

PHARMACOTHERAPY

Beneficial Effect of Anti-Interleukin-33 on the Murine Model of Allergic Inflammation of the Lower Airway

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Objective. Interleukin (IL)-33, which mediates the T_H2 allergic pathway, may play a key role in allergic airway inflammation. This study was conducted to evaluate the therapeutic potential of anti-IL-33 antibody for treatment of allergic inflammation of the lower airway in a murine model.

Methods. Twenty-four BALB/c mice were used in this study. Saline was used for sensitization and challenge of mice in Group A (control group, $n = 6$). Mice in Group B (ovalbumin (OVA) group, $n = 6$) received intraperitoneal (ip) and intranasal OVA challenge. In Group C (control IgG group, $n = 6$), mice received ip injection with control IgG prior to OVA challenge. Mice in Group D (anti-IL-33 group, $n = 6$) received an ip injection of anti-IL-33 prior to challenge. Measurements of serum total and OVA-specific IgE and the number of eosinophils, neutrophils, and lymphocytes in bronchoalveolar lavage (BAL) fluid were performed. We performed histopathologic examination to evaluate the degree of eosinophilic infiltration in lung tissue. Airway hyperreactivity was measured according to change of enhanced pause (Penh). **Results.** A significant decrease in serum total and OVA-specific IgE and the number of eosinophils and neutrophils in BAL fluid was observed in Group D, compared with Group B or Group C ($p < .05$). In Group D, treatment with anti-IL-33 resulted in a significant decrease in eosinophilic infiltration in lung tissue, compared with Group B and Group C ($p < .05$). Degree of airway hyperreactivity, measured by Penh, showed a significant decrease in the anti-IL-33 treatment group, compared with the OVA group or the control IgG treatment group ($p < .01$, at 50 mg/mL of methacholine). **Conclusions:** Anti-IL-33 has therapeutic potential for treatment of allergic inflammation of the lower airway.

Keywords airway, allergy, anti-IL-33, bronchoalveolar lavage, murine, ovalbumin

INTRODUCTION

The nuclear factor for high endothelial venule (NF-HEV), identified as a member of the cytokine interleukin (IL)-1 superfamily, has subsequently been renamed as IL-33 (1, 2). Its molecular properties are similar to those of closely related cytokines, including IL-1 α/β , IL-1Ra, and IL-18 (3).

IL-33 and its receptor, ST2, promote various activities of different cell types, including eosinophils, mast cells, T_H2 lymphocytes, basophils, and structural cells, similar to those of airway epithelium (3). These activities are related to upregulation of a systemic T_H2 response (4). Because the membrane-bound form of ST2 is expressed mainly on mast cells and T_H2 lymphocytes, an association of the IL-33–ST2 axis with T_H2-type pathological effects, such as bronchial asthma, has been reported (5, 6).

The site for cleavage by enzymes, such as caspase 3, caspase 7, or calpain, is located within the cytokine domain of IL-33; therefore, the cleavage products are biologically inactive; this process generally occurs during apoptosis (7, 8). Therefore, the presence and action of intact IL-33 is limited during apoptosis. However, as a consequence of infection, inflammation, or trauma, prior to cleavage, intact IL-33 can be released from

damaged cells undergoing necrotic cell death. This suggests that one of the functions of IL-33 is as an endogenous danger signal, or “alarmin”, particularly in epithelial or endothelial cells directly exposed to the external environment (such as those of the skin, gastrointestinal tract, or airway epithelium) (9, 10).

In a murine model of allergic asthma, blockade of the IL-33–ST2 cascade resulted in reduced severity of disease (11–15). On the other hand, intranasal instillation of IL-33 resulted in induction of allergic pathologic features (16). These findings suggest that IL-33 could also play a key role in allergic inflammation of the lower airway. However, the precise role of IL-33 in a murine model of allergic asthma remains to be determined (4).

This study was conducted to evaluate the therapeutic potential of anti-IL-33 antibody for treatment of allergic inflammation of the lower airway by measurement of levels of serum total IgE and ovalbumin (OVA)-specific IgE, number of eosinophils in bronchoalveolar lavage (BAL) fluid, histopathology of lung tissue, and change of airway hyperreactivity to methacholine.

Blockade of IL-33 by anti-IL-33 prior to sensitization events in mice was found to result in a significant reduction in the aforementioned allergic manifestations. This finding suggests that further exploration of the roles of IL-33, as well as its blockade, could have therapeutic potential for treatment of allergic disease and asthma.

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METHODS

Animals

Twenty-four female BALB/c mice, 8–10 weeks old and free of murine-specific pathogens, were obtained from Orient Bio (Seongnam, Korea). Mice were housed in a controlled environment with a 12 hour light/dark cycle with free access to food and water and were maintained on an OVA-free diet. The animal protocol used in this study has been reviewed with regard to ethical procedures and scientific care by the INHA University–Institutional Animal Care and Use Committee (INHA-IACUC), and it has been approved (approval number INHA-110614-100).

Sensitization and Antigen Challenge

Sensitization and antigen challenge for the murine model of allergic rhinitis was performed using a previously described method, with slight modification (17). Mice were sensitized using OVA (Sigma-Aldrich, St Louis, MO, USA) under pathogen-free conditions. OVA (40 µg/kg) diluted in sterile normal saline was administered along with aluminum hydroxide gel (alum adjuvant, 40 mg/kg) to unanesthetized animals 4 times by intraperitoneal (ip) injection on days 1, 5, 14, and 21. This was followed by daily intranasal (in) challenge with OVA diluted in sterile normal saline (20 µL of 25 mg/mL OVA per mouse) from days 22 to 35 (Figure 1). Twenty-four hours after the last OVA challenge, blood, BAL fluid, and lung tissues were collected from each mouse.

For blocking experiments, mice received anti-mouse IL-33 antibody (R&D Systems, Minneapolis, MN, USA), which was produced in goats immunized with purified *Escherichia coli*-derived recombinant mature mouse interleukin-33 (rmIL-33). Affinity chromatography was performed for purification of mouse IL-33-specific IgG. Intraperitoneal injection of anti-IL-33 antibody (3.6 µg per mouse) was administered 30 minutes before ip OVA sensitization and in OVA antigen challenge. As the control, OVA-challenged mice were given the same amount of rabbit control IgG (purified normal rabbit IgG; R&D Systems) by ip injection. As the control group, mice in Group A ($n = 6$) were sensitized and challenged with ip

and in normal saline. Mice in Group B ($n = 6$) received similar ip and in OVA challenge. In Group C ($n = 6$; control IgG group), ip injection of rabbit control IgG was administered prior to every OVA challenge. In Group D ($n = 6$; anti-IL-33 group), pretreatment with ip injection of anti-IL-33 antibody was administered prior to every OVA challenge.

Serum Collection and Cell Count in BAL Fluid

After sacrifice, aortic puncture was performed for collection of serum from mice. BAL was obtained immediately after the last measurement of airway responsiveness. Cannulation of the trachea was performed using a polyethylene tube through which the lungs were lavaged 4 times with 1.0 mL of saline (total 4 mL). BAL fluid was then filtered through wet gauze. The Trypan blue exclusion test was performed for determination of cell viability and a total cell count was performed. BAL fluid was centrifuged for 10 minutes at $150\times g$. The pellet was suspended immediately in 4 mL of saline, and total cell numbers in BAL fluid were counted in duplicate using a hemocytometer. Then, a 100 µL aliquot was centrifuged in a Cytospin 2 cytocentrifuge (Shandon Scientific, Pittsburgh, PA, USA). Differential cell counts were obtained from centrifuged preparations stained with Diff-Quick, counting 500 or more cells in each animal at a magnification $\times 1000$ (oil immersion).

Histopathology

Using standard methods, lung tissues were fixed in 10% formalin for 24 hours and embedded in paraffin. Four micrometer-thick sections were stained with hematoxylin and eosin (H&E) for detection of eosinophil infiltration.

Measuring Airway Hyperreactivity

Using a previously described method, measurement of airway responsiveness was performed in unrestrained and conscious mice one day after the last in challenge (18, 19). Mice were placed in a barometric plethysmographic chamber (All Medicus, Seoul, Korea) and baseline readings were taken and averaged for a period of 3 minutes. Aerosolized methacholine (0–50 mg/mL) was nebulized

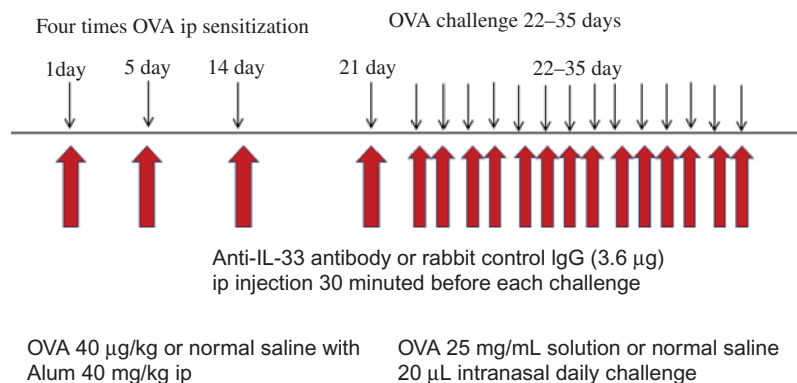


FIGURE 1.—Protocol for induction of allergic inflammation of the lower airway. Black arrows: intraperitoneal (ip) or intranasal (in) ovalbumin (OVA) challenge; red block arrows: treatment with anti-IL-33 or control IgG by ip injection (3.6 µg per mouse), 30 minutes prior to each OVA challenge.

for 3 minutes through an inlet of the main chamber. Readings were taken and averaged for a period of 3 minutes after each nebulization and enhanced pause (Penh) was determined. Penh, calculated as $((\text{expiratory time})/(\text{relaxation time} - 1)) \times ((\text{peak expiratory flow})/(\text{peak inspiratory flow}))$, according to the manufacturer's protocol, is a dimensionless value that represents a function of the proportion of maximal expiratory to maximal inspiratory box pressure signals, and is a function of the timing of expiration. Penh is used as a measure of airway responsiveness to methacholine. Results are expressed as the percentage increase of Penh following challenge with each concentration of methacholine, where the baseline Penh (after saline challenge) is expressed as 100%. Penh values averaged for a period of 3 minutes after each nebulization were evaluated.

Statistical Analyses

The Kruskal–Wallis test and Mann–Whitney *U* test were used for comparison of serum levels of total and OVA-specific IgE, number of eosinophils in BAL fluid, and change of Penh between groups. A *p*-value <.05 was considered statistically significant.

RESULTS

Serum Total and OVA-Specific IgE

Significantly elevated titers of total and OVA-specific IgE were observed in serum of Group B (OVA-sensitized group, total IgE = 19.4 ± 8.5 $\mu\text{g/mL}$, OVA-specific IgE = 0.47 ± 0.22 $\mu\text{g/mL}$) and Group C (OVA-sensitized group treated with rabbit control IgG, total IgE = 19.9 ± 9.6 $\mu\text{g/mL}$, OVA-specific IgE = 0.44 ± 0.02 $\mu\text{g/mL}$), compared with Group A (the normal saline control group, total IgE = 0.8 ± 0.6 $\mu\text{g/mL}$, OVA-specific IgE = 0.08 ± 0.00 $\mu\text{g/mL}$) (all *p*-values <.01), which returned to baseline value in Group D (OVA-sensitized group treated with anti-IL-33, total IgE = 7.7 ± 4.4 $\mu\text{g/mL}$, OVA-specific IgE = 0.23 ± 0.09 $\mu\text{g/mL}$, *p* < .05, Figure 2).

Number of Differential Cells in BAL Fluid

The number of eosinophils and neutrophils in BAL fluid showed a significant increase in Group B (eosinophil: $8.2 \pm 3.3 \times 10^4$ cells/mL, neutrophil: $2.9 \pm 0.5 \times 10^4$

cells/mL) and Group C (eosinophil: $6.9 \pm 2.6 \times 10^4$ cells/mL, neutrophil: $3.2 \pm 1.9 \times 10^4$ cells/mL), compared with Group A (eosinophil: $0.2 \pm 0.2 \times 10^4$ cells/mL, neutrophil: $0.1 \pm 0.1 \times 10^4$ cells/mL), and a significant decrease was observed in Group D (eosinophil: $2.9 \pm 1.3 \times 10^4$ cells/mL, neutrophil: $1.6 \pm 0.5 \times 10^4$ cells/mL), compared with Group B and Group C (*p* < .05, Figure 3). However, no significant decrease in the number of lymphocytes was observed in Group D, compared with Group B and Group C (*p* > .05, Figure 3).

Histopathologic Examination

Significantly greater pulmonary infiltration of eosinophils was observed in Group B and Group C, compared with Group A. Treatment with anti-IL-33 resulted in significantly decreased eosinophilic infiltration (Figure 4).

Airway Hyperreactivity

Compared with the control group (Group A, $676.1 \pm 381.2\%$ increase of %Penh, compared with baseline), the OVA group (Group B, $1735.8 \pm 497.2\%$ increase) and the control IgG group (Group C, $1503.0 \pm 926.5\%$ increase) showed significantly increased airway sensitivity to methacholine at a dose of 50 mg/mL. Decreased airway hyperreactivity was observed in the anti-IL-33 treatment group (Group D), compared with Group B and Group C ($1064.6 \pm 322.1\%$ increase); these differences were statistically significant at doses as high as 50 mg/mL (*p* < .01, Figure 5).

DISCUSSION

Pretreatment with anti-IL-33 resulted in decreases of serum IgE and eosinophils in BAL fluid. Decrease of IgE could, in turn, result in a reduction of mast cell degranulation. Anti-IL-33 can also inhibit adhesion of eosinophils, inhibit expression of surface molecules such as CD11b, prolong survival of eosinophils, and increase degranulation (20, 21). These actions in mast cells and eosinophils are thought to result in downregulation of the systemic allergic response, which, in turn, could have a therapeutic effect on allergic inflammation of the lung.

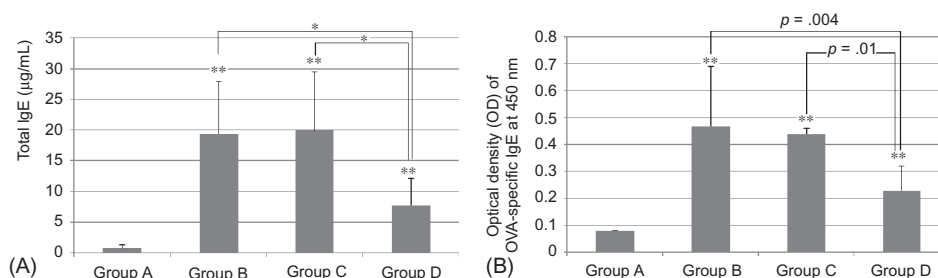


FIGURE 2.—Serum (A) total IgE and (B) ovalbumin (OVA)-specific IgE. Group A: control group; Group B: OVA-induced allergic group; Group C: allergic group + treatment with control IgG; Group D: allergic group + treatment with anti-IL-33 antibody. (Kruskal–Wallis test and Mann–Whitney *U* test; **p* < .05, **significant difference with Group A, *p* < .01.)

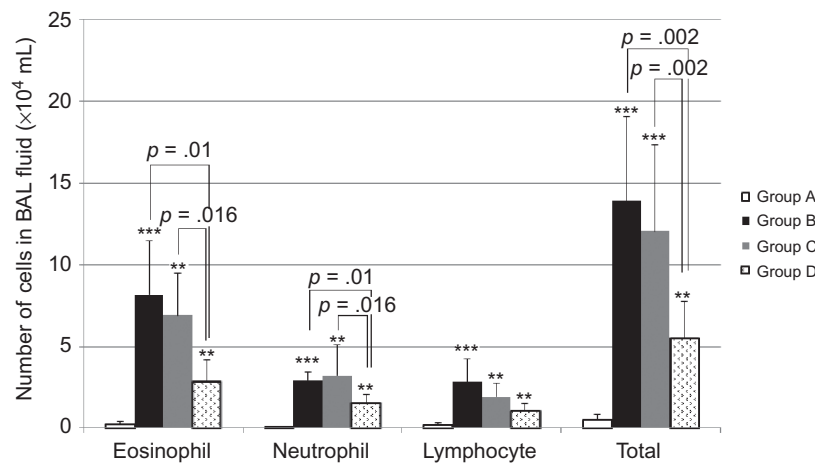


FIGURE 3.—Number of eosinophils in bronchoalveolar lavage (BAL) fluid. Group A: control group; Group B: ovalbumin-induced allergic group; Group C: allergic group + treatment with control IgG; Group D: allergic group + treatment with anti-IL-33 antibody. (Kruskal–Wallis test and Mann–Whitney *U* test; **significant difference with Group A, $p < .01$.)

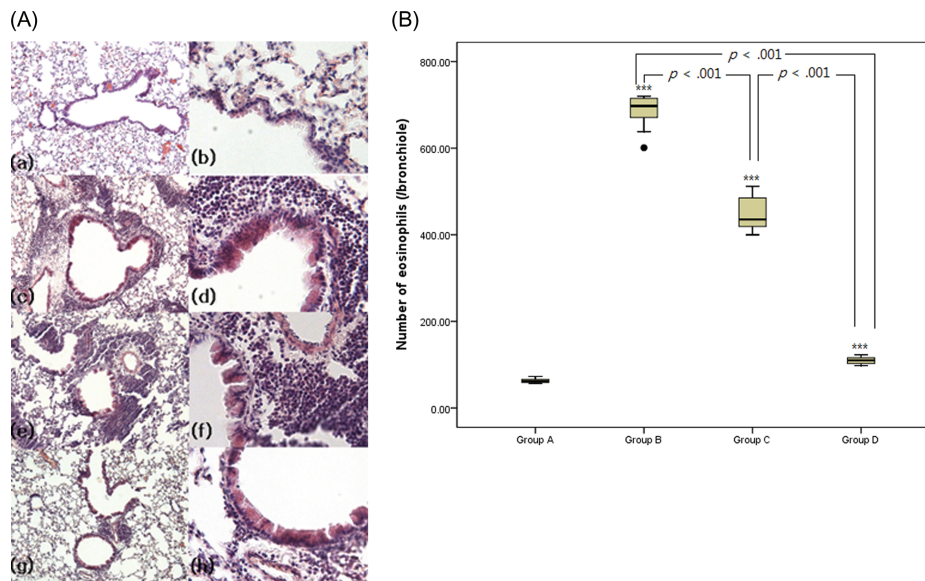


FIGURE 4.—(A) Histopathologic examination of lungs. Group A: control group; Group B: ovalbumin-induced allergic group; Group C: allergic group + treatment with control IgG; Group D: allergic group + treatment with anti-IL-33 antibody. (a, b: Group A; c, d: Group B; e, f: Group C; g, h: Group D. H&E stain, a, c, e, and g: $\times 200$; b, d, f, and h: $\times 400$). (B) Comparison of the number of eosinophils (per bronchiole). (Kruskal–Wallis test and Mann–Whitney *U* test; ***significant difference with Group A, $p < .001$.)

Histological examination of the target organ is mandatory to confirm the effect of treatment with anti-IL-33 on experimental airway inflammation. In this study, by providing proof of decreased eosinophilic infiltration into lung tissues, we can conclude that anti-IL-33 has a therapeutic effect on experimental airway inflammation. Our results are consistent with those of a prior study (13). Liu and researchers reported reduced infiltration of eosinophils and lymphocytes, less mucus and fewer goblet cells, and less damage to airway epithelium (13).

In a previous study, the effect of treatment with anti-IL-33 on airway inflammation was assessed by histopathologic examination (13). However, prior to conduct of this study, no studies reporting on the effect of anti-IL-33 on airway hyperreactivity using dynamic study tools, such as measurement of Penh, have been conducted. In this study, airway hyperreactivity showed a significant decrease after

treatment with anti-IL-33, as evidenced by the decrease in %Penh change with methacholine challenge.

In our prior study, we evaluated the therapeutic effect of anti-IL-33 antibody in a murine model of allergic rhinitis (22). Mice treated with anti-IL-33 antibody showed improvement of systemic allergy, which was evaluated by serum total and OVA-specific IgE. In our consecutive studies using the same provocative protocol, we attempted to find significant correlation between the number of nose-scratching behaviors (for evaluation of the severity of allergic rhinitis in our prior study) and the degree of airway hyperreactivity (evaluated by change of Penh). Results demonstrating some correlations between these two parameters might support the notion that there is a significant relationship between the allergic inflammation of the upper and lower airways consistent with the united airways hypothesis.

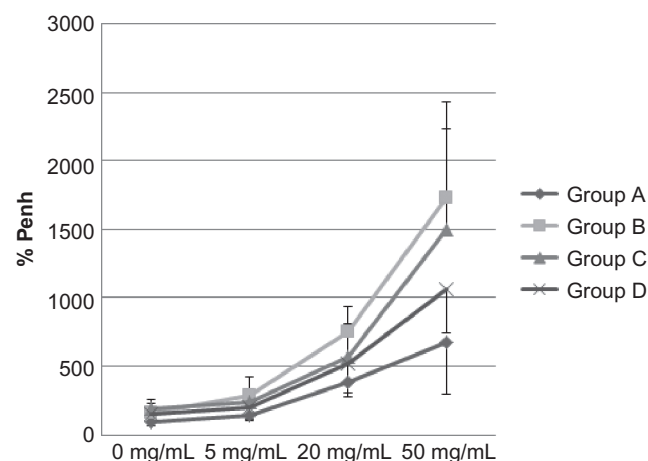


FIGURE 5.—Change of %Penh with increasing concentration of methacholine. Group A: control group; Group B: ovalbumin-induced allergic group; Group C: allergic group + treatment with control IgG; Group D: allergic group + treatment with anti-IL-33 antibody. (Kruskal–Wallis test and Mann–Whitney U test, $p < .05$.)

Although not included in this study, mast cells are also important in allergic inflammation. Further studies to identify changes in the number of mast cells or change of their mediators could yield other meaningful results. In addition, serum OVA-specific IgG could be measured to examine the effect of anti-IL-33 antibody on natural helper cells, which are important for production of serum immunoglobulin.

In conclusion, treatment with anti-IL-33 has several effects, which include decrease of serum IgE, decrease of eosinophils in BAL fluid, decrease of eosinophilic infiltration into the lung, and decreased airway hyperresponsiveness. These effects were implicated in results showing alleviation of allergic inflammation of the lower airway in a murine model. Further studies are needed in order to identify the exact mechanism of anti-IL-33 in treatment of allergic inflammation.

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DECLARATION OF INTEREST

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

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