Administration of Pentoxifylline During Allergen Sensitization Dissociates Pulmonary Allergic Inflammation from Airway Hyperresponsiveness

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Administration of Pentoxifylline During Allergen Sensitization Dissociates Pulmonary Allergic Inflammation from Airway Hyperresponsiveness

Carolyn M. Fleming,* Hongzhen He,† Alex Ciota,‡ David Perkins,§ and Patricia W. Finn²†

Asthma, a chronic inflammatory disease characterized by intermittent, reversible airflow obstruction and airway hyperresponsiveness (AHR), is classically characterized by an excess of Th2 cytokines (IL-4, IL-13) and depletion of Th1 cytokines (IFN-γ, IL-12). Recent studies indicating an important role for Th1 immunity in the development of AHR with allergic inflammation suggest that Th1/Th2 balance may be important in determining the association of AHR with allergic inflammation. We hypothesized that administration of pentoxifylline (PTX), a phosphodiesterase inhibitor known to inhibit Th1 cytokine production, during allergen (OVA) sensitization and challenge would lead to attenuation of AHR in a murine model of allergic pulmonary inflammation. We found that PTX treatment led to attenuation of AHR when administered at the time of allergen sensitization without affecting other hallmarks of pulmonary allergic inflammation. Attenuation of AHR with PTX treatment was found in the presence of elevated bronchoalveolar lavage fluid levels of the Th2 cytokine IL-13 and decreased levels of the Th1 cytokine IFN-γ. PTX treatment during allergen sensitization leads to a divergence of AHR and pulmonary inflammation following allergen challenge. The Journal of Immunology, 2001, 167: 1703–1711.

Asthma is a chronic inflammatory disease characterized by intermittent and reversible airflow obstruction and airway hyperresponsiveness (AHR). In both asthma and allergic inflammation, a relative excess of Th2 cytokines (IL-4, IL-13) and depletion of Th1 cytokines (IFN-γ, IL-12) has been proposed to mediate disease (1–5). However, recent studies suggest that Th2 and Th1 cytokines may not have such simple dichotomous functions in allergic inflammation and AHR. Anti-inflammatory properties for IL-13 have been demonstrated in a model of pulmonary allergic inflammation in the guinea pig (6), and attenuation of AHR has been correlated with inhibition of IFN-γ in a murine model of allergic pulmonary inflammation (7). Furthermore, increased bronchoalveolar lavage (BAL) fluid expression of IFN-γ, although to a lesser degree than Th2 cytokines, is seen in animal models of allergic inflammation and AHR and in patients with asthma (8–10). These findings underscore a possible role for both Th1 and Th2 cytokines in the development of AHR and allergic inflammation.

A role for Th1/Th2 balance in AHR and allergic inflammation is suggested by a recent study focusing on NF-κB (11), a transcription factor that regulates the gene expression of many inflammatory proteins including mediators important in asthma (12–17). Transgenic mice that express a dominant mutant form of IκB (IκBΔN), an in vivo inhibitor of NF-κB, exhibit inhibition of Th1 immunity, as evidenced by attenuation of delayed-type hypersensitivity. However, in a Th2-characterized model of OVA-induced pulmonary inflammation, IκBΔN mice exhibit decreased AHR despite typical signs of pulmonary allergic inflammation compared with wild-type mice (11). These findings suggest that a Th1 component of the immune response to allergen may be required for the development of AHR in association with allergic inflammation (11). Thus, pharmacologically altering the Th1/Th2 balance during an immune response could lead to divergence of AHR and allergic inflammation.

To address this possibility, we analyzed the effects of altering Th1/Th2 cytokine balance on pulmonary allergic inflammation using pentoxifylline (PTX), a methylxanthine derivative, which has been shown, both in vitro and in vivo, to inhibit Th1 cytokine production (IFN-γ, IL-2, TNF-α, IL-1, IL-2) with either no effect or a stimulatory effect on Th2 cytokines (IL-4, IL-10, IL-13) (18–30). In animal models, PTX also inhibits development of Th1-mediated disorders such as autoimmune diabetes mellitus and experimental allergic encephalomyelitis (20, 25, 29, 30). In addition, PTX has been shown to inhibit the activation of NF-κB by unclear mechanisms (31–37).

We hypothesized that administration of PTX during allergen sensitization and challenge would lead to attenuation of AHR in a murine model of allergic pulmonary inflammation. We found that PTX treatment led to attenuation of AHR when administered at the time of allergen sensitization, without affecting other aspects of typical pulmonary allergic inflammation, including NF-κB activation, following allergen challenge. Previous investigations have reported separation of allergic inflammation from AHR (38). However, while these studies have focused primarily on interventions at the time of allergen sensitization, our findings suggest that pharmacologically altering the Th1/Th2 balance may be important in determining the association of AHR with allergic inflammation.
challenge, our results suggest that the pathways leading to alergic inflammation and AHR can diverge at the time of allergen sensitization. The attenuation of AHR after PTX treatment at the time of allergen sensitization was associated with elevated levels of the Th2 cytokine IL-13 and relatively decreased levels of the Th1 cytokine IFN-γ. This suggests that a component of a Th1 immune response may be critical for development of AHR.

Materials and Methods
Protocol for allergen sensitization and challenge
Mice were sensitized and challenged with OVA as previously described (39). Briefly, female BALB/cjy mice, 4 wk old (The Jackson Laboratory, Bar Harbor, ME) were sensitized by i.p. injection of 10 μg of chicken OVA and 2 mg of Al(OH)₃ (OVA/Alum) on days 0 and 7. On days 14–20, mice received aerosolized OVA challenge with 6% OVA for 25 min/day. OVA was dissolved in 0.5× PBS (saline). Control mice received 2 mg of alum in saline, i.e., on days 0 and 7 and were nebulized with saline on days 14–20. An ultrasonic nebulizer (model 5000; DeVilbiss, Somerset, PA) was dissolved in 0.5

In vivo administration of PTX
PTX (100 mg/kg) was administered by i.p. injection 5 min before allergen sensitization on days 0 and 7 and/or 5 min before aerosolized allergen challenge on days 14–20. Mice that did not receive PTX received an equal volume (0.2 ml) of saline. Six groups of mice were analyzed as defined in Fig. 1. Groups 1–4 were sensitized and challenged with OVA. Group 1 was treated with saline before sensitization and challenge. Group 2 was treated with saline before sensitization and with PTX before challenge. Group 3 was treated with PTX before sensitization and with saline before challenge. Group 4 was treated with PTX before both sensitization and challenge. Groups 5 and 6 were sensitized and challenged with saline. Group 5 received treatment with saline before sensitization and challenge. Group 6 received treatment with saline before sensitization and with PTX before challenge.

Determination of AHR
Twenty-four hours after the last aerosol challenge, AHR was assessed using whole-body plethysmography (Buxco Electronics, Birmingham, U.K.) (40). Mice were placed in individual chambers. Increasing doses of methacholine (0–100 mg/ml) were nebulized into the chambers via an inlet for 45 s. Readings were averaged over 8 min from the beginning of the nebulization. The whole-body plethysmography system measures changes in box pressure during expiration and inspiration, peak expiratory and peak inspiratory pressures (PEP andPIP, respectively), inspiratory time (Ti), expiratory time (Te), and a relaxation time (Tr = time of the pressure decay to 36% of total box pressure during expiration), and generates a value called enhanced pause (PEF = PEEP × Ti/(Te – Tr)) which directly correlates with airway resistance (40).

BAL and histologic analysis
Following measurement of AHR, mice were anesthetized with i.p. Nembutal (0.35 ml of 25% solution) and sacrificed. Three mice from each group underwent BAL as previously described (39). BAL cells were pelleted and supernatant was stored at –70°C until analyzed. For analysis of cytokine production, cell culture supernatants were obtained following 60 h of stimulation with Con A. For all assays, cells were incubated for 5 min in the absence or presence of PTX (100 μg/ml) before addition of Con A (10 μg/ml).

BAL and cell activation assay cytokine ELISAs
BAL and cell culture supernatant cytokine concentrations were measured by ELISA according to the manufacturer’s specifications (R&D Systems, Minneapolis, MN). To optimize BAL cytokine detection, BAL fluid samples were combined and concentrated using a speed vacuum. Samples were resuspended in assay diluent (R&D Systems); 330 μl per combined sample of original BAL fluid. BAL fluid samples and cell culture supernatants were aliquoted into microplates precoated with Ab to IL-13 or IFN-γ. After a 2-h room temperature incubation, mouse cytokine conjugate (IL-13 or IFN-γ) was added followed by incubation for 2 h at room temperature. Substrate solution was added and OD at 450 nm was measured. Plates were washed in between each step. Cytokine levels were determined by comparison with known standards. For BAL fluid samples, cytokine levels measured were corrected for original volume of BAL fluid before concentration (measured concentration (picograms per milliliter) × (330 per original volume)).

Statistical analysis
Data analysis was performed using Sigma Stat. Parametric data were analyzed with the Tukey-Kramer test and nonparametric data by the Wilcoxon/Kruskal-Wallis rank sum test. Data are reported as means ± SE. Statistical significance was defined by p < 0.05.

Results
PTX treatment before allergen sensitization attenuates AHR
To determine whether PTX treatment before allergen sensitization and/or challenge influenced physiologic outcomes associated with OVA-induced pulmonary allergic inflammation, we measured AHR. Six groups of mice were analyzed. The agent with which each group was sensitized and challenged (OVA or saline) and the time point, before sensitization and/or challenge, that each group received treatment with PTX or saline are defined in Fig. 1.

OVA-sensitized and -challenged mice that received no treatment with PTX (group 1) had significantly greater AHR than saline control mice (groups 5 and 6, *p < 0.02). OVA-sensitized
and challenged mice that received PTX treatment before allergen challenge (group 2) also developed AHR significantly greater than saline control mice (groups 5 and 6, \( p, p < 0.04 \); Fig. 1A). In contrast, development of AHR was attenuated in mice that received PTX treatment at the time of allergen sensitization (groups 3 and 4, respectively). AHR was diminished in these PTX treatment groups to a level not significantly different from that of saline control mice (Fig. 1A). In addition, AHR in OVA-treated mice that received PTX at the time of allergen sensitization only (group 3) was significantly less than that of OVA-treated mice that did not

### Sensitization and Challenge

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**FIGURE 1.** PTX treatment before allergen sensitization attenuates development of AHR but does not affect development of BAL fluid eosinophilia or serum IgE levels. Mice in groups 1–4 were sensitized with OVA/alum and challenged with aerosolized 6% OVA. Saline control mice (groups 5 and 6) were sensitized with saline/alum and challenged with aerosolized saline. The agent with which each group was sensitized and challenged (saline or OVA) as well as the agent with which each group was treated (saline or PTX (100 mg/kg)) at the time of sensitization and/or challenge is summarized in the table. All results shown are representative of two independent experiments. A. PTX treatment before allergen sensitization attenuates development of AHR. Penh, determined by whole body plethysmography, correlates directly with airway resistance and was measured in response to increasing doses of methacholine. Penh following 100 mg/ml methacholine is shown. *, \( p < 0.04 \) compared with saline controls (groups 5 and 6). #, \( p < 0.05 \) compared with group 3. Data are expressed as average proportion of baseline Penh \( \pm \) SD (\( n = 5 \) mice per group). B. PTX treatment before allergen sensitization and/or challenge does not affect development of BAL fluid eosinophilia. After measurement of AHR, mice were sacrificed by cardiac puncture and BAL was performed by instillation of 1 ml of BAL fluid followed by lavage three times. Cells were counted and slides prepared by Cytospin. Slides were fixed and stained using Diff-Quik. For differential cell counts, 200 cells were counted by an investigator blinded to sample identity. The percentage of alveolar macrophages, neutrophils, eosinophils, and lymphocytes found in the different groups is shown. All OVA-sensitized and -challenged mice (groups 1–4) had increased BAL eosinophilia compared with saline control mice (groups 5 and 6). Data are presented as mean \( \pm \) SD (\( n = 3 \) mice per group). *, \( p < 0.05 \) compared with groups 5 and 6. C. PTX treatment before allergen sensitization and/or challenge does not affect serum IgE levels. Total serum IgE levels were measured by ELISA. All OVA-sensitized and -challenged mice (groups 1–4) had significantly greater serum IgE levels than saline control mice (groups 5 and 6). Data are expressed as mean \( \pm \) SD (\( n = 5 \) mice per group). *, \( p < 0.05 \) compared with groups 5 and 6.
PTX treatment does not affect pulmonary allergic inflammation

BAL fluid cells, serum IgE levels, and lung histology were assessed to determine the effects of PTX treatment on allergic inflammation. BAL fluid eosinophilia was significantly increased in all OVA-sensitized and -challenged mice (groups 1–4) compared with saline control mice (groups 5 and 6) in which eosinophils were not detected (Fig. 1B). There were no significant differences in quantitative or qualitative amounts of BAL cell types between any of the OVA-sensitized and -challenged mice (groups 1–4).

All OVA-sensitized and -challenged mice (groups 1–4) had significantly elevated serum IgE levels compared with saline control mice (groups 5 and 6). Serum IgE levels for groups 1–4 were 208 ± 39, 236 ± 88, 250 ± 84, and 231 ± 83 ng/ml, respectively, vs 35 ± 11 and 88 ± 60 ng/ml for groups 5 and 6, respectively (p < 0.02, Fig. 1C). There were no significant differences in serum IgE concentrations between any of the OVA-sensitized and -challenged mice (groups 1–4).

Histologic sections of lungs from all groups were examined. Results for groups 1, 3, and 5 are shown (Fig. 2). Lungs from OVA-sensitized and -challenged mice that received no treatment with PTX (group 1) developed inflammatory changes typical of this model: peribronchial and perivascular infiltrates composed of eosinophils, neutrophils, and lymphocytes (39, 42) (Fig. 2A). Similar inflammatory changes were noted in all other groups of OVA-sensitized and -challenged mice (groups 2–4) (results for group 3 shown, Fig. 2B). Both groups of saline control mice (groups 5 and 6) showed no evidence of abnormal histology (results for group 5 shown, Fig. 2C).

PTX does not alter NF-κB activation in thoracic lymphocytes following in vivo allergen challenge, but does inhibit NF-κB activation in naive BALB/c spleen cells stimulated in vitro

We and others have previously reported a critical role for NF-κB in OVA-induced allergic pulmonary inflammation (44, 46) and AHR (44). We investigated whether alterations in NF-κB activation could be important to the attenuation of AHR seen in mice treated with PTX before OVA sensitization (groups 3 and 4, respectively). Increased activation of NF-κB in nuclear extracts from thoracic lymphocytes, as detected by EMSA analysis, was found in all OVA-sensitized and -challenged mice (groups 1–4) compared with saline control mice (groups 5 and 6; results not shown). Competition assays using homologous unlabeled NF-κB oligonucleotide and a non-NF-κB oligonucleotide (AP-1) revealed specific binding to the NF-κB probe (Fig. 3). In our in vivo model, PTX treatment at the time of allergen sensitization and/or challenge did not result in inhibition of NF-κB activation following allergen challenge.

To determine whether PTX could have altered NF-κB function at the time of initial allergen challenge, NF-κB activation was assessed in naive BALB/c spleen cells stimulated in vitro in the absence and presence of PTX. These studies demonstrated activation of NF-κB in naive spleen cells following stimulation with Con A. Treatment of cells with PTX, at concentrations achieved in the serum of mice at the dose given, inhibited this activation by 40%, as determined by densitometry analysis (Fig. 4).

PTX treatment before allergen sensitization alters BAL cytokine response

To determine whether an alteration in the phenotype of the local cytokine response might account for the effects of PTX on AHR, BAL cytokines were analyzed. BAL supernatant was assayed for IL-13 and IFN-γ, cytokines representative of Th2 or Th1 response, respectively. All OVA-sensitized and -challenged mice (groups 1–4) had elevated levels of IL-13 compared with saline control mice (groups 5 and 6; groups 1–4 = 33.6, 35.1, 78.6, and 46.9 pg/ml, respectively, vs groups 5 and 6 = 4 and 0.5 pg/ml, respectively) (Fig. 5A).
Levels of IFN-γ were highest in the OVA-sensitized and -challenged mice that had significant AHR (groups 1 and 2, 1.9, and 2.3 pg/ml, respectively; Fig. 5B). IFN-γ levels in OVA-sensitized and -challenged mice that did not have significant AHR (groups 3 and 4, 0.4, and 0.8 pg/ml, respectively) and in saline control mice (groups 5 and 6, 0.2 and 0.3 pg/ml, respectively) were below the limits of reliable detection (Fig. 5B).

To determine the effects of PTX on Th1 vs Th2 cytokine production, at levels of PTX achieved in the serum of mice at the dose given, IFN-γ and IL-13 concentrations were measured in cell culture supernatants following in vitro stimulation of spleen cells from naive BALB/c mice, with Con A (10 μg/ml) for 60 h, in the absence and presence of PTX (100 μg/ml) (Fig. 6). Con A stimulation led to increases in both IFN-γ and IL-13 expression (Fig. 6). In the presence of PTX (100 μg/ml), IFN-γ production, but not IL-13 production, was significantly inhibited (p < 0.05; Fig. 6).

**Discussion**

Treatment with PTX before allergen sensitization attenuates the development of AHR without significantly affecting the development of pulmonary allergic inflammation (pathological changes, BAL fluid eosinophilia, and elevated serum IgE). Although differences in NF-κB activation following OVA challenge were not associated with alterations in AHR, alterations in the local cytokine milieu were detected, suggesting potential mechanisms for attenuation of AHR. Analysis of BAL cytokine expression revealed increased levels of IL-13 in all OVA-sensitized and -challenged mice compared with saline control mice. However, IFN-γ levels were highest in OVA-sensitized and -challenged mice that had AHR while IFN-γ levels in OVA-sensitized and -challenged mice that had attenuation of AHR were similar to those of saline control mice.

**FIGURE 4.** PTX inhibits NF-κB activation in BALB/c spleen cells stimulated in vitro. Spleen cells (5 ml/well at 10^6 cells/ml) isolated from 4- to 6-wk-old female BALB/c mice were stimulated in vitro with Con A (10 μg/ml) for 45 min in the presence/absence of PTX (100 μg/ml). Nuclear extracts were isolated and EMSA was conducted as described in Materials and Methods. Activation of NF-κB was detected following Con A stimulation. This activation was inhibited by treatment of cells with PTX (100 μg/ml). The density of NF-κB bands from stimulated cells and/or cells treated with PTX (lanes b–d) relative to untreated cells (lane a) (relative density) is noted at the bottom of each lane. Arrow, NF-κB bands.

Although PTX, a methylxanthine derivative and phosphodiesterase (PDE), could potentially exert bronchodilator effects, our data indicate that PTX is not acting as a bronchodilator. PTX treatment before aerosolized OVA challenge did not affect development of pulmonary allergic inflammation or AHR. These data are consistent with PTX’s in vivo half-life of only a few minutes when administered by the i.p. route in mice (47, 48).

The PDEs are a family of isozymes with at least seven known members. PDEs 3 and 4 are the most abundant PDEs in immune cells (49). Although some PDE inhibitors, in particular PDE 4 isozyme-specific inhibitors, have been reported to attenuate allergic inflammation and AHR when given at the time of allergen challenge in animal models (50–60), the data for nonspecific PDE inhibitors are more equivocal (51, 54, 61, 62).

In our study, the lack of an inhibitory effect for PTX on inflammation and AHR when administered at the time of allergen challenge could be due to a number of factors. Most studies with PDE inhibitors have analyzed responses in rabbits, rats, and guinea pigs. There are minimal data in mice; therefore, species variability could be a factor. In addition, PTX nonspecifically inhibits most PDE isozymes. The activity of different PDE isozymes can be associated with different and sometimes opposing functions (49). Thus, the relative ability of PTX to inhibit different PDE family members may account for its lack of inhibition of inflammation and AHR when administered to allergen-sensitized mice before allergen challenge.

The half-life of PTX is very short (minutes) with the likely possibility that differences in drug dose, route of administration, and frequency of administration in our study as compared...
with other studies accounts for the lack of attenuation of allergic inflammation and AHR by PTX treatment at the time of allergen challenge.

In contrast to its lack of effect when administered at the time of allergen challenge, PTX treatment at the time of allergen sensitization decreased AHR without significantly effecting allergic inflammation in OVA-sensitized and -challenged mice. This finding suggests an early divergence of the allergic immune response. Since the cytokine milieu at the time of initial allergen sensitization can determine the phenotype of the immune response to an allergen, alterations in this milieu by PTX could account for its effect on AHR. Although PDE 4-specific inhibitors have been shown to inhibit Th2 cytokine expression by Th2 cells and by PBMCs from atopic individuals, they have also been shown to inhibit Th1 cytokine production by Th1 cells in vitro and in animal models of Th1-mediated disease in vivo(63–72). For the nonspecific PDE inhibitor PTX, data indicate that both in vitro and in vivo, in naive and differentiated cells, PTX inhibits Th1 cytokine production and has either no effect or an enhancing effect on Th2 cytokine production (18–22, 24–30).

**FIGURE 5.** PTX alters local cytokine expression. Levels of BAL fluid IL-13 and IFN-γ were measured by ELISA (R&D Systems). A. IL-13 levels were elevated in all OVA-sensitized and -challenged mice (groups 1–4) compared with saline control mice (groups 5 and 6). B. BAL fluid IFN-γ levels (pg/ml) were highest in OVA-sensitized and -challenged mice with AHR, i.e., those that received no treatment with PTX (group 1) or that received treatment with PTX before allergen challenge only (group 2). BAL fluid IFN-γ concentrations in allergen-sensitized and -challenged mice that received PTX before sensitization and had attenuation of AHR (groups 3 and 4) were similar to IFN-γ levels in saline control mice (groups 5 and 6). The results shown are representative of two separate experiments.

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Given the ability of PTX to inhibit Th1 cytokine production (18–30), we speculate that a Th1 component of the immune response may be critical for development of AHR. This possibility is supported by findings in mice that transgenically express a transdominant mutant form of the in vivo NF-κB inhibitor, IκB (IκBΔN mice) (11). IκBΔN mice exhibit inhibition of Th1 immunity, as evidenced by attenuation of delayed-type hypersensitivity. However, in a Th2-characterized model of OVA-induced pulmonary inflammation, IκBΔN mice exhibit decreased AHR when compared with wild-type mice despite typical signs of pulmonary allergic inflammation (11). Thus, attenuation of AHR in IκBΔN mice may be due to inhibition of a Th1 component of the allergic immune response. Interestingly, PTX has been shown, under certain circumstances, to inhibit NF-κB activation (31–37). Given the findings in IκBΔN mice, inhibition of NF-κB may be one explanation for the ability of PTX to inhibit Th1 immune responses.

We have previously shown activation of NF-κB in thoracic lymphocytes from OVA-sensitized mice following allergen challenge (44). In addition, we have shown that absence of the NF-κB family member c-Rel (c-Rel knockout mice) prevents the development of allergic pulmonary inflammation and AHR following allergen sensitization and challenge (44). In the current study, mice that received PTX treatment before allergen sensitization developed a phenotype similar to that of IκBΔN mice. However, at the time point analyzed, all groups of OVA-sensitized and -challenged mice had increased NF-κB activation in thoracic lymphocytes. Thus, differential regulation of NF-κB activation following allergen challenge does not account for the differences in AHR. However, PTX did inhibit NF-κB activation in naive BALB/c spleen cells stimulated in vitro (Fig. 4), which supports the possibility that PTX may have had effects on NF-κB activation at the time of allergen sensitization.

An important role for Th2 cytokines in the development of allergic inflammation and AHR in murine models has previously been shown (73–83). To determine whether an alteration in the local cytokine milieu might account for the effects of PTX on AHR, BAL fluid Th1/Th2 cytokine balance was assessed. For Th2, IL-13 was chosen because of recent reports indicating its importance in the evolution of allergic inflammation and AHR (2, 3, 5). For Th1, IFN-γ was assessed because of its well-characterized role as a Th1 cytokine (84). BAL fluid cytokine analysis was able to differentiate the groups based on allergic inflammation and AHR. IL-13 levels were elevated in all OVA-sensitized and -challenged mice compared with saline control mice, irrespective of treatment with PTX. Furthermore, the BAL fluid levels of IL-13 in OVA-sensitized and -challenged mice that had AHR (no PTX at sensitization) and those that had attenuation of AHR (PTX treatment at sensitization) were similar. In contrast, IFN-γ levels were elevated only in OVA-sensitized and -challenged mice that had AHR (no PTX treatment at sensitization). OVA-sensitized and -challenged mice that had attenuation of AHR (PTX treatment at sensitization) had BAL fluid IFN-γ levels similar to those of saline control mice.

The similar levels of IL-13 in BAL fluid from OVA-sensitized and -challenged mice with and without AHR is in contrast to reports that increased IL-13 levels may promote allergic inflammation and AHR and suggests that the role of IL-13 in allergic inflammation may be multifactorial. IL-13 has had both pro- and anti-inflammatory properties described. Increased levels of IL-13 are found in the BAL fluid of atopic asthmatics following allergen challenge (85–87), although its role is not clearly defined. Attenuation of allergic inflammation and AHR in OVA-sensitized mice is seen with inhibition of IL-13 during allergen challenge and direct induction of an asthma-like phenotype is found in naive mice with application of IL-13 to the airways (2, 3). In addition, naive IL-13-transgenic mice chronically exposed to high levels of IL-13 (2 ng/ml in BAL fluid) develop AHR and airway pathology similar to allergic inflammation (5). Although these findings suggest that IL-13 is important in the development of both allergic inflammation and AHR, an anti-inflammatory role for IL-13 in the lung has also been reported. Intratracheal administration of human recombinant IL-13 (1–100 ng/animal) before allergen challenge has been shown to reduce BAL fluid eosinophilia and eosinophil-stimulating activity in OVA-sensitized guinea pigs (6).

These reports of both inflammatory and anti-inflammatory properties for IL-13 indicate that the physiologic actions of IL-13 are likely quite complex. IL-13 may exhibit concentration-dependent physiologic with anti-inflammatory or inflammatory functions in different concentration ranges. In studies demonstrating an asthma-like phenotype with direct intratracheal application of IL-13, the concentrations of intratracheal IL-13 applied were greater, on a per weight basis, than in the study that demonstrated an anti-inflammatory role for IL-13 in OVA-induced pulmonary inflammation (2, 3, 6) and were ~1000-fold higher than the concentration of IL-13 we detected in the BAL fluid of OVA-sensitized and -challenged mice.

Alternatively, the physiologic actions of IL-13 may depend on other mediators present at its site of action. The findings of relatively higher levels of IFN-γ in OVA-sensitized and -challenged mice with AHR suggest that rather than depending on any one mediator, AHR may be induced when the right combination of mediators is present, including both Th1 and Th2 cytokines. Our data indicate that elevation of IL-13 during an in vivo response to allergen cannot by itself account for the AHR detected in OVA-exposed mice.

The finding of elevated IL-13, but decreased IFN-γ, in BAL fluid of OVA-sensitized and -challenged mice with allergic inflammation but attenuated AHR supports the possibility that, in addition to the established role of Th2 immunity, Th1 immunity may also play an important role in the development of AHR in association with allergic inflammation. In concert with our in vitro results showing preferential inhibition by PTX of Th1 relative to Th2 cytokine expression, these in vivo data support the possibility that PTX treatment at sensitization may have produced long-term inhibition of a Th1 component of the immune response required for development of AHR.

Administration of PTX at the time of allergen sensitization leads to a divergence of AHR and allergic inflammation following allergen challenge. Separation of allergic inflammation from AHR has been previously reported (38, 88–90). However, many of these
studies have focused primarily on interventions at the time of allergen challenge. The results presented here suggest that the evolution of pulmonary allergic inflammation and AHR can diverge early in the immune response, at the time of allergen sensitization. Opportunities to analyze divergence between the induction of al-lergic pulmonary inflammation and AHR may provide clues to dissect the early signals that lead to downstream events of physiologic and pathologic change.

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References


