The Role of IL-13 in Established Allergic Airway Disease

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The effectiveness of targeting IL-13 in models where airway hyperresponsiveness (AHR) and airway inflammation have already been established is not well-described. We investigated the effects of blocking IL-13 on the early and late phase airway responses and the development of AHR in previously sensitized and challenged mice. BALB/cByJ mice were sensitized (days 1 and 14) and challenged (days 28–30) with OVA. Six weeks later (day 72), previously sensitized/challenged mice were challenged with a single OVA aerosol and the early and late phase response and development of AHR were determined. Specific in vivo blockade of IL-13 was attained after i.p. injection of a soluble IL-13Ra2-IgG fusion protein (sIL-13Ra2Fc) on days 71–72 for the early and late responses and on days 71–73 for the development of AHR. sIL-13Ra2Fc administration inhibited the late, but not early, phase response and the OVA challenge-induced changes in lung resistance and dynamic compliance; as well, sIL-13Ra2Fc administration decreased bronchoalveolar lavage eosinophilia and mucus hypersecretion following the secondary challenge protocols. These results demonstrate that targeting IL-13 alone regulates airway responses when administered to mice with established allergic airway disease. These data identify the importance of IL-13 in the development of allergen-induced altered airway responsiveness following airway challenge, even when administered before rechallenge of mice in which allergic disease had been previously established. The Journal of Immunology, 2002, 169: 6482–6489.

Clinical and experimental investigations have identified CD4+ Th2 cells as crucial in orchestrating the allergic inflammatory response leading to airway hyperresponsiveness (AHR) (1–3). Over the past several years, emphasis has been placed on the role of IL-13 in the development of AHR (4–8). IL-13 is a pleiotropic cytokine that is secreted by activated Th2 cells with immunoregulatory activities that partially overlap with those of IL-4 (9). The redundancy in biologic responses to IL-13 and IL-4 may be explained by shared components in the receptors for IL-4 and IL-13 (10). IL-13 can regulate IgE isotype switching and IL-4 may be explained by shared components in the receptors for IL-4 and IL-13 (9). The redundancy in biologic responses to IL-13 and IL-4 may be explained by shared components in the receptors for IL-4 and IL-13 (10). IL-13 can regulate IgE isotype switching in B cells, MHC class II and low affinity IgE receptor (CD23, FceRII) expression on B cells and monocytes, chemokine production, activation of mast cell, eosinophil, and neutrophil function, as well as the inhibition of proinflammatory gene expression by monocyte/macrophage populations (8, 11–15). IL-13 also increases expression of VCAM-1 on endothelial cells, facilitating the preferential recruitment of eosinophils (and T cells) to the airway tissues (16), and airway mucus secretion, which can exacerbate airway responsiveness (8, 17). Although not necessary for, or even capable of inducing Th2 development, IL-13 plays a regulatory role in Th2 cell activation (18), and in Th1 differentiation indirectly through its down-regulatory effects on production of proinflammatory cytokines, particularly on monocyte production of IL-12 (19). Administration of IL-13, or over expression of IL-13 in the airways, induced airway eosinophilia, mucus production, and AHR to various degrees (4, 7, 8).

The role of IL-4 in allergen-induced AHR development has been extensively studied. Using neutralizing Ab to IL-4 administered during the sensitization phase, or in mice deficient in IL-4, the development of airway eosinophilia, AHR, and increases in serum IgE seen following sensitization and allergen provocation are markedly reduced or abolished (20–22). However, administration of Ab to IL-4 after sensitization but during the allergen challenge phase only partially reduces the response suggesting alternate mechanisms or even a sequential requirement for IL-4 then IL-13 (20, 21, 23). Inhibition of both IL-4 and IL-13-transduced signals in STAT-6-deficient mice or in mice treated at the time of the challenge with an IL-4R antagonist inhibited AHR and airway eosinophilia, suggesting the importance of blocking not only IL-4 but also IL-13 (24, 25). The activity of IL-13 can be specifically blocked by administration of a soluble fusion protein comprised of the extracellular domain of the IL-13 high affinity receptor (IL-13Ra2) fused to the Fc portion of human IgG1, which specifically binds to and neutralizes IL-13 (26). Blockade of IL-13 at the time of allergen challenge in this way inhibited OVA-induced AHR with variable results on bronchoalveolar lavage (BAL) eosinophilia (4, 6, 7).

To date, most of the studies investigating the role of IL-13 have been performed in models of primary allergen challenge and the role of IL-13 in already established allergic airway disease is not well-defined. In patients with allergic asthma, allergen challenge leads to an early phase response (EPR), occurring within 15–30
min following allergen challenge. About 60% of patients also develop a late phase response (LPR), occurring about 3–5 h after allergen challenge, and characterized by airway obstruction and increased airway inflammation (27, 28). Similarly in mice with already established airway disease, allergen challenge can evoke EPR and LPR (29), followed by the development of sustained AHR (30). We previously showed important differences when a primary challenge approach was compared to mice which had previously been sensitized and challenged and later provoked with a single airway challenge (secondary challenge) (30). We also showed that lung resistance (RL, thought to reflect central airway function) and dynamic compliance (Cdyn; thought to reflect peripheral airway function) are differentially regulated in the latter model (30). The aim of the present study was to evaluate the role of IL-13 on airway function and lung inflammation in a model of allergen-induced AHR after re-exposure to allergen in previously sensitized mice assessed by monitoring EPR and LPR and the development of AHR.

Materials and Methods

Animals
Female BALB/cByJ mice were obtained from The Jackson Laboratory (Bar Harbor, ME). The mice were maintained on 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 National Jewish Medical and Research Center (Denver, CO).

Experimental protocol
Ten- to 12-week-old mice were sensitized by i.p. injection of 20 µg of OVA (Grade V; Sigma-Aldrich, St. Louis, MO) emulsified in 2.25 mg of aluminum hydroxide (AlumInject; Pierce, Rockford, IL) in a total volume of 100 µl on days 1 and 14. Mice were challenged (20 min) via the airways with OVA (1% in saline) (endotoxin concentration, 0.307 ELISA units/µg protein; BioWhittaker, Walkersville, MD) for 3 days (days 28, 29, and 30; primary challenge) using ultrasonic nebulization (AeroSonic Medical and Research Center (Denver, CO). The experimental animals used in this study were under a protocol approved by the Institutional Animal Care and Use Committee of the National Jewish Medical and Research Center (Denver, CO).

The aim of the present study was to evaluate the role of IL-13 on airway function and lung inflammation in a model of allergen-induced AHR after re-exposure to allergen in previously sensitized mice assessed by monitoring EPR and LPR and the development of AHR.

Determination of Airway Function
To assess airway function following secondary challenge, airway responsiveness was assessed as a change in airway function after challenge with aerosolized metacholine (MCh) administered for 10 s (60 breaths/min, 500-µl tidal volume) in increasing concentrations (1.5625, 3.125, 6.25, and 12.5 µg/ml). Anesthetized (pentobarbital sodium, i.p., 70–90 mg/kg), tracheostomized (18G cannula) mice were mechanically ventilated (160 breaths/min, 150 ml tidal volume to 150 ml, tidal volume to 150 ml, positive end-expiratory pressure of 12.5 mg/ml). Anesthetized (pentobarbital sodium, i.p., 70

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For detection of mucus-containing cells in formalin-fixed airway tissue, sections were stained with periodic acid Schiff (PAS), H&E, and quantified as previously described (32).

Measurement of cytokines
Cytokine levels in the BAL fluid were measured by ELISA as previously described (32). IFN-γ, IL-4, IL-5, IL-10, IL-12 (BD Pharmingen, San Diego, CA) and IL-13 (R&D Systems, Minneapolis, MN) ELISAs were performed according to the manufacturer’s directions. The limits of detection were 4 pg/ml for IL-4, IL-5, and IL-13 and 10 pg/ml for IL-10, IL-12, and IFN-γ.

Measurement of total and OVA-specific Antibody
Serum levels of total IgE and OVA-specific IgE, IgG1, and IgG2a were measured by ELISA as previously described (32). Briefly, 96-well plates (Immulon 2; Dynatech, Chantilly, VA) were coated with either OVA (5 µg/ml) or purified anti-OVA (02111D; BD Pharmingen). After addition of serum samples, a biotinylated anti-IgE Ab (02112D; BD Pharmingen) was used as detecting Ab, and the reaction was amplified with avidin-HRP (Sigma-Aldrich). IgG2a was detected using alkaline phosphatase-labeled anti-IgG2a (02013E; BD Pharmingen). The OVA-specific Ab titers of the samples were related to pooled standards that were generated in the laboratory and expressed as EU per milliliter. Total IgE levels were calculated by comparison with known mouse IgE standards (BD Pharmingen). The limit of detection was 100 pg/ml for total IgE.

Statistical analysis
ANOVA was used to determine the levels of difference between all groups. Comparisons for all pairs were performed by Tukey-Kramer honest significant difference test. Values of p for significance was set to 0.05. Values for all measurements were expressed as the mean ± SEM.

Results
Levels of IL-13 are increased in BAL fluid after airway challenge of mice with already established airway disease
To determine whether IL-13 is released after allergen challenge in the lungs of mice with already established airway disease, levels of IL-13 were assessed in BAL fluid. Eight hours after the 5% OVA challenge in previously sensitized and challenged mice, levels of IL-13 were significantly (p < 0.001) increased in BAL fluid compared to nonsensitized control mice (Fig. 1A). In addition, 48 h
if neutralization of IL-13 affects inflammatory changes in the lung, we assessed tissue inflammation 8 h after the 5% OVA challenge. Lung tissue was stained with H&E, PAS, and anti-MBP. H&E-stained slides showed a slight increase in peribronchial inflammation in sensitized and challenged animals (Fig. 3C) compared to the nonsensitized animals (Fig. 3A). Mice treated with sIL-13Rα2-Fc demonstrated similar increases in tissue inflammation (Fig. 3E). MBP staining of lung tissue revealed a significant (p < 0.01) increase of eosinophils in peribronchial lung tissue in sensitized and challenged mice treated with the control Ab (mean ± SEM: 43 ± 7 eosinophils/mm basement membrane (BM)) or sIL-13Rα2-Fc (36 ± 5 eosinophils/mm BM) (Fig. 4C), compared to the nonsensitized mice (1 ± 0 eosinophils/mm BM) (Fig. 4A).

To assess goblet cell hyperplasia tissue, slides were stained with PAS. At this time point, challenged only mice showed no PAS-positive cells (mean ± SEM: 0 ± 0 PAS positive cell/mm BM) (Fig. 3B), whereas sensitized and challenged mice treated with control Ab showed goblet cell hyperplasia (67.4 ± 11.7 PAS-positive cells/mm BM) (Fig. 3D). Few PAS-positive cells were detected in mice treated with the sIL-13Rα2-Fc (4.2 ± 2.8 PAS-positive cells/mm BM) (Fig. 3F).

**sIL-13Rα2-Fc inhibits the development of AHR in established airway disease**

To evaluate the effect of sIL-13Rα2-Fc on the development of nonspecific AHR to inhaled MCh in fully allergic mice, we waited 6 wk after the primary challenge and then rechallenged the animals. We have previously shown that at this time point, the inflammatory reaction and AHR developing after primary challenge are resolved but that a secondary airway challenge induces a strong inflammatory reaction with development of AHR (30). Indeed, previous sensitized and challenged mice showed an increase in RL and a decrease in Cdyn to MCh (Fig. 5) 48 h after the secondary challenge. Under these conditions, sIL-13Rα2-Fc treatment inhibited both the increases in RL and decreases in Cdyn (Fig. 5). Baseline RL and Cdyn were not affected by allergen or sIL-13Rα2-Fc treatment (data not shown).

**sIL-13Rα2-Fc decreases airway inflammation after secondary challenge**

In sensitized mice, inflammatory cell recruitment into the airways was increased after secondary airway challenge (Fig. 6). Increased total cell numbers was largely due to increased numbers of eosinophils. There was also a small but significant (p < 0.05) increase

![FIGURE 1](http://www.jimmunol.org/)

Levels of IL-13 are increased in BAL fluid following secondary airway challenge. Levels of IL-13 were measured in BAL fluid by ELISA 8 h after the secondary 5% OVA challenge (A) or 48 h after the secondary 1% OVA challenge (B). Means ± SEM of either challenged only mice (neb) (n = 4 in A and B) or sensitized and challenged mice treated with control Ab (huIgG) (n = 4 in A and n = 8 in B) or treated with sIL-13Rα2-Fc (sIL-13 Re) (n = 8 in A and B) are shown. *, p < 0.001 compared to neb and sIL-13Rα2Fc, #, p < 0.01 compared to neb.

![FIGURE 2](http://www.jimmunol.org/)

Treatment with sIL-13Rα2Fc inhibits LPR after allergen challenge. After the secondary allergen provocation with 5% OVA, airway changes were monitored using whole-body plethysmography. EPR were detected in sensitized and challenged mice treated with the control Ab (huIgG) (n = 8) as well as in mice treated with sIL-13Rα2Fc (sIL-13 Re) (n = 12). No response in challenged only control mice was detected (neb) (n = 8). A LPR following allergen challenge was only present in the huIgG group whereas sIL-13Rα2Fc-treated mice did not develop this response. Means ± SEM are shown. #, p < 0.05 compared to neb; *, p < 0.05 compared to neb and sIL-13 Re.
in the number of lymphocytes and neutrophils compared to the challenged only mice (Fig. 6). Administration of sIL-13Ra2-Fc at the time of the secondary challenge led to a significant ($p < 0.05$) decrease in total cell numbers as well as in eosinophil numbers (Fig. 6).

**Effect of sIL-13Ra2-Fc on tissue inflammation and goblet cell hyperplasia**

Lung tissue was obtained and processed 48 h after the secondary allergen provocation. Increased peribronchial and perivasculary inflammatory infiltrates were seen in sensitized and challenged mice treated with the control Ab (Fig. 3G). Mice treated with sIL-13Ra2-Fc still demonstrated an inflammatory infiltrate, albeit reduced overall (Fig. 3J). To specifically quantitate the infiltration of eosinophils, tissue sections, were stained with anti-MBP Ab. After the secondary challenge, sensitized and challenged mice treated with the control Ab (Fig. 4E) demonstrated a significant increase in peribronchial MBP-positive cells compared to control mice (Fig. 4D) (means ± SEM: 89 ± 5 eosinophils/mm BM in mice treated with the control Ab compared to 3 ± 0 in challenged only mice $p < 0.001$). The number of peribronchial MBP-positive cells after the secondary challenge was significantly lower in sIL-13Ra2-Fc-treated mice (Fig. 4F) (55 ± 7 eosinophils/mm BM), but were still significantly higher when compared to challenged only mice ($p < 0.01$, respectively).

To assess the degree of goblet cell hyperplasia, tissue sections were stained with PAS. After secondary challenge, challenged only mice showed no PAS-positive cells (mean ± SEM: 0 ± 0 PAS-positive cells/mm BM), whereas sensitized and challenged mice treated with control Ab showed many PAS-positive cells (122.7 ± 4.7 PAS-positive cells/mm BM) (Fig. 3H). In contrast, sensitized and challenged mice treated with sIL-13Ra2-Fc showed only scattered PAS-positive cells (10.5 ± 5.9 PAS-positive cells/mm BM) (Fig. 3K).

**Effect of sIL-13Ra2-Fc on cytokine production following secondary challenges**

Forty-eight hours after secondary allergen challenge, BAL fluid was obtained to assess Th1 (IFN-γ), pro-Th1 (IL-12), Th2 (IL-4, IL-5), and anti-inflammatory cytokine (IL-10) levels. After the secondary challenge, Th1 (IFN-γ) and pro-Th1 (IL-12) cytokines, as well as IL-10, were decreased in sensitized and challenged mice treated with the control Ab compared to challenged-only mice (Fig. 7). IL-5 production was increased in sensitized and challenged mice treated with control Ab. Treatment with sIL-13Ra2-Fc did not significantly affect IFN-γ, IL-12, or IL-10 production, but significantly inhibited IL-5 production (Fig. 7). IL-4 was not detectable in the BAL fluid of challenged only mice. In sensitized and challenged mice treated with the control Ab, mean (±SEM) levels were 38.8 ± 12.1 pg/ml. Levels of IL-4 were lower in sensitized and challenged mice treated with sIL-13Ra2-IgG mice (23.2 ± 2.2 pg/ml) but did not achieve statistical significance ($p = 0.12$) compared to the control Ab-treated mice.

**sIL-13Ra2-Fc treatment does not modify Ig production**

Serum from sensitized and challenged mice treated with control Ab showed elevated Ig levels compared to nonsensitized control mice following the secondary challenge protocol (Table I). Treatment with sIL-13Ra2-Fc did not significantly alter levels of total IgE, OVA-specific IgE, IgG1, or IgG2a (Table I).

**Discussion**

The exact mechanisms underlying the development of AHR are still incompletely understood and there are several areas of controversy surrounding murine models of allergen-induced AHR. This is perhaps not surprising given the findings that different strains of mice, different experimental protocols, and different read-outs of airway function have been used in response to bronchoconstrictive agents administered via different routes. In this study, we evaluated the role of IL-13 in the development of allergen-induced EPR and LPR and AHR in sensitized and challenged mice after secondary allergen challenge. We demonstrate that treatment with a specific inhibitor of IL-13 effectively prevents development of the LPR as well as development of AHR after allergen re-exposure in previously sensitized mice, a model that perhaps resembles more closely human exposure history.

Blockade of IL-13 was achieved following systemic administration of a soluble fusion protein (sIL-13Ra2-Fc) consisting of...
the extracellular domain of the murine IL-13 high affinity receptor
fused to the Fc portion of human IgG1; this fusion protein specifically binds to and neutralizes IL-13 (26) and as demonstrated in
the present study, lowered IL-13 levels in BAL fluid of sensitized
and challenged mice following treatment compared to mice treated
with a control Ab. It has been shown previously that administration
of this fusion protein in OVA-sensitized and challenged AJ mice
inhibited the development of AHR and mucus production, but did
not affect airway inflammation or OVA-specific IgE (7). In a dif-
ferent mouse strain (BALB/c mice), treatment with the same fu-
sion protein inhibited AHR, mucus production, and BAL eosino-
philia, without any effect on BAL neutrophilia (4). These results
highlight some of the strain-to-strain differences following a pri-
mary challenge protocol. As a corollary, when administered intra-
nasally, rIL-13 induces AHR, BAL eosinophilia and neutrophilia,
and mucus production (7).

Previous work from this laboratory, using similar approaches,
demonstrated temporal differences in the up-regulation of IL-4

FIGURE 4. Tissue infiltration with MBP+ eosino-
phils 8 and 48 h after secondary challenge. Immunohiso-
tochemical (MBP) localization of lung tissue eosinophils
was determined 8 h after the 5% OVA challenge (see
Materials and Methods) in challenged only mice (A),
sensitized and challenged mice receiving the control Ab
(B), and sensitized and challenged mice treated with sIL-
13Rα2Fc (C) and 48 h after the secondary 1% OVA
challenge (see Materials and Methods) in challenged
only mice (D), sensitized and challenged mice receiving the
control Ab (E), and sensitized and challenged mice treated
with sIL-13Rα2Fc (F) (final magnification, ×64).

FIGURE 5. sIL-13Rα2Fc inhibits both RL and Cdyn changes in sensitized and challenged mice in the secondary challenge protocol. RL (A) and Cdyn
(B) were measured in sensitized and challenged mice 48 h after the secondary challenge. Sensitized and challenged mice treated with control Ab
(IPN/huIgG) showed increased RL and decreased Cdyn to inhaled MCh compared to challenged only mice treated with sIL-13Rα2Fc (nbeh/sIL-13 Rc) or
a human IgG control (nbeh/huIgG). Sensitized and challenged mice treated with sIL-13Rα2Fc (IPN/sIL-13 Rc) showed no hyperresponsiveness. *, p < 0.05
compared to all other groups.
When compared to control Ab (human IgG) treated mice, numbers of inflammatory changes were only reduced, airway inflammation following secondary challenge, whereas in IL-13-deficient mice (which fail to develop airway eosinophilia) demonstrated the same degree of airway inflammation as wild-type mice, but IL-13-deficient mice failed to develop AHR (6), suggesting that airway inflammation or at least the accumulation of inflammatory cells in the airways in the absence of IL-13 is not sufficient for the development of AHR. This was certainly true in the present study for mice in which airway disease had already been established. Treatment with sIL-13Rx2-Fc completely prevented the development of the LPR and AHR after secondary challenge, whereas inflammatory changes were only reduced. These data suggest that IL-13 is critical to the development of AHR, perhaps beyond the association with numbers of inflammatory cells, eosinophils, lymphocytes, or neutrophils.

In agreement with previous studies (4, 7), treatment with sIL-13Rx2-Fc abolished goblet cell hyperplasia and induced mucus secretion, probably by direct reduction of MUC-5 gene expression in epithelial cells (17). These responses are not affected in IL-5-deficient mice (which fail to develop airway eosinophilia), indicating that IL-13-induced mucus secretion is dissociated from airway eosinophilia (14). This dissociation of airway inflammation, mucus cell hyperplasia, and altered airway function has been described (34). In STAT6-deficient mice, reconstitution of STAT6 only in epithelial cells was sufficient for IL-13-induced AHR and mucus production in the absence of inflammation, demonstrating the importance of IL-13 directly on airway epithelial cells for mucus production and development of AHR.

IL-13 shares structural characteristics and functional properties with IL-4. The IL-4Rx chain is a component of the IL-4 and the IL-13 receptors (12). Signaling through the IL-4Rx chain induces the absence of IL-13 is not sufficient for the development of AHR. The inflammatory infiltrate, in the read-out of airway function monitored in the response to interventions when primary and secondary challenge protocols were compared (33). Given the suggestive potency of IL-13 blockade in effectively preventing a number of these responses in primary challenge models (4, 7) and the increased levels of IL-13 in BAL fluid following secondary challenge, we examined the effects of the IL-13 inhibitor in a secondary exposure model of previously sensitized mice. Treatment with sIL-13Rx2-Fc decreased, but did not completely abolish, airway inflammation following secondary challenge. When compared to control Ab (human IgG) treated mice, numbers of eosinophils and lymphocytes were decreased about 60% in the BAL fluid. Nonetheless, despite the presence of residual inflammatory infiltrates following treatment, AHR was virtually abolished following secondary challenge. This absence of a direct correlation between BAL and tissue eosinophilia numbers and AHR is now well-described in many species, including humans. Although there is a clear-cut relationship between AHR and eosinophil numbers (perhaps more in tissue than in BAL; Ref. 32) in many studies, a number of exceptions have now been described. A major deficiency in trying to correlate eosinophil numbers and airway function is the absence of a reliable marker of eosinophil activation. The findings in the present study are also similar to results described in IL-13-deficient mice. After sensitization and airway challenge, IL-13-deficient mice demonstrate the same degree of airway inflammation as wild-type mice, but IL-13-deficient mice failed to develop AHR (6), suggesting that airway inflammation or at least the accumulation of inflammatory cells in the airways in the absence of IL-13 is not sufficient for the development of AHR. This was certainly true in the present study for mice in which airway disease had already been established. Treatment with sIL-13Rx2-Fc completely prevented the development of the LPR and AHR after secondary challenge, whereas inflammatory changes were only reduced. These data suggest that IL-13 is critical to the development of AHR, perhaps beyond the association with numbers of inflammatory cells, eosinophils, lymphocytes, or neutrophils.

**Table I.** sIL-13Rx2-Fc does not affect serum Ig levels in sensitized and challenged mice

<table>
<thead>
<tr>
<th></th>
<th>Nonsensitized</th>
<th>Sensitized</th>
<th>Challenged</th>
<th>sIL-13Rx2-Fc</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total IgE (ng/ml)</td>
<td>41 ± 14</td>
<td>223 ± 43</td>
<td>210 ± 38</td>
<td>190 ± 32</td>
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<td>OVA-specific IgE (EU/ml)</td>
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<td>287 ± 34</td>
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<td>OVA-specific IgG1 (EU/ml)</td>
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<td>OVA-specific IgG2a (EU/ml)</td>
<td>25 ± 23</td>
<td>1103 ± 190</td>
<td>1138 ± 418</td>
<td>1138 ± 418</td>
</tr>
</tbody>
</table>

*Mice were sensitized and challenged as described in Materials and Methods. Serum levels of Ig were assessed 48 h after the last challenge. Mean values ± SEM are given: sIL-13Rx2-Fc treatment with soluble IL-13Rx2 fusion protein. p < 0.05, compared to the nonsensitized group.
IL-13 ROLE IN ESTABLISHED ALLERGIC AIRWAY DISEASE

STAT6 activation, which is critical for the development of AHR and airway inflammation (25). Despite these similarities, IL-4 and IL-13 have differences in their function in allergen-induced airway disease. IL-4 is critical for Th2 cell induction (35), especially during the sensitization phase (20–22), but it has been shown that in the absence of IL-4, AHR, lung eosinophilia and mucus production can still be induced (36). In contrast, IL-13 appears to be critical during the airway challenge phase, at least for the development of AHR (6, 7). IL-13-deficient mice develop airway inflammation, without developing AHR, whereas mice lacking both IL-4 and IL-13 neither develop airway inflammation nor AHR (6). It has been proposed that IL-4 is required for the persistence of Th2 cells in vivo (37) and the presence of IL-4 might be more important in chronic airway disease. In the present study, following the secondary challenge protocol, sIL-13Ra2-Fc was effective in preventing the development of AHR after allergen provocation, without significantly altering IL-4 levels in BAL fluid. In the same model, neutralization of IL-4 using a soluble IL-4R (which targets only IL-4 signaling), had little effect on development of AHR or airway inflammation (38), implying that IL-13, more than IL-4, is important in the development of AHR in already established allergic airway disease.

A direct effect of IL-13 on airway epithelial cells has been proposed with induction of MUC-5 gene expression. IL-13 may also induce neutrophil recruitment (4) and activation (15) in the airways. Interestingly, in allergic airway disease neutrophil recruitment may be directly associated with goblet cell metaplasia at pretreatment with an IL-8-blocking Ab prevented both IL-13-induced neutrophil recruitment and mucin production (15). In the present study, inhibition of IL-13 led to a small reduction of neutrophil numbers in BAL fluid, but whether this neutrophil influx plays a role in the development of AHR remains to be elucidated. Under some conditions, mucus production may not be directly associated with alterations in airway function (24).

In humans, the measurement of early and late phase airway responses following allergen challenge is often used to assess the effectiveness of treatment interventions (39, 40). Murine models of allergic airway disease demonstrate an early and late airway response to inhaled allergen. Previous studies have shown that the early response following allergen challenge is dependent on the presence of allergen-specific IgG (41) and can be abolished using β2-adrenoceptor antagonists or cromoglycates (29). In the present study, neutralization of IL-13 had no effect on the early airway response. This is not surprising as neutralization of IL-13 did not have any effect on serum levels of allergen-specific Abs. It has been shown that the LPR is associated with an increase in IL-5 levels in BAL fluid and tissue eosinophilia and can be suppressed by treatment with either anti-IL-5, cromoglycates, or hydrocortisone (29). We found increased levels of IL-13 in BAL fluid at the time of the late airway response and treatment with the sIL-13Ra2-Fc completely abolished the development of the LPR. Interestingly, IL-13 neutralization showed no effect on tissue eosinophil inflammation at this time point, supporting the possibility of a direct effect of IL-13 on airway function, independent of airway inflammation.

Secondary exposure to a single provocative OVA aerosol in sensitized mice elicited airway changes similar to those obtained after a series of primary challenges, confirming previous results (30). We previously showed a differential regulation of AHR in the central and peripheral airways monitoring these two parameters of airway function (30, 33). It has been proposed that changes in dynamic compliance reflect narrowing of peripheral airways, whereas changes in airway resistance represent airflow obstruction of central airways (42–44). In previous studies, we have shown that eosinophilic inflammation might relate to changes in the central airways while changes in the epithelium of peripheral airways, including mucus production, may relate to changes in dynamic compliance (30, 33). Interestingly, and different from anti-IL-5 and anti-very late Ag-4 treatment, sIL-13Ra2-Fc is capable of inhibiting both changes in airway resistance and dynamic compliance, suggesting an effect on central and peripheral airway function following secondary challenge.

In summary, our results show that IL-13 is essential to the development of a LPR following airway challenge of mice with established allergic disease. In addition, we demonstrate that blockade of IL-13 can prevent changes in central and peripheral airway physiology following secondary allergen challenge. The data suggest that targeting IL-13 may be important in the treatment of chronic allergic asthma.

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References


