

Glucocorticoids Inhibit MUC5AC Production Induced by Transforming Growth Factor- α in Human Respiratory Cells

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ABSTRACT

Background: Mucus hypersecretion from airway epithelium is a characteristic feature of severe asthma. Glucocorticoids (GCs) may suppress mucus production and diminish the harmful airway obstruction. We investigated the ability of GCs to suppress mRNA expression and protein synthesis of a gene encoding mucin, *MUC5AC*, induced by transforming growth factor (TGF)- α in human mucoepidermoid carcinoma (NCI-H292) cells and the molecular mechanisms underlying the suppression.

Methods: We determined if GCs such as dexamethasone (DEX), budesonide (BUD), and fluticasone (FP) could suppress *MUC5AC* production induced by a combination of TGF- α and double-strand RNA, polyinosinic-polycytidylic acid (polyI:C). *MUC5AC* mRNA expression and *MUC5AC* protein production were evaluated. The signaling pathways activated by TGF- α and their inhibition by GCs were tested using a phosphoprotein assay and *MUC5AC* promoter assay.

Results: DEX significantly suppressed the expression of *MUC5AC* mRNA and *MUC5AC* protein induced by TGF- α . The activation of the *MUC5AC* promoter by TGF- α was significantly inhibited by DEX. DEX did not affect activation of downstream pathways of the EGF receptor or mRNA stability of *MUC5AC* transcripts. DEX, BUD, and FP suppressed *MUC5AC* protein expression induced by a combination of TGF- α and polyI:C in a dose-dependent manner.

Conclusions: GCs inhibited *MUC5AC* production induced by TGF- α alone or a combination of TGF- α and polyI:C; the repression may be mediated at the transcriptional but not post-transcriptional level.

KEY WORDS

asthma, glucocorticoids, mucin, transcription, viral infection

INTRODUCTION

Excessive mucus production, which contributes to sputum accumulation and resultant airway obstruction, is a major clinical manifestation of chronic airway inflammatory diseases such as asthma. As is observed in patients with asthma, goblet cell hyperplasia is accompanied by changes in stored and secreted mucin, which leads to mucus hypersecretion.^{1,2} Recent studies have revealed an important role of the

epidermal growth factor receptor (EGFR) and its ligands in goblet cell hyperplasia in asthma patients.³⁻⁵ The critical ligand for the EGFR is transforming growth factor (TGF)- α , which is released from neutrophils or from the airway epithelium itself. An increasing body of evidence indicates that EGFR activation plays an essential role in asthma pathogenesis.⁴

Viral infection by viruses such as human rhinovirus, respiratory syncytial virus, influenza virus, and

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human metapneumovirus induces hypersecretion of mucus and exacerbates asthma.^{6,7} Among such viruses, certain RNA viruses synthesize double-stranded (ds) RNA during replication in infected cells.⁸ In a previous study, we have shown that a synthetic analog of viral dsRNA, polyinosinic-polycytidylic acid (polyI:C), does not induce the production of the mucin MUC5AC itself, but synergistically induces it in combination with TGF- α .⁸ These findings may help explain the excessive production of mucus in asthmatic patients during viral infection.

Glucocorticoids (GCs) are first-line drugs for controlling airway inflammation in asthmatic patients. GCs can suppress the mucus hypersecretion associated with airway inflammation and alleviate the associated detrimental clinical manifestations during an asthmatic attack.³ The mechanism of GC-mediated inhibition of mucus hypersecretion is mainly thought to be secondary to the anti-inflammatory properties of GCs. Alternatively, GCs may inhibit mucus production by their direct action on airway epithelial cells. In fact, it has been demonstrated that dexamethasone (DEX) inhibits the expression and production of MUC2 and MUC5AC in cells of the lung cancer cell line NCI-H292⁹ in a resting state. DEX has been shown to repress MUC5AC expression at the transcriptional level.¹⁰ Previous studies have investigated the effect of GCs on unstimulated epithelial cells or tissues.^{9,10} Recently, it has been shown that DEX can inhibit expression of *MUC5AC* mRNA induced by TGF- α in NCI-H292 cells.¹¹ However, little is known about the effect of GCs on mucin production induced by stimuli such as growth factors and viral infection, which mimic airway inflammation.

We hypothesized that the production of MUC5AC mucin by TGF- α and polyI:C in human airway epithelial cells (NCI-H292) could be repressed with DEX or inhaled GCs, including budesonide (BUD) and fluticasone (FP), which are used in asthma treatment. In the present study, we investigated the mechanisms whereby DEX regulates mucin genes induced by TGF- α in NCI-H292 cells.

METHODS

CELL CULTURE AND STIMULATION

A human pulmonary mucoepidermoid carcinoma cell line (NCI-H292) was maintained in RPMI 1640 medium supplemented with 10% fetal bovine serum (FBS), penicillin (100 U/mL), and streptomycin (100 μ g/mL) at 37°C in a humidified atmosphere with 5% CO₂. NCI-H292 cells were seeded into 12-well plates for the ELISA and luciferase assay and into 6-cm dishes for mRNA analysis. Cells were grown until 70% confluence and were maintained overnight in serum-free medium before stimulation. The cells were exposed to TGF- α (R&D Systems, Minneapolis, MN, USA) at 4 or 40 ng/mL with or without polyI:C (Sigma-Aldrich, St. Louis, MO, USA) at 25 μ g/mL;

cells were pretreated with dexamethasone (DEX, 10⁻⁶ to 10⁻¹⁰ M) (Sigma-Aldrich), BUD (10⁻⁹ to 10⁻¹³ M) (Astra-Zeneca, Osaka, Japan), or FP (10⁻⁹ to 10⁻¹³ M) (Sigma-Aldrich).

ANALYSIS OF MUCIN BY ELISA

MUC5AC protein was measured as previously described.⁵ In brief, supernatants were collected 24 h after stimulation, and 50 μ L of each sample was incubated overnight with bicarbonate buffer (50 μ L) at 40°C in a 96-well plate (Nunc A/S, Roskilde, Denmark). Plates were washed 3 times with PBS and blocked with 2% bovine serum albumin (BSA) for 1 h at 37°C. The plates were again washed 3 times with PBS and then incubated with 50 μ L of mouse monoclonal anti-MUC5AC antibody (1 : 100) (Lab Vision-Neomarkers, Fremont, CA, USA), which was diluted with PBS containing 0.05% Tween 20 and dispensed into each well. After 1 h, the plates were washed 3 times with PBS, and 100 μ L of horseradish peroxidase-sheep anti-mouse IgG conjugate (1 : 10,000) (Amersham Biosciences, Buckinghamshire, UK) was added to each well. After 1 h, the plates were washed 3 times with PBS. Color was developed with 3,3',5,5'-tetramethylbenzidine (TMB) peroxidase solution (Kirkegaard & Perry Laboratories, Gaithersburg, MD, USA), and the reaction was stopped with 1 M H₂SO₄. The data are expressed as fold induction on the same experimental day as mucin production with cell passage in NCI-H292 cells.

REAL-TIME QUANTITATIVE PCR ANALYSIS

The expression of *MUC5AC* mRNA in NCI-H292 cells was determined by reverse transcription (RT), followed by real-time quantitative PCR. Total RNA was extracted from lysates of NCI-H292 cells by using Iso-gen (Nippon Gene, Tokyo, Japan) at 12 h after stimulation. Reverse transcription was performed with 1 μ g of total RNA and oligo (dT) primers in a 25- μ L reaction mixture using the High Capacity cDNA Reverse Transcription Kit, according to the manufacturer's protocol (Applied Biosystems, Branchburg, NJ, USA). The sequences of the specific primer sets that were used for PCR are listed in Table 1.^{12,13} Real-time PCR was performed with an ABI Prism 7900HT sequence detection system (PE Applied Biosystems, Foster City, CA, USA) using the SYBR Green PCR Master Mix (Applied Biosystems). For *MUC5AC* and β -actin, initial denaturation was done at 95°C for 10 min, followed by 40 cycles of denaturation at 95°C for 15 s, and annealing and extension at 60°C for 1 min. The threshold cycle (CT) was recorded for each sample to reflect the level of mRNA expression. A validation experiment confirmed the linear dependence of the CT value on the concentrations of *MUC5AC* and β -actin and the consistency of Δ CT (Δ CT = mean CT β -actin - mean CT *MUC5AC*) in a given sample at different RNA concentrations. Therefore, Δ CT was used

Table 1 Primers used for quantitative real-time polymerase chain reaction analysis of gene expression

Target mRNA	Forward Primer (5' to 3')	Reverse Primer (3' to 5')
MUC5AC	TCA CAG CCG GGT ACG CGT TGG CAC AAG TGG	TGC TAT GCC CTG TGT AGC CAG GAC TGC
β -actin	GTG GGG CGC CCC AGG CAC CA	CTC CTT AAT GTC ACG CAC GAT TTC
EGFR	TGC GTC TCT TGC CGG AAT	GGC TCA CCC TCC AGA AGG TT

Abbreviations: mRNA, messenger RNA; EGFR, epidermal growth factor receptor.

as an indicator of relative mRNA expression. To determine the effects of different stimuli on *MUC5AC* gene expression compared with unstimulated cells, $\Delta\Delta CT$ was calculated ($\Delta\Delta CT = \Delta CT_{\text{stimulus}} - \Delta CT_{\text{unstimulated cells}}$). *MUC5AC* mRNA was indexed to β -actin using the formula $1/(2^{\Delta\Delta CT}) \times 100\%$; $2^{\Delta\Delta CT}$ was calculated to demonstrate the fold change of *MUC5AC* gene expression in stimulated cells relative to that of unstimulated ones. The expression of *EGFR* mRNA in NCI-H292 cells was determined in the same manner.

REPORTER ASSAY FOR THE *MUC5AC* PROMOTER

To investigate the effects of DEX (10^{-7} to 10^{-9} M) on *MUC5AC* promoter activity induced by TGF- α , the human *MUC5AC* promoter region (1330 bp upstream of the transcriptional start site; pGL3-MUC5AC1330) was cloned from human genomic DNA into the pGL3-Basic luciferase plasmid (Promega, Madison, WI, USA) using standard DNA ligation techniques.¹⁴ The truncated forms pGL3-MUC5AC192 and pGL3-MUC5AC63, containing -192 and -63 bp upstream of the transcriptional start site, respectively, were also constructed by restriction enzyme digestion.

NCI-H292 (0.8×10^5) cells were seeded into 12-well plates and grown overnight in complete medium. At 60% confluence, cells were rinsed and incubated for 1 h with 1 mL of serum-free medium. Then, the cells were transfected with 4 μ g of pGL3-MUC5AC using 1.3 μ L of Fugene 6 (Roche Applied Science, Indianapolis, IN, USA) in 50 μ L of RPMI medium. After 12 h, the cells were stimulated with TGF- α (40 ng/mL). The cells were then lysed, and luciferase activity was determined using a Luciferase Assay Kit (Promega). The total protein concentration of samples was measured by spectrophotometry (Nanodrop, Wilmington, DE, USA) to adjust for variations in cell harvesting.

PHOSPHOPROTEIN ASSAY

Protein phosphorylation was determined using the Bioplex Phosphoprotein Assay (Bio-Rad, Hercules, CA, USA). Briefly, NCI-H292 cells (3.0×10^5) were seeded into 6-cm dishes and treated with TGF- α for 15 min. Protein lysates were prepared using a cell lysis kit (Bio-Rad); phosphorylated EGFR, ERK, p38MAPK, JNK, CREB, and NF- κ B were detected using the appropriate assay kit (Bio-Rad) and the Phosphoprotein Testing Reagent kit (Bio-Rad) according

to the manufacturer's protocol. Briefly, 50 μ L of cell lysate (adjusted to a protein concentration of 200-400 μ g/mL) was plated into a 96-well filter plate coated with the appropriate antibody-coupled beads and incubated overnight on a platform shaker at 300 rpm at room temperature. Then, 25 μ L of detection antibody (1 $\times$) was added and incubated for 30 min. Streptavidin-PE (50 μ L; 1 $\times$) was added and incubated for 10 min in the dark. The phosphoproteins of EGFR, MAPKs, CREB, and NF- κ B were analyzed by a Luminex 100TM analyzer (Bio-Rad).

STATISTICAL ANALYSIS

All data are expressed as the mean $\pm$ SD. Results were analyzed using the Student's *t*-test or ANOVA as appropriate. Analyses were performed with SPSS II software (SPSS Japan, Tokyo, Japan); *P* values less than 0.05 were considered significant.

RESULTS

DEXAMETHASONE INHIBITS *MUC5AC* mRNA EXPRESSION AND PROTEIN PRODUCTION

We first examined the effects of TGF- α on *MUC5AC* production in human mucoepidermoid NCI-H292 cells. Stimulation with TGF- α (40 ng/mL) significantly increased *MUC5AC* mRNA expression (Fig. 1A) and *MUC5AC* protein production (Fig. 1B) compared with that of unstimulated cells as was previously observed.⁸ We next investigated the effects of DEX (10^{-7} to 10^{-9} M) on *MUC5AC* production induced by TGF- α . DEX significantly suppressed *MUC5AC* mRNA expression and protein production induced by TGF- α (Fig. 1A, B) at 10^{-7} M, while it did not affect the basal level of *MUC5AC* mRNA or *MUC5AC* protein. The range of DEX concentrations used approximates the glucocorticoid levels in the airway epithelium microenvironment after aerosol delivery of glucocorticoids.¹⁵ The highest concentration (10^{-7} M) did not affect the viability of the cells (data not shown). The time-course study also demonstrated that DEX (10^{-7} M) completely inhibited TGF- α -induced *MUC5AC* mRNA expression from 12 to 36 h after stimulation (Fig. 1C). In addition, the protein production of *MUC5AC* was significantly inhibited in the presence of DEX from 24 to 48 h after stimulation (Fig. 1D). Taken together, these results clearly demonstrate that DEX can directly repress TGF- α -induced *MUC5AC* gene expression and protein production in airway cells without the presence of inflam-

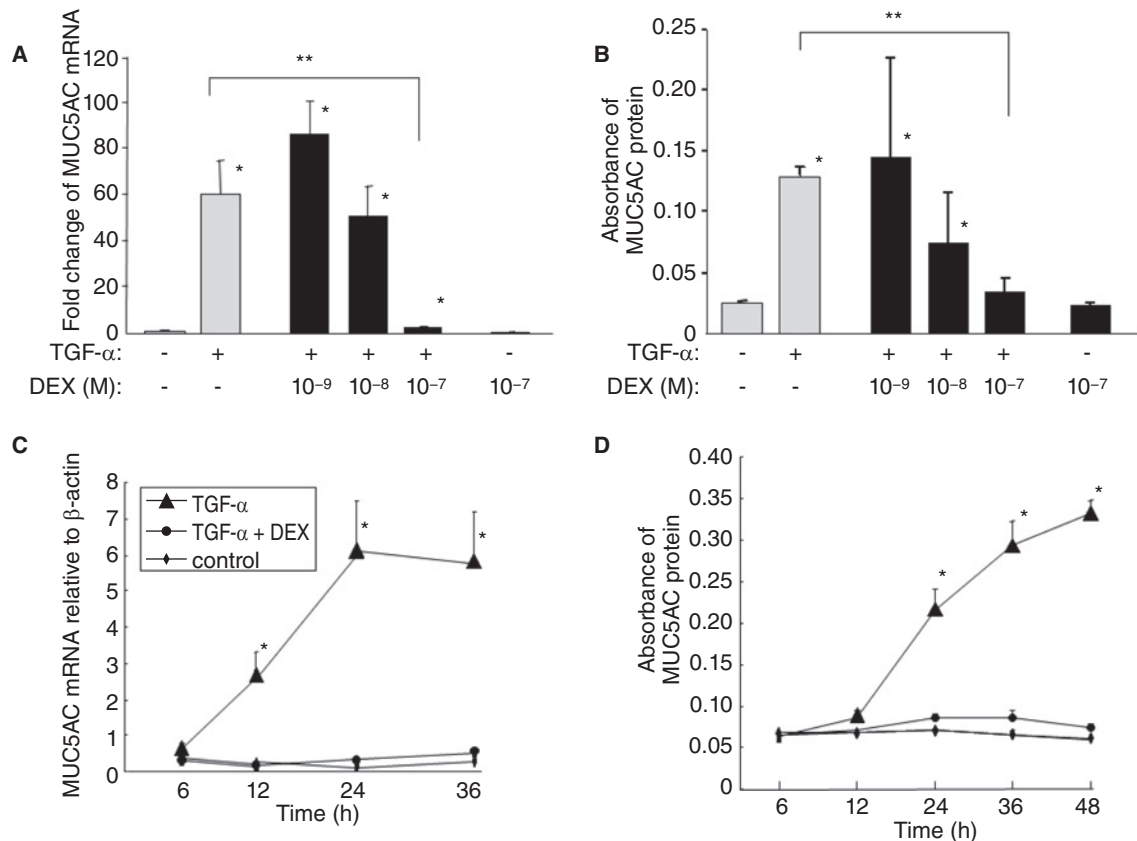


Fig. 1 *MUC5AC* mRNA Expression and Protein Production. (A) Effects of dexamethasone (DEX) on TGF- α -induced *MUC5AC* mRNA expression in NCI-H292 cells ($n = 6$). *MUC5AC* mRNA expression 24 h after incubation with TGF- α (40 ng/mL) and/or DEX (10^{-7} to 10^{-9} M) was analyzed by quantitative real-time PCR. Expression levels of *MUC5AC* mRNA were normalized to β -actin and expressed as the relative quantity to unstimulated controls. Data are presented as the mean $\pm$ SD of 6 independent experiments. (B) Effects of DEX on TGF- α -induced *MUC5AC* protein production in NCI-H292 cells ($n = 6$). Cells were incubated without or with TGF- α (40 ng/mL) and/or DEX (10^{-7} to 10^{-9} M) for 24 h. The concentration of *MUC5AC* protein in the medium was analyzed by ELISA. Data are presented as the mean $\pm$ SD of 6 independent experiments. (C and D) Time-course of the effect of DEX (10^{-7} M) on *MUC5AC* mRNA expression (C) and protein production (D) induced by TGF- α (40 ng/mL) in NCI-H292 cells ($n = 6$). Data were normalized to β -actin (C) and are shown as the mean $\pm$ SD of 6 independent experiments (C and D). DEX significantly suppressed TGF- α -induced *MUC5AC* mRNA expression and protein production at 12 h and 24 h after stimulation, respectively. * $P < 0.05$ compared with unstimulated control cells; ** $P < 0.05$.

matory cells.

DEX DOES NOT AFFECT *EGFR* mRNA EXPRESSION AND PHOSPHORYLATION OF *EGFR*

To obtain mechanistic insight into the inhibitory roles of DEX on TGF- α -induced *MUC5AC* production, we examined the involvement of *EGFR* activation in the inhibition of *MUC5AC* mucin production by DEX. *EGFR* has been shown to play a critical role in TGF- α -induced *MUC5AC* mucin production via ligand-dependent transactivation in NCI-H292 cells.⁵ We first determined *EGFR* mRNA expression by quantitative real-time RT-PCR. As shown in Figure 2A, DEX

did not affect *EGFR* mRNA expression, suggesting that DEX is not involved in the expression of *EGFR*. Since the phosphorylation of *EGFR* is essential for its signal transduction, we next tested phosphorylation of *EGFR* by the Bioplex Phosphoprotein Assay. TGF- α dramatically induced the phosphorylation of *EGFR* within 15 min after stimulation, but DEX did not affect the phosphorylation (Fig. 2B). These results suggest that DEX inhibits *MUC5AC* production through other mechanisms than inhibition of *EGFR* phosphorylation.

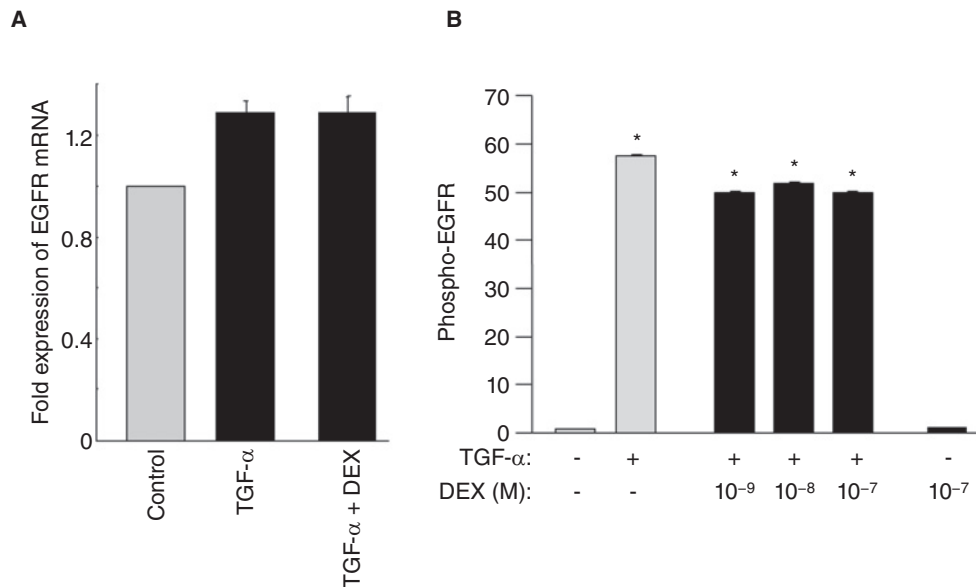


Fig. 2 Effect of DEX on *EGFR* mRNA Expression and Phospho-EGFR. **(A)** *EGFR* mRNA expression was determined by quantitative real-time PCR 12 h after incubation with TGF- α (40 ng/mL) and/or DEX (10^{-7} M). The fold expression compared to controls was obtained after normalization to β -actin. Data are presented as the mean $\pm$ SD of 6 independent experiments. **(B)** Quantification of phosphorylated EGFR in NCI-H292 cells treated with TGF- α with or without DEX. NCI-H292 cell lysates 15 min after TGF- α (40 ng/mL) treatment with or without DEX (10^{-7} to 10^{-9} M) were tested for the quantification of phosphorylated EGFR by using the Bio-Plex phosphoprotein assay kit using the Bio-Rad Luminex machine. The values plotted show phosphorylated EGFR levels expressed as the fold increase over unstimulated control ($n = 6$). * $P < 0.05$ compared with unstimulated control cells.

ERK AND CREB ARE ACTIVATED BY TGF- α , WHICH IS NOT SUPPRESSED BY DEX

Since EGFR activation is essential for TGF- α to induce MUC5AC but DEX did not affect phosphorylation of EGFR, we reasoned that DEX could inhibit downstream signaling pathways of EGFR. To determine which signaling pathways are activated by TGF- α , we investigated the phosphorylation of ERK1/2, p38MAPK, JNK, CREB, and NF- κ B, after treating cells with TGF- α , using the Bioplex Phosphoprotein Assay. We found that TGF- α activated ERK1/2 and CREB, but not p38MAPK, JNK, or NF- κ B (Fig. 3A). However, DEX (10^{-7} to 10^{-9} M) did not inhibit the phosphorylation of ERK1/2 or CREB (Fig. 3B, C) at 15 min after stimulation.

TRANSIENT TRANSFECTION AND LUCIFERASE ASSAY

Since DEX did not affect the activation of EGFR or its downstream signaling pathways, we next analyzed the effects of DEX directly on the transcriptional activity of the *MUC5AC* promoter. TGF- α (40 ng/mL) activated pGL3-MUC5AC1330 and pGL3-MUC5AC192 (Fig. 4A, B), but not pGL3-MUC5AC63 (Fig. 4C). DEX (10^{-8} or 10^{-9} M) significantly inhibited pGL3-MUC5AC1330 activation

(Fig. 4A). DEX (10^{-7} M) alone did not affect basal *MUC5AC* promoter activity (Fig. 4A), which is consistent with endogenous mRNA expression (Fig. 1A). Taken together with the data from the signaling pathways above, these data suggest that DEX specifically suppresses *MUC5AC* promoter activation rather than inhibiting signaling pathways. The inhibition is less apparent with pGL3-MUC5AC192 (Fig. 4B), indicating that inhibitory elements reside between the -192 to -1330 region in the promoter (Fig. 4). It is worth noting that the inhibition of pGL3-MUC5AC1330 activation by DEX is partial (Fig. 4A). These data suggest that DEX inhibits *MUC5AC* mRNA expression not only at a transcription level, but also through other mechanisms such as stability of mRNA, since endogenous mRNA levels were completely repressed by DEX (10^{-7} M) (Fig. 1A). We hypothesized that DEX might affect post-transcriptional regulation of MUC5AC. We analyzed the effect of DEX on the stability of *MUC5AC* transcripts. The decay in *MUC5AC* transcripts was monitored by the inhibition of transcription by actinomycin D (ACD) 8 h after stimulation by TGF- α (40 ng/mL) with or without DEX (10^{-7} M). The rates of *MUC5AC* mRNA degradation after ACD exposure were similar between cells with or without DEX (data not shown), suggesting that DEX

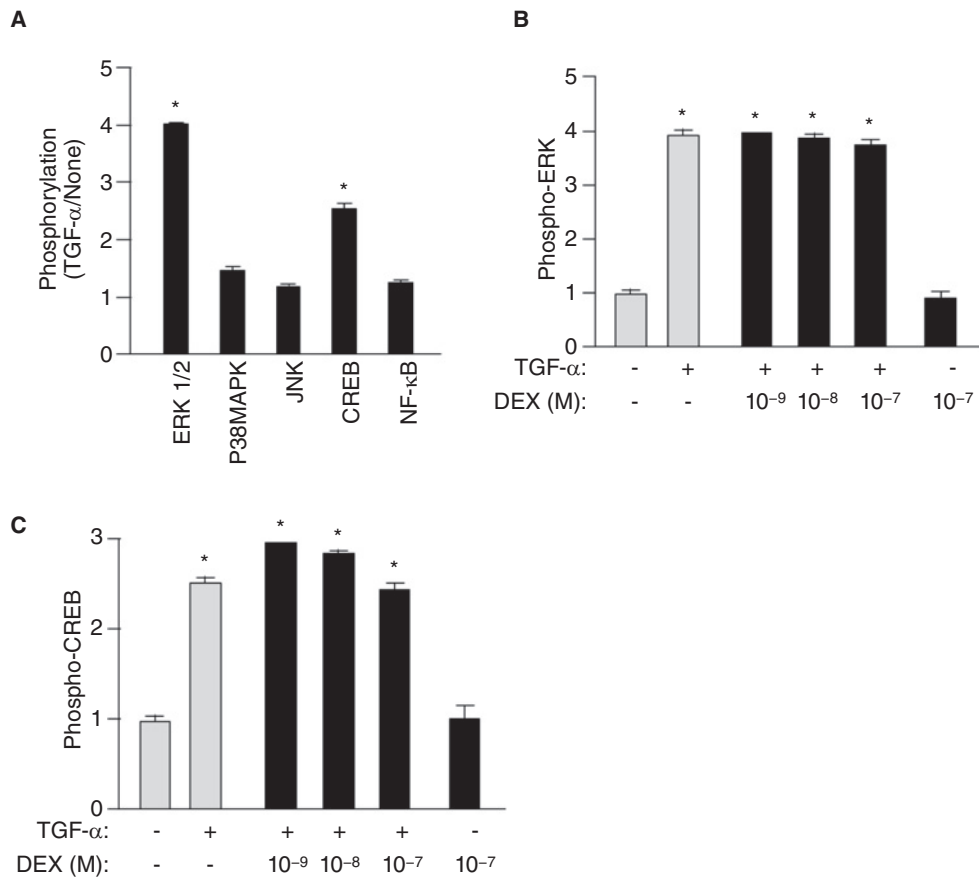


Fig. 3 Phosphorylation of ERK1/2, p38MAPK, JNK, CREB, and NF- κ B in the EGFR Signaling Pathway and the Effect of DEX. **(A)** Phosphorylated ERK1/2, p38MAPK, JNK, CREB, and NF- κ B in NCI-H292 cell lysates collected 15 min after incubation with TGF- α (40 ng/mL) were quantified by the Bio-Plex phosphoprotein assay kit. The values plotted show the phosphoprotein level expressed as the fold increase over unstimulated controls ($n = 6$). * $P < 0.01$ compared with unstimulated controls. **(B and C)** Effects of DEX (10^{-7} to 10^{-9} M) on the phosphorylation of CREB **(B)** and ERK1/2 **(C)** induced by TGF- α in NCI-H292 cells ($n = 6$).

did not affect the stability of *MUC5AC* mRNA, which is consistent with a previous study.¹⁰

DEX, BUD, AND FP INHIBIT *MUC5AC* mRNA AND PROTEIN PRODUCTION INDUCED BY TGF- α AND POLYI:C

In clinical situations, viral airway infection is known to cause enhanced mucin secretion. We have previously shown that polyI:C mimics viral RNA by synergistically inducing mucin secretion in the presence of TGF- α , while polyI:C alone does not induce *MUC5AC* expression.⁸ We investigated whether the synergistic induction could be repressed with steroids, including BUD and FP, which are commonly used in asthma treatment. As expected, polyI:C (25 μ g/mL) alone did not significantly induce *MUC5AC* protein production, but it strongly enhanced the effects of TGF- α (Fig. 5A). Co-incubation with DEX, BUD, or FP suppressed the production of *MUC5AC*

protein induced by TGF- α and polyI:C in H292 cells in a dose-dependent manner (Fig. 5A, B, C). These data demonstrate that DEX, BUD, and FP can directly repress *MUC5AC* mRNA expression or *MUC5AC* protein production in airway cells during inflammation and viral infection.

DISCUSSION

In the present study, we found that GCs suppressed *MUC5AC* gene expression and *MUC5AC* protein production induced by TGF- α in the epidermoid cell line NCI-H292. Furthermore, BUD and FP, commonly used clinically as inhaled GCs, also suppressed *MUC5AC* protein production induced by TGF- α and polyI:C, which represents the virus infection model.⁸ Activation of EGFR, the main receptor for TGF- α , and downstream signaling pathways were not inhibited by DEX, while *MUC5AC* promoter activity was suppressed. In addition, the stability of

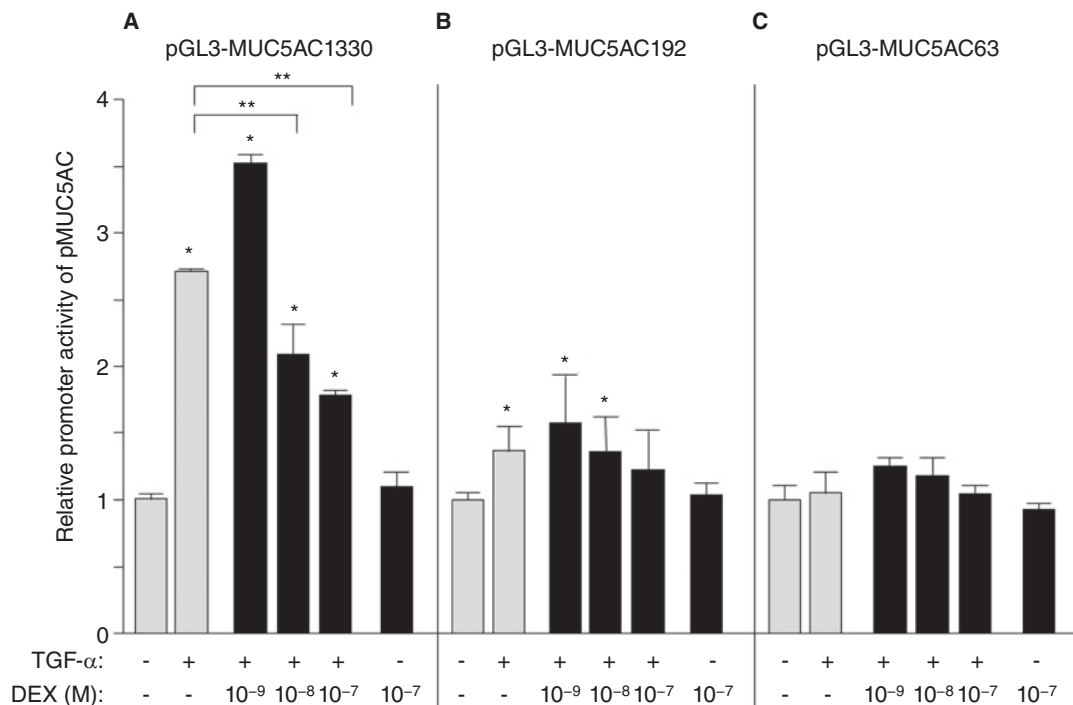


Fig. 4 Promoter Activity of MUC5AC. Effects of DEX (10^{-7} – 10^{-9} M) on TGF- α (40 ng/mL)-induced activation of the full-length (pGL-MUC5AC1330) and deletion mutants (pGL-MUC5AC192 and pGL-MUC5AC63) of the *MUC5AC* promoter in NCI-H292 cells. Cells were stimulated with TGF- α with or without DEX for 8 h before the lysates were collected. Data are shown as the mean $\pm$ SD of 6 independent experiments. * P < 0.05 compared with unstimulated control cells; ** P < 0.05.

MUC5AC mRNA was not affected by DEX. We concluded that the repression of *MUC5AC* expression by DEX in airway epithelial cells is at least in part regulated at the transcriptional level.

Mucin hypersecretion and goblet cell hyperplasia are characteristic features of airway obstructive diseases such as asthma.¹ Since hypersecretion of mucus is associated with abnormal epithelial cell growth and differentiation, both inflammatory mediators and growth factors may be involved in the stimulation of mucin production from goblet cells.³ It has been reported that GCs suppress *MUC5AC* mRNA expression, which was initially reported in NCI-H292 cells⁹ and later in another respiratory tract-derived cancer cell line, A549.¹⁶ In addition, Chen and coworkers found that DEX repressed *MUC5AC* mRNA expression in primary differentiated NHBE cells.¹⁰ However, these results were obtained from cells at steady-state equilibrium. In the present study, we have shown that the induction of *MUC5AC* mucin production and mRNA expression by TGF- α was suppressed by DEX at a concentration (10^{-7} M) that is within the range estimated for GC levels in human lung epithelium after aerosol delivery.¹⁷ This result is consistent with the previous observations made by Nishimoto *et al.*¹¹ We also showed that DEX did not inhibit *MUC5AC* mRNA expression and *MUC5AC* protein

production at steady-state equilibrium. This discrepancy from previous findings may be explained by the different methodology used. Chen *et al.* quantified *MUC5AC* and cyclophilin mRNA levels by northern blot analysis and did not measure *MUC5AC* protein production.¹⁰

Previous studies have demonstrated that both NCI-H292 and NHBE cells share key components of the signaling pathways downstream of EGFR, which are responsible for mucin production.¹⁸ Therefore, we hypothesized that NCI-H292 cells would provide a valid model of mucin production in normal cells, and thus, focused on investigating the mechanisms of the signaling pathway using NCI-H292 cells. We extensively studied the downstream signaling of EGFR and found that ERK and CREB were activated by TGF- α (Fig. 3A). These data are in partial agreement with previous findings reported by Hewson and coworkers that showed increased production of *MUC5AC* mucin protein after activation of the EGFR signaling pathway, which was exclusively dependent on MEK/ERK.¹⁴ However, we found that DEX did not affect the TGF- α -induced phosphorylation of ERK or CREB. The GC-glucocorticoid receptor (GR) complex can affect the activation of transcription factors, including NF- κ B. However, our results suggest that NF- κ B was not activated by TGF- α , indicating that DEX repres-

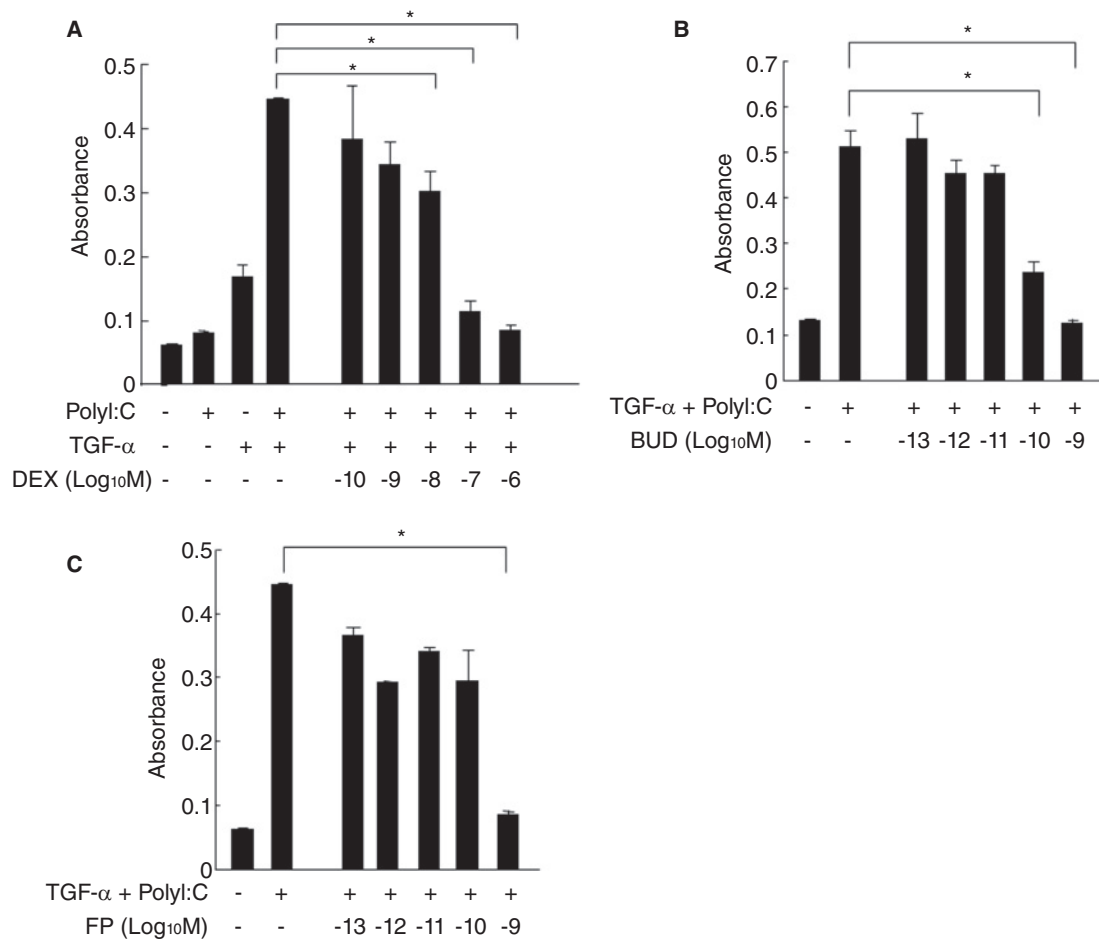


Fig. 5 Effect of DEX, BUD, and FP on MUC5AC Protein Production Induced by a Combination of TGF- α and PolyI:C. Effects of DEX (10^{-7} to 10^{-9} M) (**A**), BUD (10^{-9} to 10^{-13} M) (**B**), and FP (10^{-9} to 10^{-13} M) (**C**) on polyI:C-(75 μ g/mL) and TGF- α -(4 ng/mL)-induced MUC5AC protein production in NCI-H292 cells ($n = 6$). Data are shown as the mean $\pm$ SD of 6 independent experiments. * $P < 0.05$.

sion of MUC5AC is not mediated by *trans*-repression through NF- κ B.

In the present study, we used a promoter assay to demonstrate that DEX suppression of MUC5AC expression was partly mediated by sequences of *MUC5AC* between -1330 to -192 bp upstream of the transcriptional start site (TSS) (Fig. 4). We have not performed a detailed analysis to identify sequences with which GR binds the *MUC5AC* promoter. Our findings are consistent with those of a previous study where Chen and coworkers demonstrated that DEX transcriptionally repressed the *MUC5AC* promoter and GR bound to 2 GRE *cis*-sites (nucleotides -930 to -912 and -369 to -351 upstream of TSS) in the *MUC5AC* promoter.¹⁰

Post-transcriptional events are also important in the regulation of gene expression. Co-incubation of TGF- α with DEX significantly decreased *MUC5AC* mRNA expression. Furthermore, analysis of mRNA stability by quantitative real-time RT-PCR demonstrated that DEX did not alter the stability of *MUC5AC* mRNA.

Accordingly, DEX persistently suppressed *MUC5AC* mRNA expression induced by TGF- α without affecting the rate of *MUC5AC* mRNA degradation, suggesting that DEX-induced repression of MUC5AC in airway epithelial cells is not mediated at the post-transcriptional level.

We have previously demonstrated that a synthetic analog of viral dsRNA, polyI:C, synergistically enhances the induction of respiratory mucin MUC5AC through EGFR stimulation by TGF- α in human airway epithelial cells, both at the level of mRNA expression and protein production.⁸ Since the activation of EGFR has been implicated in the pathogenesis of asthma,^{4,5} these data suggest that viral infection might synergistically amplify respiratory mucin gene expression and protein production induced by growth factors that are involved in the pathogenesis of asthma.⁸ In the present study, we found that the synergistic induction of MUC5AC mucin production by TGF- α and polyI:C was inhibited by BUD and FP at a lower concentration than DEX, independent from in-

flammatory cells (Fig. 5B, C). These data might be relevant to the clinical situation where excessive mucin production caused by viral infection often triggers the exacerbation of asthma. Our data show that inhaled GCs can directly suppress the hypersecretion of mucus from airway epithelial cells caused by viral infection.

Receptor regulation has an important role in controlling the actions of several mediators. Yamamoto *et al.* demonstrated that IL-4-induced production of eotaxin-3 in the airway epithelium was enhanced by the upregulation of the IL-4 receptor by IFN- γ .¹⁹ In the present study, we evaluated whether the inhibitory effect of DEX was due to the downregulation of the EGFR or decreased EGFR phosphorylation. However, the downregulation of *EGFR* mRNA expression or inhibitory effects of phosphorylation of this receptor were not observed by co-incubation with DEX (Fig. 2).

The molecular mechanisms in the treatment of asthma by GCs are not completely understood, especially with respect to how specifically GCs repress gene expression in each cell type in tissues. An understanding of how GCs regulate mucin gene expression in cells exposed to inflammatory mediators or pathogens is important because inhaled GCs are used to treat patients with asthma where inflammation and mucin overproduction are characteristic findings. Our data suggest that steroids control *MUC5AC* gene expression by *cis*-repression in respiratory cells in the presence of TGF- α with or without dsRNA, which may provide a potential strategy for therapy.

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