

A time course study on the development of allergen-induced nasal airway remodeling in a rat model

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ABSTRACT

Background: Only a few studies have investigated the airway remodeling process in allergic rhinitis (AR), and the results reported are conflicting. We established an allergen-induced nasal remodeling model for AR using brown Norway rats and investigated time-dependent histological changes and the reversibility of the epithelial and subepithelial changes.

Methods: Ovalbumin (OVA)-sensitized rats were exposed to OVA daily and then assigned to one of five groups depending on the duration of the challenge. Groups I, II, III, and IV rats were exposed for 1, 4, 8, and 12 weeks, respectively. Group V rats were exposed for 12 weeks and then protected from challenge for 4 weeks. Matched control rats were exposed to saline. Histological parameters of the nasal mucosa such as epithelial and subepithelial thickness, goblet cell hyperplasia, eosinophil infiltration, submucosal gland hypertrophy, and expression of matrix metalloproteinase-9 (MMP-9) and tissue inhibitor of metalloproteinase-1 (TIMP-1) were compared between groups.

Results: Repeated challenges for 12 weeks resulted in the characteristic features of nasal airway remodeling. All parameters except epithelial thickness increased markedly. Goblet cell hyperplasia and eosinophil infiltration decreased to control group levels after cessation of challenge for 4 weeks. Subepithelial changes such as subepithelial thickening, submucosal gland hypertrophy, and increased expression of MMP-9 and TIMP-1 were still observed after 4 weeks without challenge.

Conclusion: Our results indicate that prolonged OVA challenge can induce nasal remodeling. Epithelial changes were minimal or absent after cessation of the challenge, but subepithelial changes were resistant to reversal.

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Interest in the structural changes that occur in the nasal cavities of patients with allergic rhinitis (AR) is increasing.¹ These structural changes in response to an inflammatory condition are collectively referred to as airway remodeling.

Airway remodeling is well known in asthmatic patients and was first reported in 1992.² Consistent findings include smooth muscle hypertrophy, goblet cell hyperplasia, submucosal gland hypertrophy, basement membrane thickening, increased collagen and matrix protein deposition, and epithelial disruption.^{2–11} However, studies on the airway remodeling in AR are lacking and contradictory results have been reported, because the nose and bronchi differ in embryological origin and the nasal cavity has a relatively smaller mucosal area compared with that of the bronchi.^{1,12}

To better understand the development of nasal airway remodeling in AR, we developed an allergen-induced nasal remodeling model using brown Norway (BN) rats and investigated histopathological changes according to the period of allergen challenge. In addition, we evaluated the reversibility of histopathological changes after cessation of allergen challenge.

MATERIALS AND METHODS

Animals

Fifty pathogen-free BN rats were used. Each rat weighed 144–168 g and was 3–4 weeks of age. Rats were assigned randomly into five different groups depending on the duration of allergen challenge.

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Groups I, II, III, and IV each comprised seven rats that were exposed to allergen for 1, 4, 8, or 12 weeks, respectively. Group V was composed of seven rats that were challenged by allergen for 12 weeks and then protected from allergen for 4 weeks (Fig. 1). Each of these experimental groups had a matching control group composed of three rats that were not challenged by allergen. Experiments were performed according to the guidelines for the care and use of experimental animals, and this experimental protocol was approved by the Laboratory Animal Research Center.

Sensitization and Allergen Challenge

All chemicals were purchased from Sigma-Aldrich Co. (St. Louis, MO) unless otherwise specified. Rats were sensitized to ovalbumin (OVA, grade V) by subcutaneous injection of 1 mg of OVA and 4.0 mg of aluminum hydroxide in 1 mL of phosphate-buffered saline (PBS) on days 0 and 7. At the same time, 50 ng of pertussis toxin in PBS was injected i.p. to enhance the allergic reaction. Starting on day 14, rats in the experimental groups repeatedly inhaled 0.1% OVA for 15 minutes daily for 1–12 weeks. The aerosolized OVA (particle size $\leq 4.6 \mu\text{m}$) was generated by a nebulizer (PulmoAide; Sunrise Medical, Hicksville, NY). Rats in the control groups received subcutaneous injections of PBS instead of OVA on days 0 and 7, followed by repetitive inhalation of aerosolized PBS without OVA. The control rats did not receive subcutaneous injections of $\text{Al}(\text{OH})_3$ and i.p. injections of pertussis toxin.

Confirmation of AR Model

The number of sneezes and the OVA-specific IgE levels were measured to confirm the establishment of a rat AR model. The number of sneezes was counted for 15 minutes after the final OVA challenge and the OVA-specific IgE level was measured using an enzyme-linked immunosorbent assay. Serum was acquired *via* centrifugation of blood, which was collected *via* cardiocentesis and kept at -80°C . Briefly, 96-well flat-bottom plates were coated with 100 μL of capture antibody and 2 $\mu\text{g}/\text{mL}$ of monoclonal mouse anti-rat IgE antibody in bicarbonate coating buffer, pH 9.6, followed by an overnight incubation at 4°C . Plates were washed with PBS containing 0.1% Tween-20 (T-PBS) and blocked with PBS containing 1% bovine serum albumin. Plates were washed with T-PBS, and 100 μL of diluted serum (1:10) was added, followed by incubation overnight at room temperature.

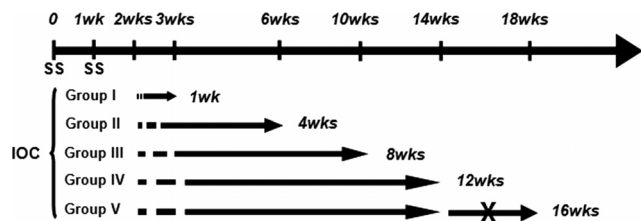


Figure 1. Experimental protocol. Rats were actively sensitized by subcutaneous injection of OVA on days 0 and 7. Starting on day 14, intranasal OVA challenges were performed for 15 minutes daily. Groups I, II, III, and IV were exposed to OVA daily for 1, 4, 8, and 12 weeks, respectively. Group V was exposed for 12 weeks and then protected from challenge for 4 weeks. Rats in the control groups were exposed to saline instead of OVA. OVA, ovalbumin; SS, systemic sensitization; IOC, intranasal OVA challenge.

After six washes with T-PBS, 100 μ L of diluted biotinylated-OVA (1:10) was added and incubated for 2 hours at room temperature. After extensive washing with T-PBS, 100 μ L of diluted peroxidase-conjugated avidin (1:10,000) was added. Plates were washed six more times with T-PBS, and 100 μ L of peroxidase substrate was added in a darkroom. The enzymic reaction was stopped through the addition of 100 μ L of stop solution (2 M of H_2SO_4) after incubation at room temperature for 30 minutes. The optical density of the reaction mixture was read at 450 nm using an automated microplate reader (Thermomax; Molecular Devices, Menlo Park, CA).

Histological Analyses

Rats in groups I, II, III, and IV were killed 24 hours after the final OVA inhalation, and rats in group V were killed 29 days after the final OVA challenge. For histological analysis of the nasal cavity, the heads were obtained and fixed in 10% formaldehyde solution, decalcified in 6% nitric acid, embedded in paraffin, sectioned coronally from the first palatal ridge of the ventral aspect, and stained with hematoxylin-eosin.

To ensure that the observation site was consistent across groups, microscopic evaluation was performed in an area where the vomeronasal organ, maxilloturbinate, and incisor could be well visualized. The epithelial and subepithelial thicknesses were measured using an image analysis system (Image Pro; Media Cybernetics, Bethesda, MD). Under light microscopic vision (400 $\times$ magnification), eosinophils were counted in the submucosal area. Goblet cells were counted, and the results are presented as the number observed per 100 μ m length. To evaluate submucosal gland hypertrophy, the diameters of the cells in the submucosal glands were measured, and the results are presented as the longest diameters. Histological analyses were repeated in the five different mucosa of maxilloturbinate by a single observer who was unaware of the experimental conditions, and average values were calculated.

Immunohistochemical Staining for Matrix Metalloproteinase 9 and Tissue Inhibitor of Metalloproteinase 1

After deparaffinization, 3% hydrogen peroxide and normal goat serum were applied. Monoclonal antibodies for either matrix metalloproteinase-9 (MMP-9) or tissue inhibitor of metalloproteinase-1 (TIMP-1) (Santa Cruz, San Diego, CA) were diluted at 1:300 and reacted at 4°C. Biotinylated secondary antibody and peroxidase-conjugated streptavidin were added for 20 minutes. Then, each section was reacted with 3,3'-diaminobenzidine tetrahydrochloride and counterstained with hematoxylin. Expression was evaluated microscopically, and the results are expressed as the percentage of the positive-stained area.

Statistical Analyses

Data were analyzed using the statistical software program SPSS for Windows (Version 10.0; SPSS Inc, Chicago, IL). The Wilcoxon rank-sum

test was used to evaluate if the differences between the experimental and matched control groups were significant. Results in the different experimental groups were analyzed using the nonparametric Kruskal-Wallis test. Values of $p < 0.5$ were considered statistically significant.

RESULTS

Confirmation of a Rat AR Model

Rats in groups I, II, III, and IV sneezed significantly more than rats in the matched control groups ($p < 0.05$; Fig. 2 A). OVA-specific IgE levels were markedly increased by repeated OVA challenge and were significantly higher in all experimental groups than the matched control groups (Fig. 2 B).

Histological Analyses

Epithelial thickness in the experimental groups was not significantly different from that in the matched control groups (Table 1), and no significant differences were noted among the experimental groups. Subepithelial thickness increased in association with the period of allergen challenge, peaked at 12 weeks of exposure, and increased in groups IV and V compared with the control groups ($p = 0.033$ and $p = 0.048$, respectively; Table 1). In addition, it was significantly thicker in group IV than groups I, II, and III ($p = 0.032$, $p = 0.032$, and $p = 0.009$, respectively). However, a significant difference was not noted between groups IV and V.

Eosinophil infiltration increased markedly after challenge, peaked at 4 weeks of exposure, and declined gradually (Fig. 3 A). Eosinophil infiltration in groups I, II, III, and IV was significantly different from that in the matched control groups ($p = 0.048$, $p = 0.024$, $p = 0.010$, and $p = 0.033$, respectively; Fig. 3 B). However, the number in group V was not significantly different from that in the matched control. The number of infiltrating eosinophils was significantly higher in group II than groups I, IV, and V ($p = 0.016$, $p = 0.015$, and $p = 0.004$, respectively). The difference between group IV and V was borderline significant ($p = 0.053$).

The numbers of goblet cells in groups I, II, and V were not significantly different from those in the matched control groups. However, groups III and IV had significantly more goblet cells than the matched control groups ($p = 0.033$ for both; Table 2). Goblet cell was significantly decreased in group V compared with the group IV ($p = 0.036$).

Submucosal gland hypertrophy increased gradually after allergen exposure and peaked at 12 weeks of exposure. It increased in groups III, IV, and V compared with the matched control groups ($p = 0.042$, $p = 0.017$, and $p = 0.024$, respectively; Table 2). Submucosal gland hypertrophy in group IV was significantly greater than that in groups I, II, and III ($p = 0.003$, $p = 0.003$, and $p = 0.013$, respectively). However, it was not significantly decreased in group V compared with the group IV.

Expression of MMP-9 and TIMP-1

The expression of MMP-9 was markedly higher in groups IV and V than the matched control groups ($p = 0.017$ and $p = 0.024$, respectively; Figs. 4). It was also significantly higher in groups IV and V than groups I, II, and III (versus group IV, $p = 0.003$, $p = 0.003$, and $p = 0.002$, respectively; versus group V, $p = 0.004$, $p = 0.004$, and $p = 0.007$, respectively). However, there was no significant difference between group IV and group V (Fig. 4 B). TIMP-1 expression was significantly higher in groups III, IV, and V than the matched control groups ($p = 0.017$, $p = 0.017$, and $p = 0.024$, respectively; Figs. 5). It was also significantly higher in groups IV and V than in groups I, II, and III (versus group IV, $p = 0.003$ for all; versus group V, $p = 0.004$, $p = 0.004$, and $p = 0.003$, respectively). However, the expression in group IV was not significantly different from that in group V (Fig. 5 B).

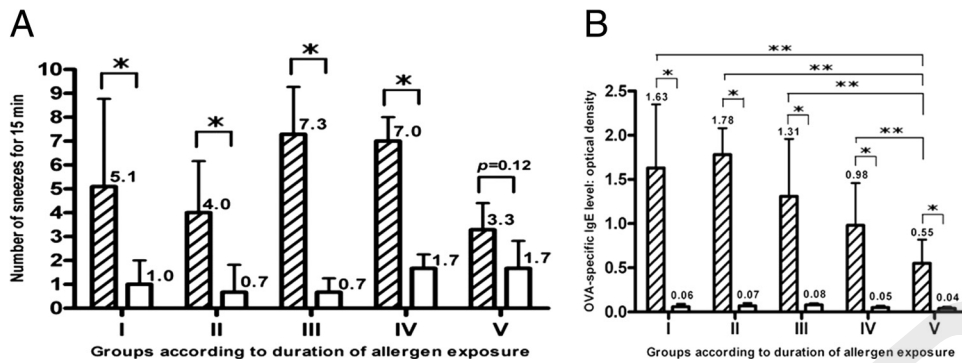


Figure 2. The number of sneezes in a 15-minute period (A) after the final OVA challenge and (B) the level of OVA-specific IgE antibody. The number of sneezes was higher in groups I, II, III, and IV than the matched control groups ($p < 0.05$). However, group V rats sneezed at a similar rate to control group rats ($p = 0.12$). The levels of OVA-specific IgE antibody in the experimental groups were significantly higher than those in the matched control groups ($*p < 0.05$). In addition, the levels of OVA-specific IgE antibody in groups I, II, III, and IV were significantly higher than that in group V ($**p < 0.05$). Diagonally striped bars indicate OVA and open bars indicate no OVA. OVA, ovalbumin; SS, systemic sensitization; IOC, intranasal OVA challenge.

Table 1 Epithelial and subepithelial thickness in the maxilloturbinate mucosa

Group	Epithelial Thickness (μm)			Subepithelial Thickness (μm)		
	Experiment	Control	p Value	Experiment	Control	p Value
I	30.9 $\pm$ 2.7	30.7 $\pm$ 2.8	0.905	90.4 $\pm$ 8.1	90.5 $\pm$ 5.8	0.905
II	32.3 $\pm$ 3.1	30.8 $\pm$ 2.0	0.714	92.6 $\pm$ 9.2	90.3 $\pm$ 5.7	0.905
III	35.4 $\pm$ 3.8	32.5 $\pm$ 1.2	0.183	89.3 $\pm$ 10.0	88.5 $\pm$ 12.8	0.667
IV	34.2 $\pm$ 1.7	32.7 $\pm$ 1.1	0.183	112.4 $\pm$ 17.6	90.2 $\pm$ 8.5	0.033
V	36.9 $\pm$ 2.2	33.5 $\pm$ 2.0	0.095	102.7 $\pm$ 6.3	87.9 $\pm$ 6.0	0.048

Groups I, II, III, and IV were exposed to OVA daily for 1, 4, 8, and 12 weeks, respectively. Group V was exposed for 12 weeks and then protected from challenge for 4 weeks. Matched control rats were exposed to saline instead of OVA. All values are expressed as mean $\pm$ SD except p values. OVA = ovalbumin.

DISCUSSION

In our study, BN rats were used to investigate the nasal remodeling process of AR, because they are a well-established model reflecting human allergic asthma.⁵ BN rats consistently show high IgE responsiveness to allergen provocation and significantly greater constitutive IgE production than other rat strains.¹³ They also require 1000-fold more antigen than low responders to induce tolerance.¹⁴ Al(OH)₃ and pertussis toxin were used as inflammatory adjuvants. It is well known that they selectively stimulate IgE responses and modulate the tolerogenic activity of inhaled antigen.^{13,15,16} OVA and Al(OH)₃ were inoculated subcutaneously in the head region, and pertussis toxin was inoculated i.p. This protocol has shown to induce the production of high levels of IgE specific for OVA and has been used in other models of allergy.¹⁷ Lymphatics of subcutaneous tissue in the head region drain to the superficial cervical nodes, and those in the peritoneal cavity drain to the parathyroid and posterior mediastinal nodes. Because these nodes have previously been identified as the initial sites of activation of IgE isotype-specific suppressor T cells during tolerogenesis in this model, these two sites were used for sensitization and inoculation of the adjuvants.¹⁸

We showed that repeated OVA challenge for 12 weeks induced features of airway remodeling in the nasal mucosa. Subepithelial thickness, goblet cell hyperplasia, eosinophilic infiltration, submucosal gland hypertrophy, and expression of MMP-9 and TIMP-1 all increased markedly after allergen exposure. Goblet cell hyperplasia and eosinophil infiltration decreased after the cessation of OVA challenge for 4 weeks and returned to the values seen in the control groups. However, subepithelial changes such as subepithelial thickening, submucosal gland hypertrophy, and increased expression of MMP-9 and TIMP-1 were still maintained after the cessation. Epithelial thickness did not change according to the duration of allergen

challenge. These results imply that epithelial changes after repeated allergen challenge may be minimal or absent after cessation of exposure, whereas subepithelial changes are resistant to reversal at least at 4 weeks protection.

Some studies have evaluated changes in the nasal epithelia of patients with AR, and contradictory results have been reported. Some researchers reported that epithelial dimensions and integrity were maintained.^{19–21} Others found a thicker epithelium characterized by enhanced epithelial shedding and intercellular edema.^{22–24} We showed that epithelial thickness did not differ significantly between the experimental and control groups and that there was no significant difference among the experimental groups. These findings suggest that epithelial dimensions are maintained regardless of allergen exposure.

The nasal mucosa contains goblet cells and submucosal glands. Conflicting findings have been reported regarding the changes in these structures in AR. Although two studies reported no change in the density of goblet cells,^{25,26} other studies showed marked hyperplasia of goblet cells and submucosal glands.^{27,28} Previous studies using BALB/c mice showed that repeated allergen challenges over 6 weeks caused hyperplasia of goblet cells in the airway.^{6,29} In our study, goblet cells increased in groups exposed to allergen for at least 8 weeks and differed significantly from that in the matched control groups. After avoidance of allergen for 4 weeks, the number of goblet cells was no longer significantly different from that in the matched control group and was different from the group exposed to allergen for 12 weeks. Submucosal gland hypertrophy was markedly increased after allergen challenge for 12 weeks, and this hypertrophy was significant compared with that seen in the groups exposed to allergen for 1–8 weeks. Hypertrophy was maintained after avoidance of allergen for 4 weeks and was found to be no different

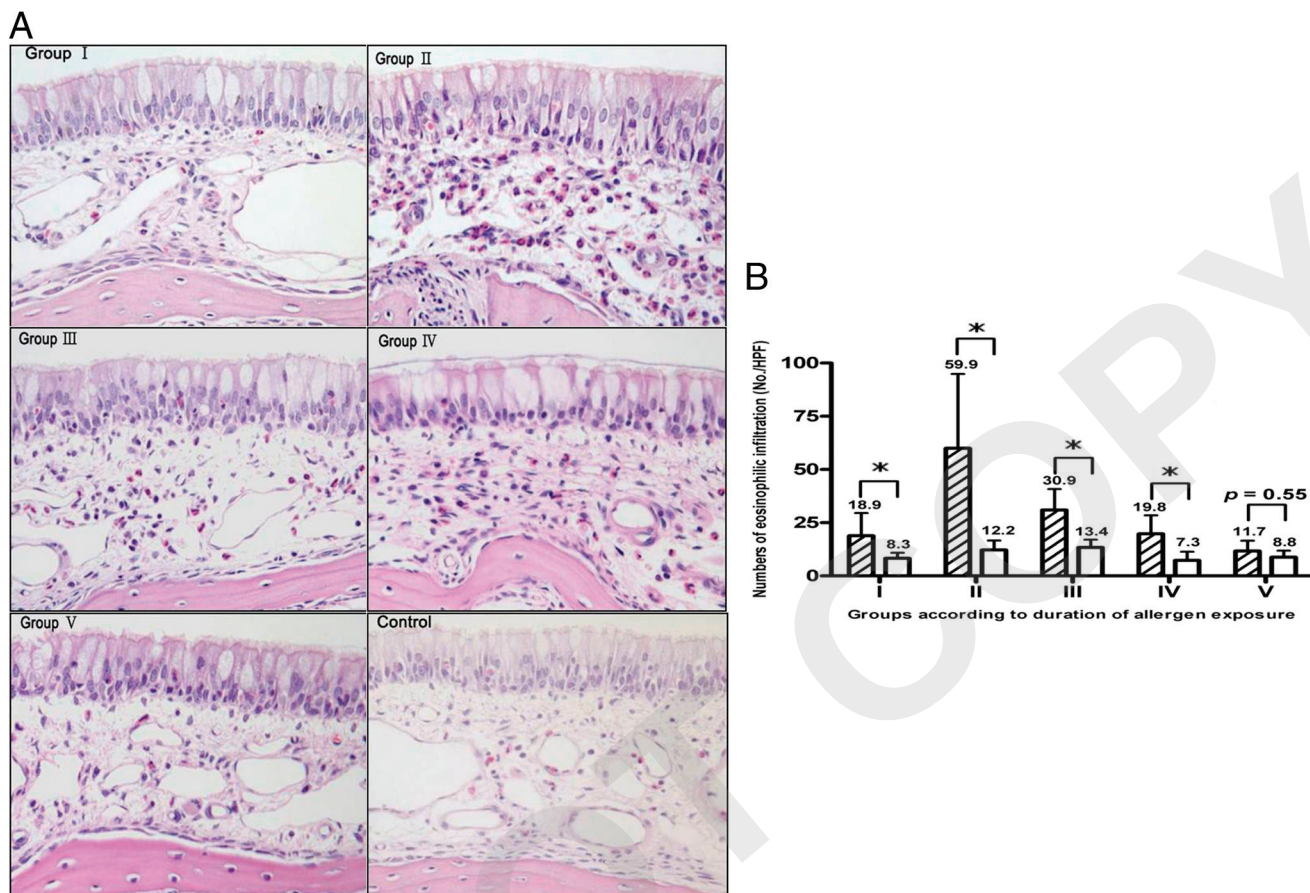


Figure 3. (A) Eosinophil infiltration in the maxilloturbinate mucosa in the experimental and control groups (H&E stain, 400 $\times$ magnification). BN rats were exposed to OVA daily for 1 (group I), 4 (group II), 8 (group III), and 12 weeks (group IV), respectively; other BN rats were exposed for 12 weeks and then protected from challenge for 4 weeks (group V). Control rats were exposed to saline instead of OVA. Eosinophil infiltration was prominent at 4 weeks of exposure and declined thereafter. The eosinophil infiltration observed in group II was significantly higher than that in groups I, IV, and V ($p = 0.016$, $p = 0.015$, and $p = 0.004$, respectively). (B) The difference in eosinophil infiltration between groups IV and V was borderline statistically significant ($p = 0.053$). Diagonally striped bars indicate OVA and open bars indicate no OVA. BN, brown Norway; H&E, hematoxylin-eosin; OVA, ovalbumin.

Table 2 Number of goblet cells and thickness of submucosal glands

Group	No. of Goblet Cell (no./100 μ m)			Thickness of Submucosal Gland (μ m)		
	Experiment	Control	<i>p</i> Value	Experiment	Control	<i>p</i> Value
I	4.22 $\pm$ 0.98	3.22 $\pm$ 0.69	0.262	11.2 $\pm$ 0.9	10.5 $\pm$ 1.1	0.548
II	4.50 $\pm$ 0.86	3.11 $\pm$ 1.50	0.167	12.0 $\pm$ 0.7	10.3 $\pm$ 1.3	0.167
III	4.76 $\pm$ 0.57	3.44 $\pm$ 0.51	0.033	12.8 $\pm$ 1.7	10.2 $\pm$ 1.3	0.042
IV	4.90 $\pm$ 0.60	3.78 $\pm$ 0.51	0.033	15.1 $\pm$ 0.9	10.4 $\pm$ 1.1	0.017
V	4.00 $\pm$ 0.67	3.89 $\pm$ 0.51	0.905	14.0 $\pm$ 0.9	10.6 $\pm$ 1.0	0.024

All values are expressed as mean $\pm$ SD except *p* values.

from that in the group exposed to allergen for 12 weeks. These results suggest that goblet cell hyperplasia and submucosal gland hypertrophy occur after allergen exposure for at least 8 weeks and that goblet cell hyperplasia is a reversible change, whereas submucosal gland hypertrophy is irreversible after avoidance of allergen for 4 weeks. These findings are similar to those of a previous study, which showed that the increase in goblet cell numbers caused by allergen exposure decreased gradually and was almost back to baseline values 4 weeks after the cessation of allergen exposure, whereas subepithelial changes did not resolve after cessation.⁹

Subepithelial thickness was markedly increased after 12 weeks of allergen challenge and did not return to the value of the control group after avoidance of allergen for 4 weeks. Previous studies in BALB/c mice also showed that the thickness of the submucosal collagen increased significantly as a result of prolonged exposure to allergens (12 weeks).^{6,29} These results, paired with our own, imply that the thickness of the subepithelial layer increases notably after exposure to allergen for at least 12 consecutive weeks and that subepithelial thickness does not return to normal.

In this study, eosinophil infiltration increased markedly after allergen challenge, peaked at 4 weeks of exposure, and declined thereafter.

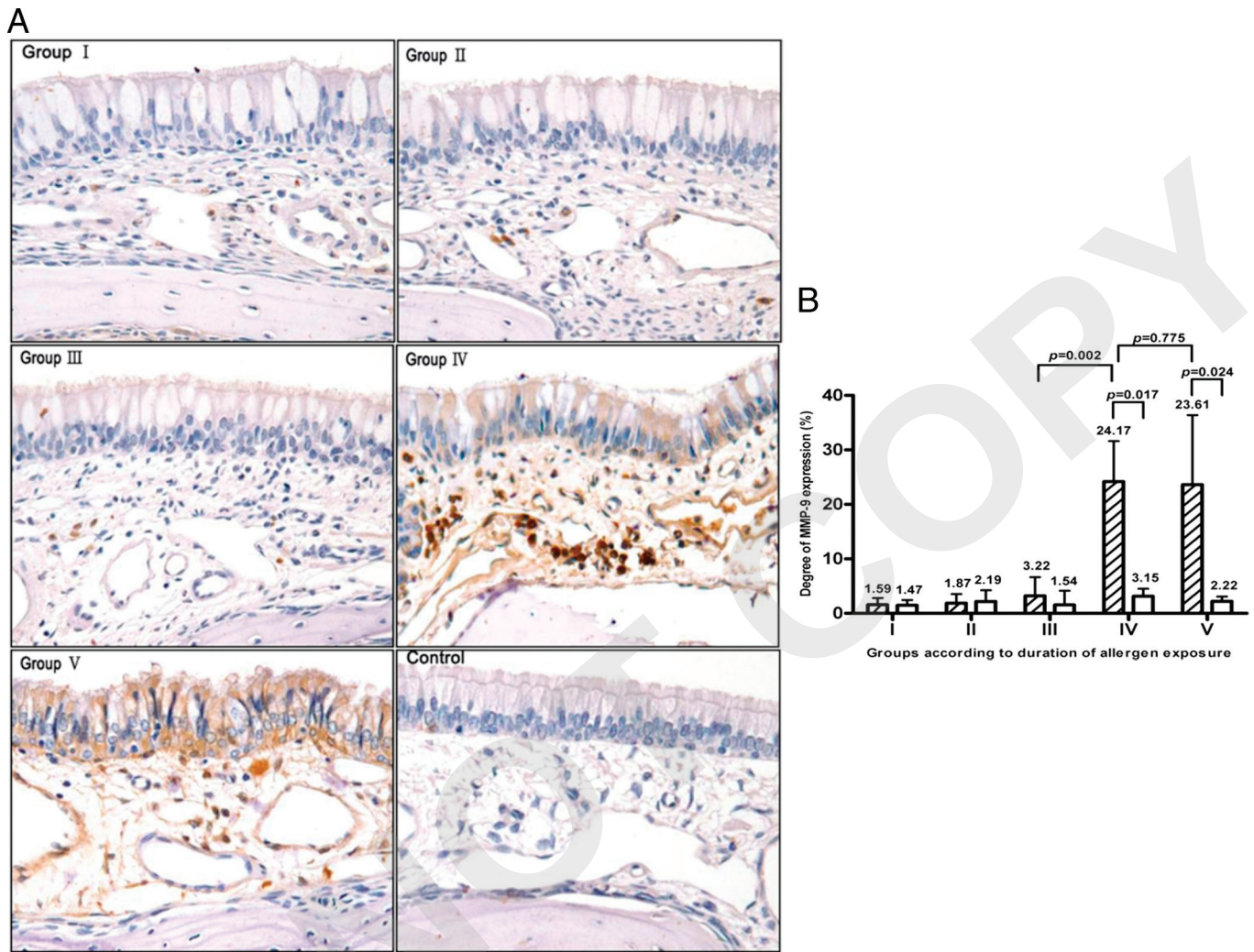


Figure 4. (A) Expression of MMP-9 in the maxilloturbinate mucosa (IHC stain, 400 $\times$ magnification). OVA exposure for 1 week (group I), 4 weeks (group II), 8 weeks (group III), and 12 weeks (group IV), respectively; rats in group V were exposed to OVA for 12 weeks, followed by avoidance of OVA for the following 4 weeks. Control rats were exposed to saline instead of OVA. (B) The expression of MMP-9 was markedly higher in groups IV and V than groups I, II, and III (versus group IV, $p = 0.003$, $p = 0.003$, and $p = 0.002$, respectively; versus group V, $p = 0.004$, $p = 0.004$, and $p = 0.007$, respectively). However, there was no significant difference in MMP-9 expression between groups IV and V ($p = 0.775$). Diagonally striped bars indicate OVA and open bars indicate no OVA. IHC, immunohistochemistry; OVA, ovalbumin; MMP-9, matrix metalloproteinase-9; TIMP-1, tissue inhibitor of metalloproteinase-1.

ter. It finally returned to the control group value after allergen avoidance for 4 weeks. Several studies have been performed to evaluate the changes of eosinophilic infiltration in the bronchoalveolar lavage fluid and the nasal mucosa after repeated allergen challenge in BALB/c mice. These studies reported that the eosinophil infiltration increased markedly during allergen exposure and peaked early during exposure and declined gradually thereafter.^{6,9} OVA-specific IgE level and eosinophil infiltration decreased together in the group exposed to allergen for 8 weeks compared with the group exposed to allergen for 4 weeks in our study, although this difference did not reach statistical significance. Some researchers reported that repetitive allergen challenge for 3 weeks induced the peak of allergic reaction and allergen exposure for 6 weeks decreased allergic reaction although inflammatory adjuvant such as Al(OH)₃ or pertussis toxin was administered.^{5,13} They indicated that allergic reaction decreased after prolonged repeated allergen challenge, because the process of tolerogenesis may begin to happen in this period. Although the peak time appears to differ according to the experimental animal and method used, eosinophil infiltration increases markedly during aller-

gen exposure and peaks early during exposure because of the possibility of tolerogenesis.

MMP-9 is a zinc- and calcium-dependent endopeptidase that induces extensive alterations of the extracellular matrix (ECM) and basement membrane proteins,³⁰ and TIMP-1 is a specific inhibitor of MMP-9. It has been suggested that imbalance of MMP-9 and TIMP-1 leads to airway remodeling by causing structural changes in the airway.³¹ Previous studies have shown that asthmatic patients have significantly higher MMP-9 and TIMP-1 immunoreactivity in their bronchial tissues and sputum, which suggests that increased expression is associated with the structural remodeling process.^{31–34} It has also been reported that overexpression of MMP-9 is associated with the pathogenesis of nasal polyps.^{35–37} However, the few studies that investigated the expression of these factors in patients with AR reported inconsistent results. One study stated that MMP-9 is not increased in perennial AR,³⁸ whereas another studies showed that MMP-9 was increased during the late-phase inflammatory response,³⁹ and large amounts of TIMP-1 were also found in the nasal mucosa of patients with perennial rhinitis.³⁸ We showed that the expression of

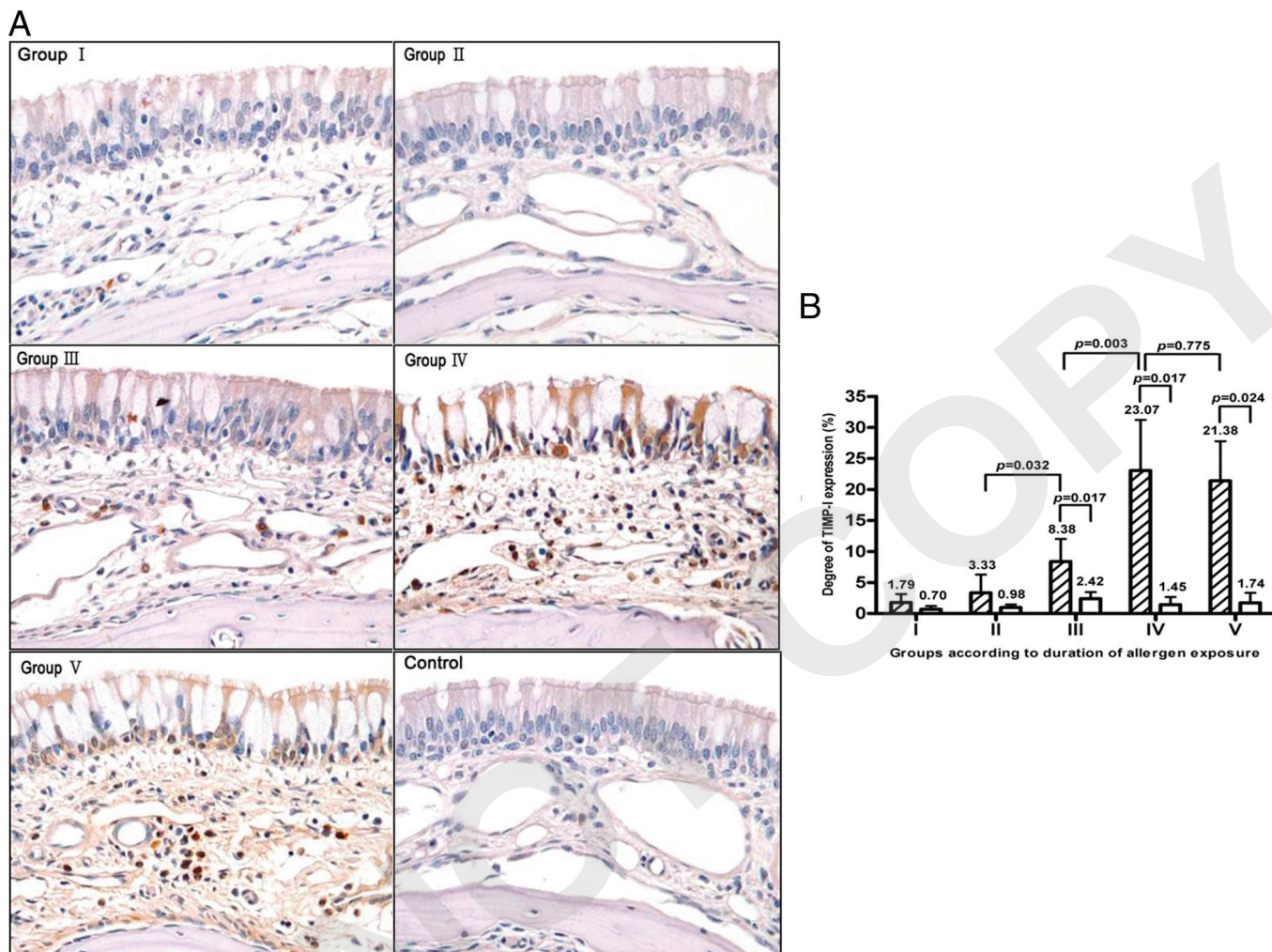


Figure 5. (A) Expression of TIMP-1 in the maxilloturbinate mucosa (IHC stain, 400× magnification). OVA exposure for 1 week (group I), 4 weeks (group II), 8 weeks (group III), and 12 weeks (group IV), respectively; rats in group V were exposed to OVA for 12 weeks, followed by avoidance of OVA for the following 4 weeks. Control rats were exposed to saline instead of OVA. (B) The expression of TIMP-1 was significantly higher in groups IV and V than groups I, II and III (versus group IV, $p = 0.003$ for all; versus group V, $p = 0.004$, $p = 0.004$, and $p = 0.003$, respectively). However, TIMP-1 expression was similar between groups IV and V ($p = 0.775$). In addition, the expression of TIMP-1 in group III was different from that in group II ($p = 0.032$). Diagonally striped bars indicate OVA and open bars indicate no OVA. IHC, immunohistochemistry; OVA, ovalbumin; MMP-9, matrix metalloproteinase-9; TIMP-1, tissue inhibitor of metalloproteinase-1.

MMP-9 and TIMP-1 increased markedly after allergen challenge for 12 weeks and did not return to baseline after cessation of the allergen challenge for 4 weeks. These findings therefore suggest that remodeling of the ECM may occur after repeated exposure to allergen and this process may be irreversible because of chronic structural changes of ECM.

In conclusion, this study suggested that the BN rat may provide a useful animal model for reflecting the nasal airway remodeling in AR and this model could be used to evaluate the mechanism and the prevention strategies for remodeling.

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