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IL-4 Up-Regulates the Expression of Tissue Inhibitor of Metalloproteinase-2 in Dermal Fibroblasts Via the p38 Mitogen-Activated Protein Kinase-Dependent Pathway

Hironobu Ihn, Kenichi Yamane, Yoshihide Asano, Masahide Kubo, and Kunihiko Tamaki

Tissue inhibitor of metalloproteinase-2 (TIMP-2) is a potent inhibitor of activated matrix metalloproteinases such as gelatinase and collagenase, and thus helps to control extracellular matrix metabolism and deposition by connective tissue cells. We examined the responsiveness of the expression of TIMP-2 to various cytokines in dermal fibroblasts and studied the regulatory and signaling mechanisms of the response. TIMP-2 protein and mRNA expression was induced by IL-4 in a dose- and time-dependent manner, but not by TGF-β, oncostatin M, or IL-6. IL-4 induction of TIMP-2 expression was dependent upon transcription. The p38 mitogen-activated protein kinase (MAPK) inhibitors SB202190 and SB203580 suppressed IL-4-induced TIMP-2 expression, suggesting the involvement of p38 MAP kinase in the signaling of IL-4 leading to TIMP-2 expression. Immunoblotting analysis using a specific Ab against phosphorylated p38 MAP kinase (Thr180/Tyr182) showed that IL-4 induced phosphorylation of p38 MAP kinase in human dermal fibroblasts. Furthermore, the p38 MAP kinase assay showed that IL-4 induces p38 MAPK activation in human dermal fibroblasts. The expression of the dominant-negative mutant p38 MAPK represses the IL-4-induced TIMP-2 expression in human dermal fibroblasts. Thus, IL-4 can potentially alter the dermal matrix metabolism by regulating TIMP-2.


Dermal wound healing is a complex biological process involving an acute inflammatory response. Transient activation of dermal fibroblasts to proliferate and produce elevated quantities of extracellular matrix (ECM) is also essential to normal fibrotic repair. Transient fibroblast activation is probably regulated by a variety of cytokines produced by infiltrating platelets, monocytes, T lymphocytes, and other inflammation-associated cells (1). Numerous in vitro and in vivo studies have suggested that some cytokines, such as TGF-α and -β, platelet-derived growth factor (PDGF), epidermal growth factor (EGF), IL-1, IL-6, and oncostatin M (OSM), regulate dermal fibroblast proliferation and ECM deposition (2–9). Dermal fibroblasts derived from patients with systemic sclerosis or keloid appear to be persistently activated and produce elevated levels of ECM components in tissue cultures. It is postulated that this persistent fibroblast activation occurs as a result of chronic exposure to various cytokines, such as TGF-β, and PDGF (10–12).

Matrix metalloproteinases (MMPs) are important breakdown enzymes of ECM components such as collagen. These enzymes consist of collagenases, stromelysins, gelatinases, and membrane-type MMPs, which degrade various components of the ECM (13). A family of tissue inhibitors of metalloproteinases (TIMPs), comprising TIMP-1, -2, -3, and -4 (14–17), is now recognized as a member of a family of intrinsic common MMP inhibitors. Pathologic turnover of ECM is believed to be a consequence of a TIMP-MMP imbalance (18).

TIMP-1 and TIMP-2 are produced in soluble form, but TIMP-3 is not soluble and is associated with ECM. Their expressions are regulated differently. The production of TIMP-1 is enhanced by cytokines, such as TGF-β and OSM (19, 20), and the expression of TIMP-3 is induced by mitogenic stimuli, such as serum, EGF, and TGF-β (21, 22). The expression of TIMP-2 mRNA was shown to be down-regulated in response to TGF-β, but is unchanged in response to phorbol ester treatment in human tumor cells (23). TIMP-2 biosynthesis was unaffected by IL-1, TNF-α, PDGF, or phorbol ester in human alveolar macrophages (24).

In this study we investigated the regulation of TIMP-2 expression by various cytokines believed to be involved in dermal fibrosis using cultured human dermal fibroblasts. We examined the effects of TGF-β, OSM, IL-6, and IL-4. The results suggest that IL-4 stimulates the expression of TIMP-2 in dermal fibroblasts. Further analyses suggested that IL-4 induces TIMP-2 synthesis via the p38 mitogen-activated protein kinase (MAPK)-dependent pathway.

Materials and Methods

Cytokines and other materials

Recombinant human IL-4, IL-6, OSM, polyclonal rabbit anti-human IL-4, and polyclonal rabbit anti-human IL-6 were obtained from Genzyme (Cambridge, MA). Recombinant human TGF-β was purchased from R&D Systems (Minneapolis, MN). SB203580, SB202190, PD98059, wortmannin, and LY294002 (Calbiochem, La Jolla, CA) were dissolved in DMSO. The p38 MAPK rabbit polyclonal Ab was purchased from Santa Cruz Biotechnology (Santa Cruz, CA). The phospho-p38 MAP kinase (Thr180/Tyr182) rabbit polyclonal Ab and activation transcription factor 2 (ATF-2) fusion protein were...
obtained from New England Biolabs (Beverly, MA). Anti-β-actin mAb was purchased from Sigma-Aldrich (St. Louis, MO).

Fibroblast cultures

Human adult skin fibroblasts were grown from explants of forearm skin biopsies from six healthy donors, with institutional approval and informed consent. Cells were seeded in MEM supplemented with 10% FCS. Fibroblast cultures independently isolated from different individuals were maintained as monolayers at 37°C in an atmosphere of 5% CO₂ and 95% air. Fibroblasts under the fifth subpassage were used for the experiments. Cell viabilities were determined by trypan blue stain.

Measurement of TIMP-2 levels in the conditioned medium

The TIMP-2 concentration was determined with a specific sandwich ELISA kit (Amersham, Arlington Heights, IL) that used two kinds of mAbs against human TIMP-2, as described previously (25). Normal human dermal fibroblasts (1 × 10⁵ cells) were seeded in six-well plates in MEM with 10% FCS and grown to confluence. Cells were then plated in 0.5 ml of MEM and 0.1% BSA for 24 h before the cytokine treatment. After incubation with various cytokines for the indicated periods, the conditioned medium was collected and clarified by centrifugation. The cells remaining in the dishes were treated with trypsin and counted electronically. The TIMP-2 concentration in the wells was determined as described above and evaluated according to cell numbers per well.

Immunoblotting

For the preparation of medium collection from fibroblasts untreated and treated with various cytokines, cells were plated in MEM and 0.1% BSA for 24 h before cytokine treatment. After incubation with various cytokines for the indicated periods, the conditioned medium was collected and clarified by centrifugation, and the cells remaining in the dishes were treated with trypsin and counted electronically. The samples were normalized for cell number, subjected to electrophoresis on 15% SDS-PAGE slab gels, and then electrotransferred from the gels onto nitrocellulose sheets. The nitrocellulose sheets were then incubated overnight with a rabbit mAb against human TIMP-2 (Fuji Chemical, Tokyo, Japan). Bound Abs were detected with HRP-conjugated anti-rabbit IgG, and immunoreactive bands were visualized with ECL (Amerham) as described previously (26).

RNA preparation and Northern blot analysis

Fibroblasts were grown to confluence in MEM with 10% FCS and then incubated for 24 h in serum-free medium (MEM plus 0.1% BSA) before addition of the cytokine (27). Total RNA was extracted and analyzed by Northern blotting as described previously (27–29). The full-length 791-bp TIMP-2 cDNA was obtained from American Type Culture Collection (Manassas, VA) and used as the probe (23). Filters were sequentially hybridized with probes for TIMP-2 and GAPDH. Autoradiography was performed, and the densities of the bands were measured using a densitometer.

Assay of p38 MAPK activation

The activation of p38 MAPK was determined by immunoblotting using Abs specific for phosphorylated, activated forms of p38 MAPK (Thr180/ Tyr182; New England Biolabs) with a p38 MAPK assay kit (New England Biolabs) (30).

In both experiments fibroblasts were serum-starved for 24 h and treated with cytokines for the indicated time. Then, the conditioned medium was removed, and the cells were washed with ice-cold PBS. The cells were lysed by scraping into solubilization buffer (50 mM of Tris-Cl (pH 8), 150 mM of NaCl, 2 mM of EDTA, 1% Triton X-100, 1 mM of sodium orthovanadate, 0.2 mM of PMSF, 10 μg/ml aprotinin, 10 μg/ml leupeptin, and 10 μg/ml pepstatin). The lysate was incubated at 4°C for 30 min and then centrifuged for 5 min at 4°C. Protein concentrations of lysates were determined using a Bio-Rad (Hercules, CA) protein assay kit as recommended by the manufacturer.

Immunoblotting was performed as described previously (26). Briefly, cell lysates (30 μg) obtained from fibroblasts were subjected to electrophoresis on 10/20% gradient SDS-PAGE slab gels, and then electrotransferred from the gels onto nitrocellulose sheets. The nitrocellulose sheets were incubated overnight with mAbs specific for phosphorylated, activated forms of p38 MAPK (Thr180/Tyr182; 1/1000 dilution). Bound Abs were detected with HRP-conjugated anti-rabbit IgG, and immunoreactive bands were visualized with ECL (Amerham, Arlington Heights, IL) as described previously (26). Abs against p38 MAPK (Santa Cruz Biotechnology) were also used to confirm that the protein concentrations of p38 MAPK were maintained.

In the p38 MAPK assay (30), 200 μg lysates were incubated with an immobilized phospho-p38 MAPK (Thr180/Tyr182) mAb overnight at 4°C for immunoprecipitation. For kinase assays the beads were incubated with 200 μM of ATP and 2 μg of ATP-2 fusion protein as a substrate for p38 MAPK at 30°C for 30 min. The reaction was terminated with 25 μl of SDS sample buffer. The samples were then boiled for 5 min, subjected to SDS-PAGE, and transferred to nitrocellulose membranes. Membranes were incubated overnight at 4°C with a phospho-ATR-2 (Thr185, Tyr185) Ab (New England Biolabs). The membranes were washed, then incubated with HRP-conjugated anti-rabbit IgG, and immunoreactive bands were visualized with ECL.

Transfections and constructs

Transient transfections were performed as described previously (26–29). Fibroblasts were transfected by the lipofection technique (FuGene 6 transfection reagent; Roche, Indianapolis, IN) with various amounts of constructs. Dominant-negative mutants (TGY-AGF) of p38β (dn p38β) and p38α (dn p38α) MAPK were provided by Dr. J. Hans (The Scripps Research Institute, La Jolla, CA) (31). The plasmid used encodes the ERK2 (p42 MAPK) cDNA in which Thr183 and Tyr185, which are required to be phosphorylated for activity, were replaced with either glutamic acid or alanine and phenylalanine, thus rendering the protein active (32–34). Plasmids used in transient transfection assays were twice purified on CsCl gradients. At least two different plasmid preparation were used for each experiment. pSV-β-galactosidase control vector (Promega, Madison, WI) was transfected to visualize for transfection efficiency. After incubation overnight, the medium was replaced with serum-free MEM and 0.1% BSA. The cells were stimulated with cytokines for 24 h in the absence of serum, and the conditioned medium was collected and clarified by centrifugation. Then, the cells remaining in the dishes were treated with trypsin and counted electronically.

Statistical analysis

Statistical analysis was conducted with the Mann-Whitney test for comparison of means. A value of p < 0.05 was considered significant.

Results

IL-4 up-regulated the expression of TIMP-2 in dermal fibroblasts

To investigate the regulation of TIMP-2 expression in dermal fibroblasts, we studied the effects of fibrogenic cytokines, TGF-β, OSM, IL-6, and IL-4, on the expression of TIMP-2 protein in normal dermal fibroblasts. Ten nanograms per milliliter of each cytokine was added for 24 h, and TIMP-2 protein levels in conditioned medium were determined using a specific TIMP-2 ELISA. As shown in Fig. 1A, TIMP-2 expression was not significantly affected by TGF-β, OSM, or IL-6. Furthermore, TIMP-2 expression was not significantly affected by higher concentrations (25 or 50 ng/ml) of TGF-β, OSM, and IL-6. On the other hand, IL-4 induced secretion of TIMP-2 into conditioned medium approximately 6-fold (Fig. 1A).

As shown in Fig. 1B, IL-4 stimulated TIMP-2 synthesis in dermal fibroblasts in a dose-dependent manner. TIMP-2 secretion induced by IL-4 reached the maximum level when the concentration of IL-4 was 25 ng/ml. Furthermore, IL-4 stimulated TIMP-2 synthesis in dermal fibroblasts in a time-dependent manner. TIMP-2 secretion induced by IL-4 reached a maximum level 24–48 h after stimulation with IL-4, and this increase diminished, but was sustained from 48–72 h (Fig. 1C).

Anti-IL-4 Ab (10 μg/ml) completely abolished the IL-4-mediated induction of TIMP-2 expression (Fig. 1D). This amount of anti-IL-4 Ab was shown to neutralize the biological activity of IL-4 (data not shown). However, anti-IL-4 Ab had little effect on basal TIMP-2 expression. As a control, anti-IL-6 Ab was also used. As shown in Fig. 1D, anti-IL-6 Ab (10 μg/ml, this amount of anti-IL-6 Ab was shown to neutralize the biological activity of IL-6) had little effect on either IL-4-induced TIMP-2 expression or basal TIMP-2 expression.
FIGURE 1. IL-4 up-regulates the expression of TIMP-2 in dermal fibroblasts. TIMP-2 expression in the culture supernatants from dermal fibroblasts with or without various cytokines was determined using a specific ELISA. A, Ten nanograms per milliliter of each cytokine was added. B, Various amounts of IL-4 were added for 24 h. C, The time course of the IL-4 effect (10 ng/ml) was determined. The mean ± SE for four separate experiments are shown. Comparisons of TIMP-2 expression were made between unstimulated and stimulated dermal fibroblasts. Asterisks indicate statistically significant results ($p < 0.001$). D, Anti-IL-4 Ab abolished the increase in TIMP-2 expression in human dermal fibroblasts stimulated with IL-4 (10 ng/ml). Addition of anti-IL-4 Ab (10 μg/ml) abolished IL-4-induced TIMP-2 expression, but anti-IL-6 Ab (10 μg/ml) did not. The mean ± SE for four separate experiments are shown. Comparisons of TIMP-2 expression were made between unstimulated and stimulated dermal fibroblasts. Asterisks indicate statistically significant results ($p < 0.01$).
To further confirm the increase in TIMP-2 secretion after IL-4 stimulation, immunoblotting was performed using a mAb against human TIMP-2. Consistent with the results obtained using a specific TIMP-2 ELISA, IL-4 induced TIMP-2 synthesis in dermal fibroblasts in a dose-dependent manner (data not shown). Furthermore, IL-4 up-regulated TIMP-2 synthesis in a time-dependent manner (data not shown).

To determine whether the IL-4-mediated induction of TIMP-2 expression was associated with the corresponding mRNA level, human dermal fibroblasts were incubated in the absence or the presence of 10 ng/ml IL-4 under the same conditions, and the expression of TIMP-2 mRNA was analyzed by Northern blotting. The expression of TIMP-2 mRNA was markedly elevated (~6-fold) after 6 h in comparison with untreated fibroblasts and gradually decreased after 12–24 h (Fig. 2, A and B). Thus, the effect of IL-4 on the expression of TIMP-2 mRNA paralleled its effect on the corresponding protein synthesis.

**IL-4-induced TIMP-2 secretion is mediated at the level of transcription in dermal fibroblasts**

To determine whether up-regulation of TIMP-2 synthesis is due to increased RNA synthesis, the effect of IL-4 on TIMP-2 expression was examined by treating human dermal fibroblasts with actinomycin D. Dermal fibroblasts were serum-starved for 24 h and incubated in serum-free medium for 12 h in the presence or the absence of actinomycin D (400 ng/ml). Cell viability was determined with trypan blue stain, which demonstrated that actinomycin D did not cause cell death. Actinomycin D completely abolished the IL-4-mediated increase in TIMP-2 mRNA levels (Fig. 3). Thus, IL-4-induced up-regulation of TIMP-2 synthesis is mediated at the level of transcription.

**Induction of TIMP-2 expression in human dermal fibroblasts is mediated by p38 MAPK**

To further understand the mechanism of IL-4-mediated induction of TIMP-2 synthesis, we investigated the roles of MAPKs using selective MAPK inhibitors. First, to block the ERK1/2 pathway, we added PD98059, a specific inhibitor of the MAPK kinase 1 (MEK1) and MEK2 activation (35, 36), to dermal fibroblasts 1 h before treatment with IL-4. Treatment with PD98059 (30 μM) did not change basal TIMP-2 expression or IL-4-mediated induction of TIMP-2 expression (Fig. 4). Next, to block the phosphatidylinositol 3-kinase (PI3 kinase) pathway, we added wortmannin or LY 294002, specific inhibitors of PI3 kinase activation (37, 38), to dermal fibroblasts. Neither wortmannin (100 nM) nor LY 294002 (10 nM) changed basal or IL-4-induced TIMP-2 expression (Fig. 4A). In contrast, addition of the selective p38 MAPK inhibitor SB203580 (10 μM) or SB202190 (10 μM) (39, 40) entirely abolished the IL-4-mediated induction of TIMP-2 expression in a dose-dependent manner (Fig. 4). Cell viabilities were determined by trypan blue stain, which demonstrated that the addition of these concentrations of the inhibitors tested did not have a cytotoxic effect. Furthermore, the synthesis of β-actin was not inhibited by SB203580 or SB202190 (Fig. 4B). These results suggest that p38 MAPK is involved in IL-4-induced up-regulation of TIMP-2 secretion in dermal fibroblasts.

**IL-4 induces p38 MAPK activation in human dermal fibroblasts**

Since inhibition of p38 MAPK significantly decreased IL-4-induced TIMP-2 expression, we determined the activation of p38 MAPK by immunoblotting using Abs specific for phosphorylated,
TIMP-2 expression (lane 5) or IL-4-mediated (10 ng/ml, for 24 h) induction of TIMP-2 expression was determined by a specific TIMP-2 ELISA. To block the MEK1 and MEK2 activation, we added PD98059 (30 μM) and SB202190 (10 μM) entirely abolished IL-4-mediated induction of TIMP-2 expression. PD98059 (30 μM) did not change IL-4-mediated induction of phosphorylated ATF-2 (Fig. 6B). These results suggest that treatment with IL-4 results in the activation of p38 MAPK in human dermal fibroblasts.

Expression of the dominant-negative mutant p38 MAPK represses IL-4-induced TIMP-2 expression in human dermal fibroblasts

To further confirm the role of p38 MAPK in IL-4-induced TIMP-2 expression in dermal fibroblasts, transient transfection of the dominant-negative mutant p38 MAPK into dermal fibroblasts was performed. As shown in Fig. 7, transient transfection of the dominant-negative mutant p38 MAPKΔ or p38 MAPKβ abolished IL-4-induced TIMP-2 expression in dermal fibroblasts in a dose-dependent manner. Cell viabilities were determined by trypan blue stain, which demonstrated that the transient transfection of these amounts of the plasmids did not have a cytotoxic effect. Furthermore, the synthesis of β-actin was not changed by the transient transfection of these plasmids. On the other hand, transient transfection of the dominant-negative mutant ERK2 in fibroblasts did not abolish IL-4-induced TIMP-2 expression.

Discussion

IL-4, a cytokine synthesized by T lymphocytes and mast cells, was originally identified as a growth regulator of B lymphocytes (41). However, IL-4 has been shown to exert a variety of effects on different types of cells. Studies have shown that fibroblasts are target cells for IL-4 (7, 42, 43) and that T lymphocyte and mast cell infiltration is observed in normal dermal repair as well as fibrotic lesions (44, 45). Recent studies demonstrated that IL-4 is a potent activator of ECM synthesis, including collagen and tenascin (7, 42, 43). Immunoblotting of whole cell extracts revealed that p38 MAPK Thr180/Tyr182 phosphorylation gradually occurred after treatment with 10 ng/ml IL-4 (Fig. 5). IL-4-induced phosphorylation of p38 MAPK reached the maximum level after 3 h and diminished after 6 h. Abs against p38 MAPK were also used to confirm that the protein concentrations of MAPKs were maintained with or without IL-4 stimulation.

In the p38 MAPK assay, IL-4 stimulation increased phosphorylation of ATF-2. The levels of phosphorylated ATF-2 were maximal 3 h after stimulation with 10 ng/ml IL-4 (Fig. 6A). In addition, SB203580 (10 μM) and SB202190 (10 μM) entirely abolished the IL-4-induced activation of ATF-2, whereas PD98059 (30 μM) did not change IL-4-mediated induction of phosphorylated ATF-2 (Fig. 6B). These results suggest that treatment with IL-4 results in the activation of p38 MAPK in human dermal fibroblasts.

FIGURE 4. The p38 MAPK inhibitor abolished the increase in TIMP-2 expression in human dermal fibroblasts stimulated with IL-4.

A. TIMP-2 expression in the culture supernatants from dermal fibroblasts was determined by a specific TIMP-2 ELISA. To block the ERK1/2 pathway, we added PD98059 (30 μM), a specific inhibitor of MEK1 and MEK2 activation, to dermal fibroblasts 1 h before treatment with IL-4 (10 ng/ml) for 24 h. To block the PI3 kinase pathway, we added wortmannin (100 nM) or LY294002 (10 nM), specific inhibitors of PI3 kinase activation, to dermal fibroblasts 1 h before treatment with IL-4 (10 ng/ml) for 24 h. To block the PI3 kinase pathway, we added wortmannin (100 nM) or LY294002 (10 nM), specific inhibitors of PI3 kinase activation, to dermal fibroblasts. Addition of the selective p38 MAPK inhibitors, SB203580 (10 μM) and SB202190 (10 μM), entirely abolished the IL-4-mediated induction of TIMP-2 expression. The mean ± SE for four separate experiments are shown. Asterisks indicate statistically significant results (p < 0.001).

B. TIMP-2 expression in the culture supernatants from dermal fibroblasts was determined by immunoblotting. PD98059 (30 μM) did not change basal TIMP-2 expression. However, addition of wortmannin (100 nM) or LY294002 (10 nM) did not change basal TIMP-2 expression.

FIGURE 5. IL-4-phosphorylated p38 MAPK in human dermal fibroblasts. Phosphorylation of p38 MAPK in dermal fibroblasts was determined by immunoblotting using Abs specific for phosphorylated, activated forms of p38 MAPK (Thr180/Tyr182). Immunoblotting of whole cell extracts revealed that p38 MAPK Thr180/Tyr182 phosphorylation gradually occurred after treatment with 10 ng/ml IL-4. Abs against p38 MAPK were also used to confirm that the protein concentrations of MAPKs were maintained with or without IL-4 stimulation. Representative results of four separate experiments are shown.
43). Therefore, IL-4 could play a role in dermal fibrotic repair or fibrosis.

The expressions of TIMPs are known to be regulated differently. For example, the production of TIMP-1 is enhanced by cytokines, such as TGF-β/H9252 or OSM (19, 20), and the expression of TIMP-3 is induced by mitogenic stimuli, such as serum, EGF, or TGF-β/H9252 (21, 22). Consistent with the previous reports (23, 24), we found no significant effect of TGF-β, OSM, or IL-6 on TIMP-2 expression in human fibroblasts. To our knowledge this is the first report that IL-4 stimulates the synthesis of TIMP-2 in human dermal fibroblasts.

IL-4 is known to use the Janus kinase-STAT signaling pathway (46, 47) and the PI3 kinase pathway (48). Recent studies demonstrated that IL-4 induces p38 MAPKs (49). This is the first report demonstrating IL-4-induced p38 MAPK activation in fibroblasts. In this study immunoblotting analyses using phospho-specific p38 MAPK Abs that detect only the Thr<sup>180</sup>Tyr<sup>182</sup>-phosphorylated forms of p38 MAPK demonstrated that IL-4-induced phosphorylation of p38 MAPK in dermal fibroblasts (Fig. 5). Furthermore, p38 MAPK activation was confirmed using a p38 MAPK assay. In the p38 MAPK assay, IL-4 stimulation increased phosphorylation of ATF-2 (Fig. 6A). These results suggest that treatment with IL-4 results in the activation of p38 MAPK in human dermal fibroblasts.

The present study showed that IL-4 up-regulates the synthesis of TIMP-2 in dermal fibroblasts via the p38 MAPK-dependent pathway. We determined whether the p38 MAPK pathway was involved in the IL-4-induced TIMP-2 synthesis in dermal fibroblasts, using approaches to block the signaling pathways. First, we used an MEK1-specific inhibitor, PD98059, which blocks MEK1 activation by Raf, thus preventing downstream activation of p42/p44 MAPKs. However, it does not inhibit c-Jun N-terminal kinase or...
p38 MAPK (35, 36). In our study pretreatment of fibroblasts with PD98059 did not abolish IL-4-stimulated TIMP-2 synthesis. Furthermore, neither wortmannin nor LY294002, specific inhibitors of activation of PI3 kinase (37, 38), affected IL-4-induced TIMP-2 synthesis in contrast, addition of selective p38 MAPK inhibitors, SB203580 and SB202190 (39, 40), entirely abolished IL-4-mediated induction of TIMP-2 expression in a dose-dependent manner (Fig. 4). Recent studies showed that collagen-dependent induction of MMP-13 requires p38 MAPK activity (50). These results suggest that p38 MAPK is involved in the modulation of ECM in dermal fibroblasts. However, the phosphorylation and activation of p38 MAPK were gradual (Figs. 5 and 6). These slow kinetics suggest that the effect may be indirect.

MAPK modules are involved in the signal transduction of a wide variety of signals in all eukaryotic organisms. In mammalian cells, three well-characterized modules coexist: p44/p42 MAPKs, p38 MAPK, and JNK cascades (51). P38 MAPK (also known as p38 MAPK) are activated by the dual-specific kinase MKK3/6, following exposure to products of microbial pathogens, environmental stress and proinflammatory cytokines, such as TNF-α or IL-1 (52, 53). It will be of great interest to determine whether similar cytokine-induced p38 MAPK-dependent signaling pathways operate in vivo to promote ECM modulation.

IL-4 is known to be a fibrogenic cytokine that promotes bio- genesis of ECM proteins in normal wound healing and in pathologic fibrosis (7, 54). The present findings potentially have important clinical implications in dermal wound healing and many fibrotic diseases.

References


