Regulation of IL-11 expression in intestinal myofibroblasts: role of c-Jun AP-1- and MAPK-dependent pathways

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Bamba, Shigeki, Akira Andoh, Hirofumi Yasui, Jin Makino, Shokei Kim, and Yoshihide Fujiyama. Regulation of IL-11 expression in intestinal myofibroblasts: role of c-Jun AP-1- and MAPK-dependent pathways. Am J Physiol Gastrointest Liver Physiol 285: G529–G538, 2003. First published May 21, 2003; 10.1152/ajpgi.00050.2003.—IL-11 inhibits the activation of NF-κB and induces the Th2 polarization of CD4+ T cells. The clinical utility of IL-11 is being investigated in Crohn’s disease. However, physiological secretion of IL-11 in the intestine remains unclear. In this study, we investigated IL-11 secretion in human intestinal subepithelial myofibroblasts (SEMFs). Intestinal SEMFs were isolated from the human colonic mucosa. IL-11 secretion and mRNA expression were determined by ELISA and Northern blot analysis. The activating protein (AP)-1-DNA binding activity was evaluated by EMSA. IL-11 secretion was induced by IL-1β and transforming growth factor (TGF)-β1. These were also observed at the mRNA level. The EMSAs demonstrated that both IL-1β and TGF-β1 induced AP-1 activation within 2 h after stimulation, and a blockade of AP-1 activation by the recombinant adenovirus containing a dominant negative c-Jun markedly reduced the IL-1β- and TGF-β1-induced IL-11 mRNA expression. IL-1β and TGF-β1 induced an activation of ERK p42/44 and p38 MAP kinases, and the MAP kinase inhibitors (SB-202190, PD-98059, and U-0216) significantly reduced the IL-1β- and TGF-β1-induced IL-11 secretion. The upregulation of IL-11 mRNA by IL-1β- and TGF-β1 was also mediated by a p38 MAP kinase-mediated mRNA stabilization. The combination of IL-1β and TGF-β1 additively enhanced IL-11 secretion. Intestinal SEMFs secreted IL-11 in response to IL-1β- and TGF-β1. Mucosal IL-11 secretion might be important as an anti-inflammatory response in the pathogenesis of intestinal inflammation.

interleukin-11; inflammatory bowel diseases; transforming growth factor-β1; interleukin-1β

IL-11 is a pleiotropic member of the IL-6-type cytokine family that mediates its biological activities via binding to a multimeric receptor complex that contains gp130 molecules (33, 40, 43). IL-11 was originally identified as a hematopoietic growth factor and stimulates multiple stages of megakaryocytepoiesis (33, 34, 40, 43). In addition, IL-11 has demonstrated anti-inflammatory properties in a variety of in vivo and in vitro models. IL-11 inhibits the production of IL-1, TNF-α, and other proinflammatory cytokines from LPS-stimulated murine peritoneal macrophages (45). This inhibition was associated with a blockade of the activation of transcription factor NF-κB (46). Furthermore, IL-11 reduces CD4+ T cell production of Th1 cytokines, such as interferon-γ induced by IL-12, while enhancing Th2 cytokines, such as IL-4 and IL-10 (4, 10).

In the gastrointestinal tract, it is of particular interest that IL-11 prevents or improves the development of acute and chronic intestinal inflammation in animal models. For example, recombinant human (rh)IL-11 downregulated the expression of mucosal proinflammatory cytokines and ameliorated the development of colitis in HLA-B27 transgenic rats (35). Recombinant IL-11 was also protective against trinitrobenzene sulfonic acid-induced colitis (37). Treatment with rhIL-11 reduced the mortality in a murine model of combined chemotherapy and irradiation, in which mortality was due to sepsis secondary to gastrointestinal mucosal damage (14, 32). These effects were explained in association with at least two distinct mechanisms. As mentioned above, IL-11 is a potent inhibitor of the activation of mucosal macrophages and T cells (4, 10, 45, 46). Another mechanism is that IL-11 enhances the integrity of the intestinal mucosa through trophic effects on the gastrointestinal epithelium (23, 32). On the basis of these studies, Sands et al. (38, 39) reported that rhIL-11 is safe and effective in inducing remission in mild to moderately active Crohn’s disease. Thus IL-11 plays an important role in the pathophysiology of intestinal inflammation and repair process.

Subepithelial myofibroblasts (SEMFs) are present immediately under the basement membrane in the normal intestinal mucosa, juxtaposed to the bottom of the epithelial cells (24, 36). These cells are specialized mesenchymal cells that exhibit the ultrastructural features of both fibroblasts and smooth muscle cells and can be characterized by positive immunoreactivity for α-smooth muscle actin but negative immunoreactivity for...
for desmin. These cells play a central role in the regulation of a number of epithelial cell functions, such as proliferation and differentiation and extracellular matrix (ECM) metabolism affecting the growth of the basement membrane (20, 24, 31, 36). Recent studies from our laboratory (18) have suggested that SEMFs might play a role in the pathogenesis of intestinal inflammation via the secretion of proinflammatory cytokines, such as IL-6 and a variety of chemokines.

Given the importance of IL-11 expression in intestinal inflammation, we were interested in the physiological secretion of IL-11 in the human intestine. Previously, IL-11 secretion has been reported in bone marrow-derived stromal cells (33, 47) and mesenchymal cells derived from the skin (42), joints (26), and lung (15). A549 lung cancer cell line also secretes IL-11 (44). However, IL-11 secretion in the intestinal mucosa remains unclear. In this study, we investigated IL-11 secretion in human intestinal SEMFs and assessed the key signaling pathways in this response. This study demonstrated that intestinal SEMFs are the local biosynthetic site for IL-11. Among the various cytokines and growth factors, IL-1β and transforming growth factor (TGF)-β1 act as potent inducers of IL-11 secretion through the transcription factor activating protein (AP)-1- and the mitogen-activated protein (MAP) kinase-dependent pathways.

MATERIALS AND METHODS

Reagents. rhIL-1β and TNF-α were obtained from R&D Systems (Minneapolis, MN). All other growth factors and cytokines were purchased from Genzyme (Cambridge, MA), and other reagents were purchased from Sigma (St. Louis, MO). The inhibitors of p42/p44 MAPK (PD-98059 and U-0216) and the inhibitor of p38 MAPK (SB-203580) were purchased from Cell Signaling Technology (Beverly, MA). All other reagents used in this study were purchased from Sigma.

Culture of human colonic myofibroblasts. The primary cultures of SEMFs were prepared according to the method reported by Mahida et al. (24), and the characters of the cells have been described in our previous reports (18, 31). The cells were cultured in DMEM containing 10% FBS. All culture media were supplemented with 50 U/ml penicillin and 50 μg/ml streptomycin. The studies were performed on passages 2–6 of myofibroblasts isolated from three resection specimens.

Quantification of human IL-11. The amount of antigenic IL-11 in the samples was determined by sandwich ELISA kits purchased from TECHNE (Minneapolis, MN). The lower detection limit was 15.6 pg/ml for human IL-11.

Northern blot analysis. Total cellular RNA was isolated by the acid guanidinium thiocyanate-phenol-chloroform method (7). Northern blot analysis was performed according to the method previously described (2, 41). The hybridization was performed with a 32P-labeled human IL-11 probe, generated by a random primed DNA labeling kit (Amer sham, Arlington Heights, IL), and evaluated by autoradiography. The human IL-11 cDNA probe was prepared from a monolayer of human umbilical vein endothelial cells by the RT-PCR method using the primers: 5'-TGAGCTGTGACCGGATG-3' corresponding to nucleotides 99–119 isolated by Paul et al. (33) and 5'-TGAGCTGTGACCGGATG-3' corresponding to nucleotides 101–119 isolated by Paul et al. (33). The PCR products were ligated into the TA cloning vector (Promega, Madison, WI) and were sequenced by the dideoxynucleotide chain termination method. The human IL-6 and IL-8 cDNA probes were described in our previous reports (2, 41).

Nuclear extracts and EMSA. Nuclear extracts were prepared from the cells exposed to IL-1β (10 ng/ml) and TGF-β1 (50 ng/ml) for 2 h by the method of Dignam et al. (12). Oligonucleotides from the AP-1 binding motif in the IL-11 promoter (5'-CTCCAGGGTCTTCAGGGAAGA-3') were end-labeled with T4 polynucleotide kinase (Promega, Madison, WI) and [γ-32P]ATP (Amersham). The binding reactions were performed according to the methods previously described. Supershift experiments were performed as described above, except that 1 μl of antibody to each transcription factor was added to the binding mixture in the absence of labeled probe. Antibodies specifically recognizing each transcriptional factor were purchased from Santa Cruz Biotechnology (Santa Cruz, CA): anti-pan Fos, sc-253x; anti-pan Jun sc-44x; anti-c-Fos, sc-7202x, anti-FosB, sc-48x; anti-Fra-1, sc-605x; anti-Fra2, sc-13017x; anti-JunB, sc-46x; anti-c-Jun, sc-1694x; and anti-JunD, sc-74x. The experiments with unlabeled oligonucleotides used a 100-fold molar excess relative to the radiolabeled oligonucleotide.

Adenovirus-mediated gene transfer. We used a recombinant adenovirus expressing the dominant negative mutant of c-Jun (Ad-DN-c-Jun) and a recombinant adenovirus containing bacterial β-galactosidase cDNA (Ad-LacZ), as described in a previous report (48). The dominant negative mutant c-Jun (TAM67) is lacking the transactivation domain of amino acids 3 to 122 of the wild-type c-Jun but retains the DNA-binding domain (5). In preliminary experiments, the Ad-LacZ infection of intestinal SEMFs at a multiplicity of infection of 200 showed a maximal expression (85% positive) for β-galactosidase. The recombinant adenovirus was transferred into the intestinal SEMFs according to the methods previously described (48). The cells were made quiescent for 48 h before being assessed for the effects of the transferred gene. The cells were then stimulated for 12 h with IL-1β (10 ng/ml) or TGF-β1 (50 ng/ml), and the IL-11 mRNA expression was determined by Northern blot analysis. The recombinant adenovirus containing the bacterial β-galactosidase gene (Ad-LacZ) was used as the negative control for Ad-DN-c-Jun.

Nuclear run-on assays. Nuclear run-on assays, using nuclei from confluent SEMFs, were performed according to the method described previously (2). In this experiment, cells were exposed to stimuli for 8 h, scraped off, and lysed in buffer (10 mM Tris, pH 7.4, 10 mM NaCl, 3 mM MgCl2, 0.5% Nonidet P-40). Empty plasmids of the TA cloning vector were also used to detect nonspecific background.

Western blot analysis. The cells were exposed to cytokines in the presence or absence of inhibitors for the indicated periods of time. The cells were then washed with PBS and lysed in SDS sample buffer containing 100 μM orthovanadate. Western blot analysis was performed according to the method previously described (41).

Measurement of radioactivity. The radioactivity of each band from the Northern blot analysis was determined by the Instant Imager electronic autoradiography system (model 2024/417257, Packard, Meriden, CT). For a comparison of the radioactivity in the stability study, each value was converted to a relative radioactivity based on the value at assay start.

Statistical analysis. The data are expressed as means ± SD. The statistical significance of the changes was determined by Mann-Whitney U-test. Differences resulting in P values <0.05 were considered to be statistically significant.
RESULTS

Effects of cytokines and growth factors on IL-11 expression. The effects of various cytokines and growth factors on IL-11 protein secretion were evaluated. The cells were stimulated for 24 h with each factor (100 ng/ml), and the IL-11 levels in the supernatant were determined by ELISA (Fig. 1A). We previously observed that each factor (100 ng/ml) is sufficient to induce cellular responses in our system (18, 31). A very small amount of IL-11 mRNA was detected in unstimulated intestinal SEMFs, but stimulation by IL-1β and TGF-β1 markedly enhanced IL-11 secretion. TNF-α, platelet-derived growth factor (PDGF)-BB, basic fibroblast growth factor (bFGF), and EGF all weakly enhanced IL-11 mRNA expression, but these stimulations were much weaker than those induced by IL-1β and TGF-β1.

The effects of IL-1β and TGF-β1 were also observed at the mRNA levels (Fig. 1B). The cells were incubated for 12 h, and the IL-11 mRNA expression was assessed by Northern blot analysis. Both IL-1β and TGF-β1 induced a marked increase in the IL-11 mRNA expression. The IL-11 mRNA consisted of two transcripts of 1.5 and 2.5 kb, which differ at their 3′-polyadenylation sites, yet encode the same function (3). Compared with the induction of IL-6 mRNA, IL-1β was a common inducer of IL-11 and IL-6, but TGF-β did not stimulate IL-6 mRNA expression. In contrast, TNF-α was a strong inducer of IL-6 mRNA, but its effect on IL-11 mRNA expression was modest. Thus it became clear that among the various factors, IL-1β and TGF-β1 were the most potent inducers of IL-11 secretion in intestinal SEMFs.

Induction of IL-11 by IL-1β and TGF-β1. Intestinal SEMFs were incubated for 12 h with increasing concentrations of IL-1β, and the IL-11 mRNA expression was analyzed by Northern blot analysis (Fig. 2A). IL-1β and TGF-β1 induced a dose-dependent increase in IL-11 mRNA expression. The effect of IL-1β was detected at as low as 0.01 ng/ml and reached a maximum at 10 ng/ml. The effect of TGF-β1 was observed at as low as 1.0 ng/ml and reached a maximum at 100 ng/ml.

These effects were also observed at the protein level (Fig. 2A, right). Incubation with IL-1β or TGF-β1 for 48 h dose dependently induced IL-11 protein secretion.

Kinetics of IL-11 induction by IL-1β and TGF-β1. The kinetics of IL-1β- and TGF-β1-induced IL-11 mRNA expression were evaluated in human SEMFs (Fig. 2B). The cells were stimulated with IL-1β (10 ng/ml) or TGF-β1 (50 ng/ml), and the sequential changes in IL-11 mRNA expression were determined by Northern blot analysis. IL-1β induced an increase in the accumulation of IL-11 mRNA, and this reached a maximum at 12 h after stimulation. Thereafter, the induced-IL-11 mRNA levels decreased. TGF-β1 also induced an increase in the accumulation of IL-11 mRNA. This reached a maximum at 12 h after stimulation and remained at the same level for a further 12 h.

The effects of IL-1β and TGF-β1 on IL-11 protein secretion were also evaluated (Fig. 2B, right). Each factor induced a time-dependent increase in IL-11 levels in the supernatant. The effects of IL-1β were noted within 24 h, and TGF-β1 induced a continuous increase in IL-11 secretion.

Modulation of transcription factor activation. Nuclear run-on assay demonstrated that IL-1β and TGF-β1 induced an increase in the transcription activity of the IL-11 gene (Fig. 3A), indicating that transcriptional activation of the IL-11 gene is involved in IL-1β- and TGF-β1-induced IL-11 mRNA expression in intestinal SEMFs.

Two AP-1 motifs are located in the promoter region of the IL-11 gene (29, 44). To elucidate the mechanisms underlying the response to IL-1β and TGF-β1 in intestinal SEMFs, we evaluated AP-1 activation. As demonstrated in Fig. 3B, stimulation with either IL-1β (10 ng/ml) or TGF-β1 (50 ng/ml) for 2 h induced an increase in AP-1-DNA binding activity. The effect of TGF-β1 was stronger than that induced by IL-1β. The specificity of this reaction was confirmed by the addition of...
cold oligo-DNA, which abolished the reactive band. The addition of anti-pan Jun and anti-pan Fos antibodies induced supershifts of the binding complexes, thus indicating that AP-1 complexes were heterodimers consisting of Jun and Fos subunits.

In previous reports, the AP-1 complex was induced in a stimulation- and cell-specific manner (3, 19, 21). In intestinal SEMFs, we analyzed the IL-1β- and TGF-β1-induced AP-1 complexes using antibodies specific for distinct Fos isoforms (c-Fos, FosB, Fra-1, and Fra-2) and Jun isoforms (JunB, c-Jun, and JunD). As shown in Fig. 3C, the supershift analysis demonstrated that in unstimulated and stimulated cells, the AP-1 complex consisted of FosB, Fra2, c-Jun, and JunD. Weak bindings of c-Fos, Fra1, and JunB were observed, but these seemed to be equivocal.

Effects of adenovirus-mediated transfer of Ad-DN-c-Jun gene. It has been reported that c-Jun is a major positive regulator of AP-1-mediated gene-transcription (19, 27), although its role in IL-11 induction remains unclear. To assess the role of the c-Jun molecule in our system, we initially assessed the activation of JNK, a member of MAPK family and a directly upstream activator of c-Jun (30). As demonstrated in Fig. 4A, IL-1β and TGF-β1 rapidly (within 5 min after stimulation) induced a phosphorylation of JNK, indicating that IL-1β and TGF-β1 induced a rapid activation of JNK. Next, we evaluated the effects of a recombinant adenovirus containing Ad-DN-c-Jun lacking the transactivation domain of the wild-type c-Jun to specifically inhibit AP-1 activity. As shown in Fig. 4B, a truncated c-Jun protein (~30 kDa) was strongly expressed in intestinal SEMFs infected with Ad-DN-c-Jun at 48 h after infection, indicating a successful gene transfer. Endogenous wild-type c-Jun protein was also detected at a low level and was not suppressed by DN-c-Jun. As shown in Fig. 4C, Ad-DN-c-Jun markedly inhibited the IL-1β- and TGF-β1-induced IL-11 mRNA expression. These inhibitory effects were not induced by Ad-LacZ. The specificity was confirmed by the responses of IL-8 gene. Induction of IL-8 gene has been reported to depend on NF-kB activation, but AP-1 plays a minor role (2, 18, 31). As shown in Fig. 4C, inhibitory effects of Ad-DN-c-Jun were modest in IL-1β-induced IL-8 mRNA expression, indicating specific inhibitory effects of Ad-DN-c-Jun on IL-11 mRNA expression. Similarly, weak effects of TGF-β1 on IL-8 mRNA expression were not affected by Ad-DN-c-Jun. Thus the activation of c-Jun AP-1 plays a major role in the induction of the IL-1β- and TGF-β1-induced IL-11 mRNA expression in these cells.

MAPK activation and MAPK inhibitors. The MAPK family has been shown to play an important role in regulating gene expression in response to inflammatory mediators (8, 17, 30). To assess whether similar responses are involved in our system, we evaluated the
Fig. 3. A: nuclear run-on assays for evaluation of transcriptional activation of IL-11 genes. Nuclei were isolated from 8 h after stimulation with IL-1β (10 ng/ml) and TGF-β (50 ng/ml), and nuclear run-on assays were performed in the presence of [α-32P]uridine triphosphate, as described in MATERIALS AND METHODS. B: EMSA for activating protein (AP)-1 DNA-binding activities. The cells were incubated with medium alone, IL-1β (10 ng/ml) or TGF-β1 (50 ng/ml) for 3 h, and then nuclear extracts were prepared. The arrows on the right represent supershift bands. Lane 1, medium alone; lane 2, IL-1β; lane 3, TGF-β; lane 4, TGF-β + cold probe; lane 5, IL-1β + anti-pan Fos antibody; lane 6, IL-1β + anti-pan Jun, lane 7, TGF-β1 + anti-pan Fos antibody; and lane 8, TGF-β1 + anti-pan Jun. C: characterization of the activating protein (AP)-1 subunit binding to the AP-1 site in the IL-11 promoter. The cells were incubated with medium alone, IL-1β (10 ng/ml) or TGF-β1 (50 ng/ml) for 2 h, and nuclear extracts were prepared. A supershift assay using the antibodies against AP-1 subunits was then performed. As a control, normal rabbit IgG was used. To detect the binding complex in the unstimulated cells, a long exposure of the X-ray film to the gel was required.

Fig. 4. JNK phosphorylation, expression of dominant negative (DN)-c-Jun protein, and the effects of DN-c-Jun on IL-11 mRNA expression. A: intestinal SEMFs were stimulated with IL-1β (10 ng/ml) or TGF-β1 (50 ng/ml), and the activation of JNK was evaluated by Western blot analysis. B: the cells were infected with an adenovirus (Ad) expressing the DN-c-Jun and were then incubated for 48 h. The expression of c-Jun protein was assessed by Western blot analysis. C: at 48 h after infection with the adenovirus, the cells were stimulated with IL-1β (10 ng/ml) or TGF-β1 (50 ng/ml) for 12 h. IL-11 and IL-8 mRNA expression was then determined by Northern blot analysis.
effects of IL-1β and TGF-β1 on the p42/44 (ERK1/2) and p38 MAPK phosphorylation in human SEMFs. As shown in Fig. 5, A and C, IL-1β and TGF-β1 induced the phosphorylation of p42/44 (ERK1/2) MAPKs as early as 15 min after the stimulation. IL-1β induced a rapid phosphorylation of p38 MAPK within 15 min (Fig. 5A), but TGF-β1 induced it at 60 min after the stimulation (Fig. 5C). These results indicate that the MAPK pathways are activated by IL-1β and TGF-β1 in human SEMFs, but the kinetics of the p38 phosphorylation were different between IL-1β and TGF-β1.

To investigate the role of MAPK activation in the induction of IL-11 secretion in intestinal SEMFs, we evaluated the effects of inhibitors of the p42/44 MAPKs (PD-98059 and U-0216) (1, 9) and an inhibitor of the p38 MAPK (SB-203580) (16). As shown in Fig. 5, B and D, these inhibitors induced specific inhibitory effects on IL-1β- and TGF-β1-induced phosphorylation of ERK1/2 and p38 MAPKs. Inhibition by U-0216 of ERK1/2 MAPKs was stronger than that induced by PD-98059.

As shown in Fig. 6, each inhibitor dose dependently blocked the IL-1β- and TGF-β1-induced IL-11 mRNA expression and significantly reduced IL-11 protein secretion. These results indicate that p42/44 and p38 MAPKs play a role in the IL-1β- and TGF-β1-induced IL-11 secretion.

Posttranscriptional regulation of IL-11 mRNA accumulation. The 3′-untranslated region of the IL-11 mRNA contains multiple repeats of the AUUUA sequence, which has been known to play a role in mRNA destabilization (47). Furthermore, it has been reported (11, 25) that the p38 MAPK is involved in the stabilization of mRNAs from various genes. To evaluate the role of p38 MAPK in the posttranscriptional regulation of IL-11 mRNA expression, we investigated the effects of IL-1β- and TGF-β1-induced IL-11 mRNA stability in the presence and absence of the p38 MAPK inhibitor SB-203580. The cells were stimulated with cytokines for 12 h, and then a chasing approach using the transcription inhibitor actinomycin D and a Northern blot analysis were performed. As shown in Fig. 7, in the unstimulated cells, IL-11 mRNA decreased immediately, and both IL-1β and TGF-β1 increased the stability of the IL-11 mRNAs. The addition of SB-203580 caused a destabilization of the IL-1β- and TGF-β1-induced IL-11 mRNAs, thus indicating that posttranscriptional mechanisms mediated by p38 MAPK activation were involved in the IL-1β- and TGF-β1-induced IL-11 mRNA expression in intestinal SEMFs.

Combined effects of IL-1β plus TGF-β1. The combined effects of IL-1β plus TGF-β1 were evaluated at the mRNA and protein levels (Fig. 8, A and B). The cells were incubated with stimulators for 12 h, and the IL-11 mRNA levels were determined by Northern blot analysis (Fig. 8A). These combinations increased the IL-11 mRNA abundance compared with the effects of individual cytokines. Furthermore, similar effects were observed for IL-11 protein secretion (Fig. 8B), and this seemed to be due to an additive effect of each cytokine. As shown in Fig. 8C, the combination of IL-1β and TGF-β1 induced additive effects on MAPK and AP-1 activation, supporting the responses in IL-11 mRNA expression and protein secretion.

**DISCUSSION**

The various functions of intestinal SEMFs are regulated by a variety of cytokines and growth factors (18, 20, 24, 31, 36). For example, PDGF-BB, bFGF, and IGF-I stimulated the proliferation of intestinal SEMFs (20, 31). ECM secretion was induced by TGF-β1 (31,
IL-1β and TNF-α are strong inducers of chemo-
kines, IL-6, and matrix metalloproteinase (MMP)-1 in
these cells (18, 31). Among these factors, we found that
IL-1β and TGF-β1 were strong inducers of IL-11 secre-
tion in intestinal SEMFs. The strong effect of TGF-β
seemed to be speci-
fi-
c for IL-11 relatively, because in
intestinal SEMFs, TGF-β1 did not alter the mRNA
expression of IL-6 and other proteins, including
MMP-1, -2, -3, tissue inhibitor of MMP-1 and -2, and
hepatocyte growth factor (data not shown). Thus, in
intestinal SEMFs, IL-11 secretion is upregulated by
the representative pro-
and anti-inflammatory cyto-
kines IL-1β and TGF-β1.

We investigated the molecular mechanisms respon-
sible for the IL-1β- and TGF-β1-induced IL-11 expres-
sion in intestinal SEMFs. Previous studies have iden-
tified two AP-1-binding motifs between −100 and −82
in the IL-11 promoter that are essential for the tran-
scriptional activation of the IL-11 gene (42, 44). AP-1
exists as a homodimer of Jun isoforms or as a het-
erodimer of Jun/Fos isoforms, both members of the
immediate early gene family (3, 21). Increasing gene
expression by AP-1 is mostly attributed to c-Jun-con-
taining complexes (19). In our experiments, both IL-1β
and TGF-β1 induced AP-1 DNA-binding activity in
intestinal SEMFs, and the supershift analyses re-
vealed that the induced-AP-1 complexes were com-
posed of FosB, Fra-2, c-Jun, and JunD. The AP-1 activa-
tion by IL-1β and TGF-β1 was accompanied by the
phosphorylation of JNK, a direct upstream activator of
c-Jun (30). Furthermore, the forced expression of a
dominant-negative c-Jun lacking the transactivation
domain of the wild-type c-Jun markedly reduced both
IL-1β- and TGF-β1-induced IL-11 mRNA expressions.
These observations suggest that c-Jun-IL-11 promoter
binding might play a major role in the IL-1β- and
TGF-β1-induced IL-11 mRNA expression in intestinal
SEMFs.

MAPK activation is an important signaling event in
response to proinflammatory stimuli (8, 17, 30), but its
role in IL-11 induction has not been clarified. The
MAPK family consists of three groups, and all are
phosphorylated on tyrosine and threonine residues by
upstream kinases, the MAPK kinases (8, 17, 30). As
demonstrated above, JNK, one of MAPK family, was
phosphorylated by IL-1β and TGF-β1 in our system.
The p44 and p42 ERK1/2 and the p38 MAPK (8, 17)
were also activated by IL-1β and TGF-β1 in human
SEMFs. Stimulation by each factor induced a rapid
activation of ERK1/2 MAPK within 15 min. However,
the apparent activation of p38 by TGF-β1 was observed
at 60 min after stimulation, whereas IL-1β induced
p38 activation within 15 min, thus indicating that

Fig. 6. The inhibitory effects of MAPK in-
hibitors on IL-11 mRNA expression and
IL-11 secretion. A: cells were stimulated
for 12 h with IL-1β (10 ng/ml) or TGF-β1
(50 ng/ml) in the presence or absence of
MAPK inhibitors (5 or 20 μM), and IL-11
mRNA expression was determined by
Northern blot analysis. B: cells were stim-
ulated for 24 h with IL-1β (10 ng/ml) or
TGF-β1 (50 ng/ml) in the presence or ab-
sence of MAPK inhibitors (20 μM) (B). The
values are expressed as means ± SD (n = 4). Significant difference from the values
in the absence of MAPK inhibitors: *P < 0.05, **P < 0.01.
Fig. 7. Changes in IL-11 mRNA stabilities. The cells were stimulated with IL-1β (10 ng/ml) or TGF-β1 (50 ng/ml) for 12 h, and then actinomycin D (2 μg/ml) was added to the cultures. Total RNA was prepared at the times indicated and was processed for Northern blot analysis. To evaluate the role of p38 MAPK, SB-203580 (20 μM) was simultaneously added with actinomycin D. To detect the binding complex in the unstimulated cells, a long exposure of the X-ray film to the gel was required.

Fig. 8. Combined effects of IL-1β and TGF-β1. A: The cells were stimulated for 12 h with IL-1β and TGF-β1, and the IL-11 mRNA expression was then determined by Northern blot analysis. B: the cells were stimulated for 24 h with IL-1β and TGF-β1, and then IL-11 levels in the supernatants were determined by ELISA. The values are expressed as means ± SD (n = 4). C: effects of IL-1β + TGF-β1 on MAPK and AP-1 activation. The cells were stimulated for 15 min for evaluation of ERK1/2, 30 min for p38, and 2 h for AP-1 activation. MAPK activation was evaluated by Western blot analysis, and AP-1 activation was analyzed by EMSA.
different mechanisms exist between IL-1β and TGF-β1 in the induction of p38 MAPK phosphorylation.

The role of MAPKs in IL-1β- and TGF-β1-induced IL-11 secretion was investigated by using specific inhibitors. The imidazole compound SB-203580 is a specific inhibitor of p38 MAPK (9). In intestinal SEMFs, SB-203580 specifically blocked the activation of IL-1β- and TGF-β1-induced p38 phosphorylation and caused a significant decrease in both IL-1β- and TGF-β1-induced IL-11 mRNA expression and secretion. These results indicate that p38 activation was involved in the IL-1β- and TGF-β1-induced IL-11 secretion. In addition, we addressed the role of ERK1/2 in our system. PD-98059 is a specific inhibitor of MEK1 (1), the kinase directly upstream of ERK1/2, and U-0216 is a specific inhibitor of MEK1 and MEK2 (16). U-0216 blocked the phosphorylation of ERK1/2 more potently than PD-98059 in intestinal SEMFs. PD-98059 and U-0216 blocked IL-1β- and TGF-β1-induced IL-11 mRNA expression and caused a significant inhibition against IL-1β- and TGF-β1-induced IL-11 secretion. Thus we concluded that ERK1/2 MAPKs also participate in the IL-11 secretion induced by both IL-1β and TGF-β1 in intestinal SEMFs.

In the mRNA expression of many genes, p38 MAPK also plays an important role at the posttranscriptional steps (11, 25). Furthermore, the 3’-untranslated region of the IL-11 mRNA contains multiple repeats of the AUUUA sequence, which has been shown to play a role in mRNA destabilization (29, 47). To assess the involvement of p38 MAPK activation in the IL-1β- and TGF-β1-induced IL-11 mRNA expression, we evaluated the changes in IL-11 mRNA stabilities. As shown in Fig. 7, the inhibitor of p38 MAPK SB-203580 markedly decreased the stabilities of IL-1β- and TGF-β1-induced IL-11 mRNAs. Thus p38 MAPK plays a role via the induction of IL-11 mRNA stabilization in the effects of IL-1β and TGF-β1.

The induction of IL-11 may potentially be advantageous in the reduction of intestinal inflammation, because IL-11 exerts anti-inflammatory actions via an inhibition of NF-κB activation in monocytes/macrophages and the induction of Th2 polarization in CD4+ T cells. In previous studies, IL-1β and TGF-β1 have been established as representative pro- and anti-inflammatory cytokines, respectively (13, 22). Increased expression of these factors has been reported in inflammatory bowel diseases (IBD) (6, 28). The IL-11 induced by IL-1β may be an autonegative regulation mechanism preventing IL-1β-induced mucosal inflammation. On the other hand, some parts of the anti-inflammatory effects of TGF-β1 may be mediated by the secondarily induced IL-11. Furthermore, previous studies (23, 32) have demonstrated that IL-11 plays a role in mucosal repair via its trophic effects on the epithelium. IL-1β and TGF-β1 have been reported to be as inducers of ECM synthesis (10, 20, 22), which is an important factor in mucosal repair. Taken together, IL-1β and TGF-β1 are involved in a complex network of IL-11 induction, which promotes both anti-inflammatory responses and mucosal repair processes.

In conclusion, this study demonstrated for the first time that IL-11 is secreted by intestinal SEMFs in response to IL-1β and TGF-β1. These responses were mediated by c-Jun AP-1- and MAPK-dependent pathways. Although the pathogenesis of IBD is becoming increasingly apparent, mucosal IL-11 secretion has not been fully investigated in IBD patients. Further investigations evaluating the in vivo IL-11 secretion of the mucosa of normal individuals and IBD patients will clarify the mechanisms responsible for the efficacy of rhIL-11 administration in IBD patients.

DISCLOSURES

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