Oncostatin M-Induced Matrix Metalloproteinase and Tissue Inhibitor of Metalloproteinase-3 Genes Expression in Chondrocytes Requires Janus Kinase/STAT Signaling Pathway[1]

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Oncostatin M (OSM), a member of the IL-6 superfamily of cytokines, is elevated in patients with rheumatoid arthritis and, in synergy with IL-1, promotes cartilage degeneration by matrix metalloproteinases (MMPs). We have previously shown that OSM induces MMP and tissue inhibitor of metalloproteinase-3 (TIMP-3) gene expression in chondrocytes by protein tyrosine kinase-dependent mechanisms. In the present study, we investigated signaling pathways regulating the induction of MMP and TIMP-3 genes by OSM. We demonstrate that OSM rapidly stimulated phosphorylation of Janus kinase (JAK) 1, JAK2, JAK3, and STAT1 as well as extracellular signal-regulated kinase (ERK) 1/2, p38, and c-Jun N-terminal kinase 1/2 mitogen-activated protein kinases in primary bovine and human chondrocytes. A JAK3-specific inhibitor blocked OSM-stimulated STAT1 tyrosine phosphorylation, DNA-binding activity of STAT1 as well as collagenase-1 (MMP-1), stromelysin-1 (MMP-3), collagenase-3 (MMP-13), and TIMP-3 RNA expression. In contrast, a JAK2-specific inhibitor, AG490, had no impact on these events. OSM-induced ERK1/2 activation was also not affected by these inhibitors. Similarly, curcumin (diferuloylmethane), an anti-inflammatory agent, suppressed OSM-stimulated STAT1 phosphorylation, DNA-binding activity of STAT1, and c-Jun N-terminal kinase activation without affecting JAK1, JAK2, JAK3, ERK1/2, and p38 phosphorylation. Curcumin also inhibited OSM-induced MMP-1, MMP-3, MMP-13, and TIMP-3 gene expression. Thus, OSM induces MMP and TIMP-3 genes in chondrocytes by activating JAK/STAT and mitogen-activated protein kinase signaling cascades, and interference with these pathways may be a useful approach to block the catabolic actions of OSM. ***The Journal of Immunology*****, 2001, 166: 3491–3498.**

Rheumatoid arthritis (RA)[3] involves systemic and chronic joint inflammation, breakdown of articular cartilage, and subchondral bone where cytokines alter connective tissue metabolism (1). Recently characterized aggrecanases along with matrix metalloproteinases (MMPs) in excess of their natural inhibitors (tissue inhibitors of metalloproteinases or TIMPs) are implicated in joint damage. The MMP enzyme family is classified into subgroups of collagenases, gelatinases, stromelysins, and membrane-type MMPs which digest various components of extracellular matrix (ECM) during physiologic and pathologic remodeling (2, 3). Stromelysin-1 (MMP-3) is responsible for degradation of cartilage aggrecan and activation of procollagenases (2, 3). Collagenase-1 (MMP-1), collagenase-2 (MMP-8), and collagenae-3 (MMP-13) are the principle neutral proteinases capable of degrading native fibrillar collagens in the ECM with similar efficiency (4); however, MMP-13 is more effective in degrading type II collagen (5). Since MMP-1, -3, and -13 are induced by proinflammatory cytokines, such as IL-1 and TNF-α (6–8), and MMPs are found elevated in patients with arthritis (8–10), they may contribute directly to progression of the disease. Identification of MMP-inducing agents and deciphering the mechanisms of induction are necessary for developing novel strategies to block cartilage resorption.

The TIMP multigene family consists of TIMP-1, -2, -3, and -4, which by complexation with MMPs regulate enzyme-inhibitor balance (3, 11). MMP inhibitors are potentially important therapeutic agents for arthritis (12). TIMPs also inhibit tumor growth, invasion, and metastasis and have growth-promoting and antiangiogenic activities (11, 13). TIMP-3 was originally isolated from chicken fibroblasts and is distinctly associated with ECM (14, 15). It is expressed in several normal and tumor tissues (16–18). By its strategic location in ECM, TIMP-3 blocks tumor growth and invasion possibly by inhibiting angiogenesis (19) and by inducing apoptosis (20). TIMP-3 inhibits MMPs similarly to TIMP-1 (21) and is extensively expressed in joint tissues (22). Due to its potential for inhibiting cartilage resorption, elucidation of signaling mechanisms regulating TIMP-3 by arthritis-associated stimuli is of particular interest.

Oncostatin M (OSM) produced by activated monocytes and T lymphocytes (23) is a multifunctional cytokine that affects the growth and differentiation of several cell types (24, 25). OSM belongs to the IL-6 family of cytokines, which includes IL-11 and LIF. They have a similar helical structure and share the receptor gp130 (25). An anti-inflammatory role for OSM in vivo was suggested because it suppressed inflammation and tissue damage in a mouse model of RA (26). OSM was undetectable in the synovial

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[3] Abbreviations used in this paper: RA, rheumatoid arthritis; ECM, extracellular matrix; ERK, extracellular signal-regulated kinase; JAK, Janus kinase, JNK, c-Jun N-terminal kinase; MAPK, mitogen-activated protein kinase, MMP, matrix metalloproteinase; OSM, oncostatin M; TIMP-3, tissue inhibitor of metalloproteinase-3.

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fluid of osteoarthritis but was present in the fluid of RA patients (27). Its mRNA and protein were elevated in the RA synovial cells (28, 29). Injection of human OSM into goat joints promoted cartilage resorption and inhibited synthesis of proteoglycans (30), suggesting its implication in the pathogenesis of rheumatoid joint lesion. OSM induced TIMP-1 in human chondrocytes (31), TIMP-1 and MMP-13 in rat osteoblasts (32), and MMP-1 in human synovial fibroblasts (33). The mechanisms by which OSM regulates TIMPs and MMPs are poorly understood. We recently showed the involvement of tyrosine kinases in OSM induction of TIMP-3 in articular chondrocytes (34); however, precise signaling pathways have not been identified. Janus kinase (JAK)/STAT signaling pathway is activated by IFNs and IL-6-type cytokines (35–37). The ligand-receptor interaction brings assembly of cytokine receptors, receptor-associated JAKs that recruit and activate STAT proteins. Phosphorylated STATs then dimerize, translocate to the nucleus, and direct transcription of the specific target genes (36, 37). In the present study, we used two important chondrocyte model systems, namely, bovine and human primary articular chondrocyte, and showed induction of multiple signaling cascades and a crucial role of the JAK/STAT pathway in the OSM induction of TIMP-3 and MMP gene expression.

Materials and Methods

Materials

Cell culture supplies such as DMEM, FCS, Fungizone, and penicillin-streptomycin were purchased from Life Technologies (Burlington, Ontario, Canada). Collagenase type II and curcumin were obtained from Sigma (St. Louis, MO). JAK3 inhibitor, 4-(4′-Hydroxyphenyl)amino-6,7-dimethoxyquinazoline (38) and AG490, α-cyano-(3,4-dihydroxy) *N*-benzylcinnamide (39), were obtained from Calbiochem (San Diego, CA). Human recombinant OSM was purchased from R&D Systems (Minneapolis, MN). The anti-human STAT1, extracellular signal-regulated kinase (ERK) 1/2, dual-phosphospecific ERK1/2 (Thr^{202}/Tyr^{204}), p38 (Thr^{180}/Tyr^{182}), c-Jun N-terminal kinase (JNK; Thr^{183}/Tyr^{185}), and phosphospecific STAT1 (Tyr^{701}) rabbit polyclonal Abs were provided by New England Biolabs (Mississauga, Ontario, Canada). The anti-mouse JAK1, dual-phosphospecific JAK1 (Tyr^{1022}/Tyr^{1023}), JAK2 (Tyr^{1007}/Tyr^{1008}), and anti-human JAK3 rabbit polyclonal Ab was purchased from Quality Controlled Biochemicals (Hopkinton, MA). Anti-mouse JAK2 rabbit polyclonal and anti-phosphotyrosine mAb 4G10 were obtained from Upstate Biotechnology (Waltham MA). Agarosre-conjugated rabbit anti-human JAK3 and STAT1-binding consensus oligonucleotide were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Protein A-Sepharose beads were obtained from Pharmacia-Amersham Biotech (Piscataway, NJ). HRP-conjugated sheep anti-rabbit IgG and a chemiluminescence detection system was purchased from Roche Molecular Biochemicals (Laval, Quebec, Canada). The gel shift assay core system was purchased from Promega (Madison, WI). [α-^{32}P]CTP was obtained from DuPont-NEN (Boston, MA).

Primary cultures of chondrocytes

Normal bovine articular cartilage was obtained from the knee and hip joints of freshly slaughtered adult animals through a local slaughterhouse. Human cartilage was from the femoral heads of patients who underwent hip replacement surgery as a result of osteoarthritis or RA at the Notre-Dame Hospital. The cartilage with bones were dipped in 1% proviodine for sterilization and washed extensively with water. The slices of cartilage were dissected out, kept for 1 h at 4°C in PBS containing 5× penicillin-streptomycin and 5× Fungizone, and washed five times with large volumes of PBS. Chondrocytes were released from bovine cartilage by digestion with Pronase (1 mg/ml) for 1 h and collagenase type II (2 mg/ml) for 12 h in DMEM at 37°C. The cells were centrifuged and washed five times with PBS and plated at high density. The cells were first allowed to adhere to the plates in DMEM alone for 4 h and then supplemented with 10% serum for confluent growth (up to 6 days). These cells in primary and first-passage cultures maintain their phenotype by expressing chondrocyte-specific type II collagen. Before different treatments, cells were made quiescent in serum-free DMEM for 24 h. The potential inhibitors were added 30 min before the OSM.

RNA extraction and Northern blot analysis

Total RNA from primary cultures of chondrocytes was extracted using the acid guanidinium method (40), and 5-μg aliquots were analyzed by electrophoretic fractionation in 1.2% formaldehyde-agarose gels and transferred to Zeta-probe membranes (Bio-Rad, Richmond, CA). Northern blot analysis of RNA was performed with bovine TIMP-3 and human MMP-1, -3, and -13 cDNA probes. The bovine TIMP-3 plasmid and human MMP-3 plasmid were linearized with *Nar*I, human MMP-13 plasmid with *Eco*RI, and RNA probes were synthesized with T7 RNA polymerase according to the protocols of Promega Biotech. The human MMP-1 (clone pSP64) was digested with *Pst*I and a probe was synthesized using T3 RNA polymerase. The human 28S ribosomal RNA plasmid (American Type Culture Collection, Manassas, VA) was cut by *Xba*I and the RNA probe was synthesized with T7 RNA polymerase. All probes were labeled to high-specific activity (1×10^8 cpm/μg) with [α-^{32}P]CTP (3000 Ci/mmol) with the RNA labeling kit from Promega Biotech according to their protocols.

Western blot analysis and immunoprecipitations

After treatment with inhibitors and OSM for various time periods, total cellular protein extracts were prepared from bovine and human chondrocytes as described before (34). Cells were lysed in lysis buffer (62.5 mM Tris-HCl (pH 6.8), 10% glycerol, 50 mM DTT, and 1% Triton X-100). Cell lysates were resolved on SDS-PAGE gels, transferred to nitrocellulose membranes by electroblotting, and reacted with phosphorylation-state-specific Abs at concentrations suggested by the manufacturers. Proteins were visualized by HRP-conjugated sheep anti-rabbit IgG and a chemiluminescence detection system. The membranes were stripped with buffer containing 100 mM 2-ME, 2% SDS, and 62.5 mM Tris-HCl (pH 6.8) at 55°C for 40 min and reprobed with total Abs.

For immunoprecipitations, chondrocytes were exposed to OSM alone or with curcumin, rinsed twice with ice-cold PBS, and then lysed in RIPA buffer (50 mM Tris-HCl (pH 7.4), 1% Nonidet P-40, 0.25% sodium deoxycholate, 150 mM NaCl, 1 mM EDTA, 1 mM Na_3VO_4, 1 mM NaF, 1 mM PMSF, and 1 μg/ml each of aprotinin, leupeptin, and pepstatin). Cell lysates were precleared with protein A-Sepharose beads for 30 min at 4°C. After removal of protein A-Sepharose, cell supernatants (400 μg of total proteins) were incubated with agarose-conjugated rabbit anti-human JAK3 Ab for 3 h at 4°C. The immune complexes were washed four times with cold RIPA buffer and the immunoprecipitated proteins were eluted into SDS-PAGE sample buffer (2% SDS, 62.5 mM Tris-HCl (pH 6.8), 50 mM DTT, and 10% glycerol) by heating at 100°C for 5 min, fractionated by 8% SDS-PAGE, and probed with anti-phosphotyrosine Ab (4G10) by immunoblotting. After stripping, these membranes were reprobed for total JAK3.

EMSA

Nuclear extracts were prepared according to details in Ref. 41, with some modifications. After exposure to inhibitors and OSM, chondrocytes were washed with cold PBS and scraped with 1.5 ml of cold PBS. Cells were centrifuged for 10 s and resuspended in 400 μl of cold buffer A (10 mM HEPES-KOH (pH 7.9), 1.5 mM $MgCl_2$, 10 mM KCl, 0.5 mM DTT, 0.1% Nonidet P-40, 1 mM PMSF, 10 μg/ml aprotinin, and 10 μg/ml leupeptin). The cells were allowed to swell on ice for 10 min and then vortexed for 10 s. Samples were centrifuged for 10 s and the supernatant was discarded. The nuclear pellets were resuspended in 50 μl of cold buffer B (20 mM HEPES-KOH (pH 7.9), 25% glycerol, 420 mM NaCl, 1.5 mM $MgCl_2$, 0.2 mM EDTA, 0.5 mM DTT, 0.5 mM PMSF, 10 μg/ml aprotinin, and 10 μg/ml leupeptin) and kept on ice for 30 min for high salt extraction. Cellular debris was removed by centrifugation for 20 min and supernatant fraction (nuclear extract) was stored at −70°C. EMSA were performed using a gel shift assay core system as described in Promega protocols. Two micrograms of nuclear proteins was mixed with ^{32}P-end-labeled dsSTAT1 (p84/p91)-binding consensus (underlined) oligonucleotide, 5′-CATGT TATGCATATTCCTGTAAGTG-3′, at room temperature for 20 min. The DNA-protein complex formed was separated from free oligonucleotides on 4.5% native polyacrylamide gel. A mutated STAT1-binding consensus oligonucleotide, 5′-CATGTTATGCATATTGGAGTAAGTG-3′, was used to examine the specificity of binding of STAT1 to the DNA. The specificity of binding was also examined by competition with the excessive unlabeled oligonucleotide.

Results

OSM induces MMP and TIMP-3 mRNAs in bovine and human chondrocytes

To examine whether chondrocytes from normal bovine and human arthritic femoral head cartilage respond to OSM, primary cultures

of bovine chondrocytes kept in serum-deprived medium for 24 h were subjected to 18-h treatment with OSM at different concentrations (0.1–50 ng/ml). In agreement with our earlier results (34), Northern blot hybridization analysis of RNA with the bovine TIMP-3 and human MMP-1, -3, and -13 probes, respectively, revealed a dose-dependent (at 10–50 ng/ml) induction of TIMP-3, MMP-1, -3, and -13 mRNAs by OSM (Fig. 1*A*). Analysis of chondrocytes from different patients revealed that MMP-1, -3, and -13 messages were also induced by the effective dose of 10 ng/ml OSM in human cells (Fig. 1*B*, also see Figs. 5*B* and 8*B*). The levels of 28S rRNA levels were even. This dose was used in all of the subsequent parts of the study.

OSM time-dependently induces activation of JAK/STAT, ERK, p38, and JNK pathways

To investigate the mechanisms of OSM signaling in chondrocytes, OSM-treated cells were analyzed for activation of JAK1, JAK2 JAK3, and STAT1. Quiescent bovine and human chondrocytes were exposed to 10 ng/ml OSM for different time periods (5–60 min), and protein extracts were analyzed by Western immunoblotting with phosphospecific Abs or immunoprecipitation with JAK-3 Ab followed by probing with anti-phosphotyrosine Ab. OSM-stimulated phosphorylation of JAK1, JAK2, JAK3, and STAT1 occurred within 5 min, peaked between 15 and 30 min, and progressively declined to different extents in bovine and human chondrocytes. JAK2 activation was more sustained in bovine chondrocytes, whereas bovine JAK3 could not be analyzed due to its nonreactivity by immunoprecipitation (Fig. 2). Similar analysis of the three subclasses of mitogen-activated protein kinases (MAPKs) revealed activation of ERKs, p38, and JNKs between 5 and 30 min that declined by 60 min (Fig. 3). The levels of respective total proteins remained generally unaffected (*lower panels* of each blot).

JAK3 inhibitor blocks OSM-induced STAT1 phosphorylation as well as TIMP-3 and MMP RNA expression

A pharmacologic JAK3 inhibitor specifically blocks its activation (38) while AG490 is a JAK2-specific inhibitor (39). To gain insight into the mechanism of OSM signaling leading to TIMP-3 and MMP RNA up-regulation, cells were exposed to OSM alone or in combination with the specific JAK3 inhibitor or JAK2 inhibitor AG490. Total cellular protein extracts prepared after 15 min of OSM treatment were analyzed for STAT1 and ERK activation. As shown by Western blotting, JAK3 inhibitor dose-dependently reduced OSM-induced phosphorylation of STAT1, but had no effect on ERK activation (Fig. 4). AG490 had no impact on OSM-induced activation of STAT1 and ERKs (Fig. 4). The levels of STAT1 and ERKs detected by total Abs remained constant and were not affected by the treatments of chondrocytes from both

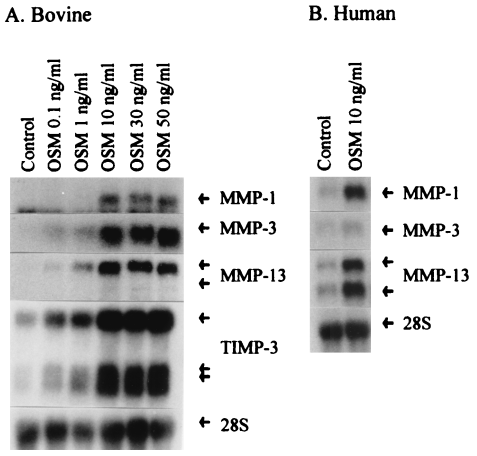

FIGURE 1. Induction of MMP and TIMP-3 RNAs by OSM in bovine and human chondrocytes. Primary cultures of bovine (*A*) or human (*B*) chondrocytes were maintained in serum-free DMEM for 24 h and then exposed to the indicated doses of OSM for 18 h. The RNA blots were hybridized sequentially with human MMP-1, -3, and -13 and bovine TIMP-3 probes. Control lane, chondrocytes treated with OSM vehicle only. Different transcripts (two for MMP-13 and three for TIMP-3) are indicated by arrows. The 28S rRNA serving as loading control is shown at the bottom.

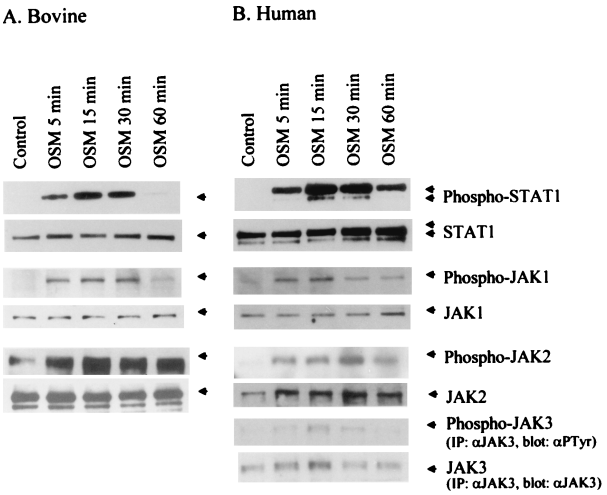

FIGURE 2. Stimulation of JAK1, JAK2, JAK3, and STAT1 phosphorylation by OSM in bovine and human chondrocytes. Quiescent chondrocytes were exposed to 10 ng/ml OSM for the indicated time periods. Cellular extracts (10 μg) were subjected to immunoblotting with phosphospecific JAK1, JAK2, and STAT1 Abs; the respective bands were detected by chemiluminescence and identified by their sizes. Phospho-JAK3 was detected by immunoprecipitation (IP) followed by Western blotting with anti-phosphotyrosine (anti-PTyr) Ab. The blots were stripped and reprobed with regular Abs which detected the total amount of the respective proteins.

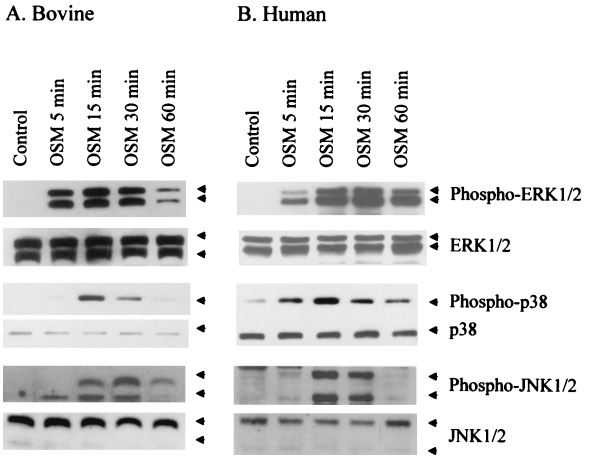

FIGURE 3. Stimulation of ERK1/2, p38, and JNK phosphorylation by OSM in bovine and human chondrocytes. Quiescent chondrocytes were treated with 10 ng/ml OSM for the indicated time periods. Cellular extracts (10 μg) were subjected to immunoblotting with phosphorylation-state-specific ERK1/2, p38, and JNK1/2 Abs; the respective bands were detected by chemiluminescence and identified by their sizes. The blots were stripped and reprobed with regular Abs which detected total amount of the respective proteins.

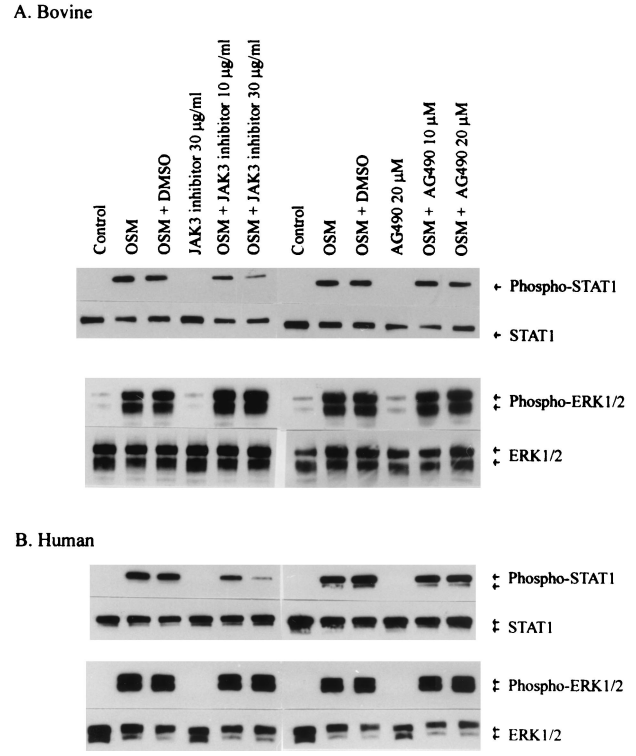

FIGURE 4. Effects of JAK3 inhibitor and AG490 on OSM-stimulated STAT1 and ERK1/2 phosphorylation in bovine and human chondrocytes. Cells maintained in the serum-free medium for 24 h were pretreated with DMSO (vehicle for the inhibitors), JAK3 inhibitor (10–30 μg/ml), or AG490 (10–20 μM) for 30 min followed by OSM (10 ng/ml) treatment for 15 min. Cell lysates were analyzed by Western blotting with phosphospecific STAT1 and ERK1/2 Abs (*upper panels*). The membranes were stripped and reprobed with total Abs (*lower panels*). The resulting bands are shown.

species. For TIMP-3 and MMP RNA analysis, total RNA was extracted from chondrocytes after treatment with OSM or OSM and the inhibitors for 20 h and probed by Northern blot hybridization. As shown in Fig. 5, JAK3 inhibitor dose-dependently suppressed the OSM-induced MMP-1, -3, and -13 gene expression and AG490 had no effect. Basal levels of TIMP-3 message were elevated; nevertheless, its induction by OSM was similarly inhibited by the JAK3 inhibitor. The 28S rRNA levels were unaffected. These results are in conformity with those observed for STAT1 activation in Fig. 4 and suggest JAK3 as a key signaling mediator for TIMP-3/MMP induction by OSM. As no JAK1-specific inhibitor is currently available, its role could not be investigated.

Curcumin inhibits OSM-stimulated phosphorylation of STAT1 and JNK but has no impact on JAK1, JAK2, JAK3, ERK, and p38 activation

To examine the effect of an anti-inflammatory agent, curcumin (see *Discussion*), on OSM-stimulated JAK/STAT and MAPK pathways, protein extracts from OSM- and curcumin-treated human and bovine chondrocytes were analyzed for activation of JAK1, JAK2, JAK3, and STAT1 as well as ERK, p38, and JNK MAPKs. Different concentrations of curcumin had no effect on the OSM-induced phosphorylation of ERKs, p38, JAK1, JAK2, and JAK3 (Fig. 6); however, phosphorylation of JNKs and STAT1 was suppressed in a dose-dependent fashion. The 15 μM dose of curcumin effectively inhibited most of the response to OSM. The levels of respective total proteins were even. Thus, curcumin mimics the effects of JAK3 inhibitor and is a potent novel inhibitor of the JAK/STAT signaling pathway.

JAK3 inhibitor and curcumin down-regulate DNA-binding activity of STAT1

To determine if by inhibiting OSM-stimulated STAT1 phosphorylation the JAK3 inhibitor and curcumin affected the DNA-binding capacity of STAT1, chondrocytes were preincubated for 30 min with curcumin followed by treatment with OSM for 25 min. Nuclear proteins extracts were then examined by EMSA for the STAT1 DNA-binding activity. Curcumin, by itself, had no effect on DNA-binding capacity of STAT1, but at concentrations of 5–15 μM, dose-dependently down-regulated OSM-induced DNA binding of STAT1. At a 15 μM concentration, curcumin completely abolished OSM-activated STAT1 binding to DNA both in bovine and human cells (Fig. 7). OSM-stimulated binding of STAT1 to its target DNA was also blocked by the specific JAK3 inhibitor but

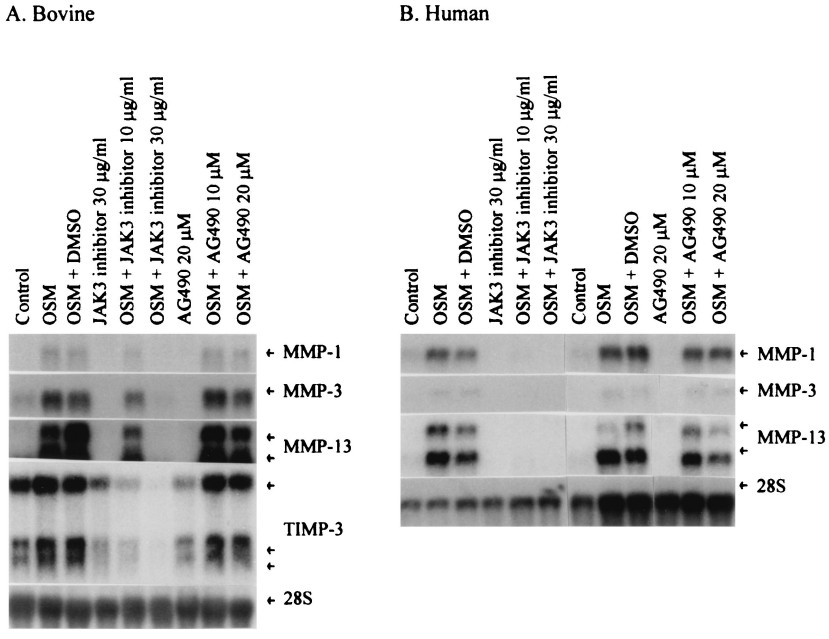

FIGURE 5. Impact of JAK3 inhibitor and AG490 on OSM-induced TIMP-3 and MMP gene expression in chondrocytes. Serum-starved chondrocytes were pretreated with DMSO (inhibitor vehicle), JAK3 inhibitor (10–30 μg/ml), or AG490 (10–20 μM) for 30 min, followed by additional treatment with 10 ng/ml OSM for 20 h. MMP-1, -3, -13, and TIMP-3 RNA were analyzed by Northern blotting. Different transcripts are indicated by arrows and the control 28S rRNA expression levels are shown at the bottom.

not by the JAK2 inhibitor AG490 (Fig. 7*A*), suggesting that JAK3 may be mediating STAT1 activation.

Curcumin down-regulates OSM-enhanced TIMP-3 and MMP gene expression

To evaluate whether proximal inhibition of STAT1 phosphorylation and DNA binding by curcumin affects OSM-induced TIMP-3 and MMP mRNA expression, chondrocytes were pretreated with curcumin for 30 min followed by OSM treatment for 20 h, and total RNA was subjected to Northern blot analysis. Curcumin, at a concentration of 15 μM, completely (bovine chondrocytes) or partially (human chondrocytes) inhibited MMP-1, -3, -13, and TIMP-3 induction by OSM without affecting 28S rRNA levels (Fig. 8). Thus, curcumin abolished the enhancement of TIMP-3 and MMP expression by OSM in a manner similar to that observed for the JAK3 inhibitor (Fig. 5).

Discussion

We show here for the first time that OSM activates JAK/STAT and MAPK pathways in primary human and bovine chondrocyte model systems. Furthermore, specific blockage of STAT1 phosphorylation either by curcumin or by upstream JAK3 inhibition results in the down-regulation of MMP and TIMP-3 gene induction by OSM. These results strongly suggest that JAK/STAT and MAPK (ERK, p38, and JNK) pathways (Ref. 34 and this study) are essential for OSM signaling responses including MMP/TIMP-3 RNA induction, which can be blocked by the specific inhibitors.

Chondrocytes have an essential role in regulating the MMP-TIMP balance and their primary function to maintain cartilage ECM integrity is significantly altered in arthritis due to changed composition of growth factors and cytokines (1, 42). The OSM-stimulated increase of all of the major MMPs implicated in cartilage degradation (MMP-1, -3, and -13) in both human and bovine chondrocytes suggests common signaling mechanisms and supports a catabolic role for OSM (29, 30, 33, 34). OSM further enhances IL-1-induced MMP-13 in human chondrosarcoma cells (43) and collagen degradation in bovine nasal cartilage (29). Induction of MMPs in chondrocytes differs from human synovial fibroblasts where MMP-1, but not MMP-3, was induced by OSM (33), suggesting cell type-specific regulation. MMP-1 and -3 induction by IL-1 and TNF-α was also enhanced by OSM in astrocytes (44). Intriguingly, TIMP-3 induction by OSM in bovine but not in human chondrocytes may be due to differences in the TIMP-3 promoter composition of two species rather than lack of OSM receptor, as human chondrocytes do respond to OSM by inducing MMPs. Induction of MMPs by OSM and noninduction of TIMP-3 could tip the enzyme-inhibitor balance in favor of degradation. In synovial fibroblasts, OSM inhibited the basal and IL-1-induced TIMP-3 RNA (45). TIMP-3 was also not induced by OSM in human astrocytes and dermal fibroblasts (44). OSM and related cytokines, IL-6 and IL-11, do induce TIMP-1 in human chondrocytes and in synovial/lung fibroblasts (31, 46–48). OSM, IL-6, and LIF expressed in cartilage and rheumatoid synovium (27, 28) could contribute to the pathogenesis of arthritis by altering TIMP-1, TIMP-3, and MMP gene expression.

The IL-6-type cytokines, including OSM, are known to induce the JAK/STAT signaling cascade in other systems (35), but this has not been shown in chondrocytes. Our demonstration of OSM-triggered phosphorylation of JAK1, JAK2, JAK3, and STAT1 in primary human femoral head chondrocytes suggests that these cells could be a source of activated STAT1 and STAT3 found in the synovial fluid and fibroblasts of patients with RA (49, 50). The JAKs family consists of JAK1, JAK2, JAK3, and Tyk2 (51). In osteoblasts, OSM induced a rapid but transient tyrosine phosphorylation of JAK1, JAK2, and Tyk2 (52). Blockade of OSM-activated STAT1 tyrosine phosphorylation by JAK3, but not by the JAK2-specific inhibitor in our study, suggests that JAK3 (and possibly JAK1) and not JAK2 may be mediating STAT1 activation and OSM signal transduction in chondrocytes. This notion was further supported by the abolishment of downstream STAT1 activation, its DNA-binding activity, and TIMP-3 and MMP RNA induction by the JAK3 and not by the JAK2 inhibitor. These results suggest the pivotal role of the JAK/STAT pathway in OSM signaling leading to TIMP-3 and MMP expression. Activation of JAKs is followed by phosphorylation of the STAT family whose homo- or heterodimers in turn are translocated to the nucleus to activate their target genes (35–37) such as TIMPs and MMPs. Although this is the first report for induction of JAK/STAT pathway in chondrocytes, STAT1 DNA-binding activity induced by OSM was previously shown in synovial lining cells where OSM inhibits IL-1-induced TIMP-3 expression (45). Maximal activation of the transcriptional capacity of STAT1 also required serine phosphorylation (53, 54) by ERK2 (55). In human primary astrocytes, Raf/ERK and JAK/STAT pathways acted synergistically to achieve maximal induction of MMP-1 expression driven by OSM (44). This is consistent with the activation of all three subclasses of MAPKs in this study and previously shown inhibition of OSM-induced TIMP-3 and MMP-1, -3, and -13 in chondrocytes by the

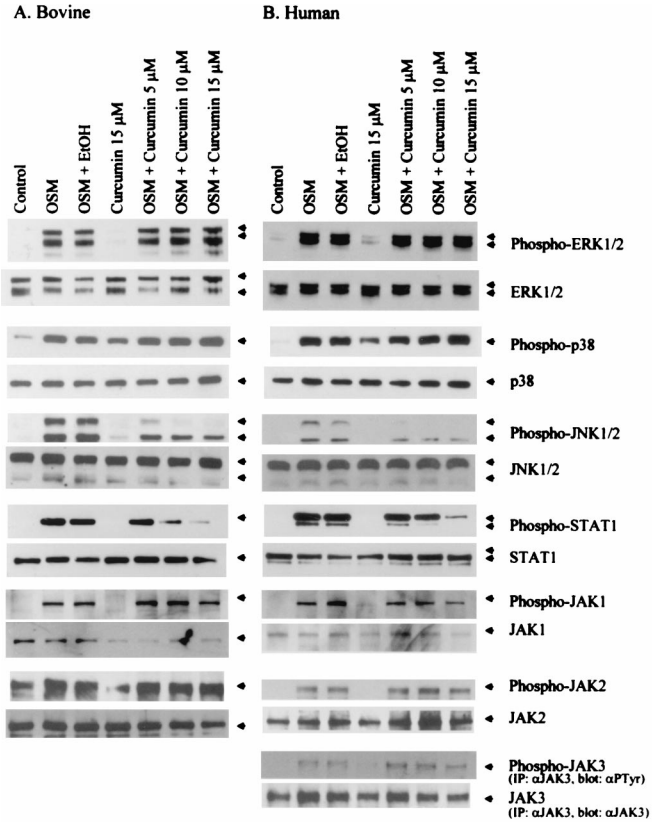

FIGURE 6. Inhibition of OSM-induced STAT1 and JNK phosphorylation by curcumin in chondrocytes. Quiescent bovine (*left panel*) or human (*right panel*) chondrocytes were exposed to ethanol (EtOH; vehicle for curcumin) or curcumin (5–15 μM) for 30 min followed by OSM (10 ng/ml) treatment for 15 min. Phospho-ERK1/2, p38, JNK, JAK1, JAK2, and STAT1 levels were measured by Western blotting. Phospho-JAK3 levels were measured by immunoprecipitation (IP) with total Ab followed by immunoblotting with anti-phosphotyrosine (anti-PTyr) Ab. Total ERK, p38, JNK, JAK1, JAK2, JAK3, and STAT1 levels are shown in the respective *lower panels*.

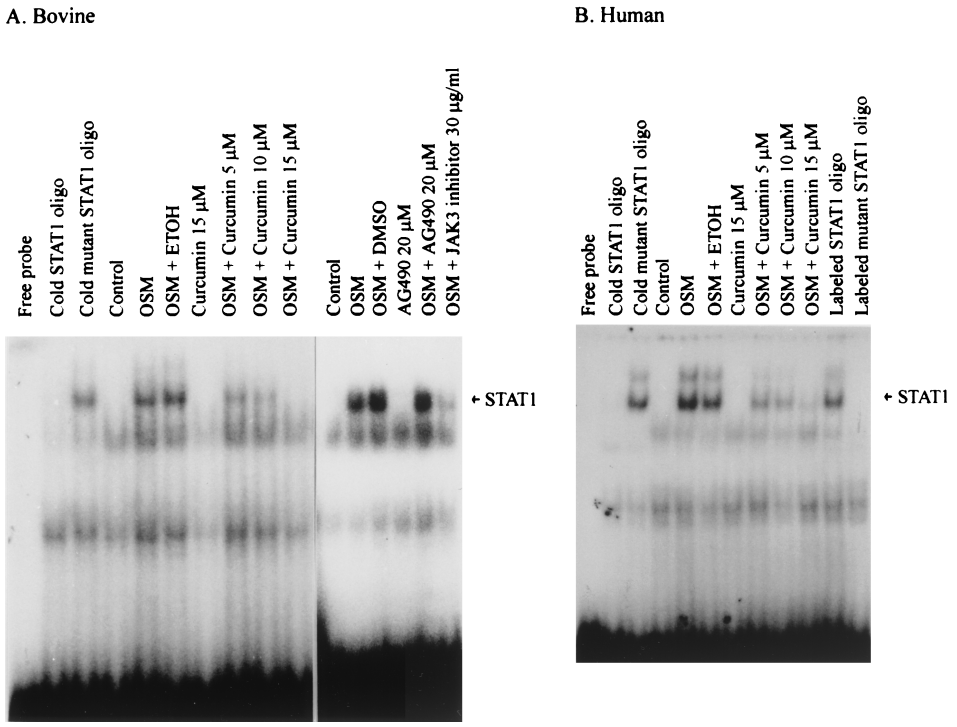

FIGURE 7. Suppression of OSM-enhanced STAT1 DNA-binding activity by curcumin. Primary bovine or human chondrocytes were pretreated with ethanol (curcumin vehicle; control) or curcumin (5–15 μM) for 30 min and then exposed to 10 ng/ml OSM for 25 min. Nuclear extracts were prepared and STAT1 DNA-binding activities were analyzed by EMSA. Free probe lane denotes ^{32}P-labeled STAT1-binding oligonucleotide without nuclear extract; cold STAT1 oligo, nuclear extracts from OSM-treated cells incubated with ^{32}P-labeled STAT1-binding oligonucleotide plus 100-fold excess of unlabeled STAT1-binding oligonucleotide (competitor); cold mutant STAT1 oligo, nuclear extracts from OSM-treated cells incubated with ^{32}P-labeled STAT1-binding oligonucleotide plus 100-fold excess of unlabeled mutant STAT1-binding oligonucleotide (noncompetitor); labeled STAT1 oligo, nuclear extracts from OSM-treated cells incubated with ^{32}P-labeled STAT1-binding oligonucleotide; labeled mutant STAT1 oligo, nuclear extracts from OSM-treated cells incubated with ^{32}P-labeled mutant STAT1-binding oligonucleotide. The other lanes indicate the nuclear protein extracts from different treatments incubated with ^{32}P-labeled STAT1-binding oligonucleotide.

specific MAPK/ERK kinase (kinases upstream of ERKs) inhibitor PD98059 (Ref. 34 and our unpublished data). Together, these results strongly support the requirement of JAK/STAT, ERK, p38, and JNK pathways and possible interaction of these cascades in the OSM-enhanced TIMP-3 and MMP gene expression.

Curcumin (diferuloylmethane), a dietary pigment from *Curcuma longa*, gives unique flavor and yellow color to curry and is a pharmacologically safe compound. Its anticarcinogenic activities are due to inhibition of tumor initiation and promotion by phorbol ester (56, 57). Curcumin inhibits JNK, c-Jun, and NF-κB activation by PMA or TNF-α treatment (58–61) and has strong anti-inflammatory and antioxidant properties (62). These therapeutic effects are believed to be due to its abilities to block MAPK kinase kinase in the JNK signaling pathway in Jurkat T cells, c-Jun expression in 3T3 cells, and IκB kinase in the NF-κB activation pathway induced by diverse agents (58–62). In agreement with these studies (58, 59), curcumin also effectively inhibits JNK activation in human and bovine chondrocytes. Here, we show for the first time that curcumin also interferes with the JAK/STAT signaling pathway specifically by inhibition of OSM-induced STAT1 Tyr^{701} phosphorylation in chondrocytes, without blocking ERK, p38, JAK1, JAK2, and JAK3 activation. The Raf/ERK pathway activation by IFN and OSM was shown to be JAK1 dependent (63). Since curcumin did not inhibit several of the OSM-induced activations, it displayed a considerable degree of selectivity. STAT1 phosphorylation and homodimerization leads to binding with its cognate DNA in the promoters of the target genes (36, 51, 53). EMSA experiments showed that indeed curcumin also prevented the binding of STAT1 to its target DNA sequence. Cur-

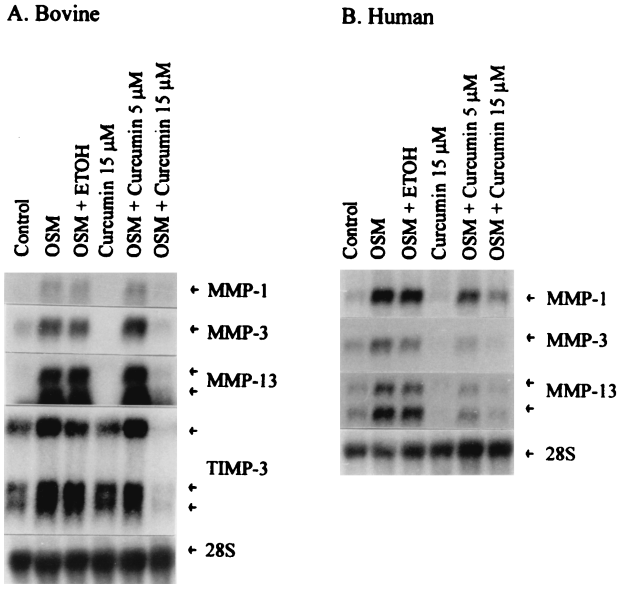

FIGURE 8. Inhibition of OSM-induced MMP and TIMP-3 gene expression by curcumin in chondrocytes. Bovine or human primary chondrocytes were preincubated with ethanol (ETOH; curcumin vehicle) or curcumin (5–15 μM) for 30 min followed by additional incubation with OSM (10 ng/ml) for 20 h. MMP and TIMP-3 RNA levels were measured by Northern blot hybridization analysis. The control 28S rRNA levels are shown at the bottom.

cumin mimicked the effects of the specific JAK3 inhibitor, which also blocked OSM-activated STAT1 phosphorylation and its DNA-binding activity. In contrast, AG490, which does not block STAT1 phosphorylation, failed to inhibit STAT1 DNA binding. Thus, our study demonstrated that OSM-stimulated JAK3-mediated Tyr^{701} phosphorylation of STAT1 plays a crucial role in the transduction of this signal and curcumin has a hitherto unknown and novel activity of specifically inhibiting STAT1 activation.

Binding of STAT1 homodimers to its cognate DNA element usually results in transcriptional up-regulation of the target genes (36, 51–53). Interestingly, by inhibiting upstream STAT1 phosphorylation and by preventing its ability to bind DNA, curcumin suppressed downstream TIMP-3, MMP-1, MMP-3, and MMP-13 gene up-regulation by OSM in chondrocytes. These results along with the analogous inhibition of TIMP-3 and MMPs by the JAK3 inhibitor further support curcumin as an effective inhibitor of the JAK/STAT pathway and implicate this pathway in the induction of these genes by OSM. The most likely target sequence of JAK/STAT signaling is the OSM-responsive element which encompasses STAT and AP-1 binding sites in the human MMP-1 and TIMP-1 and rat TIMP-1 gene promoters. For achieving maximal response by OSM, cooperation and cross-talk between STAT and AP-1 elements and the respective signaling cascades are needed via ERK2-MAPK-mediated STAT1 phosphorylation at Ser^{727} (44, 64). Consistent with the AP-1 involvement, OSM transiently increases early response genes such as c-*fos* which cooperates with the Ets and Sp1 factors to maximally induce transcription from the murine TIMP-1 promoter in hepatoma cells (65). Similar sequences may be present in the bovine and human MMP-3 and MMP-13 and possibly bovine TIMP-3 promoters which remain to be characterized. The human MMP-3 and MMP-13 promoters do contain AP-1 and Ets binding, PEA3 elements in close proximity (2), and PEA3 has sequence similarity with STAT binding sites (65). The human TIMP-3 promoter contains several Sp1 binding sites but no obvious STAT1 or STAT3 binding sites (45), which is consistent with lack of its induction by OSM in human chondrocytes. Abrogation of all of the OSM target genes, MMP-1, MMP-3, MMP-13, and TIMP-3 by the JAK3 inhibitor and curcumin suggest that common signaling or regulatory steps may be impaired, which seems to be STAT1 phosphorylation in the JAK/STAT and JNK activation in the MAPK pathway.

The ability of curcumin (and JAK3 inhibitor) to block OSM-induced JAK/STAT and JNK pathways and MMPs may have potentially several therapeutic applications. In arthritis, along with its known capacity to inhibit AP-1 and NF-κB transcription factors, curcumin may prevent inflammation and protect cartilage from the OSM-, IL-1-, and TNF-α -stimulated degradation by MMPs. Furthermore, inhibition of fibroblast growth factor 2-induced angiogenesis (a process where MMPs play an important role) and MMP-9 by curcuminoids (66) can have therapeutic benefits in RA and growth of tumors. Indeed, curcumin inhibits cancer cell metastasis by inhibiting MMP-9 (67). Curcumin can also be useful for blocking OSM-stimulated proinflammatory responses in endothelial cells (68) and promotion of vascular smooth muscle cell proliferation, the key events in atherosclerosis.

In summary, we demonstrated that OSM activates JAK/STAT and MAPK (ERK, p38, and JNK) signaling pathways in primary chondrocytes, leading to induction of MMPs and TIMP-3 genes. Blocking STAT1 phosphorylation either by curcumin or by JAK3 inhibition results in abrogation of OSM-responsive MMP-1, MMP-3, MMP-13, and TIMP-3 genes. Therefore, JAK/STAT and MAPK pathways are essential in the transduction of OSM signal and regulation of MMP and TIMP-3 genes. The catabolic responses of OSM such as promotion of cartilage degradation in arthritis could possibly be blocked by the inhibitors of JAK/STAT and MAPK signaling cascades such as JAK3 inhibitor and curcumin.

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References

1. Poole, A. R. 1994. Immunological markers of joint inflammation, skeletal damage and repair: where are we now? *Ann. Rheum. Dis. 53:3.*
2. Westermarck, J., and V-M. Kähäri. 1999. Regulation of matrix metalloproteinase expression in tumor invasion. *FASEB J. 13:781.*
3. Nagase, H., and J. F. Woessner, Jr. 1999. Matrix metalloproteinases. *J. Biol. Chem. 274:21491.*
4. Knauper, V., C. Lopez-Otín, B. Smith, G. Knight, and G. Murphy. 1996. Biochemical characterization of human collagenase-3. *J. Biol. Chem. 271:1544.*
5. Mitchell, P. G., H. A. Magna, L. M. Reeves, L. L. Lopresti-Morrow, S. A. Yocum, P. J. Rosner, K. F. Geoghegan, and, J. E. Hambor. 1996. Cloning, expression, and type II collagenolytic activity of matrix metalloproteinase-13 from human osteoarthritic cartilage. *J. Clin. Invest. 97:761.*
6. Mengshol, J. A., M. P. Vincenti, C. I. Coon, A. Barchowsky, and C. E. Brinckerhoff. 2000. Interleukin-1 induction of collagenase 3 (matrix metalloproteinase 13) gene expression in chondrocytes requires p38, c-Jun N-terminal kinase and nuclear factor κB: differential regulation of collagenase 1 and collagenase 3. *Arthritis Rheum. 43:801.*
7. Borden, P., D. Solymar, A. Sucharczuk, B. Lindman, P. Cannon, and R. A. Heller. 1996. Cytokine control of interstitial collagenase and collagenase-3 gene expression in human chondrocytes. *J. Biol. Chem. 271:23577.*
8. Lark, M. W., E. K. Bayne, J. Flanagan, C. F. Harper, L. A. Hoerrner, N. I. Hutchinson, I. I. Singer, S. A. Donatelli, J. R. Weidner, H. R. Williams, et al. 1997. Aggrecan degradation in human cartilage: evidence for both matrix metalloproteinase and aggrecanase activity in normal, osteoarthritic, and rheumatoid joints. *J. Clin. Invest. 100:93.*
9. Tetlow, L. C., and D. E. Woolley. 1998. Comparative immunolocalization studies of collagenase 1 and collagenase 3 production in the rheumatoid lesion, and by human chondrocytes and synoviocytes in vitro. *Br. J. Rheumatol. 37:64.*
10. Lindy, O., Y. T. Konttinen, T. Sorsa, Y. Ding, S. Santavirta, A. Ceponis, and C. Lopez-Otín. 1997. Matrix metalloproteinase 13 (collagenase 3) in human rheumatoid synovium. *Arthritis Rheum. 40:1391.*
11. Brew, K., D. Dinakarpandian, and H. Nagase. 2000. Tissue inhibitors of metalloproteinases: evolution, structure and function. *Biochim. Biophys. Acta 1477: 267.*
12. Vincenti, M. P., I. M. Clark, and C. E. Brinckerhoff. 1994. Using inhibitors of metalloproteinases to treat arthritis: easier said than done? *Arthritis Rheum. 37: 1115.*
13. Gomez, D. E., D. F. Alonso, H. Yoshiji, and U. P. Thorgeirsson. 1997. Tissue inhibitors of metalloproteinases: structure, regulation and biological functions. *Eur. J. Cell Biol. 74:111.*
14. Pavloff, N., P. W. Staskus, N. S. Kishnani, and S. P. Hawkes. 1992. A new inhibitor of metalloproteinases from chicken: ChIMP-3: a third member of the TIMP family. *J. Biol. Chem. 267:17321.*
15. Leco, K. J., R. Khokha, N. Pavloff, S. P. Hawkes, and D. R. Edwards. 1994. Tissue inhibitor of metalloproteinases-3 (TIMP-3) is an extracellular matrix-associated protein with a distinctive pattern of expression in mouse cells and tissues. *J. Biol. Chem. 269:9352.*
16. Apte, S. S., M. G. Mattei, and B. R. Olsen. 1994. Cloning of the cDNA encoding human tissue inhibitor of metalloproteinases-3 (TIMP-3) and mapping of the TIMP3 gene to chromosome 22. *Genomics 19:86.*
17. Silbiger, S. M., V. L. Jacobsen, R. L. Cupples, and R. A. Koski. 1994. Cloning of cDNAs encoding human TIMP-3, a novel member of the tissue inhibitor of metalloproteinase family. *Gene 141:293.*
18. Uría, J. A., A. A. Ferrando, G. Velasco, J. M. P. Freije, and C. Lopez-Otín. 1994. Structure and expression in breast tumors of human TIMP-3, a new member of the metalloproteinase inhibitor family. *Cancer Res. 54:2091.*
19. Anande-Apte, B., L. Bao, R. Smith, K. Iwata, B. R. Olsen, B. Zetter, and S. S. Apte. 1996. A review of tissue inhibitor of metalloproteinases-3 (TIMP-3) and experimental analysis of its effect on primary tumor growth. *Biochem. Cell Biol. 74:853.*
20. Ahonen, M., A. H. Baker, and V.-M. Kähäri. 1998. Adenovirus-mediated gene delivery of tissue inhibitor of metalloproteinases-3 inhibits invasion and induces apoptosis in melanoma cells. *Cancer Res. 58:2310.*
21. Apte, S. S., B. R. Olsen, and G. Murphy. 1995. The gene structure of tissue inhibitor of metalloproteinases (TIMP)-3 and its inhibitory activities define the distinct TIMP gene family. *J. Biol. Chem. 270:14313.*
22. Su, S., J. Grover, P. J. Roughley, J. A. DiBattista, J.-M. Martel-Pelletier, J.-P. Pelletier, and M. Zafarullah. 1999. Expression of the tissue inhibitor of metalloproteinases (TIMP) gene family in normal and osteoarthritic joints. *Rheumatol. Int. 18:183.*
23. Zarling, J. M., M. Shoyab, H. Marquardt, M. B. Hanson, M. N. Lioubin, and G. J. Todaro. 1986. Oncostatin M: a growth regulator produced by differentiated histiocyte lymphoma cells. *Proc. Natl. Acad. Sci. USA 83:9739.*

24. Rose, T. M., D. M. Weiford, N. L. Gunderson, and A. G. Bruce. 1994. Oncostatin M (OSM) inhibits the differentiation of pluripotent embryonic stem cells in vitro. *Cytokine 6:48.*
25. Zhang, X. G., J. J. Gu, Z. Y. Lu, K. Yasukawa, G. D. Yancopoulos, K. Turner, M. Shoyab, T. Taga, T. Kishimoto, R. Bataille, et al. 1994. Ciliary neurotropic factor, interleukin 11, leukemia inhibitory factor, and oncostatin M are growth factors for human myeloma cell lines using the interleukin 6 signal transducer gp130. *J. Exp. Med. 179:1337.*
26. Wallace, P. M., J. F. MacMaster, K. A. Rouleau, T. J. Brown, J. K. Loy, K. L. Donaldson, and A. F. Wahl. 1999. Regulation of inflammatory responses by oncostatin M. *J. Immunol. 162:5547.*
27. Hui, W., M. Bell, and G. Carroll. 1997. Detection of oncostatin M in synovial fluid from patients with rheumatoid arthritis. *Ann. Rheum. Dis. 56:184.*
28. Okamoto, H., M. Yamamura, Y. Morita, S. Harada, H. Makino, and Z. Ota. 1997. The synovial expression and serum levels of interleukin-6, interleukin-11, leukemia inhibitory factor, and oncostatin M in rheumatoid arthritis. *Arthritis Rheum. 40:1096.*
29. Cawston, T. E., V. A. Curry, C. A. Summers, I. M. Clark, G. P. Riley, P. F. Life, J. R. Spaull, M. B. Goldring, P. J. Koshy, A. D. Rowan, and W. D. Shingleton. 1998. The role of oncostatin M in animal and human connective tissue collagen turnover and its localization within the rheumatoid joint. *Arthritis Rheum. 41: 1760.*
30. Bell, M. C., G. J. Carroll, H. M. Chapman, J. N. Mills, and W. Hui. 1999. Oncostatin M induces leukocyte infiltration and cartilage proteoglycan degradation in vivo in goat joints. *Arthritis Rheum. 42:2543.*
31. Nemoto, O., H. Yamada, M. Mukaida, and M. Shimmei. 1996. Stimulation of TIMP-1 production by oncostatin M in human articular cartilage. *Arthritis Rheum. 39:560.*
32. Varghese, S., K. Yu, and E. Canalis. 1999. Leukemia inhibitory factor and oncostatin M stimulate collagenase-3 expression in osteoblasts. *Am. J. Physiol. 276:E465.*
33. Langdon, C., J. Leith, F. Smith, and C. D. Richards. 1997. Oncostatin M stimulates monocyte chemoattractant protein-1 and interleukin-1-induced matrix metalloproteinase-1 production by human synovial fibroblasts in vitro. *Arthritis Rheum. 40:2139.*
34. Li, W. Q., and M. Zafarullah. 1998. Oncostatin M up-regulates tissue inhibitor of metalloproteinases-3 gene expression in articular chondrocytes via de novo transcription, protein synthesis, and tyrosine kinase- and mitogen-activated protein kinase-dependent mechanisms. *J. Immunol. 161:5000.*
35. Heinrich, P. C., I. Behrmann, G. Müller-Newen, F. Schaper, and L. Graeve. 1998. Interleukin-6-type cytokine signaling through the gp130/Jak/STAT pathway. *Biochem. J. 334:297.*
36. Ihle, J. N. 1996. STATs: signal transducers and activators of transcription. *Cell 84:331.*
37. Schindler, C., and J. E. Darnell, Jr. 1995. Transcriptional responses to polypeptide ligands: the JAK-STAT pathway. *Annu. Rev. Biochem. 64:621.*
38. Goodman, P. A., L. B. Niehoff, and F. M. Uckun. 1998. Role of tyrosine kinases in induction of the c-jun proto-oncogene in irradiated B-lineage lymphoid cells. *J. Biol. Chem. 273:17742.*
39. Meydan, N., T. Grunberger, H. Dadi, M. Shahar, E. Arpaia, Z. Lapidot, J. S. Leeder, M. Freedman, A. Cohen, A. Gazit, A. Levitzki, and C. M. Roifman. 1996. Inhibition of acute lymphoblastic leukaemia by a Jak-2 inhibitor. *Nature 379:645.*
40. Chomczynski, P., and N. Sacchi. 1987. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal. Biochem. 162: 156.*
41. Andrews, N. C., and D. V. Faller. 1991. A rapid micropreparation technique for extraction of DNA-binding proteins from limiting numbers of mammalian cells *Nucleic Acids Res. 19:2499.*
42. Muir, H. 1995. The chondrocyte, architect of cartilage-biomechanics, structure, function and molecular biology of cartilage matrix macromolecules. *BioEssays 17:1039.*
43. Cowell, S., V. Knauper, M. L. Stewart, M. P. D'Ortho, H. Stanton, R. M. Hembry, C. Lopez-Otín, J. J. Reynolds, and G. Murphy. 1998. Induction of matrix metalloproteinase activation cascades based on membrane-type 1 matrix metalloproteinase associated activation of gelatinase A, gelatinase B and collagenase 3. *Biochem. J. 331:453.*
44. Korzus, E., H. Nagase, R. Rydell, and J. Travis. 1997. The mitogen-activated protein kinase and JAK-STAT signaling pathways are required for an oncostatin M-responsive element-mediated activation of matrix metalloproteinase 1 gene expression. *J. Biol. Chem. 272:1188.*
45. Gatsios, P., H. D. Haubeck, E. Van de Leur, W. Frisch, S. S. Apte, H. Greiling, P. C. Heinrich, and L. Graeve. 1996. Oncostatin M differentially regulates tissue inhibitors of metalloproteinases TIMP-1 and TIMP-3 gene expression in human synovial lining cells. *Eur. J. Biochem. 241:56.*
46. Richards, C. D., M. Shoyab, T. J. Brown, and J. Gauldie. 1993. Selective regulation of metalloproteinase inhibitor (TIMP-1) by oncostatin M in fibroblasts in culture. *J. Immunol. 150:5596.*
47. Maier, R., V. Ganu, and M. Lotz. 1993. Interleukin-11, an inducible cytokine in human articular chondrocytes and synoviocytes, stimulates the production of the tissue inhibitor of metalloproteinases. *J. Biol. Chem. 268:21527.*
48. Silacci, P., J.-M. Dayer, A. Desgeorges, R. Peter, C. Manueddu, and P.-A. Guerne. 1998. Interleukin (IL)-6 and its soluble receptor induce TIMP-1 expression in synoviocytes and chondrocytes, and block IL-1-induced collagenolytic activity. *J. Biol. Chem. 273:13625.*
49. Yokota, A., M. Narazaki, N. Murata, T. Tanaka, and T. Kishimoto. 1997. Persistent activation of the STAT1 in rheumatoid synovial fluid cells. *Arthritis Rheum. 40(Suppl. 9):1461.*
50. Wang, F., T. K. Sengupta, Z. Zhong, and L. B. Ivashkiv. 1995. Regulation of the balance of cytokine production and the signal transducer and activator of transcription (STAT) transcription factor activity by cytokines and inflammatory synovial fluids. *J. Exp. Med. 182:1825.*
51. Leonard, W. J., and J. J. O'Shea. 1998. Jaks and STATs: biological implications. *Annu. Rev. Immunol. 16:293.*
52. Levy, J. B., C. Schindler, R. Raz, D. E. Levy, R. Baron, and M. C. Horowitz. 1996. Activation of the JAK-STAT signal transduction pathway by oncostatin-M in cultured human and mouse osteoblastic cells. *Endocrinology 137:1159.*
53. Wen, Z., Z. Zhong, and J. E. Darnell, Jr. 1995. Maximal activation of transcription by Stat1 and Stat3 requires both tyrosine and serine phosphorylation. *Cell 82:241.*
54. Stark, G. R., I. M. Kerr, B. R. G. Williams, R. H. Silverman, and R. D. Schreiber. 1998. How cells respond to interferons. *Annu. Rev. Biochem. 67:227.*
55. David, M., E. Petricoin III, C. Benjamin, R. Pine, M. J. Weber, and A. C. Larner. 1995. Requirement for MAP kinase (ERK2) activity in interferon α- and interferon β-stimulated gene expression through STAT proteins. *Science 269:1721.*
56. Huang, M. T., Y. R. Lou, W. Ma, L. H. Newmark, K. R. Reuhl, and A. H. Conney. 1994. Inhibitory effects of dietary curcumin on forestomach, duodenal, and colon carcinogenesis in mice. *Cancer Res. 54:5841.*
57. Rao, C. V., A. Rivenson, B. Simi, and B. S. Reddy. 1995. Chemoprevention of colon carcinogenesis by dietary curcumin, a naturally occurring plant phenolic compound. *Cancer Res. 55:259.*
58. Huang, T.-S., S.-C. Lee, and J.-K. Lin. 1991. Suppression of c-Jun/AP-1 activation by an inhibitor of tumor promotion in mouse fibroblast cells. *Proc. Natl. Acad. Sci. USA 88:5292.*
59. Chen, Y. R., and T.-H. Tan. 1998. Inhibition of the c-Jun N-terminal kinase (JNK) signaling pathway by curcumin. *Oncogene 17:173.*
60. Singh, S., and B. B. Aggarwal. 1995. Activation of transcription factor NF-κB is suppressed by curcumin (diferuloylmethane). *J. Biol. Chem. 270:24995.*
61. Jobin, C., C. A. Bradham, M. P. Russo, B. Juma, A. S. Narula, D. A. Brenner, and R. B. Sartor. 1999. Curcumin blocks cytokine-mediated NF-κB activation and proinflammatory gene expression by inhibiting inhibitory factor I-κB kinase activity. *J. Immunol. 163:3474.*
62. Ammon, H. P., and M. A. Wahl. 1991. Pharmacology of *Curcuma longa. Planta Med. 57:1.*
63. Stancato, L. F., M. Sakatsume, M. David, P. Dent, F. Dong, E. F. Petricoin, J. J. Krolewski, O. Silvennoinen, P. Saharinen, J. Pierce, et al. 1997. β interferon and oncostatin M activate Raf-1 and mitogen-activated protein kinase through a JAK1-dependent pathway. *Mol. Cell. Biol. 17:3833.*
64. Bugno, M., L. Graeve, P. Gatsios, A. Koj, P. C. Heinrich, J. Travis, and T. Kordula. 1995. Identification of the interleukin-6/oncostatin M response element in the rat tissue inhibitor of metalloproteinases-1 (TIMP-1) promoter. *Nucleic Acids Res. 23:5041.*
65. Botelho, F. M., D. R. Edwards, and C. D. Richards. 1998. Oncostatin M stimulates c-fos to bind a transcriptionally responsive AP-1 element within the tissue inhibitor of metalloproteinase-1 promoter. *J. Biol. Chem. 273:5211.*
66. Mohan, R., J. Sivak, P. Ashton, L. A. Russo, B. Q. Pham, N. Kasahara, M. B. Raizman, and M. E. Fini. 2000. Curcuminoids inhibit the angiogenic response stimulated by fibroblast growth factor-2, including expression of matrix metalloproteinase, gelatinase B. *J. Biol. Chem. 275:10405.*
67. Lin, L. I., Y. F. Ke, Y. C. Ko, and J. K. Lin. 1998. Curcumin inhibits SK-Hep-1 hepatocellular carcinoma cell invasion in vitro and suppresses matrix metalloproteinase-9 secretion. *Oncology 55:349.*
68. Modur, V., M. J. Feldhaus, A. S. Weyrich, D. L. Jicha, S. M. Prescott, G. A. Zimmerman, and T. M. McIntyre. 1997. Oncostatin M is a proinflammatory mediator: in vivo effects correlate with endothelial cell expression of inflammatory cytokines and adhesion molecules. *J. Clin. Invest. 100:158.*