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Effects of Th2 Cytokines on Chemokine Expression in the Lung: IL-13 Potently Induces Eotaxin Expression by Airway Epithelial Cells¹

Li Li,* Yiyang Xia,* Andrea Nguyen,* Yew Hon Lai,† Lili Feng,* Tim R. Mosmann, † and David Lo²*²

Airway inflammation associated with asthma is characterized by massive infiltration of eosinophils, mediated in part by specific chemoattractant factors produced in the lung. Allergen-specific Th2 cells appear to play a central role in asthma; for example, adoptively transferred Th2 cells induced lung eosinophilia associated with induction of specific chemokines. Interestingly, Th2 supernatant alone administered intranasally to naïve mice induced eotaxin, RANTES, monocyte-chemotactoic protein-1, and KC expression along with lung eosinophilia. We tested the major cytokines individually and found that IL-4 and IL-5 induced higher levels of macrophage-inflammatory protein-1α and KC; IL-4 also increased the production of monocyte-chemoattractoic protein-1; IL-13 and IL-4 induced eotaxin. IL-13 was by far the most potent inducer of eotaxin; indeed, a neutralizing anti-IL-13 Ab removed most of the eotaxin-inducing activity from Th2 supernatants, although it did not entirely block the recruitment of eosinophils. While TNF-α did not stimulate eotaxin production by itself, it markedly augmented eotaxin induction by IL-13. IL-13 was able to induce eotaxin in the lung of JAK3-deficient mice, suggesting that JAK3 is not required for IL-13 signaling in airway epithelial cells; however, eosinophilia was not induced in this situation, suggesting that JAK3 transduces other IL-13-mediated mechanisms critical for eosinophil recruitment. Our study suggests that IL-13 is an important mediator in the pathogenesis of asthma and therefore a potential target for asthma therapy. The Journal of Immunology, 1999, 162: 2477–2487.

Allergic asthma is characterized by airway hyperresponsiveness and inflammation with tissue and bronchial infiltration by activated eosinophils, T cells, mast cells, and macrophages (1). The extensive infiltration of eosinophils into the lung is not only a hallmark of allergic asthma but also contributes to much of the damage of respiratory epithelium during late phase airway responses (2, 3). There is accumulating evidence that chemokines, especially the C-C subclass, are involved in both the migration and the activation of eosinophil and other leukocytes during asthma responses (4–6). Chemokines implicated in asthma include RANTES, macrophage-inflammatory protein-1α (MIP-1α),3 monocyte-chemotactic protein-3 (MCP-3), and MCP-5 (7, 8). Furthermore, the recently characterized C-C chemokine eotaxin was the major eosinophil chemoattractant found in bronchovascular lavage (BAL) fluid from rodent models of allergic inflammation (9, 10) and was also up-regulated in BAL of asthma patients (11). The selective recruitment of eosinophils by eotaxin suggests that this chemokine is crucial in asthma inflammation.

In vitro studies have shown that chemokines involved in asthma are regulated by certain cytokines. Thus, RANTES produced by human airway muscle cells is stimulated by TNF-α plus IFN-γ but inhibited by IL-4, IL-10, and IL-13 (12). Eotaxin expression by human dermal fibroblasts (13) and lung epithelial cells (14) was stimulated by IFN-γ in the presence of IL-1 and TNF-α, while eotaxin produced by human fibroblasts was induced by IL-4 (15). Th2 cytokines have been implicated as playing a central role in eosinophil recruitment in murine models of allergic asthma, and adoptively transferred Th2 cells induced eotaxin expression in the lung as well as a lung eosinophilia (16–18). We therefore sought to examine the notion that while Th2 cytokines stimulate the growth and activation of eosinophils, they may also stimulate the production of eosinophil chemoattractants in the lung during asthma responses.

In this study, we tested the effects of Th2 supernatant and some individual Th2 cytokines, IL-3, IL-4, IL-5, and IL-13, on chemokine production in the lung. We found that Th2 supernatant significantly induced the expression of eotaxin as well as RANTES, MCP-1, and KC in the lung. Individual cytokines showed different abilities to up-regulate expression of the various chemokines; interestingly, IL-13 was the most potent inducer of eotaxin expression at both the mRNA and protein levels, with lung expression mainly in epithelial cells. The induction of eotaxin was found to be Janus family kinase-3 (JAK3) independent, although eosinophilia remained JAK3 dependent.

Materials and Methods

Mice

TCR-SFE × BALB/c transgenic mice were previously described (19). These mice are transgenic for a TCR specific for influenza PR8 hemagglutinin peptide 110–119 (SFERFEIFPK) presented on I-E². Naive BALB/
The gel was dried and exposed to film. 

extraction and sodium acetate-ethanol precipitation, the protected hybrid-

rIL-12 was purchased from Genzyme (Cambridge, MA), and the rIL-13 used in this study was produced by a stably transfected BWS147 cell line and assayed as previously described (21). Anti-IL-12 was a monoclonal rat IgG, clone C17.8.20 (kind gift of Dr. G. Trinchieri). The goat anti-mouse IL-13 polyclonal Ab was purchased from R&D Systems (Minneapolis, MN) and required 1 μg to neutralize 10 ng of IL-13. The goat anti-mouse eotaxin polyclonal Ab was also purchased from R&D. The coding region of murine eotaxin (GenBank accession no. U77462) without signal peptide was generated by PCR, subcloned into pETM1, and expressed in 

JAK3 (referred to as SCID) mice (6 – 8 wk) were provided by the Rodent Breeding Colony in accordance with National Institutes of Health and TSRI institutional guidelines.

Cytokines and Abs

Murine rIL-2, rIL-3, rIL-4, and rTNF-α were purchased from Pepro Tech (Rochester, NY), rIL-7 was purchased from Pharmingen (San Diego, CA), rIL-12 was purchased from Genzyme (Cambridge, MA), and the rIL-13 used in this study was produced by a stably transfected BWS147 cell line and assayed as previously described (21). Anti-IL-12 was a monoclonal rat IgG, clone C17.8.20 (kind gift of Dr. G. Trinchieri). The goat anti-mouse IL-13 polyclonal Ab was purchased from R&D Systems (Minneapolis, MN) and required 1 μg to neutralize 10 ng of IL-13. The goat anti-mouse eotaxin polyclonal Ab was also purchased from R&D. The coding region of murine eotaxin (GenBank accession no. U77462) without signal peptide was generated by PCR, subcloned into pETM1, and expressed in

Checmokine detection by RNase protection assay

Total RNA was isolated using Trizol reagent. Probes for a panel of chemokines were described by Xia et al. (23). The assay was performed as described by Xia et al. (24). Briefly, RNA was dissolved in 80% form- and eu. Small volumes (0.4 M NaCl, 1 mM EDTA, and 40 mM piperazine-N,N’-bis(2-ethanesulfonic acid), heated to 85°C for 5 min, and hybridized for 10 h with corresponding [α-32P]UTP-labeled antisense probes at 55°C. The unhybridized RNA was digested with 50 U/ml RNase T1 (Life Technologies) and 24 μg/ml RNase A (Sigma) for 1 h at 30°C. After phenol-chloroform extraction and sodium acetate-ethanol precipitation, the protected hybridized RNA was denatured and electrophoresed on 10% polyacrylamide gel. The gel was dried and exposed to film.

Eotaxin detection by ELISA

BAL collected from mice was spun, and supernatants were used to test BAL eotaxin levels. The left lobe of the lung were cut into small pieces in 0.5 ml of RPMI medium, incubated in 37°C for 30 min, and spun; the supernatants were used to test eotaxin levels in lung tissue. Eotaxin was detected by ELISA (R&D Systems).

Histology

Cytospins of BAL cells were fixed with methanol and stained with eosin and methylene blue (Fisher, Pittsburgh, PA). Leukocytes were analyzed by

IL-3 INDUCES EOTAXIN EXPRESSION IN THE LUNG

Table 1. Cytokine levels in Th1 or Th2 supernatants

<table>
<thead>
<tr>
<th>Cytokine (ng/ml)</th>
<th>Th1</th>
<th>Th2 (1)</th>
<th>Th2 (2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-3</td>
<td>8</td>
<td>160</td>
<td>62</td>
</tr>
<tr>
<td>IL-4</td>
<td>&lt;0.06</td>
<td>296</td>
<td>213</td>
</tr>
<tr>
<td>IL-5</td>
<td>&lt;0.06</td>
<td>232</td>
<td>101</td>
</tr>
<tr>
<td>IL-10</td>
<td>&lt;0.6</td>
<td>63</td>
<td>132</td>
</tr>
<tr>
<td>IL-13</td>
<td>0.18</td>
<td>245</td>
<td>177</td>
</tr>
<tr>
<td>GM-CSF</td>
<td>&lt;2.5</td>
<td>11</td>
<td>8</td>
</tr>
<tr>
<td>IFN-γ</td>
<td>48</td>
<td>&lt;0.6</td>
<td>&lt;0.6</td>
</tr>
<tr>
<td>TNF-α</td>
<td>3</td>
<td>2</td>
<td>ND</td>
</tr>
</tbody>
</table>

a Day 5 Th1 or Th2 cells (3 x 10⁶/ml) were stimulated with con A (5 μg/ml) for 24 h, and supernatants were collected, treated with 20 mM methyl-α-D-mannopyranoside to bind Con A. Cytokines in the supernatants were tested by ELISA.

Results

Th2 supernatants induce eotaxin expression and eosinophil infiltration in the lung

In our previous studies (17), we found that adoptively transferred Th1 and Th2 cells, stimulated in the lung, induced distinct patterns of chemokines and immunoreactive cell recruitment in lung parenchyma within 3 days. Thus, Th1 cells specifically expressed IFN-γ in the lung and were potent inducers of interferon-γ-inducible protein and neutrophil recruitment. In contrast, Th2 cells expressing IL-4 and IL-5 (but not IFN-γ) in the lung specifically induced eotaxin correlating with eosinophil recruitment. Interestingly, a mix of Th1 and Th2 cells showed a codominant expression of both Th1 and Th2 effects. Th1 and Th2 cells stimulated in vitro showed nearly identical patterns of chemokine production (lymphotactin, MIP-1α, MIP-1β, and T cell activation gene-3), suggesting that the differential effects on chemokine expression and cell recruitment were mediated through lung parenchymal cells. To determine the basis of these effects, we tested whether specific cytokines produced by T cells could account for both the induction of specific chemokine patterns and the apparent codominant effects in inflammation.

To study the effects of Th1 or Th2 cytokines on chemokine production in the lung, supernatants were generated from mature Th1 or Th2 cells by stimulating these cells with ConA. The relative amounts of the various cytokines in the supernatants were tested by ELISA (Table I); among different batches of supernatants, variation in individual cytokine concentrations was less than two- to threefold. These supernatants were used to guide BALB/c mice intranasally, 50 μl/treatment and three treatments/day. Although we cannot be certain what amounts of intranasally applied cytokines would be equivalent to in vivo T cell production in the lung, we used this frequent treatment over 2.5 days to reproduce the kinetics of inflammation seen in our adoptive transfer studies (17). After 2.5 days of treatment, mice were sacrificed, and chemokine mRNAs were assayed from lung tissue using an RNase protection assay. Equally high levels of RANTES and KC mRNAs were detected in the lung tissues from Th1 or Th2 supernatant-treated mice, compared with those in the control mice (Fig. 1, A and B). However, interferon-γ-inducible protein was detectable only in
differential count of a total of 200–300 cells on coded slides. Lung was perfused, injected with OCT through the trachea, and frozen in OCT. Frozen lung sections were fixed with cold acetone (Fisher) and stained with 1 μg/ml of goat anti-mouse eotaxin Ab (R&D Systems) overnight. In the case of Ab blocking, 30 μg/ml recombinant eotaxin were added. The staining was followed by 1 μg/ml biotin-Ab), horseradish peroxidase (Jackson ImmunoResearch, West Grove, PA) and streptavidin-horseradish peroxi-
dase (Jackson ImmunoResearch), and was visualized by 3-aminobenzidine carbazol (Sigma) substrate. Eosinophils were stained for cyanide-resistant eosinophil peroxidase activity as described by Li et al. (17).
FIGURE 1. Th2 supernatants preferentially induced eotaxin expression and lung eosinophil infiltration. Naive BALB/c mice were treated with Th1 or Th2 (batch 1) supernatant, 50 μl/treatment, three treatments/day for 2.5 days. mRNA levels for a panel of chemokines were detected by RNase protection, each lane representing an individual mouse (A). The expression of each chemokine was also measured by the density ratio between chemokine and L32 bands from the same mouse, each bar representing the mean ± SD of three (medium-treated) or four (Th supernatant-treated) mice. *, p < 0.05, compared with the medium-treated group (B). Cytospin slides were prepared for BAL cells from each individual mouse, and the infiltrated cells were analyzed by different counts of 200–300 cells/slide. Each point represents one mouse (C).
Th1 supernatant-treated lungs, while higher expression of eotaxin and MCP-1 mRNAs was found in Th2 supernatant-treated lungs (Fig. 1, A and B). These results suggested that some chemokines were preferentially induced during Th1- or Th2-biased responses and therefore may be responsible for the differences in patterns of cellular infiltration during these responses.

Lung cellular infiltrates induced by the supernatant treatments were assessed by doing cell counts and cytospin preparations of BAL cells. An average of $1.08 \times 10^6$ and $1.37 \times 10^6$ cells were collected from BAL of Th1 and Th2 supernatant-treated mice, respectively (an average of $0.24 \times 10^6$ BAL cells were collected from control mice, $n = 4$). These numbers were comparable with those from Th1 and Th2 cell-transferred mice ($1.66 \times 10^6$ vs $1.78 \times 10^6$) (17). Interestingly, while the Th1 supernatant preferentially induced neutrophil infiltration, the Th2 supernatant induced a high percentage of eosinophils in the BAL (Fig. 1C), correlating with an increase in eotaxin expression (Fig. 1, A and B). These results demonstrated that Th2 cytokines, in relatively physiological proportions, were able to induce a moderate lung eosinophilia, which probably was mediated by the up-regulation of certain eosinophil chemoattractants, including eotaxin.

While the treatments with T cell supernatants were able to induce marked recruitment of inflammatory cells into BAL, histological observations revealed that the peribronchial and perivascular infiltrates were not as severe as those characteristic of T cell-mediated inflammation (not shown) (17). This contrast illustrates the point that while cytokine effects can be revealed in the lung using intranasal administration, anatomic considerations may influence tissue pathology and animal physiology. Thus, activation of allergen specific T cells in the peribronchial spaces may cause highly localized induction of chemokine expression and subsequent preferential recruitment of eosinophils into peribronchial spaces. Activated eosinophils in these areas are likely to have more significant effects on airway hyperreactivity than those activated in alveolar spaces.

**Individual Th2 cytokines induce different chemokine expression patterns**

We next determined the relative contributions of various Th2 cytokines to the induction of eotaxin and eosinophilia. The major cytokines produced by Th2 cells are IL-3, IL-4, IL-5, IL-10, and IL-13 (Table I). Since previous studies reported that IL-10 is an inhibitory
rather than a stimulatory cytokine on lung eosinophilia and asthma (25, 26), we only tested the effects of IL-3, IL-4, IL-5, and IL-13 on chemokine expression and cellular infiltration. As noted above, we cannot be certain of the exact amounts and kinetics of T cell production of cytokines in the lung, so we aimed to at least retain the relative proportions of Th2 cytokines in these studies, based on the ELISA analysis of Th2 supernatant preparations (Table I). In addition, we again kept with a protocol limited to a 2.5-day stimulation to mimic the previous studies on adoptively transferred T cells. Recombinant cytokines were used, either individually or in combination, at concentrations double those in the first batch of Th2 supernatant to treat naive BALB/c mice. We found that these cytokines induced different patterns of chemokine production (Fig. 2, A and B). Compared with controls treated with culture medium alone, IL-4 and IL-5 induced...
significantly higher levels of MIP-2 and KC production; IL-4 also increased the production of MCP-1 (Fig. 2, A and B). Eotaxin was mainly induced by IL-13 and to a lesser extent by IL-4 (Fig. 2, A–C). In combination, these cytokines did not induce significantly higher expression of any chemokine compared with that induced by individual cytokines (Fig. 2, A–C), suggesting that there is no synergy among these Th2 cytokines, at least for the induction of chemokine expression. To rule out a role for host lymphocytes in any secondary cytokine or chemokine induction, SCID mice were treated and found to have the same response as normal BALB/c mice (Fig. 2 C).

Most cytokines induced a slightly increased cellular infiltration in the BAL of mice given these brief (2.5-day) treatments. Compared with an average of $2.4 \times 10^5$ BAL cells/mouse from medium-treated mice, IL-3 induced $3.8 \times 10^5$ BAL cells, with $2.2 \times 10^5$ in IL-4, $2.8 \times 10^5$ in IL-5, and $3.3 \times 10^5$ BAL cells in IL-13-treated mice. When these cytokines were used together, an average of $5.1 \times 10^5$ cells was found in the BAL of treated mice. The proportions of different types of infiltrating cells were analyzed by differential counts of cytospin preparations. All cytokines except for IL-5 induced a moderate neutrophil infiltration in BAL (Fig. 2D). Although IL-13 and IL-4 induced eotaxin expression, these cytokines alone induced only a small increase in eosinophil numbers in BAL (Fig. 2D), suggesting either that the levels of chemokine expressed were not high enough, or other signals were required to induce a significant lung eosinophilia. The latter explanation seems more likely, as a combination of all four cytokines produced a moderate infiltration of eosinophils (Fig. 2D). Thus, while synergistic effects were not seen in the case of eotaxin induction, some synergy among cytokines was evident with regard to eosinophil recruitment.

**IL-13 is the major eotaxin-inducing cytokine produced by Th2 cells**

Eotaxin has been shown to be a potent eosinophil chemoattractant, so we focused further on the regulation of its production in the
Eotaxin was detected from BAL fluid and lung supernatants by ELISA were minced in RPMI with 10% FBS, and the supernatants were collected. Mice were sacrificed, lungs were perfused, BAL was collected, the left lungs were minced in RPMI with 10% FBS, and the supernatants were collected. Eotaxin was detected from BAL fluid and lung supernatants by ELISA (n = 3).

Among the Th2 cytokines tested, IL-13 was by far the most effective cytokine inducing eotaxin in the lung, up-regulating both the eotaxin mRNA expression and protein production (Fig. 2, A–C). Similar levels of eotaxin were detected in the BAL of mice treated with a combination of four cytokines (IL-3, IL-4, IL-5, and IL-13) as those in IL-13-treated mice, suggesting that IL-13 was the major eotaxin-inducing factor without any obvious synergistic effects among the cytokines tested (Fig. 2C).

In the lung, eotaxin was mainly produced by bronchial and alveolar epithelial cells (27, 28). Positive staining for eotaxin was detected in these cells, especially the bronchial epithelial cells, of both medium-treated (Fig. 3B) and IL-13-treated (Fig. 3F) mice, but the intensity of staining was much stronger in IL-13-treated lung. Low level eotaxin staining could also be detected in lung tissue from untreated normal mice, similar to the medium control (not shown). This result indicated that eotaxin protein was constitutively produced in the lung as previously reported (29, 30), and the level was increased by IL-13 treatment. The specificity of the staining was confirmed by the fact that adding an excess amount of recombinant eotaxin to the detecting Ab (Fig. 3, C and G) blocked the positive staining. Correlating with the increased eotaxin expression, IL-13 also caused a low but definite peribronchial and perivascular infiltration of eosinophils (Fig. 3, D and H), but the intensity of infiltration was much lower compared with that in the lung with an ongoing Th2 cell-mediated inflammation (17).

IL-4 has been reported to induce eotaxin production in human fibroblasts (15). To compare the effects of IL-4 and IL-13 on eotaxin induction in the lung, naive BALB/c mice were treated with different concentrations of IL-4 and IL-13, and eotaxin in lung tissues or in BAL were measured by ELISA. The levels of eotaxin produced in lung tissue or in BAL showed a dose-dependent response to the amounts of IL-4 or IL-13 administered. Compared with IL-13, IL-4 induced much lower levels of eotaxin even at higher doses (Fig. 4). This result indicates that IL-13 is much more potent than IL-4 in the induction of eotaxin in the lung.

The importance of IL-13 in stimulating eotaxin production in the lung was further tested by blocking its activity in Th2 supernatant using a neutralizing Ab. Addition of anti-IL-13 Ab to the Th2 supernatant effectively blocked the induction of eotaxin mRNA, with levels equivalent to those in control mice (Fig. 5A).

By contrast, anti-IL-4 or anti-IL-5 Ab treatment of the Th2 supernatant did not alter the level of eotaxin expression (not shown). In confirmation of the effects on mRNA levels, eotaxin protein levels in both lung tissue and BAL were also reduced when IL-13 was blocked in the supernatant (Fig. 5B); in lung tissue, where higher concentrations of eotaxin were detected, the levels of eotaxin protein were also reduced close to the control levels (Fig. 5B). This result again suggested that IL-13 is the major eotaxin-inducing cytokine produced by Th2 cells. Correlating with the reduced eotaxin expression in the BAL, a slight reduction (29%) in eosinophil infiltration was noted in the BAL of mice given anti-IL-13-treated Th2 supernatant (Fig. 5C). Although anti-IL-4 or anti-IL-5 Abs did not inhibit eotaxin expression induced by Th2 supernatant, these Abs significantly reduced eosinophil infiltration in BAL by 26 and 58%, respectively. This result suggests that while IL-4 and IL-5 may have important roles in the induction of lung eosinophilia, they may act through mechanisms distinct from those involving IL-13. This would help explain why the IL-4, IL-5, and IL-13 appear to have synergistic effects in eosinophil recruitment.

**FIGURE 4.** IL-13 is more potent than IL-4 in eotaxin induction in the lung. Naive BALB/c mice were treated with different concentrations of rIL-4 or rIL-13, 50 μg treatment, three treatments/day for 2.5 days. Mice were sacrificed, lungs were perfused, BAL was collected, the left lungs were minced in RPMI with 10% FBS, and the supernatants were collected. Eotaxin was detected from BAL fluid and lung supernatants by ELISA (n = 3).

**TNF-α synergizes with IL-13 for eotaxin induction in the lung**

In lung epithelial cells and human fibroblasts, TNF-α substantially increased the level of eotaxin expression induced by IFN-γ and IL-4, respectively, suggesting a synergistic effect between TNF-α and those cytokines (13–15). Although Th2 cells produce very low concentrations of TNF-α, larger amounts of TNF-α are likely to be produced by macrophages, endothelial cells, and other cell types in response to inflammatory stimuli. In addition, clinical studies have shown that elevations of TNF-α are readily detected in BAL from patients with allergic lung inflammation (31–34). To study the potential effect of TNF-α on IL-13-induced eotaxin in lung tissue, TNF-α and IL-13 were used in combination to treat naive BALB/c mice. Although TNF-α by itself did not induce much eotaxin, it significantly increased the eotaxin release induced by IL-13 at two different concentrations (200 or 600 ng/ml IL-13 and TNF-α) (Fig. 6). The cellular infiltration in BAL was analyzed in mice given a low dose of cytokines (200 ng/ml). In combination, these two cytokines also induced more eosinophil infiltration, 7.6% of total BAL cells vs 0.6% in IL-13 alone and 0.3% in TNF-α alone group (n = 4).

**IL-13-induced eotaxin production by lung epithelial cells is JAK3 independent**

IL-13 mediates biological functions on multiple cell types through a receptor that shares a chain with IL-4 (35). The signaling pathway of IL-13 is not always similar to that of IL-4, and different JAKs are phosphorylated among different cell types treated with IL-13 (36–38). To test whether JAK3 is important for IL-13 signal transduction in airway epithelial cells, we treated JAK3-deficient mice (20) with IL-13 and measured eotaxin expression and eosinophil infiltration in the lung. After 2 days of treatment, similar levels of eotaxin were detected from the lung tissue of JAK3−/− and JAK3+/− littermate control mice (Fig. 7A), suggesting that JAK3 was not required for IL-13-mediated eotaxin production by lung epithelial cells. Curiously, however, eosinophilia was almost entirely absent in the JAK3−/− mice (Fig. 7B), suggesting that JAK3 is still critical in mediating signaling of another IL-13-inducible component required for eosinophil recruitment in the lung.

**Discussion**

Eotaxin belongs to the C-C chemokine subfamily and specifically serves as an eosinophil chemoattractant. It elicits a signal for eosinophil localization to the site of inflammation (39, 40) as well as...
initiating blood eosinophilia in the early phases of allergic inflammation (39, 41). Its involvement and contribution in the pathogenesis of allergic asthma therefore make it a critical target in the treatment of this disease. Eotaxin is produced by multiple cell types including epithelium, fibroblasts, smooth muscle cells, and eosinophils (42). In vitro studies have shown that eotaxin production by lung epithelial and dermal fibroblasts cells is up-regulated by IFN-γ in the presence of IL-1 or TNF-α (13, 14), and IL-4 also...
IL-13 is produced by Th2 cells and shares many of its biological functions with IL-4 in immune regulation. Accordingly, associations between IL-13 and asthma have been suggested by various studies. IL-13 was produced by BAL cells of atopic asthma patients after allergen challenge (43), and increased IL-13 mRNA was detected in the bronchial mucosa of asthma patients (44). Furthermore, IL-13 could be detected in BAL fluid or in the lung tissue in mouse asthma models induced either by OVA priming or by Th2 cell adoptive transfer (our unpublished data). Unlike IL-4, the production of IL-13 can be sustained through the late asthmatic response, and the concentration of IL-13 secreted strongly correlates with the number of eosinophils in BAL and in bronchial submucosa (45). Together, these results suggested that IL-13 might play an important role in asthma pathogenesis. Our finding that IL-13 potently induced eotaxin production in the lung therefore provided one mechanism of its action during asthma responses. Furthermore, the synergistic effects between IL-13 and TNF-α suggest a more potent effect of IL-13 in up-regulating eotaxin expression during allergic lung inflammation, under conditions where TNF-α will be present (31–34).

IL-13 exhibits pleiotropic biological functions on multiple cell types, and it shares one chain of its receptor with IL-4. While JAK3 is one of the kinases transducing signals by the IL-4 receptor (46), the signaling pathway of IL-13 seems to be quite variable, given that different JAKs are phosphorylated in different cell types treated with IL-13. For example, IL-13 induces JAK3 phosphorylation in primary human NK and T cells (36), but phosphorylation was increased instead on Tyk2 in EBV-immortalized B cells treated by IL-13 (37), and in human colon carcinoma cell lines IL-13 induced phosphorylation and activation of JAK2 (38). In the present study, we tested the potential involvement of JAK2 in IL-13 signaling in airway epithelial cells. We found that IL-13 could induce eotaxin in the lung of JAK3-deficient mice at levels similar to those in the control mice, but it failed to induce eosinophil infiltration. This result suggested that JAK3 was not the kinase transducing IL-13 signals in airway epithelial cells, but this applied only to the induction of eotaxin expression. Since the recruitment of eosinophils involves multiple steps and factors, the lack of eosinophil infiltration in JAK3−/− mice could be due instead to a lack of adhesion molecule up-regulation, lack of eosinophil activation, or reduced eosinophil viability. It is known that IL-13 induces VCAM-1 expression on vascular endothelium (47), and IL-13 may also act directly on eosinophils (48). Either or both of these effects may depend on JAK3-mediated signaling.

Although eotaxin is very potent in local recruitment of eosinophils and contributes largely to the development of lung eosinophilia (4), its up-regulation alone is not sufficient to induce severe eosinophilia in the lung. Thus, neutralization of IL-13 and abrogation of eotaxin induction still allowed for significant eosinophilia induced by IL-4 and IL-5. Eotaxin is constitutively expressed in a number of tissues, including the lung and intestine. Constitutive expression of eotaxin in intestine was required to maintain a baseline level of tissue eosinophils, which may serve as a defense against parasite infection (49). However, unlike the intestine, constitutive expression of eotaxin in the lung was not sufficient to induce eosinophil infiltration into the lung tissue, and even at an up-regulated level only a minor eosinophilia was induced (Figs. 2 and 3H). This might be due to a lack of certain additional signals in the lung required for large numbers of eosinophil infiltration, including the possible direct role of IL-4 and IL-5 in eosinophil recruitment and survival, and secondary induction of additional chemokines. It is likely that the observed synergy between Th2 cytokines is critical in efficient induction of eosinophilia, since recombinant IL-13 alone was clearly not sufficient to induce maximal eosinophil recruitment, even allowing for possible secondary induction events during the 2.5-day treatment protocol.

Allergic asthma is often associated with Th2-biased responses and can be induced by adoptively transferred Th2 cells in animal models. It has been reported that the eotaxin-specific receptor CCR3 is expressed on Th2 cells, and eotaxin is chemotactic for Th2 cells in vitro (50, 51). It is possible that besides recruiting eosinophils, eotaxin also induces local migration and activation of allergen-specific Th2 cells during asthma. The recruited Th2 cells could produce additional IL-13 and IL-4 to induce more eotaxin expression. Therefore, during allergic inflammatory responses, there may be a positive loop formed between Th2 cells and lung epithelial cells that amplifies the intensity of the inflammation. A break in this vicious cycle, especially in IL-13-mediated eotaxin expression, could help stop disease progression.

It has been reported that Th2 cytokines induce tissue eosinophilia through several different functions. Thus, IL-3 activates eosinophils and promotes their differentiation (52, 53). IL-4 induces endothelial VCAM-1 expression, which together with β1 integrin very late Ag-4 expression on eosinophils increases eosinophil adherence to the vessels (54, 55). IL-5 induces differentiation and proliferation of bone marrow eosinophils, inducing blood eosinophilia, and activates or primes eosinophils and prolongs their survival in vitro (56–59). GM-CSF also stimulates the proliferation, differentiation, migration, activation, and survival of eosinophils in vitro (53, 60, 61). Since all these cytokines can be produced by Th2 cells and were detected in BAL, they could show synergistic effects on the induction of lung eosinophilia. This is consistent
with the observation in our study that none of the cytokines alone (GM-CSF also tested at 500 ng/ml, data not shown) was sufficient to induce a severe eosinophilia, but in combination they induced a moderate eosinophil infiltration in BAL. It has been reported that the expression of an IL-4 transgene in the lung (62), or administration of microgram concentrations of IL-5, induced significant lung and BAL eosinophilia (63, 64). While it suggests that higher concentrations of certain cytokines may overcome the requirement of other cytokines in inducing eosinophil infiltration, these cytokines may also induce secondary production of the additional cytokines necessary for eosinophil recruitment.

IL-13 is an important Th2 cytokine that regulates immune responses and affects functions of many immune effector cells (65). In addition to other studies showing an association between IL-13 and asthma, our result provides a direct link between IL-13 and eotaxin production by airway epithelial cells, making it another potential target for asthma therapy. Additionally, the finding that knockout of JAK3-dependent mechanisms can prevent eosinophilia even in the face of eotaxin expression suggests additional directions for therapies.

References