Inhibition of p38 Mitogen-Activated Protein Kinase Blocks Activation of Rat Pancreatic Stellate Cells

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ABSTRACT

Activated pancreatic stellate cells (PSCs) have recently been implicated in the pathogenesis of pancreatic fibrosis and inflammation. However, the signal transduction pathways in PSCs remain largely unknown. We examined the role of p38 mitogen-activated protein (MAP) kinase in the activation of PSCs. PSCs were isolated from rat pancreas tissue and used in their culture-activated, myofibroblast-like phenotype. Activation of p38 MAP kinase was determined by Western blotting using anti-phosphospecific antibody. The effects of two p38 MAP kinase inhibitors, 4-(4-fluorophenyl)-2-(4-methylsulfinylphenyl)-5-(4-pyridyl)imidazole (SB203580) and 4-(4-fluorophenyl)-2-(4-hydroxyphenyl)-5-(4-pyridyl)1H-imidazole (SB202190), on the parameters of PSC activation, including proliferation, expression of α-smooth muscle actin, α(I) procollagen, and prolyl 4-hydroxylase (α) genes, and monocyte chemoattractant protein-1 production were evaluated. Interleukin-1β and platelet-derived growth factor-BB activated p38 MAP kinase. Platelet-derived growth factor-induced PSC proliferation was inhibited by SB203580 and SB202190. These reagents decreased α-smooth muscle actin protein expression, and α(I) procollagen and prolyl 4-hydroxylase (α) mRNA levels. Treatment with these p38 MAP kinase inhibitors also resulted in inhibition of monocyte chemoattractant protein-1 expression. In addition, SB203580 inhibited spontaneous activation of freshly isolated PSCs in culture on plastic. Thus, inhibition of p38 MAP kinase modulated profibrogenic and proinflammatory actions in PSCs, implying a potential application of p38 MAP kinase inhibitors for the treatment of pancreatic fibrosis and inflammation.

Recently, star-shaped cells in the pancreas, namely pancreatic stellate cells (PSCs), have been identified and characterized (Apte et al., 1998; Bachem et al., 1998). They are morphologically similar to the hepatic stellate cells that play a central role in inflammation and fibrosis of the liver (Friedman, 1993). In normal pancreas, stellate cells are quiescent and can be identified by the presence of vitamin A-containing lipid droplets in the cytoplasm. In response to pancreatic injury or inflammation, they are transformed (“activated”) from their quiescent phenotype into highly proliferative myofibroblast-like cells that express the cytoskeletal protein α-smooth muscle actin (α-SMA), and produce type I collagen and other extracellular matrix components. Many of the morphological and metabolic changes associated with the activation of PSCs in animal models of fibrosis also occur when these cells are grown in culture on plastic. There is accumulating evidence that PSCs, like hepatic stellate cells, are responsible for the development of pancreatic fibrosis (Apte et al., 1998; Bachem et al., 1998; Haber et al., 1999). Furthermore, PSCs might participate in the pathogenesis of acute pancreatitis (Andoh et al., 2000; Masamune et al., 2002c). In view of their importance in pancreatic fibrosis and inflammation, it is of particular importance to elucidate the mechanisms underlying their activation. Clearly, the signaling mechanisms regulating the activation of PSCs are potential targets for the development of new treatments for pancreatic fibrosis and inflammation, but the precise intracellular signaling pathways in PSCs remain largely unknown.

Members of the mitogen-activated protein kinase (MAP kinase) family, extracellular signal-regulated kinases, c-Jun N-terminal kinase, and p38 MAP kinase, are central elements that transduce the signal generated by growth factors, cytokines, and stresses (Robinson and Cobb, 1997; Kyriakis and Avruch, 2001). Each member of this kinase family is activated by phosphorylation and subsequently translocates...
into the cell nucleus. Once in the nucleus, it phosphorylates and activates transcription factors, ultimately resulting in the transcription of specific genes. MAP kinases play a role in a variety of cellular processes including cell proliferation, cell survival, and cytokine production (Robinson and Cobb, 1997; Kyriakis and Avruch, 2001). Among these members, mammalian p38 MAP kinase was originally identified in murine pre-B cells transfected with the lipopolysaccharide complex receptor CD14 and in macrophages, where it is activated in response to lipopolysaccharide (Han et al., 1994). p38 MAP kinase plays a key role in several cellular functions such as apoptosis and inflammatory responses (Martin-Blanco, 2000). However, roles of p38 MAP kinase in PSCs remain unknown.

In this study, we examined the role of p38 MAP kinase in the activation of PSCs. We here report that inhibition of p38 MAP kinase with specific inhibitors, 4-(4-flurophenyl)-2-(4-methylsulfinylphenyl)-5-(4-pyridyl)imidazole (SB203580) and 4-(4-flurophenyl)-2-(4-hydroxyphenyl)-5-(4-pyridyl)1H-imidazole (SB202190), modulated profibrogenic and proinflammatory actions in activated PSCs. In addition, SB203580 inhibited spontaneous activation of freshly isolated PSCs in culture on plastic. Taken together, our results suggest a role of p38 MAP kinase in the activation of PSCs and a potential application of p38 MAP kinase inhibitors for the treatment of pancreatic inflammation and fibrosis.

Materials and Methods

Materials. poly(dI-dC)-poly(dI-dC), [α-32P]dCTP, and [γ-32P]ATP were purchased from Amersham Biosciences UK, Ltd. (Little Chalfont, Buckinghamshire, UK). Recombinant interleukin (IL)-1β was from Roche Applied Science (Mannheim, Germany). Rat recombinant platelet-derived growth factor (PDGF)-BB was from R & D Systems (Minneapolis, MN). Rabbit antibodies against phosphospecific p38 MAP kinase, total p38 MAP kinase, phosphospecific MAP kinase-activated protein kinase-2 (MAPKAPK-2) (Thr229), total MAPKAPK-2, phosphospecific Akt (Ser473), total Akt, and inhibitor of nuclear factor κB (IκB)-α were purchased from Cell Signaling Technology, Inc. (Beverly, MA). SB203580, SB202190, an inactive analog, 4-ethyl-2-(4-methoxyphenyl)-5-(4-pyridyl)1H-imidazole (SB202474), and wortmannin were from Calbiochem (La Jolla, CA). All other reagents were from Sigma-Aldrich (St. Louis, MO) unless specifically described.

Cell Culture. Rat PSCs were prepared as previously described using Nycodenz solution (Nygomed AS Pharma, Oslo, Norway) (Apte et al., 1998). All animal procedures were performed in accordance with the National Institutes of Health Animal Care and Use Guidelines. Isolated stellate cells were cultured in Ham’s F-12 containing 10% heat-inactivated fetal bovine serum (ICN Biomedicals, Costa Mesa, CA), penicillin sodium, and streptomycin sulfate. All experiments were performed using cells between passages two and five except for those using freshly isolated PSCs. In all experiments using culture-activated PSCs, we incubated PSCs in serum-free medium for 24 h before the addition of experimental reagents.

Western Blotting. The level of activated, phosphorylated p38 MAP kinase was determined by Western blotting as previously described (Masamune et al., 2002d). Briefly, cells were lysed in SDS buffer, and cellular proteins (approximately 100 μg) were fractionated on a 10% SDS-polyacrylamide gel. They were transferred to a nitrocellulose membrane (Bio-Rad, Hercules, CA), and the membrane was incubated overnight with anti-phosphospecific p38 MAP kinase antibody. After incubation with peroxidase-conjugated goat anti-rabbit secondary antibody, proteins were visualized by using an enhanced chemiluminescence kit (Amersham Biosciences UK, Ltd.). Levels of total p38 MAP kinase, phosphorylated MAPKAPK-2, total MAPKAPK-2, phosphorylated Akt, total Akt, α-SMA, and 1κB-α were determined in a similar manner.

Cell Proliferation Assay. Serum-starved PSCs (approximately 20–30% density) were treated with SB203580 or SB202190 at various concentrations for 30 min and then stimulated with PDGF-BB (at 25 ng/ml) in serum-free medium. Cell proliferation was assessed using a commercial kit (CellTiter nonradioactive cell proliferation assay; Promega, Madison, WI) according to the manufacturer’s instruction. Briefly, after 72-h incubation with PDGF-BB, the dye solution was added to cells and incubation continued at 37°C for 4 h. Then, the formazan product was solubilized with the solubilization/stop solution. Cell viability was determined by differences in absorbance at wavelength 570 versus 690 nm.

Enzyme-Linked Immunosorbert Assay. After a 24 h-incubation, cell culture supernatants were harvested and stored at −80°C until the measurement. Monocye chemoattractant protein-1 (MCP-1) levels in the culture supernatants were measured by enzyme-linked immunosorbert assay (Pierce Endogen, Rockford, IL) according to the manufacturer’s instruction.

Northern Blotting. Total RNA was isolated using the RNaseasy total RNA preparation kit (QIAGEN, Valencia, CA). Ten micrograms of total RNA was separated on a 1% agarose-2.2 M formaldehyde gel and transferred to a nylon membrane filter (Amersham Biosciences UK, Ltd.). Blots were hybridized for 16 h at 42°C to the 32P-labeled DNA probes of α(I) procollagen, prolyl 4-hydroxylase (α), or MCP-1 generated by polymerase chain reaction. Specific primer sets were as follows (listed 5′-3′; sense and antisense, respectively; α(I) procollagen: CCTGCTGAGCCCGAGGAAC and TACACACTGTCACTCAT, prolyl 4-hydroxylase (α): TACCTCTCAGTTCTTGTCGACC and CATCCAGAGTTGTGTGGT, MCP-1: AGCCAGATGAGTTAATGCC and GGAAAAGAGAGTGGATGCAT. Polymerase chain reaction procedure consisted of 30 cycles at 94°C (for 1 min), at 55°C (for 1 min), and at 72°C (for 1 min). The identity of the reverse transcription-polymerase chain reaction was confirmed by direct sequencing. After the hybridization, the filter was washed three times with 2 × standard saline citrate and 0.1% SDS at 42°C for 10 min. The washed filter was subjected to autoradiography at −80°C overnight.

Nuclear Extract Preparation and Electrophoretic Mobility Shift Assay. Nuclear extracts were prepared, and electrophoretic mobility shift assay was performed as previously described (Masamune et al., 1998). Double-stranded oligonucleotide probe for nuclear factor κB (NF-κB) (5′-AGTTAGGGGAGGGATTCCTGGG-3′) was end-labeled with [γ-32P]ATP. Nuclear extracts (approximately 5 μg) were incubated with the labeled oligonucleotide probe for 20 min at 22°C and electrophoresed through a 4% polyacrylamide gel. Gels were dried and autoradiographed at −80°C overnight. A 100-fold excess of unlabeled oligonucleotide was incubated with nuclear extracts for 10 min before the addition of the radiolabeled probe in the competition experiments.

Statistical Analysis. Differences between experimental groups were evaluated by the two-tailed unpaired Student’s t test. A p value less than 0.05 was considered statistically significant.

Results

IL-1β and PDGF-BB Activated p38 MAP Kinase. It has been shown that PSCs are activated (express α-SMA) after approximately 48 h of culture on plastic (Apte et al., 1998). As mentioned earlier, most experiments used passaged stellate cells that were considered activated. Indeed, the increased expression of α-SMA was confirmed by immunostaining (data not shown). We first determined whether IL-1β and PDGF-BB, which play important roles in proinflammatory responses (Masamune et al., 2002c) and proliferation (Apte et al., 1999; Luttonberger et al., 2000), respec-
tively, activated p38 MAP kinase in PSCs. We assessed the activation of p38 MAP kinase by Western blotting using anti-phosphospecific p38 MAP kinase antibody. The antibody recognizes only the phosphorylated form of p38 MAP kinase, thus allowing the assessment of activation of this kinase. Both IL-1β and PDGF-BB induced phosphorylation of p38 MAP kinase in a time-dependent manner (Fig. 1, A and B).

**SB203580 Inhibited Phosphorylation of MAPKAPK-2.** To elucidate the roles of p38 MAP kinase for cell functions in PSCs, we employed two specific inhibitors of p38 MAP kinase: SB203580 (Cuenda et al., 1995) and SB202190 (Lee et al., 1994). We first examined whether SB203580 inhibited p38 MAP kinase activity. It has been shown that the inhibition of p38 MAP kinase by SB203580 is in a reversible manner in vivo that cannot be detected by in vitro immune complex kinase assays of p38 MAP kinase (Young et al., 1997). Therefore, we examined the effects of SB203580 on the phosphorylation of MAPKAPK-2, which is a downstream target of p38 MAP kinase (Rouse et al., 1994; Ben-Levy et al., 1995). IL-1β induced phosphorylation of MAPKAPK-2 at Thr222, as shown by Western blotting using anti-phosphospecific antibody (Fig. 2). SB203580 inhibited phosphorylation of MAPKAPK-2 in a dose-dependent manner. At 25 μM, IL-1β-induced phosphorylation of MAPKAPK-2 was almost completely abolished. In contrast, an inactive analog, SB202474, did not alter the phosphorylation of MAPKAPK-2 (data not shown). These results suggested that SB203580 inhibited the IL-1β-induced p38 MAP kinase activity.

**SB203580 and SB202190 Inhibited PDGF-Induced Proliferation.** It has been shown that PDGF-BB is the most potent mitogen for PSCs (Apte et al., 1999; Luttenberger et al., 2000). In agreement with previous reports, PDGF-BB induced an approximately 5-fold increase of cell proliferation in serum-free medium after 72 h (Fig. 3). To clarify the role of activation of p38 MAP kinase in PDGF-BB-induced proliferation of PSCs, we examined the effects of SB203580 and SB202190 on PDGF-BB-induced PSC proliferation. Both SB203580 and SB202190 inhibited PDGF-BB-induced cell proliferation in a dose-dependent manner (Fig. 3). The inhibitory effects were significant as low as 2.5 μM. At 25 μM, SB203580 and SB202190 abolished the stimulation of cell proliferation by PDGF-BB. In contrast, an inactive analog, SB202474, did not affect the proliferation up to 25 μM (data not shown). In these experiments, SB203580 and SB202190 up to these concentrations did not affect cell viability and morphology during the incubation as assessed by the trypan blue exclusion test (data not shown). However, when PSCs were treated with these inhibitors above 25 μM, cytotoxic effects were observed during the incubation.

**SB203580 and SB202190 Decreased Expression of α-SMA, α1(I) Procollagen, and Prolyl Hydroxylase (α).** It has been shown that culture-activated PSCs express α-SMA and produce type I collagen (Apte et al., 1998). Indeed, α-SMA expression has been accepted as a marker of PSC activation (Apte et al., 1998), and in situ hybridization techniques showed that α-SMA-positive cells were the principal source of collagen in the fibrotic pancreas (Haber et al., 1999). In agreement with the result of immunostaining, α-SMA expression was confirmed in culture-activated PSCs by Western blotting, and the treatment of PSCs for 48 h with SB203580 and SB202190 significantly reduced α-SMA expression (Fig. 4A). In contrast, SB202474 was ineffective. We also examined the effects of SB203580 and SB202190 on the expression of α1(I) procollagen and prolyl 4-hydroxylase (α) genes, both of which play a central role in the collagen synthesis. Steady-state mRNA levels of α1(I) procollagen and prolyl 4-hydroxylase (α) were very high in culture-activated cells. However, SB203580 and SB202190 inhibited α1(I) procollagen and prolyl 4-hydroxylase (α) expression in a dose-dependent manner (Fig. 4B). The inhibitory effects were significant as low as 2.5 μM. At 25 μM, SB203580 and SB202190 abolished the stimulation of cell proliferation by PDGF-BB. In contrast, an inactive analog, SB202474, did not affect the proliferation up to 25 μM (data not shown). In these experiments, SB203580 and SB202190 up to these concentrations did not affect cell viability and morphology during the incubation as assessed by the trypan blue exclusion test (data not shown). However, when PSCs were treated with these inhibitors above 25 μM, cytotoxic effects were observed during the incubation.
SB203580 and SB202190 Inhibited IL-1β-Induced MCP-1 Expression. Activated PSCs may acquire the ability to modulate the recruitment and activation of inflammatory cells. One candidate may be MCP-1, a potent chemoattractant for monocytes and T lymphocytes (Ben-Baruch et al., 1995). Indeed, we have previously shown that IL-1β and tumor necrosis factor-α induced MCP-1 expression in activated PSCs (Masamune et al., 2002a). To clarify the role of activated p38 MAP kinase in MCP-1 expression, PSCs were treated with IL-1β in the presence of SB203580 or SB202190. IL-1β induced MCP-1 production in PSCs, and SB203580 and SB202190 decreased the inducible MCP-1 expression in a dose-dependent manner (Fig. 5A). The inhibitory effect was significant as low as 1 μM. At 25 μM, the MCP-1 induction was decreased to 40% of the control (IL-1β only) by SB203580 and to 35% by SB202190. The dose dependence of MCP-1 inhibition was roughly consistent with that of p38 MAP kinase inhibition, suggesting a role of p38 MAP kinase in the IL-1β-induced MCP-1 production. We also examined the effect of SB203580 and SB202190 on the MCP-1 gene expression by Northern blotting. IL-1β increased the level of MCP-1 mRNA, and the level was decreased in the presence of SB203580 and SB202190 (Fig. 5B), indicating that SB203580 and SB202190 inhibited IL-1β-induced MCP-1 expression, at least in part, at the transcriptional level.

Activation of NF-κB plays a central role in MCP-1 expression in PSCs (Andoh et al., 2000). In addition, the p38 MAP kinase pathway and the NF-κB pathway may reportedly “cross-talk” in several experimental systems (Lee et al., 1998; Nemoto et al., 1998). The inhibition of IL-1β-induced MCP-1 expression by SB203580 prompted us to examine whether SB203580 inhibited MCP-1 expression through altered NF-κB activation. SB203580 did not alter IL-1β-induced NF-κB binding activity (Fig. 6A) and IκB-α degradation (Fig. 6B), indicating that the p38 MAP kinase pathway does not interfere with release of NF-κB from IκB, nor with its nuclear translocation and DNA binding.

Wortmannin failed to inhibit proliferation and MCP-1 production. Because SB203580 was shown to inhibit the Akt pathway independently of p38 MAP kinase (Lali et al., 2000), it was possible that the effects of SB203580 observed in this study were through the inhibition of Akt pathway. To clarify this issue, we compared the effects of a specific inhibitor of phosphatidylinositol 3-kinase, wortmannin (Ui et al., 1995), with those of SB203580 and SB20190. First, we examined whether PDGF-BB and IL-1β activated Akt in PSCs. Because phosphorylation at serine 473 correlates with Akt activity (Chan et al., 1999), we assessed the activation of Akt by Western blotting using anti-phosphospecific Akt antibody at Ser 473. Phosphorylated Akt was barely detected in unstimulated cells but was readily apparent within 5 min of PDGF-BB treatment (Fig. 7A). IL-1β also

![Fig. 4](image-url) - SB203580 and SB202190 decreased expression of α-SMA, α1(I) procollagen, and prolyl hydroxylase (α). A, PSCs were left untreated (control) or were treated with SB203580, SB202190, or SB202474 at 25 μM for 48 h. Then, total cell lysates (approximately 100 μg) were prepared, and the level of α-SMA was determined by Western blotting. B, after treatment with SB203580, SB202190, or SB202474 at 25 μM for 24 h, total RNA was extracted, and mRNA levels of α1(I) procollagen and prolyl hydroxylase (α) [PH(α)] were determined by Northern blotting.

![Fig. 5](image-url) - Inhibition of p38 MAP kinase decreased MCP-1 expression. A, PSCs were treated with SB203580 or SB202190 at the indicated concentrations for 30 min, and then stimulated with IL-1β (at 2 ng/ml). After 24 h, MCP-1 levels in the culture supernatant were determined by enzyme-linked immunosorbent assay. Data shown are expressed as means ± S.D. (n = 6 for each data point; **, p < 0.01 versus control). B, PSCs were treated with SB203580 or SB202190 at the indicated concentrations for 30 min, and then stimulated with IL-1β (at 2 ng/ml). After 6 h, total RNA was extracted, and the level of MCP-1 mRNA was determined by Northern blotting.
induced phosphorylation of Akt, but the activation was delayed and less evident than that by PDGF-BB. Wortmannin at 100 nM completely blocked the phosphorylation of Akt (Fig. 7B) but failed to inhibit PDGF-BB-induced proliferation or IL-1β-induced MCP-1 expression (Fig. 7, C and D). Neither SB203580, SB202190, nor SB202474 inhibited PDGF-BB-induced activation of Akt in our experimental system (Fig. 7B). Taken together, it was unlikely that SB203580 inhibited these cell functions through inhibition of Akt.

**SB203580 Blocked Spontaneous Activation of Freshly Isolated PSCs.** We finally examined whether SB203580 blocked the transformation of PSCs from a quiescent to a myofibroblast-like phenotype. PSCs isolated from rat pancreas were incubated with or without SB203580 for 7 days, and morphological changes and α-SMA expression were assessed under phase contrast microscopy and Western blotting, respectively. PSCs on culture day 1, or so-called quiescent PSCs, were small and circular, contained lipid droplets in the cytoplasm and dendritic processes, and had a star-like configuration (data not shown). On day 7, PSCs showed enlarged cell bodies and transformation into cells with a myofibroblast-like phenotype (Fig. 8A). In contrast, PSCs cultured in the presence of SB203580 on culture day 7 were smaller, with slender dendritic processes, and lipid droplets present in the cytoplasm in a significant number of cells (Fig. 8B). Significant expression of α-SMA was observed on day 7 in culture-activated PSCs, and the expression was lower in the presence of SB203580 (Fig. 8C). To rule out the possibility that the effects of SB203580 might have been due to cytotoxicity, SB203580 was withdrawn from PSCs that had been treated with it for 7 days. Two days after the withdrawal of SB203580, PSCs showed the typical phenotype of activated PSCs (Fig. 8D). Taken together, our results suggested that activation of p38 MAP kinase was in part involved for spontaneous transformation from a quiescent to a myofibroblast-like phenotype in PSCs.

**Discussion**

After pancreatic injury, PSCs undergo a transformation from quiescent cells to activated myofibroblast-like cells, which produce cytokines and extracellular matrix proteins. There is accumulating evidence that activated PSCs play critical roles in the pathogenesis of pancreatic fibrosis and inflammation (Apte et al., 1998; Bachem et al., 1998; Haber et al., 1999; Andoh et al., 2000; Masamune et al., 2002c). The present study demonstrated that p38 MAP kinase was activated in response to IL-1β and PDGF-BB in PSCs, and that p38 MAP kinase inhibitors SB203580 and SB202190 inhibited many key parameters of PSC activation including cell proliferation, α-SMA expression, α1(I) procollagen and prolyl 4-hydroxylase (α) gene expression, and MCP-1 production. In addition, SB203580 blocked spontaneous activation of freshly isolated PSCs in culture on plastic, suggesting a role of activated p38 MAP kinase in the activation process of PSCs. These effects appear not through the potential cyto-
Stellate cell proliferation and the expansion of their pool are a fundamental feature of pancreatic fibrosis (Haber et al., 1999). Recent studies suggest that PDGF-BB is the most potent mitogen of PSCs and is likely to be an important mediator of the increased proliferation of the cells both in vivo and in vitro (Apte et al., 1999; Luttenberger et al., 2000). Haber et al. (1999) reported that PDGF-β receptor is closely associated with desmin staining in areas of fibrosis, suggesting that PSCs expressed PDGF-β receptor. In fibrous tissue sections from patients with chronic pancreatitis, the concomitant overexpression of PDGF-BB and PDGF receptor-β points to the existence of autocrine and paracrine PDGF-dependent loops in human chronic pancreatitis. In this study, PDGF-BB-induced proliferation was abolished by SB203580 and SB202190, suggesting a major role of this kinase in PDGF-BB-induced proliferation of PSCs. Although PDGF has been shown to activate p38 MAP kinase in several types of cells, roles of activated p38 MAP kinase in cellular function are cell type-specific. For example, p38 MAP kinase mediated PDGF-induced cell growth in rat cultured vascular smooth muscle cells (Yamaguchi et al., 2001). In porcine aortic endothelial cells, activation of p38 MAP kinase was required for PDGF-induced cell migration and actin reorganization, but not for PDGF-stimulated DNA synthesis (Matsumoto et al., 1999). The signal transduction pathways involved in the proliferative response of PSCs to PDGF are currently unknown, but they have been characterized in detail in hepatic stellate cells; PDGF-induced proliferative response is mediated by the rapid activation of the extracellular signal-regulated kinases (Marra et al., 1999) and, in part, by the activation of phosphatidylinositol 3-kinase (Marra et al., 1997). In this study, wortmannin, an inhibitor of phosphatidylinositol 3-kinase, did not affect the PDGF-BB-induced PSC proliferation, suggesting cell type-specific variations in signal transduction between PSCs and hepatic stellate cells.

Activated PSCs may acquire the ability to modulate the recruitment and activation of inflammatory cells. MCP-1 expression by myofibroblasts is shown to be increased in fibrous tissue sections from patients with chronic pancreatitis (Saurer et al., 2000). Furthermore, MCP-1 may also act as a fibrosis-promoting chemokine; MCP-1 stimulated collagen gene expression via endogenous up-regulation of transforming growth factor β in rat lung fibroblasts (Gharaei-Kermani et al., 1996). Therefore, control of MCP-1 expression is an important therapeutic target for pancreatic fibrosis as well as inflammation. We have previously shown that IL-1β and tumor necrosis factor-α induced expression of MCP-1 (Masamune et al., 2002a) and intercellular adhesion molecule-1 (Masamune et al., 2002c) in PSCs. SB203580 and SB202190 here inhibited IL-1β-induced MCP-1 expression, indicating that p38 MAP kinase is required for optimal MCP-1 expression in PSCs. This is in agreement with the previous report showing that p38 MAP kinase was necessary for IL-1β-induced MCP-1 expression in human mesangial cells (Rovin et al., 1999). In contrast, inducible ICAM-1 expression was not inhibited by SB203580 (Masamune et al., 2002c), suggesting a differential role of p38 MAP kinase in proinflammatory responses in PSCs. We have also reported that ethanol and acetaldehyde at clinically relevant concentrations activated p38 MAP kinase but failed to induce MCP-1 expression in PSCs (Masamune et al., 2002b). Thus, activation of p38 MAP kinase is required, but not sufficient for MCP-1 expression in PSCs.

Cross-talk between the MAP kinase and NF-κB pathways has been demonstrated in a number of recent studies. For example, the MAP kinase family members MAP kinase kinase and NF-κB-inducing kinase can each directly activate the IκB kinase signalsome, resulting in IκB phosphorylation and release of activated NF-κB (Lee et al., 1998; Nemoto et al., 1998). Thus, we examined whether p38 MAP kinase activation by IL-1β was an upstream signaling event in the pathway leading to NF-κB activation and consequent MCP-1 production. Although SB203580 was effective in blocking MCP-1 production, it did not have any apparent effect on IL-1β-induced NF-κB binding activity or IκB-α degradation. These data suggested that activation of p38 MAP kinase is not required for IL-1β-induced NF-κB activation. Thus, the p38 MAP kinase and NF-κB pathways may exert independent regulatory effects on IL-1β-induced MCP-1 production. Although Bergmann et al. (1998) reported that a p38 MAP kinase inhibitor reduced NF-κB-mediated transactivation in response to tumor necrosis factor-α without affecting NF-κB translocation, our results are in agreement with other studies showing that p38 MAP kinase and NF-κB have been shown to regulate cytokine gene expression by independent pathways (Beyaert et al., 1996; Yamakawa et al., 1999). However, the mechanism by which p38 regulates cytokine production without altering NF-κB activation and DNA binding is not known.

In this study, SB203580 inhibited spontaneous activation of freshly isolated PSCs in culture on plastic, suggesting a role of p38 MAP kinase for the activation process of PSCs.
This is in agreement with the previous study by Reeves et al. (2000) showing that p38 MAP kinase was in part responsible for activation of hepatic stellate cells. They reported that constitutive activity of p38 MAP kinase was higher in transformed versus quiescent cells, and that another p38 MAP kinase inhibitor, SB202190, reduced activation of hepatic stellate cells in culture as assessed by α-SMA expression. In vascular smooth muscle cells, α-SMA expression is regulated through the activation of α-SMA promoter, and inhibition of p38 MAP kinase decreased arginine vasopressin-stimulated α-SMA promoter activity (Garat et al., 2000). Furthermore, transfection of a constitutively active form of a specific p38 MAP kinase kinase increased α-SMA promoter activity in the absence of vasoconstrictor stimulation. Along this line, it appears that α-SMA expression is regulated in part through the activation of p38 MAP kinase in PSCs.

Because p38 MAP kinase inhibitor blocked profibrogenic and proinflammatory actions in activated PSCs, it would be interesting to examine whether inhibition of p38 MAP kinase might provide new therapeutic strategies for pancreatic fibrosis and inflammation. We previously reported that troglitazone, a ligand of the peroxisome proliferator-activated receptor-γ, modulated profibrogenic and proinflammatory actions in a manner similar to that of the inhibitors of p38 MAP kinase (Masamune et al., 2002a). Very recently, Shimizu et al. (2002) have reported that troglitazone prevented the progression of pancreatic inflammatory process and fibrosis in an animal model of chronic pancreatitis, suggesting that PSCs are potential targets of antifibrogenic and anti-inflammatory strategies in vivo. Experiments designed to test this hypothesis are under way in our laboratory.

References


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