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IL-13 Fusion Cytotoxin Ameliorates Chronic Fungal-Induced Allergic Airway Disease in Mice

Kate Blease,* Claudia Jakubzick,* Jane M. Schuh,* Bharat H. Joshi,† Raj K. Puri,† Cory M. Hogaboam2*

IL-13 has emerged as a major contributor to allergic and asthmatic responses, and as such it represents an attractive target in these diseases. In this study, IL-13-responsive cells in the lung were targeted via the intranasal administration of IL-13-PE38QQR (IL-13-PE), comprised of human IL-13 and a derivative of Pseudomonas exotoxin, to Aspergillus fumigatus-sensitized mice challenged with A. fumigatus spores, or conidia. Mice received 50, 100, or 200 ng of IL-13-PE or diluent alone (i.e., control group) on alternate days from day 14 to day 28 after the conidia challenge. The control group of mice exhibited significant airway hyperreactivity, goblet cell hyperplasia, and peribronchial fibrosis at day 28 after conidia. Although the two lower doses of IL-13-PE had limited therapeutic effects in mice with fungal-induced allergic airway disease, the highest dose of IL-13-PE tested significantly reduced all features of allergic airway disease compared with the control group. Whole lung mRNA expression of IL-4Rα and IL-13Rα1 was markedly reduced, whereas bronchoalveolar lavage and whole lung levels of IFN-γ were significantly elevated in mice treated with 200 ng of IL-13-PE compared with the control group. This study demonstrates that a therapy designed to target IL-13-responsive cells in the lung ameliorates established fungal-induced allergic airway disease in mice. The Journal of Immunology, 2001, 167: 6583–6592.

Considerable clinical (1–4) and experimental (5–7) evidence illustrates that asthma and atopy are characterized by the prominent expression of type 2 cytokines such as IL-4, IL-5, and IL-13 and a relative paucity of counterregulatory Th1 cytokines such as IFN-γ (8, 9). Consequently, this paradigm of cytokine imbalance during allergic airway disease has spawned numerous therapeutic strategies directed at the attenuation of the Th2 response and/or the enhancement of the Th1 response (10–12). Therapeutic strategies include regulation of the activation of signal transducers and activators of transcription and NF-κB, as well as Abs and soluble receptors directed against IgE, IL-4, IL-5, and the unmethylated CpG oligodeoxynucleotides (13, 14). Experimental studies have also shown that the systemic administration of anti-IL-4 (15), anti-IL-13 Ab (5, 16), or the IL-13 inhibitor, soluble IL-13Rα2-Fc (6), successfully abolishes the airway hyperresponsiveness and remodeling associated with allergic airway disease. However, one concern regarding all of these strategies is that simply targeting a single transcription or cytokine pathway may not be sufficient to effectively eradicate asthmatic or allergic symptoms in all patients (17), particularly in light of recent experimental evidence that IL-4 and IL-13 appear to have redundant proinflammatory roles during aeroallergen challenge (18).

IL-4 shares receptor components and signaling pathways with IL-13, including the α-chain of the IL-4R and IL-13Rα1 (19–21). However, IL-13 can also selectively bind to specific IL-13 receptors, including IL-13Rα1 and IL-13Rα2 (22, 23). Cells that respond to IL-13 are also excellent sources of the same cytokine. These cells include activated Th2 cells (2, 24), B cells (24), mast cells (25, 26), basophils (2), and alveolar macrophages (3, 27). Given that the number of IL-4- and IL-13-producing cells are markedly increased during the course of airway inflammation associated with asthma and allergy (2), it is conceivable that adequate immunoneutralization of IL-4 or IL-13 may be difficult to maintain in these chronic diseases. An alternative strategy for targeting IL-13R-positive cells during asthma and allergy may involve the use of a fusion protein comprising IL-13 and a mutated form of Pseudomonas exotoxin, IL-13-PE38QQR (IL-13-PE) (3). This fusion protein has been used to selectively target and eradicate solid tumor cells with endogenous (28) and induced (29) IL-13R expression. Importantly, mice did not exhibit any adverse effects from the prolonged systemic in vivo administration of IL-13-PE during tumor treatment (28). In the present study, we investigated the dose-dependent therapeutic effects of IL-13-PE in a model of chronic fungal-induced allergic airway disease that is characterized by chronic airway hyperreactivity, goblet cell hyperplasia, peribronchial fibrosis, and elevated pulmonary levels of IL-4 and IL-13 (7). Our previous studies have shown that development of these pulmonary features is IL-13 dependent but partly IL-4 dependent (16). IL-13-PE was administered via the intranasal route to mice with established allergic airway disease, and the findings presented in this paper show that this therapy effectively ameliorated all the chronic features of this model.

Materials and Methods

Murine model of chronic fungal-induced allergic airway disease
Specific pathogen-free (SPF) female CBA/J mice were purchased from The Jackson Laboratory (Bar Harbor, ME) and were maintained in a SPF facility for the duration of this study. Prior approval for mouse usage was

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2 Abbreviations used in this paper: IL-13-PE, IL-13-PE38QQR; SPF, specific pathogen-free; BAL, bronchoalveolar lavage; s, soluble; PAS, periodic acid Schiff.

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IL-13-PE therapy during fungal-induced allergic airway disease

IL-13-PE is a recombinant chimeric fusion protein comprising human IL-13 and a mutated Pseudomonas exotoxin, and it has been previously used to target IL-13R-expressing tumor cells (28, 29). These previous studies have demonstrated that IL-13-PE is cytotoxic when incorporated into cells expressing IL-13Rα1 and IL-13Rα2. Because this chimeric molecule had not been previously used in a model of allergic airway disease, we conducted a pilot study to determine a range of doses of IL-13-PE that were appropriate for in vivo testing. This preliminary study showed that 50 ng of IL-13-PE in 1 ml of tissue culture medium had a minor effect on the survival of cultured pulmonary fibroblasts, whereas 200 ng/ml of this chimeric protein markedly attenuated fibroblast survival by >60% (data not shown). Based on these preliminary observations, groups of five to ten A. fumigatus-sensitized mice received 50, 100, or 200 ng of IL-13-PE dissolved in 20 μl of PBS containing 0.25% human serum albumin (HSA-PBS or diluent) via an intranasal bolus. IL-13R-positive cells were targeted with IL-13-PE from day 14 to day 28 after the conidia challenge to coincide with marked increases in IL-13 and protein levels in this model (16). In addition, because A. fumigatus conidia are typically absent in the airways of A. fumigatus-sensitized mice at day 14 after an intratracheal conidia challenge (7), this time was selected as the starting point for IL-13-PE treatment to avoid any confounding effects of IL-13-PE on the innate immune response required for the clearance of conidia from allergic mice (30, 31). Day 14 after conidia also corresponds with peak bronchial accumulations of eosinophils and CD4+ T cell, significant airway hyperresponsiveness to methacholine, goblet cell hyperplasia, and subepithelial collagen deposition in A. fumigatus-sensitized mice (7). Another group of 10 A. fumigatus-sensitized mice received 20 μl of diluent via the same route beginning at day 14 and concluding on day 28 after conidia.

Measurement of bronchial hyperresponsiveness

At day 28 after the A. fumigatus conidia challenge, bronchial hyperresponsiveness in IL-13-PE-treated and control mice was measured in a Bucox plethysmograph (Bucox Electronics, Troy, NY) as previously described (7). Sodium pentobarbital (0.04 mg/g of mouse body weight; Butler, Columbus, OH) was used to anesthetize each mouse before its intubation for ventilation with a Harvard pump ventilator (Harvard Apparatus, Renno, NV). The following ventilation parameters were used: tidal volume = 0.25 ml/breath; breathing frequency = 120/min; and positive end-expiratory pressure = 3 cm of H2O. Within the sealed plethysmograph mouse chamber, transrespiratory pressure (Δ tracheal pressure) and inspiratory volume or flow were continuously monitored online by an adjacent computer, and airway resistance was calculated by the division of the transpulmonary pressure by the change in inspiratory volume. Following a baseline period in the mouse chamber, each mouse received doses of methacholine ranging from 62.5 to 250 μg/kg of methacholine by tail vein injection, and airway responsiveness to this bronchoconstrictor was again calculated online. The data shown in this manuscript are focused on a dose of 125 μg/kg methacholine because this methacholine dose failed to elicit a response in nonsensitized mice but elicited maximal changes in airway hyperresponsiveness in A. fumigatus-sensitized mice after the conidia challenge. At the conclusion of the assessment of airway responsiveness, a bronchoalveolar lavage (BAL) was performed with 1 ml of normal saline. Approximately 500 μl of blood was then removed from each mouse and transferred to a microcentrifuge tube. Sera were obtained after the sample was centrifuged at 10,000 rpm for 5 min. Whole lungs were finally dissected from each mouse and snap frozen in liquid N2 or prepared for histological analysis.

Morphometric analysis of leukocyte accumulation in BAL samples

Lymphocytes and macrophages were enumerated in BAL samples cytospun (Thermo Shandon, Runcorn, U.K.) onto coded microscope slides. Each slide was stained with a Wright-Giemsa differential stain and the average number of each cell type was determined after counting a total of 300 cells in 10–20 high-powered fields (×1000) per slide. A total of 1 × 106 BAL cells were cytospun onto each slide to compensate for differences in cell retrieval.

Whole lung histological analysis

Whole lungs from both groups of mice at day 28 after A. fumigatus conidia challenge were fully inflated with 10% formalin, dissected, and placed in fresh formalin for 24 h. Routine histological techniques were used to paraffin-embed the entire lung, and 5-μm sections of whole lung were stained with H&E or with periodic acid Schiff (PAS). Inflammatory infiltrates and structural alterations were examined under standard light microscopes and adjacent blood vessels using light microscopy at a magnification of ×200.

Preparation of cDNA and RT-PCR amplification

Total RNA was prepared from whole lung samples removed from mice in the IL-13-PE-treated and control groups at day 28 after the conidia challenge. RNA was isolated using TRIzol reagent (Life Technologies, Rockville, MD) according to the manufacturer’s directions. The purified RNA was subsequently reverse transcribed into cDNA using a Gibco reverse transcription kit (Life Technologies, Rockville, MD) and oligo(dT) 12–18 primers. The amplification buffer contained 50 mM KCl, 10 mM Tris-HCl (pH 8.3), and 2.5 mM MgCl2. Specific oligonucleotide primers were added (200 ng/sample) to the buffer along with 5 μl of reverse transcribed cDNA sample. The following murine oligonucleotide primers were used: IL-4Rα sense, GAGTGAAGGGATCTTAGACATC; IL-4Rα antisense, GCTGAGTGACAGAACAGGC (32); IL-13Rα1 sense, GAATTTGAGCGCTCTGCAGAA; IL-13Rα2 antisense, GTTATTGACAAATGACTTGAAG; IL-13Rα2 sense, ATGGCTTTTTGTCATACGATGCT; and IL-13Rα2 antisense, CAGTGTGCTCATTCTTCTTAT.

These mixtures were then first incubated for 5 min at 94°C and amplified using the following cycling parameters: IL-4Rα, cycled 38 times at 94°C for 30 s and at 58°C for 45 s, and elongated at 72°C for 70 s; IL-13Rα1, cycled 38 times at 94°C for 30 s and at 66°C for 60 s, and elongated at 72°C for 70 s; IL-13Rα2, cycled 38 times at 94°C for 30 s and at 66°C for 60 s, and elongated at 72°C for 70 s. After amplification the samples were separated on a 2% agarose gel containing 0.3 μg/ml ethidium bromide and bands were visualized and photographed using a translucent UV source.

ELISA and total soluble collagen analysis

Murine IL-13, IL-4, IL-12, IFN-γ, and C10 chemokine levels were measured in 50-μl samples from whole lung homogenates using a standardized sandwich ELISA technique previously described in detail (33). BAL fluids from the diluent and IL-13-PE groups were also screened for IFN-γ. Each ELISA was screened to ensure Ab specificity and recombinant murine cytokines, and chemokines were used to generate the standard curves from which the concentrations present in the samples were derived. The limit of ELISA detection for each cytokine was consistently above 50 pg/ml. The sieved collagen assay (Bicrocolor, Belfast, Ireland) was used to measure the soluble forms of collagen present in the same lung homogenates. This assay was developed from the Sirius red-based histochemical procedure. The cytokine and collagen levels in each sample were normalized to total protein levels measured using the Bradford assay.

Serum levels of IgE, IgG1, and IgG2a at day 28 after conidia in the diluent and IL-13-PE treatment groups were analyzed using complementary capture and detection Ab pairs for IgE, IgG1, and IgG2a (BD PharMingen, San Diego, CA). Ig ELISAs were performed according to the manufacturer’s directions. Duplicate sera samples were diluted to 1/100 for IgE determination and 1/10 for determination of IgG levels. Ig levels were then calculated from OD readings at 492 nm, and Ig concentrations were calculated from a standard curve generated using rIgG1, rIgG1, or rIgG2a (both standard curves ranged from 5 to 2000 pg/ml).

Statistical analysis

All results are expressed as mean ± SEM. A one-way ANOVA and a Dunnett’s multiple comparisons test were used to reveal statistical differences between the control group and the IL-13-PE treatment groups at day 28 after the conidia challenge. A value of p < 0.05 was considered statistically significant.

Results

IL-13-PE therapy significantly reduced airway hyperresponsiveness during chronic fungal-induced allergic airway disease

Airway hyperresponsiveness following a systemic methacholine challenge is a persistent feature of chronic fungal allergic airway disease in mice (7). As shown in Fig. 1, the airway resistance measured in the group of mice that received diluent from day 14 to day 28 after the conidia challenge was 19.2 ± 4.6 cm H2O/ml/sec, obtained from the University Laboratory of Animal Medicine facility at the University of Michigan Medical School (Ann Arbor, MI). Systemic sensitization of mice to a commercially available preparation of soluble Aspergillus fumigatus Ags was performed as previously described in detail (7). Seven days after the third intranasal challenge, each mouse received 5.0 × 106 A. fumigatus conidia suspended in 30 μl of 0.1% Tween 80 via the intratracheal route (7).
and this represented an ~8-fold increase above the baseline resistance (dashed line shown in Fig. 1). Mice that received 50 ng of IL-13-PE from day 14 to day 28 after conidia exhibited airway hyperresponsiveness following methacholine provocation that was similar to that elicited in the control group at day 28. However, airway resistance in mice that received 100 ng of IL-13-PE over the same time period was significantly (p ≤ 0.05) lower than that measured in the control group (19.2 ± 4.6 vs 9.4 ± 2.9 cm H 2 O/ml/s; Fig. 1). Likewise, mice that received 200 ng of IL-13-PE exhibited significantly lower (p ≤ 0.01) methacholine-induced airway resistance compared with the control group (19.2 ± 4.6 vs 5.4 ± 1.4 cm H 2 O/ml/s; Fig. 1). These data suggested that, in a dose-dependent manner, IL-13-PE inhibited airway hyperresponsiveness associated with chronic fungal-induced allergic airway disease.

The 200-ng IL-13-PE therapy significantly reduced the numbers of T lymphocytes in the BAL from mice with chronic fungal-induced allergic airway disease

Previous studies have demonstrated that T lymphocytes are the primary effectors of airway hyperresponsiveness (15) and that both IL-4 (34) and IL-13 (5) have major, and possibly distinct, roles in this response. Th2 lymphocytes appear to be the primary source of IL-4 (15), whereas alveolar macrophage appears to be the primary lung source of IL-13 during atopic asthma (3). Because both types of cells also respond to IL-4 and IL-13 in a receptor-dependent manner (22, 24), we next examined whether IL-13-PE therapy during chronic allergic airway responses to Aspergillus affected the numbers of T cells and macrophages in the airways of these mice. T lymphocytes (Fig. 2A) and macrophages (Fig. 2B) were enumerated in BAL samples from all groups of mice at day 28 after conidia challenge. Only the 200-ng IL-13-PE treatment significantly reduced the number of T lymphocytes present in BAL samples compared with numbers of these cells in similar samples from the control group (Fig. 2A). None of the IL-13-PE treatments significantly altered the numbers of BAL macrophages compared with number of BAL macrophages in the diluent group (Fig. 2B).

Furthermore, few eosinophils and neutrophils were identified in BAL samples from all groups at day 28 after the conidia challenge (data not shown), and these findings were consistent with our previous observations in this model (7, 30, 31, 35). Thus, the 200-ng IL-13-PE treatment significantly reduced T lymphocyte but not macrophage numbers in the airways of A. fumigatus-sensitized mice exposed to A. fumigatus conidia.

IL-13-PE therapy significantly attenuated the peribronchial inflammation and goblet cell hyperplasia characteristic of chronic fungal-induced allergic airway disease

As we have previously reported (7), the introduction of conidia into A. fumigatus-sensitized mice promotes a marked and persistent peribronchial accumulation of T lymphocytes and mononuclear cells. In the present study, pronounced airway inflammation was observed in allergic mice that received diluent alone (Fig. 3A). In contrast, the airways of mice in all three IL-13-PE treatment groups exhibited a clear paucity of inflammatory leukocytes (Fig. 3, B–D), and the greatest reduction in peribronchial inflammation was observed in whole lung sections from mice that received 200 ng of IL-13-PE (Fig. 3D). The airways of SPF mice have few, if
any, mucus-producing goblet cells, so an increase in the number of
these cells reflects an induction of mucin-gene expression (36). In the
present study, goblet cells were easily identified in the bronchial
epithelium of allergic mice that received diluent (Fig. 4A) or
50 ng of IL-13-PE (Fig. 4B). However, only scattered goblet cells
were detected in the airways of mice that received 100 ng of IL-
13-PE (Fig. 4C), and the airways of mice treated with the highest
dose of IL-13-PE completely lacked PAS-positive goblet cells
(Fig. 4D). Therefore, the therapeutic effects of IL-13-PE were
manifest at a histological level as evidenced by decreased peri-
bronchial inflammation and goblet cell hyperplasia/mucus
production.

The 200-ng IL-13-PE therapy significantly attenuated
peribronchial fibrosis in mice with chronic fungal-induced
allergic airway disease

Peribronchial fibrosis is another prominent feature of the remod-
eled airway in mice with chronic fungal-induced allergic airway
disease (7), and previous studies have shown that IL-13 is a major
mediator of fibroblast activation and tissue fibrosis (37–39). In
the present study, the peribronchial distribution of extracellular
matrix and fibroblasts was pronounced around the airways of the control
group of mice at day 28 after the conidia challenge (Fig. 5A). Conversely,
peribronchial fibrosis was markedly diminished around the airways of mice that received 200 ng of IL-13-PE from
day 14 to day 28 after the conidia challenge (Fig. 5B). Histological
examination of lungs from the other two IL-13-PE treatment
groups revealed little effect of lower doses of this chimeric protein
on peribronchial fibrosis (data not shown). Analysis of total collage-
ne levels in whole lung homogenates from the diluent and
200-ng IL-13-PE treatment groups of mice confirmed that less
peribronchial fibrosis was present in IL-13-PE-treated mice at day 28
after the conidia challenge (Fig. 6). Again, the two lower doses of IL-13-PE did not reduce total soluble collagen levels in whole
lung samples (data not shown). Taken together, these data suggest
that targeting lung cells that recognize IL-13 significantly reduced
the degree of peribronchial fibrosis associated with chronic fungal
allergic airway disease in mice.

The 200-ng IL-13-PE therapy during chronic fungal-induced
allergic airway disease dramatically reduced whole lung mRNA
levels of IL-13Rα1 and IL-4Rα

We have previously observed whole lung mRNA levels of IL-
13Rα1 and IL-4Rα at days 21 and 30 after a conidia challenge in
A. fumigatus-sensitized mice (16). Given these previous findings,
it was determined whether the 200-ng IL-13-PE treatment modu-
lated mRNA levels of these receptors in the lungs of A. fumigatus-
sensitized mice challenged with conidia 28 days previously. As

FIGURE 4. Representative photomicrographs of PAS-stained whole lung sections at day 28 after A. fumigatus conidia challenge in the control (HSA-PBS vehicle or diluent) and IL-13-PE treatment groups. A. fumigatus-sensitized mice received diluent or 50, 100, or 200 ng of IL-13-PE every other day starting at day 14 and concluding at day 28 after a conidia challenge (see Materials and Methods). Goblet cells (stained dark magenta) were readily apparent in whole lung sections from the diluent treat-
ment group (A) and the 50-ng (B) and 100-ng (C) IL-13-PE treatment
groups. However, goblet cells were not observed in the group of mice that
received 200 ng of IL-13-PE (D). Original magnification was ×200 for
each photomicrograph.

FIGURE 5. Histological evidence of peribronchial fibrosis at day 28 after an A. fumigatus conidia challenge in the control HSA-PBS diluent (A) and 200-ng IL-13-PE (B) treatment groups. In H&E-stained whole lung sections, pink-colored and spindle-shaped fibroblasts were prominent around the airways of mice from the diluent treatment group (A). In con-
trast, a build-up of fibroblasts and extracellular matrix was absent around
the airways of mice that received 200 ng of IL-13-PE from day 14 to day
28 after the conidia challenge (B). Original magnification was ×200 for
each photomicrograph.
shown in Fig. 7, whole lung mRNA levels from control and IL-13-PE-treated A. fumigatus-sensitized mice were analyzed for IL-13Rα1, IL-13Rα2, and IL-4Rα (both soluble and membrane-associated isoforms of IL-4R) expression using RT-PCR (Fig. 7A). The whole lung levels of IL-13Rα1 mRNA were markedly reduced, whereas mRNA for soluble (Fig. 7A, top band) and membrane-associated (Fig. 7A, bottom band) IL-4Rα were absent in mice that received the 200-ng IL-13-PE treatment from day 14 to day 28 compared with mice that received diluent over the same time (Fig. 7A). The ratio of cytokine receptor:β-actin based on densitometry analysis is shown in Fig. 7B. In contrast, the 50- and 100-ng IL-13-PE treatments did not markedly reduce mRNA levels of IL-13Rα1 and IL-4Rα in whole lung samples compared with the respective diluent alone group (data not shown). Interestingly, no IL-13Rα2 was detected in any group at day 28 after the conidia challenge, whereas this IL-13R chain was detected in whole lungs taken from nonallergic mice (data not shown). Taken together, these data showed that the IL-13-PE treatment abolished the whole lung mRNA for IL-13Rα1 and IL-4Rα, suggesting that this chimeric protein therapy markedly reduced the numbers of IL-13-responsive cells in the lung.

The 200-ng IL-13-PE therapy did not significantly affect circulating levels of IgE, but significantly reduced IgG1 and significantly increased IgG2a

Serum levels of total IgE, IgG1, and IgG2a are summarized in Fig. 8. Following the diluent or IL-13-PE treatment at day 28 after conidia, all groups of mice exhibited similar levels of serum IgE, suggesting that the IL-13-PE treatments did not affect the production of IgE in this model. IgG1 and IgG2a levels are shown in Fig. 8, B and C, respectively. At day 28 after conidia, IgG1 levels were significantly decreased in the 200-ng IL-13-PE treatment group, but not the other IL-13-PE treatment groups, compared with the day 28 diluent group (Fig. 8B). However, IgG2a levels were significantly higher in the 200-ng IL-13-PE treatment group than IgG2a levels measured at day 28 in the diluent group (Fig. 8C).

Thus, these data suggested that the 200-ng IL-13-PE treatment inhibited the production of IgG1, which is normally associated with a Th2 immune response, and promoted the production of IgG2a, which is normally associated with a Th1 immune response.

The 200-ng IL-13-PE therapy significantly increased whole lung levels of IL-13, but not IL-4 or IL-12

Whole lung levels of IL-13, IL-4, and IL-12 were measured in all four treatment groups at day 28 after the conidia challenge, and these ELISA results are summarized in Fig. 9. All three cytokines were elevated above baseline levels detected in A. fumigatus-sensitized mice before the conidia challenge (Fig. 9, dashed lines). Neither the 50-ng IL-13-PE treatment nor the 100-ng IL-13-PE treatment significantly altered whole lung levels of IL-13 (A), IL-4 (B), and IL-12 (C) compared with the control group. Whole lung IL-13 levels were significantly elevated in the 200-ng IL-13-PE treatment group compared with the control group (Fig. 9A), but IL-4 and IL-12 levels did not differ between this IL-13-PE treatment group and the control group (Fig. 9, B and C, respectively). Thus, these data suggested that IL-13-PE therapy did not inhibit lung levels of IL-13, IL-4, and IL-12 in this model. In addition, it appeared that 200-ng IL-13-PE treatment reduced the number of IL-13-responsive cells in the lung, considering that whole lung levels of IL-13 were significantly increased in this treatment group.

The 200-ng IL-13-PE therapy significantly increased BAL and whole lung levels of IFN-γ

Previous studies have shown that IFN-γ is a potent inhibitor of many of the physiological and histological features of allergic airway disease induced by ovalbumin (40) and A. fumigatus conidia.
The effect of the diluent and IL-13-PE therapies on whole lung and BAL levels of IFN-$\gamma$ is shown in Fig. 10. Markedly greater amounts of IFN-$\gamma$ were detected in both compartments at day 28 after the conidia challenge compared with IFN-$\gamma$ levels measured in similar samples from A. fumigatus-sensitized mice.

FIGURE 8. Total serum IgE (A), IgG1 (B), and IgG2a (C) levels in A. fumigatus-sensitized mice at day 28 after A. fumigatus conidia challenge in the control (HSA-PBS vehicle) and IL-13-PE treatment groups. A. fumigatus-sensitized mice received diluent or 50, 100, or 200 ng of IL-13-PE every other day starting at day 14 and concluding at day 28 after a conidia challenge (see Materials and Methods). Total IgE, IgG1, and IgG2a were measured using specific ELISAs as described in Materials and Methods. No differences in serum IgE levels were detected between the two groups at any point before or after the conidia challenge (A). Data are expressed as mean $\pm$ SE; $n$ = 5–10 mice per group. Statistical differences between the control (HSA-PBS vehicle or diluent) and 200-ng IL-13-PE treatment groups at day 28 after conidia are indicated in B and C.

FIGURE 9. IL-13 (A), IL-4 (B), and IL-12 (C) levels in whole lung homogenates at day 28 after A. fumigatus conidia challenge in the control (HSA-PBS vehicle or diluent) and IL-13-PE treatment groups. A. fumigatus-sensitized mice received diluent or 50, 100, or 200 ng of IL-13-PE every other day starting at day 14 and concluding at day 28 after a conidia challenge (see Materials and Methods). Immunoreactive levels of IL-13, IL-4, and IL-12 were measured using a specific ELISA as described in Materials and Methods. The dashed lines indicate cytokine levels in whole lung samples from A. fumigatus-sensitized mice before the conidia challenge. Values are expressed as mean $\pm$ SE; $n$ = 5–10 mice per group. *, $p \leq 0.05$ compared with levels measured in the control group at day 28 after the conidia challenge.
before the conidia challenge. Whole lung IFN-γ levels were increased in all of the IL-13-PE treatment groups, but this elevation reached statistical significance in the 200-ng IL-13-PE treatment alone (Fig. 10A). Likewise, immunoreactive IFN-γ levels were increased in BAL samples from the 100- and 200-ng IL-13-PE treatment groups, but only BAL IFN-γ levels in the latter group reached statistical significance compared with BAL levels from the control group (Fig. 10B). These data demonstrated that the Th1 cytokine response in A. fumigatus-sensitized mice was significantly increased at day 28 after the conidia challenge and intranasal IL-13-PE chimeric protein therapy.

Discussion
There is considerable evidence that asthma is an inflammatory disease of the airways that is caused by an imbalance in the activity of lung-associated T lymphocytes (3, 8, 41). Specifically, asthma is characterized by a predominance of Th2 lymphocytes in the airways that appear to generate abnormal quantities of IL-4, IL-5, and IL-13 (9). The effect of increased levels of Th2 cytokines in the asthmatic airways appears to be magnified by a relative deficiency in the levels of the Th1 cytokine, IFN-γ, produced by Th1 lymphocytes (42). Nevertheless, previous attempts to amplify Th1-mediated responses using rIL-12 (43) or inhibit Th2-mediated responses using anti-IL-5 therapy (44) were modestly successful in the treatment of clinical asthma. Clinical asthmatic responses triggered by A. fumigatus mirror this abnormal cytokine pattern because pulmonary levels of Th2 cytokines are substantially higher than levels of Th1 cytokines (45). Because of our recent replication of this and other features of fungus-induced allergic airway disease in mice (7), we examined the therapeutic utility of selective targeting of lung cells that bind IL-13. This was facilitated by the delivery of a chimeric protein that contained human IL-13 and a derivative of Pseudomonas exotoxin, PE38QQR. IL-13-PE has been used successfully in the eradication of IL-13R-positive tumors (28, 29). In the present study, we observed that IL-13-PE therapy in mice with established fungal allergic airway disease markedly reduced mRNA levels of IFN-γ, suggesting that the targeting of IL-13-responsive cells was effective in promoting levels of this antiallergic cytokine (40).

Lung cells that bind IL-13 were specifically targeted during chronic airway response to Aspergillus in mice because of the evidence that this Th2 cytokine is prominently expressed during clinical asthma (1, 46) and has a major and distinct role during experimental allergic airway disease (5, 6, 18). It is important to note that IL-4 and IL-13 share heteromultimeric receptor complexes of variable composition. The biologic responses induced by IL-4 or IL-13 require a complex interaction of signaling pathways and regulators (47). The classical IL-4R is found on hematopoietic cells and consists of IL-4Rα and IL-2Rγ (or γ-chain), whereas the alternative form of IL-4R consists of the IL-4Rα and IL-13Rα chains (48). Because the alternative IL-4R contains IL-13Rα1 it can also recognize IL-13, and it appears that this receptor is the major IL-13 receptor in hematopoietic and nonhematopoietic cells (49, 50). An additional receptor, IL-13Rα2, binds to IL-13 with 100-fold higher affinity than IL-13Rα1 but appears to lack the cytoplasmic domain necessary for intracellular signaling (51). Previous studies have demonstrated that IL-13-PE effectively binds to both IL-13 receptors but exhibits a much higher affinity for IL-13Rα2 than for IL-13Rα1 (52). In the present study, we observed that the 200-ng IL-13-PE therapy markedly reduced mRNA levels of membrane and soluble IL-4Rα, as well as IL-13Rα1, and increased whole lung IL-13 levels in the lungs of allergic mice. These findings suggested that the 200-ng IL-13-PE therapy successfully targeted cells that normally express IL-13Rα1 as well as lung-associated cells that are expressing IL-4Rα. Interestingly, IL-13Rα2 mRNA was only detected in whole lung samples from nonsensitized, SPF mice; mRNA for this IL-13R was not detected in any lung samples from A. fumigatus-sensitized mice before or after conidia challenge. This finding is intriguing in light of growing evidence that IL-13Rα2 may function as a decoy receptor for IL-13, thereby limiting the biologic effects of IL-13 (53). Furthermore, the lack of IL-13Rα2 in the lungs of allergic mice may be one explanation for the persistence of allergic airway disease in A. fumigatus-sensitized mice exposed to fungal conidia.

Given that T lymphocytes are the primary effectors during a wide array of allergic airway responses (15, 54, 55), it is clear that...
this cell and the mediators it produces are appropriate targets during the treatment of atopic asthma (56). We too have previously observed that despite the fact that eosinophils are the most abundant and major effector cell type in the airways up to day 14 after a conidia challenge in A. fumigatus-sensitized mice, T lymphocytes are the predominant cellular effectors in this model at later times (7). Furthermore, enhancing IL-12 (43) or neutralizing IL-5 (44) during clinical asthma has been shown to have little effect on airway responsiveness to spasmogen or Ag challenge, despite the fact that these treatments markedly reduced blood and sputum eosinophil numbers. In the present study, a prominent consequence of IL-13-PE treatment was the marked and dramatic reduction in the numbers of T lymphocytes in and around the airways of A. fumigatus-sensitized mice challenged with conidia 28 days previously. Activated T lymphocytes highly express IL-4Ra, but unlike B lymphocytes and monocytes, resting or activated T cells exhibit little or no surface expression of IL-13Ra1 (22, 24). Therefore, it is not immediately clear from the current study whether the IL-13-PE treatment directly killed T lymphocytes present in the lung, altered the apoptotic status of these cells, or inhibited mechanisms that promote the emigration of these cells into the lungs. Support for the latter possibility comes in the form of preliminary observations that the IL-13-PE therapy significantly inhibited the production of a potent T cell chemotractant, C10 chemokine (data not shown). Because C10 chemokine has a major proinflammatory role during acute A. fumigatus-induced allergic airway disease (57), further studies are necessary to determine whether a reduction in C10 levels could account for the decreased numbers of T lymphocytes in this allergic airway model.

The importance of airway remodeling during clinical asthma remains controversial (58–60), but postmortem studies clearly reveal that airway wall thickening is present in asthmatic patients, and this observation appears to correlate to the severity of airway hyperresponsiveness and airflow obstruction (61–63). Airway remodeling is normally characterized by the activation of cells that form the structural and support elements of the airway, including epithelial cells, smooth muscle, fibroblasts, and endothelial cells (58). Asthma is also characterized by increased mucus production that in turn can contribute to airway obstruction (64). Cohn and colleagues (65) have shown that although IL-5, eosinophils, or mast cells are not essential, signaling through the IL-4R is critical (66). Airway wall thickening is present in asthmatic patients, and this observation appears to correlate to the severity of airway hyperresponsiveness and airflow obstruction (61–63). Airway remodeling is normally characterized by the activation of cells that form the structural and support elements of the airway, including epithelial cells, smooth muscle, fibroblasts, and endothelial cells (58).

In conclusion, IL-13 has been shown to be a central mediator in airway inflammation, hyperreactivity, increased number of goblet cells, and excess mucus, all pulmonary features that characterize asthma (5, 6, 36, 40). Given its distinct and dynamic role in the development and maintenance of so many features of asthma, it is clearly important to characterize strategies that safely and effectively target cells that respond to this cytokine. In the present study, we observed that the prolonged intranasal instillation of a chimeric protein that selectively targeted lung cells expressing IL-13 was well tolerated and effective in the treatment of asthmatic airway disease, thereby obviating the need for Ab or receptor antagonist therapies (73). These findings provide impetus to explore the efficacy of this treatment in clinical asthma and atopy.

References
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