Interleukin-1β-induced Cyclooxygenase-2 Expression Requires Activation of Both c-Jun NH₂-terminal Kinase and p38 MAPK Signal Pathways in Rat Renal Mesangial Cells*

Zhonghong Guan‡§, ShaAvhree Y. Buckman, Brent W. Miller, Lisa D. Springer, and Aubrey R. Morrison§

From the Department of Medicine and Molecular Biology and Pharmacology, Washington University School of Medicine, St. Louis, Missouri 63110

The inflammatory cytokine interleukin-1β (IL-1β) induces cyclooxygenase-2 (Cox-2) expression with a concomitant release of prostaglandins from glomerular mesangial cells. We reported previously that IL-1β rapidly activates the c-Jun NH₂-terminal/stress-activated protein kinase (JNK/SAPK) and p38 mitogen-activated protein kinase (MAPK) and also induces Cox-2 expression and prostaglandin E₂ (PGE₂) production. The current study demonstrates that overexpression of the dominant negative form of JNK1 or p54 JNK2/SAPKβ reduces Cox-2 expression and PGE₂ production stimulated by IL-1β. Similarly, overexpression of the kinase-dead form of p38 MAPK also inhibits IL-1β-induced Cox-2 expression and PGE₂ production. These results suggest that activation of both JNK/SAPK and p38 MAPK is required for Cox-2 expression after IL-1β activation. Furthermore, our experiments confirm that IL-1β activates MAP kinase kinase-4 (MKK4)/SEK1, MKK3, and MKK6 in renal mesangial cells. Overexpression of the dominant negative form of MKK4/SEK1 decreases IL-1β-induced Cox-2 expression with inhibition of both JNK/SAPK and p38 MAPK phosphorylation. Overexpression of the kinase-dead form of MKK3 or MKK6 demonstrated that either of these two mutant kinases inhibited IL-1β-induced p38 MAPK phosphorylation and Cox-2 expression but not JNK/SAPK phosphorylation and activation. This study suggests that the activation of both JNK/SAPK and p38 MAPK signaling cascades is required for IL-1β-induced Cox-2 expression and PGE₂ synthesis.

Prostaglandin (PG)¹ H synthase is a homodimer that catalyzes the rate-limiting step in prostaglandin biosynthesis. This bifunctional enzyme forms prostaglandins by catalyzing the conversion of arachidonic acid to prostaglandin G₂ (PGH₂) by its inherent cyclooxygenase activity. PGH₂ is subsequently reduced to PGH₁ by the peroxidase activity of this enzyme. PGH₁ serves as a common precursor for prostaglandins, prostanoids, and thromboxanes (1). Prostaglandins are arachidonic acid metabolites that influence inflammatory responses, bone development, wound healing, hemostasis, reproductive function, glomerular filtration, and renal homeostasis. Furthermore, alterations in prostaglandin production have been linked to cardiovascular disease, chronic and acute inflammation, atherosclerosis, and colon cancer (2, 3).

There are two PGH synthase (also known as cyclooxygenase) isozymes that have been identified in humans bearing 60% homology: cyclooxygenase-1 (Cox-1) and cyclooxygenase-2 (Cox-2). Cox-2 can be up-regulated by various stimuli including tumor promoters (4), v-sr transformation (5), lipopolysaccharide (6), interleukin (IL)-1 (7), platelet-derived growth factor (8), and serum (9). The molecular mechanisms by which this up-regulation is mediated remain unclear. Recent papers have linked Cox-2 expression with activation of the mitogen-activated protein kinase (MAPK) pathway (10, 11). In mammalian cells, several subfamilies of MAPKs have been identified. These MAPK family members include the extracellular signal-regulated kinases (ERKs) p44 MAPK (ERK1) and p42 MAPK (ERK2); stress-activated protein kinases (SAPKs), also referred to as c-jun-NH₂-terminal kinases (JNKs), which include p54 SAPKα/JNK2 and p46 SAPKγ/JNK1 and the p38 MAPKs (α, β, δ, γ, and β). Phosphorylated MAPKs phosphorylate and activate downstream targets such as transcription factors and regulators of cell function, growth, and differentiation. Activation of these kinases involves a cascade in which the upstream activator MAPK kinase kinase (MEKK, α, β, γ, δ, and ε) or Raf in the case of ERK phosphorylates and activates MAPK/ERK kinases/MAPK kinases (MKKs) which include MKK1–4, and which phosphorylate and activate ERKs, JNKs, and p38 MAPks (12).

Previous work has demonstrated that both the JNK/SAPK and p38 MAPK cascades are activated by the inflammatory cytokines IL-1 and tumor necrosis factor-α as well as by a wide variety of cellular stresses such as ultraviolet light, ionizing radiation, hyperosmolality, heat shock, and oxidative stress (13). These findings suggest a role for these two kinase pathways as important signaling mechanisms underlying the inflammatory process. We demonstrated previously that p38 MAPK activation is linked to IL-1β-induced prostaglandin biosynthesis in renal mesangial cells (11). In addition, we have demonstrated that overexpression of a constitutively active truncation mutant of MEKK1, a putative upstream kinase of MKK4/SEK1, can induce Cox-2 expression and prostaglandin biosynthesis (14). Earlier studies demonstrated activation of
JNK1 by the v-src oncogene and induction of Cox-2 expression (15). In the current study we have expressed either wild type or dominant negative constructs of both p38 MAPK and JNK, as well as their immediate upstream activators, to evaluate their role in IL-1β-induced Cox-2 expression.

The data presented in this manuscript suggest a requirement for both p38 MAPK and JNK activity for cytokine-induced Cox-2 expression. Control of Cox-2 expression by IL-1β may be linked to elements within the Cox-2 promoter which require activated transcription factor binding (16). In conjunction with previous findings, our observations suggest a potential mechanism for transcriptional activation of the Cox-2 gene which involves the activation and binding of transcription factors induced by both p38 MAPK and JNK to facilitate full expression of Cox-2 in response to IL-1β stimulation.

**EXPERIMENTAL PROCEDURES**

**Materials—**Human recombinant IL-1β and restriction enzymes were purchased from Boehringer Mannheim. Myelin basic protein (MBP) was purchased from Sigma. Fetal bovine serum was purchased from Life Technologies, Inc. Polyclonal or monoclonal rabbit or mouse IgG antibodies against Cox-2 and Cox-1 were from Cayman Chemical Co. Inc. MKK3, MKK4, MKK6, JNK, phospho-specific JNK, ERK, and p38 MAPK antibodies were from Santa Cruz Biototechnology Inc. Phospho-specific p38 MAPK, MKK4/SEK, and MKK3/MKK6 antibodies were from New England Biolabs. Phospho-specific ERK antibody was from Promega. Anti-FLAG-M2 antibody was from IBID Kobi. pET28gb-Δ, a histidine-tagged fusion protein expression plasmid that encodes e-cjun (1–79) which contains the NH-terminal activation domain of c-jun and a mutant c-jun (1–79), in which Ser63 and Ser73 of c-jun (1–79) were mutated to alanine, were generously provided by Dr. Maryann Gruda (Department of Molecular Biology, Bristol Myers Squibb Pharmaceutical Research Institute, Princeton, NJ). Both wild type His-cjun (1–79) and mutant His-cjun (1–79, Ala63–Ala73) were expressed as histidine-tagged mutant of JNK1 (T183A/Y185F) in pCMV5 and p38 MAPK (T180A/Y182F) in pCMV. His-cjun was coupled to horseradish peroxidase (Amersham Pharmacia Biotech) at a concentration of 75 μg/ml. pGEX ATF-2 (1–96) was obtained from Dr. J. Silvio Gutkind (Molecular Signaling Unit, Laboratory of Cellular Development and Oncology, NIH). MKK4/SEK1 wild type (pCMV SEK1-WT), a constitutively active mutant form of SEK1 (pCMV SEK1-M), and dominant negative mutant of JNK (p38 MAPK SEK1) were kindly donated by Dr. Roger Davis, Howard Hughes Medical Institute, University of Massachusetts Medical Center.

**Cell Culture—**Primary mesangial cell cultures were prepared from male Sprague-Dawley rats as described previously (17). Cells were grown in RPMI 1640 medium supplemented with 10% heat-inactivated fetal calf serum, 0.3 IU/ml insulin, 100 μg/ml penicillin, 100 μg/ml streptomycin, 250 μg/ml amphotericin B, and 25 μg/ml HEPES. Induction of Rat Mesangial Cells and NIH 3T3 Cells by the Retroviral Vector—p54 SAPKβ was subcloned into retrovector vector pLXSN, and 10 μg of plasmid DNA was purified and used to transfect PA317 retroviral packaging cells (American Type Culture Collection CRL 9078) with LipofectAMINE (Life Technologies, Inc). Transfected clones were selected in Dulbecco’s modified Eagle’s medium supplemented with 10% (v/v) fetal calf serum (Life Technologies, Inc.), 100 units/ml penicillin, 100 μg/ml streptomycin, and 500 μg/ml G418 (Life Technologies, Inc.) and then isolated with sterile glass cloning rings.

**Transfection of Rat Mesangial Cells and NIH 3T3 Cells with LipofectAMINE—**MKK4/SEK1 wild type (SEK1-WT), a constitutively active mutant form of SEK1 (pCMV SEK1-ED), the dominant negative mutant of SEK1 (SEK1-AL), MKK3 wild type, kinase-dead form of MKK3, MKK6 wild type, or dominant negative mutant MKK6 was subcloned into the popRSV1 mammalian expression vector (Stratagene). The wild type or dominant negative mutant form of p38 MAPK or JNK1 was subcloned into the pcDNA3 mammalian expression vector. Primary cultures of rat mesangial cells were plated and transfected at 50–80% confluence with LipofectAMINE—

**Western Blot Analysis—**At the time of harvest, cells were washed with ice-cold phosphate buffered and lysed in whole cell extract (WCE) buffer (25 mM HEPES-NaOH, pH 7.7, 0.3 mM NaCl, 1.5 mM MgCl2, 0.2 mM EDTA, 0.1% Triton X-100, 0.5 mM dithiothreitol (DTT), 20 mM β-glycerophosphate, 100 μM NaVO4, 2 μg/ml leupeptin, and 100 μg/ml phenylmethylsulfonyl fluoride) to which 8 μL Laemmli sample buffer (6 M urea, 2% sodium dodecyl sulfate, 10% glycerol, 0.01% bromophenol blue, and 0.02% SDS) was added before heating. After boiling for 5 min, equal amounts of protein were run on 10% SDS-polyacrylamide gel electrophoresis. Proteins were transferred to polyvinylidene difluoride membranes (Immobilon-P; Millipore Corp., Bedford, MA). The membranes were saturated with 5% fat-free dry milk in Tris-buffered saline (50 mM Tris-HCl, pH 7.4) for 1 h. After washing with 5% fat-free milk Tris-buffered solution for 1 h, primary antibody (1:1000 dilution in 5% BSA) was added for 1 h at room temperature. Blots were then incubated overnight with primary antibody. After washing with 5% fat-free milk Tris-buffered solution for 1 h, antibodies were incubated for 1 h at room temperature with goat anti-rabbit or mouse IgG antibody coupled to horseradish peroxidase (Amersham Pharmacia Biotech) at a 1:3000 dilution in TBS-T. Blots were then washed five times in TBS-T before visualization. An Enhanced Chemiluminescence (ECL) kit (Amersham Pharmacia Biotech) was used for detection.

**Immunocomplex p38 MAPK or JNK Activity Assay—**The cell extracts were immunoprecipitated by incubation overnight with anti-p38 MAPK or anti-JNK antibody and then with protein A-Sepharose beads for 3 h at 4°C. Beads were washed three times with 1 ml of extraction buffer. The supernatant was then added to 1 ml of WCE buffer. p38 MAPK activity was assayed using MBP or GST-ATF-2 (1–96) as the substrate at 30°C for 20 min in 30 ml of kinase reaction buffer (5 μg of MBP or GST-ATF-2 (1–96) for p38 activity assay or His-cjun (1–79) for JNK activity assay, 20 μM ATP, 10 μCi of [γ-32P]ATP, 25 mM HEPES, and 20 mM MgCl2). The reaction was terminated with Laemmli sample buffer, and the products were resolved by 10% SDS-polyacrylamide gel electrophoresis. The phos-
phosphorylated His-c-jun, MBP, or GST-ATF-2 was visualized by autoradiography.

Immunocomplex MKK3, MKK4, and MKK6 Activity Assay—Cell extracts were immunoprecipitated by incubation overnight with anti-MKK3, MKK4, or MKK6 antibody and then incubated with protein A-Sepharose beads for 3 h at 4 °C. The beads were washed three times with 1 ml of ice-cold WCE buffer. The immunocomplex MKK3, MKK4, or MKK6 activity assay using GST-p38 MAPK (10 μg/reaction) as the substrate was performed at 30 °C for 20 min in 30 μl of kinase reaction buffer (10 μg of GST-p38 MAPK, 100 μμμ ATP, 25 μμμ HEPES, and 20 μμμ MgCl2). The reaction was terminated with Laemmli sample buffer, and the products were resolved by 10% SDS-polyacrylamide gel electrophoresis. Phosphorylated p38 MAPK was analyzed by Western blot using anti-phospho-specific p38 MAPK antibody and detected by enhanced chemiluminescence. The phosphorylation level of p38 MAPK was used to reflect MKK3, MKK6, or MKK4/SEK1 activity.

Statistical Analysis—Data were expressed as the mean ± S.E. Statistical analysis was performed using paired or unpaired Student's t test. A difference with a p value of 0.05 was considered statistically significant.

RESULTS

JNK/SAPK Mediates IL-1β-induced Cox-2 Expression—To determine whether the activation of JNK/SAPK in response to IL-1β is required for induction of Cox-2 protein expression and PGE2 biosynthesis, stably transfected cells overexpressing JNKs/APK in rat glomerular mesangial cells as well as NIH 3T3 cells were used. We first investigated whether a catalytically inactive form of JNK1 would function as a dominant inhibitor of IL-1β induction of Cox-2 expression. Overexpression of both wild type and dominant negative mutant JNK1 in pcDNA3 was verified by a Western blot assay using an anti-JNK antibody. An immunocomplex JNK activity assay demonstrated that overexpression of the kinase-dead form of JNK1 resulted in decreased IL-1β-induced JNK activity (data not shown). As shown in Fig. 1, the kinase-dead mutant JNK1 inhibited Cox-2 protein expression and PGE2 production in response to IL-1β stimulation. In our experiments, we also evaluated whether the kinase-negative mutant of JNK2/p54 SAPKβ could inhibit Cox-2 expression and PGE2 production after IL-1β stimulation. Rat mesangial cells and NIH 3T3 cells transfected with either wild type JNK2/p54 SAPKβ or the JNK2/p54 SAPKβ kinase-inactive mutant were stimulated with IL-1β. Overexpression of JNK2/p54 SAPKβ was verified by the Western blot analysis followed by immunocomplex JNK activity assays that revealed that the kinase-negative form of p54 SAPKβ inhibited total JNK activity induced by IL-1β (data not shown). Similar to JNK1, the dominant negative JNK2/p54 SAPKβ expressed in pLXSN blocked IL-1β-induced Cox-2 expression and PGE2 production in renal mesangial cells (Fig. 2). This finding was also demonstrated in NIH 3T3 cells in which Cox-2 expression and PGE2 production were inhibited by the kinase-dead form of JNK2/p54 SAPKβ (data not shown). In experiments in which mesangial cells were infected with empty pLXSN we observed enhanced basal levels of Cox-2 expression and PGE2 production and a blunting of the response to IL-1β. Nevertheless kinase-dead JNK2 inhibited both basal and IL-1β-induced Cox-2 expression and PGE2 production. These results demonstrate that JNKs/APK is important for IL-1β activation of Cox-2 protein expression and that the activation of JNKs/APK is necessary for IL-1β-induced Cox-2 expression and PGE2 production.

p38 MAPK Is Involved in the Regulation of Cox-2 Expression Induced by IL-1β—Previously we demonstrated that IL-1β increases p38 MAPK phosphorylation and activation. Pharmacological inhibition of p38 MAPK can effectively block Cox-2 expression and PGE2 release stimulated by IL-1β in renal mesangial cells. To confirm further the physiological function of p38 MAPK in the regulation of Cox-2 protein expression, we analyzed the effects of overexpression of the kinase-inactive p38α MAPK mutant on IL-1β-induced Cox-2 expression and PGE2 production. As shown in Fig. 3, the dominant negative mutant form of p38α MAPK blocked Cox-2 expression and PGE2 production after IL-1β stimulation. These results demonstrate further the physiologic function of p38 MAPK in the regulation of IL-1β-stimulated Cox-2 induction and PGE2 synthesis.

MKK3 and MKK6 Function Regulate Cox-2 Expression Stimulated by IL-1β—MKK3 and MKK6 are upstream kinases that activate and phosphorylate p38 MAPK in vitro and in vivo. To verify whether MKK3 and MKK6 are involved in IL-1β signaling, we first measured MKK3 and MKK6 activity by an immunocomplex kinase assay using GST-p38 MAPK as the substrate and phosphorylation of p38 MAPK with an anti-phospho-specific p38 MAPK antibody. Fig. 4, A and B, demonstrates that IL-1β increases both MKK3 and MKK6 activity, suggesting that MKK3 and MKK6 may function as important mediators of IL-1β signaling. To determine whether MKK3 and MKK6 mediate IL-1β-induced p38 MAPK activation and Cox-2 expression in mesangial cells, we transfected mammalian expression
plasmids encoding either epitope-tagged MKK3 or MKK6 wild type or the kinase-negative mutant. Stable overexpression of MKK3 (Fig. 5A) and MKK6 (data not shown) in popRSV1 were detected by Western blot analysis using an anti-FLAG tag antibody. Transfection of cells with dominant negative MKK3 (Fig. 5B) or MKK6 (data not shown) inhibited p38 MAPK after IL-1β stimulation. Importantly however, JNK phosphorylation was unaffected (data not shown). These data verify that MKK3 and MKK6 can activate p38 MAPK after IL-1β stimulation in renal mesangial cells. Furthermore, we examined the effects of the kinase-inactive mutant forms of MKK3 or MKK6 on Cox-2 expression and PGE2 production stimulated by IL-1β. Interestingly, we observed that overexpression of either kinase-negative mutant (MKK3 or MKK6) resulted in the inhibition of IL-1β-induced Cox-2 expression and PGE2 synthesis in renal mesangial cells (Figs. 5 and 6). One explanation for these results is that the expressed dominant negative protein binds the p38 MAPK substrates and prevents phosphorylation by either of the activated native MKK3 or MKK6 proteins. These results demonstrate that both MKK3 and MKK6 may mediate IL-1β-induced p38 MAPK activation as well as Cox-2 protein expression and PGE2 production.

MKK4/SEK1 Mediates IL-1β-induced Cox-2 Expression through Both JNK/SAPK and p38 MAPK Mechanisms—Our previous studies have demonstrated that MKK4/SEK1 activates and phosphorylates both JNK/SAPK and p38 MAPK. Overexpression of the kinase-negative mutant form of MKK4/SEK1 inhibits basal Cox-2 expression and PGE2 production in NIH 3T3 cells cultured in serum-containing media (14). To determine whether MKK4/SEK1 was involved in the IL-1β signal transduction mechanism, we measured MKK4/SEK1 activity after IL-1β stimulation. We analyzed the MKK4 activity by an immunocomplex kinase assay using GST-p38 MAPK as the substrate. We found that IL-1β can enhance MKK4/SEK1 activity in mesangial cells (Fig. 7A). This finding suggests that MKK4/SEK1 may be involved in IL-1β signaling. We therefore tried to determine whether activation of MKK4/SEK1 is required for activation of JNK/SAPK and p38 MAPK and subsequent prostaglandin biosynthesis in mesangial cells. We established permanently transfected mesangial cells containing the
wild type (SEK-WT), dominant negative mutant form (SEK-AL), or the constitutively active mutant form (SEK-ED) of MKK4/SEK1. When stimulated with IL-1β, we found that the dominant negative mutant form of MKK4/SEK1 (SEK-AL) inhibited both JNK/SAPK and p38 MAPK phosphorylation. In contrast, the constitutively active mutant form of MKK4/SEK1 enhanced IL-1β-induced JNK/SAPK and p38 MAPK phosphorylation (Fig. 7, B and C). These results suggest that MKK4/SEK1 can mediate IL-1β-induced JNK/SAPK and p38 MAPK activation in the intact mesangial cell. More importantly, our experiments show that the kinase-negative mutant form of MKK4/SEK1 (SEK-AL) inhibits IL-1β-induced Cox-2 expression and PGE2 production. By comparison, the constitutively active mutant form of MKK4/SEK1 (SEK-ED) enhanced IL-1β-induced Cox-2 expression and PGE2 production (Fig. 7, D and E). Together, these results suggest a role for MKK4/SEK1 in IL-1β-induced JNK/SAPK and p38 MAPK activation and modulation of prostaglandin biosynthesis in renal mesangial cells.

DISCUSSION

The inflammatory cytokine IL-1 is a potent immunoregulatory and proinflammatory agent involved in a variety of pathological processes such as the response to infection, activated lymphocyte products, microbial toxins, and other stimuli (19). In glomerular inflammation, infiltrating macrophages produce IL-1, which activates renal mesangial cells and promotes glomerular injury. Our laboratory has reported previously that IL-1β induces Cox-2 protein expression with concomitant synthesis of prostaglandins such as PGE2 in renal mesangial cells (11, 20, 21). The induction of this key mediator may provide a critical mechanism involved in IL-1-induced renal inflammation. Recent studies suggest that activation of Cox-2 is not only involved in the pathogenesis of renal inflammatory diseases but may also play a critical role in normal kidney development, differentiation, and function (22).

Although much effort has been made to identify the intracellular signaling pathways triggered by IL-1, the signal trans-
duction mechanisms by which IL-1 induces Cox-2 protein expression and prostaglandin production are still unclear. Several recent reports indicate that an important group of protein kinases, the MAPKs, may be involved in these signaling processes. The MAPK pathways have been implicated as a mechanism by which signals are transduced from the cell surface to the nucleus in response to a variety of different stimuli and participate in intracellular processes by further inducing the phosphorylation of intracellular substrates such as other protein kinases and transcription factors. This signaling mechanism is believed to control a wide spectrum of cellular physiological and pathophysiological processes including cell growth, differentiation, and stress responses (12). Recent work has demonstrated that both JNK/SAPK and p38 MAPK cascades are activated by the inflammatory cytokines IL-1β and tumor necrosis factor-α as well as by a wide variety of cellular stresses such as ultraviolet light, ionizing radiation, hyperosmolarity, heat shock, and oxidative stress (13). These findings suggest an important role for these two kinase pathways in the signaling mechanisms recruited as part of the inflammatory process.

Recent work suggests that the MAPK pathway is also involved in regulating prostaglandin biosynthesis. For example, activation of cytosolic phospholipase A2 by thrombin involves activation of both ERK and p38 MAPK (23, 24). Furthermore, we have shown previously that IL-1 stimulation of renal mesangial cells increases PGE2 production and Cox-2 expression concomitant with activation of the p38 MAPK and JNK signaling pathways. We have demonstrated that pharmacological inhibition of p38 MAPK dose-dependently inhibits IL-1β-mediated Cox-2 expression and PGE2 production (11). In the current study, overexpression of the kinase-inactive mutant p38α MAPK inhibits IL-1β-induced Cox-2 expression and PGE2 production, thus confirming further that activation of p38α MAPK is required for Cox-2 expression and PGE2 production.

Herschman and his colleagues (15, 16) reported previously that activation of the JNK/SAPK pathway plays an important role in v-src-induced PGH synthase-2 (Cox-2) gene expression. To elucidate the physiological function of JNK/SAPK, we overexpressed both wild type and kinase-dead forms of JNK1 and JNK2/p54 SAPKβ in renal mesangial cells. The kinase-dead form of both JNK constructs markedly inhibits IL-1β-induced Cox-2 expression and PGE2 release, thus confirming the requirement of JNK/SAPK activity for cytokine-induced prostaglandin biosynthesis.

Previous data have suggested that MKK4/SEK1 is an immediate upstream kinase activating the JNK pathway (25, 26). We recently reported that overexpression of a constitutively active mutant form of MKK4/SEK1 increases both JNK and p38 MAPK activity and phosphorylation (14). Because IL-1β can activate MKK4/SEK1, we tested the effects of transfection of either constitutively active or dominant negative MKK4/SEK1. We observed that the dominant negative mutant MKK4/SEK1 inhibited IL-1β-induced JNK/SAPK and p38 MAPK activation, whereas the constitutively active form activated both JNK and p38 MAPK. Furthermore, overexpression of dominant negative MKK4/SEK1 resulted in inhibition of IL-1β-induced cyclooxygenase expression and prostaglandin biosynthesis.
Therefore, we believe that MKK4/SEK1 is an important upstream kinase that influences both p38 MAPK and JNK/SAPK activity, resulting in regulation of Cox-2 expression.

Previous data have indicated that both MKK3 and MKK6 can activate and phosphorylate p38 MAPK (27, 28). Our experiments demonstrate that IL-1β increases the activity of both MKK3 and MKK6 in renal mesangial cells. To ascertain whether MKK3 and MKK6 function and p38 MAPK activation and Cox-2 expression induced by IL-1β, wild type and kinase-dead MKK3 or MKK6 constructs were utilized. The data presented demonstrate that IL-1β induces p38 MAPK activation and Cox-2 expression induced by IL-1β, wild type and kinase-dead MKK3 or MKK6 constructs were utilized. The data presented demonstrate that IL-1β induces p38 MAPK activation and Cox-2 expression induced by IL-1β, wild type and kinase-dead MKK3 or MKK6 constructs were utilized. The latter seems unlikely because dominant negative mutant forms of any of these MAPK kinases may result in inhibition of p38 MAPK activity by either 1) competing with endogenous MAPK kinases for binding and activation of p38 MAPK or 2) by competing for activation by a putative upstream MAPK kinase protein. The latter seems unlikely because dominant negative p38α does not affect MKK4 and JNK activation. The consequence of inhibition of MKK3, MKK4, or MKK6 activity is inhibition of IL-1β-induced Cox-2 expression.

The aforementioned results suggest that the activation of JNK/SAPK and p38 MAPK is necessary both for induction of Cox-2 protein expression and for PGF_2α production in the renal mesangial cells when induced by IL-1β. This conclusion is based on the observation that the inhibition of either p38α MAPK or the JNK/SAPK pathway results in significant inhibition of IL-1β-induced Cox-2 expression and PGF_2α production. Based on previous observations and our current findings, Fig. 8 depicts a hypothetical model for the combined role of p38 MAPK and JNK activation in the modulation of Cox-2 expression when mesangial cells are exposed to IL-1β.

In summary, we demonstrate that activation of both SAPK/JNK and p38 MAPK is required for Cox-2 expression and PGF_2α production after IL-1β stimulation. Furthermore, we demonstrate that MKK4/SEK1, MKK3, and MKK6 are all involved in IL-1β-induced prostaglandin biosynthesis. MKK3 and MKK6 function as upstream regulators of p38 MAPK, whereas MKK4/SEK1 can function as the upstream kinase of both p38 MAPK and SAPK/JNK. We believe that the activation of both SAPK/JNK and p38 MAPK signaling cascades together are crucial intracellular mechanisms that mediate Cox-2 expression and PGF_2α synthesis induced by cytokine stress.

REFERENCES
1. DeWitt, D., and Smith, W. L. (1995) Cell 83, 345–348
2. Smith, W. L., and Dewitt, D. L. (1996) Adv. Immunol. 62, 167–215
3. Marnett, L. J. (1992) Cancer Res. 52, 5575–5589
4. Koubou, D. A., Fletcher, B. S., Varmus, B. C., Lim, R. W., and Herschman, H. R. (1991) J. Biol. Chem. 266, 12866–12872
5. Han, J. W., Sadowski, H., Young, D. A., and Macara, I. G. (1990) Proc. Natl. Acad. Sci. U. S. A. 87, 3373–3377
6. Lee, S. H., Sayoolla, F., Chanmugam, P., Hart, S., Sun, W., Zhong, H., Liu, S., Simmons, D., and Hwang, D. (1992) J. Biol. Chem. 267, 25934–25938
7. Maier, J. A., Ha, T., and Maciag, T. (1990) J. Biol. Chem. 265, 10805–10808
8. Habenstein, A. J., Goerig, M., Grohlich, J., Rotte, D., Gronwald, R., Loth, U., Schettler, G., Kommerell, B., and Ross, R. (1985) J. Clin. Invest. 75, 1381–1387
9. O’Banion, M. K., Winn, V., and Young, D. A. (1992) Proc. Natl. Acad. Sci. U. S. A. 89, 4888–4892
10. Xie, W., and Herschman, H. R. (1996) J. Biol. Chem. 271, 31742–31748
11. Guan, Z., Baier, L. D., and Morrison, A. R. (1997) J. Biol. Chem. 272, 8083–8089
12. Kyriakis, J. M., and Avruch, J. (1996) Bioessays 18, 567–577
13. Kyriakis, J. M., and Avruch, J. (1996) J. Biol. Chem. 271, 24313–24316
14. Guan, Z., Buckman, S. Y., Pentland, A. P., Templeton, D. J., and Morrison, A. R. (1998) J. Biol. Chem. 273, 12981–12986
15. Xie, W., and Herschman, H. R. (1995) J. Biol. Chem. 270, 27622–27628
16. Xie, W., Fletcher, B. S., Andersen, R. D., and Herschman, H. R. (1994) Mol. Cell. Biol. 14, 6531–6539
17. Guan, Z., Tetsuka, T., Baier, L. D., and Morrison, A. R. (1996) Am. J. Physiol. 270, F634–F641
18. Miller, B. W., Baier, L. D., and Morrison, A. R. (1997) Am. J. Physiol. 273, C130–C136
19. Taub, D. D., and Oppenheim, J. J. (1994) Ther. Immunol. 1, 229–246
20. Tetsuka, T., Daphna-Iken, D., Miller, B. W., Guan, Z., Baier, L. D., and Morrison, A. R. (1996) J. Clin. Invest. 97, 2051–2056
21. Tetsuka, T., Baier, L. D., and Morrison, A. R. (1996) J. Biol. Chem. 271, 11669–11673
22. Morham, S. G., Langenbach, R., Lofin, C. D., Tian, H. F., Vouloumanos, N., Jenuwein, N., Faucher, M. J., Bois, M. L., Lefort, A. L., Lee, C. A., and Smithies, O. M. (1995) Cell 83, 473–482
23. Lin, L. L., Wartmann, M., Lin, A. Y., Knoep, J. L., Seth, A., and Davis, R. J. (1993) Cell 72, 269–278
24. Kramer, R. M., Roberts, E. F., Um, S. L., Borsch-Haubold, A. G., Watson, S. P., Fisher, M. J., and Jakubowski, J. A. (1996) J. Biol. Chem. 271, 27723–27729
25. Kyriakis, J. M., Banerjee, P., Nikolakaki, E., Dai, T., Rubie, E. A., Ahmad, M. F., Avruch, J., and Woodgett, J. R. (1994) Nature 369, 156–160
26. Derijard, B., Ringeaud, J., Barrett, T., Wu, I. H., Han, J., Ulevitch, R. J., and Davis, R. J. (1995) Science 267, 682–685
27. Ensen, H., Ringeaud, J., and Davis, R. J. (1998) J. Biol. Chem. 273, 1741–1748
28. Ringeaud, J., Whitmarsh, A. J., Barrett, T., Derijard, B., and Davis, R. J. (1999) Mol. Cell. Biol. 19, 2421–2425
29. Jiang, Y., Chen, C., Li, Z., Gao, W., Gegner, J. A., Lin, S., and Han, J. (1996) J. Biol. Chem. 271, 17920–17926