

Niflumic Acid Inhibits Goblet Cell Degranulation in a Guinea Pig Asthma Model

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ABSTRACT

Background: Human Ca^{2+} -activated Cl ion channel 1 (hCLCA1) is expressed in goblet cell hyperplasia in the airway of asthmatics, and murine CLCA3 is associated with antigen-sensitized and IL-13-induced goblet cell metaplasia in mice. However, the role of CLCA in goblet cell degranulation is not fully investigated. Niflumic acid (NFA), a relatively specific CLCA inhibitor, inhibits goblet cell metaplasia, but the effect of NFA on goblet cell degranulation has not been determined in an asthma model.

Methods: Guinea pigs were sensitized with ovalbumin (OA) twice and then challenged with saline, OA, histamine, and one of the Ca^{2+} -dependent secretagogues, UTP. The PAS/AB-stained mucus area in the tracheal epithelium was measured with a computer image analysis system, and the morphology of mucus granules was examined by transmission electron microscopy. In the *in vitro* experiment, goblet cells cultured with IL-13 at the air-liquid interface were stimulated with UTP in the presence or absence of NFA, and the MUC5AC level in cell lysates was measured by ELISA.

Results: The mucus areas were smaller in the OA-, histamine-, and UTP-challenged animals than in the saline-challenged animals. NFA inhibited the decrease in mucus area and morphological changes in mucus granules. UTP caused swelling and exocytosis of mucus granules and MUC5AC secretion by cultured goblet cells, and NFA inhibited these changes.

Conclusions: NFA inhibited the secretory response of mucus granules in an asthma model, suggesting that CLCA may be associated with goblet cell degranulation and that CLCA inhibitors may be useful for the treatment of hypersecretion in asthma.

KEY WORDS

calcium-activated chloride channel, degranulation, goblet cells, MUC5AC, niflumic acid

INTRODUCTION

Goblet cell hyperplasia is one of the central features of asthma. Mucus hypersecretion due to goblet cell hyperplasia causes airway narrowing, loss of lung function, and, occasionally, asthmatic death.¹ However, the specific treatments for mucus hypersecretion are not currently available.

There is increasing evidence of a relationship between a calcium activated chloride channel and mucus production. hCLCA1 is expressed in goblet cell hyperplasia in the airway of asthmatics.² Nakanishi *et al.* reported finding that mCLCA3 (the mouse

counterpart of hCLCA1, alias gob-5) is associated with goblet cell metaplasia in a murine model of asthma.³ Long AJ reported observing decreased goblet cell metaplasia in ovalbumin-challenged *gob-5*-knockout mice.⁴ Th2 cytokines, especially IL-13, are central mediators of asthma,⁵ and IL-13 potently induces both hCLCA1 expression and goblet cell metaplasia by human airway epithelial cells.⁶ Niflumic acid (NFA) is a relatively specific CLCA inhibitor and has been shown to inhibit IL-13-induced goblet cell metaplasia in a murine model,⁷ in which mCLCA3 had been specifically induced by IL-13. These findings suggest that CLCA expression may be strongly asso-

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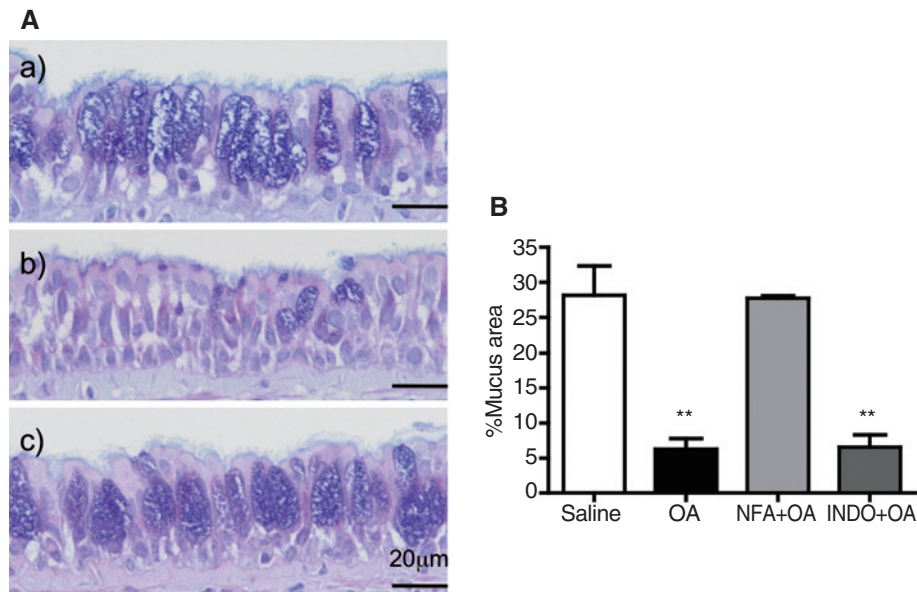


Fig. 1 Effect of niflumic acid (NFA) on goblet cell degranulation induced by OA challenge. **A)** Photomicrographs of tracheal epithelium (PAS/alcian blue stain). **a)** Saline challenge, **b)** OA challenge, **c)** NFA-pretreated, OA challenge. Scale bar = 20 μ m. **B)** Comparison of the PAS/alcian-blue-positive mucus area as a percentage of the total area of the tracheal epithelium measured with computer image analysis software. $n = 4-6$. ** $p < 0.01$, OA challenge or indomethacin (INDO)-pretreated, OA challenge vs. saline challenge or NFA-pretreated, OA challenge.

ciated with antigen-sensitized- or IL-13-induced goblet cell metaplasia. We previously reported finding that NFA inhibits the UTP-induced increase in chloride ion transport caused by IL-13-induced hCLCA1 expression in a human bronchial epithelial cell line.⁸ Although our findings suggested that hCLCA1 modulates chloride ion transport, the role of hCLCA1 in the goblet cell degranulation in the pathophysiology of asthma remains unclear. mCLCA3 has been found to be localized to mucus granule membranes by immunoelectron microscopy in mice,⁹ suggesting that mCLCA3 is related to goblet cell secretion. In the present study we investigated whether NFA affects the secretory response of the mucus granules in goblet cells in an asthma model. We used the OA sensitization model of asthma in guinea pigs *in vivo* and the culture model of goblet cell metaplasia induced by IL-13 *in vitro*, because the guinea pig model is suitable for assessing goblet cell degranulation *in vivo*,^{10,11} and because human IL-13 has the ability to induce guinea pig tracheal epithelial cells to differentiate into goblet cells *in vitro*.¹²

METHODS

OA CHALLENGE EXPERIMENT

All protocols were approved by the Animal Care and Use Committee of Tokyo Women's Medical University. Pathogen-free male Dunkin-Hartley guinea pigs

weighing between 250 and 300 g were sensitized by intraperitoneal injection of OA (70 mg/kg, Sigma Chemical Co., St. Louis, MO, USA) on the Day 1 and Day 8. On the Day 22, after anesthetizing the animals with pentobarbital (50 mg/kg) intraperitoneally, the larynx and upper trachea were exposed, and the trachea was incised just below the larynx, and an intubation tube was inserted 3 mm into the trachea, and the guinea pigs were artificially ventilated (frequency, 70 breaths/min; tidal volume, 10 ml/kg) with a constant-volume ventilator (model SN-480-7, Shinano Co., Tokyo, Japan). To investigate the effect of NFA on goblet cell degranulation in response to OA challenge, aerosols were generated with an ultrasonic nebulizer (Pulmo-Sonic model-25, De Vilbiss Co., Somerset, PA, USA) at 0.2 ml/min and delivered via the tracheal tube during mechanical ventilation. NFA (Sigma Chemical Co.) was dissolved in 0.4 M NaHCO₃ in 5% glucose.⁷ NFA (30 mg/kg) or vehicle solution was administered by inhalation for 2 min, and 10 min later, 5% OA or saline was administered by inhalation for 2 min. Because NFA has the ability to inhibit cyclooxygenase, we evaluated the effect of indomethacin (1 mg/kg) on OA-induced goblet cell degranulation by the same protocol as used for NFA.

Although we did not use an adjuvant for sensitization in this model, in preliminary experiment we confirmed an increase in the serum guinea pig OA-

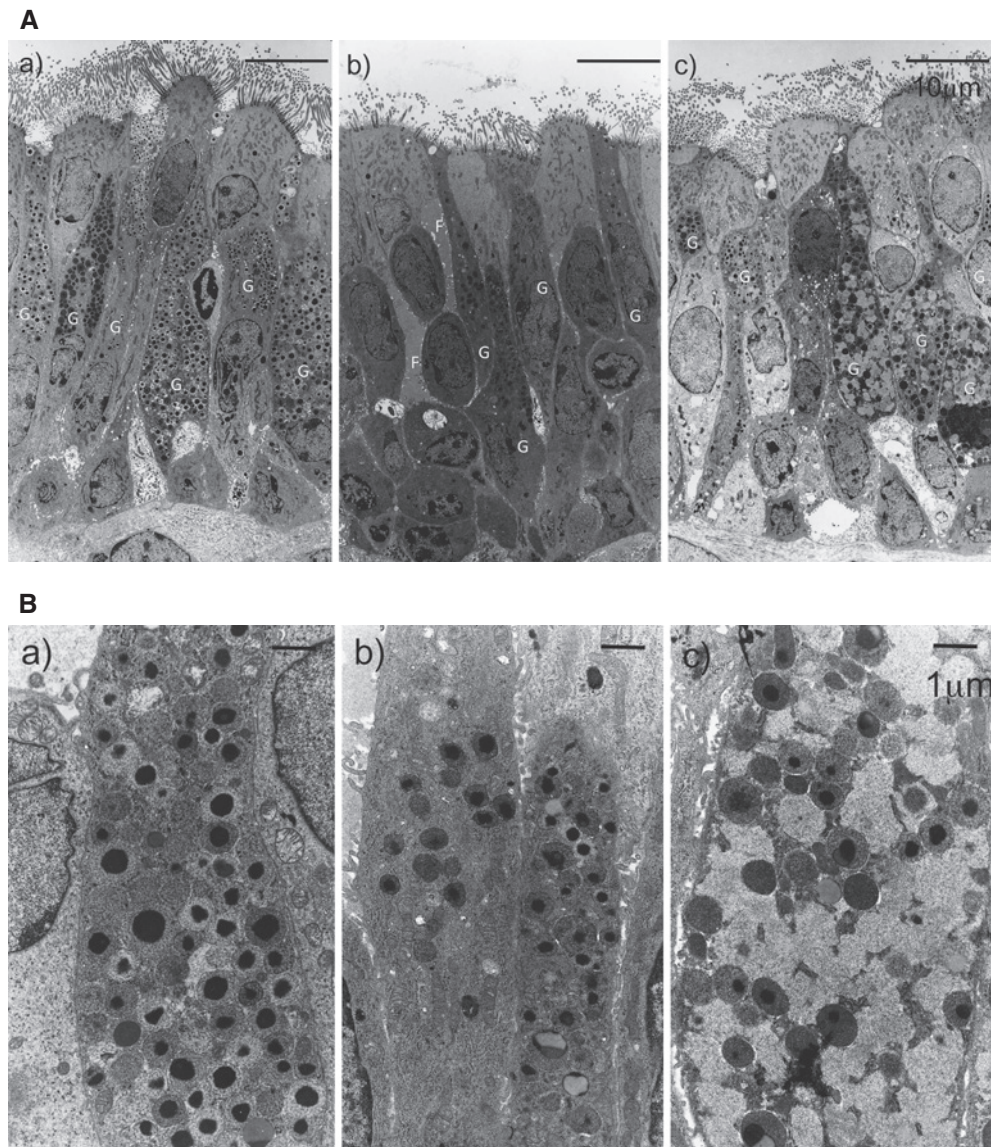


Fig. 2 Transmission electron micrographs of tracheal epithelium after OA challenge. **A)** Tracheal epithelium at low magnification. **a)** Saline challenge, **b)** OA challenge, **c)** NFA-pretreated, OA challenge. G: goblet cells. F: intercellular fluid. Scale bar = 10 μ m. After OA challenge, goblet cell (G) volume reduced by degranulation, and intercellular fluid (F) appeared (b). NFA inhibited these changes (c). **B)** Goblet cells at high magnification. **a)** Saline challenge, **b)** OA challenge, **c)** NFA-pretreated, OA challenge. Scale bar = 1 μ m. OA challenge induced disappearance of mucus granules. NFA-pretreated, OA-challenged goblet cells contained a large number of fused granules without dense cores.

specific IgE level on Day 22 in all sensitized animals by ELISA (Cusabio Biotech Co., Newark, DE, USA) (OA-sensitized vs. non-sensitized animals, 0.116 ± 0.007 vs. 0.064 ± 0.005 OD; $p < 0.0005$, $n = 7$). The results showed that the OA-sensitized guinea pigs ventilated with the respirator exhibited acute respiratory distress at ~ 1 min after OA challenge, but that the non-sensitized guinea pigs did not, indicating that sensitization with OA alone was sufficient to induce

an immediate asthmatic response in guinea pigs, the same as reported by others.¹³

For the morphological assessment, the trachea was removed and fixed for 24 h with 10% formal saline. The tracheal sections were then cut longitudinally in the coronal plane (5 μ m thick) and stained with periodic acid-Schiff (PAS) and alcian blue (AB) to demonstrate the acidic and neutral intracellular glycoprotein in the goblet cells. A semiautomatic image analysis

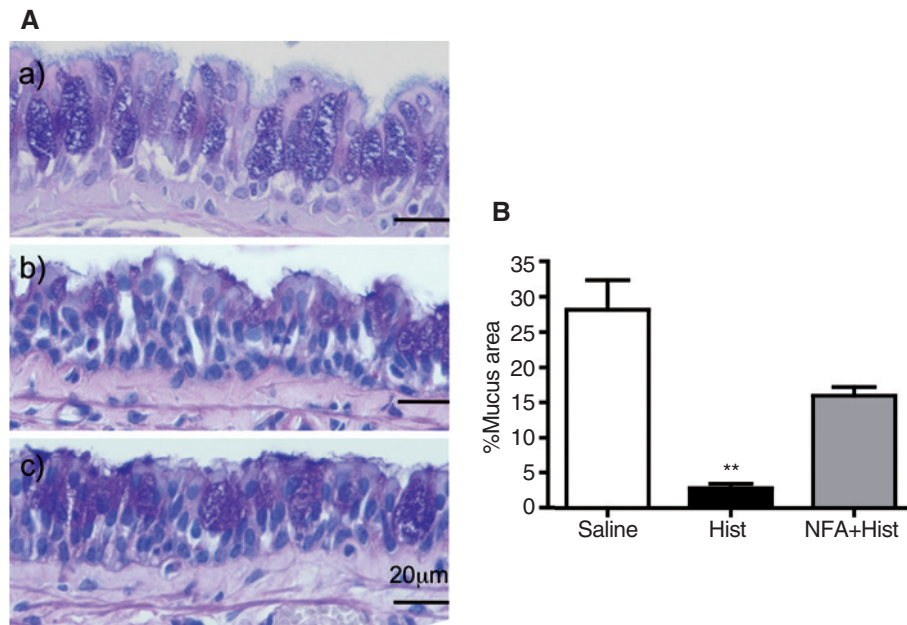


Fig. 3 Effect of niflumic acid (NFA) on goblet cell degranulation induced by histamine (Hist) challenge in OA-sensitized trachea. **A)** Photomicrographs of tracheal epithelium (PAS/alcian blue stain). **a)** Saline challenge, **b)** histamine challenge, **c)** NFA-pretreated, histamine challenge. Scale bar = 20 μ m. **B)** Comparison of the PAS/alcian-blue-positive mucus area as a percentage of the total area of the tracheal epithelium measured with computer image analysis software. $n = 4$, ** $p < 0.01$, histamine challenge vs. saline challenge or NFA-pretreated, histamine challenge.

system (Win Roof, Mitani Co., Fukui, Japan) was used to measure the PAS/AB-positive area and the total tracheal epithelial area, and the mucus area stained with PAS/AB was calculated as a percentage of the total area of tracheal epithelium. For electron-microscopic examination, the tissues were fixed with glutaraldehyde/osmium tetroxide and embedded in epon. The sections (with a silver interference color) were stained with lead citrate and uranyl acetate.

HISTAMINE CHALLENGE EXPERIMENT

Since in a previous study we reported that the histamine level in airway tissue increased after an OA challenge in the same guinea pig model of asthma used in the present study,¹⁴ next we investigated the effect of NFA on histamine-induced goblet cell degranulation in the OA-sensitized model. NFA or vehicle solution was administered by inhalation for 2 min, and 10 min later histamine (2 mg/ml) was administered by inhalation during 30 breaths as previously reported.¹⁴ The mucus area was measured as described above.

UTP CHALLENGE EXPERIMENT

Since UTP directly stimulates the P2Y2 receptor in airway epithelial cells and causes mucin secretion via a Ca^{2+} -dependent pathway,¹⁵⁻¹⁷ we used UTP as a se-

cretagogue to clarify the Ca^{2+} -dependent action of CLCA in goblet cells in this model. NFA or vehicle solution was administered by inhalation for 2 min, and 10 min later UTP (10^{-4} M) or saline was administered by inhalation for 5 min. The mucus area was assessed as described above.

In vitro UTP EXPERIMENT

IL-13-induced cultured goblet cells were used to clarify the mechanism of the direct effect of NFA on UTP-induced goblet cell degranulation *in vitro*. Tracheal epithelial cells from Dunkin-Hartley guinea pigs were isolated by digestion with 0.05% protease (type XIV, Sigma Chemical Co.) at 4°C overnight, and after pelleting (200 g, 10 min) the cells and suspending them in a 50 : 50 mixture of Dulbecco's modified Eagle medium (DMEM) and Ham's F12 medium (GIBCO, Grand Island, NY, USA) containing 5% fetal calf serum, they were plated at 2.5×10^5 cells per cm^2 onto polycarbonate inserts having a diameter of 12 mm, pore size of 0.4- μ m, and thickness of 10- μ m (Costar Transwell, Cambridge, MA, USA) and that had been coated with human placental collagen (type XVI, Sigma Chemical Co.). After 24 hours, the cells were cultured with serum-free, hormonally defined medium as previously reported,¹² and when they had reached confluence, the apical medium was removed,

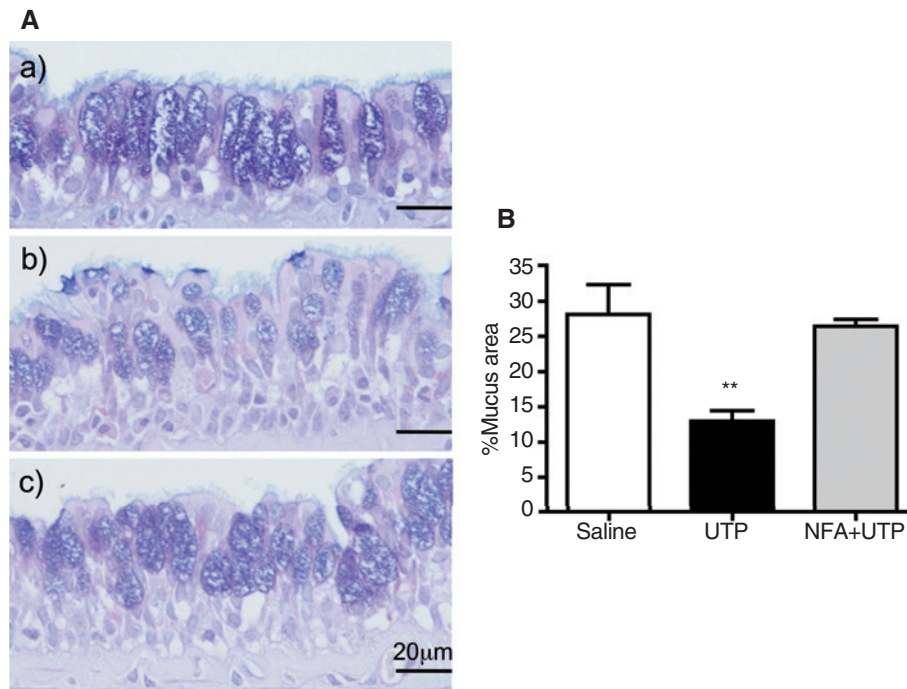


Fig. 4 Effect of niflumic acid (NFA) on goblet cell degranulation induced by UTP challenge in OA-sensitized trachea. **A)** Photomicrographs of tracheal epithelium (PAS/alcian blue stain). **a)** Saline challenge, **b)** UTP challenge, **c)** NFA-pretreated, UTP challenge. Scale bar = 20 μ m. **B)** Comparison of the PAS/alcian-blue-positive mucus area as a percentage of the total area of the tracheal epithelium measured with computer image analysis software. $n = 4$, ** $p < 0.01$, UTP challenge vs. saline challenge or NFA-pretreated, UTP challenge.

and the cells were fed from the basolateral side alone with 1 ml of medium containing human recombinant IL-13 (10 ng/ml, Biosource International, Camarillo, CA, USA). The cells were cultured at the air-liquid interface for 14 days with medium containing IL-13. On day 14 a large number of mature goblet cells were present as reported previously.¹²

The cells were washed with Hank's balanced salt solution (HBSS) containing 10 mM N-2-hydroxyethyl-piperazine-N'-2-ethane sulfonic acid (HEPES) (pH 7.4). A 0.2 ml volume of HBSS was added to the Transwell on the apical side and 1.0 ml of HBSS added on the basolateral side. The cells were preincubated in the presence or absence of NFA (10^{-4} M, apical side and basolateral side) for 20 min at 37°C, and then stimulated with UTP (10^{-4} M, apical side and basolateral side) for 20 min at 37°C. The cell lysate was used for MUC5AC protein analysis. Some cell sheets on the filters were used for morphological examination as previously reported.¹²

MUC5AC protein was measured by using ELISA as reported previously.¹² The cell lysate was prepared with phosphate buffered saline (PBS) at multiple dilutions, and 50 μ l of each sample was incubated with bicarbonate-carbonate buffer (50 μ l) at 40°C in a 96-

well plate (Nunc) until dry. The plates were washed three times with PBS and then incubated with 50 μ l of mouse monoclonal MUC5AC antibody (clone 45 M1, 1 : 100, New Markers, Fremont, CA, USA), which was diluted with PBS containing 0.05% Tween 20 (Sigma Chemical Co.). After 1 h, the wells were washed three times with PBS, and 100 μ l of horseradish peroxidase-goat anti-mouse IgG conjugate (1 : 10,000, Sigma Chemical Co.) was added. After 1 h, the plates were washed three times with PBS, color was developed with 3,3', 5,5'-tetramethylbenzidine (TMB) peroxidase solution (Kirkegaard & Perry Laboratories, Gaithersburg, MD, USA) and stopped with 1 M H_2SO_4 . Absorbance was read at 450 nm.

STATISTICS

All data are expressed as means $\pm$ SEM. Student's unpaired t-test was used for comparisons between two groups. One-way ANOVA was used for comparisons among three groups. Scheffe's F test was used to correct for multiple comparisons when statistical significance was identified by the ANOVA. A probability of <0.05 for the null hypothesis was accepted as indicating a statistically significant difference.

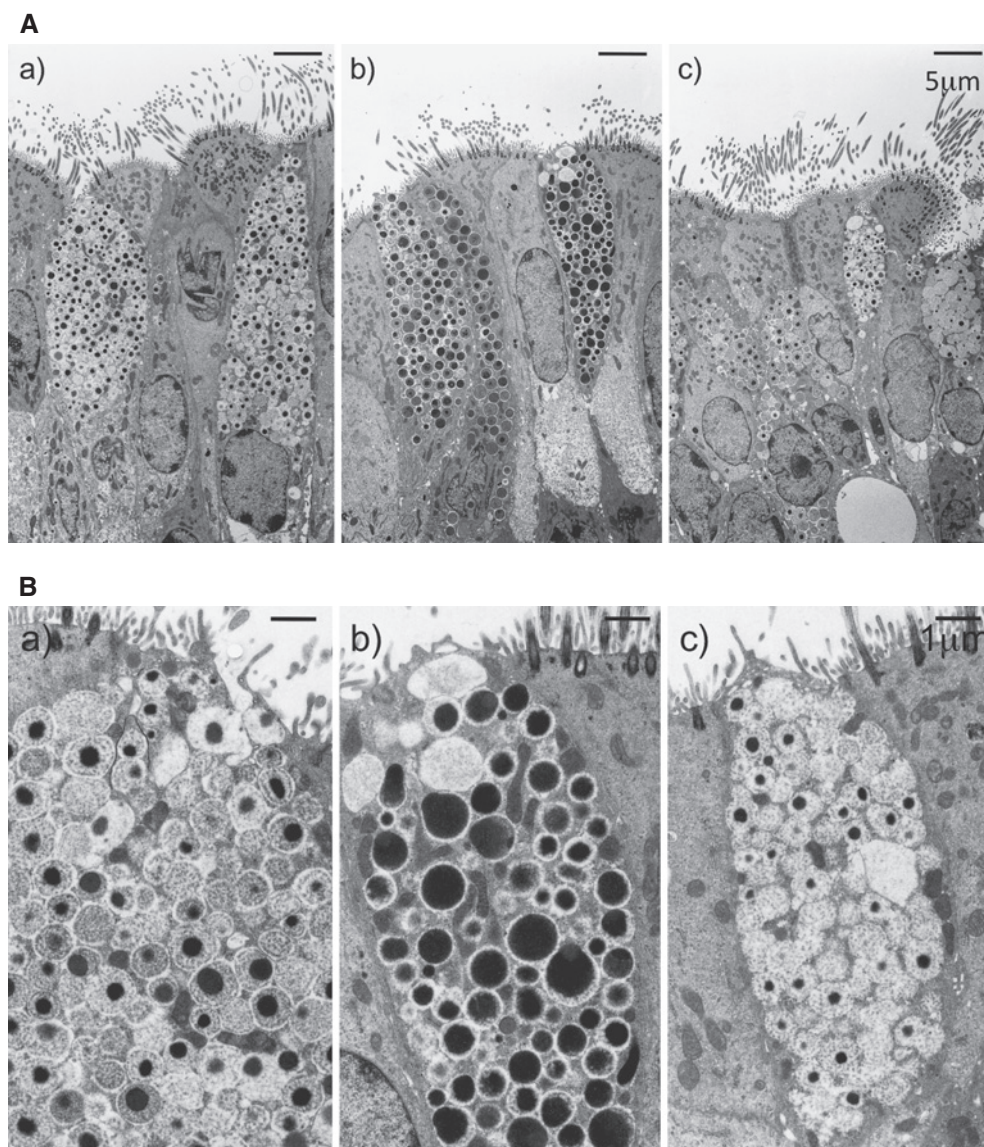


Fig. 5 Transmission electron micrographs of tracheal epithelium after UTP challenge. **A)** Tracheal epithelium at low magnification. **a)** Saline challenge, **b)** UTP challenge, **c)** NFA-pretreated, UTP challenge. Scale bar = 5 μ m. **B)** Goblet cells at high magnification. **a)** Saline challenge, **b)** UTP challenge, **c)** NFA-pretreated, UTP challenge. Scale bar = 1 μ m. Some of UTP-challenged goblet cells contained swollen granules containing a large dark area. In contrast, NFA-pretreated goblet cells contained the fused mucus granules containing a small dense core.

RESULTS

OA CHALLENGE EXPERIMENT

As shown in Figure 1A, OA challenge induced goblet cell degranulation in the OA-sensitized animals, and NFA inhibited the degranulation response. The area of PAS/AB-stained mucus in the tracheal epithelium calculated by computer image analysis was smaller in the OA-challenged animals than in the saline-challenged animals (OA $6.3 \pm 1.5\%$ vs. saline $28.1 \pm 4.2\%$, $n = 4-6$, $p < 0.01$, Fig. 1B). Pretreatment with

NFA inhibited the decrease in mucus area after OA-challenge (OA $6.3 \pm 1.5\%$ vs. NFA+OA $28.0 \pm 0.7\%$, $n = 4-6$, $p < 0.01$, Fig. 1B). By contrast, pretreatment with indomethacin did not prevent the decrease in mucus area after OA challenge ($6.4 \pm 1.8\%$, $n = 4$, Fig. 1B). TEM photographs of goblet cells after OA-challenge are shown in Figure 2. The goblet cells in the saline-challenged animals contained numerous mucus granules containing a dense core (Fig. 2Aa, Ba), whereas the goblet cells in the OA-challenged animals were smaller and contained only a small number of mucus

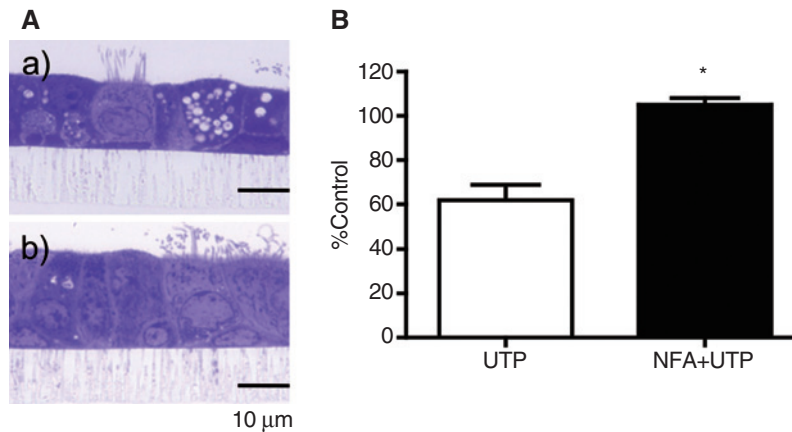


Fig. 6 Effect of NFA on UTP-induced mucus secretion by cultured tracheal epithelial cells. **A)** Photomicrographs of cultured tracheal epithelial cells (Toluidine blue stain). **a)** UTP-stimulated epithelial cells, **b)** NFA-pretreated, UTP-stimulated epithelial cells. Scale bar = 10 μm. UTP-stimulated cells contained numerous vacuoles, whereas NFA-pretreated, UTP-stimulated cells had few vacuoles. **B)** MUC5AC level in the cell lysate after UTP stimulation. $n = 4$. * $p < 0.05$, UTP-stimulated cells vs. NFA-pretreated, UTP-stimulated cells. Data are shown as a percentage of the level in the non-stimulated control cells.

granules. Thus, OA challenge had induced strong degranulation (Fig. 2Ab, Bb). In addition, intercellular fluid collection was observed in the OA-challenged epithelium. By contrast, after the OA challenge the NFA-pretreated goblet cells did not exhibit any change in cell size and contained a large number of mucus granules with or without a dense core (Fig. 2Ac, Bc).

HISTAMINE CHALLENGE EXPERIMENT

Histamine (2 mg/ml, 30 breaths) induced robust goblet cell degranulation and an increase in the intracellular space of epithelium in the OA-sensitized guinea pigs (Fig. 3A). The area of PAS/AB-stained mucus in the histamine-challenged animals was smaller, and pretreatment with NFA significantly inhibited the histamine-induced decrease in mucus area (histamine $2.8 \pm 0.6\%$ vs. NFA+histamine $16.0 \pm 1.2\%$, $n = 4$, $p < 0.01$, Fig. 3B).

UTP CHALLENGE EXPERIMENT

The area of PAS/AB-stained mucus in the UTP-challenged animals was smaller, and pretreatment with NFA significantly inhibited the decrease in mucus area induced by UTP (UTP $13.0 \pm 1.5\%$ vs. NFA+UTP $26.5 \pm 1.0\%$, $n = 4$, $p < 0.01$, Fig. 4). TEM photographs of goblet cells after UTP-challenge are shown in Figure 5. Some of the goblet cells in the UTP-challenged epithelium contained swollen mucus granules that contained a large dense core (Fig. 5Ab, Bb), whereas the NFA-pretreated UTP-challenged goblet cells contained mucus granules that had a small dense core (Fig. 5Ac, Bc), the same as the

saline-challenged goblet cells (Fig. 5Aa, Ba).

In vitro UTP EXPERIMENT

The tracheal epithelial cells cultured in the presence of IL-13 for 14 days contained a large number of mature goblet cells. After UTP stimulation, the number of mucus granules decreased, and light microscopy revealed many vacuoles in the goblet cells (Fig. 6Aa). NFA inhibited these changes (Fig. 6Ab). The MUC5AC level in the cell lysates was lower after UTP stimulation for 20 min, indicating that UTP induced a secretory response. NFA significantly inhibited the UTP-induced decrease in MUC5AC level (Fig. 6B).

The TEM photographs of the UTP-stimulated goblet cells showed swelling of the granules, exocytosis, and vacuole formation (Fig. 7a, b), whereas the mucus granules in the NFA-pretreated UTP-stimulated cells were not swollen (Fig. 7c).

DISCUSSION

The results of this study demonstrated that NFA inhibits antigen-, histamine- and UTP-stimulated goblet cell degranulation in a guinea pig asthma model, and the transmission electron microscopy findings demonstrated that NFA inhibits the morphological changes in mucus granules.

Since antigen sensitization induces CLCA expression, we speculated that NFA might have inhibited the goblet cell degranulation induced by OA challenge via a CLCA-dependent pathway. The TEM photographs (Fig. 2) of OA-challenged goblet cells showed loss of mucus granules and a decrease in cell size, suggesting that strong degranulation had occurred,

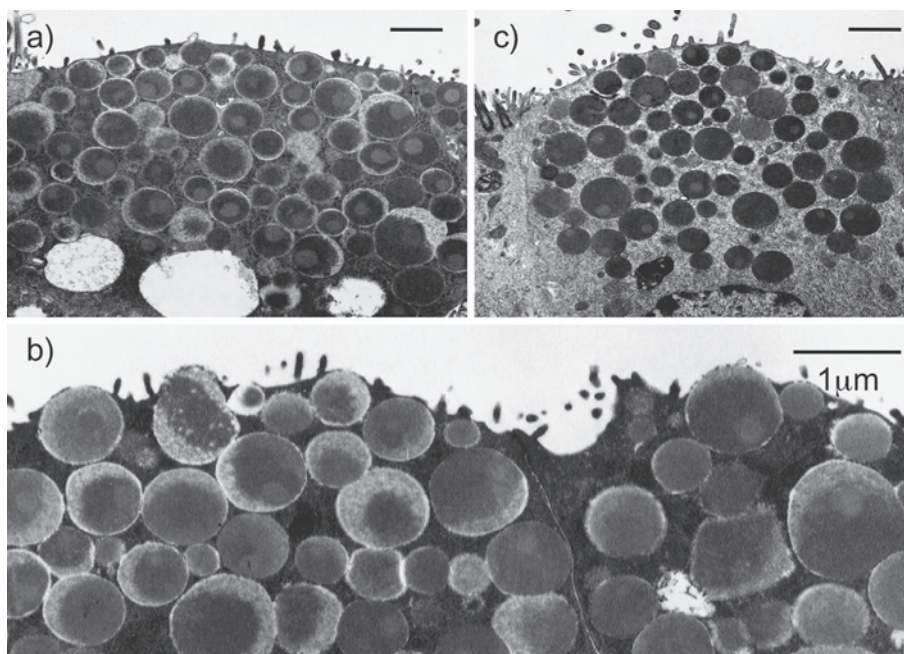


Fig. 7 Transmission electron micrographs of mucus granules in cultured goblet cells. **a)** , **b)** UTP-stimulated goblet cells, **c)** NFA-pretreated, UTP-stimulated goblet cells. Scale bar = 1 μ m. UTP-stimulated goblet cells contained swollen of granules and exhibited vacuole formation (**a**) and exocytosis (**b**). NFA-treated, UTP-stimulated goblet cells contained no swollen granules (**c**).

whereas NFA-pretreated, OA-challenged goblet cells retained a large number of mucus granules. Interestingly, the TEM photographs showed that some granules did not have a dense core. These findings suggested that NFA inhibits the secretory response by affecting mucus granules directly.

Antigen challenge causes the mast cells of sensitized animals to release chemical mediators such as histamine and cysteinyl-leukotrienes. These mediators are reported to induce goblet cell degranulation in guinea pigs.^{11,18,19} We previously reported that the histamine level of airway tissue increased after OA challenge in the same guinea pig asthma model.¹⁴ In the present study NFA inhibited histamine-induced goblet cell degranulation (Fig. 3), suggesting that NFA inhibits OA-challenge-induced goblet cell degranulation via a histamine-dependent pathway. However, it is still unclear whether agonists such as histamine or acetylcholine directly activate receptors on airway goblet cells or induce airway smooth-muscle contraction leading to ATP.²⁰ UTP as well as ATP is a well-known mediator that directly stimulates mucin secretion activated via P2Y2 receptors followed by intracellular Ca^{2+} elevation in airway goblet cells,¹⁵⁻¹⁷ and UTP could be used as a secretagogue with less effect on bronchospasm than hypertonic saline in asthma patients.²¹ Therefore, we next investigated the effect of NFA on UTP-induced goblet cell degranulation in OA-sensitized trachea to more clearly

identify the role of CLCA in Ca^{2+} -dependent secretory response.

As shown in Figure 4, Figure 5, NFA inhibited UTP-induced goblet cell degranulation. The TEM photographs showed swollen mucus granules containing a large dense core in UTP-stimulated goblet cells, and that NFA inhibited these morphological changes. The results of the *in vitro* experiment showed that NFA had a direct inhibitory effect on the secretory response of IL-13-induced goblet cells. As shown in Figure 6A, Figure 7, UTP induced swelling of mucus granules containing a large dense core, exocytosis, and vacuole formation in the cultured goblet cells, whereas NFA inhibited these changes. Since it has been reported that IL-13 induces hCLCA1 and mCLCA3 expression in human and murine goblet cells *in vitro* and that mCLCA3 is located in murine mucus granule membranes, the inhibitory effect of NFA on goblet cell degranulation might be associated with blockade of granule-located CLCA. Bertrand *et al.* showed that NFA inhibits ATP-stimulated exocytosis in colon cancer epithelial cell line, HT29-CL6E, which differentiates into a goblet-cell-like epithelium containing a large number of mucin granules in their apical cytoplasm.²² Gibson *et al.* reported that hCLCA1 and mCLCA3 are secreted, non-integral membrane proteins, not ion channels, and that they detected N-terminal cleavage products of hCLCA1 and mCLCA3 in bronchoalveolar lavage fluid from

asthma patients and OA-challenged mice.²³ Hamann M *et al.* demonstrated that hCLCA1 is not an ion channel *per se* but a modulator of anionic conduction of Ca²⁺-dependent chloride currents.²⁴ NFA is a non-steroidal anti-inflammatory agent that was originally determined to be a potent, reversible blocker of endogenous calcium-activated Cl⁻ currents in *Xenopus* oocytes.²⁵ Our findings suggest that the CLCA may be involved in goblet cell degranulation, but it is still uncertain whether the action of NFA is dependent on blockade of Ca²⁺-dependent Cl⁻ currents. Furthermore, because NFA has the ability to inhibit cyclooxygenase, the inhibition of mucus secretion by NFA may be mediated by a cyclooxygenase-dependent pathway.²⁶ However, that seems unlikely because indomethacin did not inhibit OA-induced goblet cell degranulation in the present study as reported by others in sensitized rats²⁷ (Fig. 1B).

Goblet cell degranulation, mucus production, and goblet cell metaplasia are different phenomena, and whether they are linked remains unknown. hCLCA1 is known to be associated with goblet cell metaplasia, and our findings suggested that CLCA, presumably type hCLCA1, may be associated with goblet cell degranulation. If that is true, CLCA may play an important role in both the rapid secretory response and delayed remodeling process of goblet cells. Unfortunately, no counterpart of hCLCA1 has yet been identified in guinea pigs, but the counterparts of hCLCA1 in mice, pigs, and horses have been reported to be mCLCA3, pCLCA1, and eCLCA1, respectively, all of which belong to CLCA cluster 1 in the phylogenetic tree,²⁸ suggesting that CLCAs, including hCLCA1, are ubiquitous molecules in animals.

Mucus hypersecretion causes airway narrowing, loss of lung function, and, occasionally, asphyxia, in asthma. However, no specific treatment for mucus hypersecretion exists. Since hCLCA1 has been reported to be associated with hypersecretion not only in asthma, but in COPD and cystic fibrosis,^{29,30} CLCA inhibitors may be useful for the treatment of mucus hypersecretion in various airway diseases.

In conclusion, NFA was found to inhibit goblet cell degranulation induced by OA-challenge, histamine, and UTP in *in vivo* and *in vitro* asthma models, suggesting that CLCA may regulate secretory responses and that inhibitors may be useful in treating the hypersecretion in asthma patients.

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CONFLICT OF INTEREST

The authors have no conflicts of interest to declare.

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