Regulation of Interleukin-1β-induced Platelet-derived Growth Factor Receptor-α Expression in Rat Pulmonary Myofibroblasts by p38 Mitogen-activated Protein Kinase*

Received for publication, December 10, 1999, and in revised form, March 31, 2000 Published, JBC Papers in Press, May 11, 2000, DOI 10.1074/jbc.M909785199

Yi-Zhe Wang, Ping Zhang, Annette B. Rice, and James C. Bonner‡

From the Laboratory of Pulmonary Pathobiology, NIEHS, National Institutes of Health, Research Triangle Park, North Carolina 27709

The potential role of p38 mitogen-activated protein (MAP) kinase in platelet-derived growth factor receptor-α (PDGF-Rα) gene expression was investigated using cultured rat pulmonary myofibroblasts. p38 MAP kinase was constitutively expressed in myofibroblasts and activated by interleukin (IL)-1β. A pyridylimidazole compound, SB203580, completely inhibited the ability of p38 MAP kinase activity to phosphorylate PHAS-1 substrate. SB203580 inhibited IL-1β-induced up-regulation of PDGF-Rα mRNA and protein in a concentration-dependent manner. Other kinase inhibitors, including the mitogen-activated protein kinase/extracellular signal-regulated kinase inhibitor PD98059, did not block up-regulation of PDGF-Rα. The IL-1β-induced increase in the number of 125I-PDGF-AA-binding sites at the cell surface was reduced >70% by pretreatment with SB203580. Accordingly, an enhancement of PDGF-AA-stimulated DNA synthesis following IL-1β pretreatment was blocked >70% by SB203580. SB203580 did not affect IL-1β-induced ERK activation, yet enhanced IL-1β-induced JNK activation approximately 2-fold. Treatment of cells with SB203580 after inhibition of transcription by actinomycin D decreased the half-life of IL-1β-induced PDGF-Rα mRNA from >4 to 1.5 h. Moreover, pretreatment of cells with cycloheximide blocked induction of PDGF-Rα mRNA by IL-1β, suggesting that de novo protein synthesis was required for PDGF-Rα mRNA stabilization. These data indicate that p38 MAP kinase regulates PDGF-Rα expression at the translational level by signaling the synthesis of an mRNA-stabilizing protein.

Platelet-derived growth factor (PDGF) is a potent mesenchymal cell mitogen and chemotactant that exists as a disulfide-linked dimer of two polypeptide chains, A or B, that form functional PDGF-AA, PDGF-BB, or PDGF-AB isoforms (reviewed in Ref. 1). Two PDGF receptor subtypes bind the three isoforms of PDGF differentially; β1-PDGF receptor (PDGF-Rβ1) can interact only with B-chain containing isoforms while α2-PDGF receptor (PDGF-Rα) can bind all three isoforms (2). PDGF binding results in receptor dimerization to form αα, αβ, or ββ combinations, followed by tyrosine kinase phosphorylation of the intracellular receptor domain and activation of a vast array of signal transduction molecules including Src family kinases, Grb2, Shc, phosphatidylinositol 3-kinase, GAP, Shb, PTP 1D, and phospholipase C-γ (reviewed in Ref. 3). The biologic activity of PDGF isoforms on rat pulmonary myofibroblasts is modulated in the extracellular microenvironment through interaction with its binding protein, α2-macroglobulin (4, 5), and by regulation of cell-surface PDGF-Rα (6, 7).

The PDGF-Rα and its ligand, PDGF-AA, are essential to lung development (8), yet induction of the PDGF-Rα also occurs in adult tissues during the pathogenesis of certain fibroproliferative diseases. For example, human fibroblasts isolated from dermal keloids express elevated PDGF-Rα (9). We and others have reported that PDGF-Rα is up-regulated during the progression of pulmonary fibrosis in rats, while the PDGF-Rβ is constitutively expressed (10, 11). Interleukin (IL)-1β is a potent inducer of the PDGF-Rα on cultured myofibroblasts isolated from rat lung and PDGF-Rα up-regulation enhances the mitogenic and chemotactic responses to PDGF isoforms (6, 12). The maximal responses of connective tissue cells to PDGF isoforms require PDGF-Rα in addition to the normally abundant PDGF-Rβ (7, 13), and this could be due to unique signal transduction events stimulated by α2 receptor dimerization, as compared with ββ receptor dimerization (14). Other mediators, including transforming growth factor-β (15) and prostaglandin E2 (16) suppress PDGF-Rα expression and counteract the up-regulatory effect of IL-1β.

It is becoming increasingly clear that IL-1β signals the production of a variety of different mediators (e.g. cytokines, metalloproteinases, prostaglandin H synthase 2, nitric oxide, and inducible nitric-oxide synthase) via the activation of p38 mitogen-activated protein (MAP) kinases (17–20). p38 MAP kinase is activated upon stimulation of cells with cytokines, bacterial lipopolysaccharide, and stress (21, 22). Several transcription factors are substrates for p38 MAP kinase isozymes, including MAP-KAP kinase-2 (23), ATF-2 (24), CHOP/GADD153 (25), MAX (26), myocyte enhancer factor 2C (27), and ternary complex factor (28). In addition to the original p38 (also termed p38α, cytokine-suppressive, anti-inflammatory drug-binding protein-2, or SAPK2A), the p38 subgroup of MAP kinases now consists of cytokine-suppressive, anti-inflammatory drug-binding protein 1 (29), Mxi2 (26), p38β (also known as SAPK2B), p38-2 (also known as p38β2) (30), p38γ (also known as ERK6 or

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‡ To whom correspondence should be addressed: Laboratory of Pulmonary Pathobiology, NIEHS, National Institutes of Health, Research Triangle Park, North Carolina 27709.

The abbreviations used are: PDGF, platelet-derived growth factor; PDGF-Rα, α-PDGF receptor; PDGF-Rβ1, β-PDGF receptor; IL, interleukin; MAP, mitogen-activated protein; ERK, extracellular signal-regulated kinase; JNK, c-Jun NH2-terminal kinase; MEK-1, MAP kinase kinase; NF-κB, nuclear factor-κB; AP-1, activator protein-1; SDFDM, serum-free defined medium; FBS, fetal bovine serum; DMEM, Dulbecco’s modified Eagle’s medium; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; LPS, lipopolysaccharide; TNF-α, tumor necrosis factor-α; C/EBP, CAAT/enhancer-binding protein; PHAS, phosphorylated heat-stable protein.
Bromphenol blue, 20% glycerol, and 50 mM 2-mercaptoethanol and fluoride, 1 mM phenylmethylsulfonyl fluoride, 0.25% sodium deoxy-

The membrane was separated by SDS-PAGE in a 10–20% Tris glycine gel for p38 MAP kinase activation following IL-1β treatment results in the stabilization of PDGF-Rα mRNA and this requires de novo protein synthesis. These findings indicate that p38 MAP kinase regulates PDGF-Rα expression at the translational level via synthesis of an mRNA-stabilizing protein.

**EXPERIMENTAL PROCEDURES**

**Reagents—**Reagents were from the indicated sources, SB203580 (CalBiochem, La Jolla, CA); PD98059 (Promega, Madison, WI); cycloheximide (Sigma); [3H]thymidine (Amersham Pharmacia Biotech); anti-phospho-p38 MAP kinase and anti-p38 (total) MAP kinase (New England Biolabs); anti-PDGF-Rα and anti-PDGF-Rβ (Santa Cruz, Santa Cruz, CA); TRI† reagent (Molecular Research Center, Cincinnati, OH); p38 MAP kinase kit (Stratagene, La Jolla, CA). The PDGF-Rα cDNA was a generous gift from Dr. Yutaka Kitami, Ehime University, Japan.

**Cell Culture—**Primary rat pulmonary myofibroblasts were isolated from male Harlan Sprague-Dawley rats as described previously (12). These cells stain positively for vimentin, desmin, and α-smooth muscle actin which indicated a myofibroblast phenotype (10). In addition, examination of glutaraldehyde-fixed cell pellets by transmission electron microscopy showed ultrastructural features consistent with a myofibroblast phenotype (abundant intermediate filaments and rough endoplasmic reticulum, and lack of Weibel-Palade bodies characteristic of endothelial cells). Cells were grown to confluence in 10% FBS/DMEM before being seeded for the assays described below.

**Western Blot Analysis—**Cells were grown to a confluent state in 10% FBS/DMEM in 75-cm² tissue culture dishes, then rendered quiescent for 24 h with serum-free defined medium (SDFM) consisting of Ham’s F-12 medium supplemented with 0.25% bovine serum albumin and an insulin/transferrin/selenium mixture (Roche Molecular Biochemicals). After treatment with agent of interest for 24 h, the cultures were washed with phosphate-buffered saline-Tween prior to a 90-min incubation with an ECL kit (Amersham Pharmacia Biotech). The membranes were washed 3 times with a 1:2,000 dilution of horseradish peroxidase-swine anti-rabbit IgG (BioLab) was used at a dilution of 1:1,000. Rabbit anti-mouse PDGF-Rβ antibodies overnight at 4 °C. Anti-phospho-p38 antibody (New England Biolabs) was used at a 1:500 dilution. The membranes were washed 3 times with a 1:500 dilution. The membranes were washed 3 times with a 1:500 dilution. The membranes were washed 3 times with a 1:500 dilution. The membranes were washed 3 times with a 1:500 dilution. The membranes were washed 3 times with a 1:500 dilution.
**RESULTS**

Temporal Activation of p38 MAP Kinase and Up-regulation of PDGF-Rα mRNA following IL-1β Treatment—Treatment of cells with IL-1β-activated p38 MAP kinase within 30 min as detected by Western blotting for the phosphorylated form of p38 (Fig. 1A). Western blotting for total p38 protein demonstrated that the amount of unactivated p38 did not significantly change during the course of the experiment. Northern blot analysis showed up-regulation of PDGF-Rα mRNA within 2 h following IL-1β treatment, which continued to increase by 24 h (Fig. 1B). GAPDH mRNA was not significantly affected by IL-1β treatment during the course of the experiment. Densitometric evaluation of p38 MAP kinase activation and PDGF-Rα mRNA induction demonstrated that phosphorylation of p38 MAP kinase peaked prior to an increase in PDGF-Rα mRNA (Fig. 1C).

**SB203580 Inhibits IL-1β-induced p38 MAP Kinase Activity**—A specific inhibitor of p38 MAP kinase, SB203580, was used to inhibit activation of p38 MAP kinase in cells stimulated with IL-1β. SB203580 does not inhibit the phosphorylation of p38 MAP kinase, but instead inhibits the kinase activity of p38 for phosphorylating substrates (33). First, we utilized a kinase assay wherein cells were pretreated with SB203580 for 1 h prior to stimulation with IL-1β, then p38 MAP kinase was immunoprecipitated from cell lysates and assayed for its ability to phosphorylate the PHAS-1 substrate (39). IL-1β strongly activated p38 kinase activity and SB203580 (50 μM) completely inhibited p38-induced phosphorylation of PHAS-1 (Fig. 2, A and B). In addition, we used a MAPKAP kinase 2 assay to measure the inhibitory effect of SB203580, as MAPKAP kinase 2 is a downstream substrate of p38 MAP kinase (23). As shown in Fig. 2C, IL-1β clearly induced MAPKAP kinase 2 activity, which was significantly inhibited by SB203580.

**SB203580 Inhibits IL-1β-induced Up-regulation of PDGF-Rα mRNA and Protein**—Pretreatment of cells with SB203580 (50 μM) reduced the basal expression of PDGF-Rα mRNA and blocked IL-1β-induced up-regulation of PDGF-Rα mRNA by >70% (Fig. 3). IL-1β-induced up-regulation of PDGF-Rα protein was also prevented by pretreatment with SB203580 as determined by Western blot analysis using an antibody specific for the PDGF-Rα (Fig. 4). In these Western blotting experiments, the level of PDGF-Rβ was not changed by IL-1β treatment or by treatment with SB203580 (Fig. 4). An 125I-PDGF-AA binding assay was used to quantitate cell surface PDGF-Rα, since PDGF-AA binds selectively to PDGF-Rα and not PDGF-Rβ (1). SB203580 inhibited IL-1β-induced up-regulation of cell surface 125I-PDGF-AA binding to cultured cells in a concentration-dependent manner with an IC_{50} between 5 and 10 μM SB203580 (Table I). IL-1β up-regulated 125I-PDGF-AA specific binding in a dose-dependent manner that was maximal at 1 ng/ml and pretreatment with 50 μM SB203580 inhibited IL-1β-stimulated up-regulation of 125I-PDGF-AA by >70% (Fig. 5A). Scatchard analysis of 125I-PDGF-AA saturation binding data demonstrated that SB203580 prevented an increase in the number of binding sites without altering receptor affinity (Fig. 5B). A variety of other kinase inhibitors, including those for MEK (PD98059), receptor tyrosine kinases (genistein), and protein kinase C (phorbol 12-myristate 13-acetate) had no inhibitory effect on IL-1β-stimulated PDGF-Rα up-regulation (Table II).

**SB203580 Inhibits the Enhanced Mitogenic Response to PDGF-AA following IL-1β-induced Up-regulation of PDGF-Rα**—Rat pulmonary myofibroblasts had a poor mitogenic response to PDGF-AA due to the low number of constitutively expressed PDGF-Rα at the cell surface, yet pretreatment with IL-1β for 24 h enhanced the concentration-dependent PDGF-AA mitogenic response severalfold. SB203580 (50 μM) alone had no effect on [3H]thymidine uptake by rat pulmonary myofibroblasts, but pretreatment of cells inhibited the IL-1β-enhanced mitogenic response to PDGF-AA by 60–70% (Fig. 6). IL-1β caused a 3-fold increase in [3H]thymidine uptake in the absence of PDGF-AA and this increased mitogenesis was also blocked by SB203580.

**Effect of SB203580 on PDGF-Rα mRNA Stability**—To determine the effect of SB203580 on the stability of PDGF-Rα mRNA, rat pulmonary myofibroblasts were stimulated with IL-1β for 4 h to up-regulate PDGF-Rα mRNA. Cells were then treated with actinomycin D, a transcriptional inhibitor, or actinomycin D plus SB203580 was added. Total cellular RNA was
isolated following various time periods after the addition of actinomycin D and examined for the presence of PDGF-Rα mRNA or GAPDH mRNA by Northern blot analysis. A representative result is shown in Fig. 7A. For correction for differences in loading, the densitometric signal of each RNA sample hybridized to the PDGF-Rα probe was divided by a GAPDH signal (Fig. 7B). IL-1β-induced PDGF-Rα mRNA had a calculated half-life of 4 h in pulmonary myofibroblasts treated with actinomycin D alone. Treatment of cells with a combination of actinomycin D and SB203580 reduced the half-life of PDGF-Rα mRNA to 1.5 h.

Requirement of de Novo Protein Synthesis for IL-1β-induced Up-regulation of PDGF-Rα mRNA—The experiments described above using actinomycin D indicated that p38 MAP kinase plays a role in the stabilization of PDGF-Rα mRNA.

**Fig. 2.** Inhibition of p38 MAP kinase activity by SB203580. Confluent cultures of rat pulmonary myofibroblasts were rendered quiescent for 24 h in SFDM and then treated for 30 min with IL-1β in the absence or presence of 50 μM SB203580. p38 MAP kinase was immuno-precipitated from whole cell lysates and a kinase assay performed using PHAS-1 as the substrate. Panel A, a representative autoradiograph showing phosphorylation of PHAS-1 by p38 MAP kinase immunoprecipitated from IL-1β-treated cells and inhibition of p38 MAP kinase activation by SB203580. Panel B, relative expression of p38 MAP kinase activity in the absence or presence of SB203580 or IL-1β were determined by densitometric scanning of PHAS-1 bands. Panel C, induction of MAPKAP kinase 2 activity by IL-1β and inhibition by SB203580. Data are expressed as the mean ± S.E. of three experiments. ***, p < 0.01 as compared with the value for IL-1β alone.

**Fig. 3.** SB203580 blocks up-regulation of PDGF-Rα mRNA expression by IL-1β. Confluent, quiescent rat pulmonary myofibroblasts were pretreated for 1 h with 5 or 50 μM SB203580 or Me2SO vehicle alone, then stimulated for 24 h with 10 ng/ml IL-1β prior to collecting cell lysates for Western blot analysis as described under “Experimental Procedures” using antibodies specific for either PDGF-Rα or PDGF-Rβ. Panel A, IL-1β pretreatment up-regulated PDGF-Rα protein 2–3-fold and SB203580 blocked the increase in PDGF-Rα levels by ~50% at 5 μM or 100% at 50 μM. PDGF-Rβ was not affected by IL-1β or SB203580. Panel B, quantitative densitometry of PDGF-Rα (gray bars) and PDGF-Rβ (black bars) levels. Data are representative of three separate experiments.

**Fig. 4.** SB203580 prevents IL-1β-induced up-regulation of PDGF-Rα protein as determined by Western blot analysis. Confluent, quiescent rat pulmonary myofibroblasts were pretreated for 1 h with 5 or 50 μM SB203580 or Me2SO vehicle alone, then stimulated for 24 h with 10 ng/ml IL-1β prior to collecting RNA for Northern blot analysis as described under “Experimental Procedures.” IL-1β-induced PDGF-Rα mRNA had a calculated half-life of ~4 h in pulmonary myofibroblasts treated with actinomycin D alone. Treatment of cells with a combination of actinomycin D and SB203580 reduced the half-life of PDGF-Rα mRNA to ~1.5 h.

**Induction of PDGF-Rα Requires p38 MAP Kinase**
Confluent, quiescent cells were treated with an increasing concentration of SB203580 or Me2SO vehicle for 1 h, then stimulated with IL-1β (10 ng/ml) for 24 h prior to performing an 125I-PDGF-AA binding assay as described under “Experimental Procedures.” Data are expressed as the mean ± S.E. of three experiments.

| SB203580 (μM) | 125I-PDGF-AA bound (cpm/culture) | −IL-1β | +IL-1β |
|---------------|----------------------------------|--------|--------|
| 0             | 1296 ± 96                       | 8880 ± 766 |
| 1             | 1274 ± 103                      | 7670 ± 801 |
| 5             | 1276 ± 68                       | 6630 ± 555 |
| 10            | 1286 ± 74                       | 4440 ± 515a |
| 50            | 1342 ± 80                       | 3000 ± 362a |
| 100           | 1381 ± 113                      | 1980 ± 209a |

a p < 0.01 as compared with the corresponding value for IL-1β plus vehicle.

However, it was unclear whether p38 MAP kinase caused mRNA stabilization by mediating the synthesis of a new protein(s). In order to determine if de novo protein synthesis was required, cells were pretreated for 1 h with 5 μg/ml cycloheximide to block protein synthesis and then treated for 4 h with IL-1β to up-regulate PDGF-Rα mRNA. Cycloheximide treatment abolished the induction of PDGF-Rα mRNA caused by IL-1β (Fig. 8).

SB203580 Does Not Affect IL-1β-induced ERK Activation but Enhances IL-1β-induced JNK Activation—To test whether SB203580 might have effects on the activity of other MAP kinases, we preincubated cells with increasing concentrations of SB203580 (1–100 μM) and then stimulated the cells with IL-1β for 30 min prior to collecting cell lysates. In kinase assays, SB203580 completely inhibited p38 MAP kinase activity (Fig. 9). However, 10 μM SB203580 inhibited IL-1β-induced up-regulation of 125I-PDGF-AA binding by 60–70%, and higher concentrations of SB203580 (50 and 100 μM) were required to completely inhibit 125I-PDGF-AA up-regulation in response to IL-1β (Table I). These data suggested that another signaling mechanism might be required to complement p38 MAP kinase to facilitate up-regulation of PDGF-Rα. ERK activation induced by IL-1β was not affected by concentrations of SB203580 as high as 100 μM, while IL-1β-induced JNK activation was enhanced approximately 2-fold by SB203580 (Fig. 9).

p38 MAP Kinase Is Necessary yet Alone May Not Be Sufficient to Cause Up-regulation of PDGF-Rα—The experiment described above in Fig. 9 suggested that activation of p38 MAP kinase alone might not be sufficient to up-regulate PDGF-Rα. Therefore we compared LPS, another known inducer of PDGF-Rα (12), and TNF-α, which has been reported to have no effect on induction of PDGF-Rα(37), for their ability to activate p38 MAP kinase, ERK, or JNK. IL-1β activated all three MAP kinases, while LPS and TNF-α activated only p38 MAP kinase (Fig. 10A). Both IL-1β and LPS, but not TNF-α, up-regulated 125I-PDGF-AA specific binding to cultured myofibroblasts (Fig. 10B). Since TNF-α activates p38 MAP kinase but does not up-regulate PDGF-Rα, these data indicate that another signaling mechanism complements p38 MAP kinase to facilitate up-regulation of PDGF-Rα in response to IL-1β.

**DISCUSSION**

IL-1β is the major factor produced by activated pulmonary macrophages that up-regulates the PDGF-Rα on lung myofibroblasts (6, 12). In this study we report that p38 MAP kinase is a required signaling intermediate for IL-1β-induced up-regulation of the PDGF-Rα, as SB203580 blocked the increase in PDGF-Rα mRNA expression (Fig. 3) and appearance of functional cell-surface PDGF-Rα protein following IL-1β treatment (Figs. 4 and 5). Moreover, pretreatment of cells with SB203580...
significantly reduced IL-1β-induced enhancement of PDGF-AA-stimulated mitogenesis (Fig. 6). We clearly showed that inhibition of p38 MAP kinase activation by SB203580 resulted in accelerated degradation of PDGF-Rα mRNA (Fig. 7), which proved that p38 MAP kinase plays a role in the stabilization of PDGF-Rα mRNA. IL-1β-induced up-regulation of PDGF-Rα mRNA was abolished by pretreatment with cycloheximide (Fig. 8), which showed that de novo protein synthesis was required for the IL-1β-stimulated increase in PDGF-Rα mRNA. Taken together, these data support the idea that IL-1β activates p38 MAP kinase, which then signals downstream events that culminate in...
the synthesis of a protein that stabilizes PDGF-Ra mRNA.

Other studies have shown that p38 MAP kinase may play a role in stabilizing mRNA or by increasing transcription. For example, Miyazawa and co-workers (18) reported that IL-1β induced IL-6 expression in human fibroblast-like synoviocytes was blocked by SB203580 (18). Similar to our observation in the present study, they observed that SB203580 increased the IL-6 mRNA degradation rate in the presence of actinomycin D and concluded that p38 MAP kinase controlled IL-6 expression at the translational level by stabilization of IL-6 mRNA (18). However, they observed that cycloheximide had no effect on the increase in IL-6 mRNA after IL-1β stimulation (18). In our hands, cycloheximide abolished the increase in PDGF-Ra mRNA following IL-1β treatment, suggesting that de novo protein synthesis was required for PDGF-Ra mRNA stabilization. Other investigators have reported that IL-6 mRNA expression and NF-κB reporter gene activation by TNF-α in murine fibrosarcoma L929 cells was completely inhibited by SB203580, leading to the conclusion that p38 MAP kinase controlled TNF-α-induced IL-6 expression at the transcriptional level (35, 36).

Our data in the present study support the concept that p38 MAP kinase signals the de novo synthesis of a protein(s) that stabilizes PDGF-Ra mRNA. Several proteins such as AUF1 (40) and TTP (41) have been reported to reduce mRNA stability, whereas other proteins including AUBF (42) and the α-globulin complex (43) increase mRNA stability. All of these factors, whether they function to stabilize or destabilize mRNAs, bind AU-rich sequences in the 3′-untranslated region of the mRNA to cause either mRNA stability or degradation. In particular, repeated AUUUA sequences in the 3′-untranslated region of many proto-oncogenes and cytokine mRNAs are the target for RNA-binding proteins (44–46). PDGF-Ra mRNA contains 10 copies of the AUUUA sequence in its 3′-untranslated region (47). Thus, it is not unexpected that PDGF-Ra mRNA would be the target for RNA-binding proteins that would influence mRNA stability.

IL-1β activates other MAP kinases in pulmonary myofibroblasts including JNK and ERK, yet activation of these kinases apparently does not result in PDGF-Ra up-regulation. For example, treatment of cells with the MEK inhibitor, PD98059, enhanced IL-1β-induced up-regulation of PDGF-Ra 2–3-fold (Ref. 37 and Table II). Thus, activation of ERK has the opposite effect of p38 MAP kinase activation on IL-1β-induced expression of PDGF-Ra. Nevertheless, we investigated the possibility that SB203580 might be affecting the activity of ERK. However, concentrations of SB203580 as high as 100 μM did not affect IL-1β-induced ERK activation (Fig. 9). We also investigated JNK as a possible signaling intermediate that might mediate the increase in PDGF-Ra following IL-1β treatment. In the present study, SB203580 enhanced IL-1β-induced JNK activity approximately 2-fold (Fig. 9). Alone these data suggest the possibility that the effect of SB203580 on IL-1β-induced up-regulation of PDGF-Ra was mediated in part by JNK activation. However, LPS strongly up-regulates PDGF-Ra in myofibroblasts without activating JNK (Fig. 10). Additionally, pyrrolidine dithiocarbamate activates JNK in myofibroblasts but does not up-regulate PDGF-Ra (37). Collectively, these findings indicate that JNK does not play a role in induction of PDGF-Ra. Finally, we excluded a role for receptor tyrosine kinases or protein kinase C, as genistein or phorbol 12-myristate 13-acetate had no effect on IL-1β-induced PDGF-Ra expression, respectively.

While p38 MAP kinase appears to be necessary for IL-1β-induced up-regulation of the PDGF-Ra, the possibility exists that p38 MAP kinase activation alone might not be sufficient to cause up-regulation of PDGF-Ra. Indeed, we found that TNF-α activates p38 MAP kinase in rat pulmonary myofibroblasts, yet TNF-α did not up-regulate PDGF-Ra (Fig. 10). These data suggest that IL-1β and other agents that cause up-regulation of PDGF-Ra following activation of p38 MAP kinase (e.g. LPS) might also activate a signaling pathway that is required to compliment p38 MAP kinase to facilitate up-regulation of PDGF-Ra. Alternatively, TNF-α could activate a signaling pathway that suppresses expression of PDGF-Ra in addition to activating p38 MAP kinase. In any case, our comparison of various inflammatory mediators in Fig. 10 suggest that p38 MAP kinase activation is necessary yet alone is not sufficient to cause up-regulation of PDGF-Ra.

Our findings do not rule out the possibility that increased PDGF-Ra mRNA expression but is also controlled at the level of PDGF-Ra transcription. Kitami and co-workers (48) recently reported that members of the CAAT/enhancer-binding protein (C/EBP) family control expression of the PDGF-Ra. Specifically, they found that a high level of C/EBP-δ expression was a major determinant for elevated gene expression of the PDGF-Ra in vascular smooth muscle cells of genetically hypertensive rats (48). Whether or not C/EBP plays a role in IL-1β-induced up-regulation of the PDGF-Ra, (i.e. transcriptional regulation) remains to be elucidated. To our knowledge, no transcription factors other than C/EBP have been linked to the regulation of the PDGF-Ra. A previous study from our laboratory addressed the possible role of NF-κB in the regulation of PDGF-Ra by IL-1β, yet IL-1β-induced up-regulation of
PDGF-Rα was independent of NF-κB since other activators of NF-κB (e.g., TNF-α) did not up-regulate PDGF-Rα. Moreover, the PDGF-Rα is up-regulated by dexamethasone (49) and staurosporine,² yet these agents do not activate NF-κB.

Several studies have shown that maximal mitogenic and chemotactic responses to PDGF isoforms require co-expression of both PDGF-Rα and PDGF-Rα (6, 7, 12, 13), yet expression of PDGF-Rα in many mesenchymal cell types is constitutively suppressed. However, the PDGF-Rα is up-regulated during the progression of several fibroproliferative diseases (9–11). During pulmonary fibrogenesis in rats, the temporal up-regulation of this receptor precedes myofibroblast hyperplasia (10, 11). Moreover, induction of PDGF-Rα in cultured myofibroblasts stimulated with IL-1α results in enhanced proliferative and chemotactic responses to all PDGF isoforms (6, 12). Collectively, these in vitro and in vivo observations indicate that induction of the PDGF-Rα is a mechanism that contributes to accelerated myofibroblast growth during pulmonary fibrogenesis. Overall, the PDGF receptor system appears to be important to the progression of lung fibrosis as this disease in rats is reduced by the administration of a PDGF-specific receptor tyrosine kinase inhibitor (50).

In summary, our findings support the idea that IL-1α induces PDGF-Rα expression in rat pulmonary myofibroblasts by activating p38 MAP kinase, which functions to stabilize PDGF-Rα mRNA by acting downstream to signal de novo synthesis of a protein(s) that stabilizes PDGF-Rα mRNA. Further investigation is warranted to identify the RNA-binding protein(s) that regulate PDGF-Rα mRNA stability. Expression of the PDGF-Rα appears to be a mechanism of fibroproliferative lung disease. Therefore, elucidation of the molecular mechanisms that control the expression of this receptor may lead to strategies for therapeutic intervention of the disease.

Acknowledgments—We thank Dr. Perry Blackshear and Dr. John O’Bryan at the National Institute of Environmental Health Sciences for helpful comments during the preparation of this manuscript.

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² P.M. Lindroos and J.C. Bonner, unpublished observation.

Induction of PDGF-Rα Requires p38 MAP Kinase