Flt-3 Ligand Reverses Late Allergic Response and Airway Hyper-Responsiveness in a Mouse Model of Allergic Inflammation

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Flt-3 Ligand Reverses Late Allergic Response and Airway Hyper-Responsiveness in a Mouse Model of Allergic Inflammation

Jehad H. Edwan,*§ Greg Perry,* James E. Talmadge,¶ and Devendra K. Agrawal2*†‡§

Flt3 ligand (Flt3-L) is a growth factor for dendritic cells and induces type 1 T cell responses. We recently reported that Flt3-L prevented OVA-induced allergic airway inflammation and suppressed late allergic response and airway hyper-responsiveness (AHR). In the present study we examined whether Flt3-L reversed allergic airway inflammation in an established model of asthma. BALB/c mice were sensitized and challenged with OVA, and AHR to methacholine was established. Then mice with AHR were randomized and treated with PBS or 6 μg of Flt3-L i.p. for 10 days. Pulmonary functions and AHR to methacholine were examined after rechallenge with OVA. Treatment with Flt3-L of presensitized mice significantly suppressed (p < 0.001) the late allergic response, AHR, bronchoalveolar lavage fluid total cellularity, absolute eosinophil counts, and inflammation in the lung tissue. There was a significant decrease in proinflammatory cytokines (TNF-α, IL-4, and IL-5) in bronchoalveolar lavage fluid, with a significant increase in serum IL-12 and a decrease in serum IL-5 levels. There was no significant effect of Flt3-L treatment on serum IL-4 and serum total IgE levels. Sensitization with OVA significantly increased CD11b+CD11c+ cells in the lung, and this phenomenon was not significantly affected by Flt3-L treatment. These data suggest that Flt3-L can reverse allergic airway inflammation and associated changes in pulmonary functions in murine asthma model. The Journal of Immunology, 2004, 172:5016–5023.

A

llergic asthma is a chronic disease of the lungs associated with bronchoconstriction and chronic inflammation of the airways. Several immune cells and mediators are responsible for aggravation and development of allergy and asthma (1). Consequently, it is not surprising that there are many possible modalities to treat the ailment (2–7). Since the identification of distinct Th cell subsets that differ in cytokine production and effector functions as they relate to allergic asthma, a challenging concept has evolved to allow the use of therapeutic modalities that will modulate Th1/Th2 balance in asthma without deleterious side effects (8, 9). Primarily, these concepts have focused on modulation of Ag presentation to T cells and inhibition of Ag-specific Th2 responses and/or increasing the Th1 response (10). Thus, the suppression of Th2 cytokines (IL-4, IL-5, and IL-13) has been implicated as an approach to suppress airway inflammation and hyper-responsiveness. In this regard, several studies have been reported that detail the prevention of Th2-mediated allergic airway inflammation.

In recent years various immunomodulators, including anti-IgE (11); anti-cytokine Abs (12, 13); probiotics (14); CpG (15); synthetic YpG, CpR, and YpR immunostimulatory motifs (16); and mycobacterial Ags (17), which can up-regulate a type 1 T cell response, have shown therapeutic activity for allergen-induced asthma in both experimental animal models (2, 18) and a few clinical studies (19, 20). However, many of these immunomodulators elicit serious side effects. For example, treatment with mycobacterial Ags may cause latent infection and loss of delayed-type hypersensitivity skin testing for mycobacterial infection (21, 22). IL-12 therapy elicits several toxic effects, including a flu-like syndrome, transient increases in serum hepatic transaminases, and cardiac arrhythmia (23).

Flt3 ligand (Flt3-L), a recently described cytokine (24), has been shown to play a central role in the proliferation and differentiation of early hemopoietic precursor stem cells in both humans and mice (25–27). It has also been shown to induce proliferation and differentiation of dendritic cells (DCs) (28, 29), which have potent immunoregulatory properties (30). One subset of DCs (CD8α+; also known as lymphoid DCs) (31) secretes relatively higher concentrations of type 1 cytokines, such as IL-2 and IL-12, and another subset (CD8α+; also known as myeloid DCs) secretes relatively higher concentrations of type 2 cytokines, such as IL-4 and IL-5 (32). Flt3-L treatment induces a preferential increase in IL-12 and Th1 responses by DCs (33, 34). As type 2 T cells predominate in asthma, and IL-12 production is clearly correlated with sensitization of Th1 lymphocytes in vitro and in vivo (35), Flt3-L has the potential to modulate the type 1/type 2 T cell profile in allergic asthma.

Recently, we reported that treatment with Flt3-L could prevent the development of asthma-like conditions in a mouse model with complete abolition of AHR to methacholine (36). However, from a clinical point of view it will be important to examine the ability

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3 Abbreviations used in this paper: Flt3-L, Flt3 ligand; AHR, airway hyper-responsiveness; BAL, bronchoalveolar lavage; BALF, BAL fluid; DC, dendritic cell; EAR, early allergic response; LAR, late allergic response; PAS, periodic acid-Schiff; Penh, enhanced pause.

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of Flt3-L to suppress pre-existing AHR and airway resistance associated with the early allergic response (EAR) and the late allergic response (LAR). We hypothesized that treatment with Flt3-L in OVA-presensitized and -challenged mice would reverse and suppress existing allergic airway inflammation and associated clinical correlates of established asthma in mice. We observed, for the first time, that previously existing AHR and airway resistance during the LAR in presensitized and challenged mice could be reversed with Flt3-L treatment.

Materials and Methods

Animals

Four- to 5-wk-old female BALB/c mice were purchased from Harlan Laboratories (Indianapolis, IN) and were housed in separate cages according to treatment protocol. Food and water were provided ad libitum. According to National Institutes of Health guidelines, the research protocol of this study was approved by the institutional animal care and use committee of Creighton University (Omaha, NE).

Sensitization and treatment

Allergic airway inflammation was induced by i.p. injection of 20 μg of grade V chicken egg OVA (Sigma-Aldrich, St. Louis, MO) emulsified in 2.25 mg of Imject alum (Pierce, Rockford, IL) in total volume of 100 μl on days 1 and 14, followed by aerosol sensitivity with 1% OVA for 20 min using an Ultra-Neb 90 nebulizer (De Villbiss, Somerset, PA) on days 28, 29, and 30. Mice were again challenged with OVA on day 32, and 24 h later AHR to methacholine was established (Fig. 1). Starting on day 33, 29, and 30. Mice were again challenged with OVA on day 32, and 24 h thereafter using an Ultra-Neb 90 nebulizer (De Villbiss, Somerset, PA) on days 28, 29, and 30. Mice were again challenged with OVA on day 32, and 24 h later AHR to methacholine was established (Fig. 1). Starting on day 33, OVA-sensitized mice with AHR were randomized into two groups; the later AHR to methacholine was established (Fig. 1).

2.25 mg of Imject alum (Pierce, Rockford, IL) in total volume of 100 μl was injected into the LAR in presensitized and challenged mice could be reversed.

Noninvasive method for measuring pulmonary function

A single-chamber, whole-body plethysmograph (Buxco Electronics, Troy, NY), without the use of anesthesia or restraint, was used to measure pulmonary functions. This method has been demonstrated to accurately reflect airway resistance, expressed as Penh units (37), in asthma models (38). On day 44, 24 h after OVA challenge, mice were again placed within the chambers and challenged with increasing doses of methacholine (Sigma-Aldrich) to measure AHR, and Penh in response to methacholine challenge was recorded and expressed as the absolute Penh units.

Serum IgE analysis

Blood collected after sacrifice on day 44 was immediately centrifuged, and serum was separated and stored at −80°C for later analysis. ELISA for total IgE was conducted as previously described (39) and according to the manufacturer’s recommendations using rat anti-mouse IgE (BD PharMingen, San Diego, CA), IgE standard (BD PharMingen), and rat anti-mouse IgE-HRP (Southern Biotechnology Associates, Birmingham, AL) for the total IgE assay. IgE assays were developed with 3,3’,5,5’-tetramethylbenzidine substrate and read at 450 nm using a microplate reader and software.

Bronchoalveolar lavage (BAL)

BAL fluid (BALF) was collected from each animal via cannulation of the exposed trachea and gentle flushing of the lungs with 1 ml of warm sterile PBS. Total cell counts were determined by counting the cells in a hemocytometer. BALF was centrifuged, and the supernatant was collected and immediately frozen for later cytokine measurement. Cells were suspended in sterile PBS, and cyto centrifuge slides were stained (Hema 3 stain set; Biochemical Sciences, Swedesboro, NJ) for differential counts.

Cytokine assays

Serum cytokines (IL-4, IL-5, IFN-γ, and IL-12) were measured by ELISA. Ab pairs for individual cytokines as well as standards were used according
with collagenase D (1 mg/ml) in HEPES medium. CD11c
removing the blood, lungs were excised, washed with PBS, and digested
y, after
fl
choline lungs of animals were processed to isolate DCs using MACS
Phenotype of lung DCs

Immediately after the last challenge with methacholine, the lungs were
fixed in 10% formalin, dehydrated, mounted in paraf
fil
sioned by the periodic acid-Schiff
stained with H&E. Airway mucus was identi
flammation, 2 for minimal accumulation of in
flammatory cells, 3
flammation by two independent examiners. A score of 1 was given
chial in
ammatory response and mucous
cells were counted (cells per milliliter

Data analysis

Data were analyzed using GraphPad PRISM statistical analysis and graph-
ing software. Unpaired Student’s t test was used to determine differences
between two groups. Multiple group comparison was made using ANOVA.
A value of p < 0.05 was considered significant.

Results

Effect of Flt3-L treatment on EAR, LAR, and AHR in
OVA-presensitized and -challenged mice

Before treatment with Flt3-L or PBS, we confi
sensitized and -challenged mice had established AHR to metha-
choline (data not shown). Treatment with Flt3-L caused signi
cant suppression of Penh values for LAR (p < 0.01; Fig. 2B), with no
significant effect on EAR (Fig. 2A). Additionally, treatment with Flt3-L
induced a significant reduction in AHR in response to methacholine to levels comparable to those in PBS-treated mice (Fig. 3). None of the sensitized nontreated mice with pre-existing
AHR exhibited change in the EAR, LAR, and AHR.

Table 1. BAL fluid total and differential cell counts (× 10−3)a

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<tr>
<th></th>
<th>PBS</th>
<th>OVA</th>
<th>OVA/FL</th>
</tr>
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<tbody>
<tr>
<td>Total cells</td>
<td>65.00 ± 35.06</td>
<td>515.00 ± 86.72b</td>
<td>200.00 ± 25.00a</td>
</tr>
<tr>
<td>Macrophages</td>
<td>54.58 ± 28.43</td>
<td>111.44 ± 20.28</td>
<td>134.38 ± 3.88</td>
</tr>
<tr>
<td>Eosinophils</td>
<td>10.38 ± 7.28</td>
<td>388.25 ± 70.19a</td>
<td>58.25 ± 25.00a</td>
</tr>
<tr>
<td>Neutrophils</td>
<td>0.00 ± 0.00</td>
<td>11.61 ± 4.32b</td>
<td>4.25 ± 2.50</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>0.05 ± 0.05</td>
<td>3.71 ± 1.81</td>
<td>3.13 ± 1.38</td>
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a BALF (0.8 ml) was collected from each animal and centrifuged. Recovered total
cells were counted (cells per milliliter × 10−3), and differential analysis was per-
formed using standard morphological criteria on cytospin slides. At least 300 cells
were examined in each cytospin slide, and absolute cell numbers were calculated per
milliliter of BALF based on the percentage of individual cell in a slide. Shown are the
means ± SEM for six animals in each group.

b p < 0.05 compared to PBS group.

c p < 0.01 compared to OVA group.
d p < 0.001 compared to OVA group.
Effect of Flt3-L treatment on total and differential cells in BALF

OVA sensitization and challenge significantly increased the total number of cells in the BALF with predominant increase in eosinophils and neutrophils (Table I). Treatment with Flt3-L significantly lowered the total number of cells and eosinophils in BALF (Table I).

Effect of Flt3-L treatment on serum cytokines, serum total IgE, and serum total and anti-OVA IgG subclasses in OVA-presensitized and -challenged mice

Treatment with Flt3-L significantly increased serum IL-12 levels (Fig. 4C) and significantly decreased serum IL-5 levels (Fig. 4B). There was no significant effect of Flt3-L on serum IL-4 levels (Fig. 4A). IFN-γ levels were not detectable in any of the samples. Treatment with Flt3-L also caused a significant reduction in total and anti-OVA IgG subclasses (Table II). However, there was no significant change in the serum total IgE concentration (Fig. 4D).

Effect of Flt3-L treatment on BALF cytokines and inflammatory cells in presensitized and challenged mice

We observed a significant increase over control values in TNF-α, IL-2, IL-4, and IL-5 concentrations in the BALF of OVA-sensitized and challenged mice (Fig. 5). Interestingly, there was a generalized decrease in all these cytokines in the BALF of Flt3-L-treated mice. IFN-γ was not detectable in BALF.

Sensitization and challenge with OVA induced a significant influx of cells into the airways. Treatment with Flt3-L significantly reduced total cellular infiltration (p < 0.01). This reduction was especially prevalent in the number of infiltrating eosinophils (p < 0.0001).

Effect of Flt3-L administration on lung histology in Ag-presensitized and -challenged mice

To assess the histological effects of Flt3-L on allergen-induced airway inflammation, we analyzed lung tissues after the last methacholine challenge on day 44. In untreated, OVA-sensitized mice, eosinophil infiltration in the bronchial interstitium together with damaged epithelial cells lining was observed (Fig. 6). In addition, many mucus-producing cells, as assessed with staining to PAS, were observed in the airways of mice sensitized to OVA (Fig. 6) compared with PBS-treated mice. The airway inflammation was reversed by treatment with Flt3-L. Also, there was a significant reduction in PAS-positive cells in the lungs of the Flt3-L-treated group (Fig. 6).

<table>
<thead>
<tr>
<th>IgG Subclass</th>
<th>PBS</th>
<th>OVA</th>
<th>OVA/FL</th>
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<tbody>
<tr>
<td>Total IgG2a (µg/ml)</td>
<td>18.4 ± 1.0</td>
<td>33.5 ± 1.6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>21.2 ± 0.8&lt;sup&gt;c&lt;/sup&gt;</td>
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<tr>
<td>Anti-OVA IgG2a (U/ml)</td>
<td>0.004 ± 0.001</td>
<td>0.27 ± 0.08&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.22 ± 0.07&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Total IgG1 (µg/ml)</td>
<td>10.8 ± 1.3</td>
<td>31.5 ± 1.9&lt;sup&gt;b&lt;/sup&gt;</td>
<td>20.1 ± 3.0&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Anti-OVA IgG1 (U/ml)</td>
<td>0.002 ± 0.0008</td>
<td>0.37 ± 0.014&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.28 ± 0.005&lt;sup&gt;c&lt;/sup&gt;</td>
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<sup>a</sup> Effect of Flt3-L and Ag sensitization and challenge on IgG subclasses in mouse serum. Data are shown as the mean ± SEM from six animals in each group.

<sup>b</sup> p < 0.001 compared to PBS group.

<sup>c</sup> p < 0.001 compared to OVA group.

<sup>d</sup> p < 0.01 compared to OVA group.
FIGURE 6. Effect of Flt3-L treatment on airway inflammation in OVA-sensitized mice. Lungs were collected and fixed in 10% buffered formalin. Thin sections (8 μm) were cut, stained with H&E or PAS, and examined at ×10 and ×40 magnification. These data are representative (of at least six mice in each experimental group) lung histology of PBS-treated mice (upper panel), OVA-sensitized and -challenged (OVA) mice (middle panel), and OVA-sensitized and -challenged mice treated with Flt3-L (OVA/FL) for 10 days (lower panel). In the OVA group, massive peribronchial infiltration with eosinophils, thickening of the basement membrane, and de-epithelialization were seen. In contrast, after treatment with Flt3-L, an intact bronchial epithelial layer and no eosinophil infiltration were observed, and histology was comparable to that in the PBS-treated group. The mucus substances are stained in magenta by the PAS reaction. In the OVA group, there was strong staining to PAS (middle panel), which was significantly reduced by Flt3-L treatment (lower panel).

Effect of Flt3-L administration on lung DCs

OVA sensitization and challenge induced a significant increase in CD11c⁺CD11b⁺ (DC2) cells in the lungs (46.2 ± 3.3%) compared with PBS treatment (11.2 ± 0.6%; Table III and Fig. 7). There was no significant change in CD11c⁺CD8α⁺ (DC1) cells in the OVA-sensitized and -challenged group. Interestingly, Flt3-L did not show any significant effect on either CD11c⁺CD11b⁺ cells or CD11c⁺CD8α⁺ (Table III). However, Flt3-L treatment induced a significant increase in CD11c⁺CD11b⁻ (10.4 ± 0.7%) and CD11c⁺CD8α⁻ (21.6 ± 2.7%) cells (Table III).

Discussion

This report presents evidence that Flt3-L is capable of modulating the immune allergic response to Ag in OVA-presensitized and -challenged mice with established AHR. This model of allergic airway inflammation has been reported to elicit type 2 T cell responses and is characterized by eosinophilic inflammation. The inability of Flt3-L to significantly attenuate EAR was consistent with the finding that Flt3-L treatment had no effect on either serum IgE or IL-4 levels. Several other immunomodulators, including mycobacterial Ags, have been reported to exert no effect on serum IgE or IL-4 levels and failed to prevent EAR (2). These data suggest that immunomodulators such as Flt3-L can significantly attenuate LAR and AHR without affecting EAR. Indeed, Kay and colleagues (41) have reported that LAR and AHR are not dependent on EAR.

Table III. Phenotypic characteristics of lung DCs

<table>
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<tr>
<th>Phenotype</th>
<th>DC Population</th>
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<th>OVA</th>
<th>OVA/FL</th>
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<tr>
<td>CD11c⁺CD11b⁻</td>
<td>11.3 ± 0.5</td>
<td>4.6 ± 0.1ᵇ</td>
<td>10.4 ± 0.7ᵇ</td>
<td></td>
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<tr>
<td>CD11c⁺CD11b⁺</td>
<td>11.2 ± 0.6</td>
<td>46.2 ± 3.3ᶜ</td>
<td>41.1 ± 1.7</td>
<td></td>
</tr>
<tr>
<td>CD11c⁻CD8α⁺</td>
<td>DC1</td>
<td>8.2 ± 0.2</td>
<td>5.1 ± 0.7ᶜ</td>
<td>4.8 ± 0.2</td>
</tr>
<tr>
<td>CD11c⁻CD8α⁻</td>
<td>8.9 ± 0.5</td>
<td>14.2 ± 0.6ᵇ</td>
<td>21.6 ± 2.7ᵇ</td>
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*Effect of Flt3-L and Ag sensitization and challenge on CD11c⁻ enriched cells in the lungs of mice discussed in Fig. 7. The percentage of cells is shown as the mean ± SEM from four to six animals in each group.

ᵇ p < 0.05 compared to PBS group.
ᶜ p < 0.05 compared to OVA group.
ᵈ p < 0.001 compared to PBS group.

FIGURE 7. Representative flow cytometric contour plots showing the fluorescence characteristics of lung DCs. Lungs from nonsensitized (PBS), OVA-sensitized and challenged (OVA), and OVA-sensitized and challenged mice treated with FL (OVA/FL) were digested with collagenase D. CD11c⁺ cells were isolated by positive selection, followed by labeling with FITC-conjugated CD8α or CD11b Ab and PE-conjugated CD11c antibody. Cumulative data from several animals are shown in Table I.
The role of IL-4 has been identified in the development of allergic inflammation. Augmented levels of IL-4 in BALF (42) and increased expression of IL-4 mRNA in BAL cells (43, 44) have been recognized in patients with allergic inflammation. In murine models, IL-4 has been reported to be important for the development of allergic airway inflammation (45–47) and AHR (48, 49). In our study we observed a significant decrease in BALF IL-4 levels. However, there was no significant effect of Flt3-L treatment on serum IL-4 and serum IgE levels. This suggests that the abrogation of AHR and asthma-like parameters is independent of IL-4 and IgE. Alternatively, it is possible that 10-day treatment with Flt3-L might not be sufficient to remove previously existing antibodies. This was supported by a recent publication by Peng and colleagues (50), who reported that vaccination with CpG oligodeoxynucleotides prevented the induction of IgE production by B cells, but did not eliminate previously produced IgE. In addition, IgE might not be required for the development of eosinophilic airway inflammation and AHR in mice, as reported by several investigators (5, 51, 52).

There have been several reports associating the elevated production of IgG2a with the Th1 response (53–55). However, IgG1 has been associated with atopic allergy and asthma (56). In this study we observed a significant increase in both total and anti-OVA IgG subclasses (IgG2a and IgG1) after OVA sensitization and challenge. Flt3L caused a significant reduction in both total and anti-OVA IgG subclasses (Table II). Our results are in agreement with a previous study (57) in which mice were treated with Flt3-L for 10 days and then immunized with hen egg lysozyme, and Flt3L treatment significantly lowered the levels of anti-hen egg lysozyme IgG subclasses in serum compared with those in the nontreated group, indicating that Flt3-L treatment modulated the induction of the Ab response. Interestingly, treatment with toxins, such as cholera toxin (58) and tetanus toxoid (58), after Flt3-L stimulation resulted in significantly higher levels of IgGs. Thus, Flt3-L has the potential to elicit immunomodulatory effects.

The role of IL-5 in the development of AHR and allergic airway inflammation has been controversial. In our study we observed a significant increase in both serum and BALF IL-5 levels after Ag sensitization and challenge, and IL-5 levels were significantly attenuated by Flt3-L treatment. These data suggest that the reduction in IL-5 levels after Flt3-L might be one of the factors involved in the attenuation of AHR to methacholine. However, we have yet to examine the mechanistic relationship between the inhibitory effects of Flt3-L on AHR, inflammation, and goblet cell hyperplasia and the decrease in IL-5 levels.

The precise mechanism by which Flt3-L treatment mediates its effects is not present not clear, but may be due to potential differences in the repertoire of the DC subsets (59). The factors regulating T cell cytokine polarization are versatile, including DCs, which have an important role in the control of the adaptive immune response (60), implying the functional flexibility of DCs to direct T cell activation into either Th1 or Th2 polarized effectors. The effect of Flt3-L administration on the development and immunogenicity of mouse spleen DCs has been reported (29, 30, 59, 61, 62). However, studies of the effect of Flt3-L on the generation and phenotype of mouse lung DCs are scarce. In a recently published study on the effect of Flt3-L on the lungs by Pabst and colleagues (58), a single dose of 100 μg of Flt3-L intratracheally resulted in an enlargement of the perivascular space in the lung and a significant and rapid expansion of DCs and T cells in the interstitial space. In the lungs, immature DCs act as a sentinel. After Ag challenge they migrate to the draining lymph nodes and efficiently present the captured and processed Ag to Ag-specific naive T cells, initiating the development of the primary effecter T cell response (63). In this study we observed that allergic airway inflammation due to sensitization and challenge with OVA induced a marked increase in the total number of lung CD11c+CD11b+ cells. This increase in DC2-type cells could be due to increased levels of cytokines, such as TNF-α and GM-CSF, that are involved in differentiation of immature DCs to mature DCs. We have not measured GM-CSF levels in this study. However, Flt3-L treatment significantly decreased BALF TNF-α levels with no effect on DC2 cells. This might suggest that cytokines other than TNF-α are involved in the differentiation and maturation of DC2 cells in the lung.

Flt3-L treatment significantly altered the profile of CD11c+ in the lungs, with an increase in the number of CD11c+CD11b− (p < 0.05) and CD11c+CD8α− cells. However, there was no effect of Flt3-L on either CD11c+CD8α− or CD11c+CD11b+ cells. Therefore, the increase in total CD11c+ cells could be due to immature DCs, which have been implicated in the induction of peripheral tolerance (64). It has recently been demonstrated that a distinct subset of DCs with the phenotype CD11c+CD45RBhigh has the ability to secrete IL-10 and induce tolerance and T regulatory cell differentiation (65). It has also been shown that IL-12 production by immature DCs is susceptible to inhibition by IL-10-producing T regulatory cells (66, 67). In addition, DCs induce selective expansion of regulatory T cells, producing immunosuppressive cytokines such as IL-10 and TGF-β (68–70). Therefore, it is reasonable to speculate that the Flt3-L-induced effects on LAR, AHR, and allergic airway inflammation are mediated via induction of immature DCs in the lungs. This could be supported by our recent finding that i.m. administration of Flt3-L plasmid increased CD11c+CD45R+ cells in the lungs by 5-fold (our unpublished observations). This suggests that marked proliferation of immature DCs expressing few or no costimulatory molecules in response to Flt3-L treatment might have the potential to induce tolerance.

In summary, we report that treatment with Flt3-L can suppress the LAR, AHR to methacholine, serum IL-5, and BALF IL-5 and IL-4, and significantly elevate serum IL-12 levels in established model of allergic airway inflammation. Although more investigation is warranted to define the duration of this effect and the dose-dependency of Flt3-L, these results support the hypothesis that Flt3-L may be an effective immunotherapeutic agent in the treatment of allergic airway inflammation and associated changes in pulmonary functions.

References