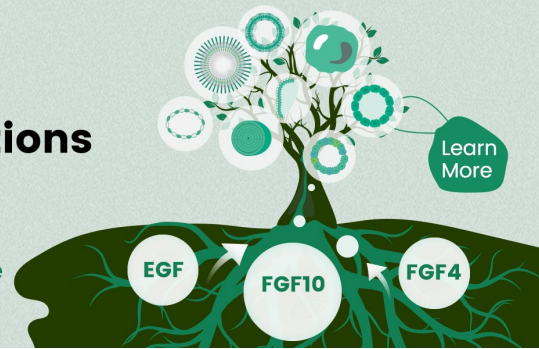


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IL-6 Secretion by Human Pancreatic Periacinar Myofibroblasts in Response to Inflammatory Mediators¹

Mitsue Shimada, Akira Andoh,² Kazunori Hata, Kazuhito Tasaki, Yoshio Araki, Yoshihide Fujiyama, and Tadao Bamba

There is increasing evidence that IL-6 plays an important role in the pathophysiology of acute pancreatitis via its broad proinflammatory actions. To identify the local biosynthetic site for IL-6 in human pancreas, we investigated IL-6 secretion in human pancreatic periacinar myofibroblasts. IL-6 secretion was determined by ELISA and Northern blotting. The activation of NF- κ B was assessed by EMSA. The activation of mitogen-activated protein kinase (MAPK) was assessed by immunoblotting. IL-6 secretion was rapidly induced by IL-17, IL-1 β , and TNF- α . EMSAs demonstrated that IL-17, IL-1 β , and TNF- α induced NF- κ B activation within 1.5 h after stimulation, and a blockade of NF- κ B activation by the pyrrolidine derivative of dithiocarbamate and tosyl-phe-chloromethylketone markedly reduced the IL-17-, IL-1 β -, or TNF- α -induced IL-6 gene expression. Furthermore, IL-17, IL-1 β , and TNF- α induced a rapid activation of extracellular signal-related kinase p42/44 and p38 MAPKs, and specific MAPK inhibitors (SB203580, PD98059, and U0216) significantly reduced IL-17-, IL-1 β -, or TNF- α -induced IL-6 secretion, indicating the role of MAPKs in the induction of IL-6. The combination of either IL-17 plus IL-1 β or IL-17 plus TNF- α enhanced IL-6 secretion and IL-6 mRNA expression; in particular, the effects of IL-17 plus TNF- α were much stronger than those induced by IL-17 plus IL-1 β . TNF- α -induced IL-6 mRNA degraded rapidly at any concentrations, and the combination of IL-17 and TNF- α markedly enhanced IL-6 mRNA stability. This indicates that the effects of IL-17 plus TNF- α were regulated at the post-transcriptional level. In conclusion, pancreatic periacinar myofibroblasts secreted a large amount of IL-6 in response to proinflammatory cytokines. These cells might play an important role in the pathogenesis of acute pancreatitis via IL-6 secretion. *The Journal of Immunology*, 2002, 168: 861–868.

Acute pancreatitis is a serious disease, with highly persistent morbidity and mortality (1). The cause of death in most of these patients seems to be related to the pancreatic inflammation itself or even to infection associated with pancreatic necrosis (1). Severe cases are often the result of multiple organ failure, similar to that occurring in patients with septic shock (1, 2). In these cases, proinflammatory cytokines (TNF- α , IL-1 β , and IL-6) may play a central role and mediate the systemic complications of the disease (1, 2). The release of these proinflammatory mediators is initially limited to the pancreas, but they subsequently extend to various distant sources during the course of the disease (1).

IL-6 plays an important role in the development of the acute phase response in various tissues via its broad proinflammatory actions (3, 4). Evidence obtained in studies of experimental animals and supported by data in humans suggests that excessive production of IL-6 is involved in the pathogenesis of acute pancreatitis. For example, in experimental acute pancreatitis, IL-6 has been reported to be associated with distant organ complications (5), and the aggravation of experimental acute pancreatitis was

observed in IL-6 transgenic mice (6). In patients with acute pancreatitis, systemic complications are associated with increased IL-6 secretion by isolated peripheral blood monocytes (7). In other studies, serum IL-6 level is a specific and sensitive marker of development of pulmonary failure, and peak IL-6 levels were increased in severe and lethal acute pancreatitis as well as in multiple organ failure (8–12).

We previously reported the isolation of human pancreatic periacinar myofibroblasts, which are located in the periacinar region of the normal human pancreas (13). These cells are characterized by expression of type I, III, and IV collagen, fibronectin, prolyl hydroxylase β , laminin, vimentin, nonmuscle myosin, and α -smooth muscle actin (13). In addition, we have recently demonstrated that these cells can secrete high amounts of IL-8, monocyte chemoattractant protein (MCP)³-1, and RANTES in response to the monocyte/macrophage-derived cytokines, IL-1 β , and TNF- α (14). These cells are likely to be important not only in the regulation of acinar cell functions such as digestive enzyme secretion, but also in the induction of inflammatory responses in the pancreas.

In this study, to further understand the extent to which periacinar myofibroblasts participate in local and systemic inflammatory responses in acute pancreatitis, we assessed the expression of IL-6 in these cells. In particular, we focused on the role of IL-17, which is a newly identified T cell-derived proinflammatory cytokine, and its secretion is strictly limited in activated CD4⁺ and CD8⁺ T lymphocytes (15–20). The secretion of a large amount of IL-6 by pancreatic periacinar myofibroblasts suggests that these cells play

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³ Abbreviations used in this paper: MCP, monocyte chemoattractant protein; MAPK,
mitogen-activated protein kinase; PDTC, pyrrolidine derivative of dithiocarbamate;
TPCK, tosyl-phe-chloromethylketone; ERK, extracellular regulated kinase; MEK,
MAPK kinase; TRAF, TNFR-associated factor.

a critical role in the initiation and maintenance of the acute inflammatory process in the pancreas. Furthermore, the regulation of IL-6 secretion in the pancreas is dependent not only on the cytokines derived from monocytes/macrophages (IL-1 β and TNF- α), but also on the T cell-derived factor (IL-17).

Materials and Methods

Reagents

Recombinant human IL-1 β , IL-17, and TNF- α were obtained from R&D Systems (Minneapolis, MN). The inhibitor of p42/44 mitogen-activated protein kinases (MAPKs; PD98059 and U0216; Refs. 21 and 22) and the inhibitor of p38 MAPK (SB203580; Ref. 23) were purchased from Cell Signaling Technology (Beverly, MA). All other reagents used in this study were purchased from Sigma-Aldrich (St. Louis, MO).

Cells

Normal human pancreatic tissue was obtained under informed consent from patients who underwent total gastropsectomy due to gastric cancer, and pancreatic periacinar myofibroblasts were isolated using the method described by Saotome et al. (13). The purity of the cells, determined by the expression of α -smooth muscle actin, was over 99%. Cells were cultured in DMEM (Nikkenkagaku, Kyoto, Japan) containing 10% FBS (Life Technologies, Grand Island, NY). All culture media were supplemented with 50 U/ml penicillin and 50 μ g/ml streptomycin. All experiments were performed using the cells of passage number 3–6. The cells were seeded at a density of 2.5×10^5 cells/ml, and the cell culture media was changed every third day. All experiments were performed after cells had achieved confluence.

Quantification of human IL-6, IL-8, and MCP-1

The amounts of antigenic IL-6, IL-8, and MCP-1 in the samples were determined by sandwich ELISA kits purchased from BioSource International (Camarillo, CA).

Northern blot analysis

Total cellular RNA was isolated by the acid guanidinium thiocyanate-phenol-chloroform method (24). Northern blot was performed according to the method described previously (14). The hybridization was performed with 32 P-labeled human IL-6 probe generated by a random primed DNA labeling kit (Amersham, Arlington Heights, IL) and evaluated by autoradiography. The human IL-6 cDNA probe was prepared from a monolayer of HUVECs by the reverse transcription-PCR method using primers: 5'-TGAGAAAGGAGACATGTAAC corresponding to nucleotides 262–282 isolated by May et al. (25), and 3'-AGTGTCTTAACGCTCATACT corresponding to nucleotides 824–803. The PCR products were ligated into the TA cloning vector (Promega, Madison, WI) and sequenced by the dideoxynucleotide chain termination method (26).

Nuclear extracts and EMSA

Nuclear extracts were prepared from cells exposed to IL-1 β , IL-17, and TNF- α for 1.5 h by the method of Dignam and Roeder (27). Consensus oligonucleotides of NF- κ B (5'-AGTTGAGGGGACTTCCAGCC; Ref. 28) were used. The consensus sequence for binding of NF- κ B is underlined. Oligonucleotides were 5' end-labeled with T4 polynucleotide kinase (Promega) and [γ - 32 P]ATP (Amersham). Binding reactions were performed according to methods described previously (14). Supershift experiments were performed as described above except that 1 μ l of Ab to each transcription factor was added to the binding mixture in the absence of labeled probe. Antisera specifically recognizing each transcriptional factor were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Experiments with unlabeled oligonucleotides used a 100-fold molar excess relative to the radiolabeled oligonucleotide.

Western blot analysis

Cells were exposed to cytokines in the presence or absence of inhibitors for the indicated periods of time. Cells were then washed with PBS and lysed in SDS sample buffer containing 100 μ M orthovanadate. Lysates were homogenized and protein content was determined by using the Bradford method. For Western blotting, 10 μ g of protein from each sample was subjected to SDS-PAGE on a 4–20% gradient gel under reducing conditions. Proteins were then electrophoretically transferred onto a nitrocellulose membrane. The Abs against phosphorylated and total MAPKs were purchased from Cell Signaling Technology, and peroxidase-conjugated

second Abs were purchased from Amersham. Subsequently, detection was performed using the ECL Western blotting system (Amersham).

Measurement of radioactivity

The radioactivity of each band of Northern blotting and EMSA was determined by the Instant Imager Electronic Autoradiography system (model no. 2024/417257; Packard Instruments, Meriden, CT). For comparison of radioactivity, each radioactivity was converted to relative radioactivity to the value of medium alone.

Statistical analysis

The data are expressed as means \pm SD. The variance was analyzed by the Bartlett test (Statview for Macintosh, Version 4.5; Abacus Concepts, Berkeley, CA). Subsequently, statistical significance of changes was determined by the Fisher's protected least significance difference test (Statview for Macintosh, Version 4.5). Differences resulting in p values < 0.01 were considered significant.

Results

Induction of IL-6 secretion by IL-17, IL-1 β , and TNF- α

Human pancreatic periacinar myofibroblasts were incubated for 24 h with increasing concentrations of IL-17, IL-1 β , or TNF- α , and secreted IL-6 levels in supernatants were determined by ELISA. As shown in Fig. 1, the addition of these cytokines induced a dose-dependent increase in IL-6 secretion. The effects of IL-17 were modest as compared with those induced by IL-1 β or TNF- α .

Induction of IL-6 mRNA expression by IL-17, IL-1 β , and TNF- α

The kinetics of the effects of IL-17, IL-1 β , and TNF- α on IL-6 mRNA expression were evaluated in pancreatic periacinar myofibroblasts (Fig. 2). The cells were stimulated with IL-17 (200 ng/

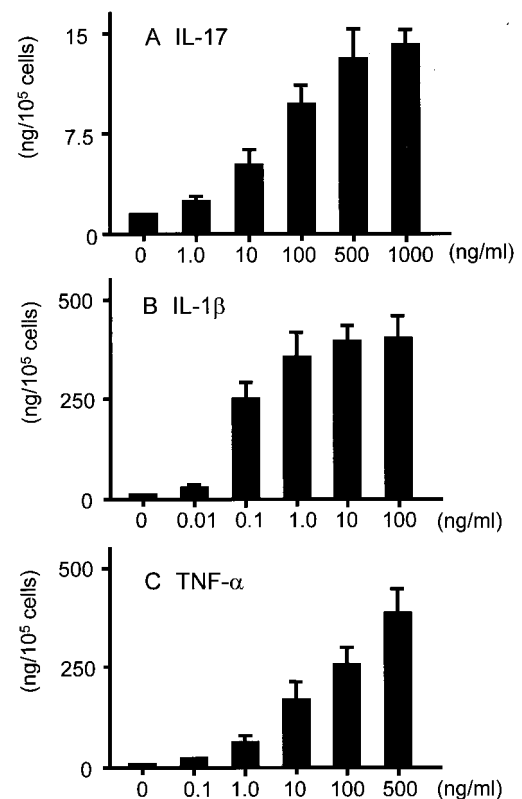


FIGURE 1. Effects of IL-17, IL-1 β , and TNF- α on IL-6 secretion in pancreatic periacinar myofibroblasts. The cells were incubated for 24 h in the presence of increasing concentrations of IL-17, IL-1 β , or TNF- α . The concentration of IL-6 was determined by ELISA. Values are expressed as mean \pm SD ($n = 4$).

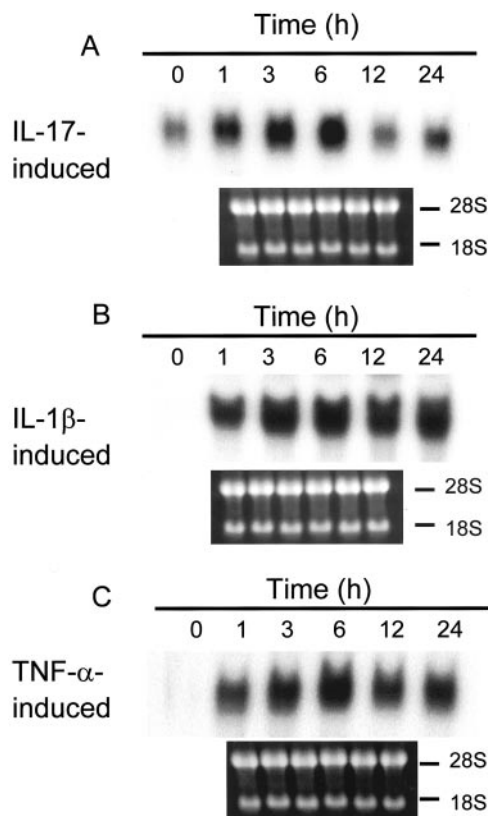


FIGURE 2. Kinetics of IL-17-, IL-1 β -, or TNF- α -induced IL-6 mRNA expression in pancreatic periacinar myofibroblasts. The cells were stimulated with IL-17 (200 ng/ml), IL-1 β (10 ng/ml), or TNF- α (100 ng/ml), and the abundance of IL-6 mRNA was sequentially determined by Northern blotting. Lower panels, Ribosomal RNA stained by ethidium bromide. Prolonged exposure time was required to get the result of IL-17.

ml), IL-1 β (10 ng/ml), or TNF- α (100 ng/ml), and IL-6 mRNA abundance was determined by Northern blotting. IL-17, IL-1 β , and TNF- α induced a rapid increase in the accumulation of IL-6 mRNA, and these reached a maximum at 6 h after stimulation. Thereafter, the induced IL-6 mRNA abundance decreased gradually. To detect the effects of IL-17, the prolonged exposure of films was required in the process of autoradiography.

Modulation of transcription factor activation

The expression of IL-6 genes is regulated by the activation of specific transcription factors such as NF- κ B (29, 30). To elucidate the mechanisms underlying the response to IL-17, IL-1 β , and TNF- α , we evaluated the activation of the transcription factors NF- κ B in pancreatic periacinar myofibroblasts. As demonstrated in Fig. 3A, stimulation with either IL-17 (200 ng/ml), IL-1 β (10 ng/ml), or TNF- α (100 ng/ml) for 1.5 h induced an increase in NF- κ B DNA-binding activity. The effects of IL-17 were rather weak as compared with those induced by IL-1 β and TNF- α . The specificity of this reaction was confirmed by the addition of cold oligo-DNA, which abolished the reactive band. The addition of Abs directed against a 50,000-Da subunit (p50) and a 65,000-Da subunit (p65) of NF- κ B induced supershifts of the binding complexes, indicating that this binding complex was a heterodimer consisting of p50 and p65 subunits.

To confirm the role of NF- κ B activation, we assessed the actions of NF- κ B inhibitors such as the pyrrolidine derivative of dithiocarbamate (PDTC) (31) and tosyl-phe-chloromethylketone (TPCK) (32) on IL-6 mRNA expression. A role for oxygen radi-

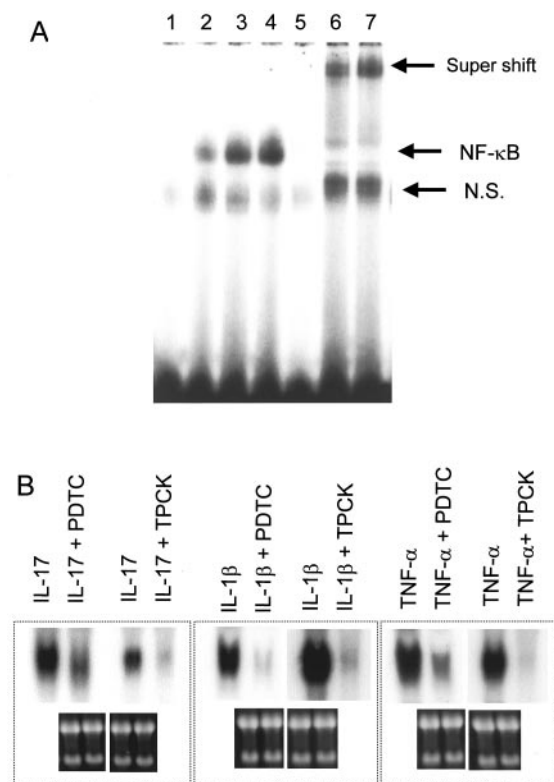


FIGURE 3. A, EMSAs for NF- κ B DNA-binding activities. The cells were incubated with medium alone, IL-17 (200 ng/ml), IL-1 β (10 ng/ml), or TNF- α (100 ng/ml) for 1.5 h, and then nuclear extracts were prepared. N.S., Nonspecific band. Lane 1, Medium alone; lane 2, IL-17; lane 3, IL-1 β ; lane 4, TNF- α ; lane 5, TNF- α plus cold probe; lane 6, TNF- α plus anti-p50 Ab; and lane 7, TNF- α plus anti-p65 Ab. B, Effects of the NF- κ B inhibitor PDTC and TPCK on IL-17-, IL-1 β -, and TNF- α -induced IL-6 mRNA expression. The cells were incubated for 3 h with IL-17 (200 ng/ml), IL-1 β (10 ng/ml), or TNF- α (100 ng/ml) in the presence or absence of either PDTC (20 μ M) or TPCK (20 μ M), and then the total cellular RNA was extracted. The IL-6 mRNA expression was analyzed by Northern blot. Lower panel, Ribosomal RNA stained by ethidium bromide.

cals in mediating NF- κ B activation has been postulated, and antioxidants such as PDTC have been shown to block NF- κ B activation in several cell lines (31). As demonstrated in Fig. 3B, the addition of PDTC blocked IL-17-, IL-1 β -, and TNF- α -induced IL-6 gene expression. TPCK blocks NF- κ B activation by preventing the degradation of the predominant inhibitory molecule, I κ B α , and inhibits the translocation of NF- κ B into the nucleus (32). TPCK potentially abrogated IL-17-, IL-1 β -, and TNF- α -induced IL-6 mRNA expression in periacinar myofibroblasts. These findings indicate that the activation of NF- κ B may play a major role in the induction of IL-6 mRNA expression in these cells.

IL-17, IL-1 β , and TNF- α induce the activation of MAPKs

In various cells, the MAPK family has been shown to play an important role in regulating gene expression in response to inflammatory mediators (33). However, it has not fully been studied whether MAPKs participate in IL-17 signaling. To assess whether similar responses are involved in our system, we evaluated the effects of IL-17, IL-1 β , and TNF- α on MAPK phosphorylation in pancreatic periacinar myofibroblasts. As shown in Fig. 4A, IL-17, IL-1 β , and TNF- α induced a phosphorylation of p42/44 (extracellular regulated kinase (ERK)) and p38 MAPKs as early as 5 min after the stimulation, and these reached a maximum at 15 min. The effects of IL-17 were weaker than those induced by IL-1 β or

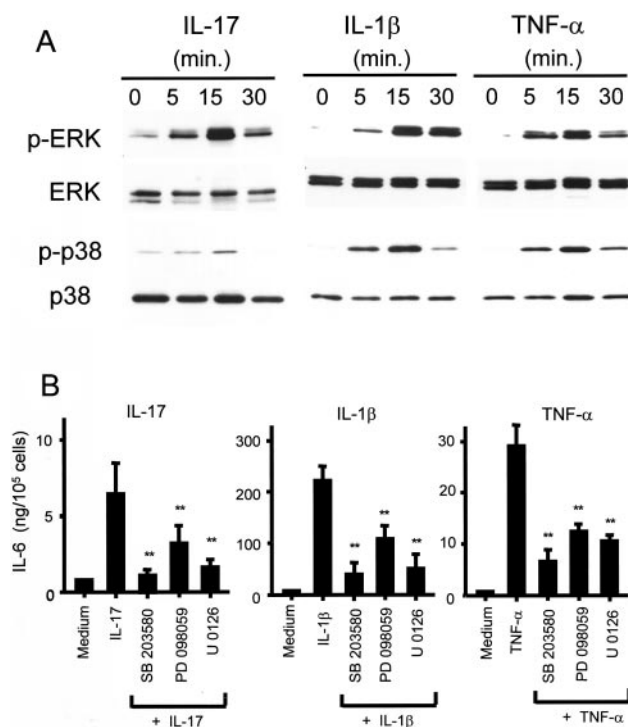


FIGURE 4. A, Kinetics of IL-17-, IL-1 β -, or TNF- α -induced MAPK activation in pancreatic periacinar myofibroblasts. The cells were stimulated with IL-17 (200 ng/ml), IL-1 β (10 ng/ml), or TNF- α (100 ng/ml), and the phosphorylated (p-) and total MAPKs (ERK1/2 and p38) were sequentially detected by Western blotting. B, Effects of MAPK inhibitors on IL-17-, IL-1 β -, or TNF- α -induced IL-6 secretion in pancreatic periacinar myofibroblasts. The cells were incubated with IL-17 (200 ng/ml), IL-1 β (10 ng/ml), or TNF- α (100 ng/ml) in the presence or absence of 20 μ M MAPK inhibitors, and the concentration of IL-6 in supernatants was determined by ELISA. Values are expressed as mean \pm SD ($n = 4$). Significant differences from the values in the absence of MAPK inhibitors. **, $p < 0.01$.

TNF- α . These results indicate that MAPK pathways are rapidly activated by IL-17, IL-1 β , and TNF- α in pancreatic periacinar myofibroblasts.

Suppression of the IL-6 induction by MAPK inhibitors

To evaluate the effects of MAPKs on the induction of IL-6 secretion by IL-17, IL-1 β , or TNF- α in pancreatic periacinar myofibroblasts, the effects of SB203580, PD98059, and U0126 were examined. As shown in Fig. 4B, each inhibitor significantly reduced IL-17-, IL-1 β -, and TNF- α -induced IL-6 secretion. The inhibitory effects of SB203580 and U0126 were stronger than those induced by PD98059.

Combination effects of IL-17 plus IL-1 β and/or IL-17 plus TNF- α

The combined effects of either IL-17 plus TNF- α or IL-17 plus IL-1 β were evaluated. The cells were incubated with stimulators for 24 h, and IL-6 levels were determined. As shown in Fig. 5, IL-17 dose-dependently enhanced TNF- α - or IL-1 β -induced IL-6 secretion. Although the effects of IL-17 itself on IL-6 secretion were modest, the enhancing effects of IL-17 on TNF- α - or IL-1 β -induced IL-6 secretion were strong. The enhancing effects of IL-17 on TNF- α -induced IL-6 secretion were much stronger than those on IL-1 β -induced IL-6 secretion. When comparing the effects of IL-17 on IL-6 secretion with those on IL-8 or MCP-1 secretion, the enhancing effects of IL-17 on TNF- α -induced IL-6 secretion were

much stronger than those on IL-8 or MCP-1 secretion (Fig. 6). In these cells, incubation with IL-1 β (1 ng/ml) and TNF- α (10 ng/ml) for 24 h induced IL-8 secretion of 528 ± 45 ng/10⁵ cells and 287 ± 23 ng/10⁵ cells, respectively. The stimulation with IL-1 β (1 ng/ml) and TNF- α (10 ng/ml) for 24 h also induced MCP-1 secretion of 305 ± 31 ng/10⁵ cells and 390 ± 39 ng/10⁵ cells, respectively.

The combined effects of IL-17 plus IL-1 β or those of IL-17 plus TNF- α on IL-6 mRNA expression were investigated. Cells were stimulated for 3 h, and then IL-6 mRNA abundance was determined by Northern blotting. As shown in Fig. 7, these combinations increased IL-6 mRNA abundance as compared with the effects of individual cytokines. The combination of IL-17 plus TNF- α exerted a stronger effect than that of IL-17 and IL-1 β . These findings were compatible with the results at a protein level.

The effects of IL-17 plus TNF- α on NF- κ B activation and IL-6 mRNA stability

To assess the molecular mechanisms involved in the combined effects of IL-17 plus TNF- α , NF- κ B activation was evaluated by EMSAs. As shown in Fig. 8A, the combination of IL-17 plus TNF- α , as well as IL-17 plus IL-1 β , exerted modest effects on NF- κ B DNA-binding activities, suggesting that this transcriptional mechanism might not play a major role in the combined effects of IL-17 plus TNF- α .

To evaluate the possibility that the effects of IL-17 plus TNF- α are dependent on a result of increased mRNA stability, the cells were stimulated with cytokines for 3 h, washed, and then treated with actinomycin D (5 μ g/ml) for various time periods to block further RNA transcription (Fig. 8, B and C). In the cells treated with TNF- α (10 ng/ml) alone, IL-6 mRNA abundance rapidly decreased and only 30% of IL-6 mRNA was remaining at 5 h after the addition of actinomycin D. A similar response was induced by high-dose TNF- α (100 ng/ml; data not shown). The combination of IL-17 (200 ng/ml) plus TNF- α (10 ng/ml) markedly prolonged the rate of IL-6 mRNA degradation; 86% of IL-6 mRNA was remaining after 5 h. IL-17 also induced a stabilization of IL-6 mRNA induced by high-dose TNF- α (100 ng/ml; data not shown). Thus, the combined effects of IL-17 and TNF- α on IL-6 mRNA abundance are associated with the increasing actions on IL-6 mRNA stabilities.

In contrast, IL-6 mRNA induced by high-dose IL-1 β (10 ng/ml) was stable for 5 h, whereas IL-6 mRNA induced by low-dose IL-1 β (0.1 ng/ml) decreased rapidly (Fig. 8B). In contrast to the effects on TNF- α -induced IL-6 mRNA, IL-17 did not affect the stability of IL-1 β (0.1 ng/ml)-induced IL-6 mRNA (Fig. 8, B and C).

Discussion

Accumulating evidence has incriminated various cytokines and their interactions in the pathophysiology of acute pancreatitis (1, 2). Among these cytokines, IL-6 is considered to play crucial roles (6, 7). Several clinical studies have reported that serum IL-6 is elevated at the early stage of acute pancreatitis (9, 10), and that the severity and systemic complications of acute pancreatitis depend on proinflammatory cytokines, including IL-6 (1, 2). However, it remains unclear which cells are secreting IL-6 in the pancreas. Recently, pancreatic acinar cells isolated from rodent were shown to express IL-6 (33). To the best of our knowledge, this study is the first report to demonstrate one of the local biosynthetic sites for IL-6 in human pancreas. It is of particular interest to note that human pancreatic periacinar myofibroblasts are capable of secreting a large amount of IL-6 (1~2 μ g per 10⁵ cells per 24 h) in response to monocyte/macrophage-derived cytokines (IL-1 β and TNF- α) and a factor derived from activated T cells (IL-17). IL-6

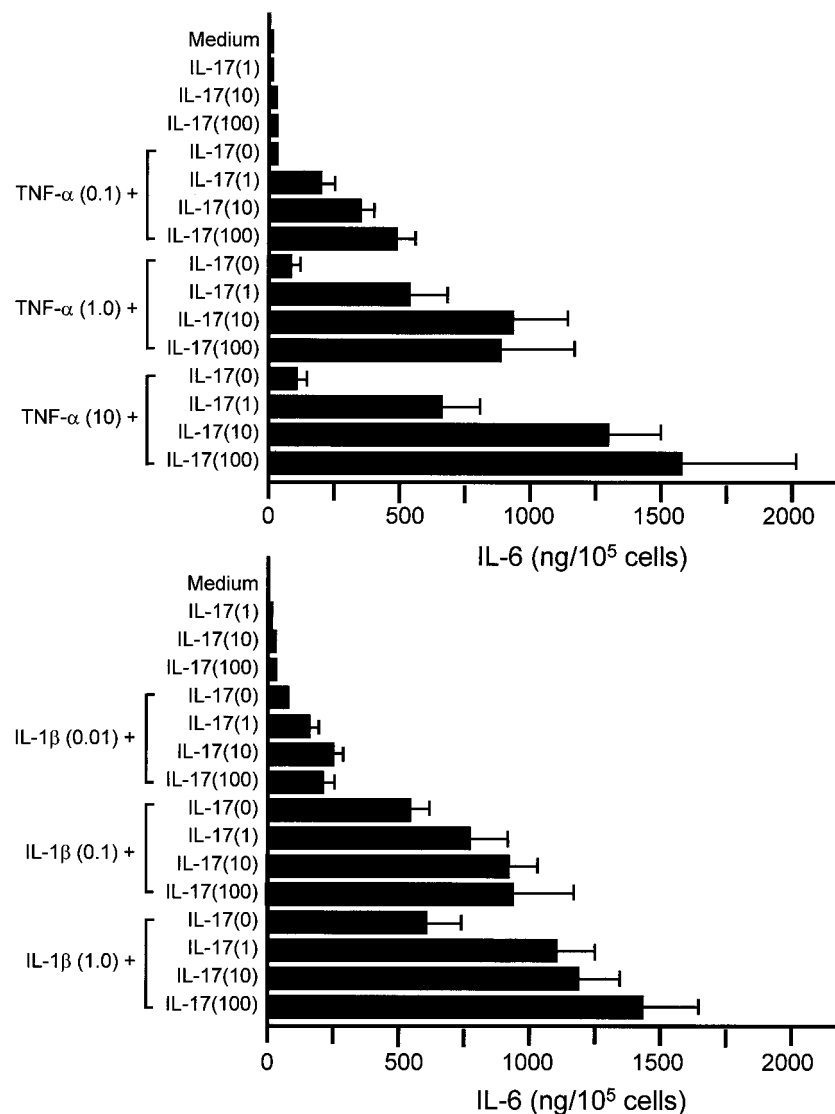


FIGURE 5. Combined effects of IL-17 plus IL-1 β or IL-17 plus TNF- α . The cells were cultured for 24 h in the presence of various concentrations of cytokines, and the concentration of IL-6 in supernatants was determined by ELISA. Values are expressed as mean \pm SD ($n = 4$).

secretion in pancreatic myofibroblasts is much stronger than that reported in other cell types (34–36). It is likely that pancreatic periacinar myofibroblasts may play a pivotal role in the pathophysiology of acute pancreatitis and in the development of systemic complications via IL-6 secretion.

IL-17 is a newly identified T cell-specific cytokine. Human IL-17 is an ~20-kDa glycoprotein of 155 amino acids, the sequence of which exhibits close homology to both CTLA Ag-8 and the open reading frame 13 of T lymphotropic *Herpesvirus saimiri* (15, 16). IL-17 secretion is strictly limited to activated CD4⁺ and CD8⁺ T lymphocytes and is predominantly secreted by the memory CD45RO⁺ cells (17–19). Both the Th1 and Th2 subsets of CD4⁺ cells release IL-17. In contrast, IL-17R is widely distributed on various cell types (20, 37), and there is increasing evidence that IL-17 is a mediator of inflammatory responses in various tissues (34, 35, 38–41). Thus, IL-6 induction by IL-17 in pancreatic periacinar myofibroblasts suggests a role for T cells in the pathogenesis of acute pancreatitis. Although the role of T cells in the pathogenesis of acute pancreatitis has not been fully investigated, recent clinical and experimental studies have demonstrated that T cells affect inflammatory responses involved in the pathophysiology of acute pancreatitis (42–44).

Previously, only one report has demonstrated the combined effects of IL-17 plus IL-1 β on IL-6 secretion in synoviocytes (35). In pancreatic periacinar myofibroblasts, the combination of IL-17 with either IL-1 β or TNF- α strongly enhanced IL-6 secretion. These responses were clearly observed even at low concentrations. For example, the combination of IL-17 (10 ng/ml) plus IL-1 β (0.01 ng/ml) and/or IL-17 (10 ng/ml) plus TNF- α (0.1 ng/ml) induced a large amount of IL-6 secretion. These responses were also confirmed at the mRNA level. The combined effects on IL-6 secretion were much stronger with the combination of IL-17 plus TNF- α than with the combination of IL-17 plus IL-1 β . Furthermore, this strong effect of IL-17 plus TNF- α was specific for IL-6 secretion. These results indicate that cytokines produced by monocytes/macrophages (IL-1 β and TNF- α) and activated T cells (IL-17) can cooperate in the induction of IL-6 secretion in pancreatic periacinar myofibroblasts at the low levels that can be easily achieved in vivo.

Many cytokine-inducible responses are mediated by one of the important DNA-binding proteins, such as NF- κ B (28). The promoter regions of the human IL-6 genes have been cloned and are shown to contain putative consensus binding motifs for NF- κ B (29, 30). Our results demonstrated that the activation of NF- κ B

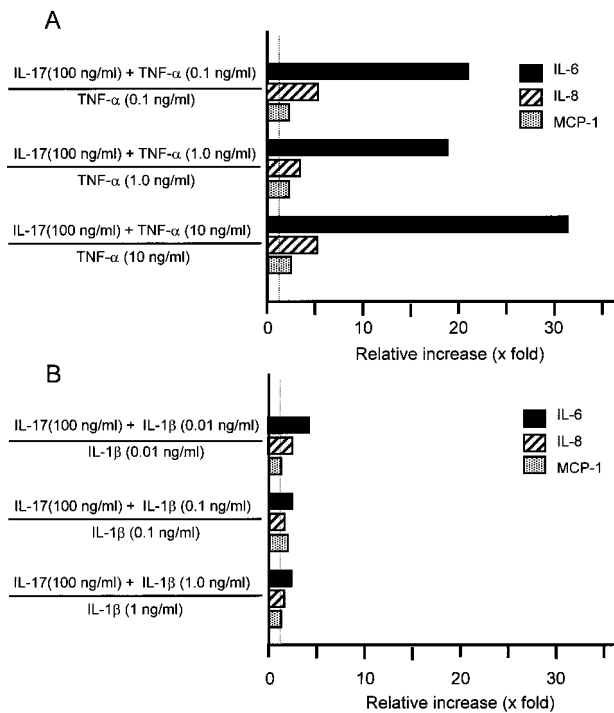


FIGURE 6. Comparison of the effects of either IL-17 plus TNF- α or IL-17 plus IL-1 β on IL-6, IL-8, or MCP-1 secretion. The cells were cultured for 24 h in the presence of cytokines, and the concentration of IL-6, IL-8, and MCP-1 in supernatants was determined by ELISA. Values are expressed as relative increase compared with the values in the absence of IL-17.

was necessary for not only IL-1 β - and TNF- α -induced IL-6 gene expression, but was also induced by IL-17 in pancreatic periacinar myofibroblasts. The evidence supporting this conclusion may be summarized as follows: 1) IL-17 rapidly induced nuclear proteins that exhibited binding to an oligonucleotide containing an NF- κ B consensus recognition motif. Binding specificity was confirmed by experiments in which the binding was blocked by the addition of excess cold-NF- κ B oligonucleotide. 2) The inhibition of NF- κ B activation by PDTC and TPCK caused a marked decrease in IL-17-induced IL-6 mRNA expression. PDTC and TPCK are potent inhibitors of NF- κ B activation (31, 32). It is likely that the effects of IL-17 on IL-6 secretion may be mainly regulated at the transcriptional levels via NF- κ B activation in pancreatic periacinar myofibroblasts.

MAPK activation has been regarded as another important signaling event in response to proinflammatory stimuli. Three subgroups of the MAPK family have been identified, and all are phosphorylated on tyrosine and threonine residues by upstream kinases, the MAPK kinases (MEKs). The p44 and p42 ERK1 and ERK2 mediate responses mainly to mitogenic stimuli, and the Jun NH₂-terminal kinases and p38 mediate responses to cellular stress (45–48). In this study, we show that IL-17, as well as IL-1 β and TNF- α , activate the two groups of MAPKs in pancreatic periacinar myofibroblasts. Similar to the responses induced by IL-1 β and TNF- α , the MAPK activation by IL-17 was maximal after 15 min and then declined. Like the effects on NF- κ B activation, the effects of IL-17 on MAPK activation were weaker than those induced by IL-1 β or TNF- α . The role of MAPKs in IL-17-, IL-1 β -, and TNF- α -induced IL-6 secretion was investigated in pancreatic periacinar myofibroblasts by using specific inhibitors. The imidazole compound SB203580 is a specific inhibitor of p38 MAPK (23). SB203580 caused a significant decrease in IL-17-, IL-1 β -, and TNF- α -induced IL-6 secretion, indicating that p38 activation is

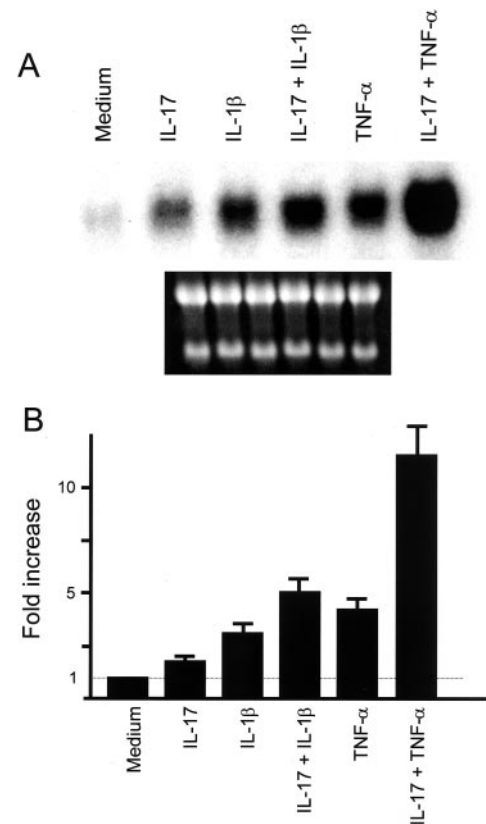


FIGURE 7. A, Northern blot analysis of combined effects of IL-17 plus IL-1 β or IL-17 plus TNF- α on IL-6 mRNA expression. The cells were stimulated for 3 h with IL-17 (200 ng/ml), IL-1 β (0.1 ng/ml), TNF- α (10 ng/ml), IL-17 plus IL-1 β , or IL-17 plus TNF- α , and then IL-6 mRNA expression was determined by Northern blotting. B, For comparison of radioactivity, each radioactivity was converted to relative radioactivity to the value of medium alone. Values are expressed as mean \pm SD ($n = 4$).

involved in IL-17, IL-1 β -, and TNF- α -induced IL-6 secretion. This observation is compatible with the recent report by Craig et al. (49) indicating that the stimulation of p38 MAPK by the MEK, MKK6, activates NF- κ B DNA-binding activity and induces IL-6 secretion. In contrast, the role of ERK1/2 MAPKs in IL-6 induction has not fully been investigated (50). In this study, we addressed the role of ERK1/2 in our system. PD098059 is a specific inhibitor of MEK1 (21), the kinase directly upstream to ERK1/2, and U0216 is a specific inhibitor of MEK1 and MEK2 (22), respectively. U0216 more potently blocked the phosphorylation of ERK1/2 as compared with PD098059. PD098059 and U0216 caused a significant inhibition on IL-17-, IL-1 β -, and TNF- α -induced IL-6 secretion. Thus, we concluded that ERK1/2 MAPKs also participate in IL-6 secretion by IL-17, IL-1 β , and TNF- α in pancreatic periacinar myofibroblasts.

The molecular mechanism involved in the strong induction of IL-6 secretion by IL-17 plus TNF- α remains to be clarified. These cytokines initiate signaling cascades leading to NF- κ B activation via distinct cell surface receptors that use the TNFR-associated factor (TRAF) family of adaptor proteins as signal transducers. TNF- α requires TRAF2 activation to induce subsequent molecular events, whereas IL-17, as well as IL-1 β , uses TRAF6 (51, 52). So, the possibility that the combination of IL-17 plus TNF- α -modulated NF- κ B DNA-binding activity was initially proposed. However, the combination of either IL-17 and TNF- α or IL-17 plus IL-1 β induced a modest increase in NF- κ B DNA-binding activities, indicating that the NF- κ B-mediated transcriptional mechanism did not play a major role. In contrast, the combination of

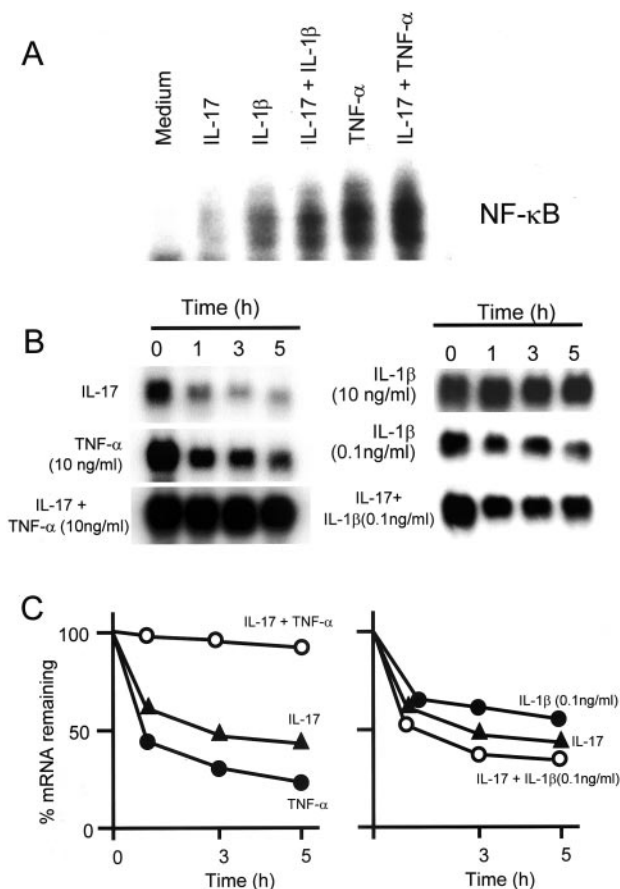


FIGURE 8. A, EMSAs for NF- κ B DNA-binding activities. The cells were incubated with medium alone, IL-17 (200 ng/ml), IL-1 β (0.1 ng/ml), IL-17 (200 ng/ml) plus IL-1 β (0.1 ng/ml), TNF- α (10 ng/ml), or IL-17 (200 ng/ml) plus TNF- α (10 ng/ml) for 1.5 h, and then nuclear extracts were prepared. Lane 1, Medium alone; lane 2, IL-17; lane 3, IL-1 β ; lane 4, IL-17 plus IL-1 β ; lane 5, TNF- α ; and lane 6, IL-17 plus TNF- α . B, Stability studies of IL-6 mRNA. Pancreatic myofibroblasts were stimulated with or without cytokines for 3 h, washed, and further incubated with actinomycin D (5 μ g/ml) for various time periods. Total RNA was sequentially extracted, and IL-6 mRNA abundance was determined by Northern blotting. IL-17 was used at 200 ng/ml. C, Percentage remaining of IL-6 mRNA. IL-6 mRNA abundance as shown above was expressed as relative to the corresponding level before addition of actinomycin D (percentage remaining). Cytokines were used at the following concentrations: IL-17 (200 ng/ml), TNF- α (10 ng/ml), and IL-1 β (0.1 ng/ml).

IL-17 plus TNF- α markedly enhanced IL-6 mRNA stabilities. These effects were not induced by the combination of IL-17 plus IL-1 β . Thus, these observations highly suggested that the strong induction of IL-6 secretion by IL-17 plus TNF- α might be closely associated with the post-transcriptional mechanisms that enhance IL-6 mRNA stabilities. The precise mechanisms involved in the enhancement of IL-6 mRNA stabilities should be clarified in the future.

In conclusion, one of the sources of IL-6 secretion in human pancreas was identified. T cell-derived IL-17 and monocyte/macrophage-derived IL-1 β and TNF- α induced IL-6 secretion in human pancreatic periacinar myofibroblasts. These findings may explain the linkage between monocytes/macrophages- and T cell-mediated immune responses in the pathophysiology of acute pancreatitis. These observations reinforce the concept that T cells can collaborate with monocytes/macrophages in the promotion and shaping of inflammatory responses in the pancreas.

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