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Anti-IL-33 antibody treatment inhibits airway inflammation in a murine model of allergic asthma

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ABSTRACT

Interleukin (IL)-33 is a recently described member of the IL-1 family and has been shown to induce production of T helper type 2 cytokines. In this study, an anti-IL-33 antibody was evaluated against pulmonary inflammation in mice sensitized and challenged with ovalbumin. The anti-IL-33 or a control antibody (150 μg/mouse) was given intraperitoneally as five doses before the sensitization and antigen challenge. Treatment with anti-IL-33 significantly reduced serum IgE secretion, the numbers of eosinophils and lymphocytes, and concentrations of IL-4, IL-5, and IL-13 in bronchoalveolar lavage fluid compared with administration of a control antibody. Histological examination of lung tissue demonstrated that anti-IL-33 significantly inhibited allergen-induced lung eosinophilic inflammation and mucus hypersecretion. Our data demonstrate for the first time that anti-IL-33 antibody can prevent the development of asthma in a mouse model and indicate that blockade of IL-33 may be a new therapeutic strategy for allergic asthma.

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Introduction

Allergic asthma is a chronic inflammatory disorder characterized by massive infiltration of eosinophils, high levels of the T helper type 2 (Th2) cytokines in bronchoalveolar lavage fluid (BALF), high levels of serum immunoglobulin (Ig) E, increasing airway hyperresponsiveness (AHR), and reversible airway obstruction. The pathologic features of allergic asthma include inflammatory cell infiltration, edema, mast cell activation, denudation of airway epithelium, and collagen deposition. Although current standard therapies (including corticosteroids and β2 receptor agonists) effectively provide symptomatic control for the majority of asthma patients, patients with severe or difficult asthma do not respond well to these therapies [1–3]. Furthermore, no therapeutic agent is effective in preventing airway remodeling, which involves epithelial damage, mucus gland hyperplasia, airway smooth muscle hypertrophy, and subepithelial fibrosis [4,5]. Therefore, developing therapeutic agents that can suppress airway inflammation and tissue remodeling would be necessary in the treatment of asthma.

Interleukin (IL)-33 is a recently described member of the IL-1 family, which mediates its biological effects as a ligand for ST2 [6]. ST2, an IL-1 receptor-related protein specifically expressed on mast cells and Th2 lymphocytes, has been shown to function as an important effector molecule of Th2 responses in some experimental settings including mouse asthma models. Binding of IL-33 to ST2 receptor activates nuclear factor-κB and mitogen-activated protein kinases and induces expression of Th2 cytokines and leads to severe pathological changes in mucosal organs [6]. IL-33 has a variety of effects on inflammatory cells. It drives production of pro-inflammatory and Th2 cytokines in mast cells and Th2 lymphocytes [6–12], induces chemotaxis of Th2 cells [13], promotes eosinophils and basophils adhesion, and enhances eosinophils survival and basophils migration [14–16]. A study on primary human cells demonstrates that basophils and eosinophils are the only direct target leukocytes for IL-33 [17]. In animal studies, administration of IL-33 induces the IL-5-producing T cells and exacerbates allergen-induced airway inflammation [18]. Recent studies have demonstrated that IL-33 administration induces AHR and goblet cell hyperplasia by induction of IL-4, IL-5, and IL-13 entirely independent of acquired immune system [19]. Moreover, blockade of IL-13 signaling using a soluble ST2 protein [10] or ST2 plasmid [20] inhibits the allergic asthma response in mice. Together, these results strongly suggest that IL-33 plays a crucial role in the pathogenesis of allergic asthma.

To investigate the therapeutic potential of IL-33 blockade in allergic asthma, we evaluated the effects of a neutralizing antibody to murine IL-33 on pulmonary inflammation in a murine experimental model of asthma. Our results suggest for the first time that anti-IL-33 antibody might inhibit allergic airway inflammation by down-regulation of antigen-driven Th2-mediated responses. Therefore administration of anti-IL-33 could prove to be a beneficial approach to the treatment of asthma.

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Materials and methods

Animals. Female BALB/c mice, 8–10 weeks of age and free of murine specific pathogens, were obtained from the Animal Center of the South Medical University (Guangzhou, China). The mice were housed in a controlled environment with a 12 h light/dark cycle and had free access to food and water, and were maintained on ovalbumin (OVA)-free diets. All experimental animals used in this study were under a protocol approved by the Institutional Animal Care and Use Committee of the Shantou University Medical College.

Sensitization and antigen challenge. BALB/c mice were sensitized and challenged as described previously with slight modification [21]. Briefly, mice were sensitized on Days 1 and 14 by intraperitoneal (i.p.) injection of 20 μg of OVA (Grade V; Sigma–Aldrich, St. Louis, MO) emulsified in 2 mg of aluminum hydroxide gel (alum, Sigma) in a total volume of 200 μl. On Days 25, 26, and 27 after the initial sensitization, the mice were challenged for 30 min daily with an aerosol of 1% (wt/vol) OVA in saline (or with saline as a control) using an ultrasonic nebulizer (Yuyue, Jiangsu, China). Twenty-four hours after the last OVA challenge, the mice were prepared for the collection of blood, BALF, and lung tissues (Fig. 1).

For blocking experiments, mice also received a neutralizing polyclonal antibody against murine IL-33. This antibody was produced in rabbits immunized with purified, *Escherichia coli*-derived, recombinant mouse IL-33. IL-33-specific IgG was purified by Protein A Sepharose (Amersham Biosciences, Piscataway, NJ). Anti-IL-33 antibody was given intraperitoneally at 30 min before sensitization and antigen challenge on Day 0 and followed with the same dose on Days 14, 25, 26, and 27. As control, OVA-challenged mice were given the same amount of rabbit control IgG (purified normal rabbit IgG) by i.p. injection.

Bronchoalveolar lavage. Mice were anesthetized with a sodium pentobarbitone, the lungs were lavaged three times with 1 ml saline. Total cells and cell differentials were counted using a hemocytometer and Diff-Quik stain (Fisher Scientific, Pittsburgh, PA), respectively. The supernatant was stored at −80 °C for cytokine analysis.

Histopathology. Lung tissues were fixed in Carnoy's solution and embedded in paraffin using standard methods. Four-μm thick sections were stained with hematoxylin and eosin (H; E) to detect cellular infiltration, and periodic acid-Schiff/alcan blue (PAS/AB) to detect mucus-secreting goblet cells.

Enzyme-linked immunosorbent assay (ELISA). Serum levels of OVA-specific IgE were measured by ELISA as previously described [22]. Total IgE was measured and compared with known mouse IgE standard (BD PharMingen, San Diego, CA). Levels of IL-4, IL-5, IL-13, and IFN-γ in BALF supernatants were measured using ELISA kits (Biosource, Camarillo, CA) according to the manufacturer's instructions, and compared with known standards. The lowest detection limits were 5 pg/ml for IL-4, 3 pg/ml for IL-5, 2 pg/ml for IL-13, and 1 pg/ml for IFN-γ.

Statistical analysis. Data are presented as mean ± standard error of the mean (SEM). The statistical significance of differences between groups was determined by analysis of variance. A p value of less than 0.05 was accepted as statistically significant.

Results

Effect of anti-IL-33 treatment on lung inflammation

Total cell numbers in BALFs were significantly increased 24 h after OVA challenge compared with saline challenge. The increase of total cell numbers was associated with macrophage, eosinophils, lymphocytes, and neutrophils (Fig. 2). As compared with control IgG, treatment of OVA-sensitized and challenged mice with anti-IL-33 (150 μg/mouse, five times intraperitoneally) significantly inhibited the increase in total cell numbers in BALF (Fig. 2). Eosinophils and lymphocytes decreased after anti-IL-33 treatment ($p < 0.05$). Treatment with the control antibody had no effect on the BAL eosinophilia and the increase in total cells of sensitized mice after OVA challenge. These data demonstrate that anti-IL-33 plays a role during the inflammatory response in allergic lungs, by reducing the number of inflammatory cells in the airways of allergic mice.

Histological examination of lung

Evidence of inflammatory cell infiltration and the effects of anti-IL-33 treatment were further investigated by histologically examining lung sections stained with H; E. OVA challenge to sensitized mice increased the numbers of eosinophils and lymphocytes in the lung tissue (Fig. 3B). Furthermore, there was damage to the airway epithelium and goblet cell metaplasia and hyperplasia with a mucus overproduction of sensitized mice after OVA challenge stained with PAS/AB (Fig. 3F). Treatment of sensitized mice with anti-IL-33 significantly reduced the number of eosinophils and lymphocytes in the peribronchial and alveolar regions (Fig. 3D), and attenuated the damage to the airway epithelium and mucus overproduction (Fig. 3H). Treatment with a control antibody had no effect on the appearance of eosinophils and lymphocytes in lung tissue (Fig. 3C), damage to the airway epithelium, or mucus hypersecretion (Fig. 3G).

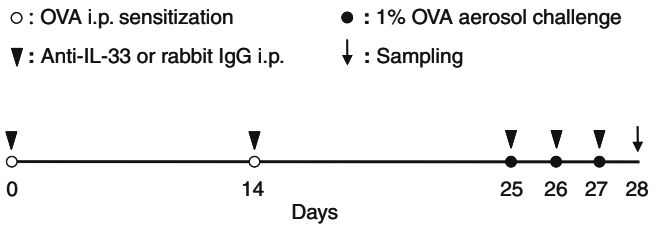

Fig. 1. Protocol for inducing experimental asthma and anti-IL-33 treatment. Mice were sensitized by two intraperitoneal (i.p.) injections of OVA/alum on Days 0 and 14, and then received three consecutive days of aerosolized OVA challenge on Days 25, 26, and 27. To assess the effect of anti-IL-33 on airway inflammation, mice received i.p. injections of the anti-mouse IL-33 (150 μg/mouse) on Days 0, 14, 25, 26, and 27. Control mice received the same dose of control IgG. On Day 28, mice were killed, blood was collected, BAL was performed, and lungs were removed for histological analysis.

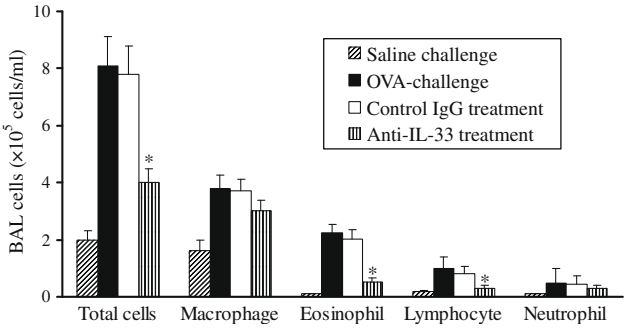

Fig. 2. Effects of anti-IL-33 on lung BAL inflammation in sensitized and challenged mice. The number of inflammatory cells in BALF was determined 24 h after the last OVA challenge, as described in Materials and methods. Data represent means ± SEM of 6 mice per group. *Significant differences ($p < 0.05$) between sensitized and challenged control groups and sensitized and challenged anti-IL-33 treatment groups.

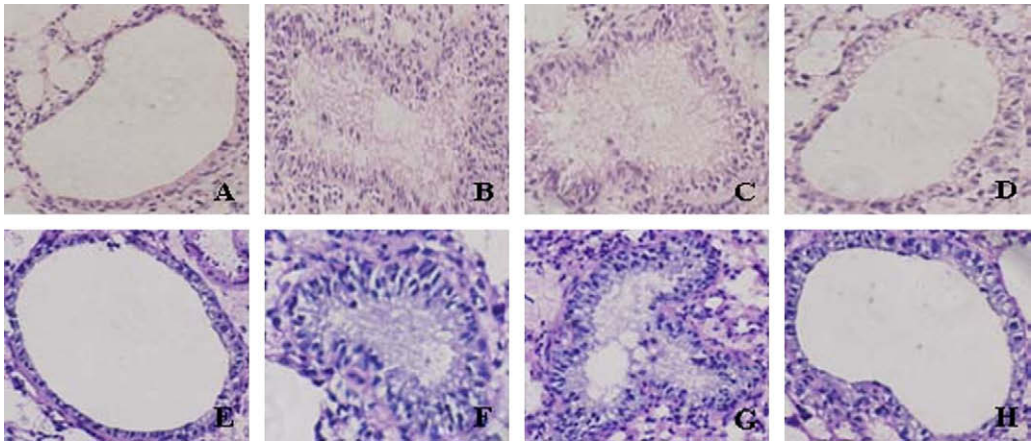

Fig. 3. Effect of anti-IL-33 treatment on airway inflammation in OVA-treated mice. Lung tissues from saline challenge (A and E), OVA-challenge (B and F), control IgG treatment (C and G) and anti-IL-33 treatment (D and H) mice were processed 24 h after the last OVA challenge. Tissue eosinophilia was detected with H and E staining (A–D) and goblet cells and mucus were detected with PAS/AB staining (E–H) by light microscopy (original magnification: 400×).

Effect of anti-IL-33 treatment on Th1/Th2 cytokines production in BALF

IL-4, IL-5, IL-13, and interferon-γ concentrations in BALF were measured by ELISA 24 h after the last challenge. After sensitization and challenge, the increases in IL-4, IL-5, and IL-13 production in BALF supernatants were significantly suppressed by anti-IL-33 treatment ($p < 0.05$) (Fig. 4). Treatment of sensitized mice with control IgG had no apparent effect on the concentrations of IL-4, IL-5, IL-13, and IFN-γ. The production of Th1 cytokine interferon-γ in BALF was not significantly changed by anti-IL-33 treatment (Fig. 4).

Serum antibody levels

Serum levels of total and OVA-specific IgE were increased dramatically in OVA-challenged mice relative to values in saline-challenged controls. Treatment with the anti-IL-33 antibody (150 μg/mouse, five times intraperitoneally) reduced serum levels of total and OVA-specific IgE. Treatment with control antibody had no effect (Table 1).

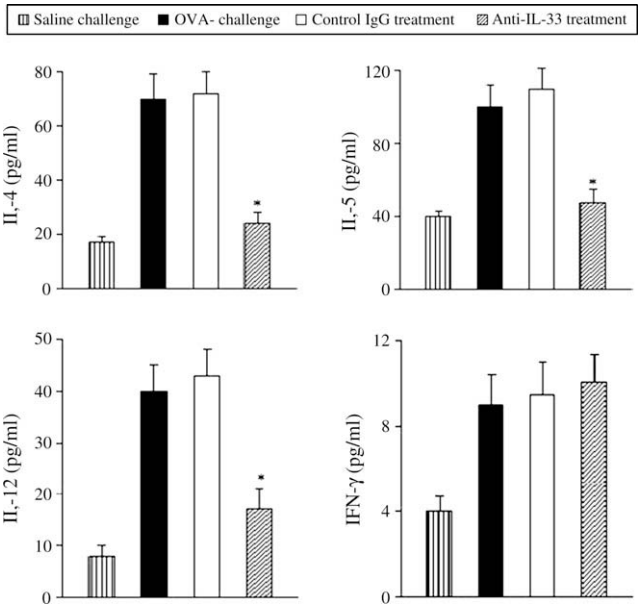

Fig. 4. Effect of anti-IL-33 treatment on Th1/Th2 cytokines production in BALF. IL-4, IL-5, IL-13, and interferon-γ concentrations in BALF were assessed 24 h after the last challenge, as described in Materials and methods. Marked increases in IL-5, IL-4 and IL-13 production in BALF after OVA sensitization and challenge were significantly suppressed by treatment with anti-IL-33 as compared with the control IgG-treated groups. Interferon-γ production in BALF was not significantly changed by anti-IL-33. *Significant differences ($p < 0.05$) between sensitized and challenged control groups and sensitized and challenged anti-IL-33 treatment groups.

Table 1
Effect of anti-IL-33 on total and OVA-specific IgE in the serum of allergic mice.

Group	Total IgE (ng/ml)	OVA-specific IgE (U/ml)
Saline challenge	28 ± 5	<10
OVA-challenge	220 ± 45	47 ± 5
Control IgG treatment	204 ± 38	51 ± 8
Anti-IL-33 treatment	67 ± 26*	14 ± 2*

Anti-IL-33 or control IgG (150 μg/mouse) was administered intraperitoneally at 30 min before antigen sensitization and challenge on Day 0 and followed on Days 14, 25, 26, and 27. Levels of serum IgE were measured 24 h after the last OVA challenge. Values represent mean ± SEM ($n = 6$ per group), and represent two separate experiments. *Significant difference ($p < 0.05$) compared with OVA-challenge.

Discussion

This study was designed to determine whether anti-IL-33 antibody attenuated airway inflammation in a murine model of allergen-induced asthma. We demonstrated for the first time that the treatment of allergic mice with anti-IL-33 given intraperitoneally before sensitization and antigen challenge inhibited Th2 cytokines (IL-4, IL-5, and IL-13) production, lung inflammatory cell infiltration, goblet cell hyperplasia and mucus hypersecretion. The serum levels of total and OVA-specific IgE were also reduced after treatment with the anti-IL-33 antibody.

Eosinophils are important effector cells in asthma and allergic diseases, but the mechanisms regulating their biological functions remain unclear. Our results showed that pulmonary eosinophilia was decreased by anti-IL-33 treatment and that such decrease in eosinophil numbers was associated with a decrease in the levels of IL-4, IL-5, and IL-13 in the airways of sensitized and challenged mice. This result is supported by the findings of several studies. Eosinophils are the direct target leukocytes for IL-33 [17]. Mice injected with IL-33 display profound mucosal eosinophilia with associated pathologic changes [6]. IL-33 potently induces human eosinophil adhesion and CD11b expression and enhances eosinophil survival [16]. IL-33 induces eosinophil superoxide anion production and degranulation, IL-33 also increases eosinophil survival and induces production of IL-8 [15]. We also showed that anti-IL-33 treatment prevented the increase in T lymphocytes in BALF. IL-33 is able to enhance antigen-dependent and -indepen-

dent T cell responses, including IL-5, IL-13, and IFN-γ production [23]. Cherry et al. [15] found that neutrophils did not express ST2, nor did they respond to IL-33. Interestingly, injection of anti-IL-33 decreased slightly the number of neutrophils in BALF but not significantly as compared with the level in control-treated mice (Fig. 2). Further studies designed to clarify the effects of IL-33 on neutrophil functions are needed. These findings indicate that the inhibition of eosinophil recruitment to the lung associated with anti-IL-33 treatment most likely directly reflects neutralization of IL-33-induced promotion of eosinophil activity.

Increased mucus production by goblet cells in the airway epithelium is associated with airway inflammation and asthma. Previous study has shown that *in vivo* IL-33 treatment induces goblet cell hyperplasia by induction of endogenous IL-13 [19]. In the present study, treatment with the anti-IL-33 reduced the increase in PAS/AB epithelial cells and mucus hypersecretion in the airways after antigen challenge. Th2 cytokines, T cells, and eosinophils are required to produce airway mucus accumulation and goblet cell degranulation [24–26]. Although a direct role of anti-IL-33 in these cells cannot be ruled out, the observed decrease in mucus production in anti-IL-33-treated mice lung tissue may be attributed to an indirect effect on goblet cells resulting from the combined effects of reduction in Th2 cytokine levels and eosinophilia in OVA-sensitized and challenged mice.

IL-4, IL-5, and IL-13 produced by Th2 cells have been postulated to have central roles in the initiation and maintenance of allergic inflammation [27]. The development of airway eosinophilia and allergen-induced AHR is also associated with increased levels of IL-4, IL-5, and IL-13 in BALF, consistent with development of a Th2-mediated allergic response. IL-4 regulates allergic inflammation by promoting Th2 cell differentiation, IgE synthesis and its receptor up-regulation, and mucus hypersecretion [28–30]. IL-5 promotes eosinophilic inflammation and infiltration into airways [31]. IL-13 promotes B cell differentiation and is capable of inducing isotype-switching in B cells to produce IgE [32]. Recent studies demonstrated that IL-33 administration induced AHR and goblet cell hyperplasia by induction of IL-4, IL-5, and IL-13 [19]. Our present data showed that treatment with anti-IL-33 significantly reduced the levels of IL-4, IL-5, and IL-13 in BALF from sensitized and challenged mice, thus indicating its effect in attenuating the inflammatory response. The inhibition of Th2 cytokine levels may be associated with decreased T cells in the lung. We speculated that anti-IL-33 treatment acted locally within the airways and adjacent parenchymal tissue to inhibited Th2 cytokines production.

IL-33-treated mice have significantly higher serum levels of IgE and IgA [6]. We found that both serum levels of total and OVA-specific IgE were reduced by treatment with the anti-IL-33 given before sensitization and antigen challenge. The increased serum IgE is likely related to the increased production of IL-4 [33]. In addition, IL-4 and IL-13 are important in directing B cell growth, differentiation, and secretion of IgE [34]. Therefore, the observed reduction in serum IgE in our asthma model by anti-IL-33 may be the result of inhibitory effects on B cell activation and reduced Th2 cytokine release. The biological activities of IgE are mediated through high-affinity IgE receptors (FcεRI) on mast cells and basophils. Cross-linking of FcεRI initiates multiple signaling cascades leading to cellular degranulation and activation [35,36]. IL-33 promotes IL-4, IL-13, and IL-8 secretion in synergy with IL-3 and/or FcεRI-activation, and enhances FcεRI-induced mediator release [17] or induces IL-13 production by mast cells independently of IgE-FcεRI signals [11]. Recently, IL-33 was reported that to be critical for allergen-IgE induced mast cell release of cytokines [8,12]. However, IL-33 neither induced nor enhanced mast cell degranulation [11]. Taken together, these results suggest that IL-33 promotes allergic inflammation and Th2 polarization mainly by the selective activation of these specialized cells of the innate immune system. Inhibition of IL-33 by anti-IL-33 therefore would attenuate not only production of IgE but also allergen-IgE induced mast cell activation during allergic diseases.

In conclusion, blockage of IL-33 by anti-IL-33 antibody can alleviate asthmatic syndromes, inhibit pulmonary eosinophilic inflammation, and decrease Th2 cytokine production in mice. These findings suggest that IL-33 plays a key role in this murine model of allergic asthma. Our results will improve the understanding of the pathogenesis of asthma and offer a novel therapeutic approach for the treatment of allergic inflammatory airway disorders.

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