Interleukin-6 Increases Rat Metalloproteinase-13 Gene Expression through Stimulation of Activator Protein 1 Transcription Factor in Cultured Fibroblasts*

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EXPERIMENTAL PROCEDURES

Cell Culture—Rat-1 fibroblasts obtained from American Type Culture Collection were grown at 37 °C in an atmosphere of 5% CO₂, 95% air in cell culture flasks using 10 ml of Dulbecco’s minimum essential medium with Earle’s salts (Life Technologies, Inc.) containing 5% fetal bovine serum (Flow, Irvine, Ayrshire, Scotland), 0.5 mg/ml L-glutamine, 100 units/ml penicillin G and 0.1 mg/ml streptomycin. In some experiments we used protein extract from J-Jahn cells (29). This is a human lymphoblastoid cell line derived from Jurkat cells that expresses large amount of c-Jun after being stimulated with phorbol 12-myristate

The role of IL-6 in collagen production and tissue remodeling is controversial. In Rat-1 fibroblasts, we measured the effect of IL-6 on matrix metalloproteinase-13 (MMP-13), c-jun, junB, and c-fos gene expression, binding of activator protein 1 (AP1) to DNA, amount of AP1 proteins, immunoreactive MMP-13 and TIMP-1 proteins, and Jun N-terminal kinase activity. We show that IL-6 increased MMP-13 mRNA and MMP-13 protein. These effects were exerted by acting on the AP1-binding site of the MMP-13 promoter, as shown by transfecting cells with reporter plasmids containing mutations in this element. Mobility shift assays demonstrated that IL-6 induced the DNA binding activity of AP1. This effect was accompanied by a marked increase in c-Jun, JunB, and c-Fos mRNA, as well as in c-Jun protein and its phosphorylated form. The latter is not due to increased Jun N-terminal kinase activity but to a decreased serine/threonine phosphatase activity. We conclude that IL-6 increases interstitial MMP-13 gene expression at the promoter level. This effect seems to be mediated by the induction of c-jun, junB, and c-fos gene expression, by the binding of AP1 to DNA, by increasing phosphorylated c-Jun, and by the inhibition of serine/threonine phosphatase activity. These effects of IL-6 might contribute to remodeling connective tissue.

Interleukin-6 (IL-6) is a multifunctional glycoprotein produced by activated monocytes, macrophages, endothelial cells, and hepatic stellate cells that induces a wide variety of biological activities on many kinds of target cells, including fibroblasts, hepatocytes, and hepatic stellate cells (1). IL-6 promotes cell growth and differentiation and regulates specific gene expression of a variety of cells (2). IL-6 induces the expression of the acute phase proteins in the liver by inducing the binding of NF-IL6 and STAT3 to the promoter region of acute phase genes and promotes a rapid and transient tyrosine phosphorylation of the cytoplasmic domains of the IL-6 receptor (gp130) (1, 3). Acute and chronic liver diseases, particularly alcoholic liver diseases, are similar to the acute phase response in some respects. Thus, patients with acute alcoholic hepatitis show fever, muscle wasting, neutrophilia, and increased production of C-reactive protein, α1-antitrypsin, and amyloid A (4, 5). High levels of IL-6 have been detected in the sera of patients with alcoholic liver cirrhosis (6–8), hepatitis B virus infection (9), and acute hepatitis (10, 11). Some authors have shown a correlation between circulating concentrations of IL-6 and serum concentrations of C-reactive protein (12, 13). Thus, IL-6 seems to be one of the most important factors regulating inflammatory responses in the liver.

Matrix metalloproteinases (MMPs) constitute a family of structurally related zymogens (collagenase-1 (MMP-1), collagenase-3 (MMP-13), gelatinases A and B (MMP-2 and MMP-9), and stromelysin (MMP-3), among others) capable of degrading a wide variety of extracellular matrix components (14). In rats and mice, there is only one interstitial collagenase (MMP-13); it shares 86% homology with human MMP-13 but not with the human or rabbit MMP-1 (15, 16). A variety of biologically active agents, such as tumor necrosis factor-α and interleukin-1, modulates the synthesis of these enzymes and their natural inhibitors, tissue inhibitors of MMP (TIMPs) (17–19). Although IL-6 shares many biological activities with IL-1, the role of IL-6 on the regulation of synthesis of MMPs and TIMPs remains controversial (17, 20–22). Whereas some authors found evidence for increased collagenase production (20, 23–25), others could not demonstrate any effect of IL-6 on the expression of MMP (21) or showed that IL-6 induces the synthesis of TIMP (17, 22, 26–28). We have undertaken the present study to elucidate the effect of IL-6 on rat MMP-13 (collagenase-3) gene expression in Rat-1 fibroblasts. We demonstrate that this cytokine stimulates MMP-13 expression by acting on an AP1-binding site in the MMP-13 promoter, after inducing the synthesis and phosphorylation of AP1 proteins.

* This study was supported in part by National Institutes of Health Grants GM41804 and DK 47361 (to D. A. B.), Fondo de Investigaciones de la Seguridad Social, and the Biomedical Research Collection were grown at 37 °C in an atmosphere of 5% CO₂, 95% air in cell culture flasks using 10 ml of Dulbecco’s minimum essential medium with Earle’s salts (Life Technologies, Inc.) containing 5% fetal bovine serum, 0.5 mg/ml L-glutamine, 100 units/ml penicillin G and 0.1 mg/ml streptomycin. In some experiments we used protein extract from J-Jahn cells (29). This is a human lymphoblastoid cell line derived from Jurkat cells that expresses large amount of c-Jun after being stimulated with phorbol 12-myristate.

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13-acetate (PMA). This cell line was a gift from Dr. J. Alcami (Madrid, Spain) (29). Recombinant Plasmids—The luciferase reporter gene p2TRE-Luc contains two copies of the 12-O-tetradecanoylphorbol-13-acetate-responsive element (TRE) upstream of the herpes simplex virus tk promoter (pRSV-tk). Chien, University of California, Santa Cruz, CA) inserted into p19-Luc. pMMP-13-CAT plasmids (p-2200) MMP-13-CAT, p-1000MMP-13-CAT, p-500MMP-13-CAT, p-284MMP-13-CAT, and p-76MMP-13-CAT contain 76 base pairs to 2.2 kilobase pairs 5'-flanking DNA of the MMP-13 gene promoter inserted into a vector that encodes the enzyme chloramphenicol acetyltransferase (CAT, pSVO-CAT) (31). pSvo-CAT vector that encodes the enzyme chloramphenicol acetyltransferase (CAT) (pSVO-CAT) (31). pSvo-CAT contains the HindIII/XbaI fragment (760 base pairs) of the human c-fos promoter inserted into p19-Luc (32). Construction of pRSV-β-galactosidase containing the Rous sarcoma virus (RSV) promoter and the β-galactosidase reporter gene has been described elsewhere (32). pJun-Luc contains the SstI fragment (2.2 kilobase pairs) of the human c-jun promoter inserted into p19-Luc vector (33). Human pBR 18 S contains the EcoRI fragment (5.8 kilobase pairs) subcloned into the pBR 322 vector.

Transfection and Luciferase Assay—Rat 1 fibroblasts were transiently transfected by the LipofectAMINE technique. Briefly, after overnight incubation, cells were washed with Opti-MEM medium (Life Technologies, Inc.) and incubated with a cotransfection mixture containing 1.5 μg of CAT plasmids and 11 μg of LipofectAMINE reagent (Life Technologies, Inc.) in Opti-MEM medium at 37 °C for 8 h. After incubation, the transfection mix was aspirated and replaced with growth medium containing 10% fetal calf serum. After 24 h, the cells were washed with phosphate-buffered saline, and a new medium without fetal calf serum was added. After 2 h, cells were treated with IL-6 for various periods of time. Afterward, cells were washed with cold phosphate-buffered saline. Cell lysates were prepared, and CAT activity was determined as described elsewhere (34). Because CAT activity in cells transfected with constructs containing the smaller portions of the MMP-13 promoter was very low, the amount of cell lysate used for the CAT assays was 2 (p-500MMP-13-CAT), 5 (p-284MMP-13-CAT), and 18 times higher (p-76MMP-13-CAT and p-76MMP-13-CATm) than the amount of cell lysate used when cells were transfected with p-1000MMP-13-CAT or p-2000MMP-13-CAT constructs. In some experiments, cells were cotransfected with 0.5 μg of luciferase reporter plasmids and 0.5 μg of β-galactosidase pRSV-β-galactosidase as an internal control of transfection efficiency. In these cases, luciferase activity was determined using the enhanced luminometer (Turnip, San Diego, CA). Cell lysates were prepared in 125 μl of cell lysis buffer. Luciferase activity was determined using 50-μl aliquots, and protein concentrations determined with 5-μl aliquots using the Bradford protein assay (Bio-Rad). Luciferase activity was standardized to the enhanced luminometer (Turnip, San Diego, CA). Cell lysates were prepared in 125 μl of cell lysis buffer. Luciferase activity was determined using 50-μl aliquots, and protein concentrations determined with 5-μl aliquots using the Bradford protein assay (Bio-Rad). Published procedures were used to measure β-galactosidase activity (35). Transfections were performed in duplicate or triplicate.

RNA Preparation and Northern Analysis—Total RNA was prepared from cultured Rat-1 fibroblasts as described by Chomczynski and Sacchi (36). Five-microgram RNA samples were separated by electrophoresis on 2.2% formaldehyde, 1% agarose gels and transferred to nylon membranes (MSI, Westboro, MA). cDNA probes for rat interstitial collagenase, c-Jun (37), JunB (38), and c-Fos (39) and 18 S RNA were synthesized by passing cell lysate through a Sephadex G-25 spin column. Protein concentration of the phosphate-reduced sample was measured using the Bradford protein assay reagent from Bio-Rad. Serum/threonine phosphatase activity was measured using a commercially available assay following the manufacturer’s instructions (Promega).

RESULTS

IL-6 Induces MMP-13 Gene Transcription—To determine the effects of IL-6 on MMP-13 gene expression, we examined the steady-state levels of MMP-13 mRNA in Rat-1 fibroblasts treated with increasing concentrations of IL-6 for 24 h. The level of MMP-13 mRNA in each sample was normalized to the level of 18 S RNA. The level of 18 S RNA that resulted in a 10-fold increase in steady-state levels of MMP-13 mRNA (Fig. 1A). Thus, 40 ng/ml IL-6 increased MMP-13 levels 3.4-fold over the control level (Fig. 1A). Likewise, these levels were increased to 1.2-, 2-, 2.9-, and 3.2-fold more than the control level after 3, 6, 12, and 24 h, respectively, of incubation with 20 ng/ml IL-6 (Fig. 1B). This increase was in the range of the increase induced with 0.6 nm tumor necrosis factor-α (2.4-fold) or 2 ng/ml IL-1 (3.8-fold) in
To determine the sequences on which IL-6 exerts its effects on MMP-13 transcription, we transiently transfected cells with a series of constructs obtained by progressively deleting more 5’-flanking sequences of the MMP-13 gene promoter and inserting them into a CAT reporter plasmid. Deletion of promoter sequences upstream of base pair –76 relative to the transcription start site did not abrogate the effect of IL-6 on MMP-13 gene expression (Fig. 4, A–E). On the contrary, this effect of IL-6 disappeared in cells transfected with a mutant construct in which the proximal AP1 binding site have been converted from GTGACTCA into GTTCCAAG (Fig. 4F).

To assess the role of the TRE sequence in the stimulation of the MMP-13 gene, we transfected Rat-1 fibroblasts with a plasmid containing two copies of the TRE upstream of the herpes simplex virus-tk promoter (p2xTRE-Luc) and measured the effect of increasing concentrations of IL-6 on the luciferase activity in cell lysates. We found that 20 ng/ml IL-6 increased luciferase activity 1.4-fold the control level at 6 h and reached 1.9- and 2.6-fold at 12 and 24 h, respectively (Fig. 5). The increased luciferase activity was more marked in cells incubated with 40 ng/ml IL-6, where there was a 1.9-, 2.7-, and 3.1-fold induction at 6, 12, and 24 h, respectively (Fig. 5).

**IL-6 Increases AP1 Binding to DNA**—To determine whether IL-6 induces the DNA binding activity of AP1, we examined the kinetics of AP1 binding activity in IL-6 stimulated cells. Nuclear extracts were prepared from Rat-1 fibroblasts treated with 20 ng/ml IL-6 for 1–6 h. A 32P-labeled oligonucleotide containing the AP1 consensus sequence was used as a probe. AP1 binding activity increased 1.9-fold (Fig. 6A) and 2.4-fold (Fig. 6B) over the control level after 6 and 24 h, respectively. Likewise, AP1 binding to DNA was only slightly enhanced (1.2-fold) in cells treated with 5 ng/ml IL-6 for 24 h, but rose to 1.8-, 2.4-, and 2.6-fold with 10, 20, and 30 ng/ml IL-6, respectively, for the same period of time (Fig. 6B). This binding was efficiently competed with 200-fold molar excess of the same unlabeled oligonucleotide (Fig. 6), but not with 200-fold molar excess of an unlabeled oligonucleotide containing a C/EBP consensus binding site (Fig. 6A). On the other hand, incubation of the nuclear extract with a phosphorylated c-Jun-specific antibody prior to the gel retardation assay led to the formation of two supershifted complexes (Fig. 6A), demonstrating that phosphorylated c-Jun was a member of this complex.

**IL-6 Enhances c-fos and c-jun Gene Expression**—Because AP1 is a collection of transcriptional factors composed of mem-
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Fig. 3. Effect of IL-6 on collagenase gene promoter in Rat-1 fibroblasts. Cells were transfected by the LipofectAMINE method with 1.5 μg of p(-1000)MMP-13-CAT reporter plasmid and 0.6 μg of pRSV-β-galactosidase plasmid. One day after transfection, the cells were incubated with 0, 10, 20, or 40 ng/ml of IL-6 in the absence of fetal calf serum for 6, 12, 24, or 48 h. After the indicated time, cells were harvested, and CAT and β-galactosidase activities were measured in the homogenates as described under “Experimental Procedures.” CAT activity was normalized to β-galactosidase activity as an internal standard for transfection efficiency. Values are given as cpm corrected by the pRSV-enhanced phosphorylated c-Jun only 1.9-fold (Fig. 10A). Treatment of cells with higher doses of IL-6 (80 ng/ml), reached its maximal density after 12 h (8.5-fold) (Fig. 10A). (20 ng/ml), reached its maximal density after 12 h (8.5-fold) (Fig. 10A), and then declined slowly over the next 12 h (Fig. 10B). Treatment of cells with higher doses of IL-6 (80 ng/ml) enhanced phosphorylated c-Jun only 1.9-fold (Fig. 10C).

Because IL-6 increased phosphorylation of the c-Jun activation domain, we measured JNK activity in Rat-1 fibroblasts treated with IL-6 for 15 min. JNK activity was measured using a previously described solid-state assay using GST-c-Jun as substrate (41). Incubation of cells with 10 and 20 ng/ml IL-6 decreased JNK activity to 80 and 60%, respectively, of that of control cells (Fig. 10D).

IL-6 Inhibits Serine/Threonine Phosphatase—Because IL-6 enhanced phosphorylation of the c-Jun activation domain without enhancing JNK activity, we wanted to determine whether IL-6 inactivates a serine/threonine phosphatase. Measurement of the serine/threonine phosphatase activity in Rat-1 fibroblasts treated with increasing concentration of IL-6 for 6 h showed that IL-6 decreased phosphatase activity in a dose-dependent fashion. Treatment of cells with 20 or 40 ng/ml IL-6 decreased this activity to 39 or 22%, respectively, of control activity (Fig. 11A). Likewise, incubation of cells with 20 ng/ml IL-6 for 3–24 h resulted in a decrease of serine/threonine phos-
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**Fig. 5.** Effect of IL-6 on TRE in Rat-1 fibroblasts. Cells were transfected as indicated under "Experimental Procedures" with 0.5 μg of p2xTRE-Luc, 0.5 μg of pRSV-β-galactosidase, and 1.1 μg of pUC19 and cultured without fetal calf serum in the absence or presence of 20 or 40 ng/ml IL-6 for 6, 12, or 24. After the indicated time, luciferase and β-galactosidase activities were measured as mentioned. Values are given as fold over the activity in cells incubated without IL-6. Results represent mean values ± SD. *, p < 0.05; **, p < 0.01; ***, p < 0.001, as compared with control cells.

**Fig. 6.** Mobility shift assays using the AP1 probe. DNA binding was analyzed by gel retardation assay as described under "Experimental Procedures." A, time course of AP1 activation in response to IL-6 for 1–6 h. A double-stranded radiolabeled AP1 oligonucleotide was incubated with nuclear proteins extracted from control cells or cells treated with 20 ng/ml IL-6. B, nuclear extracts from cells treated for 24 h with increasing concentrations of IL-6 were incubated with radiolabeled AP1 probe. Lanes P, AP1 radiolabeled probe without addition of nuclear extract. Lanes C, probe incubated with nuclear proteins extracted from control cells. Lanes 6a and 6a, nuclear protein extract from cells treated for 6 and 24 h, respectively, with 20 ng/ml IL-6 incubated with radiolabeled AP1 probe in the presence of 200-fold molar excess of unlabeled AP1 oligonucleotide. Lane 6h, supershift after incubation of the reaction mix with specific phosphorylated c-Jun antibody prior to the gel retardation assay. Lane Ca, nuclear protein extract from control cells incubated with radiolabeled AP1 probe. Lane Cb, nuclear protein extract from control cells in the presence of 200-fold molar excess of unlabeled C/EBP oligonucleotide. Lane Cc, supershift after incubation of the reaction mix with specific C/EBP antibody (αebp). The results presented are representative of four separate experiments. NE, nuclear protein extract; comp., competitor; Ab., antibody; αpjd, anti-phosphorylated c-Jun antibody; AP, unlabeled AP1 oligonucleotide, ebp, C/EBP oligonucleotide; probe, radiolabeled probe.

**Fig. 7.** c-Jun, JunB, and c-Fos mRNA are induced by IL-6. Total RNA was isolated from Rat-1 fibroblasts cultured with 0–30 ng/ml IL-6 for 24 h and analyzed by Northern blotting as described under "Experimental Procedures." The blot was hybridized with 32P-labeled probes specific for c-Jun, JunB, c-Fos, and 18 S RNA, which served as a control for sample loading. Autoradiograms were quantitated by scanning laser densitometry. The level of mRNA in each sample was normalized to the level of 18 S RNA. Blots are representative of at least three separate experiments.

**DISCUSSION**

We show that IL-6 increased the steady-state levels of MMP-13 mRNA in a dose- and time-dependent manner (Fig. 1) and that this effect mediated within the gene promoter (Fig. 3). This effect was evident after 6 h of treatment but was particularly marked after 24 h. This prolonged incubation time required by IL-6 to stimulate MMP-13 gene expression and MMP-13 mRNA levels suggests that de novo synthesis of a protein may be required for this effect. This requirement is supported by the fact that the IL-6-induced increase in MMP-13 mRNA levels was blocked by inhibiting protein synthesis with cycloheximide (Fig. 1D). As expected, the enhanced MMP-13 gene expression was associated with a striking increase in the immunoreactive MMP-13 protein (Fig. 2A). These results agree with those reported by Franchimont et al. (25) and Kusano et al. (45), who demonstrated that IL-6, in the presence of its soluble receptor, increased MMP-13 mRNA levels (25, 45), immunoreactive MMP-13 (25), and its biological activity (25). Our study demonstrated that the effect of IL-6 on MMP-13 secreted into the culture medium was much higher than that induced on the steady-state levels of MMP-13 mRNA. We speculate that this difference might be ascribed to an effect of IL-6 on the extracellular metabolism of secreted MMP-13. In fact, Sehgal and Thompson (46) recently showed that transforming growth factor β1 induced a marked increase in stability of MMP-2 protein, resulting in a significantly enhanced MMP-2 protein level in culture medium, despite an unchanged steady-state level of MMP-2 mRNA. Although some authors have shown that IL-6 significantly enhanced TIMP-1 production and TIMP-1 mRNA expression in human fibroblasts and other cell lines (17, 22, 25–28), we found that IL-6 increased immunoreactive TIMP-1 only slightly (Fig. 2B).

Rat MMP-13 gene displays a general organization similar to that of other members of the MMP family (47, 48), particularly to human (49) and rabbit MMP-1 genes (50). All share a common 10-exon organization (48, 49, 51) and contain a typical TATA box in addition to TRE and polyomavirus enhancer activator 3 (PEA-3) consensus sites in their promoter region (15, 48, 52–57), suggesting a common regulatory mechanism of gene transcription. Our study showed that sequences upstream

- Phatase activity, which was particularly marked at 6 h (44%). At 12 and 24 h, this activity was higher than at 6 h (50 and 53%, respectively, of control) (Fig. 11B).
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**Fig. 8. Effect of IL-6 on c-jun and c-fos promoter activity.** Rat-1 fibroblasts were transfected as indicated in Fig. 2 with 0.5 μg pJun-Luc (A) or pFos-Luc (B) as reporter plasmids plus 0.5 μg of pRSV-β-galactosidase and 1.1 μg of pUC19 plasmids. Transfected cells were incubated with 0–30 ng/ml IL-6 for 3–24 h. After the indicated time, cells were harvested and luciferase, and β-galactosidase activities were measured in homogenates. Luciferase values were normalized for differences in transfection efficiencies. Fold represents luciferase/β-galactosidase ratio in the presence of IL-6 divided by that obtained in the absence of IL-6. Data are the means ± SD of one experiment with triplicate determinations. *p < 0.05; **p < 0.01; ***p < 0.001, as compared with the control cells.

The stimulatory effect of IL-6 on the TRE site was also confirmed in cells transfected with a luciferase construct containing two copies of the TRE upstream of a minimal promoter (Fig. 5). Very little information exists about the effect of IL-6 on the activation of genes with a TRE. Daffada et al. (62) found that IL-6 had no effect on the expression of pTRE-CAT in transient transfected cells, suggesting that AP1 is not induced by IL-6 treatment. On the contrary, Melamed et al. (63) showed that IL-6 induced a TRE-binding complex, which was abolished by anti-Jun specific antibodies.

To confirm that IL-6 promotes the binding of nuclear proteins to the TRE, gel retardation experiments were performed (Fig. 6). Treatment of cells with IL-6 induced a dose- and time-related increase in the formation of a TRE-protein complex (Fig. 6) that contained phosphorylated c-Jun. These results support the role played by TRE and AP1 in mediating the effect of IL-6 on rat MMP-13 gene expression.

The AP1 transcription factor actually represents a heterogeneous group composed of members of the Jun and Fos families. These proteins form a variety of homo- and heterodimers that bind to a common DNA recognition site (65–74) by acting on a region containing an ETS and a STAT3 binding site (67, 69, 72). More recently, Cressman et al. (75) have shown that the expression of junB gene expression in a variety of cells (65–74) by acting on a region containing an ETS and a STAT3 binding site (67, 69, 72). More recently, Cressman et al. (75) have shown that the expression of junB and STAT3 are markedly reduced in the liver of IL-6-deficient mice.

The increase in c-Fos mRNA seems to be only partially due to enhanced transcriptional activity, because luciferase activity in cells transiently transfected with the plasmid pFos-Luc increased only slightly after IL-6 treatment. There are few studies concerning the effect of IL-6 on c-fos gene expression or Fos protein. However, a number of authors have found the induction of c-fos gene in a variety of cells (71, 76, 77) and Cressman et al. (75) reported that hepatectomy induced the expression of c-Fos protein in the liver of control mice but was reduced or absent in the livers of IL-6-knockout mice. Nevertheless, other
Separate experiments.

Recombinant GST-c-Jun was incubated with 25 μg of whole cell extracts from J-Jhan cells treated with 25 ng/ml PMA was used as positive control of phosphorylated c-Jun (Junkt PMA). D, effect of IL-6 on JNK activity in Rat-1 fibroblasts. Recombinant GST-c-Jun was incubated with 25 μg of whole cell extracts from Rat-1 fibroblasts treated without or with 10 or 20 ng/ml IL-6 for 15 min. JNK-mediated phosphorylation of GST-c-Jun was assessed by incorporation of [γ-32P]ATP, followed by SDS-polyacrylamide gel electrophoresis. Autoradiograms were quantitated by scanning laser densitometry. The single band represents GST-c-Jun. P-cJun, phosphorylated c-Jun. Blots are representative of at least three separate experiments.

Authors, working on a variety of cell lines, could not demonstrate any effect of IL-6 on c-fos. These studies showed that only some early response genes, such as the jun family, but not c-myc or c-fos, were stimulated by the addition of IL-6 (66–68).

Transcriptional activity of AP1 depends not only on the abundance of AP1 components and their ability to bind DNA but also on the degree of phosphorylation of these proteins (64). Phosphorylation of c-Jun in its activation domain at serine 63 and 73 prolongs its halflife and potentiates the ability of c-Jun to activate transcription as either a homodimer or as a heterodimer with c-Fos (64). Western blots using a specific monoclonal antibody for serine 63 phosphorylated c-Jun demonstrated that IL-6 induces an increase in this form of c-Jun, which was particularly marked after 12 h of treatment (Fig. 10). This result concurred with the study of Lüttkien et al. (70), who showed that IL-6 triggers a delayed phosphorylation of STAT3 at serine residues. A variety of protein kinases, including pp42, pp54, and pp44 mitogen-activated protein kinases, p34cdc2, protein kinase C, casein kinase II, efficiently phosphorylates c-Jun (78). JNK, also known as stress-activated protein kinase, is a member of the mitogen-activated protein kinase family that phosphorylates serines 63 and 75 of c-Jun and potentiates its transactivation function (42). However, our study indicates that IL-6 does not induce the phosphorylation of c-Jun by stimulating JNK activity (Fig. 10D). Therefore, we have to consider that the increase in phosphorylated c-Jun is the result of either a decrease in protein phosphatase activity or an activation of another protein kinase involved in the phosphorylation of c-Jun (78). Thus, a number of studies have clearly demonstrated that inhibition of protein phosphatases 1 and 2A by okadaic acid results in an induction of collagenase, JunB, and c-Fos mRNA and a potent activation of AP1, through serine/threonine phosphorylation (79–81). The results of our study concur with these reports by demonstrating that treatment of cells with IL-6 decreased serine/threonine phosphatase activity in a dose-dependent manner and that this effect was particularly marked at 6 h of treatment (Fig. 11). Despite that, we cannot exclude the participation of another protein kinase. In fact, Belka et al. (82) found that IL-6-mediated phosphorylation of the small heat shock protein 27 was the result from activation of the mitogen-activated protein-kinase-activated protein kinase 2, a serine/threonine kinase that is activated by mitogen-activated protein kinase.

In conclusion, this study shows that treatment of Rat 1 fibroblasts with IL-6 stimulated MMP-13 gene expression in a time- and dose-dependent manner. This effect was associated with an enhanced expression of jun and fos genes, an increase in the DNA-binding activity of API, and an elevation of phospho-c-Jun. The latter increase was not mediated by enhanced JNK activity but was associated with decreased serine/threonine phosphatase activity.

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