

Original article

Blocking IL-25 prevents airway hyperresponsiveness in allergic asthma

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Background: IL-25 (IL-17E), a member of the IL-17 family of immunoregulatory cytokines, has been implicated in the regulation of type 2 immunity. Its roles in antigen-driven airway inflammation and airway hyperresponsiveness (AHR) remain to be fully established.

Objective: We sought to determine whether a neutralizing antibody against IL-25 represents a novel therapeutic for airway inflammation and hyperresponsiveness.

Methods: We generated a neutralizing mAb against IL-25 and used this to inhibit IL-25 in a mouse model of allergic airway disease.

Results: Blocking IL-25 in an experimental model of allergic asthma prevented AHR, a critical feature of clinical asthma. Administration of anti-IL-25 mAb during the sensitization phase resulted in significantly reduced levels of IL-5 and IL-13 production, eosinophil infiltration, goblet cell hyperplasia, and serum IgE secretion, and prevented AHR. Even more striking was the ability of anti-IL-25 mAb, administered only during the challenge phase of the response, specifically to prevent AHR even during an ongoing type 2 inflammatory response in the lungs.

Conclusion: IL-25 is critical for development of AHR.

Clinical implications: We define a novel pathway for the induction of AHR and suggest that IL-25 represents an important therapeutic target for the treatment of asthma. Significantly, our antibody also blocks the binding of human IL-25 to its receptor. (J Allergy Clin Immunol ■■■■;■■■:■■■-■■■.)

Key words: IL-25, airway hyperresponsiveness, allergic inflammation, type 2 immunity, IL-13, IL-17

Abbreviations used

5-HT: 5-Hydroxytryptamine
AHR: Airway hyperresponsiveness
BAL: Bronchoalveolar lavage
IL-25R: IL-25 receptor
OVA: Ovalbumin
rIL-25: Recombinant IL-25
rmIL-25: Recombinant mouse IL-25

Allergic asthma is characterized by uncontrollable airway hyperresponsiveness (AHR) induced by a variety of provocative stimuli and is associated with type 2 inflammatory infiltrates into the lungs. The number of patients has increased dramatically over recent decades, and the World Health Organization estimates that in the region of 300 million people worldwide have this disorder. Type 2 immune responses are characteristic of immunity generated to combat parasitic helminth infection and are characterized by the presence of a CD4⁺ T_H2 cell subset producing cytokines including IL-4, IL-5, IL-9, and IL-13. It is these cytokines that regulate effector functions such as stimulating B-cell growth, initiating immunoglobulin isotype switching to IgE, and inducing goblet cell hyperplasia and associated mucus production, eosinophilia, mastocytosis, and fibrosis.¹ It is the central roles played by these cytokines that have made them key therapeutic targets in asthma. Indeed, mouse models in which these cytokines are overexpressed show significant characteristics of asthma. Surprisingly then, efforts to ameliorate experimental asthma by blocking specific type 2 cytokines have proven unsuccessful, with the exception of inhibiting IL-13. In contrast with the other type 2 cytokines, inhibition of IL-13 suppresses both AHR and airway inflammation, although the mechanism remains unclear.^{2,3}

Recently, overexpression of IL-17E/IL-25 has been shown to induce type 2 responses *in vivo*.⁴⁻⁶ IL-25/IL-17E is a member of the structurally related IL-17 cytokine family.⁷ IL-17B, IL-17C, IL-17D, IL-17E/IL-25, and IL-17F share between 16% and 50% sequence homology with IL-17A.^{8,9} Transgenic expression of both human IL-25⁵ and mouse IL-25,⁶ or administration of recombinant mouse IL-25 (rmIL-25),⁴ has been shown to induce type

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2 biased responses with increased IL-4, IL-5, and IL-13 production, eosinophilia, and elevated IgE. Native IL-25 expression has been detected from *in vitro* differentiated T_H2 cells⁴ and *in vitro* cultured mast cells.¹⁰ The receptor for IL-25, termed EVI27/IL-17BR, was originally found to bind IL-17B,⁹ but IL-25 binds with higher affinity than IL-17B; therefore, this is now termed IL-25R.

It is noteworthy that *il25*^{-/-} mice failed to expel the helminth parasite *Nippostrongylus brasiliensis* efficiently, a key indicator of an ineffectual type 2 response. The observed defect correlated with a delay in the upregulation of type 2 cytokine production that was not a result of an inability to generate T_H2 cells but correlated with a deficit in a population of non-B/non-T (NBNT), c-kit⁺, FcεR1⁻, IL-4-producing, IL-5-producing, IL-13-producing cells in the mesenteric lymph nodes of the infected *il25*^{-/-} mice.¹¹ Similar importance was ascribed to IL-25 in the protection against *Trichuris muris*.¹² These studies revealed a pivotal role for IL-25 as a mediator of type 2 immunity and suggested that IL-25 lies upstream of the classic T_H2 cytokines.^{4,11} Furthermore, IL-25 has been shown to control IL-17-mediated autoimmunity during experimental autoimmune encephalomyelitis.¹³ Treatment with IL-25 was found to induce elevated IL-13 production, and this suppressed T_H17 responses, leading to the suggestion that IL-25 and IL-17 play opposing roles in autoimmunity.

Overexpression of IL-25 has been shown to induce type 2 inflammation in the lung with associated eosinophilia, mucus production, and increased responsiveness to airway agonists.^{5,6,14} Recent studies have also indicated that pulmonary administration of exogenous IL-25 induces airway inflammation and eosinophil infiltration via a type 2 cytokine-dependent mechanism.^{14,15} However, these studies did not assess the requirement for endogenous IL-25 production in inducing or maintaining allergic airway responses.

We now demonstrate that IL-25 plays a critical role in both airways inflammation and AHR. By using a blocking antibody to IL-25 in a mouse model of asthma, we show that IL-25 acts initially to enhance type 2 cytokine-mediated inflammation. However, in addition, and unexpectedly, IL-25 plays a critical role in the induction of AHR that is independent of the inflammatory response. Our data suggest that IL-25 does not act directly on the lung smooth muscle but is required to upregulate the levels of IL-13 locally within the asthmatic lung, which can in turn activate smooth muscle responses. However, we also demonstrate that although IL-25 regulates the level of type 2 cytokines and their downstream effectors, these are not essential for the ability of IL-25 to induce the AHR reaction.

METHODS

Mice

BALB/c mice were obtained from Harlan UK (Bicester, UK) and maintained in the Small Animal Barrier Unit and Central Biomedical

Services or National Heart and Lung Institute facilities in specific pathogen-free environments. *il4*^{-/-}*il5*^{-/-}*il9*^{-/-}*il13*^{-/-} mice and *il13*^{-/-} mice on a BALB/c background (6th generation backcross) were as described.^{1,16} *Il25*^{-/-} mice on a C57BL/6 × 129 background were as described.¹¹ All animal experiments outlined in this report were undertaken with the approval of the UK Home Office.

Generation of anti-IL-25 antibodies

An *il25*^{-/-} mouse was immunized with recombinant mouse IL-25 (R&D Systems, Minneapolis, Minn) intraperitoneally on days 0 (5 μg) and 10 (5 μg), and then intravenously on day 14 (2.5 μg). On day 21, the spleen was isolated, a single cell suspension prepared, and 1 × 10⁸ splenocytes mixed with 1 × 10⁷ NS0 myeloma cells. Cell fusion was initiated with PEG 1500 (Roche Diagnostics, Nonnenwald, Germany), and clones were grown out in the presence of hypoxanthine, aminopterin, and thymidine by standard protocols.

Antibody screening (ELISA)

Recombinant mouse or human IL-25 (R&D Systems) was directly plate-bound. After blocking, recombinant mouse IL-17BR-Fc fusion protein (R&D Systems) was added and incubated for 2 hours at room temperature. A biotinylated antihuman IgG antibody (BD Pharmingen, San Jose, Calif) was used to bind the fusion protein and was detected by using a standard ELISA protocol. Supernatants from anti-IL-25 producing hybridomas were screened in this assay for ability to block binding of IL-25 to the IL-17BR-Fc fusion protein.

Antibody screening (*in vitro* cellular assay)

Sorted non-B/non-T (CD19⁺CD4⁺CD8⁻) cells were plated in either media alone or media + rIL-25. IL-25 induced IL-13 production from non-B/non-T cells. Purified IgG from anti-IL-25 hybridomas was screened for ability to inhibit IL-13 production.

Sensitization and allergen exposure

BALB/c mice (6–12 weeks) were sensitized by intraperitoneal administration of ovalbumin in PBS (20 μg/injection) complexed with alum, or PBS and alum only (controls), at days 0 and 12. Aerosol administration of 1% ovalbumin was undertaken on days 19, 20, and 21 for 20 minutes per day. Control animals received PBS. Anti-IL-25 mAb (500 μg/dose) was administered intraperitoneally the day before each ovalbumin (OVA) sensitization and 4 hours before each OVA aerosolization. In further experiments, anti-IL-25 mAb (500 μg/dose) was administered before each aerosolization only. Control mice received either saline or isotype control (500 μg/dose) instead of anti-IL-25 mAb. Isotype control was anti-c-myc (mouse IgG₁, clone 9E10.2). On day 22, the animals were analyzed by using restrained plethysmography to assess AHR.

Measurement of AHR

Animals were anesthetized, tracheostomized, and placed on a ventilator (SAR-830 series; CWE Inc, Ardmore, Pa) at a rate of 150 breaths/min, with a tidal volume of 0.2 mL. Mice were monitored in a whole-body plethysmograph (EMKA Technologies, Paris, France), and transpulmonary pressure was assessed via an inline transducer. After recording stable baseline pulmonary resistance, increasing concentrations of acetyl-β-methylcholine chloride (methacholine; Sigma, Dorset, United Kingdom) were administered by aerosol for 10 seconds with an ultrasonic nebulizer, and pulmonary resistance was recorded for a 5-minute period. IOX software (EMKA Technologies, Paris, France) was used to analyze airways resistance, compliance, and standard pulmonary parameters.

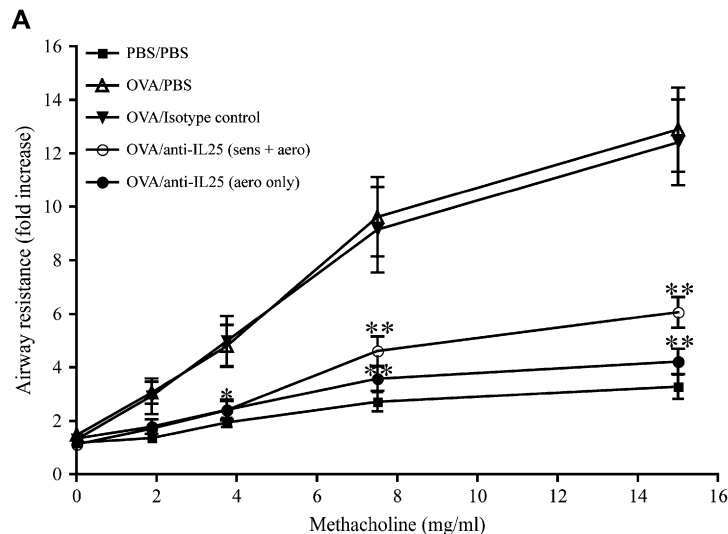


FIG 1. Neutralization of IL-25 prevents AHR. Pulmonary resistance in response to methacholine in OVA-sensitized mice. Data are combined from 2 experiments and represent the means $\pm$ SEMs of 14 to 18 mice/group. (* P < .05 vs isotype control; ** P < .01 vs isotype control). *sens*, Antibody administered before sensitization; *aero*, antibody administered before aerosol challenges.

Bronchoalveolar lavage

Mice were killed by cervical dislocation, and $4 \times 500 \mu\text{L}$ aliquots of PBS were injected through the tracheostomy and retrieved. Differential cell counts on 150 cells were performed on cytopins stained with Giemsa.

Lung tissue collection and histology

Lungs were fixed in formalin (10% formaldehyde in 0.9% saline solution) and stained with Giemsa for inflammatory infiltrate and periodic acid-Schiff for goblet cells. Inflammation and mucus production were evaluated blindly by using numerical scoring expressed in arbitrary units.

Restimulation of draining lymph nodes

Mediastinal lymph node cells were harvested, counted, and plated at 3×10^6 cells/mL (3×10^5 cells per well in a round-bottom 96-well plate; final volume, $200 \mu\text{L}$ per well) with $100 \mu\text{g/mL}$ OVA (Sigma, St Louis, Mo). Supernatants were harvested after 72 hours and cytokine levels analyzed by ELISA.

Intranasal IL-25 administration

Wild-type BALB/c mice, *il4^{-/-}il5^{-/-}il9^{-/-}il13^{-/-}* mice, and *il13^{-/-}* mice on a BALB/c background were administered $1.8 \mu\text{g}$ recombinant (rIL-25) (R&D Systems) in $40 \mu\text{L}$ PBS per mouse intranasally. Control animals received PBS only.

ELISA

IL-13 ELISA was performed by using the Quantikine Murine IL-13 Kit (R&D Systems). All other cytokines were measured by sandwich ELISA with antibodies purchased from Becton Dickinson (Minneapolis, Minn). Serum levels of anti-OVA IgE were measured by ELISA, and antibody titers were compared with a pooled standard generated in the laboratory, which was then assigned arbitrary units.

Preparation of lung slices

Female Sprague-Dawley rats 6 to 8 weeks old were killed by lethal exposure to CO_2 . The trachea was cannulated, followed by instillation of 2% low-melting-point agarose solution into the airways.

After the tissue was cooled, cores were taken, and slices ($250 \mu\text{m}$) were prepared using a Krumdieck tissue slicer (Alabama Research and Development, Munford, Ala). These slices were placed in Dulbecco modified Eagle medium supplemented with 100 U/mL penicillin, 0.1 mg/mL streptomycin, 4 mmol/L L-glutamine, and $2.5 \mu\text{g/mL}$ amphotericin B and incubated on a roller system. Adjacent slices containing the same airway were paired (1 slice was treated with cytokine and the other with carrier protein alone). The slices were incubated overnight in media containing recombinant mouse IL-13 (100 ng/mL; Peprotech, London, UK), rmIL-25 (100 ng/mL; R&D Systems), or carrier protein (BSA, Sigma). We first assessed the cross-species biological activity of rmIL-25 on rat lymph node cells by assessing their expression of IL-13 after stimulation.

Image acquisition and analysis

Airways were identified and imaged with a video camera (Image Associates, Westlake Village, UK). After preincubation for 5 minutes with Dulbecco modified Eagle medium, the first image was acquired (baseline image). The slices were then incubated with increasing concentrations of 5-hydroxytryptamine (Sigma) for 5 minutes at 37°C and reimaged. Responses were analyzed by using an image analysis program (ZEISS KS 300 3.0, Zeiss, Thornwood, NY). The luminal area was taken as the area enclosed by the epithelial luminal border and was quantified after setting the appropriate threshold value. Baseline area was defined as 100%.

Statistical analysis

The significance of the differences between experimental groups was analyzed by using an ANOVA (for dose response to methacholine) and/or Student unpaired *t* test.

RESULTS

Generation of blocking anti-rmIL-25 mAbs

To study the biology of IL-25, we generated anti-IL-25 mAbs by immunizing *il25^{-/-}* mice with rmIL-25. Two of these anti-IL-25 antibodies (2C3 and 5C1) blocked the interaction between rmIL-25 and a soluble mouse

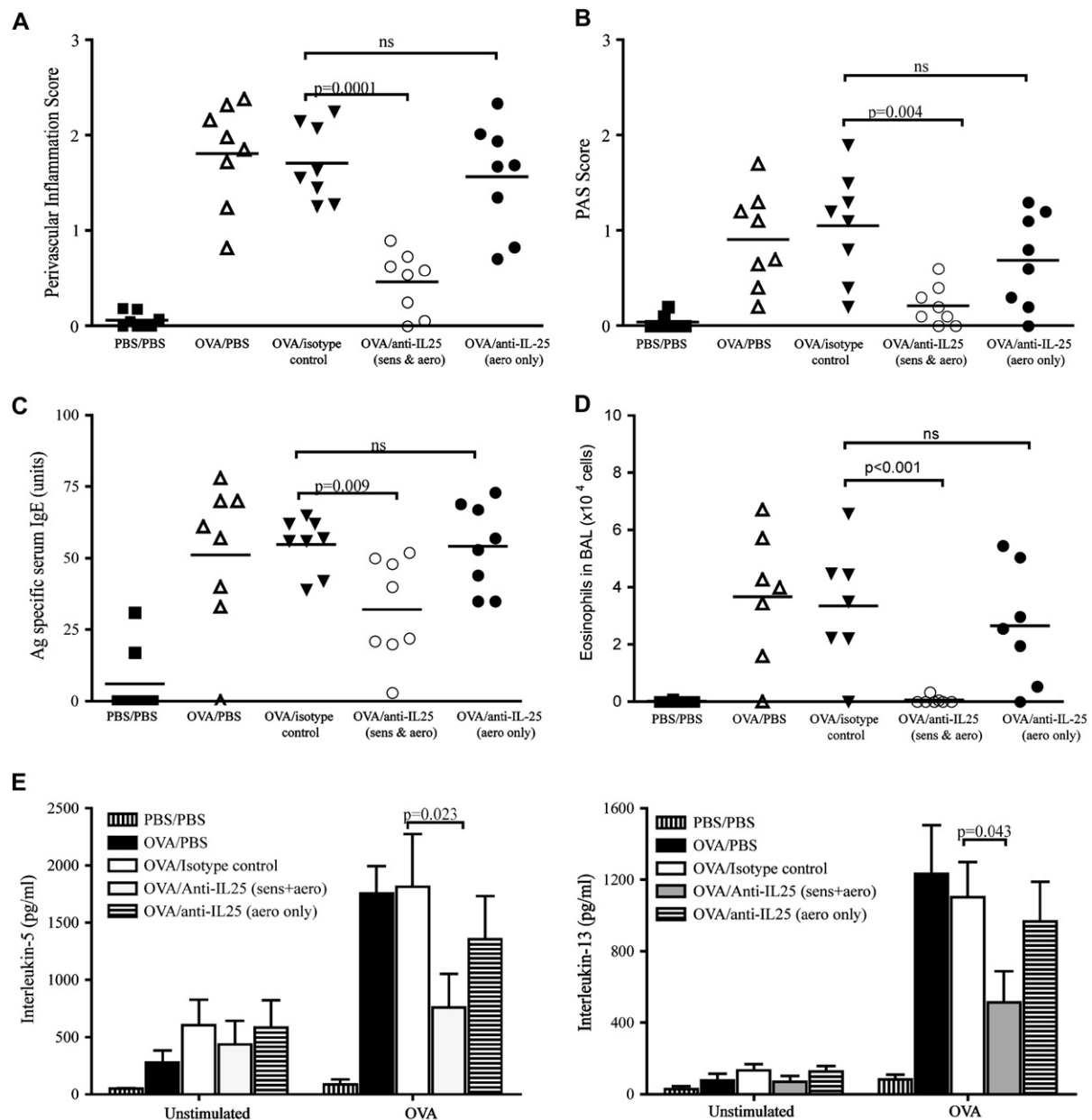


FIG 2. Type 2 responses after neutralization of IL-25. **A**, Perivascular infiltration in lung sections. **B**, Mucus content. **C**, Antigen-specific serum IgE. **D**, Eosinophils in BAL. **E**, Antigen-induced cytokines. Symbols represent individual mice; bar = mean. Data are representative of at least 2 independent experiments. *aero*, Antibody administered before aerosol challenges; *sens*, antibody administered before sensitization.

IL-25R-Fc fusion protein dose-dependently (data not shown) and inhibited IL-25-dependent production of IL-13 by primary mouse non-B, non-T cells in an *in vitro* bioassay (data not shown).

Neutralization of IL-25 during sensitization and airway challenge prevents development of allergic airway inflammation and AHR

Our studies and those of others suggest that IL-25 acts upstream of the type 2 cytokines and that by inhibiting the initial production of IL-25, we may be able to abrogate

type 2 mediated pathology. We tested this supposition using an experimental mouse model of allergic asthma. BALB/c mice were sensitized with the antigen OVA before being challenged with aerosolized OVA. Sensitized and challenged BALB/c mice developed a distinctive asthma phenotype characterized by increased AHR after exposure to the provocative agent methacholine, eosinophil infiltration of the airways, goblet cell hyperplasia, and serum IgE secretion, compared with control BALB/c mice challenged with PBS (Figs 1 and 2). By contrast, administration of anti-IL-25 mAb before each sensitization and

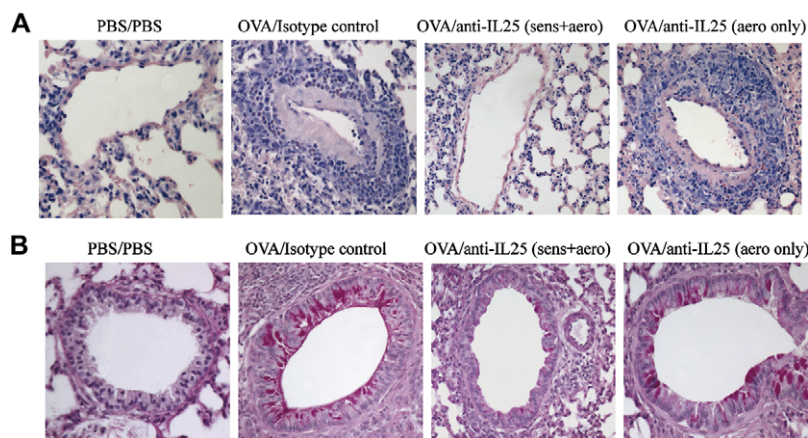


FIG 3. Lung histology after anti-IL-25 mAb treatment. **A**, Lung sections from anti-IL-25—treated and control animals stained with Giemsa to demonstrate infiltration of inflammatory cells. Representative sections are shown for each group. **B**, Lung sections from anti-IL-25—treated and control animals stained with periodic acid-Schiff to visualize mucin and mucous secreting cells. Representative sections are shown for each group. *sens*, Antibody administered before sensitization; *aero*, antibody administered before aerosol challenges.

aerosolization resulted in a marked abrogation in AHR after challenge with methacholine. This is evidenced by a reduction in lung resistance with values comparable to those of the PBS control mice (Fig 1). Administration of an isotype-matched control mAb did not suppress AHR (Fig 1). The anti-IL-25 mAb also significantly reduced the levels of cellular infiltration around the lung vasculature (Fig 2, A; Fig 3, A), goblet cell hyperplasia in the airways (Fig 2, B; Fig 3, B), and levels of antigen-specific serum IgE (Fig 2, C) and eosinophil infiltration (Fig 2, D). Because type 2 cytokines are known to regulate these effector functions, we determined the levels of cytokines secreted from cells isolated from the draining lymph nodes after antigen restimulation. In contrast with the elevated levels of IL-5 and IL-13 induced by OVA sensitization and challenge in BALB/c mice, administration of anti-IL-25 mAb resulted in a significant reduction in the levels of these cytokines (Fig 2, E).

Blocking IL-25 during airway challenge of OVA-sensitized mice inhibits AHR

To assess whether the anti-IL-25 mAb could inhibit the AHR response when given after OVA sensitization, we also administered antibody only before the airway challenge. Unexpectedly, even when given later in the response, treatment with the anti-IL-25 mAb prevented the airways resistance induced by methacholine (Fig 1). Significantly, analysis of lung histology sections showed no changes in the levels of cellular infiltrate (Fig 2, A; Fig 3, A) or airway goblet cell hyperplasia (Fig 2, B; Fig 3, B) between anti-IL-25 mAb—treated mice and the OVA-challenged BALB/c controls or the isotype-matched mAb-treated controls. Furthermore, there was no reduction in the levels of antigen-specific serum IgE (Fig 2, C) or the number of eosinophils in the bronchoalveolar lavage (BAL) fluid (Fig 2, D) after anti-IL-25 mAb administration. Strikingly, the levels of IL-5 and IL-13,

produced by antigen-restimulated cells from the draining mediastinal lymph nodes, remained comparable to those of the OVA-challenged BALB/c controls or the isotype-matched mAb-treated controls (Fig 2, E). Thus, anti-IL-25 mAb administration during the challenge phase of the response can specifically abrogate AHR even during an ongoing type 2 inflammatory response.

IL-25 regulates localized pulmonary IL-13 and IL-17 but does not directly induce smooth muscle contraction

Although the levels of type 2 cytokines secreted by restimulated lymph node cells were similar between anti-IL-25 mAb and control-treated mice, we also assessed cytokine levels in BALs. In contrast with the levels of cytokines expressed by the draining lymph node cells, we detected a significant reduction in the levels of IL-13 and IL-5 in the BALs harvested from the lungs of anti-IL-25 mAb—treated animals (Fig 4, A). These results demonstrate that anti-IL-25 mAb treatment is able to suppress locally expressed type 2 cytokines during an ongoing inflammatory response. Furthermore, the reduced levels of IL-13 and IL-5 were accompanied by a significant elevation in the levels of IL-17 (Fig 4, A).

We also tested the ability of IL-25 to induce airway smooth muscle contraction directly. Lung slices were prepared from adult rats and incubated with IL-25 or IL-13 before airway contractility was measured in the presence of increasing concentrations of 5-hydroxytryptamine (5-HT). Although IL-13 was able to enhance airway contraction (Fig 4, B), we were unable to detect a demonstrable effect with IL-25 after overnight incubation (Fig 4, B). Furthermore, in the absence of 5-HT, we did not observe any IL-25-induced contraction after either overnight (data not shown) or short-term incubation with rIL-25 (Fig 4, C). These data suggest that IL-25 does not directly enhance muscular contraction, but that blocking IL-25 can

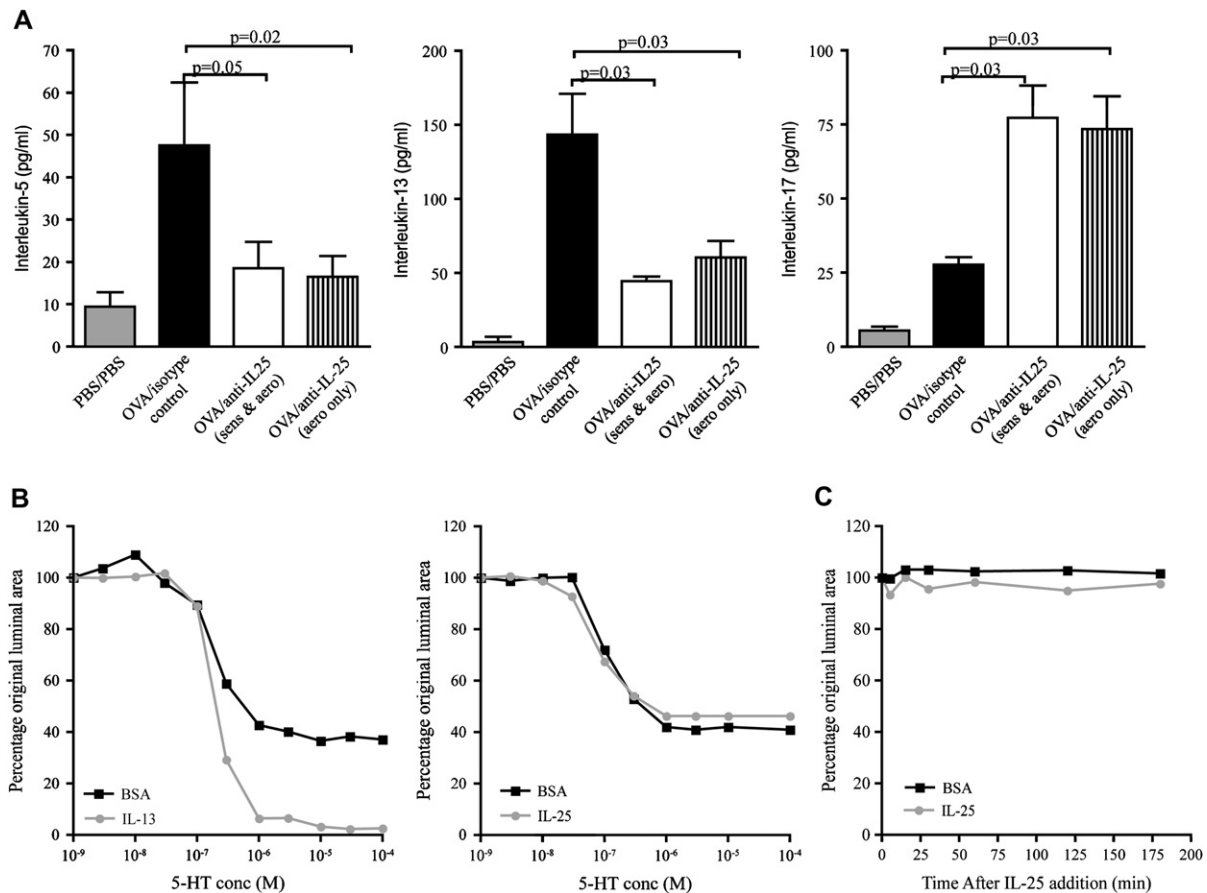


FIG 4. IL-25 regulates BAL IL-13 and IL-17, but does not directly induce smooth muscle contraction. **A**, Cytokine levels in BAL. **B**, Responses of rmIL-13 and rmIL-25-treated rat lung slices to 5-HT; representative slices shown. **C**, Response of rat lung slices to rmIL-25; representative slices shown. $n = 6-12$. Data are representative of at least 2 independent experiments.

suppress smooth muscle contraction by limiting agents such as IL-13.

Intranasal administration of IL-25 can also induce AHR independently of the classic type 2 cytokines

To assess whether the role of IL-25 in regulating type 2 cytokines was essential for the development of AHR, we challenged naive wild-type and cytokine-deficient BALB/c mice intranasally with rmIL-25. Even by 16 hours post-intranasal administration of rmIL-25 to BALB/c mice, we detected significantly elevated airways resistance (Fig 5, A). Furthermore, despite the absence of IL-13, *il13*^{-/-} mice also exhibited elevated AHR after rmIL-25 treatment (Fig 5, B). Because the other type 2 cytokines have also been shown to contribute to the asthma phenotype, we assessed the response of *il4*^{-/-}*il5*^{-/-}*il9*^{-/-}*il13*^{-/-} mice to rmIL-25 administered intranasally. Even in the absence of all of the classic type 2 cytokines, IL-25 treatment enhanced AHR after methacholine provocation (Fig 5, C). These data demonstrate that IL-25 can exacerbate AHR through a mechanism that is independent of the classic type 2 cytokines.

DISCUSSION

Using an antagonist of IL-25 to block IL-25 bioactivity, our data highlight for the first time the critical and functionally distinct roles played by endogenous IL-25 in a mouse model of allergic asthma. We have shown that IL-25 acts first at the initiation of the type 2 cytokine-dependent induction of airway inflammation, but also plays an essential role in the development of AHR independently of the inflammatory response, through both IL-13-dependent and independent pathways.

When administered before and during the sensitization and challenge phases of the experimental allergic asthma model, anti-IL-25 mAb inhibits both type 2 inflammation and AHR. We detected profoundly reduced levels of IL-5 and IL-13, eosinophil infiltration into the lung tissue, goblet cell hyperplasia, and serum IgE secretion. These changes were accompanied by the prevention of AHR. These data support the hypothesis that by blocking IL-25 signaling, we have constrained the production of type 2 cytokines leading to the abrogation of the type 2 effector functions including inflammation and AHR. Thus,

antagonists of IL-25 effectively suppress type 2 inflammation if administered from the initiation of the response, supporting a role for IL-25 in the initiation of type 2 responses as reported previously.^{4-6,11,15,17} These findings are also consistent with the report that IL-25-deficient mice infected with *Nippostrongylus brasiliensis* took longer to start producing IL-4, leading to a delay in worm expulsion.¹¹ However, once the type 2 response developed, the mice were able to produce normal levels of cytokines and clear the parasite.

Unexpectedly, when we administered anti-IL-25 antibody only during the challenge phase of the response, we found that this also prevented airways resistance induced by methacholine provocation. This observation contrasts with the previously described roles for IL-25 and suggests that IL-25 plays a more integrated role in lung inflammation and AHR. Thus, even during a robust type 2 response, anti-IL-25 mAb is a potent inhibitor of AHR. These data identify a novel pathway through which IL-25 regulates AHR. Although the levels of type 2 cytokines secreted by restimulated mediastinal lymph node cells were similar between anti-IL-25 mAb and control-treated mice, we detected a significant reduction in the levels of IL-13 and IL-5 in the BALs harvested from the lungs of anti-IL-25 mAb-treated animals. Interestingly, this was also accompanied by an elevation in the levels of IL-17 in the lungs.

A recent report has demonstrated the importance of the IL-13/IL-17/IL-25 axis in the regulation of experimental autoimmune encephalomyelitis. Our data similarly underline the importance of IL-25 in regulating IL-13 and IL-17 levels during the challenge phase of experimental allergic asthma. The upregulation of IL-13 is a major contributory factor to the pathogenesis of asthma, regulating inflammatory cell recruitment and AHR.^{2,3} It is likely that anti-IL-25 mAb treatment suppresses both the effector functions directly downstream of IL-13 and IL-5 and the cross-regulatory role that IL-13 plays in controlling the expression of IL-17.¹³ IL-17 has recently been reported to ameliorate established allergic asthma,¹⁸ and it is possible that by blocking IL-25 and reducing the levels of IL-13, we have provided an environment in which the elevated levels of IL-17 suppress allergic asthma still further. Analysis of such cross-regulatory pathways will form an important subject of further investigation.

We also tested, using lung slices *in vitro*, the ability of IL-25 to induce airway smooth muscle contraction directly because of the presence of IL-25R on airway smooth muscle cells.¹⁹ However, although IL-13 was able to enhance smooth muscle contraction, as suggested previously,²⁰ we were unable to detect a demonstrable effect with IL-25 after overnight incubation. Thus, unlike IL-13, IL-25 does not appear to act directly on the airways smooth muscle.

Although our data support a role for IL-25 acting by modulating the IL-13 pathway, we have also detected IL-13-independent AHR after IL-25 administration, suggesting that there is additional complexity involved in the control of IL-25-induced AHR. Indeed, using mice deficient in IL-4, IL-5, IL-9, and IL-13, IL-25 administration induced AHR independently of the classic type 2

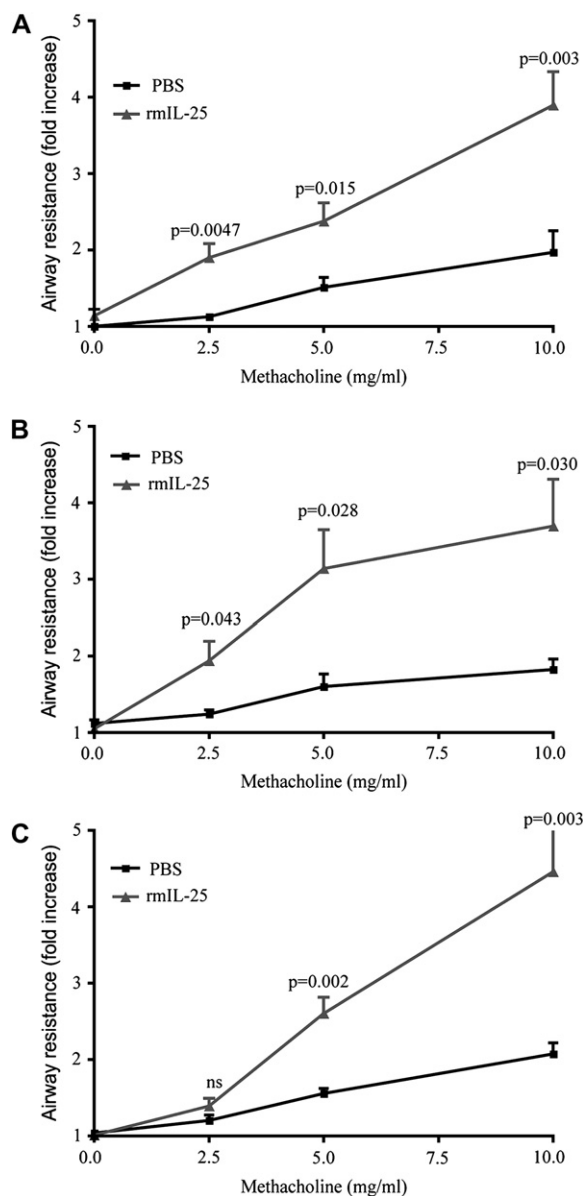


FIG 5. Administration of rmIL-25 to naive mice. Wild-type (A), *il13*^{-/-} (B), or *il4*^{-/-}*il5*^{-/-}*il9*^{-/-}*il13*^{-/-} (C) mice were administered 1.8 μ g rIL-25 or PBS intranasally. Methacholine sensitivity was determined 16 hours after challenge. n = 4-8 per group. Data are representative of at least 2 independent experiments.

cytokines. Further investigation will be required to determine whether IL-25 acts through the induction of known bronchoconstrictors such as the leukotrienes, or via molecules such as the recently described acidic chitinase.²¹

Recent reports have suggested that IL-25 may play a role in asthma in human beings. A recent study indicated that IL-25 is found in biopsy tissue from patients with asthma, associated with eosinophilic infiltration.²² In addition, IL-25 has been reported to induce inflammatory cytokine and chemokine production from lung fibroblasts²² and components of extracellular matrix from airway smooth muscle cells.^{19,22} It is also noteworthy that the

IL25 gene maps to a postulated asthma susceptibility locus on chromosome 14q11.2 with linkage to the T-cell receptor α/δ loci.^{23,24} However, a causative link to asthma has not been identified.

The efficacy with which anti-IL-25 antibodies can independently prevent AHR and reduce type 2 cytokine-induced inflammation *in vivo* suggests IL-25 is a good therapeutic target in asthma. Furthermore, it is noteworthy that our antibody also blocks the interaction between human IL-25 and its receptor and therefore can be used to generate humanized anti-IL-25 antibodies.

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