF production in monocytes and IL-8 production in neutrophils.
- IL-8 increased MIF production in monocytes while MIF increased IL-8 production in neutrophils.
- The inhibition of reciprocal interaction between MIF and IL-8 could be a new therapeutic option for acute gout.

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