# NF-κB Mediates IL-1β– and IL-17A–Induced *MUC5B* Expression in Airway Epithelial Cells

**Tomoyuki Fujisawa[1]*, Mary Mann-Jong Chang[1], Sharlene Velichko[1], Philip Thai[1], Li-Yin Hung[1], Fei Huang[1], Newton Phuong[1], Yin Chen[2], and Reen Wu[1]**

[1]Center of Comparative Respiratory Biology and Medicine, University of California at Davis, Davis, California; and [2]Department of Pharmacology and Toxicology, College of Pharmacy, University of Arizona, Tucson, Arizona

**A major pathological feature of chronic airway diseases is the elevated expression of gel-forming mucins. NF-κB activation in airway epithelial cells has been shown to play a proinflammatory role in chronic airway diseases; however, the specific role of NF-κB in mucin gene expression has not been characterized. In this study, we show that the proinflammatory cytokines, IL-1β and IL-17A, both of which use the NF-κB pathway, are potent inducers of MUC5B mRNA expression in both well differentiated primary normal human bronchial epithelial cells and the human bronchial epithelial cell line, HBE1. *MUC5B* induction by these cytokines was both time- and dose-dependent, and was attenuated by the small molecule inhibitor, NF-κB inhibitor III, as well as p65 small interfering RNA, suggesting that the regulation of *MUC5B* expression by these cytokines is via an NF-κB–based transcriptional mechanism. Deletion analysis of the *MUC5B* promoter demonstrated that IL-1β– and IL-17A–induced promoter activity resides within the −4.17-kb to −2.56-kb region relative to the transcriptional start site. This region contains three putative κB-binding sites (NF-κB-1, −3,786/−3,774; NF-κB-2, −3,173/−3,161; and NF-κB-3, −2,921/−2,909). Chromatin immunoprecipitation analysis confirmed enhanced binding of the p50 NF-κB subunit to the NF-κB-3 site after cytokine stimulation. We conclude that an NF-κB-based transcriptional mechanism is involved in *MUC5B* regulation by IL-1β and IL-17A in airway epithelium. This is the first demonstration of the participation of NF-κB and its specific binding site in cytokine-mediated airway *MUC5B* expression.**

**Keywords:** cytokines; gene regulation; mucin; transcription factors; lung

**CLINICAL RELEVANCE**

Mucin overproduction is a major clinical hallmark associated with airway inflammation. The elucidation of the molecular mechanism involved in cytokine-induced MUC5B expression will provide a therapeutic basis for the treatment to reduce the overproduction.

Homeostasis in mucus production is essential for proper mucociliary clearance and innate immune function in normal airways (1, 2); excessive production of mucin in inflamed airways can increase morbidity and mortality by obstructing mucociliary clearance and air flow (3, 4). To date, 11 mucin (MUC) genes (*MUC1*, *2*, *3*, *4*, *5AC*, *5B*, *6*, *7*, *8*, *13*, and *19*) have been described as being expressed in the lung (2). Among these, MUC5AC and MUC5B are the most prominent mucins in the airway. MUC5B is mainly expressed in mucous glands under normal conditions; however, the airway epithelium is also known to express MUC5B as well as MUC5AC, particularly in disease states, such as chronic obstructive pulmonary disease (COPD) and usual interstitial pneumonia (5, 6). In a mouse model of asthma, airway epithelial cells have also been shown to up-regulate *Muc5b* expression in association with mucous cell hyperplasia (7). Although extensive work has been done to elucidate the molecular mechanism of cytokine-induced *MUC5AC* expression (8–11), there are few studies describing the regulation of cytokine-mediated *MUC5B* gene expression. We have previously reported that IL-6 and IL-17A could stimulate *MUC5B* gene expression via a c-Jun kinase/extracellular signal–regulated kinase signaling pathway in normal human bronchial epithelial (NHBE) cells (12); however, the transcription factors involved in cytokine-induced *MUC5B* expression still remain to be determined.

NF-κB is a pleiotropic transcription factor with multiple critical roles in regulation of immune responses (13–15). NF-κB becomes activated in response to inflammatory cytokines, mitogens, physical and oxidative stress, infection, and microbial products (16). Before stimulation, NF-κB subunits are sequestered in the cytoplasm by IκB. After cell stimulation, IκB-α is phosphorylated by IκB kinase (IKK) 2. Phosphorylation of IκB-α results in the ubiquitination and degradation of IκB-α, leading to the nuclear localization of NF-κB, and transcriptional activation of target genes (14). The proinflammatory role of NF-κB in chronic airway diseases has been well documented. Enhanced activation of NF-κB has been implicated in both asthma and COPD (17, 18). Through the use of transgenic mice and conditional ablation strategies, activation of NF-κB within the airway epithelium has been shown to be necessary to induce airway inflammation and mucus overproduction (19, 20). However, little is known about the direct involvement of NF-κB in airway mucin gene regulation. The few studies published point to the involvement of NF-κB in *MUC5B* up-regulation by cigarette smoke (21) and *MUC5AC* up-regulation by lipoproteins of *Haemophilus influenza* or *Mycoplasma pneumonia* (22, 23). However, these NF-κB studies were not performed in a primary human cell system, nor did they address the role of NF-κB in proinflammatory cytokine–induced *MUC5B* expression.

IL-1β is a proinflammatory cytokine that has been shown to play an important role in airway diseases characterized by increased mucus production (24–26), and has been shown to be capable of activating the classical NF-κB signaling pathway (27). IL-17A is a member of a novel family of proinflammatory cytokines that is composed of six members: IL-17A, -B, -C, -D,

(*Received in original form August 24, 2009 and in final form August 31, 2010*)

*Current address: Dr. Tomoyuki Fujisawa. Department of Laboratory Medicine, Hamamatsu University School of Medicine, 1-20-1 Handayama Higashi-ku Hamamatsu, Shizuoka 431-3192, Japan.

This work was supported by in part by National Institutes of Health (NIH) grants HL-77902, HL77315, and ES00628, by California Tobacco-Related Disease Research Program grant 16RT-0127), and by NIH training grant T32 HL07013 (S.V.).

Correspondence and requests for reprints should be addressed to Reen Wu, Ph.D., Center for Comparative Respiratory Biology and Medicine, GBSF Room 6523, University of California at Davis, 451 Health Sciences Drive, Davis, CA 95616. E-mail: rwu@ucdavis.edu

**Am J Respir Cell Mol Biol Vol 45. pp 246–252, 2011**
**Originally Published in Press as DOI: 10.1165/rcmb.2009-0313OC on October 22, 2010**
**Internet address: www.atsjournals.org**

-E, and -F (28). IL-17A has been shown to play important roles in a variety of inflammatory lung conditions, including asthma, COPD, and Gram-negative bacterial pneumonia infection (29–31). IL-17A stimulates the production of inflammatory cytokines and chemokines, and mediates pulmonary neutrophil migration (32, 33). Our recent studies have demonstrated that IL-17A stimulates the degradation of IκB-α, followed by the nuclear translocation of p50 and p65 subunits of NF-κB (34, 35), and induces mucin gene expression (11, 12) and production of human β-defensin-2 (36), CCL-20 (37), CXCL-1, -2, -3, -5, -6, and IL-19 (34) production by primary NHBE cells. Although NF-κB plays a central role in both IL-1β and IL-17A signaling cascades, no evidence is available about an NF-κB–based transcriptional mechanism in IL-1β– and IL-17A–induced *MUC5B* expression.

The purpose of this current study was to elucidate the molecular events associated with IL-1β– and IL-17A–induced *MUC5B* expression. Here, we demonstrate that IL-1β and IL-17A induced *MUC5B* expression in an NF-κB–dependent manner. Using promoter analysis and chromatin immunoprecipitation (ChIP) studies, we have identified an NF-κB–binding element in the promoter region of the *MUC5B* gene.

## MATERIALS AND METHODS

### Culture Conditions

Human bronchial tissues were obtained with informed consent from patients of the University of California–Davis Medical Center (Sacramento, CA) and the National Disease Research Interchange (Philadelphia, PA). The University Human Subjects Review Committee approved and periodically reviewed the protocol. Primary NHBE cells were isolated and cultured under an air–liquid interface condition, as described previously (11) The immortalized NHBE cell line, HBE1 (38), was used for most of the transfection experiments that were performed with a Lipofectamine 2000–mediated protocol (Invitrogen, Carlsbad, CA). Culture conditions for the HBE1 cell line have been described previously (11).

### Cytokine and Inhibitor Treatments

Recombinant human cytokines, IL-1β and IL-17A or IL-17F, were obtained from Invitrogen and R&D systems (Minneapolis, MN), respectively. NF-κB activation inhibitor III (20 μM; Calbiochem, San Diego, CA), a thiazoloamide, was dissolved in DMSO before use. We observed no cytotoxic effects of the inhibitor (determined by trypan blue exclusion) at the dose used in this study (data not shown).

### Real-Time RT-PCR

Isolated DNA-free RNA was used for quantitative real-time RT-PCR to obtain relative mRNA amounts of each gene after normalizing to the β-actin or GAPDH message abundance, as described previously (33–36). The primer sequences were as follows: GAPDH forward, TGGGCTACACTGAGCACCAG; GAPDH reverse, GGGTGTCG CTGTTGAAGTCA; β-actin forward, AGTCGGTTGGAGCGAG CAT; β-actin reverse, AAAGTCCTCGGCCACATTGT; MUC5B forward, GTGAGGAGGACTCCTGTCAAGT; MUC5B reverse, CCTCGCAGAAGGTGATGTTG; p65 forward, AGCTCAAGAT CTGCCGAGTG; p65 reverse, ACATCAGCTTGCGAAAAGGA.

### Mucin ELISA and Western Blot Analysis

Mucin secreted by primary NHBE cells was measured by a double-sandwich ELISA method using a monoclonal antibody specific to airway sputum mucin, 17B1, as described previously (39). The amount of mucin secreted in the culture was expressed as nanogram protein per million cells per day.

For Western blot analysis of MUC5B expression in NHBE cells, a deglycosylation step was performed on the blotted membrane before immunoreaction with monoclonal antibody 5B19-2E (Santa Cruz Biotechnology, Santa Cruz, CA), as described previously (40, 41).

### Promoter–Reporter Constructs and Small Interfering RNA

Two *MUC5B* 4.17 kb and 2.56 kb promoter-luciferase reporter constructs previously constructed in our laboratory (7, 40) were used for the *MUC5B* promoter study. Cells were cotransfected with pRL-TK (Promega, Madison, WI) to control for transfection efficiency. NF-κB p65 small interfering RNA (siRNA) and random oligomer negative control were purchased from Ambion Biotech (Austin, TX).

### ChIP Assay

ChIP assays were performed according to the ChIP protocol from Millipore (Billerica, MA), with minor modifications as described previously (34, 40, 41). Primers used for putative NF-κB sites of *MUC5B* were: NF-κB-1 (from −3,861 to −3,712): forward primer, 5′-GTG CGTCTGGCCTGGTAAG-3′; reverse primer, 5′-CCCAGGATGTG TACTCAGAGC-3′; NF-κB-2 (from −3,195 to −3,070): forward primer, 5′-GCAAGTTCCTGGCACGTC-3′; reverse primer, 5′-AAG GCGCTGAAAACAGAAGA-3′; NF-κB-3 (from −3,006 to −2,851): forward primer, 5′-CCGGGATGTCTCAATAGCTG-3′; reverse primer, 5′-GGCACACAGTGACACCAAAC-3′.

### Statistical Analysis

Data are expressed as means (±SE). Experiments were performed in triplicate, and performed in at least two independent cultures. Group differences were calculated using the Student's *t* test. Differences were considered significant for *P* values less than or equal to 0.05.

## RESULTS

### IL-1β and IL-17A Stimulate MUC5B Gene Expression in NHBE Cells

We examined the potency of IL-1β and IL-17A stimulation of *MUC5B* expression in well differentiated NHBE cells cultured under air–liquid interface conditions. As shown in Figure 1, both IL-1β and IL-17A induced *MUC5B* mRNA expression in primary NHBE cells in both a dose- and time-dependent manner. For IL-1β, a significant stimulation of *MUC5B* was observed at concentrations as low as 0.2 ng/ml (Figure 1A). A time-course analysis indicated that maximum stimulation of MUC5B expression occurred at 24 hours (Figure 1B) after addition of 10 ng/ml IL-1β. A similar dose–response curve was seen for IL-17A, except that a decrease in MUC5B gene expression occurred with concentrations higher than 10 ng/ml (Figure 1C). Maximum stimulation was seen 24 hours after treatment with 10 ng/ml of IL-17A (Figure 1D). These results confirm that both IL-1β and IL-17A are potent stimulators of *MUC5B* gene expression in well differentiated NHBE cells. Similar time- and dose-dependent results were seen in studies with the HBE1 cell line (data not shown).

We performed both mucin ELISA and Western blot analysis to examine effects on protein expression. As shown in Figure 2A, both IL-17A and IL-17F were able to stimulate mucin production significantly, approximately threefold, over untreated cells, from 30 to nearly 100 ng/$10^6$ cells/day, whereas IL-1β provoked a twofold increase in mucin production. Western blot analysis using a MUC5B N-terminal–specific antibody confirmed the enhanced accumulation of high–molecular weight MUC5B protein after cytokine stimulation (Figure 2B).

### NF-κB Is Required for Both IL-1β– and IL-17A–Induced MUC5B Expression

To evaluate the involvement of NF-κB in cytokine-induced *MUC5B* expression, an NF-κB activation inhibitor and NF-κB siRNA were used. As shown in Figures 3A and 3B, both IL-1β– and IL-17A–induced *MUC5B* expression was sensitive to the NF-κB activation inhibitor in NHBE cells. To further these results confirm, HBE1 cells were transfected with p65 NF-κB siRNA. As shown in Figure 4A, transfection with p65 NF-κB

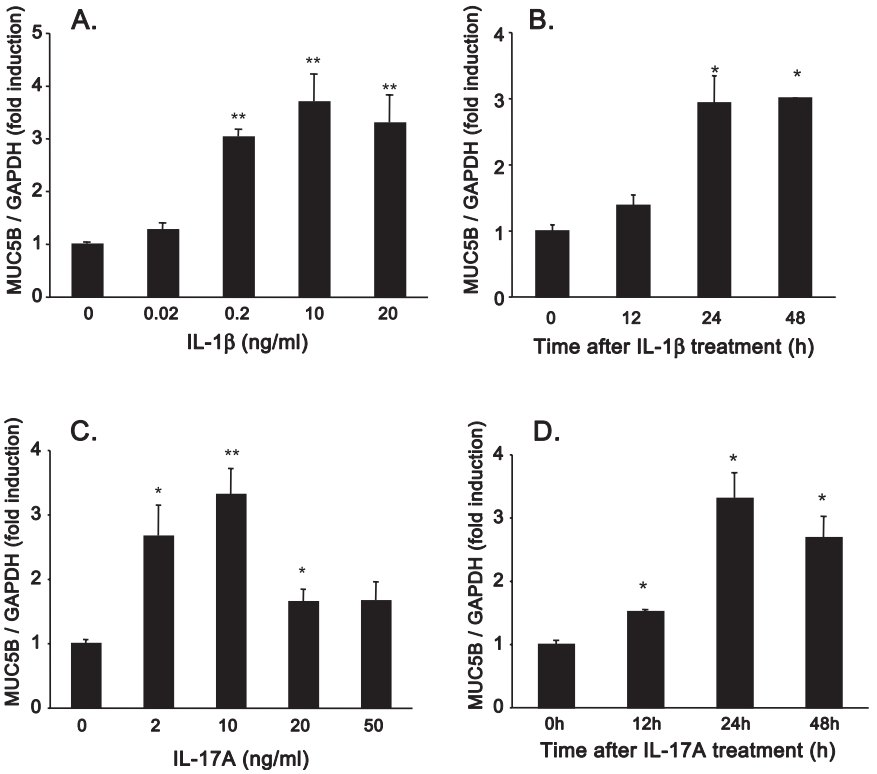


***Figure 1.*** Stimulation of *MUC5B* mRNA expression by IL-1β and IL-17A. (*A* and *C*) Dose–response effects of IL-1β and IL-17A on *MUC5B* mRNA expression. Normal human bronchial epithelial (NHBE) cells grown in air–liquid interface (ALI) conditions for 1 week were starved for 16 hours (without growth factors) before being treated with various concentrations of IL-1β (0–20 ng/ml) and IL-17A (0–50 ng/ml). At 24 hours after cytokine treatment, total RNA was harvested, and *MUC5B* mRNA was analyzed using SYBR Green quantitative real-time PCR and normalized to a housekeeping gene, *GAPDH*, as described in MATERIALS AND METHODS. (*B* and *D*) Time course effects of IL-1β and IL-17A treatment on *MUC5B* mRNA expression. NHBE cells were treated with 10 ng/ml IL-1β (*B*) or 20 ng/ml IL-17A (*D*). RNA samples were harvested from these cultures at different time points (0, 12, 24, 48 h) after treatment. *MUC5B* and *GAPDH* mRNA levels were quantified using real-time PCR. Triplicate dishes were used for each experiment, and experiments were repeated with cultures derived from two different human donors. Statistically significant: $*P < 0.05$, $**P < 0.01$, compared with unstimulated control.

siRNA significantly attenuated p65 message in HBE1 cells. A similar inhibition at the protein level was seen with p65 siRNA treatment (11). p65 siRNA also attenuated IL-1β–induced *MUC5B* expression, compared with the negative control (Figure 4B). A similar attenuation was seen for IL-17A–induced *MUC5B* expression (Figure 4C). These results demonstrate that NF-κB is involved in IL-1β– and IL-17A–induced *MUC5B* expression.

### Deletion Analysis of Cytokine-Induced MUC5B Promoter Activity

As shown in Figure 5A, IL-1β significantly increased luciferase activity of the *MUC5B* 4.17-kb promoter construct; however, there was no significant induction of the *MUC5B* 2.56-kb promoter construct. Similar results were observed for IL-17A (Figure 5B). Together, these results indicate that the region of the *MUC5B* promoter spanning −4.17 kb to −2.56 kb contains *cis*-acting element(s) that are required for both IL-1β– and IL-17A–stimulated gene expression.

### Demonstration of NF-κB Binding to MUC5B Promoter by ChIP Assay

To identify the putative enhancer element(s) in the *MUC5B* promoter, sequence analysis using MatInspector (Genomatix Software GmbH, Ann Arbor, MI) revealed three putative κB-binding sites between −4.17 kb and −2.56 kB of the *MUC5B* promoter (Figure 6A). These are as follows: NF-κB-1, antisense (−) 5′ gtGGGAccctcca 3′ (−3,786/−3,774); NF-κB-2, sense (+) 5′ gtGGGAggctcct 3′ (−3,173/−3,161); and NF-κB-3, sense (+) 5′ cgGGGAggtgcct 3′ (−2,921/−2,909). To determine if these putative NF-κB sites are involved in cytokine-induced MUC5B expression, a ChIP assay was performed. Because of the difficulty in obtaining consistent results with the anti-p65 antibody during ChIP analyses, an antibody to the p50 subunit of NF-κB was used for these experiments. As shown in Figure 6B, IL-1β enhanced the binding of the p50 subunit of NF-κB to the promoter region containing the NF-κB-3 site, which was detected by real-time PCR quantification. On the other hand, the binding of p50 to either the NF-κB-1 or the NF-κB-2 site was not enhanced by cytokine treatment (Figure 6B). IL-17A treatment resulted in similar p50 binding patterns (data not shown). These results confirm the presence of an NF-κB–binding element in the *MUC5B* promoter region, and show that cytokine treatment enhances binding of the p50 subunit of NF-κB to this region.

## DISCUSSION

In the present study, we demonstrate that NF-κB plays a role in both IL-1β– and IL-17A–induced *MUC5B* expression in both well differentiated primary NHBE cells and the HBE1 cell line. Both IL-1β and IL-17A stimulated *MUC5B* gene expression in a time- and dose-dependent manner. Attenuation of NF-κB activity by use of a specific inhibitor or treatment of the cells with a p65 siRNA both suppressed *MUC5B* induction by either cytokine. Using a reporter-based promoter study, we showed that enhancer elements located in the *MUC5B* promoter between −4.17 kb and −2.56 kb of the transcriptional start site play a critical role in IL-1β– and IL-17A–induced promoter activation. Importantly, we also provide an *in vivo* ChIP evidence to demonstrate an enhanced physical interaction between the NF-κB p50 subunit and the *MUC5B* promoter after cytokine treatment. This is the first report describing a critical role for NF-κB in transcriptional regulation of airway *MUC5B* expression by IL-1β and IL-17A, as well as the identification of an NF-κB–binding element in the promoter of the *MUC5B* gene. A similar demonstration has been recently reported for induced *MUC5AC* gene expression (11). For IL-1β, this is the first demonstration that IL-1β is capable of stimulating *MUC5B* expression in addition to *MUC5AC*. This is in contrast to a previous report (42), which could only demonstrate the stimulation of *MUC5AC*, but not *MUC5B* message by

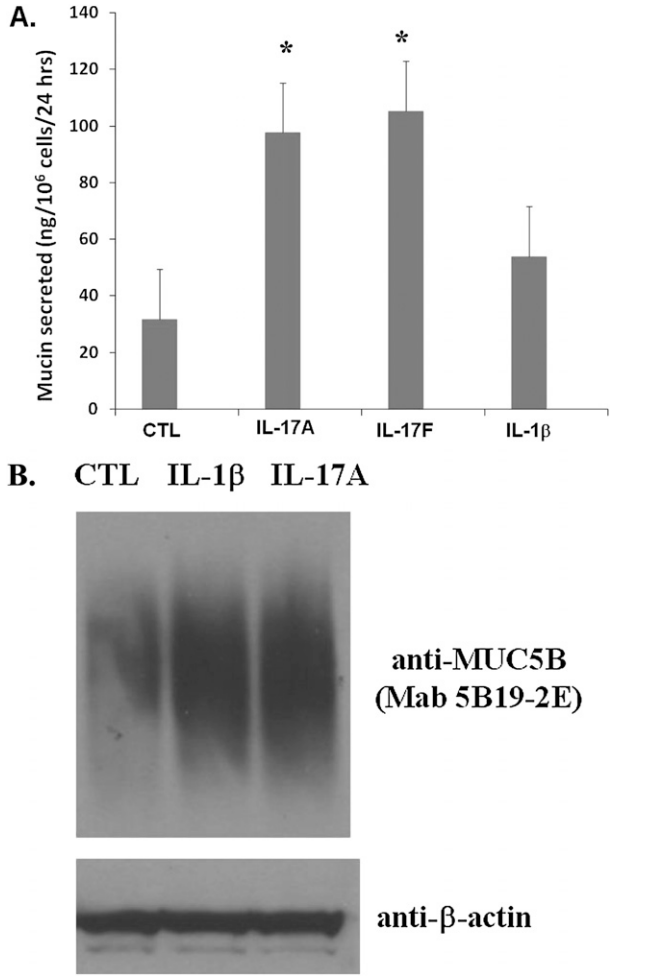


*Figure 2.* Effects of cytokines on mucin production and MUC5B glycoprotein expression in NHBE cells. NHBE cells were treated with 10 ng/ml IL-1β and 20 ng/ml IL-17A/F. (*A*) Medium was collected 24 hours after stimulation for mucin ELISA quantification, as described in the text and Ref. 39. (*B*) Cells were harvested 24 hours after stimulation and lysed for Western blot analysis with monoclonal antibody (Mab) 5B19-2E specific for MUC5B N-terminal peptide (40) and anti–β-actin antibody, which was used to monitor the protein input. $*P < 0.05$. CTL, unstimulated control.

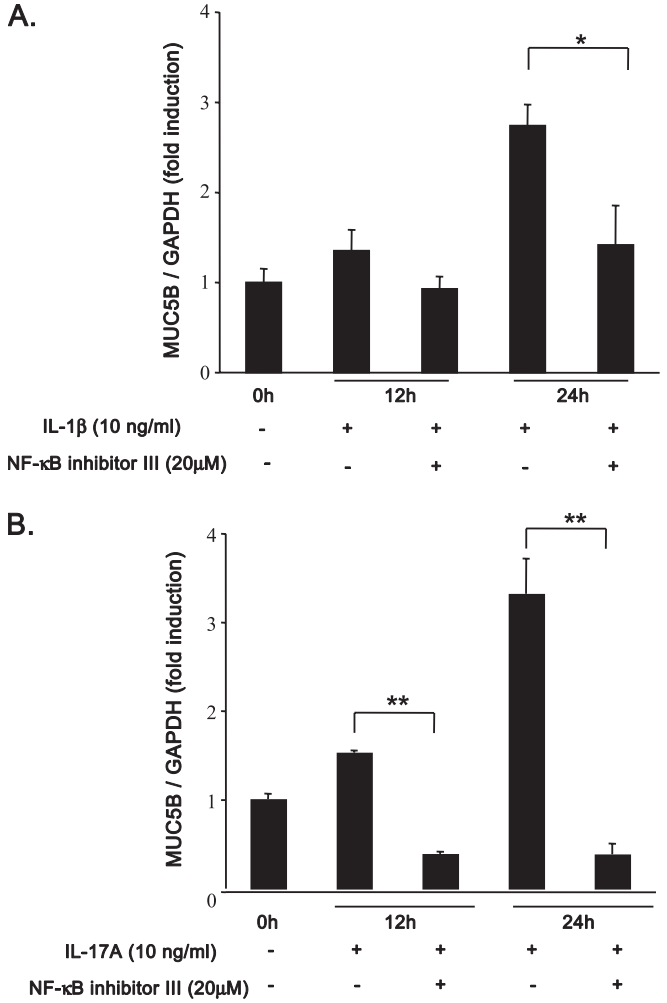


*Figure 3.* Effects of NF-κB inhibitor III on cytokine-induced *MUC5B* expression. NF-κB inhibitor III (20 μM) or an equal amount of vehicle (DMSO) was added 1 hour before 10 ng/ml IL-1β (*A*) or 20 ng/ml IL17A (*B*) treatment on primary NHBE cells, as described in MATERIALS AND METHODS. At 12 and 24 hours after treatment, RNA samples were collected from these cultures. SYBR Green quantitative RT-PCR was used to quantify the message levels of MUC5B and GAPDH (or β-actin) in these RNA samples. Triplicate dishes were used for each time point, and experiments were repeated three times for different cultures derived from different donors. Statistically significant: $*P < 0.05$, $**P < 0.01$.

IL-1β. This difference may be difficult to explain due to the variation in different culture conditions among different laboratories.

We previously reported that IL-17A could stimulate *MUC5B* expression in primary NHBE cultures (12), and that this stimulation could be partially blocked by an anti–IL-6 receptor neutralizing antibody. This result suggests that an IL-17A–mediated IL-6 autocrine/paracrine loop could be involved in regulation of mucin gene expression. Further studies have shown that this mechanism reached a maximum stimulation at 48–72 hours (Y.C., unpublished data). In contrast, the IL-1β and IL-17A induction of *MUC5B* in this study is an early event that occurs within 24 hours after treatment.

NF-κB activation in airway epithelial cells plays a central role in airway inflammation (20, 43–45); however, no report has demonstrated the involvement of an NF-κB–based mechanism in cytokine-stimulated *MUC5B* up-regulation. Here, we show that NF-κB activation is indispensable for IL-1β– and IL-17A–induced *MUC5B* gene expression. Our findings indicate that NF-κB activation in airway epithelium results in airway inflammation and mucus overproduction, which are two major features of chronic airway disease, and highlight the potential clinical benefit of focused targeting on the NF-κB pathway in inflamed airways.

Few published studies have addressed involvement of NF-κB in *MUC5B* induction. Preciado and colleagues (21) reported that cigarette smoke could activate NF-κB and induce *Muc5b* expression in mouse middle ear cells, but they did not address whether or not NF-κB is directly responsible for *Muc5b* induction. In the present study, attenuation of NF-κB activity using both inhibitor and siRNA approaches significantly decreased IL-1β– and IL-17A–induced *MUC5B* gene expression in NHBE and HBE1 cells. In addition, we recently demonstrated that both IL-1β and IL-17A stimulation of HBE1 cells induced the degradation of IκB-α, and led to nuclear localization of NF-κB subunits, p50 and p65 (35).

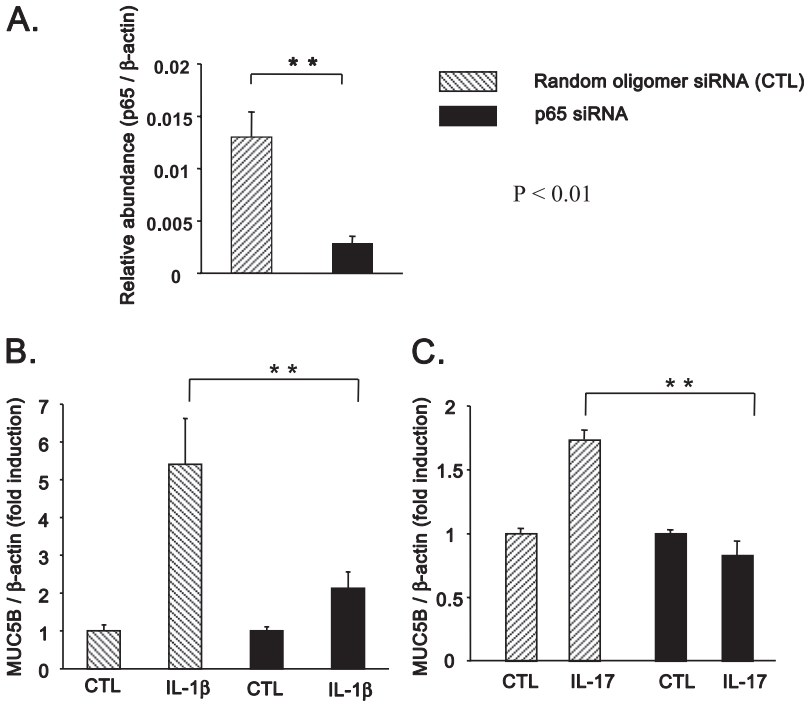


***Figure 4.*** Effects of p65 small interfering RNA (siRNA) on cytokine-induced *MUC5B* expression. HBE1 cells were treated with p65 siRNA or random oligomer (RO) siRNA as the control treatment (CTL), as described in the text. Two days after siRNA treatment, cells were starved for 6 hours, then treated with IL-1β (*B*) or IL-17A (C) for 16 hours. The efficiency of siRNA-p65 in reducing endogenous p65 mRNA was confirmed by performing real-time PCR (*A*). Relative MUC5AC message levels were averaged from triplicate dishes, and the experiment was repeated three times with HBE1 cells from different passage numbers. Statistically significant: $^{**}P < 0.01$.

These findings provide evidence that NF-κB activation is involved in IL-1β– and IL-17A–stimulated *MUC5B* induction. Furthermore, using *MUC5B* promoter luciferase constructs, we also demonstrate that cytokine-mediated transcriptions act on *cis* element(s) located within the −4.17 kb to −2.56 kb region of the *MUC5B* promoter. Importantly, sequence analysis revealed three potential κB-binding sites located within this region. Using ChIP analysis, we demonstrated that IL-1β stimulation enhanced the binding of p50 to the region of *MUC5B* promoter from −3,006 to −2,851, which contains the κB-binding site (NF-κB-3 site, −2,921/−2,909). Further experiments, such as site-directed mutagenesis, should been done to verify the functionality of the NF-κB–binding site in *MUC5B* promoter activation.

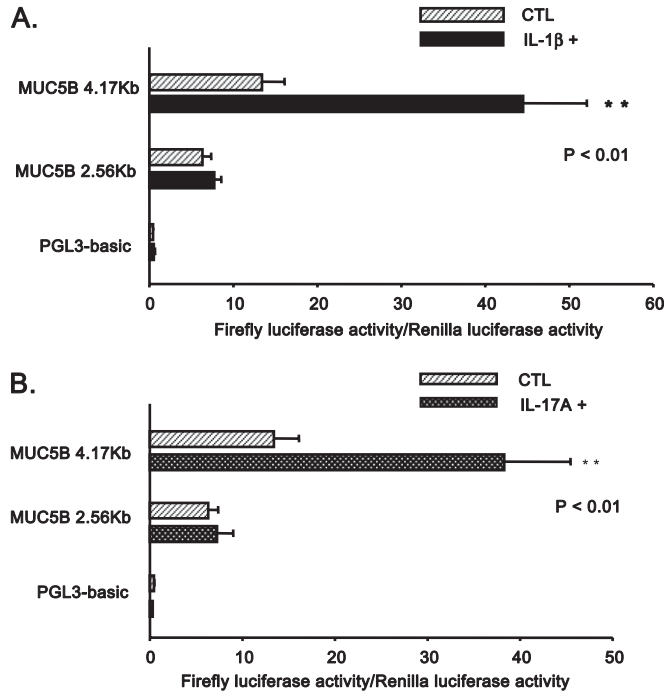


***Figure 5.*** Deletion analysis of MUC5B promoter–reporter gene activities in response to cytokines. HBE1 cells were cotransfected with various *MUC5B* promoter luciferase reporter constructs and the control pRL-TK plasmid, as described in MATERIALS AND METHODS. At 2 days after transfection, cells were left unstimulated (CTL) or simulated with IL-1β (*A*) or IL-17A (*B*) at the 10-ng/ml level for 16 hours. Luciferase activities in these cells were measured and normalized as described in MATERIALS AND METHODS. Data from triplicate dishes were averaged and experiments performed in three independent cultures. Statistically significant: $^{**}P < 0.01$ compared with the CTL case without cytokine treatment.

Although a significant role for NF-κB in the transcriptional regulation of IL-1β– and IL-17A–stimulated *MUC5B* expression has been shown in this study, the possibility that other transcription factors are involved cannot be ruled out. Recently, we reported that specificity protein 1 (SP1) activation plays an important role in both basal *MUC5B* promoter activity and phorbol 12-myristate 13-acetate–induced *MUC5B* gene expression in NHBE cells (41). Choi and coworkers (46) reported that the cAMP response element–binding protein and a cAMP-response element site on the −956 region of the *MUC5B* promoter is required for 17β-estradiol–induced *MUC5B* expression in normal human nasal epithelial cells and NCI-H292 cells. It is possible that an intricate network of transcriptional factors is involved in the regulation of *MUC5B* expression under various conditions. Given the broad nature of NF-κB activation, it seems likely that other factors might be involved in the regulation of IL-1β– and IL-17A–induced *MUC5B* expression, which will be a topic of exploration in future studies.

In conclusion, we have found that both IL-1β and IL-17A, two prominent proinflammatory cytokines associated with chronic airway inflammation, can mediate *MUC5B* induction in airway epithelial cells. We further show that NF-κB activation is an essential mechanism for both IL-1β– and IL-17A–induced *MUC5B* expression, and we have identified the functional region (from −4.17 kb to −2.56 kb) of the *MUC5B* promoter that contains the NF-κB–binding site (−2,921/−2,909). As IL-1β and IL-17A have both been demonstrated as positively promoting airway inflammation in various disease states, our results are

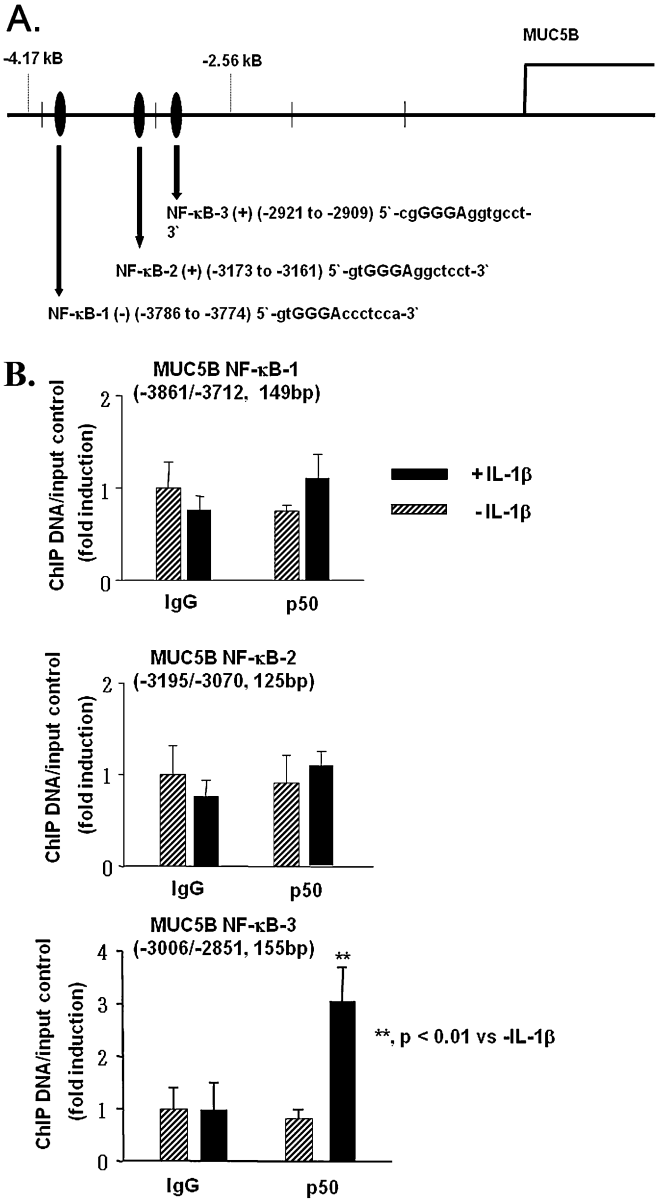


*Figure 6.* Chromatin immunoprecipitation (ChIP) assays on p50 protein binding on *MUC5B* promoter region in cells after IL-1β treatment. (*A*) Identification of putative κB-binding sites in the 5′-flanking region of *MUC5B* promoter. (*B*) Quantitative PCR analysis of anti-p50 antibody–precipitated and control IgG antibody–precipitated chromatin from HBE1 cells treated with 10 ng/ml of IL-1β for 1 hour. Coprecipitated DNA and the corresponding input DNA before precipitation were quantified by real-time PCR analysis using primers to amplify the *MUC5B* promoter DNA, which contain different κB-binding sites (*top*, NF-κB-1; *middle*, NF-κB-2; *bottom*, NF-κB-3). Results from the quantitative real-time PCR after normalization with input control DNA template were averaged from three independent experiments. $^{**}P <$ 0.01 compared with non–cytokine-treated control.

consistent with these findings, and further suggest one manner in which these cytokines contribute to the pathogenesis of airway inflammatory diseases. This study highlights the importance of NF-κB as a transcriptional regulator of mucin gene expression in airway epithelium, and may provide new strategies for controlling mucus overproduction in chronic airway diseases.

***Author Disclosure*:** None of the authors has a financial relationship with a commercial entity that has an interest in the subject of this manuscript.

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