

Prolonged Allergen Challenge in Murine Nasal Allergic Rhinitis: Nasal Airway Remodeling and Adaptation of Nasal Airway Responsiveness

Muneo Nakaya, MD; Makoto Dohi, MD, PhD; Katsuhide Okunishi, MD, PhD;
Kazuyuki Nakagome, MD, PhD; Ryoichi Tanaka, MD; Mitsuru Imamura, MD;
Kazuhiko Yamamoto, MD, PhD; Kimitaka Kaga, MD, PhD

Background: Nasal airway remodeling exists in allergic rhinitis, but it appears to be far less extensive than in asthma. However, there has been little study about nasal airway remodeling and no study using mice models. It has been reported that airway hyperresponsiveness decreased after prolonged allergen challenge in a chronic murine asthma model together with the progression of remodeling. However, there has been no study of the relation of remodeling and airway responsiveness in nasal allergy. Therefore, we have undertaken this investigation to characterize nasal airway structural changes after prolonged allergen challenge and to examine the relationship between nasal airway hyperresponsivity and remodeling. **Methods:** We prepared murine allergic rhinitis for ovalbumin. Mice were subsequently challenged three times a week with ovalbumin from day 19 to days 53, 88, and 130. We examined allergen-induced nasal symptoms and objective nasal hyperresponsiveness using the enhanced pause system. Moreover, the pathologic changes were investigated after allergen challenge. **Results:** The extended allergen challenge protocol caused significant nasal airway remodeling. Specifically, remodeling was characterized by goblet cell hyperplasia and deposition of collagen in the sub-mucosal area. Allergen-induced nasal hyperresponsiveness was first increased but gradually decreased in nasal symptoms and Penh after prolonged allergen challenge. **Conclusions:** We have demonstrated that a remodeling of nasal mucosa in a murine allergic rhinitis model prolonged allergen exposure. Moreover,

prolonged allergen exposure induced a reduction of nasal hyperresponsiveness together with a progression of nasal remodeling. **Key Words:** Remodeling, nasal allergy, allergic rhinitis, nasal hyperresponsiveness, adaptation, murine model, Penh. *Laryngoscope*, 117:881–885, 2007

INTRODUCTION

Remodeling is defined as “model again or differently, to reconstruct.” It is now well accepted that in remodeling within a lower airway in asthma, there are structural change in addition to the classical inflammatory infiltration.¹ These changes in asthma include edema; inflammatory cell infiltration; mucous gland hyperplasia; reticular basement membrane thickening; subepithelial fibrosis; vascular smooth muscle cell proliferation, hyperplasia, and hypertrophy; and myofibroblast and goblet cell hypertrophy. These are encompassed within the term airway remodeling.² On the other hand, nasal airway remodeling exists in rhinitis, but it appears to be far less extensive than in asthma.^{1,3} Even though inflammation is similar in allergic rhinitis and asthma, the pathologic extent of nasal remodeling, as well as its clinical consequences, might be different from those of bronchi.³ However, there are only a few studies^{4–6} concerned with nasal remodeling, and almost all of them use human specimens. Therefore, there is no study concerned with nasal remodeling using an animal-model-induced prolonged nasal allergen challenge. In addition, it is believed that a remodeling of bronchial mucosa would induce additional bronchial hyperresponsivity and affect the severity or refractoriness of asthma.⁷

However, lower airway hyperresponsiveness has recently decreased after prolonged allergen challenge in chronic murine asthma models.^{8,9} Moreover, prolonged ozone exposure decreases lower airway hyperresponsiveness in murine asthma models.¹⁰ These studies indicate that airway remodeling and adaptation after repeated

From the Department of Otolaryngology (M.N., M.D., K.K.), Faculty of Medicine, University of Tokyo, Tokyo, Japan; and the Pulmonary Division (K.O., K.N., R.T., K.I., K.Y.), Department of Allergy and Rheumatology, Graduate School of Medicine, University of Tokyo, Tokyo, Japan.

Editor's Note: This Manuscript was accepted for publication January 11, 2007.

Send correspondence to Dr. Muneo Nakaya, Department of Otolaryngology, Faculty of Medicine, University of Tokyo, Hongo 7-3-1, Bunkyo-ku, Tokyo, 113-8655 Japan. E-mail: muneo@interlink.or.jp

DOI: 10.1097/MLG.0b013e318033f9b0

exposure to air pollutants might provide protection against airway hyper-responsiveness.

The effects of long-term, repeated allergen exposure on nasal airway structural changes and nasal airway hyperresponsivity remain poorly defined. The aims of this study were to characterize nasal airway structural changes after prolonged allergen challenge and to examine the relationship between nasal airway hyperresponsivity and remodeling.

MATERIALS AND METHODS

Mice

Male BALB/cAJcl mice (5 wk of age) were obtained from CLEA Corp. (Tokyo, Japan) and housed in an air-conditioned room maintained at 22°C to 24°C and 50% to 60% humidity. All the animal experiments carried out in this study were approved by the Animal Research Ethics Board of the University of Tokyo.

Immunization of Mice and Nasal Challenge

Ovalbumin (OVA)-induced (Worthington Biochemical Corp., Lakewood, NJ) airway inflammation was induced in mice as previously described.¹¹ Male mice 5 weeks of age were immunized by an intraperitoneal injection of 2 µg OVA/2 mg of aluminum hydroxide gel on days 0 and 5, followed by daily intranasal challenge (from day 12 to day 19) with 3% (w/v) OVA diluted in 40 µL of sterile normal saline. Prolonged inflammation was induced by the subsequent exposure of mice to 3% OVA three times a week after day 19 until groups of mice were killed on days 19, 53, 88, and 130. The data were obtained from eight mice per group. The control mice received saline injections and intranasal challenge instead of the OVA solution.

Evaluation of Allergen-Induced Nasal Symptoms

Nasal symptoms were evaluated by counting the number of sneezes and nasal itching motions (nasal rubbing) that occurred within a 10-minute interval after daily OVA intranasal provocation. Observations were performed in a cage, as previously described.¹¹ Examinations were performed on days 19, 53, 88, and 130. The data were obtained from eight mice per group.

Measuring of Nasal Response by Noninvasive Enhanced Pause System

OVA-induced nasal responsiveness by enhanced pause (Penh) in mice was measured as previously described.¹¹ Response to a nasal challenge of 3% OVA was measured for 35 minutes. The results are shown for Penh on days 0, 12, 14, 19, 26, 33, 53, 88, and 130. The data were obtained from eight mice per group. The percent Penh was calculated as Penh value/prevalue (before nasal challenge). The following changes of Penh after nasal challenge is biphasic: first peak (1–8 min), maximum peak (1–35 min), as previously described.¹² Percent Penh (first peak) was calculated as the Penh value of peak from 1 to 8 minutes after nasal challenge/prevalue. Percent Penh (maximum) was calculated as the Penh value of peak from 1 to 30 minutes after nasal challenge/prevalue.

Histology

The experimental mice were sacrificed after being anesthetized by an intraperitoneal injection of sodium pentobarbital. The decapitated heads were fixed in 10% formaldehyde for 48 hours at 20°C. Specimens were then decalcified for 7 days in 20% EDTA-Na and embedded in paraffin. The paraffin-embedded nasal cavity was transversely sectioned at the level of the maxillary sinus by continuous microtomy at a thick-

ness of 4 µm, and specimens were then deparaffinized and stained with hematoxylin-eosin to evaluate general morphology, and goblet cells were visualized on periodic acid Schiff (PAS)-stained nasal mucosa. Specimens were also stained with Masson-Goldner to visualize collagen distribution in nasal mucosa. Goblet cells were counted by light microscopy.

The number of goblet cells was quantified per unit (1 mm²) of nasal mucosa by eyepiece reticule. The average of cells counted in at least six sections was used for data analysis. The data were obtained from eight mice per group.

An image analysis was performed on Masson-Trichrome-stained lateral nasal mucosa using a Scion Image Analysis software package (Scion Corp., Frederick, MD). Digital photographs of the nasal mucosa of the nasal lateral wall per tissue section were taken at ×400 magnification. Four measurements of the height of the collagen area from the basement membrane of the epithelial into the bone membrane were calculated and averaged.

Data Analysis

The data were expressed as mean ± standard error of the mean. A statistical analysis was evaluated by using a two-way analysis of variance followed by Student's *t* test for comparison between two groups; a critical value of less than .05 was considered significant.

RESULTS

Change of Allergen-Induced Nasal Symptoms

Figure 1 shows the change of allergen-induced nasal symptoms. The average sneezing on day 53 slightly increased compared with that of day 19, although there was no significance. Afterward, average sneezing gradually decreased, and on day 130, it was significantly lower than on day 19 ($P < .01$). Moreover, the change in average nasal rubbing was the same as that of sneezing. However, there was no significance between the average rubbing on day 130 and on day 19 ($P = .09$). These results indicate that allergen-induced nasal symptoms were decreased during prolonged allergen challenge.

Change of Penh After Nasal Allergen Challenge

Figure 2A shows the change in percent Penh after nasal challenge on days 19, 53, 88, and 130. This change was gradually decreased as the duration of nasal allergen exposure was increased.

Figure 2B shows the change in percent Penh (first peak) and percent Penh (maximum) on days 0, 12, 14, 19, 26, 33, 53, 88, and 130. Percent Penh (first peak) was gradually increased until day 26 and slightly decreased afterward. Percent Penh (first peak) on day 130 was not significantly different from that of day 0. The change in percent Penh (maximum) was same as that of percent Penh (first peak). Percent Penh (maximum) on day 130 was also not significantly different from that of day 0. These results indicate that nasal hyperresponsivity was decreased as the duration of nasal allergen exposure was increased.

Change of Goblet Cells

Figure 3 shows the changes in goblet cell. An increase in PAS-positive cells was observed in the nasal mucosa as the duration of nasal allergen exposure was increased. The number of goblet cells on days 53, 88, and 130 was

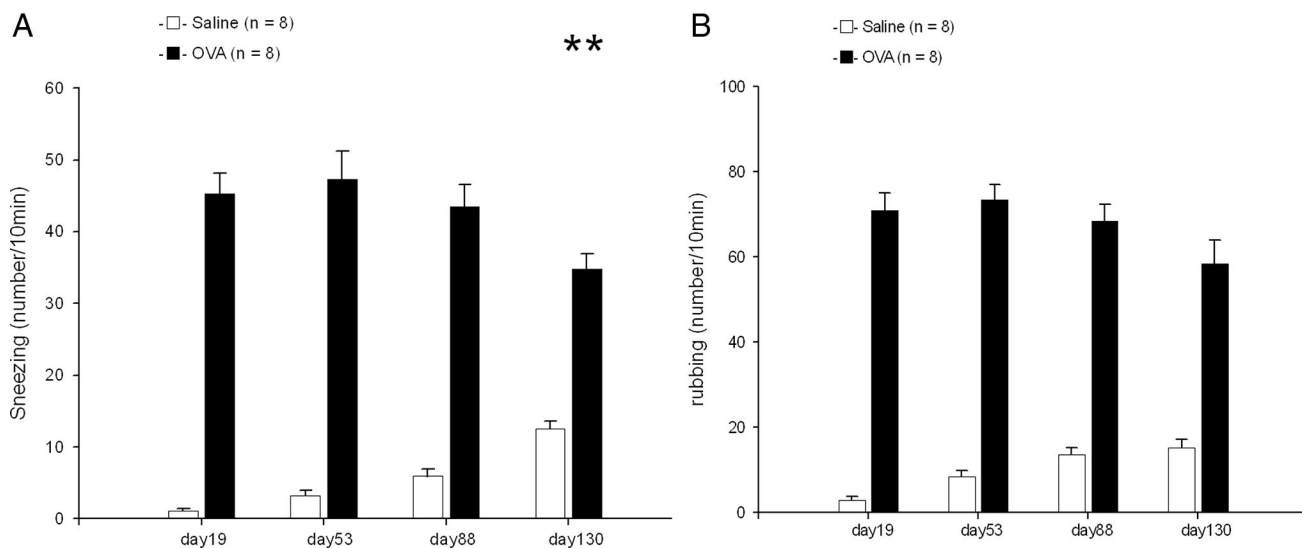


Fig. 1. Change in allergen-induced nasal symptoms. (A) The change in sneezing rate after the nasal challenge. The average sneezing rate gradually decreased after day 53. The average sneezing rate on day 130 was significantly decreased from day 19. $**P < .01$. (B) The change in rate of nasal rubbing after the nasal challenge. The average rate of nasal rubbing, like sneezing, gradually decreased after day 53.

significantly increased compared with that of day 19 ($P < .05$, $P < .001$, $P < .001$, respectively). These results indicate that excessive mucus secretion from hyperplastic goblet cells occurred after prolonged allergen challenge.

Change of Thickness of Submucosal Collagen

Figure 4 shows the change in thickness of submucosal collagen. This thickness on day 53 was not different from that of day 19. However, the thickness on day 88 was significantly increased compared with day 19 ($P < .05$). Moreover, on day 130, it was significantly increased compared with day 19 ($P < .01$). These results indicate that submucosal collagen was increased after prolonged allergen challenge.

Other Histologic Changes After Prolonged Allergen Challenge

Nasal epithelium gradually dropped away, and some cilium of the epithelium disappeared after prolonged allergen challenge. In addition, some ciliated columnar cell disappeared, and squamous metaplasia occurred on day 130. Moreover, submucosal vessels were increased, and dilatation of vessels occurred after prolonged allergen challenge (data not shown).

DISCUSSION

It is known that remodeling exists in the nasal mucosa of patients with allergic rhinitis,^{5,6} although it appears that remodeling in the nose is less extensive than in

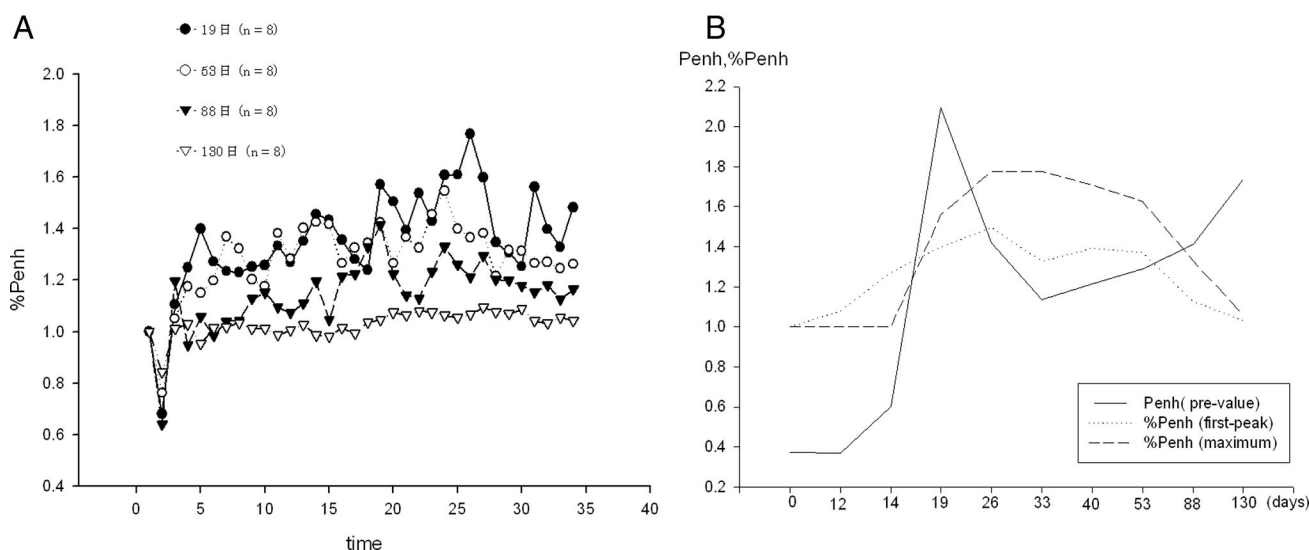


Fig. 2. (A) The change in percent Penh on days 19, 53, 88, and 130. The change gradually decreased as the duration of the allergen exposure increased. (B) The change in percent Penh (first peak) and percent Penh (maximum). Percent Penh (first peak) and percent Penh (maximum) increased until day 26 and decreased thereafter. They were not significantly different on day 130 than on day 0.

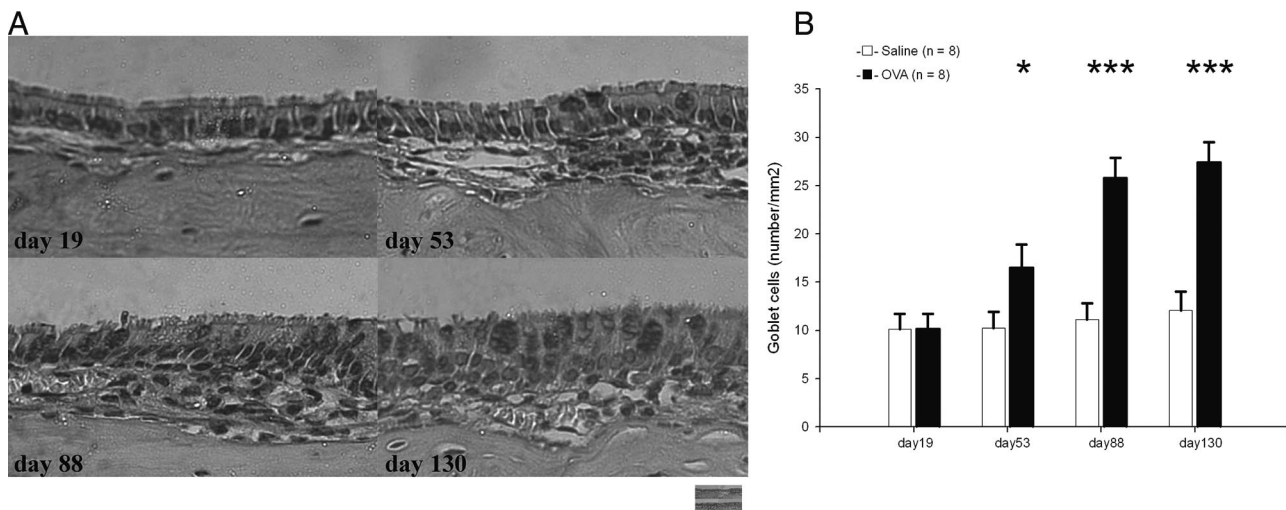


Fig. 3. Changes in goblet cells. (A) A representative example of the days after the nasal challenge (PAS-stained $\times 400$). The number of goblet cells increased with increased nasal allergen exposure. (B) Changes in the number of goblet cells. There was a hyperplasia of goblet cells as nasal allergen exposure increased. The number of goblet cells on days 53, 88, and 130 was significantly higher compared to day 19. $*P < .05$, $***P < .001$.

the lungs.³ However, the details of remodeling nasal mucosa in nasal allergy are unclear compared with that in asthma because there have been few studies concerning the remodeling of the nasal mucosa, and there has been no study using a planned animal model of nasal allergy. Therefore, we set out to establish a model of prolonged allergen-induced nasal airway inflammation to try to mimic the tissue remodeling observed in allergic rhinitis. We found that extending the number and period of allergen challenges induced nasal airway remodeling.

In this study, goblet cells in the epithelium were increased after prolonged allergen challenge. The changes of goblet cells in nasal remodeling are unclear in the past study, though there is a hyperplasia of goblet cells in severe asthma¹² or murine remodeling model of asthma.⁹ During pollen exposure, allergic patients present marked

goblet cell hyperplasia.¹³ In another study of patients with seasonal allergic rhinitis, no significant differences were found in the numbers of goblet cells before and during the pollen season.¹⁴

In addition, in this study, the submucosal collagen area was increased after prolonged allergen exposure. Sanai et al.⁶ reported that the thickness of the basement membrane zone underlying the epithelia was statistically significantly greater in allergic subjects than in nonallergic subjects and corresponds to regions positive for type I and III collagen antibodies. The pseudothickening of the reticular basement membrane caused by collagen and fibrous protein deposition occurred as remodeling in bronchial and nasal mucosa, although the extent was different between these mucosae.² In the present murine model, we confirmed remodeling in the nasal submucosal area.

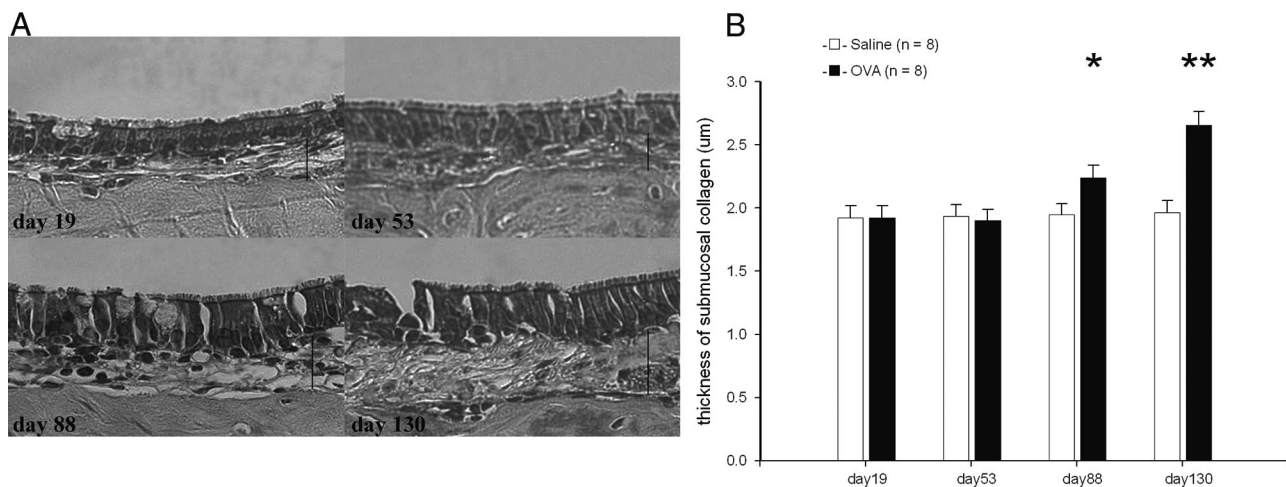


Fig. 4. Changes in the thickness of submucosal collagen. (A) A representative example from the days following the nasal challenge (Masson-Trichrome stained $\times 400$). (B) The submucosal collagen gradually increased as nasal allergen exposure increased. The thicknesses on days 88 and 130 were significantly increased compared to day 19. $*P < .05$, $**P < .01$.

On other hand, airway hyperresponsiveness is a hallmark feature of human asthma and nasal allergy. However, prolonged allergen challenge reduced nasal hyperresponsiveness in this study. The symptoms of sneezing and nasal rubbing after nasal allergen challenge were increased until day 53, but then gradually decreased (Fig. 1). Moreover, percent Penh (maximum) and percent Penh (first peak) were increased until day 26, but gradually decreased afterward (Fig. 2). These results indicate that objective nasal hyper-responsiveness is decreased after prolonged nasal allergen challenge. Palmans et al.⁸ reported a decrease of airway hyper-responsiveness in prolonged allergen exposure in sensitized rats. They also indicated airway remodeling such as goblet cell hyperplasia and thickening of the airway inner wall area in that model. Therefore, remodeling in asthma might be related to the development of a compensatory mechanism. McMillan and Lloyd⁹ similarly reported that a murine asthma model of prolonged allergen challenge indicated bronchial remodeling and a reduction of airway hyper-responsiveness after prolonged allergen challenge. They also showed a reduction of both Th2 cytokines, such as interleukin -4, 5, and 13, and Th1 cytokines, such as interferon- γ , after prolonged allergen challenge. Furthermore, Jangs et al.¹⁰ prolonged the exposure of ozone, the photochemical oxidation products of substrates emitted as air pollution, in a murine asthma model of induced bronchial remodeling and reduction of airway responsiveness. They hypothesized that airway remodeling and adaptation after repeated exposure to air pollutants can provide protection against airway hyper-responsiveness. In consideration of this finding, remodeling in nasal mucosa might be associated with the adaptation for nasal hyper-responsiveness by a defensive mechanism the same as in remodeling of bronchial mucosa. However, the details of the mechanism between remodeling and the adaptation for airway hyper-responsiveness are unclear, and further studies are needed to clarify the potential mechanisms.

In conclusion, we have demonstrated that a remodeling of nasal mucosa in a murine allergic rhinitis model prolonged allergen exposure. Moreover, prolonged allergen exposure induced the reduction of nasal hyper-

responsiveness together with a progression of nasal remodeling.

BIBLIOGRAPHY

1. Salib RJ, Howarth PH. Remodeling of the upper airways in allergic rhinitis: is it a feature of the disease? *Clin Exp Allergy* 2003;33:1629–1633.
2. Jeffery PK. Remodeling in asthma and chronic obstructive lung disease. *Am J Respir Crit Care Med* 2001;164:s28–s38.
3. Bousquet J, Jacquot W, Vignola M, et al. Allergic rhinitis: a disease remodeling the upper airways? *J Allergy Clin Immunol* 2004;113:43–49.
4. Watanabe K, Kiuna C. Epithelial damage of nasal mucosa in nasal allergy. *Ann Otol Rhinol Laryngol* 1998;107:564–570.
5. Bavbek S, Sencer S, Misirligil Z, et al. Light and electron microscope study in allergic rhinitis patients (ARP) with or without bronchial hyperreactivity (BHR). *J Investig Allergol Clin Immunol* 1996;6:172–182.
6. Sanai A, Nagata H, Konno A. Extensive interstitial collagen deposition on the basement membrane zone in allergic nasal mucosa. *Acta Otolaryngol* 1999;119:473–478.
7. Rasmussen F, Taylor DR, Flannery EM, et al. Risk factors for airway remodeling in asthma manifested by a low post-bronchodilator FEV1/vital capacity ratio: a longitudinal population study from childhood to adulthood. *Am J Respir Crit Care Med* 2002;165:1480–1488.
8. Palmans E, Kips JC, Pauwels RA. Prolonged allergen exposure induces structural airway changes in sensitized rats. *Am J Respir Crit Care Med* 2000;161:627–635.
9. McMillan SJ, Lloyd CM. Prolonged allergen challenge in mice leads to persistent airway remodeling. *Clin Exp Allergy* 2004;34:497–507.
10. Jang AS, Choi I, Lee JH, et al. Prolonged ozone exposure in an allergic airway disease model: adaptation of airway responsiveness and airway remodeling. *Respir Res* 2006;7:24.
11. Nakaya M, Dohi M, Okunishi K, et al. Noninvasive system for evaluating allergen-induced nasal hypersensitivity in murine allergic rhinitis. *Lab Invest* 2006;86:917–926.
12. Akikawa T, Shimura S, Sasaki H, et al. Marked goblet cell hyperplasia with mucus accumulation in the airways of patients who died of severe acute asthma attack. *Chest* 1992;101:916–921.
13. Karlsson G, Pipkorn U. Natural allergen exposure does not influence the density of goblet cell in the nasal mucosa of patients with seasonal allergic rhinitis. *ORL J Otorhinolaryngol Relat Spec* 1989;51:171–174.
14. Gluck U, Gebbers J. Epithelial changes in seasonal allergic rhinitis throughout the year: evidence of coexistent air pollution and local secretory IgA deficiency? *ORL J Otorhinolaryngol Relat Spec* 2000;62:68–75.