

ASTHMA MECHANISMS

Inhibitory Effects of Anti-Immunoglobulin E Antibodies on Airway Remodeling in A Murine Model of Chronic Asthma

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Background: Airway remodeling is one of the cardinal features of asthma and is thought to play a pivotal role in refractory or persistent asthma. Immunoglobulin E (IgE) has a major effect on the pathogenesis of asthma. The aim of this study was to investigate the effects of anti-IgE antibody not only on airway inflammation and bronchial hyperresponsiveness, but also on airway remodeling in a murine model of chronic asthma. **Methods:** The authors developed a mouse model of chronic asthma in which ovalbumin (OVA)-sensitized female BALB/c-mice were exposed to intranasal OVA administration twice a week for 3 months. Anti-IgE antibodies were administered intravenously starting on the 38th day and once a month thereafter for 3 months during the intranasal OVA challenge. **Results:** Mice that were chronically exposed to OVA developed sustained eosinophilic airway inflammation and airway hyperresponsiveness (AHR) to methacholine and showed increased levels of collagen, hydroxyproline, and α -smooth muscle actin, as compared with control mice. Treatment with anti-IgE antibody inhibited the development of AHR, eosinophilic inflammation, and airway remodeling. Moreover, anti-IgE antibody treatment reduced the levels of interleukin (IL)-5 and IL-13 in the bronchoalveolar lavage fluids, although it did not affect the levels of IL-10, transforming growth factor- β , and activin A. **Conclusion:** These results suggest that anti-IgE antibody treatment modulates the airway inflammation and remodeling associated with chronic allergen challenge. The inhibition of inflammation may be related to the regulation of Th2 cytokines. However, the mechanisms underlying the blocking of airway remodeling by anti-IgE antibody remain to be elucidated.

Keywords airway remodeling; asthma; immunoglobulin E; monoclonal antibody

INTRODUCTION

Asthma is a disease characterized by chronic inflammation, hyperresponsiveness, and structural alterations in the airway. These structural changes in asthma are termed airway remodeling. Clinically, airway remodeling may be linked to persistent increases in airway responsiveness and mucus production, variable or fixed airflow limitation, and possibly declines in pulmonary function (1). Histologically, the characteristic features of airway remodeling include epithelial denudation, goblet cell metaplasia, subepithelial fibrosis, smooth muscle hypertrophy/hyperplasia, increased angiogenesis, and alterations to extracellular matrix (ECM) components (2). Although the mechanism of remodeling in asthma has not been fully clarified, it appears that persistent inflammation and/or aberrant tissue repair are the underlying causes of airway remodeling, and that several important inflammatory cell types and mediators may be involved in mediating airway remodeling (3). Therefore, both classical asthma drugs, such as corticosteroids and leukotriene antagonists, and agents directed against the above candidate mediators have been investigated whether to prevent or reverse remodeling in asthma.

Immunoglobulin E (IgE) plays a key role in the pathogenesis of allergic diseases and asthma. In asthma, IgE initiates the allergic response by inducing mast cells to release inflammatory mediators and by stimulating the recruitment of eosinophils, which are major effector cells (4). Therefore, attention has been focused in recent years on the regulation of IgE production as an important strategy for the treatment of allergic diseases. Recently, the nonanaphylactogenic anti-human IgE antibody omalizumab was approved for use in adults and children over 12 years of age as an add-on therapy in severe asthma. In asthmatic subjects, the administration of omalizumab has been shown to reduce the plasma-free IgE levels, reduce early- and late-phase allergic responses after allergen provocation, improve symptoms, reduce rescue medication, and decrease the rate of acute exacerbation (5). In addition, anti-IgE antibody exerts an anti-inflammatory effect in acute asthma by suppressing inflammatory cytokines (6). However, to the best of our knowledge, there are few reports on whether or not anti-IgE antibody has any effect on airway remodeling, especially in models of chronic asthma.

The objective of the present study was to determine whether anti-IgE therapy used for chronic asthma has anti-inflammatory and anti-remodeling effects. Furthermore, to explore the underlying mechanism, we measured the levels of Th2 cytokines and regulatory cytokines, including interleukin (IL)-10, transforming growth factor (TGF)- β , and activin A, after anti-IgE treatment in a murine model of chronic asthma.

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MATERIALS AND METHODS

Sensitization and Antigen Challenge Protocol

Female BALB/c mice (The Dae-Han Experimental Animal Center, Daejeon, Korea), 8 to 10 weeks of age, were used in the experiments and divided into three groups ($n = 16$ per group), i.e., control, chronic asthma, and chronic asthma with anti-IgE treatment. The mice were immunized on days 0, 7, 14, and 21 by subcutaneous injection of 25 μ g ovalbumin (OVA; grade V; Sigma-Aldrich, St. Louis, MO, USA), which was adsorbed to 1 mg of aluminum hydroxide (Aldrich, Milwaukee, WI, USA) in 200 μ l of normal saline. Intranasal OVA challenges (20 ng/50 μ l in phosphate-buffered saline [PBS]) were administered on days 27, 29, and 31 under isoflurane (Vedco, St. Joseph, MO, USA) anesthesia. From day 38, the intranasal OVA challenges were repeated twice a week for 3 months, in accordance with the chronic asthma protocols used in other studies (7–10). Control mice were treated in the same way with PBS without OVA. Mice were killed 24 h after the final OVA challenge and the bronchoalveolar lavage (BAL) fluid and lungs were analyzed. All animal experimental protocols were approved by the Animal Subjects Committees of the Catholic University of Korea.

Administration of Anti-IgE Antibody

Twenty minutes before challenge of OVA inhalations, nonanaphylactic anti-mouse IgE monoclonal antibodies (clone R35–92; Pharmingen, San Diego, Calif., USA) were administered intravenously (100 μ g/200 μ l in normal saline), starting on day 38 and repeated once a month for 3 months, referring to the previous study (11).

Measurement of Airway Hyperresponsiveness (AHR)

Airway responsiveness was assessed 24 h after the final OVA inhalation using a single-chamber, whole-body plethysmograph (OCP 2000; Allmedicus, An Yang, Korea). The enhanced pause (Penh), which is a dimensionless value that correlates well with pulmonary resistance measured by conventional two-chamber plethysmograph in ventilated mice, was used to monitor airway responsiveness. In the plethysmograph, the mice were exposed for 3 min to nebulized PBS, to establish the baseline Penh values, and were subsequently exposed to increasing concentrations of nebulized methacholine (Mch; Sigma-Aldrich), i.e., 3.125, 6.25, 12.5, 25, and 50 mg/ml in PBS, using the Aerosonic Ultrasonic Nebulizer (DeVilbiss, Somerset, PA, USA). The Penh values were measured during each 3-min sequence, averaged, and expressed for each Mch concentration.

Bronchoalveolar Lavage (BAL)

Mice were subjected to thoracotomy under anesthesia, induced by intraperitoneal injection of ketamine and xylocaine, after measuring airway responsiveness. The trachea was exposed and cannulated with silicone tubing attached to a 23-gauge needle on an 800- μ l tuberculin syringe. The left main bronchus was tied and following instillation of 1.2 ml of sterile PBS through the trachea into the right lung, the BAL fluid was withdrawn. Recovered BAL fluids were cytospun (7 min at 2000 rpm at 4°C) and the supernatants were stored at –70°C. The total cell counts in the BAL fluids were counted using a hemocytometer. After adjusting the

cell number to 1×10^6 /ml, 50- μ l aliquots of BAL fluid were centrifuged for 5 min at 700 rpm onto microscope slides and stained with Diff-Quick (Sysmax, Japan). The percentages of macrophages, eosinophils, lymphocytes, and neutrophils in the BAL fluids were obtained by counting 500 leukocytes in randomly selected fields of the slide under a light microscope.

ELISA

The levels of cytokines, IL-5, IL-10, IL-13, TGF- β , and total IgE in the supernatants of BAL fluids, and the levels of activin A in the lung tissues, were measured by enzyme-linked immunosorbent assay (ELISA) (R&D Systems, Minneapolis, MN, USA, and BioSource International, Camarillo, CA, USA). The samples were assayed according to the manufacturers' recommendations.

Immunohistochemistry

Sections (4- μ m thickness) cut from lung tissues embedded in paraffin blocks were deparaffinized with xylene and hydrated in ethanol. The presence of α -smooth muscle actin, collagens III and V were confirmed by immunohistochemistry. For immunohistochemical detection of α -smooth muscle actin, the lung sections were incubated overnight at 4°C with either a primary monoclonal antibody directed against α -smooth muscle actin (Sigma-Aldrich), or as a negative control mouse serum instead of the primary antibody. Immunoreactivity was detected by sequential incubations of the lung sections with a biotinylated secondary antibody, followed by incubation with peroxidase reagent and AEC (3-amino-9-ethylcarbazole) chromogen. Similar methods were used for the immunohistochemical detection of collagens III and V. The primary monoclonal antibodies against mouse collagens III and V were obtained from Sigma-Aldrich.

Assay for Hydroxyproline

Measurements of total collagen content in the peripheral portions of the lung were based on estimations of hydroxyproline content. A saline solution (1.5 ml) was added to approximately 30 mg of tissue sample collected from the peripheral part of the left lower lobe. Then, the sample was homogenized on ice and the hydroxyproline content was measured. The assay was conducted using the spectrophotometric method.

Measurement of Smooth Muscle Area

The immunostained area corresponding to α -smooth muscle actin in each paraffin-embedded lung was outlined and quantified using a light microscope attached to an image analysis system (BX50; Olympus, Tokyo, Japan). The results are expressed as the area of immunostaining per micron length of basement membrane of the bronchioles (150- to 200- μ m internal diameter). At least 10 bronchioles were counted in each slide.

Data Analysis

The results for each group were compared by the non-parametric Kruskal-Wallis test, followed by post hoc Dunn's multiple comparison. All results are presented as mean $\pm$

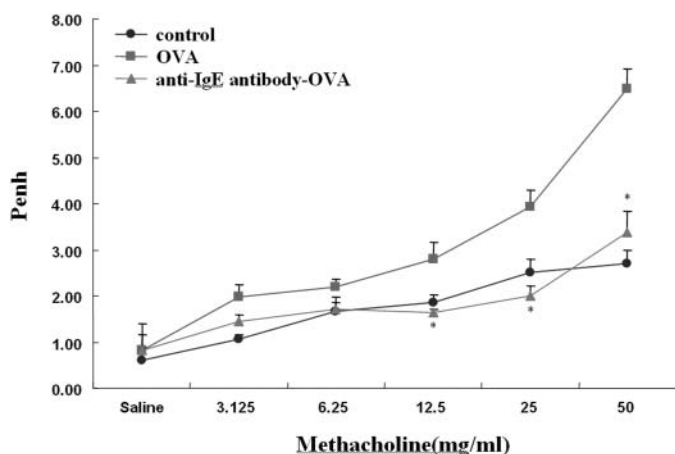


FIGURE 1.—Effect of anti-IgE antibody on airway hyperresponsiveness to inhaled methacholine. Values shown are mean $\pm$ SEM ($n = 10$ – 15 /group). * $p < .05$, compared with the OVA group.

standard error of the mean (SEM). A p value $< .05$ was considered statistically significant.

RESULTS

Effect of Anti-IgE Antibody on AHR

The mice in the OVA group showed bronchial hyperresponsiveness, as compared with the control group. After the injection of anti-IgE antibody, the Penh value to Mch decreased significantly in the treatment group, as compared with the OVA group (Figure 1).

Effects of Anti-IgE Antibody on Airway Inflammation and Total IgE in Serum

The numbers of total cells, macrophages, lymphocytes, and eosinophils in the BAL fluids were significantly increased in the OVA group. There were significant reductions in the total cell counts and eosinophil counts in the treatment group, as compared with the OVA group (for total cells, $262.6 \pm 17.5 \times 10^4/\text{ml}$ versus $157.5 \pm 11.7 \times 10^4/\text{ml}$, $p < .01$; for eosinophils, $108.9 \pm 11.4 \times 10^4/\text{ml}$ versus $58.2 \pm 7.0 \times 10^4/\text{ml}$, $p < .01$) (Figure 2). The total IgE level in the serum of the group treated with anti-IgE antibody was 3-fold higher than that of the OVA group (6.558 ± 1.866 versus $5.008 \pm 0.54 \mu\text{g/ml}$), although insignificant. Compared with the control group, the OVA group had a significantly higher concentration of total IgE.

Effects of Anti-IgE Antibody on Cytokines Levels in BAL Fluids

The levels of IL-5 and IL-13 were significantly lower in the anti-IgE antibody treated group than in the OVA group (for IL-5, 6.3 ± 2.0 versus $13.6 \pm 2.4 \text{ pg/ml}$, $p < .05$; for IL-13, 20.5 ± 4.3 versus $35.0 \pm 2.7 \text{ pg/ml}$, $p < .05$). In contrast, there was no significant difference in the IL-10 level between these groups (5.2 ± 1.2 versus $7.3 \pm 0.9 \text{ pg/ml}$, $p > .05$). As for TGF- β , the levels in BAL fluids increased after the administration of anti-IgE antibody, although the difference was not statistically significant (442.1 ± 91.8 versus $311.9 \pm 27.3 \text{ pg/ml}$, $p > .05$) (Figure 3). Activin A, which is a member

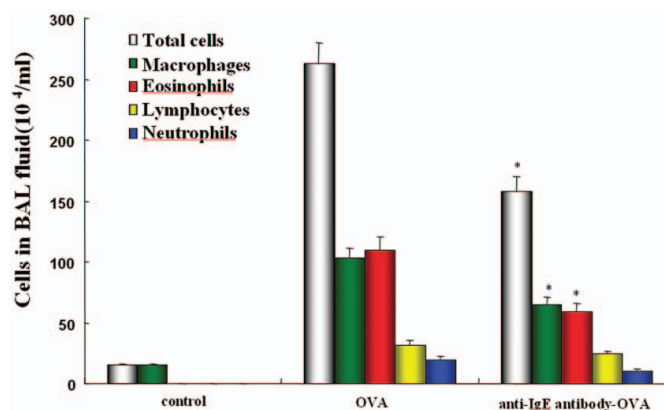


FIGURE 2.—Effect of anti-IgE antibody on total and differential cell counts in BAL fluids. Values shown are mean $\pm$ SEM ($n = 10$ – 15 /group). * $p < .01$, compared with the OVA group.

of the TGF- β family, showed results similar to those observed for TGF- β (anti-IgE antibody treatment group versus OVA group: 4941.2 ± 142 versus $4600 \pm 137 \text{ pg/ml}$, $p > .05$) (Figure 4).

Effects of Anti-IgE Antibody on Collagen Expression in Lung Tissue

Because peribronchial fibrosis is one of the cardinal structural alterations associated with remodeling in asthma, we quantitated it using immunostaining for peribronchial III and V collagens and assay for hydroxyproline level in lung tissues. The administration of anti-IgE antibody significantly decreased the area of peribronchial collagen III and V expression, as compared with the OVA group. In addition, there was a statistically meaningful reduction in hydroxyproline content after treatment with anti-IgE antibody (anti-IgE treatment group versus OVA group: 3.86 ± 0.09 versus $7.14 \pm 0.85 \text{ pg/ml}$, $p < .01$) (Figure 5).

Effect of Anti-IgE Antibody on Thickening of Airway Smooth Muscle Layer

The area of peribronchial myofibroblast immunostained for α -smooth muscle actin was quantified by image analysis, and is expressed as the stained area in $\mu\text{m}^2/\mu\text{m}$ circumference of a bronchiole. The immunostained area for peribronchial α -smooth muscle actin in mice repetitively challenged with OVA was significantly greater than that in control mice. Intravenous administration of anti-IgE antibody to mice challenged repetitively with OVA significantly reduced the area of α -smooth muscle actin immunostaining, as compared with the corresponding area in the OVA group (0.21 ± 0.02 versus $0.34 \pm 0.03 \mu\text{m}^2/\mu\text{m}$, $p < .01$) (Figure 6).

DISCUSSION

The results in this study show that anti-IgE treatment induces a significant reduction of peribronchial fibrosis in chronic asthma of murine model. In addition, we demonstrate that therapeutic intravenous administration of anti-IgE antibody ameliorates airway inflammation and AHR. Some experiments with mice have been conducted on the effects and underlying mechanisms of anti-IgE antibody on asthma

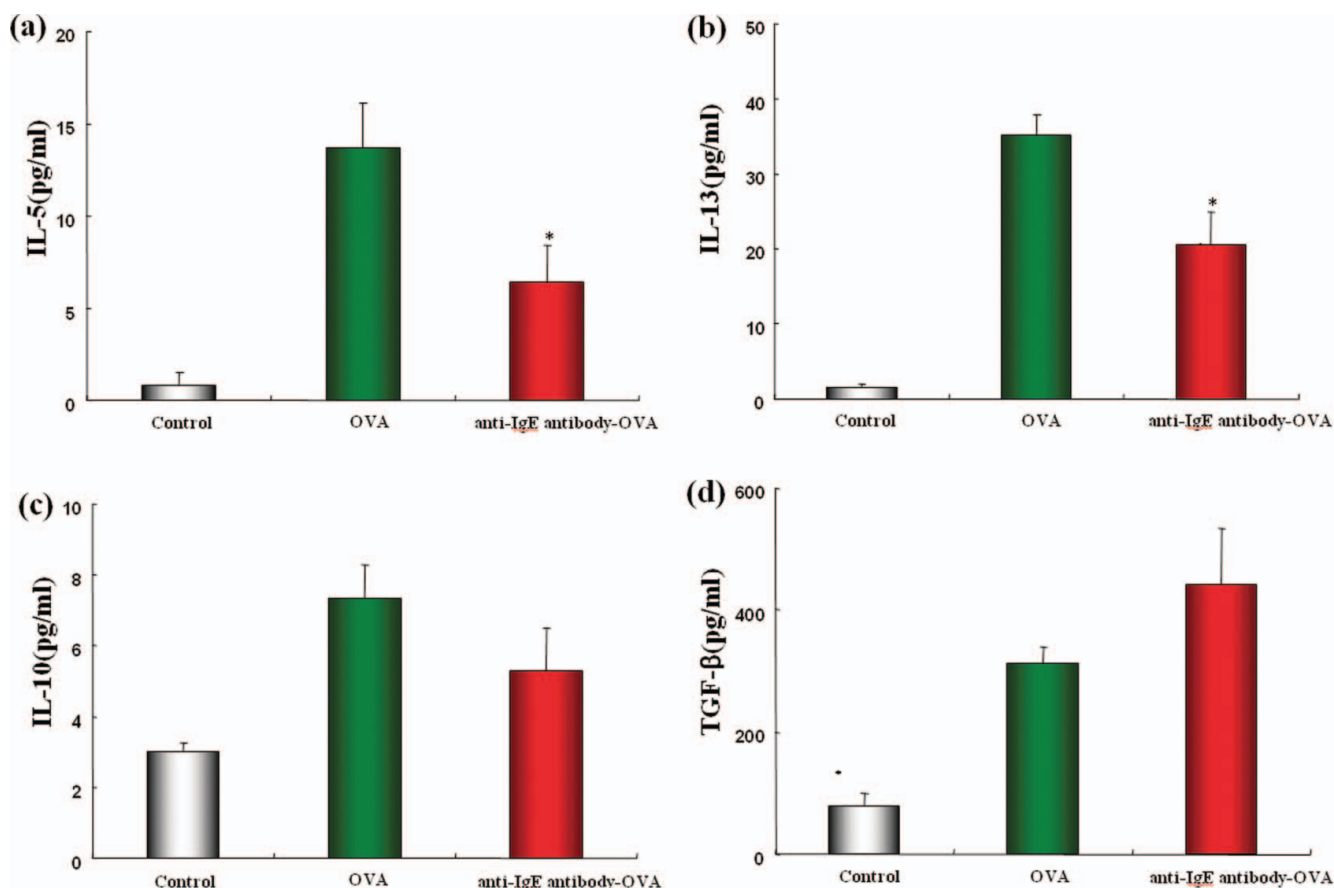


FIGURE 3.—Effect of anti-IgE antibody on the levels in BAL fluid of (a) IL-5, (b) IL-13, (c) IL-10, and (d) TGF- β . Values shown are mean $\pm$ SEM ($n = 10$ – 15 /group). * $p < .05$, compared with the OVA group.

(12–14). However, these previous studies used models of acute asthma induced by exposure to ovalbumin for a short period of time; these models fail to replicate the characteristic features of human asthma, i.e., intraepithelial recruitment of eosinophils, inflammatory involvement of the lamina propria in the airways, and subepithelial fibrosis (15). In the present study, through repetitive challenges over more than

3 months, we induced chronic inflammation and remodeling of the airway in a murine model, similar to chronic asthma in humans. In our chronic asthma model, we show that anti-IgE antibody is protective against airway inflammation and AHR. Regarding the total IgE levels in BAL fluids or serum, the literature contains disparate findings (12–14, 16–19). In the present study, we observed the increase in the total IgE concentration. There are two possible explanations for that phenomenon. First, the total IgE comprises both the free form of IgE, and complexes of IgE and anti-IgE monoclonal antibody. It is known that the latter complexes are processed through an IgG clearance pathway and are removed more slowly than free IgE (20). Therefore, the increase in the total IgE observed in the present study may reflect the predominance of the complex forms over the free, active form of IgE. Second, although previous mouse studies resemble the present study in terms of experimental setting, i.e., similar animal and anti-IgE monoclonal antibodies, the duration of anti-IgE antibody treatment and measurement time of total IgE differ between the studies.

To explore the mechanism underlying the anti-inflammatory effects of anti-IgE antibody in asthma, we measured the levels of cytokines in BAL fluids. In the treated group, the concentrations of Th2 cytokines, such as IL-5 and IL-13, were reduced significantly compared with the OVA group. In contrast, the level of IL-10, which may have

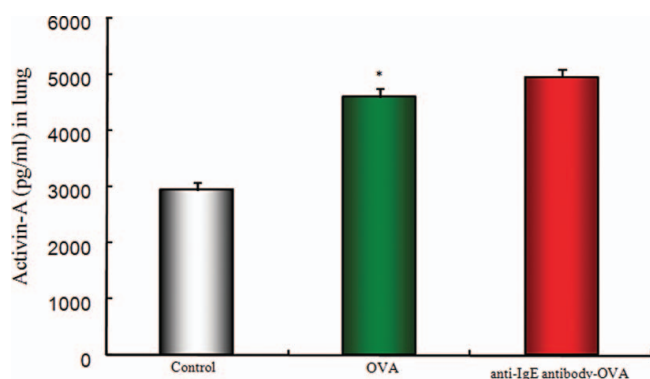


FIGURE 4.—Effect of anti-IgE antibody on levels of activin A in lung tissues. Values shown are mean $\pm$ SEM ($n = 10$ – 15 /group). * $p < .01$, compared with the control group.

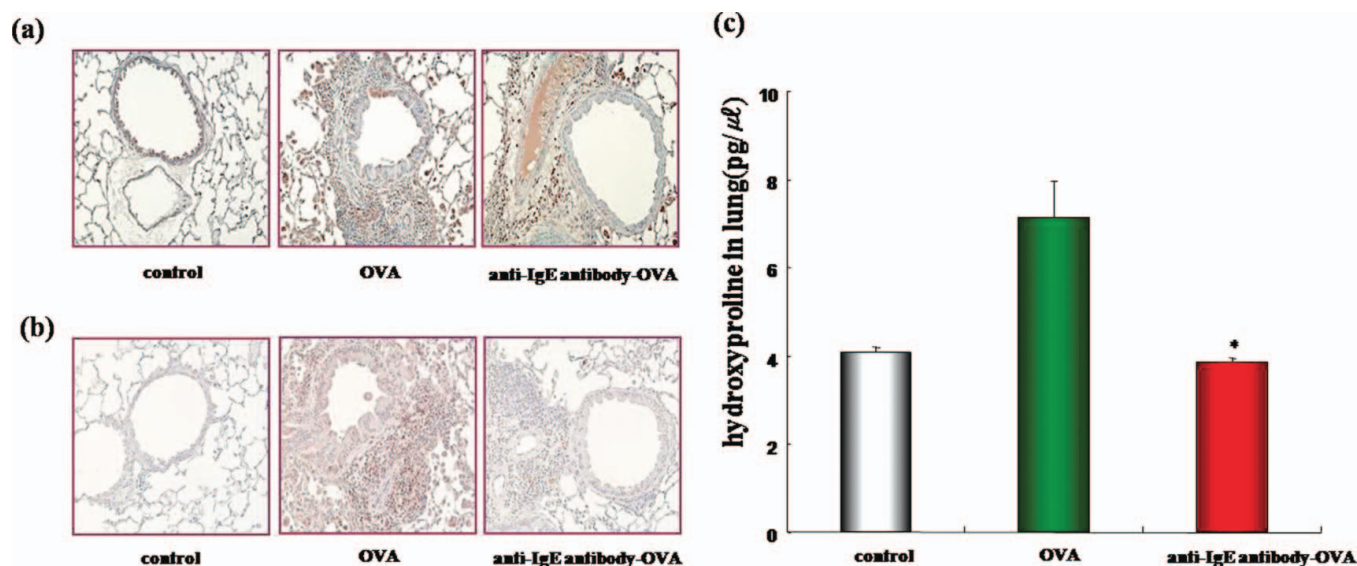


FIGURE 5.—Effect of anti-IgE antibody on collagen expression in lungs. Representative photomicrographs of lung sections stained for (a) collagen III and (b) collagen V. Anti-IgE administration in OVA group reduced peribronchial collagen staining (dark brown color) compared with the OVA group (original magnification $\times 200$). (c) Hydroxyproline levels in the lung tissues. Values shown are mean $\pm$ SEM ($n = 10$ – 15 /group). * $p < .01$, compared with the OVA group.

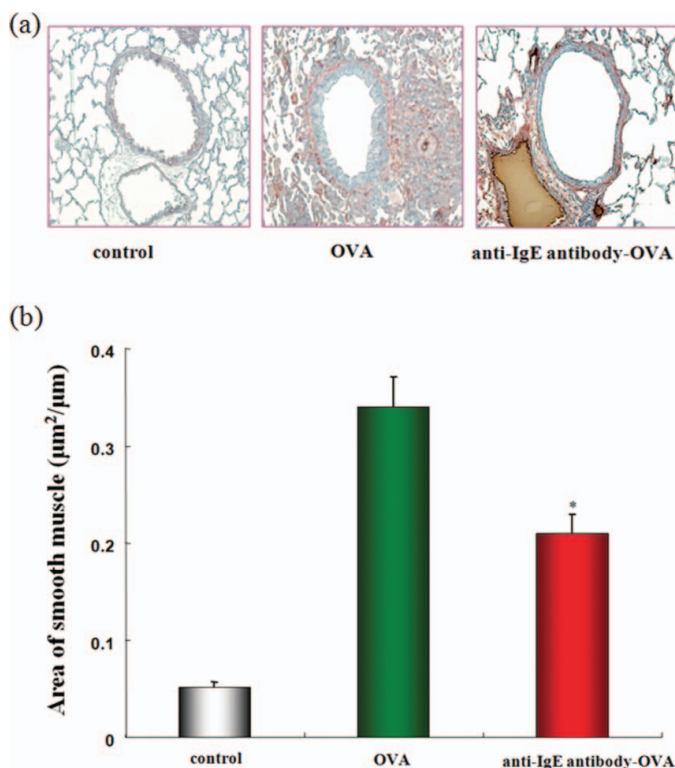


FIGURE 6.—Effect of anti-IgE antibody on airway smooth muscle area. (a) Representative photomicrographs of smooth muscle area-stained lung sections. Anti-IgE administration in OVA group developed decreased thickness of the peribronchial α -smooth muscle layer (brown color) compared with the OVA group (original magnification $\times 200$). (b) Smooth muscle areas represent the areas of immunostaining per micron length of basement membrane of the bronchi, as assessed using an image analyzer. Values shown are mean $\pm$ SEM ($n = 10$ – 15 /group). * $p < .01$, compared with the OVA group.

immunotolerogenic effects of immunotherapy in allergic diseases (21, 22), was not increased in the treated group. Consequently, we assume that the protective effect of anti-IgE antibody against airway inflammation is associated with Th2 cytokine inhibition, and is not linked to IL-10 production. With regard to airway hyperresponsiveness, we observed that the treatment group had a significantly better Penh value than the OVA group. This is probably due to the decrease in the level of IL-13, which is responsible for allergen-induced AHR (23, 24).

Airway remodeling has been proposed as a mechanism that explains many of the clinical features of asthma, such as persistent bronchial hyperresponsiveness and impaired lung function (1). Although some clinical studies have shown anti-inflammatory effects of anti-IgE antibody in asthma that are compatible with our results (4–6, 25–28), there has not been any experiment to examine its influence on airway remodeling. Therefore, we investigated whether or not anti-IgE antibody, which has beneficial effects on moderate to severe asthma, plays a protective role in airway remodeling. Administration of the anti-IgE monoclonal antibody significantly reduced peribronchial fibrosis, as assessed by measuring the hydroxyproline content and immunohistochemical staining for collagen III and V deposits. In addition, the anti-IgE antibody-treated mice showed decreased thickness of the smooth muscle layer, as compared with the OVA-challenged mice. These findings verify the inhibition by anti-IgE therapy of airway remodeling in chronic asthma.

TGF- β is a well-known fibrogenic cytokine. Therefore, we examined the levels of TGF- β and activin A, as one of TGF- β superfamily and a linker to TGF- β -mediated airway remodeling in asthma (29). Interestingly, the concentrations of both TGF- β and activin A increased after anti-IgE antibody treatment. Regardless of TGF- β , there are some possible explanations of the anti-IgE antibody effects against airway remodeling in this study. First, the anti-remodeling

effect of anti-IgE treatment appears to be mediated, either directly or indirectly, by Th2 cytokines, such as IL-5 and IL-13. IL-5 and IL-13 are associated with structural alterations in asthmatic airways (3, 8, 30–32). Second, we assume that the anti-IgE antibody acts directly on structural cells, such as smooth muscle cells and fibroblasts. Regarding the association between smooth muscle cells and IgE, recent studies have shown that activation of the Fcε receptors/IgE network could have an effect on remodeling of smooth muscle cells in asthma (33–35). Specially, some reports have demonstrated that expression of low-affinity receptor (FcεRII, CD23) was significantly increased in smooth muscle cells of asthma by IgE immune complexes/FcεRII and up-regulation of FcεRII provoked hyperplasia of airway smooth muscle cells. However, these experiments were performed on the human smooth muscle cells only. Therefore, in this study based on the mouse model, there might be limitations in explaining the effect of anti-IgE antibody, which influences airway remodeling of smooth muscle cells. Fibroblasts have crucial roles in altering airway structures, including changes in collagen content and composition. Abel et al. (36) investigated mast cell–fibroblast interactions and a role for IgE-mediated mast cell activation. Therefore, it is suggested that the inhibition of the IgE action by anti-IgE antibody has a protective effect on airway remodeling through the suppression of interactions between mast cells and fibroblasts. However, further research is required to explore the mechanism underlying airway remodeling inhibition by anti-IgE antibody.

In conclusion, this is the first study to demonstrate the effects of anti-IgE antibody, focusing on airway remodeling in a murine model of chronic asthma. Our results show that anti-IgE antibody treatment modulates not only airway inflammation and AHR, but also some parts of remodeling in chronic asthma. Although the mechanism underlying the inhibition of remodeling by anti-IgE antibody remains to be resolved, we speculate that this phenomenon is related to Th2 cytokine production.

DECLARATION OF INTEREST

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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