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Interleukin-1β and Tumor Necrosis Factor-α Induce *MUC5AC* Overexpression through a Mechanism Involving ERK/p38 Mitogen-activated Protein Kinases-MSK1-CREB Activation in Human Airway Epithelial Cells*

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Mucin hypersecretion is commonly observed in many inflammatory diseases of the respiratory tract. *MUC5AC* is generally recognized to be a major airway mucin because *MUC5AC* is highly expressed in the goblet cells of human airway epithelium. Moreover, it is regulated by various inflammatory cytokines. However, the mechanisms by which the interleukin (IL)-1β and tumor necrosis factor (TNF)-α induce *MUC5AC* gene expression in normal nasal epithelial cells, and the signal molecules involved, especially in the downstream signaling of mitogen-activated protein (MAP) kinases, remain unclear. Here we show that pharmacologic or genetic inhibition of either ERK or p38 MAP kinase pathway abolished IL-1β- and TNF-α-induced *MUC5AC* gene expression in normal human nasal epithelial cells. Our results also indicate that the activation of mitogen- and stress-activated protein kinase 1 (MSK1) and cAMP-response element-binding protein and cAMP-response element signaling cascades via ERK and p38 MAP kinases are crucial aspects of the intracellular mechanisms that mediate *MUC5AC* gene expression. Taken together, these studies give additional insights into the molecular mechanism of IL-1β- and TNF-α-induced *MUC5AC* gene expression and enhance our understanding on mucin hypersecretion during inflammation.

Mucin hypersecretion is commonly observed in many respiratory diseases, such as rhinitis, sinusitis, otitis media, nasal allergy, chronic bronchitis, and cystic fibrosis (1–4). Eighteen types of mucin genes have been discovered to date: *MUC1* (5), *MUC2* (6), *MUC3* (7), *MUC4* (8), *MUC5AC* (9), *MUC5B* (10), *MUC6* (11), *MUC7* (12), *MUC8* (13), *MUC9* (14), *MUC10* (15), *MUC11* (16), *MUC12* (16), *MUC13* (17), *MUC15* (18), *MUC16* (19), *MUC17* (20), and *MUC18* (21). Of these, *MUC5AC* and *MUC5B* are generally recognized to be the major airway mucins (22–24) because *MUC5AC* is highly expressed in the goblet cells of the human airway epithelium. Moreover, *MUC5AC* gene expression is known to be regulated by oxidative stress (25) and retinoic acid (26). In addition, *MUC5AC* is regulated by various inflammatory cytokines such as neutrophil elastase (27), IL[1]-9 (28), and IL-4 (29). Given that mucin hypersecretion is an uncontrolled mucin expression during inflammation, unveiling of the signal transduction pathway for inflammatory cytokine-induced *MUC5AC* gene expression would give an important clue to the understanding of airway mucus hypersecretion.

It is well documented that mitogen-activated protein (MAP) kinase pathways are thought to be most important in transmitting inflammatory signals from the cell surface to the nucleus (30). After being triggered by growth factors, cytokines, UV rays, or other stress-inducing agents, a signal is delivered down the MAPKKK → MAPKK (in the cases of ERK, JNK, and p38, the signal is delivered through MEK1/2, MKK4/7, and MKK3/6, respectively) to the MAP kinase cascade. The MAP kinases play a role in cell proliferation, differentiation, apoptosis, cytoskeletal remodeling, and the cell cycle (31–36). Mitogen- and stress-activated protein kinase 1 (MSK1) is a recently identified enzyme that is widely distributed in mammalian cells (37–39). MSK1 is activated *in vitro* and *in vivo* by two different classes of MAP kinases, ERK and p38 MAP kinases (37). Moreover, MSK1 is localized in the nuclei of stimulated or unstimulated cells (39), and two potential *in vivo* substrates are the cAMP-response element-binding protein (CREB) and the closely related activating transcription factor 1 (ATF1) (39).

Recently, reactive oxygen species were reported to increase the expression of the *MUC5AC* gene, by activating the ERK

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[1] The abbreviations used are: IL, interleukin; MUC, mucin; MAP kinase, mitogen-activated protein kinase; ERK, extracellular signal-regulated kinase; JNK, c-Jun N-terminal kinase; MEK1, MAPK/ERK kinase 1; SAPK, stress-activated protein kinase; MSK1, mitogen-and stress-activated protein kinase 1; CRE, cAMP-response element; CREc, consensus CRE; CREs, specific CRE; CREm, mutant CRE; CREB, CRE-binding protein; EMSA, electrophoretic mobility shift analysis; DN, dominant-negative; NF-κB, nuclear factor κB; TNF, tumor necrosis factor; RT, reverse transcriptase; PMSF, phenylmethanesulfonyl fluoride; DTT, dithiothreitol; ATF2, activating transcription factor 2; IBMX, 3-isobutyl-1-methylxanthine; NHNE, normal human nasal epithelial; W/T, wild type; KD, kinase dead; FAM, carboxyfluorescein; TAMRA, carboxytetramethylrhodamine.

MAP kinase pathway (25), and non-typeable *Haemophilus influenzae* was reported to regulate *MUC5AC* transcription via p38 MAP kinase in human epithelial cells (40). In a study of *MUC2*, *Pseudomonas aeruginosa* was found to activate NF-κB through Ras-MAPK-pp90rsk, which led to the increased expression of *MUC2*, but p38 was not involved in this pathway (41). However, the mechanism of *MUC5AC* gene expression during inflammation in normal airway epithelial cells, and the signal molecules involved, especially in the downstream signaling of MAP kinases have not yet been demonstrated.

Because *MUC5AC* hypersecretion during inflammation plays an important role in the pathogenesis of airway diseases, we hypothesized that major inflammatory cytokines, IL-1β and TNF-α, up-regulate *MUC5AC* gene expression by activating specific signal transduction pathways in airway epithelial cells. Here we show that two different MAP kinases, ERK and p38 MAP kinases, are essential for IL-1β- and TNF-α-induced *MUC5AC* gene expression in normal human nasal epithelial (NHNE) cells. We also show that MSK1 mediates the IL-1β- and TNF-α-induced phosphorylation of CREB and the transcription of *MUC5AC*. Furthermore, the cAMP-response element (CRE) in the *MUC5AC* promoter appears to be important for IL-1β- and TNF-α-induced *MUC5AC* gene expression in NCI-H292 cells. These pathways provide new insights into molecular mucous hypersecretion and may open up novel targets for therapeutic intervention.

EXPERIMENTAL PROCEDURES

Materials—PD98059, SB203580, and anti-α-tubulin antibody were purchased from Calbiochem. Anti-phospho-p44/42 MAP kinase (Thr^{202}/Tyr^{204}) antibody, anti-phospho-p38 MAP kinase (Thr^{180}/Tyr^{182}) antibody, anti-phospho-SAPK/JNK MAP kinase (Thr^{183}/Tyr^{185}) antibody, anti-phospho-MSK1 (Thr^{581}) antibody, and anti-phospho-CREB (Ser^{133}) antibody were purchased from Cell Signaling (Beverly, MA). Plasmid encoding kinase-deficient MEK1 mutant (*pcDNA5-MEK1DN*) and p38 mutant (*pcDNA3-p38AGF*) were kindly provided by Dr. Jian-Dong Li (House Ear Institute, Los Angeles, CA) and Dr. Yoshiyuki Kuchino (National Cancer Center Research Institute, Saitama, Japan), respectively. Wide-type MSK1 and N- and C-terminal kinase dead MSK1 mutant constructs were kindly provided by Dr. Dario Alessi (University of Dundee, Dundee, UK). Reporter construct, the 3.8-kb *MUC5AC* 5′-flanking region fused to a luciferase reporter gene, was kindly provided by Dr. Carol Basbaum (University of California, San Francisco).

Cell Cultures—The culture system used for the normal human nasal epithelial (NHNE) cells was as described previously (42). The human lung mucoepidermoid carcinoma cell line (NCI-H292) was purchased from the American Type Culture Collection (CRL-1848; Manassas, VA) and cultured in RPMI 1640 (Invitrogen) supplemented with 10% fetal bovine serum in the presence of penicillin/streptomycin at 37 °C in a humidified chamber with 5% CO_2. For serum deprivation, confluent cells were washed twice with phosphate-buffered saline and recultured in RPMI 1640 with 0.2% fetal bovine serum.

RT-PCR—Total RNA was isolated using TRIzol (Invitrogen) from NCI-H292 cells treated with IL-1β (10 ng/ml) or TNF-α (10 ng/ml). cDNA was synthesized with random hexamers (PerkinElmer Life Sciences and Roche Applied Science) using Moloney murine leukemia virus-reverse transcriptase (PerkinElmer Life Sciences). Oligonucleotide primers for PCR were designed based on the GenBank™ sequence of *MUC5AC* (GenBank™ accession number AJ001402, 5′ primer CGACAACTACTTCTGCGGTGC; 3′ primer GCACTCATCCTTCCTGTCGTT). The following PCR conditions used involved 35 cycles: denaturation at 94 °C for 30 s, annealing at 60 °C for 30 s, and polymerization at 72 °C for 30 s. The oligonucleotide primers for β_2-microglobulin (used as a control gene for the RT-PCR) were designed based on the GenBank™ human sequence (GenBank™ accession number XM007650, 5′ primer CTCGCGCTACTCTCTCTTTCTGG; 3′ primer GCTTACATGTCTCGATCCCACTTAA). PCR parameters used involved 23 cycles as follows: denaturation at 94 °C for 30 s, annealing at 55 °C for 30 s, and polymerization at 72 °C for 30 s. The PCR products were run in a 1.5% agarose gel and visualized with ethidium bromide under a transilluminator.

Real Time—PCR Primers and probes were designed with PerkinElmer Life Sciences Primer Express® software and purchased from PE Biosystems. Commercial reagents (TaqMan PCR Universal PCR Master Mix, PerkinElmer Life Sciences) and conditions according to the manufacturer's protocol were applied. An amount of 1 μg of cDNA (reverse transcription mixture) and oligonucleotides at a final concentration of 800 nM of primers and 200 nM of TaqMan hybridization probes were analyzed in a 25-μl volume. The probe of real-time PCR was labeled with carboxyfluoroscein (FAM) at the 5′ end and with the quencher carboxytetramethylrhodamine (TAMRA) at the 3′ end. The following primers and TaqMan probes were used: *MUC5AC*, forward 5′-CAGCCACGTCCCCTTCAATA-3′ and reverse 5′-ACCGCATTTGGGCATCC-3′; TaqMan probe 6FAM-CCACCTCCGAGCCCGTCACTGAG-TAMRA; β_2-microglobulin, forward 5′-CGCTCCGTGGCCTTAGC-3′ and reverse 5′-GAGTACGCTGGATAGCCTCCA-3′; and TaqMan probe 6FAM-TGCTCGCGCTACTCTCTCTTTCTGGC-TAMRA. Real time RT-PCR was performed on a PerkinElmer Life Sciences ABI PRISM® 7700 Sequence Detection System. The thermocycler (ABI PRISM® 7700 Sequence Detection System) parameters were 50 °C for 2 min, 95 °C for 10 min, followed by 40 cycles of 95 °C for 15 s and 60 °C for 1 min. All reactions were performed in triplicate. Relative quantity of *MUC5AC* mRNA was obtained using a comparative cycle threshold method and was normalized using β_2-microglobulin as an endogenous control.

Western Blot Analysis—NCI-H292 cells were grown to confluence in 6-well plates. After 15 or 45 min of treatment with IL-1β or TNF-α, respectively, the cells were lysed with 2× lysis buffer (250 mM Tris-Cl (pH 6.5), 2% SDS, 4% β-mercaptoethanol, 0.02% bromphenol blue, 10% glycerol). Equal amounts of whole cell lysates were resolved by 10% SDS-PAGE and transferred to a polyvinylidene difluoride membrane (Millipore, Bedford, MA). Membranes were blocked with 5% skim milk in Tris-buffered saline (50 mM Tris-Cl (pH 7.5), 150 mM NaCl) for 2 h at room temperature. This blot was then incubated overnight with primary antibody in TTBS (0.5% Tween 20 in Tris-buffered saline). After washing with TTBS, the blot was further incubated for 45 min at room temperature with anti-rabbit or anti-mouse antibody (Cell Signaling) in TTBS and then visualized by using the ECL system (Amersham Biosciences).

In Vitro p38 Kinase Assay—p38 kinase activity was measured using a p38 MAP kinase assay kit (Cell Signaling) according to the manufacturer's instructions. Briefly, confluent cells were rendered quiescent for 24 h and then incubated with or without 20 μM SB203580 for 1 h prior to being stimulated with IL-1β or TNF-α for 15 min. Cells lysates were scraped off the dish with 500 μl of lysis buffer and 1 mM PMSF, sonicated 4 times for 5 s each on ice, and centrifuged for 10 min at 4 °C. Supernatants were then transferred to a new tube, and 400 μg of the cell lysates and 20 μl of immobilized phospho-p38 MAP kinase monoclonal antibody were incubated with gentle rocking overnight at 4 °C. The pellet was washed twice with lysis and kinase buffer and then resuspended in kinase buffer containing 200 μM ATP and 2 μg of activating transcription factor 2 (ATF2) fusion protein. It was then incubated for 30 min at 30 °C and immunoblotted with phospho-ATF2 antibody.

Preparation of Inducible Dominant-negative Mutant Stable Cell Lines—Plasmid encoding the kinase-deficient MEK1 mutant (*pcDNA5-MEK1DN*) was cut with *Bam*HI and ligated with *pBluescript* (Stratagene, La Jolla, CA). This clone was cut with *Hin*dIII, filled in with Klenow, cut with *Sac*II (Promega), and then ligated with *pTRE* vector. Plasmid encoding kinase-inactive p38 mutant (*pcDNA3-p38*(*AGF*)) was cut with *Bam*HI, filled in with Klenow, cut with *Xba*I (Promega, Madison, WI), and then ligated with *pTRE* vector (Clontech, Palo Alto, CA). NCI-H292 cells were 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 replaced with G418 and doxycycline every 3 days.

Electrophoretic Mobility Shift Analysis (EMSA)—Cells were washed with ice-cold phosphate-buffered saline and pelleted. Pellets were then resuspended in nuclear extraction buffer I (10 mM HEPES (pH 7.9), 10 mM KCl, 1.5 mM $MgCl_2$, 0.5 mM DTT, 0.5% Nonidet P-40, 1 mM PMSF, 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 DTT, 0.1 mM PMSF, 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′-AGAGATTGCCTGACGTCAGAGAGCTAG-3′), CRE-specific sequences in the *MUC5AC* promoter region −878 to −871

TABLE I

Sequences of the pairs of oligonucleotides used in PCR to produce deletion mutant covering MUC5AC5′-flanking region

*Sac*I (GAGCTC) and *Hin*dIII (AAGCTT) sites were added at the end of the primers to direct subcloning and are underlined. Positions of the DNA fragments relative to the published transcription initiation sites (50) are indicated. S, sense; AS, antisense.

Position of the DNA fragment	Orientation	Oligonucleotide used for PCR
−929	S	CGC GAG CTC GTC CAG AGG GTA CTG AGC
−776	S	CGC GAG CTC CAT TTG CCT GGA GGC TGC
−486	S	CGC GAG CTC CTCCCTCCC AGG CAG CCA
+4	AS	CGC AAG CTT GAG GGA CCC AAG GTG GCA

(5′-AGAGATTGCCTGACTTGAAGAGCTAG-3′), and the CRE mutant sequence (5′-AGAGATTGCCTGACTGACAGAGCTAG-3′) were synthesized, annealed, and end-labeled with [γ-^{32}P]ATP using T4 polynucleotide kinase. Nuclear extract was incubated at room temperature for 30 min with the ^{32}P-labeled CRE probe in binding buffer (20% glycerol, 5 mM $MgCl_2$, 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 gel was dried and autoradiographed using an intensifying screen at −70 °C.

Plamids, Transient Transfection, and Luciferase Assay—Cells were transiently transfected with plasmids containing wide-type MSK1, N-terminal kinase dead MSK1 mutant (D195A), C-terminal kinase dead MSK1 mutant (D565A), CREB DN (S133A), and reporter constructs, and the *MUC5AC* 5′-flanking region was fused to a luciferase reporter gene using FuGENE 6 transfection reagent (Roche Applied Science) according to the manufacturer's instructions. Deletion mutants covering promoter regions of *MUC5AC* were generated by PCR using pairs of primers bearing specific restriction sites at their 5′ and 3′ ends (Table I) and then were constructed in promoterless pGL3 basic vector. Cells were 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 efficiency of each sample.

RESULTS

IL-1β and TNF-α Can Induce the Gene Expression of MUC5AC through ERK and p38 MAP Kinases Signaling in NHNE Cells—To determine whether IL-1β and TNF-α can induce *MUC5AC* gene expression within NHNE cells, we carried out RT-PCR after treatment with IL-1β or TNF-α for 24 h. The results showed that *MUC5AC* mRNA was significantly increased after treatment with IL-1β or TNF-α in NHNE cells (Fig. 1*A*). No corresponding change was found in the expression of internal control, β_2-microglobulin. As a next step, to determine which MAP kinase signal pathway is activated within NHNE cells stimulated by IL-1β and TNF-α, we performed a Western blot analysis using phospho-specific antibodies. ERK and p38 MAP kinases were maximally activated at 15 min, and this effect decreased after 45 min (Fig. 1*B*). However, no change was detected in the activation of JNK. A549 cells were used as a positive control for JNK activation. It thus appeared that stimulation by IL-1β and TNF-α induced the ERK and p38 MAP kinases pathways in NHNE cells. In order to investigate the possible involvement of ERK and p38 MAP kinases in IL-1β- and TNF-α-induced *MUC5AC* gene expression, 20 μM PD98059, specific MEK1/2 inhibitor, or 20 μM SB203580, p38 inhibitor, were applied before treatment with IL-1β and TNF-α. The Western blot and *in vitro* kinase assays showed that PD98059 and SB203580 clearly inhibited ERK and p38 MAP kinases, respectively, in NHNE cells (Fig. 2, *A* and *B*). Under this experimental condition, we checked the expression level of *MUC5AC* by performing real time quantitative PCR analysis. These results showed that pretreatment with PD98059 or SB203580 for 1 h inhibited *MUC5AC* gene expression (Fig. 2, *C* and *D*). Interestingly, the inhibition of either ERK or p38 MAP kinase pathway inhibited *MUC5AC* mRNA in NHNE cells. Thus, the activation of ERK and p38 MAP kinases appeared to be closely related to the signaling pathways activated by IL-1β or TNF-α.

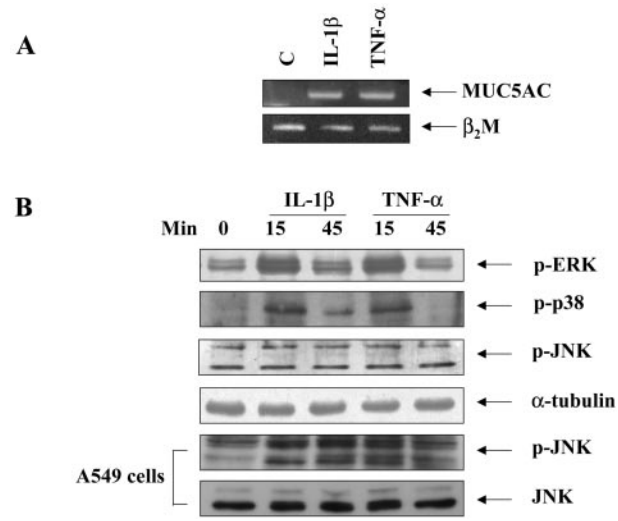

FIG. 1. **Effect of IL-1β and TNF-α on *MUC5AC* gene expression in NHNE cells.** Confluent cells were treated with IL-1β (10 ng/ml) or TNF-α (10 ng/ml) for 24 h, and cell lysates were harvested for RT-PCR (*A*). *C*, control. β_2-Microglobulin (β_2M) was employed as an internal control. Confluent cells were treated with IL-1β (10 ng/ml) or TNF-α (10 ng/ml) for 15 or 45 min, and cell lysates were harvested for Western blot analysis. Representative Western blots (*B*) using phospho-specific antibodies showed transient activation of ERK and p38 but not of JNK, and the maximum effect is at 15 min. The figures shown are representative of three independent experiments.

Both ERK and p38 MAP Kinases Are Essential for IL-1β- or TNF-α-induced MUC5AC Gene Expression—When the same experiments (Figs. 1 and 2) were performed using NCI-H292 cells, human lung mucoepidermoid carcinoma cell line, the results were the same in the NCI-H292 cells as in the normal cells (data not shown). In order to confirm the significance of ERK and p38 kinases upon the cellular level of *MUC5AC* gene expression, cells stably expressing dominant-negative (DN) mutant under the control of the Tet-off system were generated. The p38 DN mutant was generated by replacing Thr^{180} and Tyr^{182} by Ala and Phe, respectively (43). This inactive form of p38 MAP kinase binds endogenous substrates, thereby inhibiting signaling by the endogenous p38 MAP kinase pathway. After removing doxycycline to induce MEK1DN, stimulation with IL-1β or TNF-α for 15 min decreased the phosphorylation of ERK (Fig. 3*A*). However, no change in ERK expression was observed. Real time quantitative PCR showed a decrease in *MUC5AC* mRNA after 24 h for IL-1β and TNF-α. In a similar way, we investigated the role of p38 MAP kinase on IL-1β- or TNF-α-induced *MUC5AC* gene expression using p38DN. An *in vitro* kinase assay showed that the activation of p38 MAP kinase in this mutant stable cell line was decreased following IL-1β and TNF-α stimulation (Fig. 3*B*). Under this condition, *MUC5AC* mRNA was decreased 24 h after treatment with IL-1β and TNF-α. These results showed that ERK and p38 MAP kinases are essential for IL-1β- and TNF-α-induced

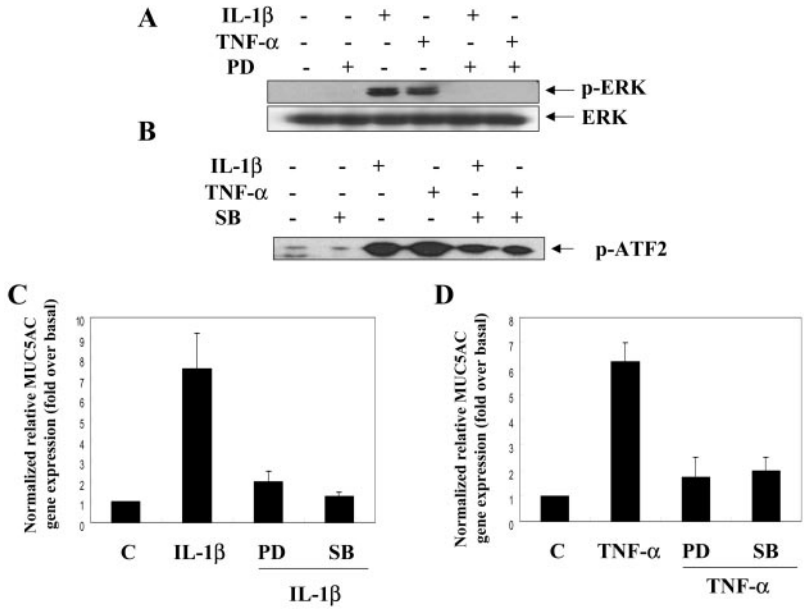

FIG. 2. **Effect of ERK and p38 MAP kinases on *MUC5AC* gene expression in NHNE cells.** Confluent cells were pretreated for 1 h with 20 μM PD98059 or 20 μM SB203580 and then stimulated for 15 min with IL-1β or TNF-α prior to collection of total proteins for kinase assays. Representative kinase assays show the phosphorylation of ERK by PD98059 (*PD*) (*A*) and ATF2 as an exogenous substrate (*B*) with p38 MAP kinase immunoprecipitated from IL-1β- or TNF-α-treated cells and the inhibition of p38 MAP kinase activation by SB203580 (*SB*). Cells were pretreated and stimulated for 24 h with IL-1β or TNF-α prior to collection of total RNA for real time quantitative PCR of *MUC5AC* (*C* and *D*). *C*, control. The figures shown are representative of three independent experiments.

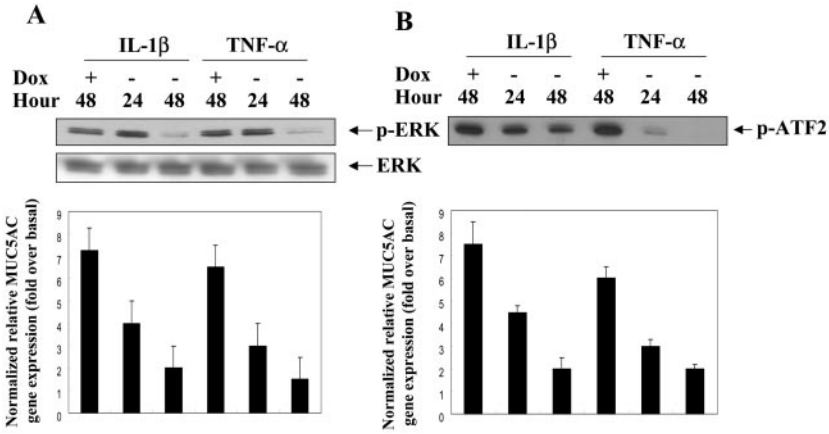

FIG. 3. ***MUC5AC* gene expression in MEK1 or p38 dominant-negative mutant stable cell lines.** Confluent, quiescent MEK1 (*A*) and p38 (*B*) dominant-negative mutant stable cells were preincubated for the indicated times to induce dominant-negative mutant protein by removed doxycycline (*Dox*) and then stimulated for 15 min with IL-1β or TNF-α prior to Western blotting and then for 24 h with IL-1β or TNF-α prior to real time quantitative PCR. The figures shown are representative of three independent experiments.

MUC5AC gene expression in NCI-H292 cells.

IL-1β- or TNF-α-induced Activation of MSK1 Is Mediated by Both ERK and p38 MAP Kinases—To determine which molecules are involved in the downstream signaling of the ERK and p38 MAP kinases within the signal pathway of *MUC5AC* gene expression induced by IL-1β or TNF-α, we investigated the phosphorylation of MSK1 by phospho-specific antibody. MSK1 is widely distributed in mammalian cells and can be activated by MAPK/ERK and SAPK2a/p38 (37–39). The result showed that the phosphorylation of MSK1 by IL-1β or TNF-α reached a maximum at 30 min and decreased at 60 min after IL-1β and TNF-α stimulation (Fig. 4, *A* and *B*). Pretreatment with 20 μM PD98059 and/or 20 μM SB203580 inhibited IL-1β- or TNF-α-induced MSK1 phosphorylation (Fig. 4, *C* and *D*), indicating that MSK1 is regulated by ERK and/or p38 MAP kinase(s). Furthermore, to determine whether MSK1 influences MAP kinases, cells were transfected with DNA expression constructs encoding a mutant MSK1 (NT-KD) that the N-terminal kinase domain was inactivating by a point mutation and a further mutant (CT-KD) that the C-terminal kinase domain was inactivating (39). Two MSK1 mutants did not affect IL-1β- and TNF-α-induced MAP kinases activation (Fig. 4*E*), indicating that MSK1 appears to be controlled by MAP kinases. These results showed that MSK1 acts as a downstream signaling mediator of ERK and p38 MAP kinases.

Effects of MSK1 and CREB on IL-1β- and TNF-α-induced MUC5AC Gene Expression—To examine the role of MSK1 on the induction of *MUC5AC* gene expression, cells were transiently transfected with DNA expression constructs encoding wild-type MSK1 (W/T), NT-, or CT-KD MSK1 mutant. IL-1β or TNF-α-induced *MUC5AC* gene expression increased in cells transfected with W/T MSK1, whereas overexpression of NT- or CT-KD MSK1 markedly suppressed IL-1β or TNF-α-induced *MUC5AC* gene expression (Fig. 5, *A* and *B*). These results showed that MSK1 appears to be closely related in the *MUC5AC* gene expression by IL-1β and TNF-α.

Because MSK1 is currently the best candidate for the mediation of cytokine-induced CREB phosphorylation at Ser^{133} (37–39), we investigated the possible implication of CREB in IL-1β- or TNF-α-induced *MUC5AC* gene expression. To examine whether IL-1β or TNF-α can induce the phosphorylation of endogenous CREB via MAP kinases and MSK1 in airway epithelial cells, we performed Western blot using phospho-specific CREB (Ser^{133}) antibody. A transient phosphorylation of CREB was observed upon the stimulation with IL-1β and TNF-α, reaching the maximum peak at 30 min (Fig. 6, *A* and *B*). In addition, pretreatment with PD98059 and/or SB203580 or NT- or CT-KD MSK1 remarkably inhibited the phosphorylation of CREB (Fig. 6, *C* and *D*). Next, to determine whether CREB plays a role in *MUC5AC* gene expression, we used forskolin (an activator of adenylate cyclase) and 3-isobutyl-1-methylxanthine (IBMX; an inhibitor of AMP phosphodiesterase). A transient phosphorylation of CREB was observed upon the stimulation with forskolin and IBMX, reaching the maximum peak

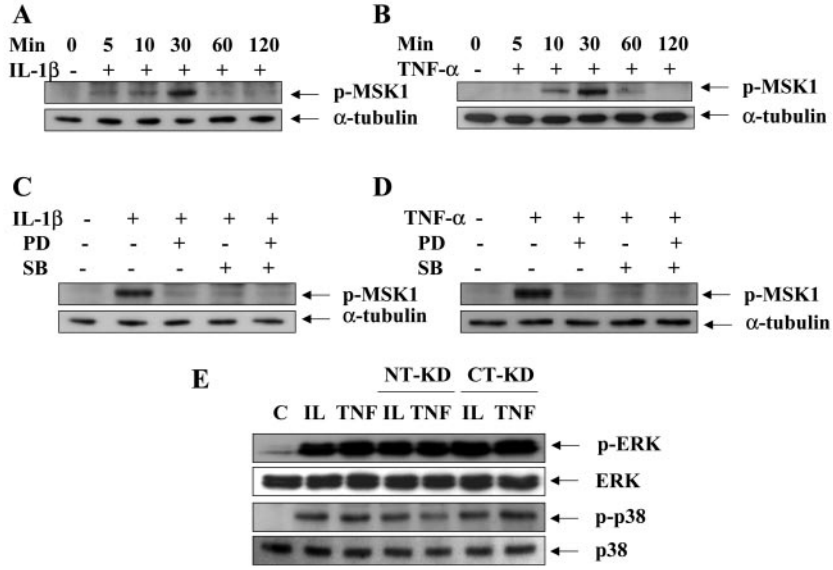

FIG. 4. **Effect of ERK and p38 MAP kinases on IL-1β- or TNF-α-induced activation of MSK1.** Confluent, quiescent cells were stimulated for the indicated times with IL-1β (*A*) or TNF-α (*B*), and then total proteins were collected for Western blot analysis. In other experiments, the cells were pretreated for 1 h with 20 μM PD98059 (*PD*) and/or 20 μM SB203580 (*SB*) and then the cells were then stimulated for 30 min with IL-1β (*C*) or TNF-α (*D*). The cells were transiently transfected with NT-KD or CT-KD MSK1 constructs and stimulated with IL-1β or TNF-α for 15 min (*E*) prior to Western blot analysis. *C*, control. The figures shown are representative of three independent experiments.

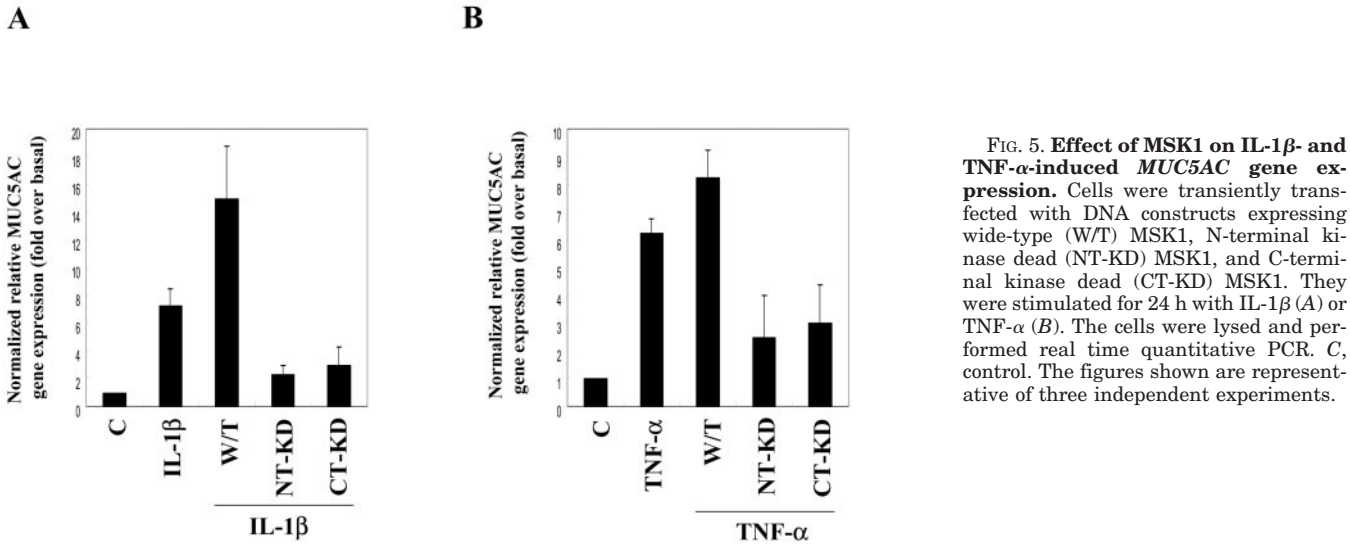

FIG. 5. **Effect of MSK1 on IL-1β- and TNF-α-induced *MUC5AC* gene expression.** Cells were transiently transfected with DNA constructs expressing wide-type (W/T) MSK1, N-terminal kinase dead (NT-KD) MSK1, and C-terminal kinase dead (CT-KD) MSK1. They were stimulated for 24 h with IL-1β (*A*) or TNF-α (*B*). The cells were lysed and performed real time quantitative PCR. *C*, control. The figures shown are representative of three independent experiments.

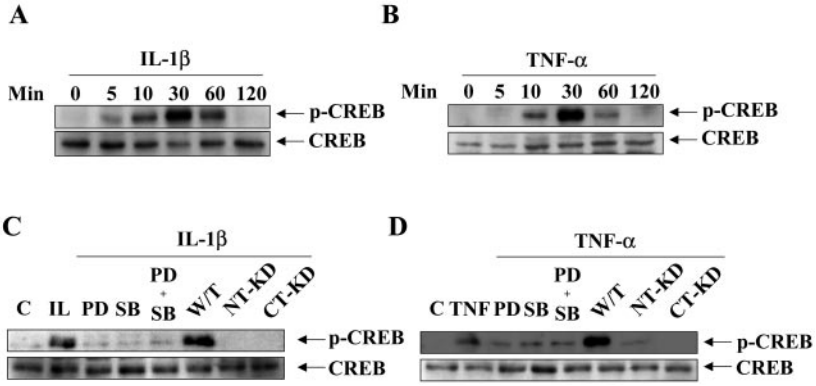

FIG. 6. **IL-1β and TNF-α can induce the activation of CREB via MAP kinases and MSK1.** Confluent, quiescent cells were stimulated for the indicated times with IL-1β (*A*) or TNF-α (*B*), and then total proteins were collected for Western blot analysis. In other experiments, the cells pretreated for 1 h with 20 μM PD98059 (*PD*) or/and 20 μM SB203580 (*SB*) and the transfected cells with NT-KD or CT-KD MSK1 constructs were stimulated for 30 min with IL-1β (*B*) or TNF-α (*D*) prior to Western blot analysis. *C*, control. The figures shown are representative of three independent experiments.

at 10 min (Fig. 7*A*). The cAMP pathway-induced CREB phosphorylation increased *MUC5AC* gene expression (Fig. 7*B*). Furthermore, IL-1β- and TNF-α-induced *MUC5AC* gene expression was significantly suppressed in cells transfected with plasmid encoding mutant CREB (*pCREB S133A*) (Fig. 7*C*). These findings suggested that the activation of MSK1 and CREB is essential for IL-1β- and TNF-α-induced *MUC5AC* gene expression via ERK and p38 MAP kinases.

Identification of the Binding Complex between CREB and CRE in Response to IL-1β and TNF-α—To analyze the DNA binding activity of IL-1β- and TNF-α-activated CREB, we performed EMSA using nuclear extracts from NCI-H292 cells after IL-1β or TNF-α treatment. As shown in Fig. 8, *A* and *B*, the activity of consensus CRE oligonucleotide (CREc) and *MUC5AC* specific CRE (CREs) remarkably increased in response to IL-1β or TNF-α but not by mutant CRE of *MUC5AC* promoter (CREm) oligonucleotide. To distinguish any specific CRE-binding complexes, competition and supershift analysis were performed using 50-fold excesses of non-radiolabeled (cold) CREs oligonucleotide and anti-phospho-CREB antibody,

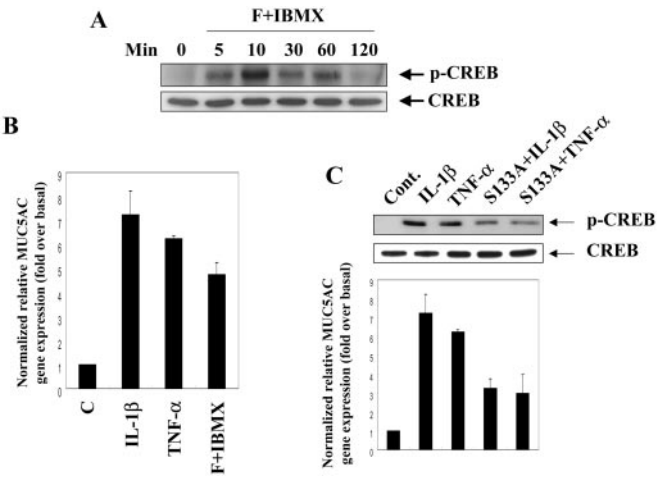

FIG. 7. **Effect of CREB on IL-1β- and TNF-α-induced *MUC5AC* gene expression.** Confluent, quiescent cells were stimulated for the indicated times with both 20 μM forskolin and 10 μM IBMX, and then total proteins were collected for Western blot (*A*). Cells were stimulated for 24 h with IL-1β, TNF-α, or both 20 μM forskolin and 5 μM IBMX, and the total RNA were then subjected to real time quantitative PCR (*B*). The cells were transiently transfected with mutant CREB (*pCREB S133A*) constructs and stimulated with IL-1β or TNF-α 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* and *Cont.*, control. The figures shown are representative of three independent experiments.

respectively. The specific band was found to be selectively inhibited by the specific CRE competitor and was supershifted by anti-phospho-CREB antibody. These results indicated that activated CREB binds to a *cis*-acting element, CRE, in the *MUC5AC* promoter.

Identification of IL-1β- and TNF-α-responsive Regions within MUC5AC Promoter—Cells were then transiently transfected with the various deletion mutants and treated with IL-1β (40 ng/ml) or TNF-α (40 ng/ml) for 24 h, respectively. As shown in Fig. 9*A*, IL-1β and TNF-α selectively increased luciferase activity of −929/+4 region of *MUC5AC* promoter. No effect was seen on fragments covering −1376/+4, −776/+4, and −486/+4 regions, indicating that the −929/−776 region of *MUC5AC* promoter may be necessary to observe a response to IL-1β or TNF-α. To further know whether CRE within the −929/−776 region of the *MUC5AC* promoter, identified using the TRANSFAC 4.0 data base, critically acts as *cis*-element, cotransfection with plasmid expression construct encoding mutant CREB was performed to study its effect on *MUC5AC* transcription activity. CREB DN suppressed luciferase activity of −929/+4 region of *MUC5AC* promoter (Fig. 9*B*). Moreover, we examined whether activation of CRE is required for IL-1β- and TNF-α-induced *MUC5AC* transcription by performing selective mutagenesis of the CREB-binding site. As a shown in Fig. 9*C*, mutant constructs M1, M2, and M3 abolished responsiveness of wild-type *MUC5AC* promoter construct (Fig. 9*C*). These results showed that CRE in the regulatory region of *MUC5AC* promoter was critical for the up-regulation of the transcriptional activity of *MUC5AC* induced by IL-1β or TNF-α.

DISCUSSION

Mucin hypersecretion causes many clinical problems, such as rhinorrhea, nasal stuffiness, and sputum in the respiratory tract. It has been reported that *MUC5AC* is the major mucin in human airways (22–24, 44). The mechanism of the regulation of *MUC5AC* secretion by inflammatory cytokines in airway is very important, and the understanding of this mechanism may offer new therapeutic strategies for the inhibition of airway mucus hypersecretion.

The molecular mechanism by which *MUC5AC* is up-regulated by IL-1β and TNF-α remains poorly understood. In the present study, we undertook to determine the involvement of IL-1β and TNF-α in the up-regulation of *MUC5AC* gene expression in normal human airway epithelial cells.

The fact that more than one MAP kinase may be necessary for the IL-1β- and TNF-α-induced *MUC5AC* gene expression in NHNE and NCI-H292 cells is an interesting finding of the present study (Figs. 2 and 3). Although *MUC5AC* is regulated by various inflammatory cytokines such as neutrophil elastase (27), IL-9 (28), and IL-4 (29), it was not shown which mechanisms are essential for cytokine-induced *MUC5AC* gene expression. Recently, Takeyama *et al.* (25) reported that epidermal growth factor increased *MUC5AC* gene expression via ERK MAP kinase but not p38 MAP kinase in NCI-H292 cells. Moreover, Wang *et al.* (40) showed that non-typeable *H. influenzae* regulated *MUC5AC* transcription via p38 MAP kinase but did not mediate ERK MAP kinase. In the present study, we showed that both ERK and p38 MAP kinase, but not JNK signaling, are essential for IL-1β- and TNF-α-induced *MUC5AC* gene expression. These suggest that the signaling pathways leading to *MUC5AC* gene expression are distinct, depending on the type of stimuli and cell lines used. We do not yet know how both pathways intervene in the activation of cytokine-induced *MUC5AC* gene expression. Recently, TNF-α-induced matrix metalloproteinase (MMP)-1 and MMP-3 gene expression is known to be regulated through AP-1-dependent transcriptional activation via the ERK pathway and AP-1-independent enhancement via p38 MAPK by mRNA stabilization in human skin fibroblasts (44). Interestingly, TNF-α has an effect on the stability of *MUC5AC* mRNA in NCI-H292 cells (45, 46). Thus, taken together, it is conceivable that the intracellular signaling coordination controlled by ERK in combination with p38 MAP kinase may be essential for IL-1β- and TNF-α-induced *MUC5AC* gene expression.

To date, signal molecules involved in the downstream signaling of MAP kinases for *MUC5AC* gene expression have not been yet demonstrated. The role of MSK1 and CREB in the downstream signaling of MAP kinases in the IL-1β- and TNF-α-induced *MUC5AC* gene expression in airway epithelial cells is a major finding of the present study. MSK1 is known to be regulated by MAPK/ERK and SAPK2a/p38 and is currently the best candidate for the mediation of cytokine-induced CREB phosphorylation at Ser^{133} (37–39, 47). Although CREB activation by MSK1 has been established by previous studies in other cells (37–39), it has remained unclear in airway epithelial cells. In addition, little is known about the involvement of CREB in *MUC5AC* gene expression. In this study, our results show that CREB activation is involved in the downstream signaling of MAP kinases and MSK1 for IL-1β- and TNF-α-induced *MUC5AC* gene expression.

Interestingly, *MUC5AC* mRNA was inhibited in *pCREB S133A* transfected cells treated by IL-1β and TNF-α, and treatment with forskolin and IBMX activated the phosphorylation of CREB and increased *MUC5AC* gene expression (Fig. 7, *A* and *B*). These suggest that CREB may be a transcription factor for IL-1β-and TNF-α-induced *MUC5AC* gene expression. However, the increase of *MUC5AC* expression by forskolin and IBMX was less than that induced by IL-1β and TNF-α, suggesting that activation by CREB alone is insufficient to induce the full expression of *MUC5AC*. This result suggests that a transcription factor, other than CREB, may be required for full expression of IL-1β- and TNF-α-induced *MUC5AC* gene expression. Previously, *P. aeruginosa*-induced *MUC2* expression is found to be regulated by NF-κB in NCI-H292 cells (41). In fact, we could find the putative NF-κB-binding site at −273 and

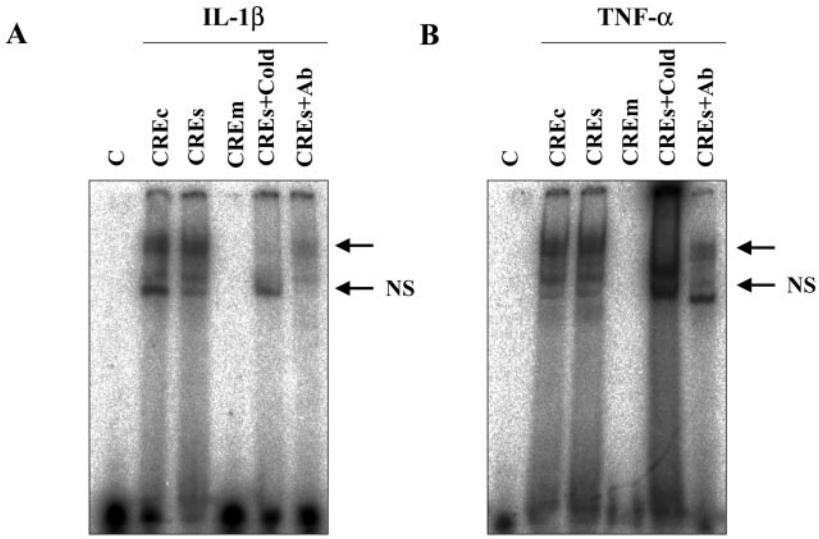

FIG. 8. **IL-1β- or TNF-α-induced nuclear binding of CRE.** Confluent, quiescent cells were stimulated for 1 h with IL-1β (*A*) or TNF-α (*B*). Nuclear protein extracts from IL-1β- or TNF-α-treated NCI-H292 cells were subjected to EMSA. Nuclear proteins were incubated with CREc, CREs, and CREm, 50-fold excess of cold probe or anti-phospho-CREB antibody before EMSA. The labeled nuclear proteins were separated by electrophoresis on 5% polyacrylamide gels, and the gels were dried and exposed to autoradiography at −70 °C overnight. *C*, control; *Ab*, antibody; *CREc,* consensus CRE; *CREs*, *MUC5AC*-specific CRE; *CREm*, *MUC5AC*-mutant CRE; *NS*, nonspecific.

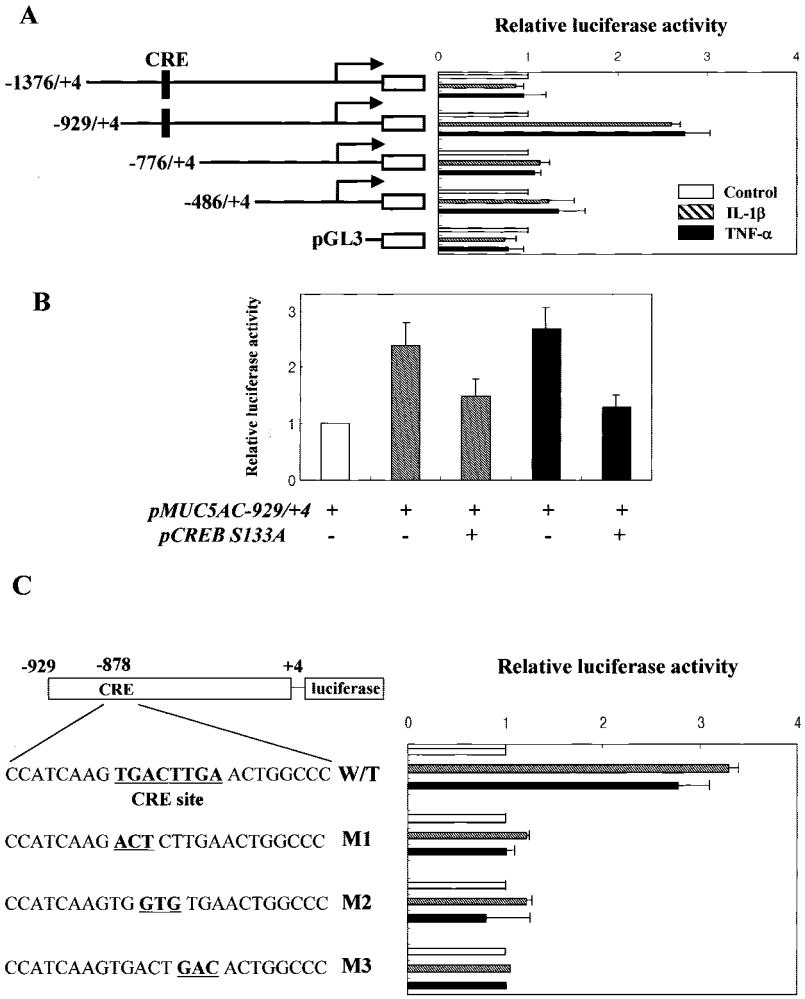

FIG. 9. **IL-1β- and TNF-α-induced activation of CRE-mediated MUC5AC transcription via the *cis*-acting regulatory CRE motif.** NCI-H292 cells were transiently transfected with various *MUC5AC* promoter luciferase reporter constructs and stimulated with IL-1β (40 ng/ml) and TNF-α (40 ng/ml) for 24 h. Luciferase activity was then assessed in IL-1β or TNF-α-treated and -untreated cells (*A*). Cells were cotransfected with a dominant-negative mutant of CREB and a reporter construct of −929/+4 region of *MUC5AC* promoter (*B*) and transfected with the *MUC5AC* promoter construct containing the various mutated CRE sites as indicated (*C*). The luciferase activities were displayed after correction for transfection efficiency using the β-galactosidase activity of the cell lysates to standardize the values. The values shown are means ± S.D. of experiments performed in triplicate.

−956 in *MUC5AC* promoter (48). Therefore, NF-κB may also regulate cytokine-induced *MUC5AC* gene expression with the cooperation of CREB. This suggestion was further supported by our recent finding that IL-1β or TNF-α can initiate IκB degradation in NHNE and NCI-H292 cells and that pretreatment of caffeic acid phenethyl ester, which is known to specifically block the translocation of p65 without affecting IκBα degradation (41), inhibits *MUC5AC* gene expression induced by IL-1β (data not shown). In fact, Gerritsen *et al.* (49) reported that p300 and CREB-binding protein (CBP) act as coactivators of p65 transactivation and may play an important role in the cytokine-induced expression of various immune and inflammation genes. Furthermore, Perrais *et al.* (50) reported that transcription factor Sp1 is essential for epidermal growth factor- and TGF-α-mediated *MUC5AC* up-regulation. Taken together, these findings suggest that CREB may interact directly or indirectly with other transcription factor(s) and that non-DNA binding transcriptional coactivators, such as p300 and CBP,

which were thought to function as bridging proteins between DNA-binding transcription factors and the basal transcription factors, play a role as integrators of diverse signaling pathways in the *MUC5AC* gene expression.

Whereas CREB has recently emerged as a potent regulator of mucins (*MUC2*, *MUC5AC*, *MUC5B,* and *MUC6*), gene expression in the p15 arm of chromosome 11 (11p15) (51), and cholera toxin A subunit, an activator of cAMP-dependent protein kinase, activates transcription of *MUC5B* promoter (52), little is known about the involvement of CRE in *MUC5AC* transcription. Our results showed that −929/+4 region of *MUC5AC* promoter was sufficient to get a response to IL-1β or TNF-α and that CRE in −878 region of *MUC5AC* promoter was critical for the up-regulation of the transcriptional activity of *MUC5AC* induced by IL-1β or TNF-α. However, Perrais *et al.* (50) reported that TNF-α did not have any significant effect of activity of −1366/+4 region of the *MUC5AC* promoter, which was in accordance with our results in −1376/+4 region of *MUC5AC* promoter. These results suggest that TNF-α-responsive repressor(s) or negative regulatory element that represses inherent basal and cAMP-inducible promoter activity (53) may be located in −1366/−929 region of *MUC5AC* promoter. Thus, it seems necessary to explore further the involvement of IL-1β- or TNF-α-responsive repressor(s) or negative regulatory element in IL-1β- or TNF-α-induced *MUC5AC* transcription.

In summary, our results showed that ERK and p38 MAP kinases, but not JNK signaling, are essential for IL-1β- and TNF-α-induced *MUC5AC* gene expression. Furthermore, the activation of MSK1 and CREB is a crucial aspect of the intracellular mechanisms that mediate *MUC5AC* gene expression. This study also demonstrated that CRE in the *MUC5AC* promoter might play a role in these processes by binding CREB. Further analysis of the signal pathways activated by various cytokines may yield deeper insights into the signal mechanism of *MUC5AC* gene expression.

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REFERENCES

1. Yuta, A., Ali, M., Sabol, M., Gaumond, E., and Baraniuk, J. N. (1997) *Am. J. Physiol.* **273,** L1203–L1207
2. Kim, S. S., Kim, K. S., Lee, J. G., Park, I. Y., Koo, J. S., and Yoon, J. H. (2000) *Laryngoscope* **110,** 276–280
3. Chung, M. H., Choi, J. Y., Lee, W. S., Kim, H. N., and Yoon, J. H. (2002) *Laryngoscope* **112,** 152–155
4. Nadel, J. A. (2001) *Respir. Res.* **2,** 85–89
5. Spicer, A. P., Parry, G., Patton, S., and Gendler, S. J. (1991) *J. Biol. Chem.* **266,** 15099–15109
6. Gum, J. R., Jr., Hicks, J. W., Toribara, N. W., Siddiki, B., and Kim, Y. S. (1994) *J. Biol. Chem.* **269,** 2440–2446
7. Van Klinken, B. J., Van Dijken, T. C., Oussoren, E., Buller, H. A., Dekker, J., and Einerhand, A. W. (1997) *Biochem. Biophys. Res. Commun.* **238,** 143–148
8. Moniaux, N., Nollet, S., Porchet, N., Degand, P., Laine, A., and Aubert, J. P. (1999) *Biochem. J.* **338,** 325–333
9. Van de Bovenkamp, J. H., Hau, C. M., Strous, G. J., Buller, H. A., Dekker, J., and Einerhand, A. W. (1998) *Biochem. Biophys. Res. Commun.* **245,** 853–859
10. Keates, A. C., Nunes, D. P., Afdhal, N. H., Troxler, R. F., and Offner, G. D. (1997) *Biochem. J.* **324,** 295–303
11. Ho, S. B., Roberton, A. M., Shekels, L. L., Lyftogt, C. T., Niehans, G. A., and Toribara, N. W. (1995) *Gastroenterology* **109,** 735–747
12. Bobek, L. A., Tsai, H., Biesbrock, A. R., and Levine, M. J. (1993) *J. Biol. Chem.* **268,** 20563–20569
13. Shankar, V., Pichan, P., Eddy, R. L., Jr., Tonk, V., Nowak, N., Sait, S. N., Shows, T. B., Schultz, R. E., Gotway, G., Elkins, R. C., Gilmore, M. S., and Sachdev, G. P. (1997) *Am. J. Respir. Cell Mol. Biol.* **16,** 232–241
14. Lapensee, L., Paquette, Y., and Bleau, G. (1997) *Fertil. Steril.* **68,** 702–708
15. Melnick, M., Chen, H., Zhou, Y., and Jaskoll, T. (2001) *Arch. Oral Biol.* **46,** 745–757
16. Williams, S. J., McGuckin, M. A., Gotley, D. C., Eyre, H. J., Sutherland, G. R., and Antalis, T. M. (1999) *Cancer Res.* **59,** 4083–4089
17. Williams, S. J., Wreschner, D. H., Tran, M., Eyre, H. J., Sutherland, G. R., and McGuckin, M. A. (2001) *J. Biol. Chem.* **276,** 18327–18336
18. Pallesen, L. T., Berglund, L., Rasmussen, L. K., Petersen, T. E., and Rasmussen, J. T. (2002) *Eur. J. Biochem.* **269,** 2755–2763
19. Yin, B. W., and Lloyd, K. O. (2001) *J. Biol. Chem.* **276,** 27371–27375
20. Gum, J. R., Jr., Crawley, S. C., Hicks, J. W., Szymkowski, D. E., and Kim, Y. S. (2002) *Biochem. Biophys. Res. Commun.* **291,** 466–475
21. Sers, C., Kirsch, K., Rothbacher, U., Riethmuller, G., and Johnson, J. P. (1993) *Proc. Natl. Acad. Sci. U. S. A.* **90,** 8514–8518
22. Hovenberg, H. W., Davies, J. R., Herrmann, A., Linden, C. J., and Carlstedt, I. (1996) *Glycoconj. J.* **13,** 839–847
23. Thronton, D. J., Howard, M., Khan, N., and Sheehan, J. K. (1997) *J. Biol. Chem.* **272,** 9561–9566
24. Wickstrom, C., Davies, J. R., Eriksen, G. V., Veerman, E. C., and Carstedt, I. (1998) *Biochem. J.* **334,** 685–693
25. Takeyama, K., Dabbagh, K., Shim, J. J., Dao-Pick, T., Ueki, I. F., and Nadel, J. A. (2000) *J. Immunol.* **164,** 1546–1552
26. Gray, T., Koo, J. S., and Nettesheim, P. (2001) *Toxicology* **160,** 35–46
27. Voynow, J. A., Young, L. R., Wang, Y., Horger, T., Rose, M. C., and Fischer, B. M. (1999) *Am. J. Physiol.* **276,** L835–L843
28. Louahed, J., Toda, M., Jen, J., Hamid, Q., Renauld, J. C., Levitt, R. C., and Nicolaides, N. C. (2000) *Am. J. Respir. Cell Mol. Biol.* **22,** 649–656
29. Dabbagh, K., Takeyama, K., Lee, H. M., Ueki, I. F., Lausier, J. A., and Nadel, J. A. (1999) *J. Immunol.* **162,** 6233–6237
30. Garrington, T. P., and Johnson, G. L. (1999) *Curr. Opin. Cell Biol.* **11,** 211–218
31. Brunet, A., and Pouyssegur, J. (1996) *Science* **272,** 1652–1655
32. Bennett, A. M., and Tonks, N. K. (1997) *Science* **278,** 1288–1291
33. Crawley, J. B., Rawlinson, L., Lali, F. V., Paga, T. H., Saklatvala, J., and Foxwell, B. M. (1997) *J. Biol. Chem.* **272,** 15023–15027
34. Foltz, I. N., Lee, J. C., Young, P. R., and Schrader, J. W. (1997) *J. Biol. Chem.* **272,** 3296–3301
35. Ichijo, H., Nishida, E., Irie, K., Dijke, P. T., Saitoh, M., Moriguchi, T., Takagi, M., Matsumoto, K., Miyazono, K., and Gotoh, Y. (1997) *Science* **275,** 90–94
36. Xia, Z., Dickens, M., Raingeaud, J., Davis, R. J., and Greenberg, M. E. (1995) *Science* **270,** 1326–1331
37. Caivano, M., and Cohen, P. (2000) *J. Immunol.* **164,** 3018–3025
38. Arther, J. S., and Cohen, P. (2000) *FEBS Lett.* **482,** 44–48
39. Deak, M., Clifton, A. D., Lucocq, L. M., and Alessi, D. R. (1998) *EMBO J.* **17,** 4426–4441
40. Wang, B., Lim, D. J., Han, J., Kim, Y. S., Basbaum, C. B., and Li, J. D. (2002) *J. Biol. Chem.* **277,** 949–957
41. Li, J. D., Feng, W., Gallup, M., Kim, J. H., Gum, J., Kim, Y., and Basbaum, C. (1998) *Proc. Natl. Acad. Sci. U. S. A.* **95,** 5718–5723
42. Yoon, J. H., Kim, K. S., Kim, S. S., Lee, J. G., and Park, I. Y. (2000) *Ann. Otol. Rhinol. Laryngol.* **109,** 594–601
43. Raingeaud, J., Gupta, S., Rogers, J. S., Dickens, M., Han, J., Ulevitch, R. J., and Davis, R. J. (1995) *J. Biol. Chem.* **270,** 7420–7426
44. Berrou, E., and Bryckaert, M. (2001) *J. Biol. Chem.* **276,** 39303–39309
45. Voynow, J. A., Selby, D. M., and Rose, M. C. (1998) *Lung* **176,** 345–354
46. Borchers, M. T., Carty, M. P., and Leikauf, G. D. (1999) *Am. J. Physiol.* **276,** L549–L555
47. Ropert, C., Almeida, I. C., Closel, M., Travassos, L. R., Ferguson, M. A., Cohen, P., and Gazzinelli, R. T. (2001) *J. Immunol.* **166,** 3423–3431
48. Li, D., Gallup, M., Fan, N., Szymkowski, D. E., and Basbaum, C. B. (1998) *J. Biol. Chem.* **273,** 6812–6820
49. Gerritsen, M. E., Williams, A. J., Neish, A. S., Moore, S., Shi, Y., and Collins, T. (1997) *Proc. Natl. Acad. Sci. U. S. A.* **94,** 2927–2932
50. Perrais, M., Pigny, P., Copin, M. C., Aupert, J. P., and Van Seuningen, I. (2002) *J. Biol. Chem.* **277,** 32258–32267
51. Van Seuningen, I., Pigny, P., Perrais, M., Porchet, N., and Aubert, J.-P. (2001) *Front. Biosci.* **6,** D1216–D1234
52. Perrais, M., Pigny, P., Buisine, M.-P., Porchet, N., Aubert, J.-P., and Van Seuningen, I. (2001) *J. Biol. Chem.* **276,** 15386–15396
53. Chung, C. C., Huang, D., Chen, Y., Short, S., Short, M. L., Zhang, Z., and Jungmann, R. A. (1995) *Mol. Cell. Biol.* **15,** 6139–6149

Interleukin-1β and Tumor Necrosis Factor-α Induce *MUC5AC* Overexpression through a Mechanism Involving ERK/p38 Mitogen-activated Protein Kinases-MSK1-CREB Activation in Human Airway Epithelial Cells

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