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Interleukin-1β Induces MUC2 and MUC5AC Synthesis through Cyclooxygenase-2 in NCI-H292 Cells

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ABSTRACT

Interleukin-1β (IL-1β) has been implicated in the pathogenesis of inflammatory diseases of the airway. In this study, we investigated the regulation of MUC2 and MUC5AC expression and of their regulatory mechanisms through cyclooxygenase-2 (COX-2) and prostaglandin E_2 (PGE_2). Cells activated by IL-1β showed increased COX-2, MUC2, and MUC5AC expressions at both the mRNA and protein levels. Mucin production was blocked by the selective COX-2 inhibitor NS398, and PGE_2 directly induced MUC2 and MUC5AC expression at both the mRNA and protein levels in a dose-dependent manner. These results suggest a role for PGE_2 in IL-1β–induced mucin synthesis in NCI-H292 cells. To investigate the roles of molecules upstream of COX-2 in mucin regulation, we examined the role of mitogen-activated protein kinases (MAPKs). Cells activated by IL-1β showed increased extracellular signal-regulated kinase (ERK)1/2 and p38 phosphorylation, and IL-1β–induced MUC2 and MUC5AC production was blocked by the ERK pathway inhibitor PD98059 or the p38 inhibitor SB203580. The inhibition of both MAPKs reduced IL-1β–induced COX-2 expression and PGE_2 synthesis. Furthermore, the addition of PGE_2 to cells overcame the inhibitory effects of both MAPK inhibitors in IL-1β–induced mucin production. These results indicate that in human pulmonary epithelial cells, IL-1β activates ERK or p38 to induce COX-2 production, which in turn induces MUC2 and MUC5AC production.

Mucins are high-molecular-weight glycoproteins that are produced by the majority of secretory epithelial cells to lubricate and protect ducts and lumina of the human body (Kim et al., 1999). However, excessive mucin secretion is a hallmark of the pathogenesis of several airway diseases, including chronic bronchitis, asthma, and cystic fibrosis (Kim, 1997). Moreover, hypersecretory diseases of the airways, such as submucosal gland hypertrophy and goblet cell hyperplasia, are associated with the abnormal growth and differentiation of mucin-synthesizing cells. In addition, histochemical studies have described abnormalities of *mucin* glycosylation in airway diseases involving increased intracellular acidic mucin levels (Jeffery and Li, 1997). Abnormalities of mucin glycoproteins have also been reported in lung cell carcinoma (Kawai et al., 1993). Thirteen human *mucin* genes have been identified (Gendler and Spicer, 1995; Basbaum et al., 1999; Lagow et al., 1999; Williams et al., 1999), although the 13th member was found only recently (Williams et al., 2001). The expressions of mucin genes, such as MUC2 and MUC5AC, in airway epithelial cells is regulated by various factors and inflammatory mediators (Takeyama et al., 2000; Gray et al., 2001; Perrais et al., 2001), and various cytokines may also induce mucin secretion (Dabbagh et al., 1999; Longphre et al., 1999; Shim et al., 2001). Moreover, levels of IL-1β are increased in inflammatory airway diseases such as asthma (Barnes, 1994), and it has been found that IL-1β increases in humans during an asthmatic attack and that this increase is related to the disease (Mattoli et al., 1991); in addition, IL-1β has been reported to regulate mucin synthesis (Yoon et al., 1999; Enss et al., 2000; Kim et al., 2000). The mechanisms controlling mucus secretion are not completely understood, but they are believed to involve lipid metabolites and cellular signaling pathways.

The PGs have many inflammatory effects (Tilley et al., 2001), and it is known that COX converts arachidonic acid to prostaglandin H_2, which is further metabolized to various PGs and thromboxanes (Smith et al., 1996). Two distinct isoforms of COX have been identified. COX-1 is expressed constitutively in many types of cells, in which it is believed to

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ABBREVIATIONS: IL-1β, interleukin-1β; PG, prostaglandin; COX, cyclooxygenase; PGE_2, prostaglandin E_2; EP, prostaglandin E_2 receptor; MAPK, mitogen-activated protein kinase; ERK, extracellular signal-regulated kinase; EGFR, epidermal growth factor receptor; RT-PCR, reverse transcription-polymerase chain reaction; PCR, polymerase chain reaction; Ab, antibody; PBS, phosphate-buffered saline; NS398, *N*-(2-cyclohexyloxy-4-nitrophenyl)methanesulfonamide; RU486, mifepristone; PD98059, 2′-amino-3′-methoxyflavone; SB203580, 4-(4-fluorophenyl)-2-(4-methylsulfonylphenyl)-5-(4-pyridyl)imidazol; HRP, horseradish peroxidase.

perform housekeeping activities; COX-2, in contrast, is not typically present, or it is present but in very low quantities. However, COX-2 is rapidly induced by cytokines, growth factors, and tumor promoters and is involved in the PG synthesis associated with inflammation and carcinogenesis. Although PGE_2 is one of the major metabolites of arachidonic acid in human pulmonary tissue, its function is not clear. Some evidence suggests that the PGs may stimulate mucin secretion (Phillips et al., 1993; Belley and Chadee, 1999), and because mucin hypersecretion is a hallmark of airway inflammation, it is possible that COX-2 and its metabolites may regulate airway mucin secretion.

Although many articles show that IL-1β induces mucin secretion by epithelial cells, the specific signaling pathways involved in the mediation of mucin production are unknown. The identification of the involvement of a PGE_2-linked signaling pathway is central to our understanding of how mucin secretion is regulated during inflammatory states. Here, we report that the stimulation of airway epithelial cells with IL-1β induces mucin production, and we show that IL-1β activates MUC2 and MUC5AC mucin production by activating either the ERK or the p38-PGE_2 pathway.

Materials and Methods

Materials. The NCI-H292 epithelial cell line was obtained from the American Type Culture Collection (Manassas, VA). Human recombinant IL-1β was obtained from R & D Systems (Minneapolis, MN). RPMI 1640 medium and the RT-PCR kits were from Invitrogen (Carlsbad, CA). Fetal calf serum was from Hyclone Laboratories (Logan, UT), enhanced chemiluminescence reagents were from PerkinElmer Life Sciences (Boston, MA), and the PGE_2 assay kit was from Amersham Biosciences Inc. (Piscataway, NJ). Rabbit polyclonal COX-2 Ab was from Cayman Chemical (Ann Arbor, MI). MUC2 and mouse and rabbit HRP-conjugated secondary Abs were from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA), and MUC5AC Ab was from NeoMarkers (Fremont, CA). Phospho-ERK1/2 and phospho-p38 Abs were from New England Bolas (Beverly, MA), and NS398 was from BIOMOL Research Laboratories (Plymouth Meeting, PA). PGE_2 and budesonide were from Sigma Chemical (St. Louis, MO) and were dissolved in dimethyl sulfoxide or ethanol before being added to cell cultures to a final dimethyl sulfoxide or ethanol concentration of 0.1% or less.

Cell Culture. NCI-H292 epithelial cells were cultured in RPMI 1640 medium supplemented with 2 mM L-glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin, and 10% fetal calf serum. Cells were grown at 37°C in 5% CO_2 fully humidified air and were subcultured twice weekly. The cells were seeded in either a 12-well plate at 5×10^5 cells/well or in a 6-well plate at 1×10^6 cells/well. When confluent, the cells were incubated in RPMI 1640 medium containing 0.5% fetal calf serum for 24 h. The cells were then rinsed with serum-free RPMI 1640 medium and exposed to the indicated concentrations of IL-1β in the presence of various inhibitors. In the case of controls, the cells were incubated with medium alone for the same times.

PGE_2 Assay. PGE_2 levels were determined using an enzyme immunoassay kit according to the manufacturer's instructions. Briefly, 100 μl of a standard or of a sample was placed into each well of a 96-well plate with the use of a pipette. Aliquots of a mouse polyclonal PGE_2 antibody and PGE_2 conjugated to alkaline phosphatase were then added to each well and incubated at room temperature for 1 h. The wells were then washed six times with 0.2 ml of PBS containing 0.05% Tween 20, and tetramethylbenzene substrate was added. Wells were read at 670 nm with an enzyme-linked immunosorbent assay reader 30 min after substrate addition.

Western Blot Analysis. The NCI-H292 cells were plated in a 6-well plate and treated with IL-1β for the indicated times. The cells were then washed with cold PBS, exposed to trypsin, and formed into pellets at 700*g* at 4°C, and the pellets were resuspended in lysis buffer (50 mM Tris-HCl, pH 8.0, 5 mM EDTA, 150 mM NaCl, 1% Triton X-100, 1 mM phenylmethylsulfonyl fluoride, and protease-inhibitor cocktail). The preparation was then clarified by centrifugation, and the supernatant was saved as a whole-cell lysate. Proteins (50 μg) were separated using 10% reducing SDS-polyacrylamide gel electrophoresis and electroblotted in 20% methanol, 25 mM Tris, and 192 mM glycine onto a nitrocellulose membrane. The membrane was then blocked with 5% nonfat dry milk in 25 mM Tris-HCl, 150 mM NaCl, and 0.2% Tween 20, and it was then incubated with the indicated antibodies for 4 h. Subsequently, the membrane was washed and incubated for 1 h with secondary antibodies conjugated to HRP, rewashed, and developed using an enhanced chemiluminescence system.

Immunoassay of MUC2 and MUC5AC Proteins. MUC2 and MUC5AC protein levels were determined by an enzyme-linked immunosorbent assay. Cell lysates were prepared in PBS at several dilutions, and each sample was incubated at 40°C in a 96-well plate until dry. Plates were then washed three times with PBS, blocked with 2% bovine serum albumin for 1 h at room temperature, washed again three times with PBS, and incubated with MUC2 or MUC5AC antibody diluted with PBS containing 0.05% Tween 20 for 1 h. The wells were then washed three times with PBS, HRP-conjugated anti-rabbit IgG was dispensed into each well, and after 4 h, the plates were washed three times with PBS. Color was developed using 3,3′,5,5′-tetramethylbenzidine peroxidase solution and stopped with 2N-H_2SO_4. Absorbance was read at 450 nm.

RT-PCR. The NCI-H292 cells were cultured, harvested, and subsequently washed three times with PBS containing 2% bovine serum albumin. RNA was isolated using an RNeasy kit (QIAGEN, Valencia, CA), and a modified RT-PCR technique was used to determine the mRNA level. Briefly, total RNA was reverse-transcribed into its cDNA using an RT-PCR kit. Oligonucleotide primers for the PCR were designed according to the published sequence for human MUC2 (sense: TGC CTG GCC CTG TCT TTG; antisense: CAG CTC CAG CAT GAG TGC) and human MUC5AC (sense: ATC ACC GAA GGC TGC TTC TGT C; antisense: GTT GAT GCT GCA CAC TGT CCA G). The PCR conditions used to produce MUC2 were 30 cycles of penetration (at 95°C/l min), annealing (at 61°C/30 s), and extension (at 72°C/1 min) in the presence of 2.5 mg $MgCl_2$, followed by a final 20-min extension at 72°C. Oligonucleotide primers for β-actin were used as a control, and the PCR products were separated by electrophoresis through a 1% agarose gel containing ethidium bromide.

Results

IL-1β Up-Regulates MUC2 and MUC5AC Gene and Protein Expression. To determine whether glycoproteins are induced by IL-1β in airway epithelial cells, we evaluated mucin gene expression by determining the mRNA expressions of MUC2 and MUC5AC in NCI-H292 cells. A representative RT-PCR analysis is shown in Fig. 1. The addition of IL-1β to NCI-H292 cells was found to increase MUC2 and MUC5AC mRNA expressions in a dose-dependent manner, and this was maximal at 0.2 ng/ml of IL-1β (Fig. 1A). The induction of MUC2 and MUC5AC by IL-1β was confirmed at the protein level by immunoassaying cell lysates using specific antibodies. Consistent with the increased gene expression data, the protein levels of MUC2 and MUC5AC were also increased in a dose- and time-dependent manner (Fig. 1, B and C).

IL-1β Induces COX-2 Expression. To determine whether COX-2 is involved in the signal-transduction pathway leading to mucin production, we assessed the ability of IL-1β to induce COX-2 protein and PGE_2 synthesis. Cultures

were incubated in serum-free medium in the presence of various concentrations of human recombinant IL-1β (0.01–5 ng/ml) for 8 h, and Western blot analysis was then performed using the human COX-2 antibody. As shown in Fig. 2A, IL-1β induced COX-2 expression and PGE_2 production. In experiments conducted to examine the time course of COX-2 expression in IL-1β–treated cells, we detected the rapid synthesis of COX-2 protein within 2 h of treatment. COX-2 levels were maximal 8 h after treatment, and this situation was maintained for at least 12 h (Fig. 2B). The influence of the glucocorticoid receptor-dependent signaling pathway on the elevated MUC2 and MUC5AC levels induced by IL-1β was also investigated. NCI-H292 cells were treated for 8 h with IL-1β and budesonide, an anti-inflammatory agent. Budesonide addition was found to abrogate IL-1β–induced MUC2 and MUC5AC protein levels and those of their genes. Moreover, RU486, which functions as a glucocorticoid receptor antagonist suppressed the effect of budesonide on MUC2 and MUC5AC expression (Fig. 3A). Consistent with the MUC expression data, budesonide completely inhibited IL-1β–induced COX-2 expression, and RU486 suppressed the effect of budesonide on COX-2 expression (Fig. 3B).

IL-1β–Induced MUC2 and MUC5AC Production Mediated through PGE_2. To test the hypothesis that the activation of COX-2 induces MUC2 and MUC5AC expression, cells were incubated with COX-2 inhibitor. The pretreatment of NCI-H292 cells with NS398, a specific COX-2 inhibitor, prevented IL-1β–induced MUC2, MUC5AC, and PGE_2 production (Fig. 4, A and B). To confirm the role of COX-2 on the synthesis of MUC2 and MUC5AC after stimulation with IL-1β, we investigated the effects of PGE_2, a product of COX-2 activity. Cells were treated with various concentrations of PGE_2 for 8 h. As shown in Fig. 5, PGE_2 was found to strongly induce MUC2 and MUC5AC gene expression in a dose-dependent manner. Furthermore, MUC2 and MUC5AC protein levels were also increased by PGE_2.

Involvement of MAPKs in MUC2 and MUC5AC Production. We studied whether MAPKs are capable of activating MUC2 and MUC5AC by examining MAPK phosphorylation in immunoblots with the use of phosphospecific antibodies. As shown in Fig. 6, ERK1/2 and p38 phosphorylation was stimulated by IL-1β to a similar extent. Maximum phosphorylation levels of ERK1/2 and p38, as induced by IL-1β and as indicated by Western immunoblotting, were observed 20 min after treatment. We also confirmed that the specific inhibitors PD98059 and SB203580 blocked the activation of the appropriate MAPKs in NCI-H292 cells activated with IL-1β. To investigate the roles of ERK1/2 and p38 on COX-2 expression after IL-1β stimulation, we examined the effects of PD98059 and SB203580 on IL-1β–induced COX-2 expression. Cells were pretreated with PD98059 (5–50 μM) or SB203580 (1–5 μM) for 30 min before adding IL-1β. Both inhibitors were found to strongly suppress IL-1β–induced COX-2 expression in a dose-dependent manner (data not shown). As shown in Fig. 7A, both PD98059 (50 μM) and SB203580 (5 μM) inhibited COX-2 expression in response to stimulation by IL-1β, although SB203580 was a less potent inhibitor of COX-2 expression than PD98059. To assess the relative roles of ERK1/2 and p38 on IL-1β–mediated MUC2 and MUC5AC production, we also investigated whether PD98059 or SB203580 affect mucin production in cells. As expected, the pretreatment of IL-1β–treated cells with PD98059 or SB203580 resulted in the significant inhibition of MUC2 and MUC5AC production (Fig. 7B).

PGE_2 Is Downstream of MAPKs during IL-1β Signaling. To investigate whether PGE_2-mediated events could be involved in the direct responses to PGE_2 stimulation, the effects of NS398, PD98059, and SB203580 on PGE_2-stimulated mucin production was examined. MUC2 and MUC5AC synthesis by PGE_2 was unaffected by these inhibitors (Fig. 8A). To further confirm that PGE_2 is downstream of IL-1β in mucin production, we tested the effect of PGE_2 on the inhibition of IL-1β–induced mucin production by COX-2, ERK, and p38. MUC2 and MUC5AC synthesis by IL-1β was inhib-

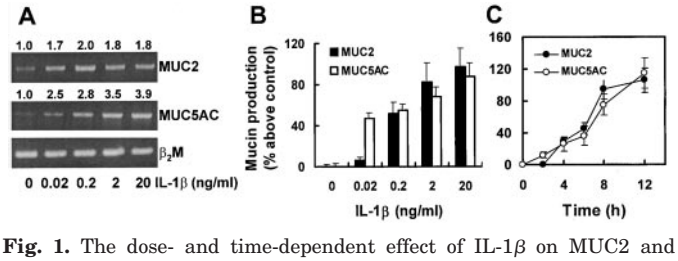

Fig. 1. The dose- and time-dependent effect of IL-1β on MUC2 and MUC5AC synthesis in NCI-H292 cells. A and B, cells were stimulated for 8 h with the indicated concentrations of IL-1β. Total RNA was isolated, and MUC2 and MUC5AC mRNA levels were analyzed by RT-PCR. MUC2 and MUC5AC proteins were measured as described under *Materials and Methods*. C, cells were stimulated with 0.2 ng/ml of IL-1β for the indicated times. The mucin mRNA levels shown are representative of three independent experiments, and the values given for mucin protein are the averages ± S.E. of three independent experiments.

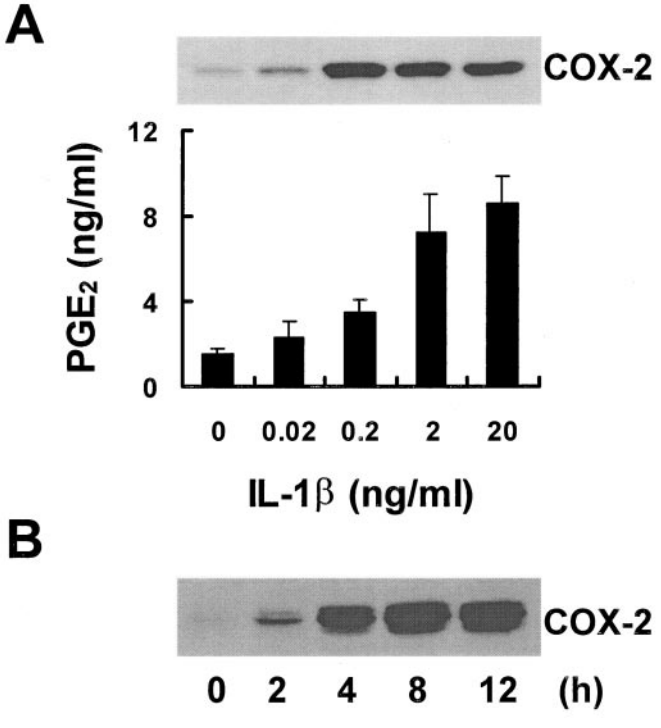

Fig. 2. Effect of IL-1β on COX-2 expression and PGE_2 production in NCI-H292 cells. A, cells were stimulated with the indicated concentrations of IL-1β for 8 h. PGE_2 production was measured in supernatant, and extracted protein was immunoblotted with COX-2–specific Ab. B, cells were stimulated with 0.2 ng/ml of IL-1β for the indicated times. COX-2 levels in the cell lysates were determined by immunoblotting. The PGE_2 production values shown are the averages ± S.E. of three independent experiments, and the COX-2 protein values are representative of three independent experiments.

ited by pretreatment with NS398, PD98059, or SB203580, but the inhibition of both mucin syntheses was overcome strongly by PGE$_2$ treatment (Fig. 8B).

Discussion

In this study, we investigated whether IL-1β induces mucin synthesis via MAPKs and PGE$_2$ in airway epithelial cells. Our results show that not only does IL-1β increase COX-2 expression and PGE$_2$ synthesis but it also increases MUC2 and MUC5AC expression. Moreover, treatment with PGE$_2$ increased MUC2 and MUC5AC expression, demonstrating a link between these species and the COX-2 signaling pathway. Moreover, the addition of the MAPK kinase inhibitor PD98059 and the p38 inhibitor SB203580 abrogated the effect of IL-1β on MUC2 and MUC5AC production and on COX-2 expression. These findings provide evidence that IL-1β induces MUC2 and MUC5AC production through a MAPK- and COX-2–dependent pathway. From these results, we infer that MAPK activation induced by IL-1β in NCI-H292 cells is necessary for COX-2 expression, for enhanced PGE$_2$ production, and for the increased mucin synthesis of these cells.

The COX-2 selective inhibitor NS398 blocked MUC2 and MUC5AC protein expression induced by IL-1β, suggesting that COX-2 and its product PGE$_2$ are involved in MUC2 and MUC5AC production. In addition, PGE$_2$ was found to markedly induce MUC2 and MUC5AC production, suggesting that PGE$_2$ directly induces mucin gene and protein production. The mechanism by which PGE$_2$ acts on mucin gene and protein expression in NCI-H292 cells requires discussion. We do not rule out the possibility that EP mediates PGE$_2$-treated mucin production. Recent studies have shown that EPs are expressed in human airway epithelial cells (Mukhopadhyay et al., 1999). Several studies have also described the functional roles of these receptors in epithelial cells (Takafuji et al., 2000; Tavakoli et al., 2001). Moreover, a signal via EP was also shown to stimulate mucin secretion (Belley and Chadee, 1999; Takahashi et al., 1999). Further studies are necessary to determine the exact role of EP in association with IL-1β–induced mucin production by NCI-H292 cells.

Glucocorticoids are the drugs of choice for the management of the inflammatory processes of asthma. Although current pharmacological approaches to airway mucus production are limited, glucocorticoids seem to be the most effective of the

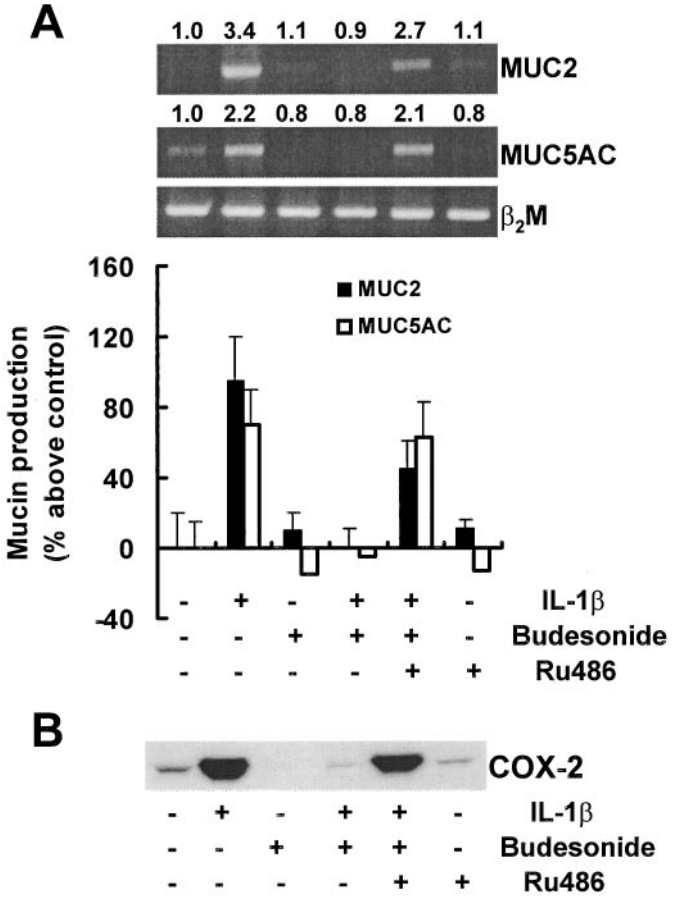

Fig. 3. Effect of budesonide on mucin gene, protein synthesis, and COX-2 expression as induced by IL-1β. Vehicle or budesonide (1 nM) or RU486 (10 nM) was added to cell culture 1 h before the addition of 0.2 ng/ml of IL-1β. A, total RNA was isolated, and MUC2 and MUC5AC mRNA levels were analyzed by RT-PCR. MUC2 and MUC5AC protein levels were determined as described under *Materials and Methods*. B, COX-2 levels in cell lysates were analyzed by immunoblotting. The mucin mRNA and COX-2 protein levels shown are representative of three independent experiments, and the mucin protein values shown are the averages ± S.E. of three independent experiments.

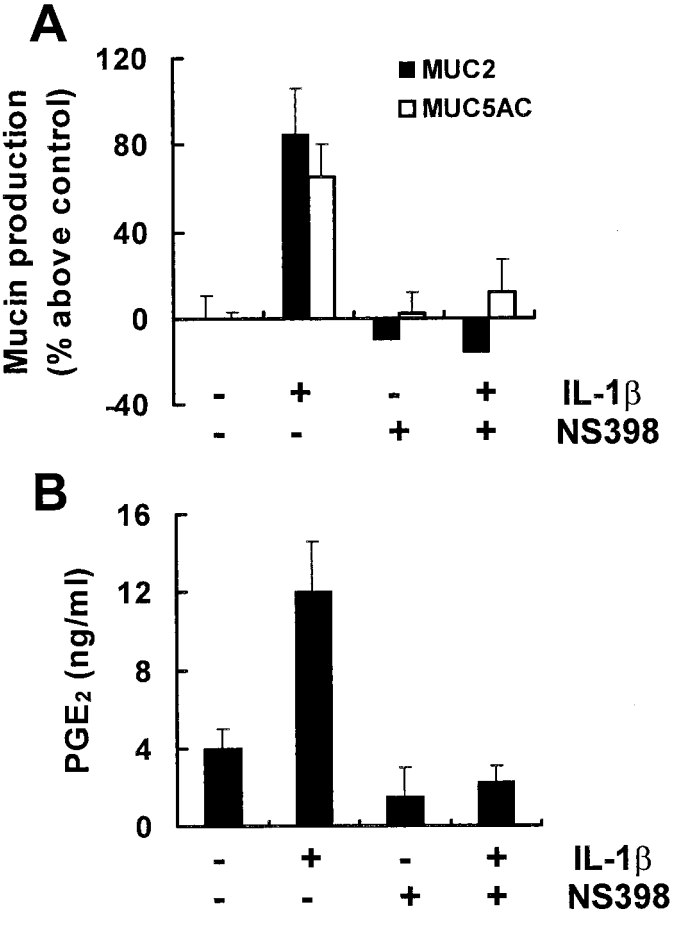

Fig. 4. The effects of NS398 on the synthesis of mucin protein, and the production of PGE$_2$. Vehicle or 5 μM NS398 was added to the cell culture 1 h before adding 0.2 ng/ml of IL-1β, as indicated. A, mucin levels were determined 8 h later in whole-cell lysates by immunoassay. B, PGE$_2$ release was measured in the supernatant. Values of mucin protein and PGE$_2$ release are the averages ± S.E. of four independent experiments.

relatively few useful drugs available. Glucocorticoids regulate the transcription of responsiveness genes via glucocorticoid receptor in the cytoplasm (Beato et al., 1995). They also constitute a range of effective drugs and have been used to reduce mucin production in vitro and in vivo (Kai et al., 1996). Moreover, budesonide is a glucocorticoid, and from the results of this study, we speculate that budesonide inhibits mucin production, thereby inhibiting COX-2 expression and PGE_2 production. Therefore, we examined the mRNA levels of MUC2 and MUC5AC after budesonide treatment. Surprisingly, we found that a very low concentration (1 nM) of budesonide attenuated the mRNA and protein levels of both MUC2 and MUC5AC and that budesonide suppressed IL-1β–induced COX-2 expression. Moreover, these inhibitory effects of budesonide were overcome by pretreating NCI-H292 cells with the glucocorticoid receptor antagonist RU486. These results suggest that the glucocorticoid receptor could be involved with IL-1β–induced MUC2 and MUC5AC production. In addition, PGE_2 overcame the inhibitory effects of budesonide on IL-1β–induced mucin production (data not shown), suggesting that PGE_2 induces mucin production directly.

Although we previously showed that IL-1β up-regulates

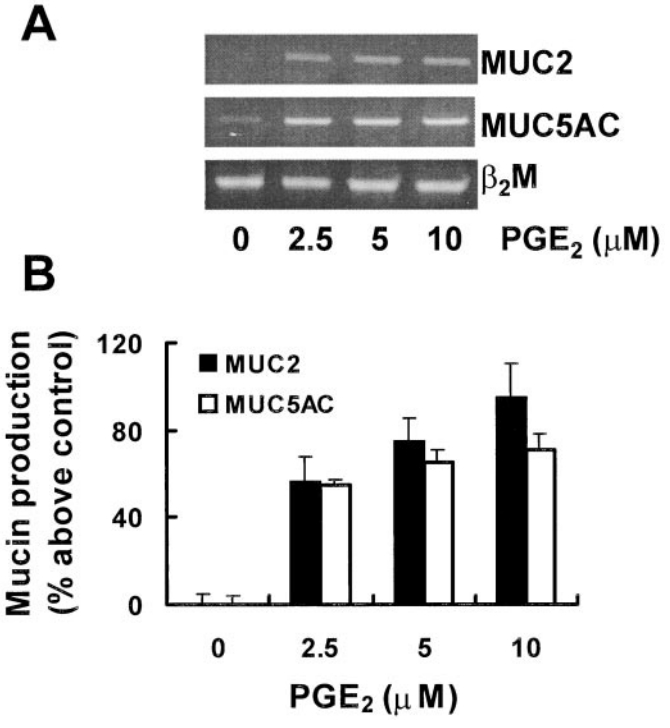

Fig. 5. Action of PGE_2 on mucin gene and protein synthesis. PGE_2 was added to NCI-H292 cells for 8 h at the indicated concentrations. A, total RNA was isolated, and MUC2 and MUC5AC mRNA levels were analyzed by RT-PCR. B, MUC2 and MUC5AC protein levels were determined as described under *Materials and Methods*. The mucin mRNA levels shown are representative of three independent experiments, and the mucin protein values given are averages ± S.E. of four independent experiments.

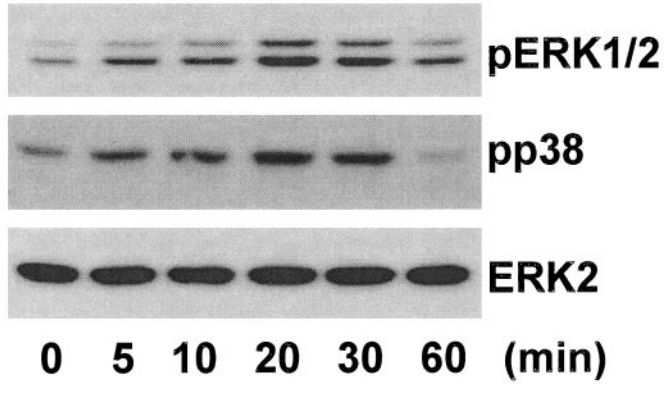

Fig. 6. Phosphorylation of ERK and p38 induced by IL-1β. NCI-H292 cells were stimulated for the indicated times with 0.2 ng/ml of IL-1β. Cells were lysed, and samples were analyzed by Western blot. Equal amounts of proteins were loaded, and ERK and p38 phosphorylation was detected by immunoblot using phosphospecific Abs. The MAPK phosphorylation levels indicated are representative of four independent experiments.

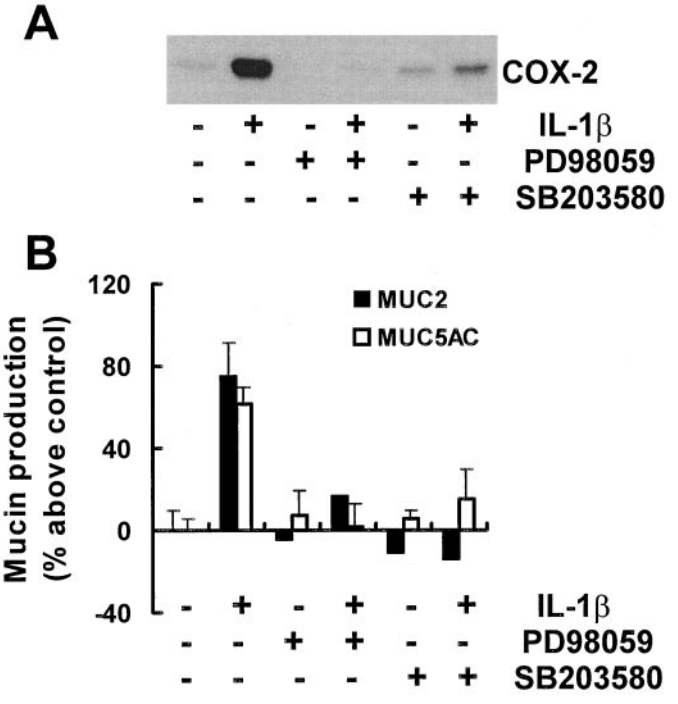

Fig. 7. Effect of MAPK inhibitor on IL-1β–induced COX-2 expression and mucin synthesis. NCI-H292 cells were pretreated with 50 μM PD98059 or 5 μM SB203580 for 1 h and then stimulated for 8 h with IL-1β. A, the COX-2 content of the cell lysates was analyzed by Western blot. The COX-2 protein levels shown are representative of three independent experiments. B, MUC2 and MUC5AC protein levels were quantified as described under *Materials and Methods*. The mucin protein values quoted are the averages ± S.E. of five independent experiments.

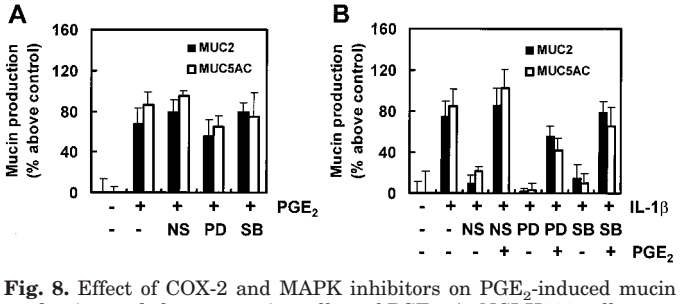

Fig. 8. Effect of COX-2 and MAPK inhibitors on PGE_2-induced mucin production and the overcoming effect of PGE_2. A, NCI-H292 cells were pretreated with 5 μM NS398 (NS), 50 μM PD98059 (PD), or 5 μM SB203580 (SB) for 1 h and then stimulated for 12 h with 5 μM PGE_2. B, cells were treated with 0.2 ng/ml of IL-1β in the presence or in the absence of various inhibitors for 6 h and then restimulated with 5 μM PGE_2 for 6 h. MUC2 and MUC5AC protein levels were determined as described under *Materials and Methods*. The mucin protein values given are the averages ± S.E. of two independent experiments.

the MUC2 gene and its protein (Kim et al., 2000), the specific signal transduction pathways and regulatory mechanisms involved are unknown. In addition, despite the extensive studies conducted on mucin, signaling events upstream of its activation are poorly understood. MAPKs have been suggested to be important molecules in the signal transduction of mucin production (Meerzaman et al., 2001) and epithelial differentiation (Taupin and Podolsky, 1999). Li et al. (1998) suggested that bacterial product-induced mucin production in epithelial cells is dependent on Ras/mitogen-activated protein kinase kinase/ERK. Takeyama et al. (2000) showed that oxidative stress induces mucin synthesis in airway epithelial cells via EGFR, which leads to the activation of the ERK signal transduction pathway. However, these studies found no direct link between ERK or p38 and mucin in IL-1β–induced NCI-H292 cells. The present study shows that two MAPKs, ERK and p38, exhibit similar activation time courses in response to IL-1β and that IL-1β treatment results in the transient activation of the ERK and p38 cascades, with maximal stimulation of both 20 min after treatment. It would seem from our studies that ERK and p38 play a significant role in the regulation of mucin production in NCI-H292 cells, because the IL-1β–induced synthesis of MUC2 and MUC5AC production is strongly inhibited by drugs that prevent the activation of the ERK cascade (PD98059) or the p38 cascade (SB203580). Interestingly, the inhibitory effects of PD98059 and SB203580 on IL-1β–induced mucin synthesis, and this correlated with their ability to suppress IL-1β–induced COX-2 expression because both of these MAPK inhibitors almost completely suppressed IL-1β–induced COX-2 expression, although the effect of PD98059 was stronger than that of SB203580. These observations support the possibility that both the ERK and p38 signaling pathways play an important role in mediating increased COX-2 expression and the subsequent synthesis of MUC2 and MUC5AC.

Cytokines are a central feature in airway inflammatory diseases, and IL-1β is one of the most important multifunctional proinflammatory cytokines that is known to have an active role in acute and chronic airway inflammation. However, relatively few reports are available on the effects of IL-1β and its signal transduction pathways on mucin production. Growth factor receptors could be involved in mucin secretion because hypersecretory diseases are associated with abnormal epithelial cell growth and proliferation, and the epidermal growth factor and its receptor (EGFR) are possible candidates. EGFR is expressed on the surface of human airway cells and is probably related to mucin production in epithelial cells (Guzman et al., 1995). The role of EGFR and of its ligand in mucin production in the airway epithelium was clarified using NCI-H292 cells, because after the induction of EGFR by tumor necrosis factor-α, the subsequent stimulation of EGFR by its ligand resulted in MUC5AC production (Takeyama et al., 1999). In addition, it was reported that the induction of COX-2 by interferon-γ is in part mediated by the activation of the EGFR signaling pathway (Matsuura et al., 1999) and that EGFR blockade reduces baseline COX-2 expression and PGE_2 production (Coffey et al., 1997). However, additional studies are required to evaluate the relationship between COX-2 and IL-1β in the EGFR system.

Currently, there is no effective therapy for relieving the symptoms or halting the progression of these diseases. Present studies show that IL-1β acts as a regulator of mucin production via MAPK activation by up-regulating COX-2 expression and PGE_2 production in airway epithelial cells. Our findings provide a mechanism and a strategy for a therapy derived from the inhibition of PGE_2 production or in the regulation of the ERK or p38 signal pathways.

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