Signaling Events Involved in Macrophage Chemokine Expression in Response to Monosodium Urate Crystals*

Received for publication, April 6, 2004, and in revised form, September 28, 2004
Published, JBC Papers in Press, October 7, 2004, DOI 10.1074/jbc.M403823200

Maritza Jaramillo‡§, Marianne Godbout¶, Paul H. Naccache**‡‡, and Martin Olivier‡§§

From the ‡Research Institute of the McGill University Health Centre, Centre for the Study of Host Resistance, Departments of Medicine, Microbiology, and Immunology, McGill University, Montréal, Québec H3A 2B4, Canada, §Centre de Recherche en Infectiologie and **Centre de Recherche en Rhumatologie et Immunologie, Centre Hospitalier Universitaire de Québec, Pavillon Centre Hospitalier de l’Université Laval et Départements de Biologie Médicale et de Médecine, Faculté de Médecine, Université Laval, Ste-Foy, Québec G1V 4G2, Canada

Chemokine production has been associated with leukocyte infiltration into the joint during gouty arthritis, and monosodium urate (MSU) crystals, the causative agent of this arthropathy, have been shown to modulate their expression. In the present study, we investigated the transductional mechanisms underlying this cellular regulation in the murine macrophage cell line R10R. We report that MSU crystals rapidly and transiently increase mRNA levels of various chemokines in a concentration-dependent manner. Examination of second messenger activation revealed that macrophage exposure to MSU crystals led to MEK1/2, ERK1/2, and inhibitory protein αβo phosphorylation as well as to NF-κB and AP-1 nuclear translocation. Of interest, specific blockade of the ERK1/2 pathway drastically reduced up-modulation of MSU crystal-mediated chemokine production and activation of nuclear factors. Similarly, selective inhibition of NF-κB suppressed NF-κB DNA binding activity and the induction of all chemokine transcripts. These findings indicate that ERK1/2-dependent signals seem to be required for AP-1 and NF-κB activation and subsequent mRNA expression of the various macrophage chemokines. In addition, transcription and stability assays performed in presence of actinomycin D showed that MSU crystal-mediated MIP-1α mRNA up-regulation resulted solely from transcriptional control, whereas that of MIP-1α, MIP-2, and MCP-1 was due to both gene transcription activation and mRNA posttranscriptional stabilization. Overall, the results of this study help to define the molecular events that govern macrophage chemokine regulation in response to MSU crystals, which is of paramount importance to better understand, and eventually to tame, the inflammatory response during acute gout.

Gouty arthritis is characterized by joint inflammation as a result of intra-articular deposition of monosodium urate (MSU) crystals in individuals with elevated serum concentrations of uric acid (1). MSU crystals initiate, amplify, and sustain intense attacks of acute inflammation because of their ability, among others, to stimulate the release of proinflammatory mediators, leading to endothelium activation and leukocyte recruitment (2). In association with aggressive and destructive outcome, high amounts of proinflammatory cytokines (e.g. tumor necrosis factor-α, IL-1β, and IL-6) have been found in various arthropathies, including gout (reviewed by Punzi et al. (3)). In addition to these cytokines, accumulating evidence indicates that chemokines and myeloid-related proteins, which are powerful leukocyte chemoattractants and activators (4, 5), also contribute to acute gout inflammation (6–9). Indeed, increased levels of chemokines IL-8/CXCL8 (6) and monocyte chemoattractant protein (MCP)-1/CCL2 (7) as well as of myeloid-related proteins S100A8/A9 (8) and S100A12 (9) have been detected in synovial fluid of patients suffering from gout.

In line with these human studies, experimental models of gouty arthritis have revealed that intra-articular injection of MSU crystals results in chemokine production (IL-8, MCP-1, and growth-related oncogene α/CXCL1) (10), leukocyte infiltration, and joint swelling (10–13). Administration of neutralizing Abs directed against IL-8 (10), MCP-1 (12), and growth-related oncogene α (13) significantly reduced MSU crystal-induced leukocyte accumulation, suggesting an important role for these chemokines in acute gout. In parallel, the use of the murine air-pouch model has further confirmed that MSU crystal-mediated leukocyte recruitment correlates with chemokine (macrophage inflammatory protein (MIP)-1α/CXCL3, MIP-2/CXCL2, MCP-1 and KC/CXCL1) and myeloid-related protein (S100A8, S100A9, and S100A8/A9) release (8, 14, 15). Consistent with these data, MSU crystals activate the expression of a number of cytokines and chemokines in various types of leukocytes. In monocytes, MSU crystal administration results in the release of tumor necrosis factor-α (16), IL-1 (17), IL-6 (18), and IL-8 (6). In macrophages, increased mRNA levels of tumor necrosis factor-α, MIP-1α, MIP-1β, and KC (14) as well as of IL-β and MIP-2 (15) have been detected upon MSU crystal

* This work was supported in part by grants from the Canadian Institutes in Health Research (CIHR) (to M. O.) and from the Arthritis Society (to P. H. N.). The costs of publication of this article were deferred in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

§§ Member of a CIHR group in host-pathogen interactions. Recipient of the Canada Research Chair on the Molecular Physiopathology of the Neutrophil.

© 2004 by The American Society for Biochemistry and Molecular Biology, Inc.

This paper is available online at http://www.jbc.org

52797
turing polyclaramide sequencing gel and exposed to radiographic film overnight at −80 °C. Laser densitometry was performed using an α Imager 2000 digital imaging and analysis system (α Innotech, San Leandro, CA).

**Western Blotting**—Cells were collected following stimulation, lysed in cold buffer containing 20 mM Tris-HCl, pH 8.0, 0.14 M NaCl, 10% glycerol (v/v), 1% (v/v) Igepal (Sigma), 25 μM nitrophenyl guanidinobenzoate, 10 μM sodium fluoride, 1 mM sodium orthovanadate, 25 μg/ml leupeptin and aprotemin. The lysates (20 μg/lane) were subjected to SDS-PAGE, and the separated proteins were transferred onto a polyvinylidene difluoride membrane (Millipore, Bedford, MA). After a 1-h blocking period in TBST containing 5% milk, the membranes were incubated overnight in TBST, 5% bovine serum albumin at 4 °C with one of the following rabbit polyclonal Abs purchased from New England Biolabs (Beverly, MA): phospho-MEK1/2 (Ser277/286), MEK1/2; phospho-ERK1/2 (Thr185/Tyr187), ERK1/2; phospho-IkBα (Ser32/36), IκBα. Proteins were then detected with an anti-rabbit horseradish peroxidase-conjugated goat Ab (Affini-pure, Jackson ImmunoResearch Laboratories, West Grove, PA) and subsequent visualization by ECL (ECL Western blotting detection system; Amersham Biosciences).

**Electrophoretic Mobility Shift Assays (EMSA)**—Cell stimulation was terminated by the addition of ice-cold PBS, and nuclear extracts were prepared, as we described previously (32). In brief, sedimented cells were resuspended in 400 μl of cold buffer A (10 mM HEPES, pH 7.9, 10 mM KCl, 1.0 mM dithiothreitol, and 0.5 mM phenylmethylsulfon fluoride). After 15 min on ice, 25 μl of 10% (v/v) Igepal were added, and the lysate was vortexed for 10 s and centrifuged for 30 s at 12,000 × g. The supernatant was discarded, and the cell pellet was resuspended in 100 μl of cold buffer B (20 mM HEPES, pH 7.9, 0.4 M NaCl, 1 mM EDTA, 1 mM EGTA, 1 mM dithiothreitol, and 1 mM phenylmethylsulfon fluoride). Nuclei were then resuspended vigorously at 4 °C for 10 min in 300 μl of cold buffer C (10 mM Tris-HCl, pH 8.0, 200 μl of poly(dI-dC), and 10 μg of nuclelease-free bovine serum albumin (fraction V) (Sigma)) containing 1.0 ng of radiolabeled double-stranded DNA oligonucleotide. The double-stranded DNA (100 ng) was end-labeled by using [γ-32P]dATP and T4 polynucleotide kinase (New England Biolabs). This mixture was incubated for 20 min at room temperature, and the reaction was stopped using 5 μl of 0.2 mM EDTA. The labeled oligonucleotide was extracted with phenol/chloroform and passed through a G-50 spin column. The double-stranded DNA oligonucleotides (Santa Cruz Biotechnology, Inc., Santa Cruz, CA), used either as probes or as competitors, were as follows: consensus binding site for AP-1 (c-Jun homodimer and Jun/Fos heterodimeric complexes, 5′-CCGTGATGACTCAGCGGCGG-3′); consensus binding site for NF-κB from B nef B nef (11); NF-κB from B nef B nef (11); NF-κB from B nef B nef (11). This mixture was incubated for 20 min at room temperature, and the reaction was stopped using 5 μl of 0.2 mM EDTA. The labeled oligonucleotide was extracted with phenol/chloroform. Poly(dI-dC) was purchased from ICN Pharmaceuticals Canada Ltd. (Montréal, Qué., Canada), and was confirmed by performing the amebocyte lysate test (E-Limulus amebocyte lysate test (E-toxate kit (Sigma)) as well as nitric oxide measurements in presence of Polynixin B (Sigma) (data not shown), as we previously described (29). Isolated [d-32P]CTP (3000 Ci/mmol) or [γ-32P]dATP (3000 Ci/mmol) were purchased from ICN Pharmaceuticals Canada Ltd. (Montréal, Qué., Canada). Actinomycin D (AD) was obtained from Sigma. Amebocyte lysate test (E-Limulus amebocyte lysate test (E-toxate kit (Sigma)) as well as nitric oxide measurements in presence of Polynixin B (Sigma) (data not shown), as we previously described (29). Isolated [d-32P]CTP (3000 Ci/mmol) or [γ-32P]dATP (3000 Ci/mmol) were purchased from ICN Pharmaceuticals Canada Ltd. (Montréal, Qué., Canada). Actinomycin D (AD) was obtained from Sigma. Amebocyte lysate test (E-Limulus amebocyte lysate test (E-toxate kit (Sigma)) as well as nitric oxide measurements in presence of Polynixin B (Sigma) (data not shown), as we previously described (29). Isolated [d-32P]CTP (3000 Ci/mmol) or [γ-32P]dATP (3000 Ci/mmol) were purchased from ICN Pharmaceuticals Canada Ltd. (Montréal, Qué., Canada). Actinomycin D (AD) was obtained from Sigma. Amebocyte lysate test (E-Limulus amebocyte lysate test (E-toxate kit (Sigma)) as well as nitric oxide measurements in presence of Polynixin B (Sigma) (data not shown), as we previously described (29). Isolated [d-32P]CTP (3000 Ci/mmol) or [γ-32P]dATP (3000 Ci/mmol) were purchased from ICN Pharmaceuticals Canada Ltd. (Montréal, Qué., Canada). Actinomycin D (AD) was obtained from Sigma. Amebocyte lysate test (E-Limulus amebocyte lysate test (E-toxate kit (Sigma)) as well as nitric oxide measurements in presence of Polynixin B (Sigma) (data not shown), as we previously described (29). Isolated [d-32P]CTP (3000 Ci/mmol) or [γ-32P]dATP (3000 Ci/mmol) were purchased from ICN Pharmaceuticals Canada Ltd. (Montréal, Qué., Canada). Actinomycin D (AD) was obtained from Sigma. Amebocyte lysate test (E-Limulus amebocyte lysate test (E-toxate kit (Sigma)) as well as nitric oxide measurements in presence of Polynixin B (Sigma) (data not shown), as we previously described (29). Isolated [d-32P]CTP (3000 Ci/mmol) or [γ-32P]dATP (3000 Ci/mmol) were purchased from ICN Pharmaceuticals Canada Ltd. (Montréal, Qué., Canada). Actinomycin D (AD) was obtained from Sigma. Amebocyte lysate test (E-Limulus amebocyte lysate test (E-toxate kit (Sigma)) as well as nitric oxide measurements in presence of Polynixin B (Sigma) (data not shown), as we previously described (29). Isolated [d-32P]CTP (3000 Ci/mmol) or [γ-32P]dATP (3000 Ci/mmol) were purchased from ICN Pharmaceuticals Canada Ltd. (Montréal, Qué., Canada). Actinomycin D (AD) was obtained from Sigma.
crease over negative control) and were more significant for MIP-2 (7-fold increase). Maximal values for MIP-1β (9-fold), MIP-1α (5-fold), MIP-2 (22-fold), and MCP-1 (6-fold) were observed upon macrophage exposure to 500 μg/ml MSU crystals (the highest concentration tested). In parallel, kinetic analyses were performed in order to establish the time required to obtain maximal chemokine modulation with an intermediate concentration of MSU crystals (100 μg/ml). As depicted in Fig. 1B, chemokine mRNA accumulation occurred very rapidly (0.5 h posttreatment), reached its peak after 1–2 h, and transiently decreased thereafter up to 8 h. Subsequent RPA experiments were thus conducted by stimulating cells for 2 h with 100 μg/ml MSU crystals.

**MSU Crystal-inducible Activation of the ERK1/2 Pathway Is Required for Chemokine mRNA Up-regulation**—Having observed that MSU crystals mediate increases in chemokine mRNA expression, we next attempted to identify the second messengers responsible for these effects. We initially examined the induction of ERK1/2 MAPK, which are known to play an important role in chemokine regulation in response to various proinflammatory stimuli (32, 33), including MSU crystals (26). As illustrated in Fig. 2, MSU crystals led to a rapid and sustained phosphorylation of the immediate upstream activator of ERK1/2, MAPK kinase 1/2 (MEK1/2) (Fig. 2A), and of ERK1/2 (Fig. 2B). Both MEK1/2 and ERK1/2 phosphorylation were already detectable after 15 min and remained detectable up to 4 h poststimulation. Next, to evaluate the involvement of the ERK1/2 signaling pathway in MSU crystal-dependent chemokine regulation, cells were incubated for 1 h with increasing concentrations of specific inhibitors directed either against MEK1/2 (PD 98059) or ERK1/2 (apigenin) before MSU crystal stimulation (2 h). It should be noted that the employed concentrations of these compounds correspond to those previously reported to specifically block this signaling cascade. In this regard, Liu et al. (26), who evaluated the effects of MSU crystals in human monocytes, showed that 50 μM PD98059 blocked ERK1/2 phosphorylation but had no effect on p38 MAPK phosphorylation. Similarly, we have previously demonstrated that PD 98059 (40 μM) and apigenin (50 μM) inhibited ERK1/2 phosphorylation but did not affect those of Jak2 Tyr1007/Tyr1008.
and STAT1α Tyr701 in interferon-γ-stimulated M6 (34). Based on these data and in agreement with other independent studies (35–38), subtoxic and specific concentrations of inhibitors against the MEK1/2-ERK1/2 pathway were selected to perform the experiments presented below. As depicted in Fig. 3A, 20 μM PD 98059 dramatically reduced mRNA levels of MIP-1β (~90% inhibition) and MIP-2 (~85%), and caused a partial but significant decrease of MIP-1α (~21%) and MCP-1 (~58%) transcripts. As expected, high concentrations (40 μM) of the MEK1/2 inhibitor abolished the expression of all four chemokines. In line with these data, cell pretreatment with apigenin (40 μM) abrogated the MSU-inducible mRNA expression of MIP-1α, MIP-1β, and MCP-1 and almost completely suppressed that of MIP-2 (~95% decrease) (Fig. 3B). This set of experiments demonstrates that the ERK1/2 pathway is activated in response to MSU crystals and indicates that this signaling cascade is functionally relevant to the modulation of chemokine induction by MSU crystals in macrophages.

**Involvement of NF-κB in MSU Crystal-dependent Chemokine Induction**—The NF-κB complex is involved in the transcriptional activation of chemokine genes (32, 39), including that of monocytic IL-8 in response to MSU crystals (26). Based on this evidence, we first set out to elucidate whether this nuclear factor was activated in B10R macrophages upon stimulation by MSU crystals. In order to be translocated into the nucleus, NF-κB must be released of its cytoplasmic inhibitor inhibitory protein κB (IκB). This occurs following IκB phosphorylation, ubiquitination, and ultimately proteolytic degradation (40). Therefore, we investigated the ability of MSU crystals to induce IκBκ phosphorylation, an indicator of NF-κB activation. Results obtained by Western blot revealed a rapid (detectable after 15 min) and transient phosphorylation of IκBκ on its Ser32 residue, which was accompanied by a decrease of IκBκ protein levels when maximal IκBκ phosphorylation was detected (1–2 h) (Fig. 4A). Next, to confirm that NF-κB activation was taking place in response to MSU crystals, EMSA experiments were undertaken. As shown in Fig. 4B, in cells stimulated with MSU crystals, NF-κB nuclear translocation was observed at 30 min posttreatment, peaked after 1 h, and progressively declined thereafter up to 4 h. To define the nature of the MSU crystal-induced NF-κB complex, supershift assays were performed using Abs directed against p50 and p65, two ubiquitous members of the NF-κB family. As illustrated in Fig. 4C, the complex binding was diminished and partially supershifted in the presence of an anti-p50 Ab and almost completely abrogated by an anti-p65 Ab. Thus, MSU crystals appear to activate DNA binding of a p50/p50 homodimer (lower band) and a p50/p65 heterodimer (upper band) in murine macrophages. The specificity of these binding complexes was demonstrated by the fact that unlabeled NF-κB oligonucleotide (100× specificity) could compete effectively for binding, whereas an unrelated Sp1 probe (100× nonspecific) could not. These data prompted us to define the functional importance of NF-κB on MSU crystal-dependent chemokine up-regulation. Knowing that selective blockage of the ERK1/2 pathway abolished chemokine expression by MSU crystals, we were interested to determine whether NF-κB activation was also under the control of this signaling cascade. As depicted in Fig. 5A, cell exposure to either apigenin or PD 98059 resulted in a concentration-dependent decrease of MSU crystal-inducible NF-κB nuclear translocation. To more directly address the putative contribution of NF-κB to chemokine modulation, macrophages were incubated for 1 h with increasing concentrations (0.5–5 μM) of BAY 11-7082, a chemical compound that blocks NF-κB expression by inhibiting IκBκ phosphorylation (41), before MSU crystal stimulation, and NF-κB translocation was monitored by EMSA (Fig. 5B). As expected, cells incubated with BAY 11-7082 showed a concentration-dependent down-regulation in the binding of the NF-κB complex, which was nearly abolished in the presence of maximal inhibitor concentrations (5 μM). Based on this control experiment, we next eval-
Fig. 4. MSU crystals induce IkBα phosphorylation and NF-κB nuclear translocation in murine macrophages. A, following cell exposure to MSU crystals over a 4-h period, protein lysates were subjected to Western blotting to evaluate IkBα phosphorylation status (upper panel). Changes in IkBα protein levels were monitored using an anti-IkBα Ab (lower panel). B, nuclear extracts from macrophages either left untreated or stimulated with MSU crystals for different time periods (0–4 h) were incubated with a γ-32P-labeled NF-κB probe and were subjected to EMSA. C, for supershift assays, nuclear proteins from cells stimulated with MSU crystals (100 μg/ml for 1 h) were incubated or not with specific Abs against the p50 and p65 NF-κB isoforms for 1 h at 4°C before EMSA. Binding specificity was tested by adding to nuclear extracts from 1-h treated cells a 100-fold molar excess of either a cold NF-κB consensus oligonucleotide (100× specific) or a nonspecific Sp1 probe (100× nonspecific). These results are representative of one of three independent experiments.

Fig. 5. NF-κB is involved in MSU crystal-inducible macrophage chemokine gene expression. Nuclear extracts from MSU crystal-stimulated cells (100 μg/ml for 1 h) pretreated or not with 5–40 μM apigenin, 1–40 μM PD 98059 (A), or 0.5–5 μM BAY 11-7082 (B) were incubated with a radiolabeled NF-κB probe, and EMSA analysis was performed. Binding specificity was tested by adding to nuclear extracts from 1-h treated cells a 100-fold molar excess of cold NF-κB oligonucleotide. C, after a 1-h exposure to BAY 11-7082, macrophages were further stimulated with MSU crystals for 2 h. Total RNA was extracted, and changes of chemokine mRNA levels were monitored by RPA (left panel). Integrated density values of chemokine mRNA levels were normalized to GAPDH (right panel). Open bars, untreated (Nil); solid bars, MSU crystals + BAY 11-7082. These results are representative of one of three separate experiments.
MSU Crystal-inducible Macrophage Chemokine mRNA Up-regulation

All chemokine transcripts, following the same pattern of inhibition as that exerted on NF-κB (Fig. 5B). In fact, intermediate concentrations of this component (3 μM), which substantially diminished NF-κB binding activity, caused a significant reduction of chemokine mRNA (−65% for MIP-1β, −36% for MIP-1α, −53% for MIP-2, and −64% for MCP-1). In line with these observations, cell exposure to 5 μM BAY 11-7082, which suppressed NF-κB translocation, also led to a total inhibition of the MIP-1α, MIP-1β, and MCP-1 transcripts and almost completely abrogated MIP-2 mRNA expression (−94% reduction). Altogether, these data indicate that ERK1/2-mediated NF-κB activation appears to be necessary for the induction of macrophage chemokine mRNA in response to MSU crystals.

MSU Crystals Induce AP-1 Nuclear Translocation via the ERK1/2 Pathway—In addition to evaluating the potential role of NF-κB, we investigated the implication of AP-1 in MSU crystal-mediated chemokine modulation. This transcription factor is activated by MSU crystals in human macrophages and is involved in IL-8 regulation in response to this proinflammatory agent (26). Thus, we initially examined whether MSU crystals led to AP-1 activation in B10R macrophages. As depicted in Fig. 6A, when oligonucleotides containing AP-1 consensus binding sequences were used to probe nuclear extracts from MSU crystal-stimulated cells, we observed a rapid (30 min poststimulation) and sustained binding activity of this transcription factor, reaching its maximal expression at 4 h. To identify the AP-1 subunits that form the MSU crystal-inducible complex, supershift assays were conducted by incubating nuclear extracts from MSU crystal-treated macrophages with specific Abs against some of the main members of the AP-1 family: Fos B, c-Fos, JunB, and c-Jun. As illustrated in Fig. 6B, the AP-1 complex binding was diminished in the presence of Abs against c-Fos, JunB, and c-Jun, but it was not affected by an anti-FosB Ab. In parallel, the specificity of this binding complex was demonstrated by the fact that unlabeled AP-1 oligonucleotide (100× specific) could compete effectively for binding, whereas an unrelated Sp1 probe (100× nonspecific) could not. These data indicate that in macrophages, MSU crystals lead to nuclear translocation and binding activity of heterodimeric AP-1 complexes composed of c-Fos, JunB, and c-Jun. The similar kinetics of stimulation of AP-1 transcription and chemokine mRNA expression suggested a possible role for this transcription factor in MSU crystal-mediated chemokine regulation. To address this question, we next evaluated the involvement of the ERK1/2 pathway on the noticed AP-1 binding activity. When cells were treated with either apigenin or PD 98059 (Fig. 6C), AP-1 nuclear translocation in response to MSU crystals was reduced in a concentration-dependent manner. Of interest, the AP-1 complex DNA binding capacity was abrogated at the same inhibitor concentrations that were found to block chemokine expression (Fig. 3). Altogether, these results suggest that MSU crystal-induced chemokine up-regulation in macrophages involves the participation of ERK1/2-mediated AP-1 transcription factor activation.

MSU Crystal-mediated Increase of Chemokine mRNA Levels Is Due to Both Transcriptional and Posttranscriptional Controls—Previous studies performed by others (39, 42) and by us (32) have indicated that both transcriptional and posttranscriptional levels of control modulate chemokine mRNA expression. Therefore, we were interested in establishing which of these mechanisms were responsible for the augmentation of macrophage chemokine mRNA levels following MSU crystal stimulation. To do so, we tested the effects of the transcriptional inhibitor actinomycin D (AD), as we described elsewhere (32). Initially, to verify whether MSU crystal-dependent chemokine regulation occurred at the transcriptional level, B10R cells were stimulated with MSU crystals for 2 h either in the absence or in the presence of AD (5 μg/ml), and chemokine mRNA levels were monitored by RPA. As shown in Fig. 7A, AD treatment completely blocked the expression of all four chemokine transcripts by MSU crystals, indicating that, at least in part, MSU crystals regulate chemokine expression at the transcriptional level. Next, the ability of MSU crystals to increase chemokine mRNA stability was monitored by measuring their effects on the half-life of the various chemokine transcripts. To test this, cells were preincubated or not with MSU crystals for 2 h, and then they were exposed to AD (5 μg/ml) over a 4-h...
period. As depicted in Fig. 7B, in the presence of AD, chemokine mRNA from nonstimulated cells (control) decayed rapidly, with a half-life of ∼30 min. MSU crystal treatment stabilized the MIP-1β, MIP-2, and MCP-1 mRNAs, increasing their half-lives to more than 4 h. In contrast, the half-life of the MIP-1α transcript was not enhanced by MSU crystals. Overall, these results indicate that MSU crystal-inducible MIP-1β mRNA expression is controlled only at the transcriptional level, whereas the regulation of MIP-1α, MIP-2, and MCP-1 seems to be due to both transcriptional activation of their genes and posttranscriptional stabilization of their mRNA transcripts.

**DISCUSSION**

Acute gouty inflammation is characterized by a massive influx of leukocytes (mostly neutrophils) into the inflamed joints of hyperuricemic patients (2), which is mediated by the production of powerful chemoattractants and activators (8, 10–15). Notably, monocyte IL-8 production has been proposed as a major mechanism mediating MSU crystal-induced neutrophil migration (6, 10, 11). Further supporting a role for mononuclear phagocytes as a source of chemokines during gout, we provide evidence for the induction of multiple CC and CXC chemokine transcripts (MIP-1α, MIP-1β, MCP-1, and MIP-2) in macrophages following stimulation by MSU crystals. Investigation of the transductional mechanisms responsible for this cellular response suggests the requirement of ERK1/2-dependent signals leading to nuclear factor activation and subsequent chemokine mRNA expression. In addition, our data from transcription and RNA decay assays indicated that the observed increase of macrophage chemokine mRNA levels occurs at the transcriptional level for MIP-1β, whereas for MIP-1α, MIP-2, and MCP-1 both transcriptional and posttranscriptional events appear to be involved.

As described by Liu and colleagues (26), ERK1/2-dependent signals are required for MSU crystal-inducible IL-8 mRNA accumulation in human monocytes. Extending these previous findings and in agreement with several studies linking ERK1/2 activity to chemokine regulation (32, 33, 43), our data indicated that the ERK1/2 pathway seems to play an important role in MIP-1α, MIP-1β, MIP-2, and MCP-1 mRNA expression by MSU crystal-stimulated macrophages. Although activation of the p38 MAPK pathway was also detected, specific inhibition of this signaling cascade had no effect on chemokine mRNA induction (data not shown), suggesting that despite the activation of at least two different MAPK, only the ERK1/2 pathway participates in this regulatory event. As to the mechanisms involved, experimental evidence suggested that this process implicates ERK1/2-mediated activation of two transcription factors, NF-κB and AP-1. Indeed, the role of these factors in the transcriptional control of chemokine genes has been extensively documented (26, 33, 39, 44) and has been linked to ERK1/2 activation (26, 32). These results are perfectly in line with our published data showing that the up-regulating effect of MSU crystals on interferon-γ-mediated nitric oxide production occurred via ERK1/2-dependent NF-κB activation (29).
MSU Crystal-inducible Macrophage Chemokine mRNA Up-regulation

Thus, it is likely that through the activation of the same second messengers, MSU crystals modulate different macrophage functions, including multiple chemokine expression and amplification of macrophage responses to interferon-γ (e.g. nitric oxide production), which would in turn contribute to the pathology related to gouty arthritis. Although the kinases through which the ERK1/2 pathway enhances NF-κB translocation need to be identified, given that MAPK/ERK kinase kinase 1 has the ability to induce IκB kinase activation (45), and both ERK1/2 and IκB kinase were required for MSU crystal-dependent NF-κB binding to the IL-8 promoter (27), it is conceivable that the ERK1/2 pathway participates in IκB kinase phosphorylation and subsequent MSU crystal-inducible NF-κB nuclear translocation and chemokine transcription. Further investigation will bring light on this important matter.

Our data showing that chemokine mRNA up-regulation in response to MSU crystals occurs both at the transcriptional and posttranscriptional levels are in agreement with previous reports by others (39, 42) and by us (32), in which the same mechanisms were found to control chemokine expression. Different lines of evidence indicate that this phenomenon could be associated with the presence of AU-rich motifs implicated in chemokine mRNA destabilization (39, 46–49), and at least two mechanisms could be involved. First, members of the MAPK family, including c-Jun N-terminal kinase (50) and ERK1/2 (51, 52) have been found to enhance the stability of certain mRNAs that bear AU-rich motifs. Since, according to previous studies (26, 27) and our present results, MAPK activation takes place in MSU crystal-treated cells, these signaling cascades are likely to participate in the observed increases of chemokine mRNA half-lives. Second, it was postulated that enhancement on mRNA stability could be caused by the binding of a redox-sensitive protein to AU-rich motifs, which in turn form stable complexes that prevent mRNA degradation (42, 53). Because MSU crystals are able to induce oxidative stress in monocytes (54), the possibility that this protein is activated should not be ruled out.

We found that MSU crystals rapidly and transiently up-regulate mRNA expression of MIP-2, a potent neutrophil chemoattractant and activator (55). These observations are perfectly in line with in vivo studies reporting early neutrophil accumulation and increased MIP-2 production in a murine air pouch model of MSU crystal-mediated inflammation (8, 15). Thus, based on these previous works, along with our data, it is plausible that MSU crystal-inducible macrophage MIP-2 contributes to neutrophil infiltration during acute gout. Although MSU neutrophils account for most of the leukocytes recruited during the spontaneous resolution of acute gout, this cellular infiltrate is predominantly mononuclear (56), by favoring monocyte infiltration into the joint, MSU crystal-induced macrophage CC chemokines could contribute to the spontaneous resolution of acute gout.

In summary, our study demonstrates that MSU crystals regulate simultaneous activation of various chemokine genes in macrophages through a mechanism that seems to require ERK1/2-dependent cellular signals leading to transcription factor activation and subsequent chemokine mRNA expression. Moreover, our data indicated that MSU crystals control chemokine induction at both transcriptional and posttranscriptional levels. Importantly, our findings suggest that by increasing CXC chemokine production, macrophages present in the joint might play a role in early neutrophil infiltration during acute gout. In parallel, the possibility that macrophages participate in the spontaneous resolution of an acute gout attack, by favoring monocyte infiltration into the synovial environment via CC chemokine up-regulation, deserves further investigation. Overall, the current study will contribute to understanding the molecular mechanisms underlying macrophage modulation by MSU crystals, which is of paramount importance to better define the role of this cellular type in gouty arthritis and to design new therapeutic strategies for the management of this disease.

Acknowledgments—We thank Drs. Rinaldo de Médicis and André Lussier (Université de Sherbrooke, Sherbrooke, Québec, Canada), who kindly provided the triclinic MSU crystals, and Dr. Danuta Radzioch (McGill University), who provided the murine macrophage cell line B10R.

REFERENCES

1. Agudelo, C. A., and Wise, C. M. (2001) Curr. Opin. Rheumatol. 13, 224–239
2. Terkeltaub, R. (1996) in Arthritis and Allied Conditions (Koopman, W. J., ed) pp. 2085–2102, Williams & Wilkins, Baltimore
3. Punzi, L., Calo, L., and Plebani, M. (2002) Crit. Rev. Clin. Lab. Sci. 39, 63–88
4. Punzi, L., Calo, L., and Plebani, M. (1997) Blood 90, 626–628
5. Ryckman, C., Vandal, K., Rouleau, P., Talbott, M., and Tessler, P. A. (2003) J. Immunol. 170, 3233–3242
6. Terkeltaub, R., Zachariae, C., Santoro, D., Martin, J., Peveri, P., and Matsushima, K. (1991) Arthritis Rheum. 34, 894–903
7. Harigai, M., Harra, M., Yoshimura, T., Leonard, E. J., Inoue, K., and Kashiwazaki, S. (1993) Clin. Immunol. Immunopathol. 69, 83–91
8. Ryckman, C., Collin, S., Vandal, K. de Médicis, R., Lussier, A., Poubelle, P., and Tessler, P. A. (2003) Arthritis Rheum. 48, 2310–2320
9. Rouleau, P., Vandal, K., Ryckman, C., Poubelle, P. E., Boivin, A., Talbott, M., and Tessler, P. A. (2000) Clin. Immunol. 107, 46–54
10. Nishimura, A., Akahoshi, T., Takahashi, M., Katagishi, K., Itoyan, M., Kondo, H., Takahashi, Y., Yokoi, K., Mukaida, N., and Matsushima, K. (1997) J. Leukocyte Biol. 62, 444–449
11. Matsuakawa, A., Yoshimura, T., Maeda, T., Takahashi, T., Ohkawara, S., and Yoshinaga, M. (1998) Lab. Investig. 78, 559–569
12. Matsuakawa, A., Miyazaki, S., Maeda, T., Tanase, S., Feng, L., Ohkawara, S., Yoshinaga, M., and Yoshinoma, T. (1998) Lab. Investig. 79, 973–985
13. Fujiwara, K., Ohkawara, S., Takagi, K., Yoshinaga, M., and Matsuakawa, A. (2002) Lab. Investig. 82, 1297–1304
14. Murakami, Y., Akahoshi, T., Kawai, S., Inoue, N., and Kitasato, H. (2002) Arthritis Rheum. 48, 2504–2513
15. Murakami, Y., Akahoshi, T., Hayashi, I., Endo, H., Hashimoto, A., Kono, S., Kondo, H., Kawai, S., Inoue, N., and Kitasato, H. (2003) Arthritis Rheum. 48, 2931–2941
16. di Giovinio, F. S., Malavista, S. E., Thornton, E., and Duff, G. W. (1991) J. Clin. Invest. 87, 1375–1381
17. di Giovinio, F. S., Malavista, S. E. Nuki, G., and Duff, G. W. (1987) Immunol. 138, 3213–3218
18. Guerne, P. A., Terkeltaub, R., Zuraw, B., and Lotz, M. (1989) Arthritis Rheum. 32, 1443–1452
19. Robege, C. J., de Médicis, R., Dayer, J. M., Rola Pleszczyznski, M., Nacache, P. H., and Poubelle, P. E. (1994) J. Immunol. 152, 5485–5494
20. Hachihira, M., Nacache, P. H., and McColl, S. R. (1995) J. Exp. Med. 182, 2019–2025
21. Abramson, S., Hoffstein, S. T., and Weissmann, G. (1982) Arthritis Rheum. 25, 174–180
22. Terkeltaub, R. A., Sklar, L. A., and Mueller, H. (1990) J. Immunol. 144, 2719–2724
23. Bomalaski, J. S., Baker, D. G., Brophy, L. M., and Clark, M. A. (1990) J. Immunol. 145, 3391–3397
24. Nacache, P. H., Bourgon, S., Plante, E., Robege, C. J., de Médicis, R., Lussier, A., and Poubelle, P. E. (1995) Arthritis Rheum. 36, 117–125
25. Gaudry, M., Gilbert, C., Barabe, F., Poubelle, P. E., and Nacache, P. H. (1995) Blood 86, 3567–3574
26. Liu, R., O’Connell, M., Johnson, K., Pritzker, K., Mackman, N., and Terkeltaub, R. (2000) Arthritis Rheum. 43, 1145–1155
27. Liu, R., Apperle, K., and Terkeltaub, R. (2001) J. Leukocyte Biol. 70, 961–968
28. Gaudry, M., Robege, C. J., de Médicis, R., Lussier, A., Poubelle, P. E., and
Naccache, P. H. (1993) *J. Clin. Investig.* **91**, 1649–1655
29. Jaramillo, M., P. H. Naccache, and M. Olivier. (2004) *J. Immunol.* **172**, 5734–5742
30. Radzioch, D., T. Hudson, M. Boule, L. Barrera, J. W. Urbance, L. Varesio, and E. Skamene. (1991) *J. Leukocyte Biol.* **50**, 263–272
31. Marshall, N. J., Goodwin, C. J., and Holt, S. J. (1995) *Growth Regul.* **5**, 69–84
32. Jaramillo, M., and Olivier, M. (2002) *J. Immunol.* **169**, 7026–7038
33. Marumo, T., Schini-Kerth, V. B., and Busse, R. (1999) *Diabetes* **48**, 1131–1137
34. Blanchette, J., M. Jaramillo, and M. Olivier. (2003) *Immunology* **108**, 513–522
35. Bhat, N. R., P. Zhang, J. C. Lee, and E. L. Hogan. (1998) *J. Neurosci.* **18**, 1633–1641
36. Kan, H., Z. Xie, and M. S. Finkel. (1999) *Am. J. Physiol.* **277**, H1641–H1646
37. Chen, E. D., K. R. Morris, J. T. Belisle, P. Hill, L. K. Remigio, P. J. Brennan, and D. W. Riches. (2001) *Infect. Immun.* **69**, 2091–2010
38. Kim, K. W., S. H. Kim, E. Y. Lee, N. D. Kim, H. S. Kang, H. D. Kim, B. S. Chung, and C. D. Kang. (2003) *J. Biol. Chem.* **278**, 13199–13191
39. Shi, M. M., Chong, I., Godleski, J. J., and Paulauskis, J. D. (1999) *Immunology* **97**, 309–315
40. Karin, M., and Ben Neriah, Y. (2000) *Annu. Rev. Immunol.* **18**, 621–663
41. Pierce, J. W., Schoenleber, R., Jesmok, G., Best, J., Moore, S. A., Collins, T., and Gerritsen, M. E. (1997) *J. Biol. Chem.* **272**, 21096–21103
42. Shi, M. M., Godleski, J. J., and Paulauskis, J. D. (1996) *J. Biol. Chem.* **271**, 5878–5883
43. Chen, X. L., Tummala, P. E., Olbrych, M. T., Alexander, R. W., and Medford, R. M. (1998) *Circ. Res.* **83**, 952–959
44. Lakshminarayanan, V., Drah-Weiss, E. A., and Roebuck, K. A. (1998) *J. Biol. Chem.* **273**, 32670–32678
45. Lee, F. S., Peters, R. T., Dang, L. C., and Maniatis, T. (1998) *Proc. Natl. Acad. Sci. U. S. A.* **95**, 9319–9324
46. Shi, M. M., Godleski, J. J., and Paulauskis, J. D. (1995) *Biochem. Biophys. Res. Commun.* **211**, 289–295
47. Widmer, U., Manogue, K. R., Cerami, A., and Sherry, B. (1993) *J. Immunol.* **150**, 4996–5012
48. Poon, M., Liu, B., and Tai, B. (1999) *Mol. Cell. Biol.* **19**, 6471–6478
49. Mukaida, N., Okamoto, S., Ishikawa, Y., and Matsushima, K. (1994) *J. Leukocyte Biol.* **56**, 554–558
50. Chen, C. Y., Del Gatto-Konzak, F., Wu, Z., and Karin, M. (1998) *Science* **280**, 1945–1949
51. Xiao, Y. Q., Soneya, K., Morita, H., Takahashi, K., and Ohuchi, K. (1999) *Biochim. Biophys. Acta* **1450**, 155–163
52. Jijon, H. B., Panenka, W. J., and Parsons, H. G. (2002) *Am. J. Physiol. Cell. Physiol.* **283**, C31–41
53. Malter, J. S., and Hong, Y. (1991) *J. Biol. Chem.* **266**, 3167–3171
54. Palit, J., di Giovine, F. S., Dickers, E., Duff, G. W., and Nuki, G. (1986) *Br. J. Rheum.* **25**, A21
55. Wolpe, S. D., Sherry, B., Juers, D., Davateles, G., Yurt, R. W., and Cerami, A. (1989) *Proc. Natl. Acad. Sci. U. S. A.* **86**, 615–616
56. Pascual, E., and Jovani, V. (1995) *Br. J. Rheumatol.* **34**, 724–726
Signaling Events Involved in Macrophage Chemokine Expression in Response to Monosodium Urate Crystals
Maritza Jaramillo, Marianne Godbout, Paul H. Naccache and Martin Olivier

J. Biol. Chem. 2004, 279:52797-52805.
doi: 10.1074/jbc.M403823200 originally published online October 7, 2004

Access the most updated version of this article at doi: 10.1074/jbc.M403823200

Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 52 references, 24 of which can be accessed free at http://www.jbc.org/content/279/50/52797.full.html#ref-list-1