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*J Immunol* 1998; 161:5000-5007; ;
http://www.jimmunol.org/content/161/9/5000

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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

Wen Qing Li* and Muhammad Zafarullah2*

Cytokines and growth factors regulate physiologic and pathologic turn-over of cartilage extracellular matrix (ECM) by altering the balance between tissue inhibitors of metalloproteinases (TIMPs) and matrix metalloproteinases (MMPs). Oncostatin M (OSM) is a cytokine of the IL-6 family whose levels are increased in the serum and synovial fluids of patients with rheumatoid arthritis. We examined responsiveness of the TIMP-3 gene to OSM in articular chondrocytes and studied the regulatory and signaling mechanisms of this response. OSM induced TIMP-3 mRNA and protein expression in a dose- and time-dependent fashion. Concomitantly, stromelysin-1 and collagenase-1 RNA and activities were also induced. A cartilage matrix growth factor, TGF-β, induced TIMP-3, but combined OSM and TGF-β did not further increase the extent of induction, suggesting a lack of synergy between the two. OSM induction of TIMP-3 gene expression was dependent upon de novo protein synthesis and transcription. RNA decay time-courses suggested that the OSM-mediated increase of TIMP-3 RNA was not due to enhanced message stability and, along with inhibition by actinomycin-D, suggested a transcriptional control. The antiinflammatory glucocorticoid, dexamethasone, down-regulated this augmentation. Investigation of the signaling mechanisms revealed that protein tyrosine kinase inhibitors genistein and herbimycin A, as well as the specific mitogen-activated protein kinase (MAPK) kinase inhibitor PD98059, suppressed OSM-induced TIMP-3 message expression, suggesting the involvement of tyrosine kinases and mitogen-activated protein kinase cascades in the signaling of OSM leading to TIMP-3 RNA enhancement. Thus OSM can potentially alter the cartilage matrix metabolism by regulating genes like TIMP-3 and matrix metalloproteinases. The Journal of Immunology, 1998, 161: 5000–5007.

Rheumatoid arthritis (RA) is a chronic disease resulting in inflammation, synovial hyperplasia, degeneration, and deformation of several joints. Osteoarthritis (OA), although localized and less inflammatory, is a prevalent joint degenerative disease, mostly associated with aging, obesity, and joint overuse (1). The two diseases cause disability among millions of people worldwide. The salient features of the diseases are the altered balance between proinflammatory and antiinflammatory cytokines, changed connective tissue metabolism, impaired joint repair, and ultimately resorption of articular cartilage (2, 3). The latter phenomenon is due to the combined actions of an characterize enzyme, aggrecanase, and overexpression of activated matrix metalloproteinases (MMPs). These enzymes consist of collagensases, stromelysins, gelatinases, and membrane-type MMPs (MT-MMPs), which degrade various components of the extracellular matrix (ECM) (4).

A family of tissue inhibitors of metalloproteinases (TIMPs), comprising TIMP-1, -2, -3, and -4, regulates the activities of MMPs by complexation and by maintaining an enzyme-inhibitor balance (5). TIMP-1 (6) and TIMP-2 (7) are the earlier members of the gene family, while TIMP-3 (8) and TIMP-4 (9) are the most recent. MMPs and TIMPs play vital roles during physiologic ECM turnover in animal development and aging. Pathologic turnover of ECM is believed to be a consequence of TIMPs-MMPs imbalance, as shown for the OA cartilage (10). Such imbalance is also implicated in retinal degeneration, periodontal diseases, atherosclerotic plaque rupture, and metastatic invasion of cancer cells. TIMPs have both growth-promoting and antimetastatic activities (11, 12).

TIMP-3, a 21-kDa ECM-associated protein from transformed chicken fibroblasts, is distinct yet related to other TIMPs (13, 14). It stimulates the proliferation of nontransformed cells (15). Human TIMP-3 cDNA was isolated from placenta, and its expression was shown in different tissues (8), such as fetal kidney (16) and breast tumors (17). Murine TIMP-3 is expressed in fibroblasts and animal tissues, and the protein is found in ECM only (18). TIMP-3 is up-regulated by mitogens at the G1 phase of cell cycle (19). Gene mutations of TIMP-3, a component of Bruch’s membrane of the
eye (20), could interfere with inhibition of MMPs, possibly causing a retinal degenerative disease (21). TIMP-3 mutations can influence its ability to inhibit angiogenesis of the eye (22). By its presence in ECM, TIMP-3 blocks tumor growth (23), possibly by inhibiting angiogenesis (24). The extent of TIMP-3 inhibition of MMPs is similar to that of TIMP-1 (25). However, TIMP-3 and TIMP-2 are better inhibitors of MT1-MMP compared with TIMP-1 (26). Recent studies have related TIMP-3 expression with chondrocyte differentiation during mouse development (27, 28).

We have observed that TIMP-3 and TIMP-1 RNA expression is increased by unknown signals in human OA synovial membranes (Su et al., unpublished observations), and the two genes are inducible in human and bovine chondrocytes by an inducer of matrix synthesis, TGF-β (29, 30). Due to their potential for inhibiting cartilage resorption, studying the regulatory mechanisms of TIMPs by pathophysiologically relevant stimuli in cartilage could be of therapeutic value in arthritis.

Oncostatin M (OSM), a 28-kDa glycoprotein produced by activated T-lymphocytes and monocytes, inhibits the growth of human melanoma cells (31) and is a mitogen for normal fibroblasts and smooth muscle cells. OSM belongs to the IL-6 family of cytokines, including leukemia inhibitory factor (LIF), that share structural features, binding to the receptor gp 130 and linkage of their genes (OSM and LIF) on the human chromosome 22 (32). OSM was undetectable in the synovial fluid of OA but was present and increased in the RA patients, suggesting its role in joint inflammation (33). The levels of TIMP-3 in OA synovial cell culture media were elevated in the human RA vs OA synovial cell lines (34). Previous studies have shown that OSM and other IL-6-type cytokines induced TIMP-1 but not MMP gene expression in human lung and synovial fibroblasts and could influence matrix degradation during chronic inflammation (35). A proinflammatory role for OSM was suggested since it stimulates degradation of porcine cartilage and, like IL-1 and TNF-α, inhibits proteoglycan synthesis (36). IL-1 and OSM, in combination, promote bovine nasal cartilage proteoglycan and collagen degradation (37). In contrast, OSM was regarded as a cartilage protective cytokine due to its induction of TIMP-1 in human chondrocytes (38).

A recent study showed that, in rheumatoid fibroblasts, OSM increased TIMP-1 and inhibited IL-1β-induced TIMP-3 RNA expression (39). Cartilage is a major target tissue for pro- and anti-inflammatory cytokines in arthritis, which could influence the outcome of the disease by regulating a plethora of genes. None of the previous studies have addressed regulation of the recently described TIMP-3 gene by OSM in chondrocytes, which is a unique cell type whose primary function is to maintain cartilage ECM (40). Due to the contradictory role of OSM in joints, this issue is of paramount biologic and pathophysiologic significance. The aims of the present study were to assess the role of OSM in cartilage by investigating its impact on TIMP-3 gene expression and the possible mechanisms of this response in articular chondrocytes. Here, we demonstrate for the first time, TIMP-3 up-regulation by OSM and inhibition by dexamethasone in primary bovine chondrocyte model of cartilage resorption and show dependence of the response on new protein and RNA synthesis. We also demonstrate suppression of TIMP-3 RNA expression by genistein, herbimycin A, and PD98059, suggesting the involvement of tyrosine kinase and MAPK pathways in OSM signaling.

**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. The cartilage-containing bones were dipped briefly in 1% providone (Rougier, Chambly, Quebec, Canada) for sterilization and washed extensively with 0.9% NaCl. The slices of cartilage were dissected out, kept for 1 to 2 h at 4°C in 5× antibiotic-antimycotic solution, and washed five times with large volumes of PBS containing 5× penicillin-streptomycin and 1× fungizone (Life Technologies). Chondrocytes were released from bovine cartilage by digestion with pronase (1 mg/ml) for 90 min and collagenase (Sigma type II) for 12 h in DMEM at 37°C. Viability by trypan blue exclusion test was about 80%. The cells were pelleted and washed three 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). Before different treatments, cells were kept in serum-free DMEM for 24 h. The potential inhibitors were added 30 min before the OSM.

**RNA extraction and Northern hybridization analysis**

Total RNA from primary cultures of chondrocytes was extracted by the acid-guainidium procedure (41), and 5-µg aliquots were analyzed by fractionation in 1.2% formaldehyde-agarose gels, transferred to Zeta-probe membranes, and hybridized as previously described (42). The quality and quantity of applied RNA were verified visually by ethidium bromide staining and photography of 28S and 18S ribosomal RNA bands. The RNA was electroblotted onto Zeta-Probe nylon membrane (Bio-Rad) using a Bio-Rad Transblot in the presence of 100 mM glycine, at 250 mA using the Bio-Rad system. The blots were UV cross-linked, washed twice with PBS, lysed in 100 µl of 0.1% SET. For the analysis of the TIMP-3 protein, chondrocytes treated with or without OSM were washed twice with PBS, lysed in 100 µl of the lysis buffer (62.5 mM Tris-HCl (pH 6.8), 10% glycerol, and 1% Triton X-100), and sonicated for 15 s; and the protein was quantified by the Bio-Rad protein assay system. Aliquots of 40-µl concentrations of recombinant oncostatin M and human platelet TGF-β, which were reconstituted as recommended, were from R&D Systems (Minneapolis, MN).

**Materials and Methods**

**Materials**

Cell culture supplies, such as DMEM, FCS, antibiotic-antimycotic agents, trypan blue, and agarose were from Life Technologies (Burlington, Ontario, Canada). Plastisware was from Nasal Nunc (Naperville, IL). Human recombinant oncostatin M and human platelet TGF-β, which were reconstituted as recommended, were from R&D Systems (Minneapolis, MN).

**Primary cultures of chondrocytes**

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10 min, the blots were incubated overnight with gentle agitation at 4°C with 5 μg/ml of the human TIMP-3 mAb (20) (Ab-1, clone 136-13H4 from Calbiochem) in TBS and 0.5% milk. The membranes were washed 3 times with TBS for 5 min each and incubated with TBS and 0.5% milk for 10 min, followed by incubation with anti-mouse secondary IgG-POD Fab fragment (peroxidase) (Boehringer Mannheim) for 1 h and 3 washes for 5 min each. The protein bands were revealed with Boehringer Mannheim chemiluminescence detection system and exposure to x-ray film for 3 min.

**Protease substrate gel electrophoresis**

Cells were exposed to OSM for 48 h, and 30 μl of the medium was either used as such or treated with 1 mM 4-aminophenylmercuric acetate (APMA), mixed with 4× sample buffer (0.25 M Tris-HCl (pH 6.8), 10% SDS, 4% sucrose, and 0.1% bromophenol blue), and applied to 10% SDS-PAGE containing 1 mg/ml gelatin or α-casein (Sigma). Following electrophoresis under nonreducing conditions (except m.w. standards), the gels were washed twice, 30 min each in 2.5% Triton X-100 (V/V) and incubated overnight in 50 mM Tris-HCl (pH 8.0) containing 5 mM CaCl2 and were washed twice, 30 min each in 2.5% Triton X-100 (V/V) and incubation with anti-mouse secondary IgG-POD Fab fragment (peroxidase) (Boehringer Mannheim) for 1 h and 3 washes for 5 min each. The protein bands were revealed with Boehringer Mannheim chemiluminescence detection system and exposure to x-ray film for 3 min.

**Results**

**OSM dose-dependently induces TIMP-3 and metalloproteinase gene expression**

To investigate whether OSM was able to regulate TIMP-3 gene in mammalian chondrocytes, primary bovine cells in two separate experiments were kept in serum-deprived medium for 24 h and then exposed for an other 24 h in the same medium to different concentrations (0.1–50 ng/ml) of recombinant human OSM. Northern hybridization analysis of RNA with the bovine TIMP-3 and 28S rRNA probes, respectively, revealed a potent dose-dependent induction of the three TIMP-3 transcripts relative to the mostly even 28S rRNA levels (Fig. 1). The maximal induction was with the doses between 10 to 50 ng/ml. Sequential hybridization with two metalloproteinase probes revealed that stromelysin-1 and collagenase-1 RNAs were also moderately inducible with OSM (6 days film exposure compared with 18 h for TIMP-3).

To examine whether the RNA induction was followed by the respective protein synthesis, total cellular extracts or the media from the untreated and OSM-treated cells were analyzed for TIMP-3 protein and MMP activities, respectively. Western immunoblot analysis with a mouse anti-human TIMP-3 mAb (20) demonstrated a dose-dependent TIMP-3 induction as revealed by a 24-kDa band equivalent to nonglycosylated bovine TIMP-3 (Fig. 2A). Zymographic analysis showed a similar pattern of caseinolytic and gelatinolytic activities induction corresponding to stromelysin (Fig. 2B), collagenases, and gelatinases (Fig. 2C). Therefore, TIMP-3 and MMP genes are clearly responsive to OSM, and their RNAs and proteins are inducible by this cytokine in articular chondrocytes.

**Combined treatment of OSM and TGF-β does not further enhance TIMP-3 gene expression**

TGF-β is an inducer of cartilage ECM, TIMP-1 (43), and TIMP-3 (29), as well as an inhibitor of MMPs (44). To examine the effect of the two factors in combination, chondrocytes were subjected to individual or combined treatments. In two separate series of cells, TGF-β was a more potent inducer of TIMP-3 message and protein (latter not shown) compared with OSM, and the combined treatments of TGF-β and OSM did not further increase the levels of induction relative to mostly constant levels of 28S rRNA. Thus, there are no synergistic or additive effects of the two factors on TIMP-3 gene expression (Fig. 3).

**TIMP-3 gene expression is induced by OSM in a temporal fashion**

To investigate the mechanism of TIMP-3 RNA enhancement by OSM, the time-course of induction was determined by maintaining chondrocytes in serum-free medium or by exposing them to OSM (10 ng/ml) for different time periods. Northern hybridization analysis revealed that the TIMP-3 message induction occurred rapidly within 3 h, peaked at 14 h, remained elevated for 24 h, and declined drastically by 36 h (Fig. 4). The 28S rRNA levels remained relatively consistent. Thus, TIMP-3 gene is regulated by OSM in a time-dependent fashion.

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**FIGURE 1.** Dose-dependent induction of TIMP-3, stromelysin-1, and collagenase-1 mRNA by OSM in bovine chondrocytes. Lane 0, Bovine chondrocytes maintained as high density primary cultures in serum-free DMEM with vehicle only. The RNA blot was hybridized with a 32P-labeled bovine TIMP-3 RNA probe that detected three transcripts. The other lanes represent RNA from cells treated with 0.1, 1, 10, 30, or 50 ng/ml of OSM, respectively, for 24 h. The blot was also sequentially hybridized with the stromelysin-1 and collagenase-1 probes. The 28S rRNA serving as loading control is shown at the bottom. The autoradiography for TIMP-3 and 28S RNAs was 18 h and for collagenase-1 and stromelysin-1 was 6 days.

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TIMP-3 regulation by OSM requires de novo protein synthesis and transcription

To gain insight into the mechanism of TIMP-3 mRNA up-regulation by OSM, the effect of protein synthesis inhibitor cycloheximide (CHX) and that of transcriptional inhibitor actinomycin-D (Act-D) on induction was studied by individual and combined treatments. OSM induced TIMP-3 mRNA while CHX (10 μg/ml) and Act-D (1 μg/ml), added 30 min before OSM, strongly inhibited this induction. The levels of 28S rRNA were invariable and demonstrated even application of RNA (Fig. 5). Thus, OSM enhancement of TIMP-3 message is dependent on new protein synthesis and transcription.

Increase in TIMP-3 message by OSM is not due to increased mRNA stability

To investigate whether TIMP-3 augmentation was due to increased RNA stability by OSM, TIMP-3 RNA was first induced in chondrocytes by a 24-h treatment with this cytokine, and medium was replaced with Act-D only or Act-D and OSM. Treatments were stopped at different time points, and TIMP-3 RNA was analyzed to follow the RNA decay time course. As shown in Figure 6, stability of the TIMP-3 RNA was unaffected by OSM. The constant levels of 28S rRNA were not affected by the treatments and also demonstrated equal application. Therefore OSM-stimulated enhancement of TIMP-3 message is not due to its increased stability.

Dexamethasone inhibits OSM-induced TIMP-3 expression

We have demonstrated that the synthetic glucocorticoid, dexamethasone (Dex) inhibits the basal and TGF-β-induced TIMP-3 RNA expression (29). To examine the effect of Dex on the OSM stimulation of TIMP-3 mRNA expression, chondrocytes were exposed in FCS-free medium to the ethanol vehicle (for Dex), OSM and Dex individually, or pretreated with Dex (1 μg/ml) for 30 min followed by OSM treatment for 24 h. Dex further reduced the basal TIMP-3 RNA levels and strongly inhibited its induction by OSM without affecting the 28S rRNA levels (Fig. 7). Thus Dex potently down-regulates OSM-induced TIMP-3 gene expression.
FIGURE 4. Time-dependent induction of TIMP-3 gene expression by OSM in bovine chondrocytes. High density bovine chondrocytes were serum starved for 24 h and either kept in the serum-free medium with vehicle (0.1% BSA in DMEM) only (control) or OSM (10 ng/ml) for different time periods, and the resulting RNA was subjected to Northern hybridization analysis. The positions of 28S and 18S rRNA bands and those of the three TIMP-3 transcripts are indicated in the upper panel. The blot was subsequently hybridized with a human 28S rRNA probe to demonstrate even application of RNA. The time of autoradiography was 18 h for TIMP-3 and 4 days for 28S probe.

Tyrosine kinase and MAPK inhibitors block TIMP-3 RNA induction by OSM

To investigate the mechanisms of OSM signaling leading to TIMP-3 RNA increase, chondrocytes were treated with vehicles (Control), OSM, genistein (50 and 100 μM), or herbimycin A (5 and 15 μM) (tyrosine kinase inhibitors), either individually or in combination (pretreatment with the inhibitors for 30 min) for 24 h. Both inhibitors dose-dependently suppressed the OSM induction of TIMP-3 gene expression while the 28S rRNA levels were unaffected (Fig. 8). A recently developed, very specific inhibitor of MAPK kinase, PD98059 (45), at a 30-μM dose, partially inhibited the OSM action (not shown), while the doses of 60 and 100 μM strongly diminished this induction to basal levels without influencing the constant levels of 28S RNA (Fig. 9). Two separate batches of cells for each of the above experiments gave similar results.

Discussion

Up-regulation of the TIMP-3 expression by OSM in primary chondrocytes was demonstrated for the first time. The dependence of this response on new transcription (but not increased RNA stability) and protein synthesis suggested a transcriptional control. Dex inhibited TIMP-3 induction. This induction involved tyrosine and MAP kinase signaling pathways.

TIMP-3 induction in bovine chondrocytes differs from a study in human rheumatoid cells where IL-1β-induced TIMP-3 RNA was inhibited by OSM, suggesting the latter’s antiinflammatory role (39). This may be due to cell-type-specific differential regulation. OSM and TIMP-3 may have different roles in cartilage and synovium. In cartilage, TIMP-3 RNA and protein increase by OSM may have a protective role, while, in synovial linings, TIMP-3 increase (Su et al., unpublished results) may be related to inflammation and cell proliferation, since it is a cell-cycle-associated gene (19). OSM and related cytokines IL-6 and IL-11 also induce TIMP-1 in human chondrocytes and in lung/synovial fibroblasts (35, 38, 46, 47). However our results suggest that, besides TIMP-1 and TIMP-3, collagenase-1 and stromelysin-1 are also coordinately regulated by OSM. Collagenase-1, but not stromelysin-1, was recently shown to be induced by OSM in human synovial fibroblasts (48). OSM, IL-6 (49), and LIF (50) are expressed in cartilage and synovium and, through autocrine mechanisms, could influence joint metabolism by regulating TIMP-3, TIMP-1, and MMPs.

The time course suggests that OSM rapidly induces TIMP-3 gene in chondrocytes. Decline in the response by 36 h may be due to degradation of the exogenous OSM. Therefore, normal chondrocytes have OSM-signaling components that lead to the TIMP-3 response. Deficiency of this response in the RA and OA chondrocytes, which are hyporesponsive to TGF-β and insulin-like growth factor (IGF)-I (51), is not known. The proposed cartilage protection by OSM may be due to its induction of TIMP-3 and TIMP-1, which could block the degradative MMPs. However, increased OSM and TIMPs in arthritic tissues may not protect individuals from arthritic damage, and their enhancement may indicate attempted repair. Simultaneous induction of MMPs by OSM questions its protective function (36) and suggests a role in cartilage remodeling.

Although OSM and TGF-β induced TIMP-3 RNA expression individually, there was no synergy or additive effect between them. TGF-β was a more potent inducer of TIMP-3 protein than OSM, suggesting different mechanisms of induction; indeed, both factors suppressed mouse B cell hybridoma proliferation through distinct mechanisms (52). Interestingly, both OSM and TGF-β also induce TIMP-1 in human chondrocytes (30, 38).

TIMP-3 inhibition by CHX and Act-D suggests the need for transcription and synthesis of intermediate products for induction by OSM. Interestingly, TGF-β augmentation of TIMP-3 RNA can
also be blocked with these reagents, suggesting partial similarities in the induction mechanisms. OSM induces TIMP-3 RNA expression at the level of transcription, a view supported by the lack of increased TIMP-3 RNA stability by this agent. The putative intermediary gene products inhibited by CHX and Act-D may be the tyrosine kinases (Janus kinases, JAKs), STATs, and the immediate-early response genes. These conclusions were supported by Dex suppression, which prevents the binding of c-jun to its target sequences in the promoter by DNA-free interactions with the glucocorticoid receptor (53). Dex may have blocked STATs in a similar fashion. Dex also suppresses the basal and TGF-β induction of TIMP-3 RNA in bovine and human chondrocytes (29). Thus, glucocorticoids can block the action of a variety of pro- and anti-inflammatory cytokines. The phospholipase gene induction by OSM in human hepatoma cells was also inhibited by Dex (54). However, in Kaposi’s sarcoma cells, Dex stimulates gp130-mediated growth by increasing the accumulation of tyrosine phosphorylated STAT3 (55). Thus, Dex may act differentially on OSM action in different cell types. If OSM contributes to cartilage resorption (36, 37), glucocorticoids may suppress its actions in chondrocytes.

The suppression of OSM-stimulated TIMP-3 gene expression by genistein and herbimycin A suggests the implication of tyrosine kinases. Genistein also inhibits the induction of low density lipoprotein and Egr-1 transcription factor by OSM (56). One possible site of genistein action is OSM activation of tyrosine kinases, Janus kinases (Tyk-1, Jak-1, Jak-2) (57) that phosphorylate STAT-1 and STAT-3 and whose homo- or heterodimers (58) in turn are translocated to the nucleus to activate the target genes, such as TIMP-3 and TIMP-1. The JAKs/STATs are involved in the IFN, IL-6, OSM, and LIF signaling (59). In other cell types, there are certain steps in OSM signaling that involve tyrosine phosphorylation. Since MAPK (possibly ERK-2) further activates STATs, the tyrosine phosphorylation steps in the MAPK pathway may also have been inhibited by herbimycin A and genistein. Another interaction between the two pathways may be activation of Raf by JAKs (60). Indeed, genistein inhibits tyrosine phosphorylation and MAPK activation in Kaposi’s sarcoma cells (61).

Inhibition of TIMP-3 RNA expression by the MAPK kinase-specific inhibitor strongly supports the involvement of the MAPK pathway in OSM signaling. Blocking of any upstream step during MAPK activation of AP-1 and OSM activation of STATs may inhibit TIMP-3. The involvement of AP-1 as targets of OSM signaling is supported by the down-regulation of TIMP-3 message induction by curcumin, a specific AP-1 inhibitor (our unpublished
FIGURE 9. Suppression of OSM-inducible TIMP-3 gene expression by MAPK kinase inhibitor. The serum-deprived chondrocytes were kept in medium with DMSO vehicle or pretreated with the MAPK kinase (MAPKK) inhibitor PD098059 (60–100 μM) for 30 min, followed by additional treatment with OSM for 24 h, and RNA levels were measured by Northern hybridization analyses.

results). Recent studies suggest that phosphorylation of a serine residue in Stat-1b by MAPKs is needed for IFN-induction of the target genes. OSM-responsive elements are found in the rat TIMP-1 promoter (62), and there is cross-talk between the MAPK and JAK-STAT pathways (59, 63).

In summary, we have demonstrated coordinate OSM up-regulation of TIMP-3, MMP-1, and MMP-3 RNA and protein, lack of synergy between TGF-β and OSM, de novo transcription and protein synthesis dependence of TIMP-3 gene induction, inhibition by Dex, and the involvement of tyrosine and MAP kinase pathways. Thus, in normal mammalian chondrocytes, OSM may contribute to cartilage ECM remodeling. Due to MMP inhibition and potent induction of TIMPs, TGF-β may be superior to OSM for cartilage protection.

Acknowledgments

We thank Dr. Kazushi Iwata (Fuji Chemical Industries, Toyama, Japan) for valuable advice on the TIMP-3 mAB. We also thank Jean Maher (Abattoir les Cedres) for bovine cartilage and Anna Chelchowska for preparing this manuscript and the figures.

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