

A mechanism of antigen-induced goblet cell degranulation in the nasal epithelium of sensitized rats

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Background: We have produced hypertrophic and metaplastic changes of goblet cells in nasal epithelium of ovalbumin (OVA)-sensitized rats by intranasal challenge with OVA. A variety of allergic mediators and inflammatory cells are capable of stimulating goblet cell degranulation (epithelial mucus secretion); however, little is known about the mechanism by which antigen induces mucus hypersecretion.

Objective: Our aim was to explain the mechanism of goblet cell degranulation in allergic inflammation.

Methods: Antigen-induced goblet cell degranulation was evaluated by the transient decrease of epithelial mucosubstance 1 to 6 hours after intranasal challenge with OVA in nasal epithelium of OVA-sensitized rats. The effects of the H₁-antagonist (*d*-chlorpheniramine malate), H₂-antagonist (cimetidine), atropine, indomethacin, cysteinyl leukotriene (cysLT) antagonist (ONO1078), and antirat PMN antiserum on OVA-induced goblet cell degranulation were examined.

Results: Goblet cell secretion 1 hour after OVA challenge was significantly inhibited by H₁-antagonist, atropine, and cysLT antagonist, whereas the secretion 6 hours after the challenge was significantly inhibited by cysLT antagonist and antirat PMN antiserum. Circulating PMN cells and mucosal infiltrating eosinophils were completely abolished by antirat PMN antiserum.

Conclusions: These results indicate the different mechanisms of goblet cell secretion between early-phase (1 hour after OVA challenge) and late-phase (6 hours after the challenge) reactions. Histamine stimulates early-phase secretion through the H₁-receptor of cholinergic nerve terminals, and infiltrating inflammatory cells (eosinophils and/or neutrophils) play a role in late-phase secretion. CysLTs (leukotrienes C₄, D₄, and E₄) are important for both early-phase and late-phase secretion. (*J Allergy Clin Immunol* 2003;112:119-25.)

Key words: mucus secretion, goblet cell, allergy, nose, rat, ovalbumin, histamine, leukotriene

Hypersecretion of mucus is an important characteristic of allergic inflammation of respiratory epithelium such as bronchial asthma. Excessive production of mucus causes plugging in the lower airways that might lead to airway obstruction and progressive respiratory

Abbreviations used

cysLT: Cysteinyl leukotriene
OVA: Ovalbumin

insufficiency. Ovalbumin (OVA)-sensitized animals have been used to study the pathophysiologic changes of allergic inflammation in lower airways, including airway hyperresponsiveness, airway eosinophilia, plasma extravasation, and bronchocontraction.¹⁻⁵ A variety of allergic mediators and inflammatory cells are capable of stimulating mucus synthesis and secretion. However, little attention has been paid to the mechanism by which antigen induces mucus hypersecretion.

In a previous study,⁶ we demonstrated that intranasal instillation of OVA induced hypertrophic and metaplastic changes of goblet cells in nasal epithelium of OVA-sensitized rats, and the precise mechanism of these changes was examined using specific inhibitors of allergic mediators and inflammatory cells. The results suggested that cysteinyl leukotrienes (cysLTs; LT C₄, D₄, and E₄) are important mediators for antigen-induced changes of goblet cells and epithelial mucus synthesis and that histamine, prostaglandins, eosinophils, and neutrophils are not essential for this change.

In this study, to explain the mechanism of goblet cell degranulation in allergic inflammation, we used a rat model of nasal allergy and examined (1) the time course of the amount of intraepithelial mucosubstance during intranasal challenge with OVA in OVA-sensitized rats, (2) the effects of H₁-antagonist, H₂-antagonist, atropine, indomethacin, and cysLTs antagonist on OVA-induced goblet cell degranulation, and (3) the effects of antirat PMN antiserum on eosinophil infiltration and goblet cell degranulation in rat nasal septal epithelium. This study will be useful in understanding the role of allergic mediators and inflammatory cells in epithelial mucus secretion of allergic inflammation.

METHODS

Sensitization and intranasal challenge with OVA

The experiments were approved by the committee of Mie University School of Medicine for the care and use of laboratory animals. Sensitization and challenge of rats were performed as previously reported.^{6,7} Rats (6-7 weeks old) were immunized with an intraperitoneal injection of 0.1 mL saline containing 200 µg of OVA (grade V; Sigma Chemical Co, St Louis, Mo) and 10 mg of Al(OH)₃ at days 1,

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2, 3, and 11. Heat-killed *Bordetella pertussis* bacilli (10^{10} in 50 μ L saline; Wako Pure Chemical Industries, Ltd, Osaka, Japan) were given by a foot pad injection on day 1 as an adjuvant.

On day 19, the rats were anesthetized with ether, and 0.1 mL of saline containing 10 mg of OVA was instilled into both airways of the nasal cavity once a day for 4 days. Rats were killed with an intraperitoneal overdose of sodium pentobarbital before and 1, 3, 6, 12, and 24 hours after the last intranasal challenge.

Morphometry

The head of each rat was removed and fixed in 10% neutral buffered formalin for 3 days, then decalcified in 5% trichloroacetic acid for 7 days. The nasal cavity was transversely sectioned at the level of incisive papilla of the hard palate.⁸ Paraffin sections 5 μ m thick were stained with Alcian blue (pH, 2.6), periodic acid–Schiff, and hematoxylin. The percent area of Alcian blue periodic acid–Schiff–stained mucosubstance was determined by an image analyzer (SP 500; Olympus, Tokyo, Japan) in nasal epithelium over 2 mm (1 mm each side of nasal septum $\times$ 2) of the basal lamina at the center of the septal cartilage.

Treatment with H₁-antagonist, H₂-antagonist, atropine, and indomethacin

Each drug was given by intraperitoneal injection 30 minutes before the last intranasal challenge. In these experiments, the rats were killed at 1 hour and 6 hours after the last OVA challenge. For the injection, indomethacin (2 mg/kg body weight; Sigma Chemical Co) was dissolved in 1% sodium bicarbonate. Other drugs were given in the form prescribed for injection: H₁-antagonist (*d*-chlorpheniramine malate, 5 mg/kg body weight) formulated as Polaramine (Schering Plough, Osaka, Japan), H₂-antagonist (cimetidine, 200 mg/kg body weight) as Tagamet (Smith Kline Beecham, Osaka, Japan), and atropine sulfate (1 mg/kg body weight; Fuso Pharmaceutical Co, Tokyo, Japan).

Treatment with cysLT antagonist, ONO1078

CysLT antagonist, ONO1078 (Pranlukast), was a gift from Ono Pharmaceutical Co, Ltd (Osaka, Japan). ONO1078 (200 mg/kg body weight) in 0.5% carboxymethyl cellulose sodium salt was given orally 1 hour before the last OVA challenge.

Treatment with antirat PMN antiserum

To induce depletion of PMNs, rabbit antirat PMN antiserum (1 mL/body; Inter-Cell Technologies, Hopewell, NJ) was given by intraperitoneal injection for 3 days, starting with a second intranasal challenge.

Statistics

The difference between variables was analyzed by the Mann-Whitney test. Probability values of $P < .05$ were considered statistically significant.

RESULTS

Intraepithelial mucus production and eosinophil infiltration

OVA-sensitized rats, challenged with OVA on each of 3 consecutive days, showed hypertrophic and metaplastic changes of goblet cells in nasal septal epithelium before the last (fourth) challenge (Fig 1). Only a few goblet cells were observed in control groups (untreated control, saline instillation, sham-sensitized rats challenged with saline or OVA, and OVA-sensitized rats challenged with

saline). Quantitative changes in the percent area of mucosubstance in this epithelium are described in Fig 2, A.

Intranasal challenge with OVA for 3 consecutive days also induced a significant eosinophil infiltration into the nasal septal mucosa of sensitized rats before the last challenge. Control groups showed no eosinophil infiltration (Fig 2, B).

Time course changes of the amount of intraepithelial mucosubstance

The time course changes of the amount of intraepithelial mucosubstance after the last (fourth) OVA challenge are described in Figs 1 and 3. The amount of intraepithelial mucosubstance decreased temporarily 1 to 6 hours after OVA challenge and recovered 12 to 24 hours after instillation. Changes in the amount of intraepithelial mucosubstance are affected by the ratio of mucus synthesis to mucus secretion. The transient decrease in epithelial mucosubstance during the 6 hours after intranasal challenge was probably caused by the excess mucus secretion, and mucus synthesis increased by 12 hours, resulting in an increase in the intracellular pool of mucosubstance. Goblet cell degranulation (epithelial mucus secretion) was evaluated by studying these transient decreases of intraepithelial mucosubstance 1 and 6 hours after the last intranasal challenge.

Effects of treatment with H₁-antagonist, H₂-antagonist, atropine, indomethacin, and cysLT antagonist

The transient decrease of the intracellular pool of mucosubstance 1 hour after the last OVA challenge was inhibited after the pretreatment with H₁-antagonist (chlorpheniramine malate), atropine, and cysLT antagonist (ONO1078). H₂-antagonist (cimetidine) and indomethacin had no significant effect on the change 1 hour after intranasal challenge (Fig 4).

The transient decrease of mucosubstance 6 hours after the last challenge was inhibited after the administration of cysLT antagonist; however, H₁-antagonist, H₂-antagonist, atropine, and indomethacin had no significant effect on this change (Fig 5).

Effect of treatment with antirat PMN antiserum

The numbers of circulating total white blood cells in rat treated with antirat PMN antiserum are described in Fig 6, A. PMN cells were depleted by the intraperitoneal injection of antirat PMN antiserum. The numbers of other white blood cells decreased significantly in these rats. Mucosal infiltrations of eosinophils (Fig 6, B) and neutrophils (data not shown) were also completely abolished in nasal epithelium.

Despite the PMN depletion and the total suppression of eosinophil infiltration, metaplastic changes in goblet cells were induced. The transient decrease of epithelial mucosubstance 1 hour after the last OVA challenge was not affected; however, the transient change 6 hours after

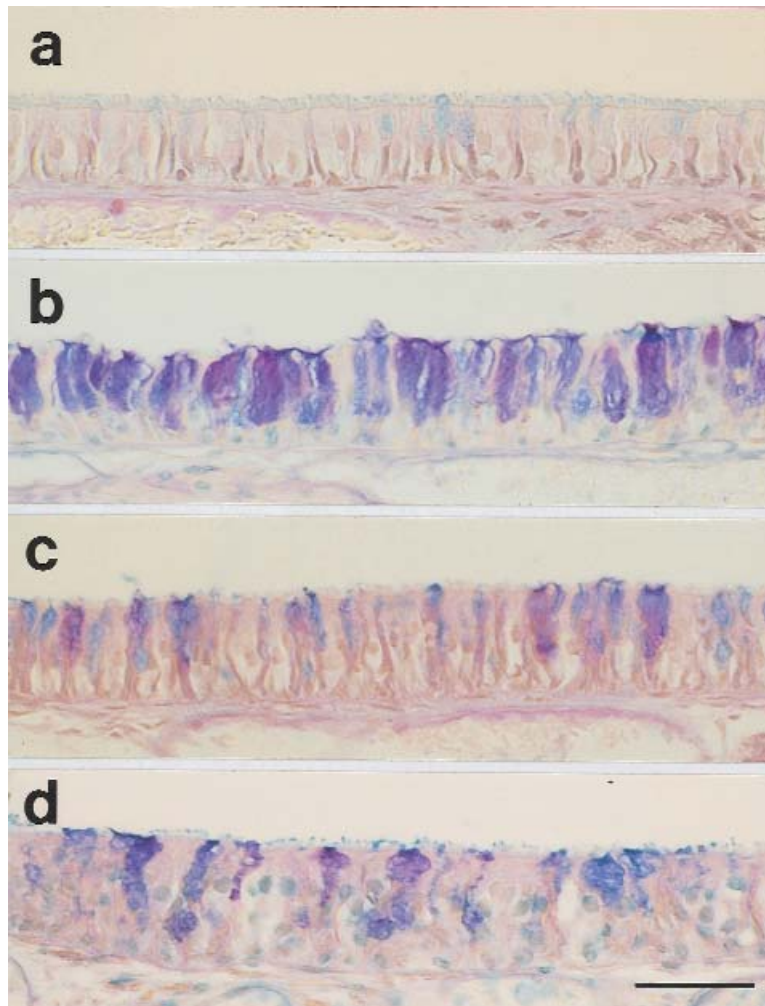


FIG 1. Effects of intranasal instillation of OVA on nasal septal epithelium in OVA-sensitized rats at different times. **a**, Control; sham-sensitized rats challenged with OVA before the last challenge. **b**, OVA-sensitized rats challenged with OVA before the last challenge. **c**, At 1 hour after the last challenge. **d**, At 6 hours after the last challenge. Bar = 30 μ m.

the challenge was inhibited after the intraperitoneal injection of antirat PMN antiserum (Fig 7).

DISCUSSION

In this study, intranasal instillation of OVA induced hypertrophic and metaplastic changes of goblet cells in nasal epithelium of OVA-sensitized rats accompanied by mucosal infiltration of eosinophils. Serum concentration of OVA-specific IgE increased significantly in this animal model, and OVA challenge induced sneezing for 15 minutes. These pathologic and symptomatic changes are similar to those of human allergic inflammation. The time course of the amount of intraepithelial mucosubstance revealed the transient decrease of the intracellular pool of mucosubstance 1 to 6 hours after OVA challenge, presumably caused by excess mucus secretion. The precise mechanism of goblet cell degranulation was examined by evaluating the effects on these transient changes of mucosubstance using specific inhibitors of allergic

mediators and inflammatory cells in the nasal epithelium of this animal model. This is the first report showing the goblet cell secretion in nasal epithelium of allergic animals, and the mechanism of mucus secretion was compared between early-phase (1 hour after OVA challenge) and late-phase (6 hours after the challenge) reactions.

Histamine is one of the most important mediators in the initiation and the development of antigen-induced airway responses. In allergic rhinitis, rhinorrhea can be induced by increased vascular permeability and gland secretion,^{9,10} and H_1 -antagonists are clinically effective for relieving the symptom. H_1 -receptors are present in endothelial cells and sensory nerves in nasal mucosa, where they increase vascular permeability and gland secretion through the parasympathetic reflex.^{11,12} However, the role of histamine on the synthesis and secretion of epithelial mucus glycoprotein is still unclear. In a previous study,⁶ we showed that antigen-induced intraepithelial mucus production was not affected by the H_1 -antagonist or H_2 -antagonist in rat nasal epithelium and that

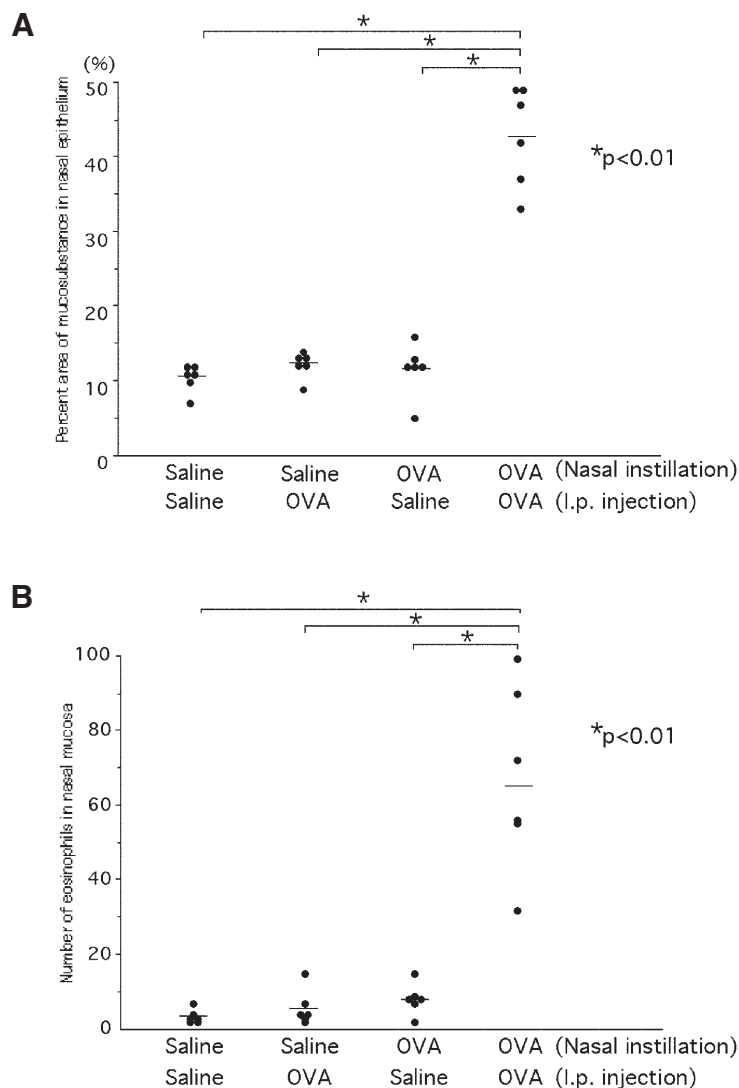


FIG 2. Effects of intranasal instillation of OVA for 3 consecutive days in OVA-sensitized allergic rats ($n = 6$). **A**, Percent area of mucosubstance in nasal epithelium. **B**, Number of infiltrating eosinophils in nasal septal mucosa. Significant increase of intraepithelial mucosubstance occurred before the last challenge with OVA in OVA-sensitized rats.

intranasal instillation of histamine did not induce goblet cell metaplasia or mucus production. These results indicate that histamine might not be involved in antigen-induced mucus synthesis of airway epithelial cells.

In this study, goblet cell secretion 1 hour after antigen challenge was significantly inhibited by H_1 -antagonist and atropine, whereas goblet cell secretion 6 hours after the challenge was not affected. These results suggest that histamine is an important mediator for early-phase secretion through the H_1 -receptor of cholinergic nerve terminals. It has been reported that goblet cell secretion is under neural control in guinea pig and rat airways.¹²⁻¹⁵ Tokuyama and coworkers¹³ showed that electrical stimulation of the cervical vagus nerve induced the goblet cell secretion in guinea pig trachea and that this effect was inhibited by atropine. Takeyama and coworkers¹⁴ reported that intravenous histamine induced goblet cell secretion in guinea pig trachea

and that histamine-induced secretion was completely blocked by atropine, by the cholinergic muscarinic receptor antagonist oxitropium, or by cutting the cervical vagus nerve. These results indicate that cholinergic mechanisms are involved in histamine-induced goblet cell secretion; however, cholinergic innervation of goblet cells in humans has not been demonstrated. The direct effect of histamine on goblet cell secretion seems to be doubtful. Liu and coworkers¹⁶ showed that histamine did not enhance SO_4 -labeled mucus secretion in vitro in guinea pig trachea and that antigen-induced mucus secretion was not affected by H_1 -antagonist or H_2 -antagonist. Takeyama and coworkers¹⁴ showed that inhaled histamine stimulated goblet cell secretion through the H_2 -receptor on goblet cells in guinea pig trachea. But, in this study, H_2 -antagonist did not affect the secretion, indicating that H_2 -receptor is not involved in antigen-induced goblet cell secretion.

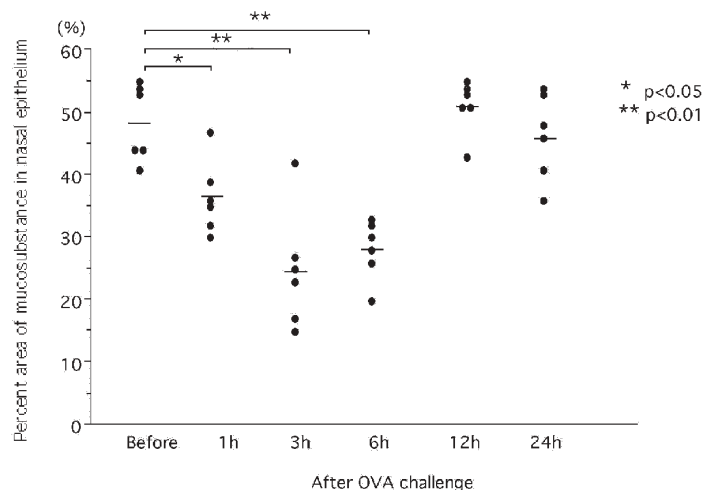


FIG 3. Time course of the percent area of mucosubstance after the last OVA challenge in nasal epithelium (n = 6). Transient decrease of epithelial mucosubstance occurred 1 to 6 hours after the challenge.

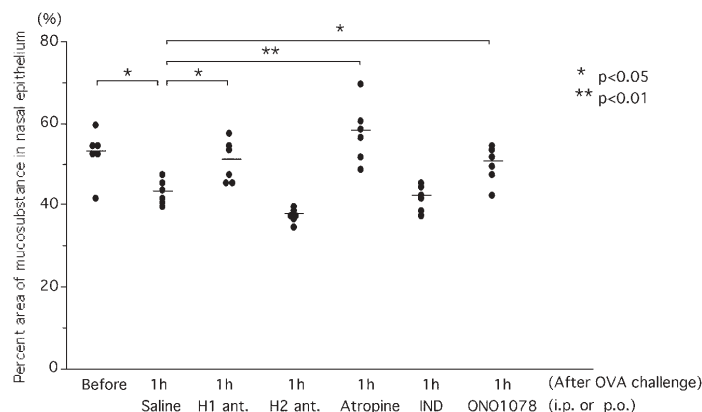


FIG 4. Effects of H₁-antagonist (*d*-chlorpheniramine malate), H₂-antagonist (cimetidine), atropine, indomethacin (*IND*), and cysLT antagonist (ONO1078) on the transient decrease of epithelial mucosubstance 1 hour after the last OVA challenge (n = 6). H₁-antagonist, atropine, and cysLT antagonist significantly inhibited this transient change of the amount of epithelial mucosubstance.

Although histamine is a major mediator for the early-phase reaction of allergic rhinitis, cysLTs (LT C₄, D₄, and E₄) are important for both early-phase and late-phase reactions causing rhinorrhea and nasal congestion. They are released from mast cells and various inflammatory cells and increase microvascular permeability, vasodilation, and mucus hypersecretion.^{9,10} Increased cysLTs have been demonstrated in nasal lavage fluid in patients with allergic rhinitis after antigen provocation.¹⁷ Nasal provocation of LT D₄ induced rhinorrhea and nasal congestion,¹⁸ and cysLT antagonist has been clinically effective in relieving these symptoms.¹⁹ The effects of cysLTs on epithelial mucus hypersecretion have been reported by in vitro studies, in which LT D₄ enhances mucus secretion, and cysLT antagonist significantly inhibited antigen-induced mucus secretion in sensitized guinea pig trachea.¹⁶ In our previous study,⁶ cysLT antagonist, ONO1078 (planlukast), significantly inhibited antigen-induced mucus production and goblet cell metaplasia 24 hours after antigen challenge in rat nasal epithelium without affecting the eosinophil infil-

tration. In this study, cysLT antagonist significantly inhibited antigen-induced goblet cell secretion 1 and 6 hours after OVA challenge. These results indicate that cysLTs are important and direct mediators for mucus synthesis and secretion of epithelial goblet cells both for early-phase and late-phase reactions.

The infiltrating inflammatory cells, including eosinophils and less dramatically, neutrophils, are involved in late-phase reactions of allergic rhinitis by releasing their own mediators. In this study, antirat PMN antiserum abolished circulating PMN cells and infiltrating eosinophils and neutrophils in nasal mucosa. Goblet cell secretion 6 hours after OVA challenge was significantly inhibited by antirat PMN antiserum, whereas the secretion 1 hour after the challenge was not affected. These results indicate that eosinophils and/or neutrophils are important for late-phase mucus secretion. Several eosinophil products, such as cysLTs, platelet-activating factor, and eosinophil cationic protein, are known to induce mucus secretion. The important role of cysLTs in

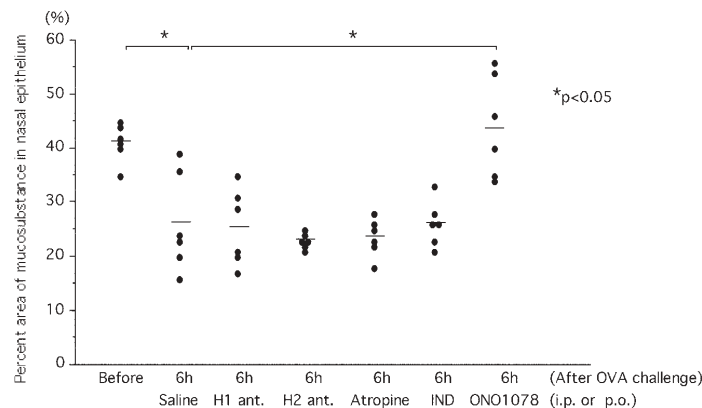


FIG 5. Effects of H₁-antagonist (*d*-chlorpheniramine maleate), H₂-antagonist (cimetidine), atropine, indomethacin (*IND*), and cysLT antagonist (ONO1078) on the transient decrease of epithelial mucosubstance 6 hours after the last OVA challenge (n = 6). CysLT antagonist significantly inhibited the transient change of the amount of epithelial mucosubstance.

mucus secretion has been demonstrated by cysLT antagonist. Infiltrating eosinophils might induce epithelial mucus secretion through the release of cysLTs.

Neutrophils, especially neutrophil elastase, can induce epithelial mucus secretion. We have shown that LPS-induced goblet cell secretion occurred 3 to 6 hours after intranasal instillation in rat nasal epithelium, coinciding with the peak of intranasal neutrophil infiltration.²⁰ Harkema and coworkers²¹ have reported that this LPS-induced secretion was significantly inhibited by neutrophil depletion. Intranasal instillation of elastase induced goblet cell secretion just after the instillation without neutrophil infiltration.²⁰ These results indicate that neutrophil elastase might be a direct mediator for mucus secretion after LPS instillation. Agusti and coworkers² have shown that antigen-induced mucus secretion 1 to 2 hours after the challenge was significantly inhibited by the inhibition of neutrophil recruitment and by neutrophil elastase inhibitor in guinea pig trachea, suggesting the important role of neutrophils and neutrophil elastase in antigen-induced goblet cell secretion.

In conclusion, we have produced OVA-sensitized rats as an animal model of nasal allergy. Intranasal instillation of OVA induced goblet cell metaplasia and epithelial mucus production in nasal epithelium. This rat model was used to study the mechanism of mucus hypersecretion in allergic inflammation. This study suggested that histamine stimulates early-phase goblet cell secretion through H₁-receptor of cholinergic nerve terminals, and infiltrating eosinophils and/or neutrophils play a role in late-phase secretion. CysLTs are important for both early-phase and late-phase secretions. These results indicate that there are different mechanisms of goblet cell degranulation between early-phase (1 hour after OVA challenge) and late-phase (6 hours after the challenge) reactions in rat model of allergic rhinitis. It is hoped that such studies will improve the understanding and the treatment of mucus hypersecretion in allergic inflammation.

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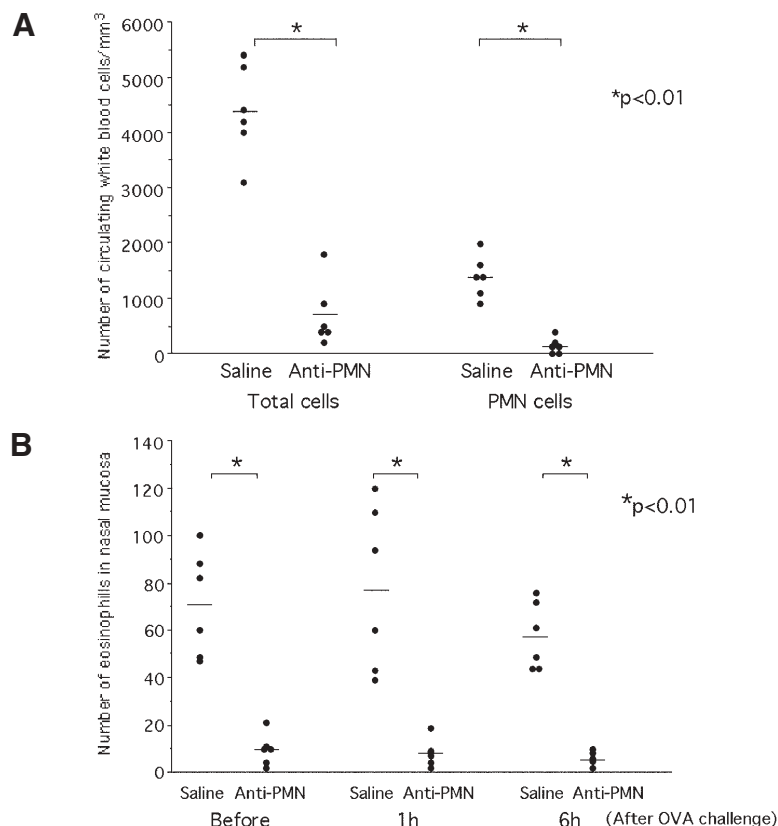


FIG 6. Effects of intraperitoneal injection of antirat PMN antiserum (*Anti-PMN*) on OVA induced changes in OVA-sensitized rats (n = 6). **A**, Numbers of circulating white blood cells and polymorphonuclear cells. **B**, Number of infiltrating eosinophils in nasal septal mucosa. Numbers of total white blood cells and PMN cells decreased significantly in Anti-PMN-treated rats. Eosinophil infiltration into nasal mucosa was completely abolished.

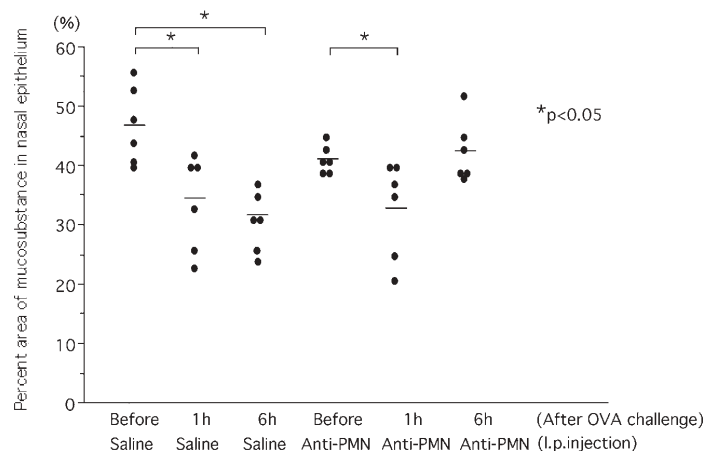


FIG 7. Effects of antirat PMN antiserum (*Anti-PMN*) on OVA induced changes in OVA-sensitized rats 1 and 6 hours after the last challenge (n = 6). The transient decrease of epithelial mucosubstance 6 hours after the challenge was significantly inhibited by Anti-PMN; however, the transient change 1 hour after the challenge was not affected.

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