IL-10 reduces Th2 cytokine production and eosinophilia but augments airway reactivity in allergic mice

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Van Scott, Michael R., J. Paul Justice, John F. Bradfield, Edward Enright, Anastasia Sigounas, and Sanjiv Sur. IL-10 reduces Th2 cytokine production and eosinophilia but augments airway reactivity in allergic mice. Am J Physiol Lung Cell Mol Physiol 278: L667–L674, 2000.—We investigated the effects of interleukin (IL)-10 administration on allergen-induced Th2 cytokine production, eosinophilic inflammation, and airway reactivity. Mice were sensitized by intraperitoneal injection of ragweed (RW) adsorbed to Alum and challenged by intratracheal instillation of the allergen. Sensitization and challenge with RW increased concentration of IL-10 in bronchoalveolar lavage (BAL) fluid from undetectable levels to 60 pg/ml over 72 h. Intratracheal instillation of 25 ng of recombinant murine IL-10 at the time of RW challenge further elevated BAL fluid IL-10 concentration to 440 pg/ml but decreased BAL fluid IL-4, IL-5, and interferon-γ levels by 40–85% and eosinophil numbers by 70% (P < 0.0001). Unexpectedly, the same IL-10 treatment increased airway reactivity to methacholine in spontaneously breathing mice that had been sensitized and challenged with RW (P < 0.001). IL-10 treatment in naive animals or RW-sensitized mice challenged with PBS failed to increase airway reactivity, demonstrating that IL-10 induces an increase in airway reactivity only when it is administered in conjunction with allergen sensitization and challenge. The results demonstrate that IL-10 reduces Th2 cytokine levels and eosinophilic inflammation but augments airway hyperreactivity. Thus, despite its potent anti-inflammatory activity, IL-10 could contribute to the decline in pulmonary function observed in asthma.

interleukin-4; interleukin-5; interferon-γ; bronchial hyperreactivity; interleukin-10

CUMULATIVE EVIDENCE SUGGESTS that Th2 cytokines play important roles in inducing eosinophilic airway inflammation and bronchial hyperresponsiveness (BHR) characteristic of asthma (1, 16, 44). Expression of Th2 cytokines is increased in allergic inflammation and asthma (44); animals with low levels of interleukin (IL)-4 and IL-5 exhibit reduced eosinophilia and BHR (8, 10, 15, 28, 29, 32), and in the case of IL-5, overexpression enhances eosinophilia and BHR (25). In contrast, the Th1 cytokine interferon (IFN)-γ inhibits Th2 responses and attenuates allergen-induced eosinophilia, thereby inhibiting the onset of allergic asthma (6, 26, 38).

IL-10 is another Th2 cytokine, but its pathophysiologic role in asthma has not been clearly elucidated. Some evidence suggests that IL-10 production is reduced in patients with asthma compared with nonasthmatic control subjects (3), and murine studies provide evidence that IL-10 suppresses development of eosinophilic inflammation in the airways. Intranasal administration of recombinant IL-10 inhibits recruitment of eosinophils in allergic mice (47), and knockout of the IL-10 gene augments allergen-induced eosinophilic airway inflammation (12). However, the role of IL-10 in the development of BHR has not been defined.

We used a murine model of allergic asthma to investigate the effects of IL-10 on eosinophilic airway inflammation, the release of IL-4 and IL-5 into the airways, and development of airway hyperreactivity. In this model, treatment of ragweed (RW)-sensitized mice with a single dose of recombinant murine IL-10 (rmIL-10) at the time of RW challenge reduced IL-4 and IL-5 concentrations and eosinophil numbers in bronchoalveolar lavage (BAL) fluid but, unexpectedly, increased airway reactivity to methacholine. The results indicate that despite its potent anti-inflammatory activity, IL-10 may contribute to the decline in pulmonary function observed in asthma.

METHODS

Allergic sensitization of animals. Six- to eight-week-old female BALB/c mice were purchased from Harlan Laboratories (Indianapolis, IN) and separated into groups defined by the sensitization, challenge, and treatment protocols (Table 1). Mice were sensitized by intraperitoneal injection of RW given on day 0 and day 4. Each injection consisted of 200 μg of RW (lot 56-129, Greer Laboratories, Lenoir, NC) in 25 μl of Alum (45 mg/ml aluminum hydroxide and 40 mg/ml magnesium hydroxide; Pierce, Rockford, IL) and 75 μl of Coca’s buffer (85 mM NaCl and 64 mM NaHCO3, pH 8.1). Endotoxin content of the RW was <2.3 ng/mg.

On day 11, the mice were anesthetized with 90 mg/kg ketamine and 10 mg/kg xylazine and challenged by intratracheal instillation of 8 μg of RW in 100 μl of PBS. This dose of RW yielded moderate increases in inflammation and airway reactivity, allowing detection of potential inhibitory and stimulatory effects of IL-10. Recombinant murine IL-10 (rmIL-10) was administered on day 11 at the time of allergen challenge. IL-10 was purchased from R&D Systems (Minne-
Table 1. Definition of study groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Intraperitoneal Sensitization (days 0 and 4)</th>
<th>Intratracheal Challenge (day 11)</th>
<th>Day Studied</th>
</tr>
</thead>
<tbody>
<tr>
<td>Naive/–</td>
<td>None</td>
<td>None</td>
<td>11</td>
</tr>
<tr>
<td>Naive/PBS</td>
<td>None</td>
<td>PBS</td>
<td>14</td>
</tr>
<tr>
<td>Naive/PBS + IL-10</td>
<td>None</td>
<td>25 ng IL-10 in PBS</td>
<td>14</td>
</tr>
<tr>
<td>Naive/RW</td>
<td>None</td>
<td>8 µg RW in PBS</td>
<td>14</td>
</tr>
<tr>
<td>Naive/RW + IL-10</td>
<td>None</td>
<td>8 µg RW + 25 ng IL-10 in PBS</td>
<td>14</td>
</tr>
<tr>
<td>RW/–</td>
<td>200 µg RW in Coca’s buffer + Alum</td>
<td>None</td>
<td>11</td>
</tr>
<tr>
<td>RW/PBS</td>
<td>200 µg RW in Coca’s buffer + Alum</td>
<td>PBS</td>
<td>14</td>
</tr>
<tr>
<td>RW/PBS + IL-10</td>
<td>200 µg RW in Coca’s buffer + Alum</td>
<td>25 ng IL-10 in PBS</td>
<td>14</td>
</tr>
<tr>
<td>RW/RW</td>
<td>200 µg RW in Coca’s buffer + Alum</td>
<td>8 µg RW in PBS</td>
<td>14</td>
</tr>
<tr>
<td>RW/RW + IL-10</td>
<td>200 µg RW in Coca’s buffer + Alum</td>
<td>8 µg RW + 25 ng IL-10 in PBS</td>
<td>14</td>
</tr>
</tbody>
</table>

All treatments were conducted using 100 µl of solution. RW, ragweed; PBS, phosphate-buffered saline; Alum, aqueous solution of aluminum hydroxide and magnesium hydroxide; IL-10, interleukin-10; –, no challenge.

RESULTS

IL-10 levels in BAL fluid and serum after intratracheal administration. Endogenous IL-10 was undetectable in BAL fluid from six naive mice (Fig. 1). RW-sensitized animals exhibited a baseline IL-10 concentration in BAL fluid of 20 ± 10 pg/ml before allergen challenge (n = 6). RW challenge of sensitized mice induced a gradual increase in endogenous IL-10 concentration in BAL fluid, resulting in a maximum concentration of 60 ± 25 pg/ml being measured 72 h post challenge.

[IL-10] in BAL Fluid (pg/ml)

Fig. 1. Interleukin (IL)-10 concentration in bronchoalveolar lavage (BAL) fluid. Lungs of naive animals that were not challenged (naive/–) and animals that were sensitized but not challenged (Rw/PBS/–) were lavaged on day 11 at a time when allergen challenge was normally performed. Lungs of sensitized and challenged animals treated intratracheally with PBS or 25 ng of recombinant murine IL-10 at the time of allergen challenge were lavaged at 12, 24, or 72 h after allergen challenge. Values are means ± SE; n, no. of animals. ND, not detectable. Groups at each time point were compared by ANOVA, and significant P values are indicated.
after the challenge. Instillation of 25 ng of rmIL-10 into the trachea at the time of RW challenge elevated total IL-10 concentration in BAL fluid to 440 ± 61 pg/ml at 12 h. By 72 h after administration, total IL-10 concentration in BAL fluid had returned to the level measured on day 11 in unchallenged animals. Instillation of 300 ng of rmIL-10, the highest dose used in this study, resulted in a total IL-10 concentration in BAL fluid of 4,600 ± 950 pg/ml 12 h after administration (n = 6). As with the 25-ng dose, the concentration returned to baseline level by 72 h after administration (28 ± 14 pg/ml; n = 6).

The concentration of endogenous IL-10 in serum was 188 ± 6 pg/ml, and no changes were observed after sensitization and challenge with RW (n = 6). No change in serum IL-10 concentration was observed in response to the 25-ng dose of rmIL-10, but the 300-ng dose elevated serum concentration to 439 ± 61 pg/ml 12 h after administration (n = 6). By 24 h after administration, the serum concentration of IL-10 had fallen to 120 ± 23 pg/ml. These data demonstrate that allergic sensitization and challenge increase endogenous IL-10 levels in the airways, and a single intratracheal treatment with 25 ng of rmIL-10 supplements local levels for 12–24 h, with minimal effects on systemic levels.

IL-10 dose-dependent effects on airway inflammation. Intratracheal administration of rmIL-10 at doses of 2.5 and 25 ng/animal reduced the number of eosinophils recovered in BAL fluid 72 h after allergen challenge by 65 and 70%, respectively (P < 0.001; Fig. 2B). Doses as low as 2.5 ng/animal also reduced the number of macrophages, lymphocytes, and neutrophils recovered in BAL fluid 72 h after allergen challenge (Fig. 2). In contrast, the 300-ng dose did not reduce the number of eosinophils, macrophages, and lymphocytes in BAL fluid. The number of neutrophils recovered in BAL fluid was increased with the 300-ng dose.

In paraffin-embedded lung sections, moderate inflammation was observed in all groups, and no differences in perivascular and peribronchial inflammation were observed. Sections were scored from 0 to 4, with 0 representing no evidence of inflammation and 4 representing cuffing of the vessels/airways. Mean perivascular and peribronchial scores from sensitized and challenged mice treated with PBS were 2.1 ± 0.2 and 1.9 ± 0.1, respectively (n = 17 animals). Corresponding scores

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**Fig. 2.** Dose-dependent effects of intratracheal IL-10 on the cellular composition of BAL fluid. RW-sensitized and challenged animals were administered either PBS or rmIL-10 intratracheally at the time of allergen challenge; 72 h later, the lungs were lavaged, total number of cells recovered in the BAL fluid was determined (A), and differential cell counts were performed to calculate the number of eosinophils (B), lymphocytes (C), neutrophils (D), and macrophages (E) recovered. Values are means ± SE for 13–25 animals. *Significant difference from 0-ng rmIL-10 group, P < 0.05.
for the 25-ng rmIL-10 treatment group were 2.0 ± 0.1 and 1.8 ± 0.1 (n = 22), and scores for the 300-ng treatment group were 1.9 ± 0.2 and 1.6 ± 0.1 (n = 21). As discussed below, the differences between BAL findings and histology may result from a single dose of IL-10 delaying the onset of airway inflammation.

The increase in neutrophils at the 300-ng dose indicated that the treatment might have nonspecifically induced inflammation, perhaps through an increase in protein levels within the airway. To control for this possibility, IL-10 was heat inactivated before administration. No differences in cell counts were observed between animals treated with 0 and 300 ng of IL-10, including neutrophil counts (1,796 ± 600 vs. 1,848 ± 875 cells/ml, respectively). These data indicated that the inflammatory activity of the 300-ng dose was not because of the protein load. rmIL-10 was obtained from a different supplier to ensure that a minor contaminant was not responsible for the inflammation observed at high doses. A 300-ng dose of rmIL-10 from Sigma reduced the number of cells in BAL fluid from RW-sensitized and challenged mice by 49 ± 1% compared with that in animals not treated with IL-10 (n = 5). Eosinophil and neutrophil numbers in BAL fluid were decreased by 81 ± 1 and 74 ± 1%, respectively. These results supported the theory that a property unique to IL-10 obtained from R&D Systems induced inflammation at high doses.

Dose-dependent effects on cytokine secretion in vivo. The 2.5-, 25-, and 300-ng doses of rmIL-10 decreased IL-4 concentration relative to the vehicle control by 81, 75, and 69%, respectively (Fig. 3A). IL-5 concentration in BAL fluid was not altered by the 2.5-ng dose of IL-10 but was decreased 61% by the 25-ng dose and 41% by the 300-ng dose (Fig. 3B). In contrast to the Th2 cytokine, IFN-γ concentration was increased 70% by the 2.5-ng dose of rmIL-10 (Fig. 3C) and decreased 80% by the 25- and 300-ng doses. These results demonstrate that low doses of IL-10 are effective in reducing Th2 cytokine levels in the airways after allergen challenge.

Paradoxical effect on airway reactivity to methacholine. Airway resistance was measured in RW-sensitized animals that had been challenged with PBS or 8 µg of RW to determine the degree of hyperreactivity induced by allergen challenge in this model (Fig. 4). Baseline resistance was not different in the PBS- and RW-challenged groups [229 ± 54 cmH₂O·l⁻¹·s⁻¹ (n = 13) and 209 ± 45 cmH₂O·l⁻¹·s⁻¹ (n = 14), respectively]. RW induced a twofold increase in airway responsiveness to methacholine compared with the PBS control (P = 0.01; Fig. 4). Variability of resistance data and potential for undesirable effects on the cardiovascular system and loss of study subjects increased with increasing doses of intravenous methacholine. Subsequent evaluation of airway reactivity in IL-10-treated animals was therefore restricted to doses of methacholine < 300 µg/kg.

The previous results demonstrated that 25 ng of rmIL-10 decreased the number of eosinophils and IL-4 and IL-5 concentrations in BAL fluid. The effect of the 25-ng dose on R aw and development of hyperreactivity was therefore assessed. Treatment of sensitized and challenged mice with rmIL-10 (RW/RW + 25 ng of IL-10) increased resting R aw relative to untreated animals (RW/RW) and shifted the methacholine dose-response curve to the left (Figs. 5A and 6C). A decline in C dyn was also observed after treatment with rmIL-10, although the change was not significant (Fig. 5B). The changes in R aw and C dyn could not be attributed to changes in respiratory rate or tidal volume (Fig. 5, C and D).

The role of sensitization and challenge in IL-10 augmentation of airway reactivity was evaluated. For this analysis, the methacholine response was expressed as a percent change in resistance to compensate for differences in baseline resistance (Fig. 6). Naive mice treated with PBS or 25 ng of rmIL-10 exhibited no difference in airway reactivity to methacholine (Fig. 6A). No difference in airway reactivity was observed in naive mice that had been challenged with RW alone or...
challenged with RW plus 25 ng of rmIL-10 (naive/RW and naive/RW + 25 ng of IL-10 groups in Fig. 6B). Likewise, sensitized animals that were not challenged with RW did not exhibit an increase in airway reactivity after treatment with 25 ng of rmIL-10 (RW/PBS + 25 ng of IL-10 group in Fig. 6C). Only animals that had been sensitized and challenged with RW exhibited an increase in reactivity to methacholine after IL-10 treatment (RW/RW + 25 ng IL-10; Fig. 6C). These results indicated that IL-10 augments airway reactivity established by allergen sensitization and challenge as opposed to inducing reactivity de novo.

DISCUSSION

The findings of this study provide evidence that allergic sensitization and challenge increases IL-10 levels in the lungs and that augmenting endogenous levels with a single dose of rmIL-10 reduces allergen-induced eosinophilic inflammation and Th2 cytokine production. In contrast to these anti-inflammatory activities, administration of IL-10 increases airway hyperreactivity, resulting in an apparent dissociation between Th2-driven inflammation and decreased pulmonary function.

The increase in IL-10 levels in BAL fluid after sensitization and challenge with RW in our study is consistent with the findings in patients with asthma, as reported by Borish et al. (3) and Robinson et al. (35). These investigators found that IL-10 expression in sensitized human skin, lungs, and peripheral blood cells increases 6–48 h after allergen challenge, perhaps as a normal negative-feedback response to allergic inflammation. Differences in IL-10 expression between asthmatic and normal individuals were also reported. A greater number of IL-10-expressing cells has been reported in the lungs of asthmatic subjects (35), but measurements of protein in the BAL fluid have revealed a lower amount of IL-10 in asthmatic subjects compared with normal subjects (3). The discrepancy between the number of IL-10-expressing cells and protein levels may reflect reduced cellular expression of IL-10 in asthma. This idea is buttressed by the observation that cellular expression of IL-10 is low in allergic and nonallergic asthmatic children compared with normal controls (20). These observations and the findings of the current study indicate that allergen challenge induces recruitment of IL-10-expressing cells to the airways and increases IL-10 release, but in asthma, the recruited cells have a reduced capacity to produce...
IL-10. Given that the severity and time course of inflammation are increased in asthmatic subjects compared with nonasthmatic individuals, it is conceivable that cumulative exposure of the airways to IL-10 is actually greater in this group. A kinetic study is required to evaluate this possibility.

In the present study, a single 25-ng dose of IL-10 administered at the time of RW challenge significantly decreased eosinophil numbers 72 h later. Prior studies have shown that recruitment of eosinophils to the lungs is inhibited by IL-10. Zuany-Amorin et al. (47) demonstrated that a 100-ng dose of IL-10 reduced eosinophil infiltration into the airway wall and BAL compartment 24 h after intranasal ovalbumin challenge. Likewise, Grunig et al. (12) demonstrated that eosinophilic airway inflammation was augmented in the absence of endogenous IL-10. These studies indicate that induction of endogenous IL-10 by allergen challenge regulates allergen-induced eosinophilic lung inflammation.

Even though 25 ng of IL-10 inhibited BAL eosinophil recruitment, the lungs of the same animals failed to demonstrate corresponding changes in tissue inflammation. One explanation for this difference is that histological evaluation is less sensitive than BAL fluid analyses in detecting relatively small differences in inflammation. Alternatively, it could reflect the timing of the tissue collection relative to the IL-10 treatment. Zuany-Amorin et al. (47) examined lung histology 6 and 24 h after treatment with IL-10 and reported significant reduction in tissue inflammation. In that same study, BAL cell counts were determined 24 and 96 h after treatment. IL-10 treatment reduced the number of eosinophils in BAL fluid at the 24-h time point, but no difference was observed 96 h after treatment (Fig. 3B in Ref. 47). These observations suggest that a single dose of IL-10 delays but does not prevent the onset of eosinophilic inflammation. This delay could explain the differences in inflammation exhibited by BAL and lung tissue samples collected 72 h after IL-10 treatment in the present study.

IL-10 treatment at the time of RW challenge modulated the levels of IL-4, IL-5, and IFN-γ in the BAL fluids. These cytokines are known to regulate eosinophilic inflammation. IL-5 plays an important role in eosinophil differentiation and recruitment to the airways and in naive guinea pigs has been shown to induce airway hyperreactivity through activation of neurokinin-2 receptors (21). However, in the present study, the 2.5-ng dose of IL-10 decreased eosinophil numbers in BAL fluid but did not change IL-5 concentration. Ochiai et al. (31) have reported that IFN-γ inhibits differentiation of eosinophils. The 2.5-ng dose of IL-10 increased IFN-γ concentration in BAL fluid. Thus high levels of IFN-γ could have counteracted the proeosinophilic activity of IL-5. Another explanation for divergence in the IL-5 levels and eosinophilia is that IL-4 production was reduced by the 2.5-ng dose of IL-10. IL-4 induces differentiation of Th2 cells and promotes eosinophil recruitment. Two studies of knockout mice have demonstrated that IL-4 attenuates allergen-induced airway eosinophilia (5, 15). The main effect of IL-4 appears to be exerted during the sensitization process, since neutralizing IL-4 during allergen challenge does not inhibit eosinophilia (5, 15). It should be
noted, however, that another study failed to demonstrate a role for IL-4 in lung eosinophilia (22).

Pulmonary function testing revealed increased airway reactivity in mice treated with 25 ng of IL-10. In a study of wild-type and IL-10 knockout mice, Grunig et al. (12) failed to detect an effect of endogenous IL-10 on airway reactivity. In that study, animals were sensitized and challenged with doses of Aspergillus fumigatus antigen that resulted in a high rate of early mortality and marked airway hyperresponsiveness to ACh. The dose of intravenous ACh that induced a 200% change in R\textsubscript{aw} was 300 µg/kg body wt in allergic animals vs. 2,240 µg/kg body wt in control animals (Table 1 in Ref. 12). In contrast, the low dose of RW used in the present study yielded moderate pulmonary inflammation and airway hyperreactivity with no premature death. The moderate degree of reactivity induced by the low-dose RW challenge in the present model may have allowed detection of an increase in airway reactivity that was masked in the previous study.

The finding that IL-10 treatment decreased BAL eosinophilia but increased airway reactivity to methacholine was surprising, since evidence from human and guinea pig studies indicates that eosinophils play a major role in development of BHR (9, 11, 42). However, the role of eosinophils in induction of hyperreactivity in mice is controversial (34). Foster et al. (10) demonstrated a correlation between eosinophilia and development of hyperreactivity in mouse airways. Other studies, however, have failed to find extracellular deposition of major basic protein or other evidence of eosinophil activation in mouse lungs (25, 40). In addition, inhibition of eosinophil infiltration into the airways is not always linked to decreased hyperreactivity to methacholine (13, 19) and, as observed in the present study, can be associated with increased airway reactivity. Finally, a recent study performed in patients with asthma also demonstrated a dissociation between eosinophil numbers and airway reactivity (7). Thus pathophysiological responses to eosinophilic inflammation appear to be complex, and consideration of alternative explanations for airway reactivity is warranted.

There is evidence that cytokines and chemokines like IFN-γ and monocyte chemoattractant protein-1 (MCP-1) play a role in development of airway reactivity. In guinea pigs, IFN-γ enhances β-adrenergic-mediated relaxation of airway smooth muscle (4), and under this scenario, reduction in IFN-γ levels by IL-10 treatment could augment airway reactivity. However, there is also evidence that IFN-γ induces airway hyperreactivity in a mouse model (14), perhaps through the release of excitatory cytokines and chemokines from smooth muscle cells (17, 18, 23, 24). Whether changes in IFN-γ levels can be the explanation for the IL-10-induced airway reactivity observed in this study is unclear. IL-10-induced airway reactivity may be linked to release of histamine. IL-10 increases murine mast cell and basophil proliferation, differentiation, and degranulation (30). IL-10 stimulates MCP-1 production (37, 39), and MCP-1 is a potent stimulator of histamine release. Depletion of MCP-1 has been shown to reduce airway reactivity without attenuating eosinophilia (27). It is therefore plausible that airway reactivity induced by the 25-ng dose of IL-10 resulted from an increase in MCP-1-stimulated histamine release from mast cells and basophils.

In summary, this study demonstrates that IL-10 accumulation in the airways is increased in response to allergic sensitization and challenge. Supplementing endogenous levels with low doses of recombinant IL-10 decreases accumulation of proinflammatory TH2 cytokines, IL-4 and IL-5, in the airways and reduces allergen-induced eosinophilic airway inflammation. In contrast to these anti-inflammatory activities, administration of IL-10 increases airway hyperreactivity. This effect could contribute to the decline in pulmonary function observed in asthma.

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REFERENCES


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