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Existence of Autocrine Loop Between Interleukin-6 and Transforming Growth Factor-β_1 in Activated Rat Pancreatic Stellate Cells

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Abstract Interleukin (IL)-6 is a proinflammatory cytokine assumed to participate in pancreatic fibrosis by activating pancreatic stellate cells (PSCs). Autocrine TGF-β_1 is to central in PSC functional regulation. In this study, we examined IL-6 secretion from culture-activated rat PSCs and its regulatory mechanism. Activated PSCs express and secrete IL-6. When anti-TGF-β_1 neutralizing antibody was added in the culture medium, IL-6 secretion from activated PSCs was inhibited, whereas exogenous TGF-β_1 added in the culture medium enhanced IL-6 expression and secretion by PSCs in a dose dependent manner. Infection of PSCs with an adenovirus expressing dominant-negative Smad2/3 attenuated basal and TGF-β_1-stimulated IL-6 expression and secretion of PSCs. We also demonstrated the reciprocal effect of PSCs-secreted IL-6 on autocrine TGF-β_1. Anti-IL-6 neutralizing antibody inhibited TGF-β_1 secretion from PSCs. Preincubation of cells with 10 nM PD98059, an extracellular signal-regulated kinase (ERK)-dependent pathway inhibitor, attenuated IL-6-enhanced TGF-β_1 expression and secretion of PSCs. In addition, IL-6 activated ERK in PSCs. These data indicate the existence of autocrine loop between IL-6 and TGF-β_1 through ERK- and Smad2/3-dependent pathways in activated PSCs. J. Cell. Biochem. 99: 221–228, 2006. © 2006 Wiley-Liss, Inc.

Key words: TGF-β_1; interleukin-6; pancreatic stellate cells; autocrine loop; Smad; ERK; IL-6; pancreatic fibrosis

Interleukin-6 is a multifunctional cytokine that regulates various immune and inflammatory responses in various tissues including gastrointestinal organs [Ishihara and Hirano, 2002]. For example, IL-6 plays a role in the perpetuation of inflammation in inflammatory bowel diseases [Ishihara and Hirano, 2002]. As to pancreas, increased serum IL-6 mediates local and systemic inflammatory responses in the early phase of acute pancreatitis [Bentrem and Joehl, 2003]. In chronic pancreatitis patients, the serum concentration of IL-6 is still elevated [Bamba et al., 1994]. In addition, IL-6 is expressed in pancreatic tissue of experimental chronic pancreatitis [Xie et al., 2001]. Thus, it has been postulated that IL-6 participates in chronic pancreatitis as well.

Pancreatic stellate cells (PSCs) are recently identified, isolated, and characterized [Apte et al., 1998; Bachem et al., 1998]. In the normal pancreas, PSCs possess fat droplets containing vitamin A and are quiescent defined with desmin positive but α-smooth muscle actin (α-SMA) negative staining [Bachem et al., 1998]. When cultured in vitro, PSCs are auto-activated (auto-transformed) changing their morphological and functional features [Apte et al., 1998]. PSCs commence losing vitamin A containing lipid droplets, highly proliferating, increasing expression of α-SMA, and producing and secreting extracellular matrix components such as collagen and fibronectin. Namely, PSCs are auto-transformed to myofibroblast-like cells. In vivo, PSCs are also activated during both human and experimental pancreatic fibrosis [Haber et al., 1999]. Therefore, PSCs are

Abbreviations used: ERK, extracellular signal-regulated kinase; IL-6, interleukin-6; Smad, Sma- and Mad-related protein; TGF-β_1, transforming growth factor β_1.

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thought to play an important role in pancreatic fibrogenesis. Recently, IL-6 has been shown to increase the expression of α-SMA, a parameter of PSC activation, and collagen in PSCs [Mews et al., 2002]. Thus, IL-6 is assumed to promote pancreatic fibrosis by stimulating PSCs.

TGF-β_1 is one of major profibrogenic cytokines that play a role in pancreatic fibrosis. TGF-β_1 expression is observed in the fibrotic regions of the pancreas of chronic pancreatitis patient [Haber et al., 1999]. Ectopic overexpression of TGF-β_1 in pancreatic islets induced severe fibrosis of pancreatic exocrine glands [Lee et al., 1995]. Furthermore, TGF-β_1 is to central in regulating PSC functions. For instance, TGF-β_1 activates PSCs, inhibits PSC growth and enhances extracellular matrix production and secretion [Apte et al., 1999; Kruse et al., 2000]. TGF-β_1 intracellular signaling is mediated through multiple pathways: Smad-2/3-dependent or -independent ones [Massague, 1998; Attisano and Wrana, 2002]. In Smad2/3-dependent pathway, upon binding to its receptor complex, TGF-β_1 activates type II receptor leading to the phosphorylation of Smad2 and Smad3. Phoshphorylated Smad2 or Smad3 (Smad2/3) binds to Smad4 and entered the nucleus, where Smad2/3-Smad4 complex promotes target gene transcription. In Smad2/3-independent pathway, various mitogen-activated protein kianses such as p38 and ERK are involved [Attisano and Wrana, 2002]. Recently, we have shown that TGF-β_1 regulates multiple PSC functions via distinct intracellular signaling pathways [Ohnishi et al., 2004]. Since TGF-β_1 is a major regulator of PSC functions and is currently assumed to be a candidate of target molecules of the treatment for pancreatic fibrosis, it is important to elucidate which pathway mediates TGF-β_1 diverse effects on PSC functions.

Activated PSCs have been shown to secrete multiple cytokines that modulate their own functions including TGF-β_1 [Kruse et al., 2000] and activin A [Ohnishi et al., 2003]. We thus hypothesized that activated PSCs may express and secrete IL-6. In this study, therefore, we investigated IL-6 expression and secretion of activated PSCs and its regulatory mechanism. We report here that TGF-β_1 increases IL-6 mRNA expression and peptide secretion of activated PSCs in an autocrine manner. Further, we show that Smad2/3-dependent signaling pathway mediates TGF-β_1 enhancement of IL-6 expression and secretion of PSCs. Finally, we demonstrate that IL-6 increases TGF-β_1 expression and secretion of PSCs via an ERK-dependent pathway, indicating the existence of autocrine loop between IL-6 and TGF-β_1 in activated PSCs

MATERIALS AND METHODS

Materials

TGF-β_1, Nycodenz, and pronase were purchased from Sigma (St. Louis, MO). IL-6, anti-IL6, and anti-TGF-β_1 neutralizing antibodies were from R&D (Abrington, UK). DNase I was from Roche (Basel, Switzerland). Collagenase P was from Boehringer Mannheim (Mannheim, Germany). PD98059 was from Calbiochem (San Diego, CA). Anti-ERK antibody was from Santa Cruz (Santa Cruz, CA). Anti-phospho-ERK antibody was from Cell Signaling (Beverly, MA). Horseradish peroxidase (HRP)-conjugated donkey anti-rabbit IgG and HRP-conjugated donkey anti-mouse IgG were from Jackson Immuno Research (West Grove, PA).

Isolation and Culture of Rat Pancreatic Stellate Cells

Rat pancreatic stellate cells were prepared as described [Apte et al., 1998]. Briefly, rat pancreas was digested in Gey's balanced salt solution supplemented with 0.05% collagenase P, 0.02% pronase, and 0.1% DNase. After filtration through nylon mesh, cells were centrifuged in a 13.2% Nycodenz gradient at 1,400*g* for 20 min. PSCs in the band just above the interface of the Nycodenz solution and the aqueous one were collected, washed and resuspended in Iscove's modified Dulbecco's medium containing 10% fetal calf serum, 100 U/ml penicillin, and 100 μg/ml streptomycin. PSCs were cultured in a 5% CO_2 atmosphere at 37°C. All experiments were carried out using culture-activated PSCs between passages two and three.

Adenovirus Infection

Recombinant adenovirus of dominant-negative Smad2/3 (AdDNSmad2/3) was kindly provided by Dr. Miyazono (University of Tokyo, Japan). For adenovirus infection, cells were infected with a recombinant adenovirus at a dose

of 10 plaque-forming units (pfu) per cell in the culture media described above. An adenovirus expressing β-galactosidase (AdLacZ) was used as an infection control.

Measurement of IL-6 and TGF-β_1 Peptides Secretion

Secretion of IL-1β and TGF-β_1 peptides was measured by determining their concentration in the culture medium using commercial ELISA kits (Biosource International, Camarillo, CA; DRG International, Mountainside, NJ), according to the manufacturers' instructions.

RT-PCR

Total RNA was isolated from PSCs using TRIzol reagent (Life Technologies BRL, Grand Island, NY). First-strand cDNA was made from total RNA using ReverTra Ace system (Toyobo, Tokyo, Japan) according to the manufacturer's instructions. PCR for TGF-β_1 was performed using PCR kit for rat TGF-β_1 (Maximbio, San Francisco, CA) according to the manufacturer's instructions. PCR for rat IL-6 and GAPDH was performed using the following primers: (a) rat IL-6: sense, 5′-CTGGTCTTCTGGAGTTCCGTTTC-3′; anti-sense, 5′-CATAGCACACTAGGTTTGCCGAG-3′; (b) rat GAPDH: sense, 5′-CATGACCACAGTCCATGCCATC-3′, anti-sense, 5′-CGTTGCT-GTAGCCATATTC-3′. The reactions were conducted with the following cycle conditions: denaturation at 94°C for 0.5 min, annealing at 45°C for 1 min, and extension at 72°C for 1 min for 30 cycles.

Western Blotting

Western blotting was carried out as described before, [Ohnishi et al., 1997] using enhanced chemiluminescence reagent to visualize the secondary antibody.

Statistical Analysis

The data was analyzed by ANOVA to determine statistical significance and $P < 0.05$ was considered significant.

RESULTS

Activated Rat PSCs Secrete IL-6

We first examined whether activated rat PSCs secrete IL-6. As shown in Figure 1, ELISA revealed that IL-6 peptide was present in PSC culture medium. IL-6 concentration in PSC culture medium increased during 2 days incubation. No IL-6 activity was detected in fresh culture medium. These data indicate that activated PSCs secrete IL-6.

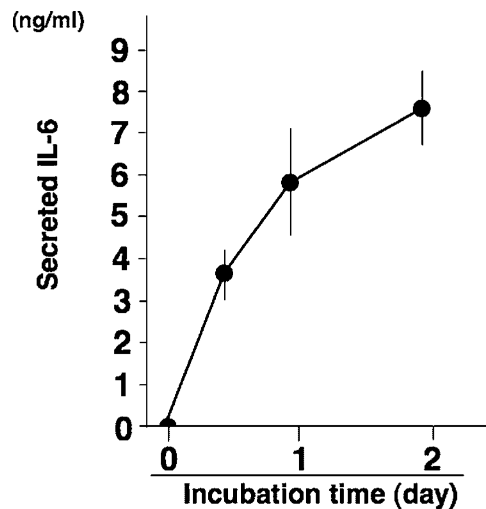

Fig. 1. IL-6 secretion from activated PSCs. IL-6 concentration in culture medium was determined with ELISA 1–2 days after the culture medium was changed. Values are mean ± SE for three independent experiments.

Autocrine TGF-β_1 Stimulates IL-6 Secretion From PSCs

Showing that IL-6 is secreted from activated PSCs, we next attempted to elucidate the mechanism that regulates IL-6 secretion from PSCs. Since autocrine TGF-β_1 plays central roles in the regulation of PSC functions, we examined the participation of autocrine TGF-β_1 in IL-6 secretion from PSCs using anti-TGF-β_1 neutralizing antibody. As shown in Figure 2, anti-TGF-β_1 neutralizing antibody added in the culture medium inhibited IL-6 secretion from activated PSCs in a dose dependent manner. In contrast, non-immune IgG did not alter it. These data indicate that autocrine TGF-β_1 stimulates IL-6 secretion from activated PSCs.

Exogenous TGF-β_1 Increases IL-6 Expression and Secretion of Activated PSCs

As an independent experiment examining TGF-β_1 stimulatory effect on IL-6 secretion from PSCs, we next examined the effect of exogenous TGF-β_1 on the IL-6 expression and

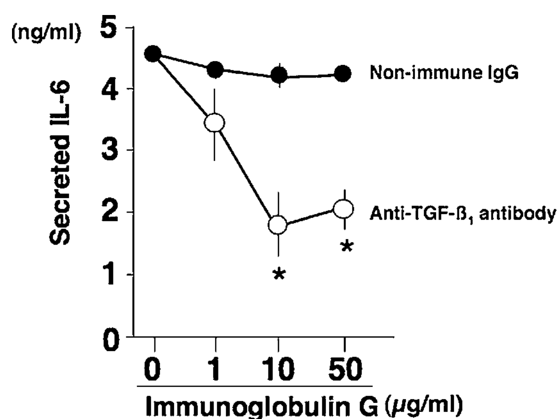

Fig. 2. Effect of anti-TGF-β_1 neutralizing antibody and non-immune IgG on IL-6 secretion from PSCs. Concentration of IL-6 secreted from PSCs into culture medium was determined with ELISA after 48 h incubation with indicated amounts of anti-TGF-β_1 antibody (open circles) or non-immune IgG (closed circles). Values are mean ± SE for three independent experiments. $^*P < 0.05$ versus control.

secretion of PSCs. As shown in Figure 3, TGF-β_1 added in the culture medium increased both IL-6 mRNA expression and peptide secretion of PSCs in a dose dependent manner. These data

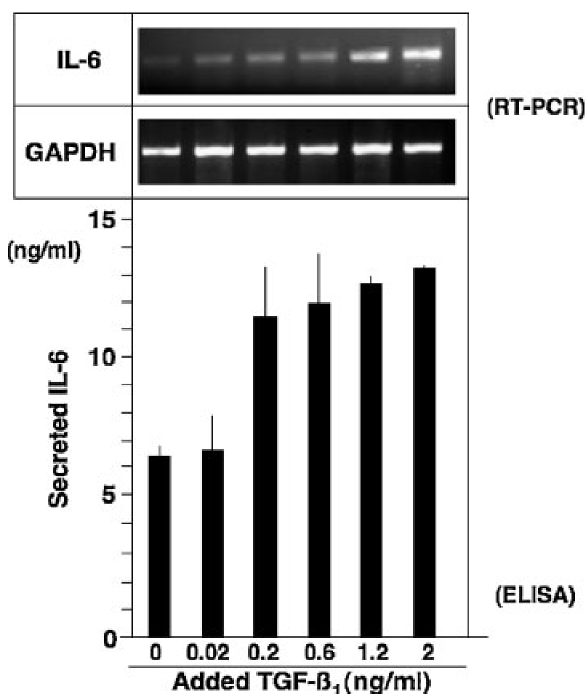

Fig. 3. Exogenous TGF-β_1 enhanced IL-6 expression and secretion by activated PSCs. Cells were incubated for 48 h with the indicated amounts of TGF-β_1. After the incubation, IL-1β mRNA expression was determined with RT-PCR using GAPDH mRNA expression as a control. Concentration of IL-6 secreted from PSCs into culture medium during the incubation was determined with ELISA. Values are mean ± SE for three independent experiments.

reinforce that autocrine TGF-β_1 enhances IL-6 secretion from PSCs.

Autocrine TGF-β_1 Enhances IL-6 Expression and Secretion by PSCs Through Smad-Dependent Pathway

We next examined the intracellular signaling pathway through which TGF-β_1 stimulates IL-6 expression and secretion by PSCs. TGF-β_1 intracellular signaling is mediated by Smad-dependent or Smad-independent pathway. We thus investigated whether TGF-β_1 stimulates IL-6 expression and secretion of PSCs through Smad-dependent pathway. For this purpose, we used adenovirus vector of dominant-negative Smad2/3 (AdDNSmad2/3). This dominant-negative Smad2/3 mutant was generated by substituting Glu for Asp-407 of smad3, which renders it defective in TGF-β receptor-dependent phosphorylation. Nevertheless, this mutant possesses a dominant-negative effect on both Smad2 and Smad3 [Goto et al., 1998]. We utilized an adenovirus expressing β-galactosidase (AdLacZ) as an infection control. We previously reported that more than 98% PSCs are infected with these adenovirus vectors and expressed sufficiently each protein [Ohnishi et al., 2004]. In addition, the infections of these adenoviruses do not affect TGF-β_1 mRNA expression or peptide secretion by PSCs [Ohnishi et al., 2004]. Therefore, we can observe the effect of these adenoviruses infections on IL-6 expression and secretion by PSCs modulated by autocrine TGF-β_1, regardless of the effect of the infection on the amount of autocrine TGF-β_1. As shown in Figure 4, when Smad-dependent pathways were blocked by AdDNSmad2/3 infection, both basal IL-6 mRNA expression (Fig. 4: RT-PCR; compare first and third lanes) and basal peptide secretion (Fig. 4: ELISA; compare first and third columns) of PSCs were attenuated compared to AdLacZ-infected controls. Since infection with AdDNSmad2/3 or AdLacZ does not alter autocrine TGF-β_1 secretion from PSCs as reported previously [Ohnishi et al., 2004], these data indicate that autocrine TGF-β_1 stimulates IL-6 mRNA expression and peptide secretion in an autocrine manner via Smad-dependent pathway. Moreover, AdDNS mad2/3 attenuated exogenous TGF-β_1 enhanced IL-6 expression and secretion (Fig. 4), thus reinforcing our suggestion that TGF-β_1 stimulates IL-6 expression and secretion via Smad-dependent pathway.

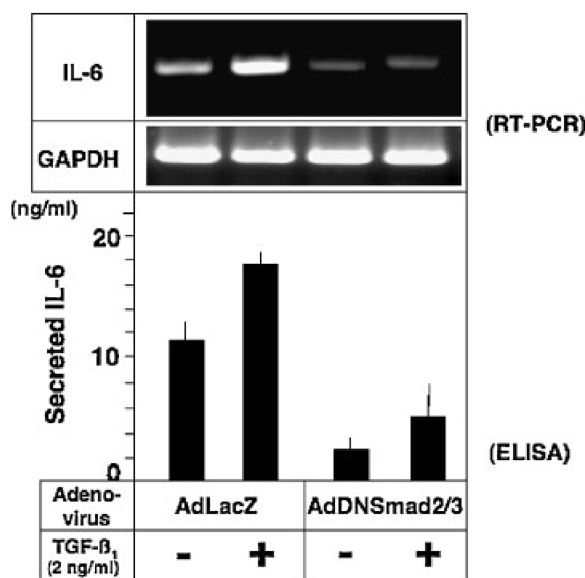

Fig. 4. Adenovirus-mediated expression of dominant-negative Smad2/3 inhibited IL-6 expression and secretion by activated PSCs. PSCs were infected with 10 pfu/cell AdLacZ or AdDNSmad2/3, then incubated for 48 h with or without 2 ng/ml TGF-β_1. After the incubation, IL-1β mRNA expression was determined with RT-PCR using GAPDH mRNA expression as a control. Concentration of IL-6 secreted from PSCs into culture medium during the incubation was determined with ELISA. Values are mean ± SE for three independent experiments.

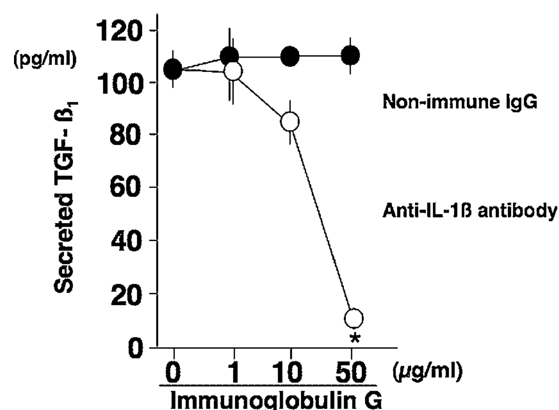

Fig. 5. Effect of anti-IL-6 neutralizing antibody and non-immune IgG on TGF-β_1 secretion from PSCs. Concentration of TGF-β_1 secreted from PSCs into culture medium was determined with ELISA after 48 h incubation with indicated amounts of anti-IL-6 antibody (open circles) or non-immune IgG (closed circles). Values are mean ± SE for three independent experiments. *$P < 0.01$ versus control.

Autocrine IL-6 Stimulates TGF-β_1 Secretion From Activated PSCs

Since exogenous IL-6 modulates PSC functions [Mews et al., 2002] and increases TGF-β_1 expression and secretion of activated PSCs (our data submitted for publication), we hypothesized that IL-6 secreted from PSCs may also increase the TGF-β_1 autocrine secretion from PSCs. To test this hypothesis, we examined the effect of anti-IL-6 neutralizing antibody on TGF-β_1 secretion from activated PSCs. As shown in Figure 5, anti-IL-6 antibody added into culture medium attenuated TGF-β_1 secretion from PSCs in a dose dependent manner. In contrast, non-immune IgG did not affect TGF-β_1 secretion from PSCs. These data indicate that autocrine IL-6 stimulated TGF-β_1 secretion from activated PSCs.

IL-6 Increases TGF-β_1 mRNA Expression and Peptide Secretion by PSCs Through ERK-Dependent Pathway

Showing that autocrine IL-6 enhances TGF-β_1 secretion from PSCs, we next attempted to elucidate the intracellular signaling pathway through which IL-6 stimulates TGF-β_1 secretion by PSCs. Since ERK is one of IL-6 signaling mediators [Ishihara and Hirano, 2002], we examined the participation of the ERK-dependent pathway in IL-6 stimulation of TGF-β_1 secretion by PSCs. For this purpose, we first examined whether exogenous IL-6 activates ERK in PSCs using western blotting with anti-phospho-ERK antibody. As shown in Figure 6A, 100 ng/ml IL-6 enhanced ERK phosphorylation and maximum enhancement was observed at 10 min incubation, indicating that IL-6 activates ERK in PSCs. We then investigated the effect of ERK-dependent pathway inhibitor, PD98059 on the IL-6 enhancement of TGF-β_1 expression and secretion in activated PSCs. We have previously demonstrated that preincubation with 10 nM PD98059 blocks ERK-dependent pathway in PSCs [Ohnishi et al., 2004]. In naive cells, 100 ng/ml exogenous IL-6 augmented TGF-β_1 expression and secretion, which is consistent with our recent data (submitted for publication). When cells were pretreated with PD98059, IL-6 could not increase TGF-β_1 expression or secretion of activated PSCs (Fig. 6B). These data suggest that IL-6 enhances TGF-β_1 mRNA expression and secretion of activated PSCs through ERK-dependent pathway.

DISCUSSION

In this study, we demonstrated that activated PSCs express and secrete IL-6. Furthermore,

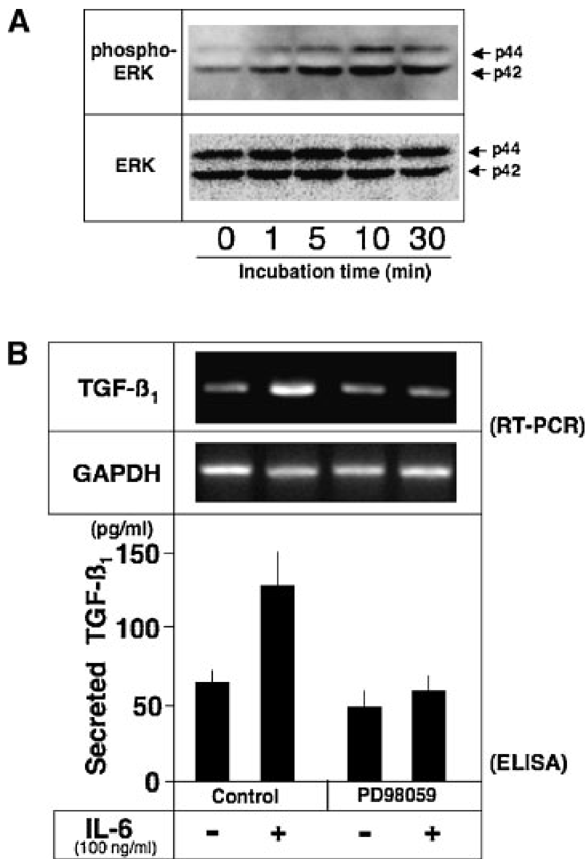

Fig. 6. ERK mediates IL-6-induced TGF-β_1 expression and secretion of activated PSCs. **A**: Effect of IL-6 on ERK activation in activated PSCs. Cells were incubated with 100 ng/ml IL-6 for indicated times. The activation of ERK was then determined with Western blotting using anti-phosphorylated ERK antibody (**upper panel**). Western blotting using anti-ERK antibody was carried out as an internal control (**lower panel**). Data are representative of three independent experiments with similar results. **B**: Effect of MEK1 inhibitor PD98059 on TGF-β_1 expression and secretion of activated PSCs. After 2 h of pretreatment with or whithout 10 nM PD98059, cultured PSCs were incubated for 48 h in the presence or absence of 100 ng/ml IL-6. After the incubation, TGF-β_1 mRNA expression was determined with RT-PCR, using GAPDH mRNA expression as an internal control. TGF-β_1 peptide secreted into culture medium from PSCs during the incubation was quantified with ELISA. Values are mean ± SE for three independent experiments.

TGF-β_1 enhances IL-6 expression and secretion through Smad2/3 dependent pathway. Reciprocally, IL-6 increases TGF-β_1 expression and secretion of activated PSCs through ERK dependent pathway. We also demonstrated that anti-TGF-β_1 and anti-IL-6 neutralizing antibodies attenuate IL-6 and TGF-β_1 secretion from activated PSCs, respectively. Accordingly, there exist an autocrine loop between IL-6 and TGF-β_1 through ERK- and Smad2/3-dependent pathways in activated PSCs.

IL-6 is a multifunctional cytokine that participates in both acute and chronic inflammation. The process of IL-6-involved inflammation has been studied mainly on the autoimmune and chronic inflammatory diseases such as rheumatoid arthritis and inflammatory bowel diseases [Ishihara and Hirano, 2002]. In the acute phase of these diseases, IL-6 mediates acute responses including production of acute-phase proteins such as C-reactive protein and the activation of the complement system [Papanicolaou et al., 1998]. In the chronic phase of the inflammation, IL-6 continuously supports the survival and the growth of lymphocytes and myeloid cells, leading to the amplification and the perpetuation of the inflammation [Ishihara and Hirano, 2002]. As to pancreatic inflammation, similar process of IL-6 participation in acute and chronic pancreatitis has been studied. In acute pancreatitis, serum IL-6 level is elevated, which is related to the severity and the systemic complications [Heresbach et al., 1998], suggesting that IL-6 directly mediates systemic inflammatory responses in acute pancreatitis. In chronic pancreatitis, although systemic inflammation is readily resolved, serum IL-6 level is still elevated [Bamba et al., 1994] and IL-6 is expressed in pancreatic tissue of experimental chronic pancreatitis, especially in infiltrated inflammatory cells [Xie et al., 2001]. In addition, IL-6 activates PSCs and enhances their collagen production [Mews et al., 2002]. Thus, in chronic pancreatitis, it has been assumed that IL-6 secreted from inflammatory cells promotes pancreatic fibrosis by activating PSCs in a paracrine mechanism. However, our current observations that activated PSCs express and secrete IL-6 indicate that activated PSCs are also the source of IL-6 in the pancreatic tissue of chronic pancreatitis. Thus, it is reasonable to speculate that IL-6 secreted from PSCs may perpetuate chronic pancreatic inflammation by stimulating inflammatory cells in chronic pancreatitis in a paracrine manner. Furthermore, IL-6 secreted from PSCs modulates TGF-β_1 secretion of PSCs (Fig. 5) indicating that IL-6 also promotes pancreatic fibrosis by regulating PSC function in an autocrine fashion.

Since both IL-6 and TGF-β_1 play pivotal roles in various inflammatory diseases, much attention has been paid to the interaction between the two cytokines. To date, IL-6 diverse effects on TGF-β_1 action has been demonstrated. In human renal epithelial cells, IL-6 augments

TGF-β_1 signaling by modulating the trafficking of TGF-β_1 receptors [Zhang et al., 2005]. Moreover, we have recently elucidated that exogenous IL-6 increases TGF-β_1 expression and secretion by activated PSCs (submitted for publication). Thus, current study has extended the knowledge obtained from previous studies by showing the autocrine loop between IL-6 and TGF-β_1 and elucidating its intracellular signaling pathways.

TGF-β_1 has been implicated in the etiology of pancreatic fibrosis. TGF-β_1 is also secreted from PSCs and regulates multiple PSC functions, including activation, proliferation, and extracellular matrix production [Kruse et al., 2000; Mews et al., 2002]. Our current data that autocrine TGF-β_1 increases IL-6 secretion of activated PSCs demonstrate another novel TGF-β_1 effect on PSC function. Since IL-6 supports the survival and the growth of inflammatory cells as described above, these data suggest that autocrine TGF-β_1 promotes pancreatic fibrosis, at least in part, by sustaining pancreatic inflammation by increasing IL-6 secretion from PSCs. Furthermore, the reciprocal effect of autocrine IL-6 on TGF-β_1 secretion from PSCs is also important in the progression of pancreatic fibrosis. TGF-β_1 is a major activator of PSCs. When intracellular TGF-β_1 signal transduction pathway toward PSC activation was blocked, α-SMA expression in PSCs, a parameter of PSC activation, was attenuated [Ohnishi et al., 2004], suggesting that autocrine TGF-β_1 stimulus is essential for PSCs to maintain their own activation. In this respect, our present data indicate that autocrine IL-6 participates in the perpetuation of pancreatic fibrosis by sustaining PSC activation by increasing autocrine TGF-β_1.

TGF-β_1 has been shown to induce IL-6 expression in various types of cells including lung fibroblasts, osteoblasts, and prostate cancer cells [Eickelberg et al., 1999; Franchimont et al., 2000; Park et al., 2003]. TGF-β_1 intracellular signaling is mediated through Smad-dependent and/or-independent pathway [Massague, 1998; Attisano and Wrana, 2002]. In lung fibroblasts, TGF-β_1 induces IL-6 expression via activating protein-1 (AP-1) dependent transcription [Eickelberg et al., 1999]. Although AP-1 is known to cooperate with Smads in the transcription of various genes, it is still uncertain whether Smad2/3 participates in IL-6 transcription in lung fibroblasts. Recently, Park et al. reported that TGF-β_1 induce IL-6 expression in prostate cancer cells through multiple signaling pathways, including Smad2 and MAPKs [Park et al., 2003]. They showed that blockade of Smad2-dependent pathway with dominant-negative Smad2 expression attenuated IL-6 expression and secretion but could not completely block TGF-β_1 effect on IL-6 expression and secretion, indicating that TGF-β_1 induces IL-6 expression and secretion through both Smad-dependent and Smad-independent pathway in prostate cancer cells. In our present study, however, expression of dominant-negative Smad2/3 completely blocked TGF-β_1 effect on IL-6 expression in activated PSCs (Fig. 4). Furthermore, expression of dominant-negative Smad2/3 strongly inhibited both basal and TGF-β_1-stimulated IL-6 secretion from activated PSCs. Thus, it is reasonable to conclude that Smad2/3 dependent pathway mainly mediates TGF-β_1 effect on IL-6 expression and secretion of activated PSCs.

In conclusion, we demonstrate the autocrine loop between TGF-β_1 and IL-6 in activated PSCs via Smad- and ERK-dependent pathways, respectively. These observations provide new insights for understanding the mechanism of pancreatic fibrosis and developing a novel therapeutic strategy for its treatment.

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