ORIGINAL CONTRIBUTION

Rhinology, 47, 199-206, 2009

Remodeling of nasal mucosa by allergen exposure in guinea pigs is suppressed by steroid and pranlukast*

Wenyu She, Kazuhiko Takeuchi, Shinya Suzuki, Hiroshi Sakaida, Hajime Ishinaga, Yuichi Majima

Department of Otorhinolaryngology, Head and Neck Surgery, Mie University Graduate School of Medicine, Edobashi, Tsu, Mie, Japan

SUMMARY

Background: *It is unclear whether remodeling exists in allergic rhinitis in man. The aim of this study was to establish a guinea pig model of allergic rhinitis with remodeling and to examine the effects of dexamethasone and pranlukast on nasal mucosa remodeling.*

Methods: *In the first experiment, three groups of ovalbumin-sensitized Hartley guinea pigs received intranasal challenges with ovalbumin for 1, 8, 12 weeks, respectively. In the second experiment, to examine the effect of dexamethasone and pranlukast, the animals were divided into 4 groups: negative control group; ovalbumin-sensitized group; ovalbumin + dexamethasone group; and ovalbumin + pranlukast group. During 12 weeks of intranasal exposure to ovalbumin, the latter two groups received daily intraperitoneal injections of dexamethasone and pranlukast, respectively.*

Results: *In the first experiment, in contrast to the negative control group, the ovalbumin-sensitized group exhibited significant goblet cell hyperplasia, epithelial damage and deposition of extracellular matrix in the nasal septal mucosa and conchae. In the second experiment, these changes were significantly inhibited by dexamethasone and pranlukast, respectively.*

Conclusions: *We have established a model of upper airway remodeling in guinea pigs. The tissue remodeling was inhibited by early intervention with the antiallergic-inflammatory agents dexamethasone and pranlukast.*

Key words: *allergic rhinitis, epithelium, extracellular matrix, guinea pigs, remodeling*

INTRODUCTION

In 1992, Bousquet reported that the chronic allergic inflammation of asthma causes structural changes in the airway, and named these changes "airway remodeling" [1]. It took several years to identify the processes that comprise airway remodeling. In asthma, airway remodeling includes epithelial shedding, thickening of the basement membrane (lamina reticularis), increased blood vessel cross-sectional area, airway smooth-muscle hyperplasia and hypertrophy, mucous gland and goblet cell hyperplasia, and increased collagen deposition [2,3].

The upper and lower airways have a similar mucosal structure, and exhibit similar inflammatory reactions to irritants and allergens. Increasing evidence indicates that there is no difference in the degree of cell infiltration or cytokine expression during allergic inflammation between nasal mucosa and bronchial mucosa [4]. Even though inflammation is similar in allergic rhinitis and asthma, the pathologic extent of nasal remodeling in patients with rhinitis seems to be far less extensive than that in the bronchi of asthaetic patients [5].

However, the structural changes of nasal mucosa in allergic rhinitis remain unclear, and conflicting data has been obtained from biopsy studies of patients with allergic rhinitis [6,7]. Many studies have shown that, although the structural changes in the upper airway may not be as distinct as those in the lower airways in asthma, the structure of the nasal mucosae in allergic rhinitis is not normal [6,8].

Allergic rhinitis is a strong risk factor for allergic asthma. It is probable that the lower airways are affected from allergic rhinitis (AR). It is reported that isolated allergic sensitization and challenge of the upper airway results in lower-airway inflammation and that IL-13 mediates inflammation of both airways in a murine model [9].

Animal models can be useful for studies of structural changes of nasal mucosa in allergic rhinitis. While most of the animal models of allergic upper airway inflammation are restricted to acute inflammatory changes following relatively short periods

*Received for publication: January 28, 2008; accepted: October 28, 2008

of allergen exposure [10], a number of other murine studies have looked at longer challenge periods [11,12]. In the present study, in order to define the features of upper airway remodeling in allergic rhinitis, we developed an experimental animal model in which ovalbumin (OVA)-sensitized guinea pigs received brief or prolonged intranasal exposure to OVA. We chose guinea pigs because we previously established a nasal allergy model in this species [13]. Secondly, using the prolonged OVA exposure murine model, we examined whether these changes would be inhibited by antiallergic-inflammatory treatment of nasal allergy. Glucocorticoids and cys-leukotriene (LT)1 receptor antagonists are both effective antiallergic-inflammatory agents for treatment of airway allergic inflammatory disease, and are both widely used to treat AR. We hypothesized that glucocorticoids and cysLT1 receptor antagonists could inhibit the remodeling changes associated with nasal allergic inflammation.

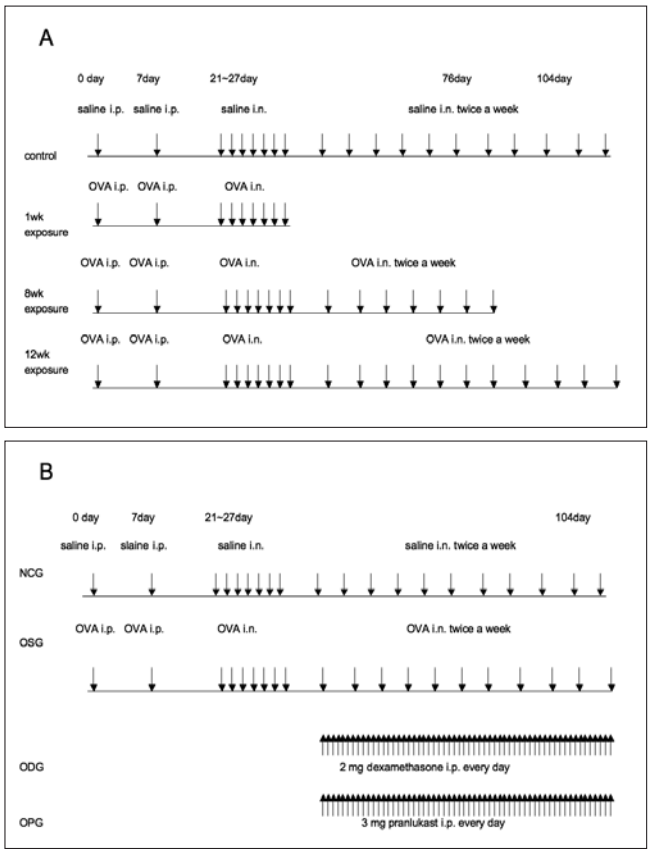

Figure 1. Outlines of study designs for (A) establishment of long-term exposure model and (B) inhibition by dexamethasone and pranlukast. (A) Three groups of ovalbumin (OVA)-sensitized Hartley guinea pigs received daily intranasal challenges with OVA for 1 week. Two of those 3 groups (groups 3 and 4) then received twice-weekly intranasal challenges with OVA, for 7 and 11 weeks, respectively. (B) In order to examine the effect of dexamethasone and pranlukast, the animals were divided into 4 groups: negative control group (NCG); OVA-sensitized group (OSG); OVA+dexamethsone group (ODG); and OVA+pranlukast group (OPG). During 12 weeks of intranasal exposure to OVA, the ODG and OPG received daily intraperitoneal injections of dexamethasone and pranlukast, respectively.

MATERIALS AND METHODS

(1) Establishment of long-term exposure model (Figure 1A)

Sensitization

We randomly divided 24 male Hartley guinea pigs, (age, 4 weeks; weight, 250~300 g; purchased from Japan SLC) into 4 groups of 6 animals each (groups 1 - 4). Animals in groups 2, 3 and 4 were sensitized to ovalbumin (OVA) by administering an intraperitoneal injection of 1 ml of a suspension of OVA (50 mg/ml) and aluminum hydroxide ($Al(OH)_3$; 20 mg/ml) in 0.9% saline on day 0. On day 7, the animals in groups 2, 3 and 4 received an intraperitoneal injection of 1 ml of a suspension of OVA (5 mg/ml) and $Al(OH)_3$ (20 mg/ml) in 0.9% saline as a booster. Animals in group 1 (control group) received intraperitoneal injections of 0.9% saline on day 0 and day 7. The entire study has been approved by the Mie University Animal Experiment Review Board. The guinea pigs were fed an OVA free diet.

Challenge

Sensitized guinea pigs were challenged with brief (group 2) or prolonged (groups 3 and 4) exposure to allergen. Groups 2, 3 and 4 received a daily intranasal challenge with 1 ml of 5% OVA in 0.9% saline from day 21 to day 27. Groups 3 and 4 also received twice-weekly intranasal challenge with 1 ml of 5% OVA in 0.9% saline for an additional 7 and 11 weeks, respectively. Control animals (group 1) received daily intranasal administration of 0.9% saline from day 21 to day 27, and received twice-weekly intranasal administration of 0.9% saline for an additional 11 weeks. The animals were kept on a standard diet with free access to water.

Tissue Preparation

At 24 h after the last intranasal challenge, guinea pigs were killed with an i.p. injection of sodium pentobarbital. The head of each animal was removed, fixed in 10% neutral buffered formalin for 24 hours, and decalcified in 5% trichloroacetic acid for 10 days. The nasal cavity was transversely sectioned at the level of the incisive papilla of the hard palate. The tissue block was embedded in paraffin and cut into sections 3 μm thick.

Morphometry

Paraffin sections were stained with hematoxylin and eosin (H&E), alcian blue (pH 2.6), periodic acid-Schiff (AB-PAS), or Masson's Trichrome (MT). We counted the eosinophils and gland cells in the nasal septum, and the AB-PAS-positive cells in the surface epithelium of the nasal septal mucosa. We determined the percentage area of MT-stained extracellular matrix (ECM) in septal mucosa and conchae and the damage to the epithelium, using an image analyzer (SP 500; Olympus, Tokyo, Japan).

Statistical analysis

The data were presented as mean ± SD. After an ANOVA was performed, individual t-tests in a multi-group study design

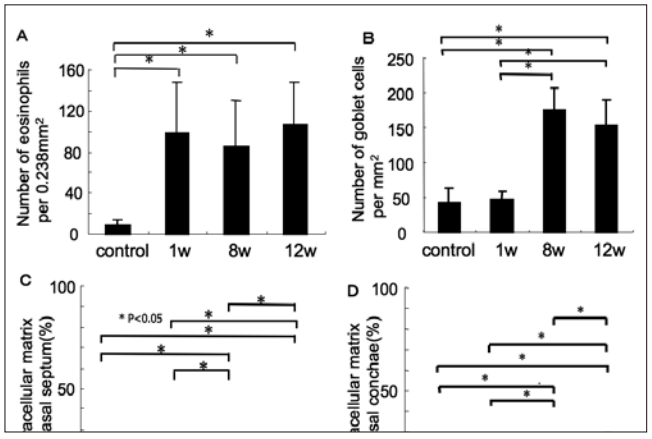

Figure 2. (A) Number of infiltrating eosinophils in nasal septal mucosa. Eosinophils were counted in at least 5 high-power fields on both sides of the septal cartilage (magnification, x400; n=6). (B) The number of AB-PAS-positive cells in nasal septal mucosa. The percent area of MT-stained ECM in mucosa of nasal septum (C) and nasal conchae (D). * indicates $p < 0.05$.

were done. All comparisons between groups were made using Student's t test. All comparisons were two-tailed, assuming equal variance. P values of < 0.05 were considered to indicate significance.

(2) Inhibition study (Figure 1B)

Sensitization

We randomly divided 18 male Hartley guinea pigs into 4 groups: negative control group (NCG) (n=6); OVA-sensitized group (OSG) (n=4); OVA + dexamethsone group (ODG) (n=4); and OVA + pranlukast group (OPG) (n=4). Animals in the OSG, ODG and OPG were sensitized to OVA as stated earlier. Animals in the NCG were injected with 0.9% saline only.

Challenge and treatment

Sensitized animals received a daily intranasal challenge from day 21 to day 27, and received a twice-weekly intranasal challenge for an additional 11 weeks. During those 11 weeks, the ODG received a daily i.p. injection of 2 mg of dexamethsone in 1 ml of 0.9% saline, OPG received a daily i.p. injection of 3 mg of pranlukast in 1 ml of 0.9% saline, and OSG and NCG received a daily i.p. injection of 1 ml of 0.9% saline only. Tissue preparation, morphometry and statistical analysis were performed as stated earlier.

Tissue preparation, morphometry, and statistical analysis

Tissue preparation, morphometry and statistical analysis were done in the same way as in section (1).

RESULTS

(1) Establishment of long-term exposure model

Eosinophil infiltration

Using the H&E-stained sections, we counted the eosinophils in nasal septal mucosa in 5 high-power fields on both sides of the septal cartilage (magnification: x400) (Figure 2). In the sensitized groups (groups 2, 3 and 4), we observed significant eosinophil infiltration. In the control group (group 1), we observed only a small number of eosinophils. There was no significant difference between any of the sensitized groups (groups 2, 3 and 4). The eosinophils were all granulated.

Epithelial damage

Epithelial damage was assessed using H&E-stained sections (Figures 3 and 4) and the staging system described by Ponikau [14]. In the 1-week exposure group (group 2), there was no significant epithelial damage. In the 8- and 12-week exposure groups (groups 3 and 4, respectively), we observed significant epithelial damage including upper layer erosion and absence of

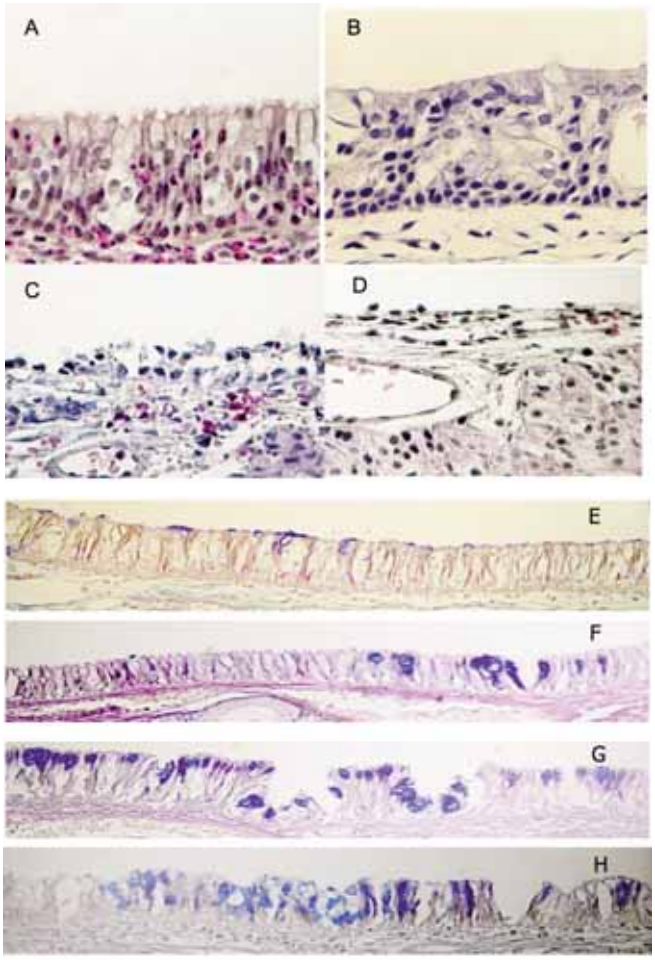

Figure 3. Representative microscopic findings of nasal septal epithelium of stage 0 (A), stage 1 (B), stage 2 (C) and stage 3 (D). Epithelial damage was assessed using H&E-stained sections and the staging system described by Ponikau [14]. The percent area of each staging of nasal septal epithelium was calculated. Comparison of goblet cell hyperplasia in the nasal septal epithelium (E-H). Paraffin-embedded sections of nasal mucosa were stained with AB-PAS. We observed an increase in the number of AB-PAS-positive cells in the nasal septum mucosa of the prolonged allergen-exposure groups (groups 3(G) and 4(H)), but not in the brief allergen-exposure group (group 2(F)), compared with the control (group 1(E)).

cilia. There was no significant difference between any of the groups in stage 3 [14], in which epithelium eroded away.

Goblet cell hyperplasia

In order to determine the extent of mucus cell metaplasia following allergen challenge, paraffin-embedded sections of nasal mucosa were stained with AB-PAS (Figures 2 and 3). We observed an increase in the number of AB-PAS-positive cells in the nasal septum mucosa of the prolongedallergen-exposure groups (groups 3 and 4), but not in the briefallergen-exposure group (group 2), compared with the control (group 1). There was no significant difference between the 2 prolongedallergen-exposure groups (groups 3 and 4).

Gland cells

The number of gland cells in the nasal septal mucosa was counted and expressed as gland cells/mm^2. There was no significant difference between any of the 4 groups (data not shown).

Extracellular matrix deposition

In order to determine whether the OVA challenge affected ECM accumulation, paraffin-embedded sections of the nasal cavity were stained with MT to visualize collagen deposition (blue colour indicates collagen) (Figures 3 and 5). The percent area of MT-stained collagen in mucosa of the nasal septum and conchae was determined to assess ECM deposition. In the control group (group 1), there was little collagen (MT staining) beneath the basement membrane of the epithelium and around the glands and vasculature. In the brief OVA-exposure group (group 2), there was no increase in collagen fibrils within the mucosa of the nasal septum or conchae. In contrast, in the prolonged OVA-exposure groups (groups 3 and 4), we observed an increase in the amount of ECM. Furthermore, in both the nasal septum and conchae, the 12-week exposure group (group 4) exhibited significantly greater ECM deposition than the 8-week exposure group (group 3).

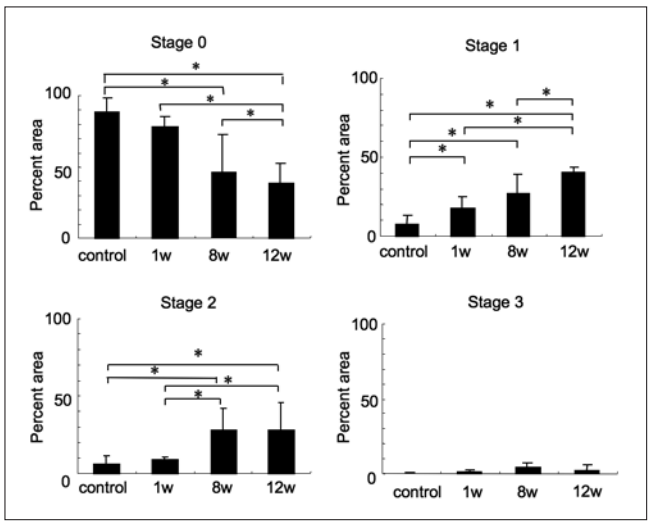

Figure 4. The percent area of each staging of epithelium in septal nasal mucosa (length of basal membrane of each stage of epithelium divided by the total length of the membrane). * indicates $p < 0.05$.

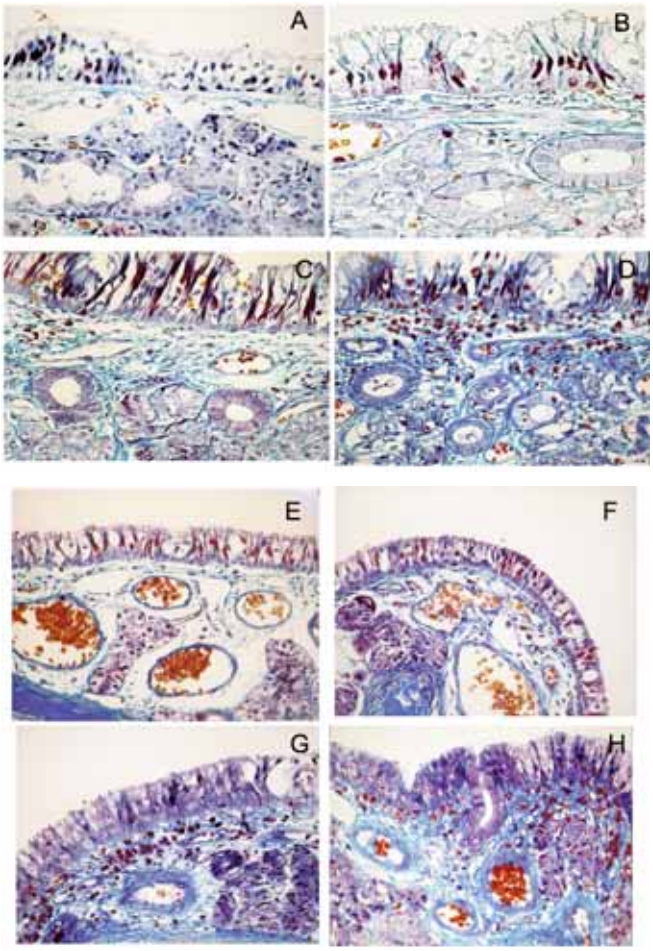

Figure 5. Airway collagen deposition/fibrosis in OVA-treated mice. Nasal septum mucosa was obtained from saline-treated guinea pigs (A) and OVA-treated guinea pigs (B-D), stained with Masson's trichrome, and examined by light microscopy. Nasal conchae tissue was obtained from saline-treated guinea pigs (E) and OVA-treated guinea pigs (F-H) and examined as above. Little collagen is observed in the nasal septum (A) and nasal conchae (E) of the control guinea pigs or of the brief (one week) OVA exposure guinea pigs (B, F). In contrast, relatively strong collagen deposition is seen in the nasal septum and nasal conchae of the prolonged OVA exposure guinea pigs (C, G for eight week OVA exposure: D, H for twelve week OVA exposure).

(2) Inhibition study

Eosinophil infiltration

Using the H&E-stained sections, we counted the number of eosinophils in nasal septal mucosa in at least 5 high-power fields on both sides of the septal cartilage (magnification: x400) (Figure 6A). The number of eosinophils was significantly higher in OSG than in NCG ($p < 0.05$). This change was significantly inhibited by administration of dexamethasone or pranlukast ($p < 0.05$ and $p < 0.05$, respectively to OSG). There was no significant difference between the two treatment groups.

Epithelial damage

In the OSG, we observed significant epithelial damage including upper layer erosion and absence of cilia compared with NCG ($p < 0.05$) (Figures 6E-H). This change was significantly inhibited by administration of dexamethasone or pranlukast ($p < 0.05$ and $p < 0.05$, respectively to OSG). There was no significant difference between the two treatment groups.

Goblet cell hyperplasia

The number of goblet cells was significantly higher in OSG than in NCG ($p < 0.05$) (Figure 6B). This change was significantly inhibited by administration of dexamethasone or pranlukast ($p < 0.05$ and $p < 0.05$, respectively to OSG). There was no significant difference between the two treatment groups. The data was expressed as goblet cells per mm basement membrane of nasal septal mucosal epithelium.

Extracellular matrix deposition

In the NCG, there was little collagen (MT staining) beneath the basement membrane of the epithelium and around the glands and vasculature (Figure 6C and D). In contrast, in the OSG, there was a significant increase in ECM deposition in the submucosa of the nasal septum and conchae ($p < 0.05$). This change was significantly inhibited by administration of dexamethasone or pranlukast ($p < 0.05$ and $p < 0.05$, respectively to OSG). There was no significant difference between the two treatment groups.

DISCUSSION

There is controversy as to whether structural changes occur in allergic rhinitis. The few published studies contain conflicting findings [6,7].

In the present study, prolonged allergen challenge of sensitized guinea pigs produced persistent allergic inflammation of the nasal mucosa with significant infiltration of eosinophils into the epithelium and submucosa. Eosinophils are thought to be a source of several molecules implicated in tissue remodeling processes, including transforming growth factor-α (TGF-α), TGF-β1, fibroblast growth factor-2 (FGF-2), vascular endothelial growth factor (VEGF), matrix metalloprotease-9 (MMP-9), tissue inhibitor of metalloproteinase-1 (TIMP-1), interleukin-13 (IL-13) and IL-17 [15]. Amin has demonstrated that the number of eosinophils is increased in patients with perennial rhinitis, and that the increased number of eosinophils is related to the increased epithelial damage observed in patients with perennial rhinitis [6].

Some authors have observed epithelial shedding in patients with allergic rhinitis [6,8]. The present results indicate that persistent epithelial damage occurs in chronic nasal allergy, although it is not as severe as the damage previously observed in the lower airway in asthma [16]. Studies suggest that lipid mediators, cytokines and eosinophil-specificgranule proteins released by activated eosinophils play important roles in the epithelial damage associated with allergic rhinitis [17]. On the other hand, some allergens that have protease activity may also have direct effects on epithelial integrity. In addition to these direct effects on epithelial structure, many of these agents also alter cell function by inducing a stress response via receptor-mediated processes or by generating reactive oxygen with activation of proinflammatory transcription factors [18].

Allergic rhinitis is also clinically associated with a significant increase in nasal mucus secretion. In healthy subjects, goblet cells (another important source of mucus) are distributed most densely in the inferior turbinate, and less densely in the nasal septum [19]. Berger observed that the number of goblet cells in the inferior turbinate is not affected by perennial allergic or non-allergic rhinitis [20]. However, Gluck reported increased

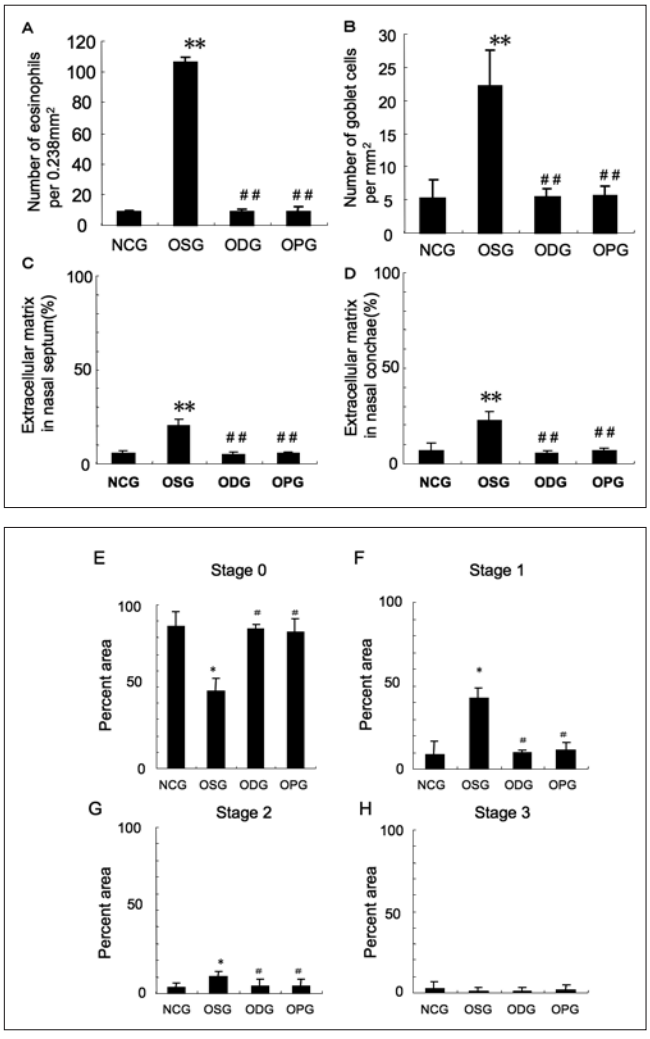

Figure 6. Both dexamethasone and pranlukast treatments inhibited increase of the number of infiltrating eosinophils (A), number of goblet cells (B), and collagen deposition in nasal septal mucosa (C) and in nasal conchae (D). ** indicates $p < 0.01$, compared with NCG. ## indicates $p < 0.01$, compared with OSG. (E-H): The percent area of each stage of epithelium in septal nasal mucosa (length of basal membrane of each stage of epithelium divided by the total length of the membrane) in the treatment groups. * indicates $p < 0.05$, compared with NCG. # indicates $p < 0.05$, compared with OSG.

recovery of goblet cells in nasal smears during the grass pollen season in subjects with pollen-sensitive rhinitis [21]. In the present study, the number of AB-PAS-positive cells increased about 4-fold in the chronic nasal allergy groups (groups 3 and 4). Goblet cell hyperplasia may be induced by allergens and T helper 2 (Th2) lymphocyte-derived cytokines. In in vitro experimental systems, the Th2 cytokines IL-4, IL-9 and IL-13 are highly effective in inducing mucin synthesis and /or goblet cell hyperplasia [22]. However, eosinophils do not appear to play a role in these effects of Th2 cytokines, because several studies [23-25] have shown that there is no association between mucus cell hyperplasia and allergen-induced or Th2 cytokine-induced eosinophilia.

In the present study, we also found that chronic allergen exposure was associated with significantly increased amounts of ECM in both the nasal septal mucosa and conchae. We have evaluated conchae as well as nasal septal mucosa, because inferior mucosal hypertrophy is clinically important. Similar results were obtained in a previous study, in which total collagen levels increased 3-fold during the chronic phase of asthma, compared with control and acute challenge groups [26]. Sanai et al. [27] studied the amount and distribution of collagen in human nasal mucosa of perennial allergic patients and non-allergic subjects and found that the thickness of the basement membrane zone was statistically significantly greater in allergic subjects than in non-allergic ones. Although the mechanism of the ECM deposition in allergic rhinitis remains unclear, it is possible that a collagen-producing system is activated in nasal allergic reactions, as occurs in asthma [27]. In asthma, studies indicate that TGF-β plays a major role in the development and maintenance of the fibrotic response [26], and that Th2 cytokines including IL-4, IL-5, IL-9, IL-11 and IL-13 can induce subepithelial fibrosis [28].

Clinical data suggest that fibrosis is a feature of mucosa remodeling in AR. In a study of turbinate biopsies from 26 patients with perennial AR, Montero Mora et al. observed enlargement of the basal membrane in 92.3% of the patients and subepithelial fibrosis in 92.4% of the patients [29].
Next we demonstrated that these remodeling changes could be inhibited by early intervention with pranlukast or dexamethasone. Leukotrienes can induce many of the abnormalities seen in airway allergic disease, including permeability of vessels, mucous gland secretion, and infiltration of inflammatory cells. Most of their effects are mediated by the cysLT1 receptor, which is a G-proteincoupled receptor [30]. CysLT1 receptor antagonists represent the first new class of anti-asthma treatment in 20 years, and have recently been used to treat nasal allergy. Considerable experience has been gained with pranlukast worldwide, and the available data regarding its clinical pharmacology, efficacy and safety indicate that leukotriene receptor antagonists hold great promise as therapeutic agents for the treatment of allergic inflammatory airway disease [31]. Glucocorticoid therapy is a very effective anti-inflammatory treatment that is suitable for AR, and has been used as a first-line treatment for AR.

Recruitment of eosinophils and nasal mucosal structural changes were significantly inhibited by administration of dexamethasone or pranlukast. These findings suggest that eosinophil infiltration is a particularly important part of the remodeling processes in AR. We do not deny the importance of mast cells in the remodeling process in this model. It is reported that Chymase-positive mast cells can play a role in the vascular component of airway remodeling in asthma, possibly through induction of VEGF [32] and that mast cells regulate procollagen I (alpha 1) production by bronchial fibroblasts derived from subjects with asthma [33].
The reduction of eosinophil activation and recruitment should help prevent epithelial damage. In a study by Druilhe et al., biopsies from steroid-treated asthma subjects exhibited increased expression of the anti-apoptotic marker Bcl-2 and proliferating cell nuclear antigen (PCNA) in epithelial cells [34]. This indicates that steroids increase both the survival and proliferation of epithelial cells, suggesting that they can promote epithelial repair under airway allergic inflammatory conditions [34]. The present finding that dexamethasone increases epithelial integrity is consistent with this hypothesis.

In the ODG and OPG, goblet cell hyperplasia was significantly inhibited by administration of dexamethasone or pranlukast. Similar phenomena have been observed in other studies. In their in vivo experiments, Liu et al. demonstrated that the CysLT1 receptor antagonists pranlukast and zafirlukast inhibited OVA-induced mucus secretion in the trachea of sensitized guinea pigs [35]. Henderson et al. found that in OVA-sensitized/challenged mice, montelukast (another cysLT1 receptor antagonist) reduced the elevated levels of IL-4 and IL-13 in the BAL fluid and reduced the allergen-induced increase in the number of goblet cells in the airways [36]. These reports are consistent with the present results.
Blyth et al. developed a murine model of atopic asthma, for use in studies of allergen-induced subepithelial fibrosis in the lower airway [37]. They demonstrated that the development of subepithelial fibrosis is associated with eosinophil infiltration into airways, and that this fibrosis is significantly suppressed by daily intraperitoneal injection of dexamethasone at a dose of 1 mg/kg [37]. Ueda et al. [38] investigated the effects of pranlukast on eosinophilic inflammation and cytokine production in human nasal mucosa. In their pranlukast-treated group, the levels of eosinophil-derived cytokines and chemical mediators (IL-4, IL-5, RANTES, cysteinyl leukotrienes, IL-1β, TNF-α, and IL-8) were significantly decreased. Their results support the hypothesis that pranlukast exerts its therapeutic effects primarily by blocking the leukotriene receptors on eosinophils.

It is generally considered that remodeling in asthma is an irreversible process. In the present study, we did not show that stopping the antigen administration after 8 or 12 weeks leads to persistent changes. This should be studied in the future research. Moreover, it is necessary to ascertain whether treatment with steroids or pranlukast reverses the process after remodeling has occurred.

In summary, the present results indicate that prolonged allergen exposure of sensitized guinea pigs causes persistent remodeling of the nasal mucosa. The features of AR remodeling that we observed were epithelial damage, goblet cell hyperplasia and ECM deposition. These structural changes were significantly inhibited by intraperitoneal injection of dexamethasone or pranlukast. These results indicate that early intervention with glucocorticoids and cysLT1 receptor antagonists can be an effective method of preventing some features of remodeling in AR.

ACKNOWLEDGEMENTS

This work was supported by Grants-in-Aid for General Scientific Research (B) 15390516, (C) 16591705 and (C) 18591864 from the Ministry of Education, Sciences, and Culture of Japan.

REFERENCES

1. Bousquet J, Chanez P, Lacoste JY, White R, Vic P, Godard P. Asthma: a disease remodeling the airways? Allergy 1992; 47: 3-11.
2. Bousquet J, Jeffery PK, Bussc WW, Jhonson M, Vignola AM. Asthma. From bronchoconstruction to airways inflammation and remodeling. Am J Crite Care Med 2000; 161: 1720-1745.
3. Vignola AM, Kips J, Bousquet J. Tissue remodeling as a feature of persistent asthma. J Allergy Clin Immunol 2000; 105: 1041-1053.
4. Togias A. Rhinitis and asthma: evidence for respiratory system integration. J Allergy Clin Immunol. 2003; 111: 1171-1183.
5. Bousquet J, Jacot W, Vignola AM, Bachert C, Van Cauwenberge P. Allergic rhinitis: a disease remodeling the upper airways? J Allergy Clin Immunol. 2004; 113: 43-49.
6. Amin K. Inflammatory cell and epithelial characteristics of perennial allergic and nonallergic rhinitis with a symptom history of 1 to 3 years' duration. J Allergy Clin Immunol. 2001; 107: 249-257.
7. Braunstahl GJ, Fokkens WJ, Overbeek SE, KleinJan A, Hoogsteden HC, Prins JB. Mucosal and systemic inflammatory changes in allergic rhinitis and asthma: a comparison between upper and lower airways. Clin Exp Allergy. 2003; 33: 579-587.
8. Ishii J, Naito K, Miyata S, Senoh Y, Yokoyama N, Baba R. An electron microscopic study of the relation between epithelial cell damage and eosinophilia in the nasal mucosa of patients with nasal allergy. Arerugi. 2000; 49: 1156-1162.
9. Wang Y, McCusker CT. Interleukin-13-dependent bronchial hyper-responsiveness following isolated upper-airway allergen challenge in a murine model of allergic rhinitis and asthma. Clin Exp Allergy. 2005; 35: 1104-1111.
10. Malm-Erjefalt M, Persson CG, Erjefalt JS. Degranulation status of airway tissue eosinophils in mouse models of allergic airway inflammation. Am J Respir Cell Mol Biol. 2001; 24: 352-359.
11. Palmans E, Pauwels RA, Kips JC. Repeated allergen exposure changes collagen composition in airways of sensitised Brown Norway rats. Eur Respir J. 2002; 20: 280-285.
12. Jain VV, Kitagaki K, Businga T, Hussain I, George C, O'shaughnessy P, et al. CpG-oligodeoxynucleotides inhibit airway remodeling in a murine model of chronic asthma. J Allergy Clin Immunol. 2002; 110: 867-872.
13. Ishida M, Amesara R, Ukai K, Sakakura Y. Antigen (DNP-As)-induced allergic rhinitis model in guinea pigs. Ann Allergy. 1994; 72: 240-244.
14. Ponikau JU, Sherris DA, Kephart GM, et al. Features of airway remodeling and eosinophilic inflammation in chronic rhinosinusitis: is the histopathology similar to asthma? J Allergy Clin Immunol. 2003; 112: 877-882.
15. Kay AB. A role for eosinophils in airway remodelling in asthma.Trends Immunol. 2004; 25: 477-482.
16. Amin K, Lúdvíksdóttir D, Janson C, et al. Inflammation and structural changes in the airways of patients with atopic and nonatopic asthma. BHR Group. Am J Respir Crit Care Med. 2000; 162: 2295-2301.
17. Makino S, Fukuda T. Eosinophils and allergy in asthma. Allergy Proc. 1995; 16: 13-21.
18. Holgate ST, Lackie P, Wilson S, Roche W, Davies D. Bronchial epithelium as a key regulator of airway allergen sensitization and remodeling in asthma.Am J Respir Crit Care Med. 2000; 162: S113-117.
19. Tos M. Distribution of mucus producing elements in the respiratory tract. Differences between upper and lower airway. Eur J Respir Dis Suppl. 1983; 128: 269-279.
20. Berger G, Marom Z, Ophir D. Goblet cell density of the inferior turbinates in patients with perennial allergic and nonallergic rhinitis. Am J Rhinol. 1997; 11: 233-236.
21. 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.
22. Rose MC, Nickola TJ, Voynow JA. Airway mucus obstruction: mucin glycoproteins, MUC gene regulation and goblet cell hyperplasia. Am J Respir Cell Mol Biol. 2001; 25: 533-537.
23. Hailé S, Lefort J, Joseph D, Gounon P, Huerre M, Vargaftig BB. Mucous cell metaplasia and inflammatory cell recruitment are dissociated in allergic mice after antibody and drug-dependent cell depletion in a murine model of asthma. Am J Respir Cell Mol Biol 1999; 20: 1-12.
24. Cohn L, Homer RJ, MacLeod H, Mohrs M, Brombacher F, Bottomly K. Th2-induced airway mucus production is dependent on IL-4Ra, but not eosinophils. J Immunol 1997; 162: 6178-6183.
25. Singer M, Lefort J, Vargaftig BB. Granulocyte depletion and dexamethasone differentially modulate airways hyperreactivity, inflammation, mucus accumulation and secretion induced by rmIL-13 or antigen. Am J Respir Cell Mol Biol 2002; 26: 74-84.
26. McMillan SJ, Lloyd CM. Prolonged allergen challenge in mice leads to persistent airway remodelling. Clin Exp Allergy. 2004; 34: 497-507.
27. 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.
28. Romagnani S. The role of lymphocytes in allergic disease. J Allergy Clin Immunol. 2000; 105: 399-408.
29. Montero Mora P, Blanco E, Matta Campos JJ, González Espinosa A, Guidos Fogelbach G, Tinajeros Castañeda OA. Nasal remodeling in patient with perennial allergic rhinitis. Rev Alerg Mex. 2003; 50: 79-82.
30. David A. pharmacology of leukotriene receptor antagonists. Am J Respir Crit Care Med 1998; 157: S214-219.
31. Salvi SS, Krishna MT, Sampson AP, Holgate ST. The anti-inflammatory effects of leukotriene-modifying drugs and their use in asthma. Chest. 2001; 119: 1533-1546.
32. Chetta A, Zanini A, Foresi A, Del Donno M, Castagnaro A, D'Ippolito R et al. Chymase-positive mast cells play a role in the vascular component of airway remodeling in asthma. J Allergy Clin Immunol. 2007; 120: 329-333.
33. Plante S, Semlali A, Joubert P, et al. Mast cells regulate procollagen I (alpha 1) production by bronchial fibroblasts derived from subjects with asthma through IL-4/IL-4 delta 2 ratio. J Allergy Clin Immunol. 2006; 117: 1321-1327.

34. Druilhe A, Wallaert B, Tsicopoulos A, et al. Apoptosis, proliferation, and expression of Bcl-2, Fas, and Fas ligand in bronchial biopsies from asthmatics. Am J Respir Cell Mol Biol. 1998; 19: 747-757.
35. Liu YC, Khawaja AM, Rogers DF. Effects of the cysteinyl leukotriene receptor antagonists pranlukast and zafirlukast on tracheal mucus secretion in ovalbumin-sensitized guinea-pigs in vitro. Br J Pharmacol. 1998; 124: 563-571.
36. Henderson WR Jr, Tang LO, Chu SJ, et al. A role for cysteinyl leukotrienes in airway remodeling in a mouse asthma model. Am J Respir Crit Care Med. 2002; 165: 108-116.
37. Blyth DI, Wharton TF, Pedrick MS, Savage TJ, Sanjar S. Airway subepithelial fibrosis in a murine model of atopic asthma: suppression by dexamethasone or anti-interleukin-5 antibody. Am J Respir Cell Mol Biol. 2000; 23: 241-246.
38. Ueda T, Takeno S, Furukido K, Hirakawa K, Yajin K. Leukotriene receptor antagonist pranlukast suppresses eosinophil infiltration and cytokine production in human nasal mucosa of perennial allergic rhinitis. Ann Otol Rhinol Laryngol. 2003; 112: 955-961.

Kazuhiko Takeuchi
Department of Otorhinolaryngology
Head and Neck Surgery
Mie University Graduate School of Medicine
2-174 Edobashi, Tsu,
Mie 514-8507
Japan

Tel: +81-59-231-5028
Fax: +81-59-231-5218
E-mail: kazuhiko@clin.medic.mie-u.ac.jp