Induction of **MUC8** Gene Expression by Interleukin-1β Is Mediated by a Sequential ERK MAPK/RSK1/CREB Cascade Pathway in Human Airway Epithelial Cells*

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Kyoung Seob Song‡, Je-Kyung Seong§, Kwang Chul Chung¶, Won-Jae Lee†, Chang-Hoon Kim‡*, Kyou Nam Cho‡, Chi-Dug Kang¶¶, Ja Seok Koo§§, and Joo-Heon Yoon‡**

From the ‡Brain Korea 21 Project for Medical Science, Yonsei University College of Medicine, Seoul 120-752, Korea, the §Department of Developmental Biology and Genomics, College of Veterinary Medicine, Seoul National University, Seoul 151-742, Korea, the ¶Department of Biostatistics, College of Sciences, Yonsei University, Seoul 120-749, Korea, the ¶¶Department of Molecular Life Science, Ewha Womans University, Seoul 120-750, Korea, the §§Department of Biochemistry, College of Medicine, Pusan National University, Pusan 609-735, Korea, the **Department of Otorhinolaryngology, Yonsei University College of Medicine, 134 Shinchon-dong, Seodaemun-gu, Seoul 120-752, Korea

Mucins are the major components of the mucus layer that covers and protects the respiratory, digestive, and reproductive tracts. Our previous studies showed that **MUC8** gene expression was overexpressed in *in vivo* polyep epithelium in chronic sinusitis and was also increased by treatment with inflammatory mediators in an *in vitro* culture condition. However, the mechanisms by which the inflammatory mediators-induced **MUC8** gene expression in normal nasal epithelial cells evolved remain unclear. We examined the mechanism by which the important proinflammatory mediator, interleukin (IL)-1β, increases **MUC8** gene expression levels. We found that pharmacologic and genetic inhibition of ERK MAPK pathway abolished IL-1β-induced **MUC8** gene expression in normal human nasal epithelial cells. Moreover, the overexpression of wide-type or of the dominant-negative mutant of p90 ribosomal S6 protein kinase 1 (RSK1) enhanced or suppressed, respectively, IL-1β-induced **MUC8** gene expression. RSK1 was found to directly phosphorylate cAMP-response element-binding protein (CREB), and this event led to the stimulation of CRE-mediated gene transcription. In conclusion, IL-1β was found to induce **MUC8** gene expression via a sequential ERK/RSK1/CREB pathway in human airway epithelial cells.

Mucins are highly glycosylated, high molecular mass glycoproteins and are major components of the mucus produced by the epithelia of the respiratory, gastrointestinal, and reproductive tracts. They are responsible for the viscoelastic properties of secreted mucus and provide lubrication and protection for mucous membranes (1). In the airway, virtually all forms of airway inflammation are associated with the overproduction of mucus, which can lead to airway obstruction (2).

Eighteen types of mucin genes have been discovered to date: **MUC1** to **MUC4**, **MUC5AC**, **MUC5B**, **MUC6**, **MUC7**, **MUC8** (2), **MUC9** (3), **MUC10** (4), **MUC11**, **MUC12** (5), **MUC13** (6), **MUC15** (7), **MUC16** (8), **MUC17** (9), and **MUC18** (10). Of these, **MUC5AC** and **MUC5B** are known to be major gel-foaming mucins secreted in the human airway. Accordingly, most studies on mucin genes have been focused on these two mucins. However, although **MUC5AC** is known to be expressed by most surface goblet cells, we found that only a portion of the goblet cells (11) expressed **MUC5AC** mRNA. This suggests that other mucin genes in addition to **MUC5AC** might be important for mucus hypersecretion. We have previously investigated the expressions of other mucin genes using various inflammatory tissues and cell lysates. Interestingly, in *in vivo*, we found that **MUC8** mRNA levels are clearly up-regulated in the polyepithelium, which is invariably stimulated by inflammatory mediators (12). In addition, in *in vitro*, interleukin-1β (IL-1β), a tumor necrosis factor-α, and a mixture of inflammatory mediators were found to up-regulate **MUC8** mRNA and to down-regulate **MUC5AC** mRNA (13, 14). These results showed that **MUC8** mRNA is increased both *in vivo* and *in vitro* during inflammatory conditions. However, the mechanisms of **MUC8** gene expression during inflammation in normal airway epithelial cells and the signal molecules involved have not been elucidated.

Mitogen-activated protein kinases (MAPKs) are ubiquitous kinases and are involved in signal transduction in eukaryotic organisms. This family of kinases is characterized by their activation by MAPKs through the dual phosphorylation of Thr and Tyr residues in their activation loop. The MAPK family includes extracellular signal-regulated kinases (ERK), which are activated in response to growth factors, via the Ras proto- oncogene. Moreover, c-Jun N-terminal kinase (JNK) and p38 MAPK constitute two other families, collectively known as stress-activated protein kinases (SAPK), because they are induced by UV radiation, heat-shock, oxidative stress, or tumor necrosis factor-α. The stimulation of ERK initiates a cascade of

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1 The abbreviations used are: IL-1β, interleukin 1β; MUC, mucin; MAPK, mitogen-activated protein kinase; ERK, extracellular signal-regulated kinase; MEK1, MAPK/ERK kinase 1; SAPK, stress-activated protein kinase; MSK1, mitogen- and stress-activated protein kinase 1; RSK1, p90 ribosomal S6 protein kinase 1; CRE, cAMP-response element; CREB, CRE-binding protein; EMSA, electrophoretic mobility shift analysis; W/T, wide-type; DN, dominant-negative; JNK, c-Jun NH2-terminal kinase; NH2, normal human nasal epithelial; FBS, fetal bovine serum; PBS, phosphate-buffered saline; FAM, carboxyfluorescein; TAMRA, carboxyltetramethylrhodamine; DTT, dithiothreitol; IBMX, isobutylmethylxanthine.
activating events, including the phosphorylation of p90 ribosomal S6 protein kinase 1 (RSK1), and its translocation to the nucleus, where RSK1 phosphorylates nuclear substrates (15). Moreover, the phosphorylation of mitogen- and stress-activated protein kinase (MSK), which localized in the nuclei (16), could lead to the phosphorylation and activation several transcription factors like cAMP-response element-binding protein (CREB) and activating transcription factor 1 (ATF1) (17).

We examined the mechanism by which the important proinflammatory mediator, IL-1β, increases MUC8 gene expression levels. Here we show that ERK MAPK is essential for IL-1β-induced MUC8 gene expression in normal human nasal epithelial (NHNE) cells. We also show that RSK1 mediates the IL-1β-induced phosphorylation of CREB and CRE-mediated transcription. Molecular cloning of the MUC8 promoter regulated by various stimuli may yield a deeper insight into cellular function.

**EXPERIMENTAL PROCEDURES**

**Materials**—PD98059, SB203580, and anti-α-tubulin antibody were purchased from Calbiochem (San Diego, CA). Anti-phospho-p42/44 MAPK (Thr202/Tyr204) antibody, anti-phospho-p38 MAPK (Thr180/Tyr182) antibody, anti-phospho-ERK1/2 (Ser202/Ser204) antibody, and anti-phospho-CREB (Ser133) antibody were purchased from Cell Signaling (Beverly, MA). The cDNA construct encoding dominant-negative Raf1 construct was kindly provided by Dr. J. H. Kim (University of Korea, Seoul, Korea).

**Cell Cultures**—The culture system used for the normal human nasal epithelial (NHNE) cells has been previously reported (15). The human lung mucociliary epithelial carcinoma cell line, NCI-H292, was purchased from the American Type Culture Collection (CRL-1848, Manassas, VA) and was cultured in RPMI 1640 (Invitrogen) supplemented with 10% fetal bovine serum (FBS) in the presence of penicillin-streptomycin at 37 °C in a 5% CO2 humidified chamber. For serum deprivation, confluent cells were washed twice with phosphate-buffered saline (PBS) and re-cultured in RPMI 1640 with 0.2% FBS.

**Real-time Quantitative PCR**—Primers and probes were designed using PerkinElmer Life Sciences Primer Express® software, purchased from PE Biosystems. Commercial reagents (TaqMan PCR Universal PCR Master Mix, PE Biosystems) and conditions were applied according to the manufacturer’s protocol. One microgram of cDNA (reverse transcription mixture), oligonucleotides at a final concentration of 800 ng/μl of primers, and 200 ng TaqMan hybridization probe were used in a 25-μl volume. The probe of real-time PCR was labeled with carboxyfluorescein (FAM) at the 5′-end and with the quencher carboxytetramethylrhodamine (TAMRA) at the 3′-end. The following primers and TaqMan probes were used: forward (5′-GACCTGGCCTCCAGGAC-3′) and reverse (5′-CAGGAGTTCCGACAGGACCCT-3′) and TaqMan probe (6FAM-CACCTTCGGGAGCCCTGACTGG-3′).

**In Vitro RSK1 Assay**—For serum deprivation, confluent cells were washed twice with PBS and recultured in RPMI 1640 containing 0.2% FBS. Cells were treated with IL-1β for 30 min, harvested, and lysed in lysis buffer. 300 μg of the protein so obtained was incubated with anti-phospho-RSK1 (Ser577) antibody overnight at 4 °C. Immunocomplexes were added to 40 μl of protein A-Sepharose beads. Samples were washed three times in lysis buffer, and kinase reactions were carried out for 1 h at 30 °C in 20 μl of kinase buffer (20 mM HEPES (pH 7.2), 5 mM MnCl2, 200 μM sodium orthovanadate, 5 μM of α-folate treated enolase, 10 μM ATP, 5 μCi of [γ-32P]ATP) and 5 μl of bacterially expressed glutathione S-transferase (GST)-CREB as a substrate. Reactions were stopped by adding SDS-PAGE sample buffer and analyzed by SDS-PAGE followed by autoradiography.

**Electrophoretic Mobility Shift Analysis**—Cells were washed with ice-cold PBS and pelleted. Pellets were then resuspended in nuclear extraction buffer I (10 mM HEPES (pH 7.9), 10 mM KCl, 1.5 mM MgCl2, 0.5 mM DTT, 0.05% Nonidet P-40, 1 mM phenylmethylsulfonyl fluoride, 2 μg/ml leupeptin, and 2 μg/ml aprotinin), incubated for 15 min on ice, and vortexed vigorously. Nuclei were pelleted, resuspended in nuclear extraction buffer II (20 mM HEPES (pH 7.9), 20% glycerol, 420 mM NaCl, 1 mM EDTA, 0.5 mM phenylmethylsulfonyl fluoride, 2 μg/ml leupeptin, and 2 μg/ml aprotinin), and vigorously vortexed. The nuclear extracts were then centrifuged for 15 min at 4 °C, and the supernatants were stored at −70 °C for EMSA. Oligonucleotides corresponding to the consensus CRE sequences (5′-AGAGATTTCTTGACGTCGAGGC-3′) were synthesized, annealed, and end-labeled with [γ-32P]ATP using T4 polynucleotide kinase. Nuclear extract was incubated at room temperature for 30 min with the 32P-labeled CRE probe in binding buffer (20% glycerol, 5 mM MgCl2, 2.5 mM EDTA, 2.5 mM DTT, 250 mM NaCl, 50 mM Tris·Cl (pH 7.5), and 0.25 mg/ml poly(dI-dC)). DNA-nuclear protein complexes were separated from the DNA probe by electrophoresis through 5% nondenaturing polyacrylamide gels in 0.5 × Tris borate EDTA (TBE) buffer. Supershift experiments were conducted using 2 μl of anti-phospho-CREB antibody. The gels were dried and autoradiographed using a intensifying screen at −70 °C.

**Transient Transfection and Luciferase Assay**—Luciferase reporter construct (pCRE-luc) and plasmid expressing mutant CREB (pCREB S133A, with serine residue 133 replaced by alanine) vector, were transiently transfected using FuGene6 transfection reagent (Roche Applied Science), according to the manufacturer’s instructions. The transfected cells were either incubated for 48 h, harvested, and assayed for luciferase activity, using a luciferase assay system (Promega), according to the manufacturer’s instructions. β-Galactosidase activity was also assayed to standardize the transfection efficiencies.

**RESULTS**

**Effects of ERK MAPK on IL-1β-induced MUC8 Gene Expression**—Previously, ERK and p38 MAPKs had shown maximum activation after 15-min treatment with IL-1β, this effect decreased at 45 min in NHNE cells. No change was detected in

**Line**—Plasmid encoding the kinase-deficient MEK1 mutant (pcDNA3-MEK1DN) was cut with BamHI (Promega, Madison, WI) and ligated with pBluescript (Stratagene, La Jolla, CA). This clone was then cut with HindIII (Promega), filled in with Klenow, re-cut with SacII (Promega), and then ligated to pTRE vector (Clontech, Palo Alto, CA). Plasmid encoding kinase-inactive p38 mutant (pcDNA3-p38ΔGF) was cut with BamHI, filled in with Klenow, re-cut with XbaI (Promega), and then ligated to pTRE vector. NCI-H292 cells were then cotransfected with pTet-off (Clontech) regulation vector and pTRE-p38DN or pTRE-MEK1DN (1:20 ratio of regulation vector to expression vector) using FuGENE 6 transfection reagent (Roche Applied Science), following the procedure recommended by the manufacturer. Stably transfected cell lines were selected with 200 μg/ml G418 (Calbiochem), and the medium was then replaced with G418 every 3 days.

**Immunoprecipitation**—One microgram of polyclonal anti-phospho CREB antibody was incubated at 4 °C overnight with 400 μg of cell extract prepared using lysis buffer (20 mM Tris·Cl (pH 7.9), 1% Triton X-100, 1 mM Na2VO4, 157 mM NaCl, 1 μg/ml leupeptin, 1 μg/ml aprotinin, 1 mM EDTA, 10 mM NaF, 1 mM Na3VO4, 5 mM Na2EDTA, 10% glycerol, 1 mM β-glycerophosphate, 0.1 mM β-nitrophenyl phosphate, 0.2 mM phenylmethylsulfonyl fluoride). Forty microliters of a 1:1 suspension of protein A-Sepharose beads was added to the cell lysates and incubated for 2 h at 4 °C, with gentle rotation. The beads were pelleted and washed extensively with cell lysis buffer. Bound proteins were dissolved by boiling the samples in PAGE sample buffer, and whole cell lysates were separated on SDS-PAGE gels. The radioactively labeled protein complex was visualized on film, and bands were quantitated using a densitometer.
the activation of JNK (18). To investigate the possible involvement of ERK or p38 MAPK pathway in IL-1β-induced MUC8 gene expression, we performed real-time PCR after pretreatment with 20 μM PD98059 or 20 μM SB203580 for 1 h. Real-time quantitative PCR showed that this treatment with PD98059 for 1 h inhibited MUC8 gene expression (Fig. 1). However, pretreatment with SB203580 did not affect MUC8 gene expression (Fig. 1). These results indicate that the activation of ERK MAPK, but not of p38 MAPK, appeared to be closely related to IL-1β-induced MUC8 gene expression.

When the same experiment (Fig. 1) was performed using NCI-H292 cells, a human lung mucoepidermoid carcinoma cell line, we obtained results that were similar to those of normal cells (data not shown). To further confirm the significance of ERK or p38 kinase upon the cellular level of MUC8 gene expression, we generated cells stably expressing dominant-negative vectors (19). These results show that the activation of ERK MAPK via MEK1 by IL-1β might occur via a Ras-independent pathway to induce MUC8 gene expression in airway epithelial cells.

**Effects of RSK1 on IL-1β-induced MUC8 Gene Expression**—To determine which molecules are involved in the downstream signaling of ERK MAPK in IL-1β-induced MUC8 gene expression, we investigated RSK1 and MSK1. RSK1 has been reported to be activated by ERK MAPK (20–22). The phosphorylation of RSK1 by IL-1β peaked at 30 min and then decreased at 60 min after IL-1β stimulation (Fig. 3A). Pretreatment with 20 μM PD98059 inhibited IL-1β-induced RSK1 phosphorylation (Fig. 3B), indicating that RSK1 is regulated by ERK MAPK. These results showed that RSK1 acts as a downstream signaling mediator of ERK MAPK. To determine whether RSK1 plays an important role in IL-1β-induced MUC8 gene expression, an RSK1 mutant study was performed. RSK1 is currently a candidate for the mediation of cytokine-induced CREB phosphorylation at Ser133 (20, 23, 24). Overexpression of wide-type (W/T) RSK1 increased IL-1β-induced CREB phosphorylation. However, the overexpression of RSK1DN (D205N) suppressed the IL-1β-induced CREB phosphorylation. Consistently, IL-1β-induced MUC8 gene expression was increased by overexpression of W/T RSK1, whereas the IL-1β-induced MUC8 gene expression was significantly suppressed by RSK1DN (D205N) (Fig. 3C). These results show that RSK1 is required for IL-1β-induced MUC8 gene expression.

**Effects of CREB on IL-1β-induced MUC8 Gene Expression**—We examined whether IL-1β-activated RSK1 specifically binds to CREB in human airway cells. Cell extracts obtained after stimulation with IL-1β were immunoprecipitated with anti-phospho CREB antibody and then blotted with an anti-phospho CREB antibody. As shown in Fig. 4A, protein complex formation was observed between RSK1 and CREB. The control IgG was used as an immunoprecipitating antibody. In addition, a transient phosphorylation of CREB was observed upon the stimulation with IL-1β, reaching the maximum peak at 30 min. These results suggest that IL-1β-induced RSK1 leads to the phosphorylation of CREB in NCI-H292 cells. To confirm whether IL-1β-induced phospho-RSK1 is enzymatically active, we performed an immunocomplex in vitro kinase assay using bacterially expressed GST-CREB as a substrate. As shown in Fig. 4B, IL-1β-induced RSK1 activity remarkably increased in cells transfected with W/T RSK1, whereas the overexpression of RSK1DN diminished IL-1β-induced RSK1 activity.

To determine whether CREB plays a role in MUC8 gene expression, we used both forskolin (an activator of adenylate cyclase) and 3-isobutyl-1-methylxanthine (IBMX, an inhibitor of AMP phosphodiesterase). The transient phosphorylation of CREB was observed after stimulation with both forskolin and IBMX, and this reached a maximum peak at 10 min (18). The cAMP pathway-induced CREB phosphorylation increased MUC8 gene expression (Fig. 4C). Furthermore, IL-1β-induced MUC8 gene expression was significantly suppressed in cells transfected with W/T RSK1, whereas the overexpression of RSK1DN diminished IL-1β-induced RSK1 activity.

**CREB Activates CRE-mediated Gene Transcription in Response to IL-1β**—To determine the DNA binding activity of IL-1β-activated CREB, we performed EMSA using nuclear extracts from NCI-H292 cells after treatment with IL-1β for 1 h. As shown in Fig. 5A, the activity of consensus CRE oligonucleotide remarkably increased in response to IL-1β. To identify specific CRE binding complex, competition and supershift analysis were performed using a 50-fold excess of nonradiolabeled (cold) CRE oligonucleotide and an anti-phospho-CREB anti-
body, respectively. Specific band was found to be selectively inhibited by the specific CRE competitor and to be supershifted by anti-phospho-CREB antibody. These results indicate that activated CREB binds to a cis-acting element, which we refer to as CRE. Next, we assayed the gene expression of the pCRE-luc construct to determine whether IL-1\(\beta\) exerts a stimulatory effect on the activation of CREB and on the subsequent CRE-mediated gene transcription. To investigate the role of CREB phosphorylation on CRE-mediated gene transcription, cells were transfected transiently with pCRE-luc vector only or both pCRE-luc vector and plasmid encoding CREB DN (S133A). Treatment of transfected NCI-H292 cells with IL-1\(\beta\) resulted in increased CRE-mediated gene transcription in a time-dependent manner (Fig. 5B). The expression of CREB containing a

**Fig. 2.** MUC8 gene expression in MEK1 or p38 dominant-negative mutant stable cell lines. Confluent, quiescent MEK1 (A) and p38 (B) dominant-negative mutant stable cells were preincubated for the indicated times to induce dominant-negative mutant protein by removing doxycycline and then stimulating for 15 min with IL-1\(\beta\) prior to Western blotting and for 24 h with IL-1\(\beta\) prior to real-time quantitative PCR. For the other experiments, cells were transiently transfected with cDNA constructs encoding dominant-negative Raf1 or Ras and stimulated with IL-1\(\beta\) for 15 min prior to Western blot analysis with phospho ERK antibody (C) and for 24 h prior to real-time quantitative PCR (D). C, control, ECV304 cells were employed as a positive control. The figures shown are representative of three independent experiments.

**Fig. 3.** Effect of RSK1 on IL-1\(\beta\)-induced MUC8 gene expression. A, confluent, quiescent cells were stimulated for the indicated times with IL-1\(\beta\), and then total proteins were collected for Western blot analysis using phospho-RSK1 antibody. B, for the other experiments, the cells were pretreated for 1 h with 20 \(\mu\)M PD98059 and then stimulated with IL-1\(\beta\) for 30 min prior to Western blot analysis. Cells were transiently transfected with wide-type or mutant RSK1 D205N construct and stimulated with IL-1\(\beta\) for 30 min prior to Western blot analysis (C, upper panel) and for 24 h prior to real-time quantitative PCR (C, lower panel). C, control. The figures shown are representative of three independent experiments.
mutation of the critical regulatory Ser\textsuperscript{133} residue was found to significantly inhibit the luciferase activity induced by IL-1\textbeta. Mock transfection, used as a negative control, showed no significant induction of CRE-mediated reporter transcription. These results suggest that IL-1\textbeta-activated CREB triggers CRE-mediated transcription by binding to CRE.

**DISCUSSION**

Mucociliary clearance is an important function of the airway epithelium. Human beings inhale noxious gases, air pollutants, bacteria, and viruses through the nose, and these are usually trapped by mucus and removed by ciliary beating toward the nasopharynx. Increased mucus secretion during inflammation may represent a defensive mechanism, and inflammatory mediators increase the ciliary beating of respiratory epithelial cells to promote mucociliary clearance (25–27).

The molecular mechanism by which MUC8 gene expression is up-regulated by IL-1\textbeta remains poorly understood. In the present study, we investigated the mechanisms by which MUC8 gene expression is up-regulated by IL-1\textbeta in normal human nasal epithelial cells. Our results show that only the activation of ERK MAPK was required for IL-1\textbeta-induced MUC8 gene expression, although several reports have concluded that more than one MAPK might be necessary for the signal transduction of various inflammatory mediators (28–30). Moreover, the ERK MAPK cascade is known to be activated by G-protein-coupled receptor via: (i) EGFR transactivation (Ras-dependent); (ii) a protein kinase C-dependent, but epidermal growth factor receptor- and Ras-independent, pathway; or (iii) a Ras-independent and cAMP/protein kinase A-mediated pathway (31). Of these pathways, the activation of ERK MAPK by MEK1, as induced by IL-1\textbeta, might occur via a Ras/Raf-independent pathway to induce MUC8 gene expression in human airway epithelial cells. Further work is necessary to explore these pathways.

**Fig. 4. Effect of CREB on IL-1\textbeta-induced MUC8 gene expression.** Confluent, quiescent cells were stimulated for the indicated times with IL-1\textbeta. A, total cell lysates were then immunoprecipitated with anti-phospho CREB antibody and blotted with anti-phospho-ERK antibody. B, an in vitro RSK1 kinase assay was performed using bacterially expressed GST-CREB as an exogenous substrate. Levels of phosphorylated CREB were visualized by autoradiography. C, cells were stimulated for 24 h with IL-1\textbeta or both 20 \muM forskolin and 5 \muM IBMX, and the total RNA were then subjected to real-time quantitative PCR. Cells were transiently transfected with mutant CREB (pCREB S133A) constructs and stimulated with IL-1\textbeta for 30 min prior to Western blot analysis (D, upper panel) and for 24 h prior to real-time quantitative PCR (D, lower panel). IP, immunoprecipitation; IB, immunoblotting; C, control. The figures shown are representative of three independent experiments.
gene expression is a major finding of the present study. The substrates of ERK MAPK are known to be the MSK and RSK family members (33). Our results show that RSK1 might be essential for IL-1β-induced MUC8 gene expression. In addition, the activation of MSK1 appears to be closely related to IL-1β-induced MUC8AC gene expression by IL-1β in human airway epithelial cells (18). Differences in the activations of these kinases by IL-1β may be responsible for differences in target genes. RSK1 phosphorylates several transcription factors, including CREB (34), c-Fos (35), CCAAT/enhancer binding protein (36), nuclear factor-κB (21), and the estrogen receptor (37), and interacts with transcriptional coactivator CREB-binding protein (also known as p300) (38). Many studies have shown that RSK1 phosphorylates Ser133 of CREB (16, 39–41). Although CREB activation by RSK1 has been established by previous studies in other cells, and CREB is a potent regulator of mucin (MUC2, MUC5AC, MUC5B, and MUC6) gene expression in the p15 arm of chromosome 11 (11p15) (17), its role remains unclear in airway epithelial cells. In addition, little is known about the involvement of CREB in MUC8 gene expression. In the present study, the activation of CREB was found, at least in part, essential for IL-1β-induced MUC8 gene expression via ERK MAPK and RSK1. Interestingly, MUC8 gene expression was inhibited in CREB DN (S133A)-transfected cells treated with IL-1β. In addition, treatment with both forskolin and IBMX activated the phosphorylation of CREB and increased MUC8 gene expression (Fig. 4, C and D). These results suggest that CREB might be a transcription factor for IL-1β-induced MUC8 gene expression. However, increased MUC8 expression induced by both forskolin and IBMX was less than that induced by IL-1β, indicating that activation by CREB alone is insufficient for IL-1β-induced MUC8 gene expression. These results suggest that a transcription factor, other than CREB, may be required for IL-1β-induced MUC8 gene expression.

We examined whether IL-1β-induced MUC8 gene expression in human airway epithelial cells is a CRE-mediated transcription. We found that the IL-1β-induced phosphorylation of CREB had the DNA binding activity to CRE (Fig. 5A). Moreover, IL-1β-induced CRE activation increased in a time-dependent manner, whereas the overexpression of CREB DN mutant led to a ~90% decrease in the response of the CRE minimal promoter to IL-1β (Fig. 5B). These results showed that the activation of the cis-element, CRE, appeared closely related to IL-1β-induced MUC8 gene expression in human airway epithelial cells. These results indicate that the MUC8 promoter might have CRE site(s) and the CRE might be an important transcription factor of the MUC8 promoter, like the mucins of 11p15 chromosome (17). However, unfortunately, the promoter and cDNA sequences of the MUC8 gene have not yet been fully identified. Thus, further studies upon the MUC8 promoter seem warranted.

In summary, IL-1β was found to induce MUC8 gene expression via the MEK1/ERK pathway. Furthermore, the activations of RSK1 and CREB are a crucial aspect of the intracellular mechanisms that mediate MUC8 gene expression in human airway epithelial cells. Molecular cloning of the MUC8 promoter regulated by various stimuli may yield a deeper insight into ciliated cell differentiation or function.

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