Effects of IL-13 on airway responses in the guinea pig

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Morse, Brian, Joseph P. Sypek, Debra D. Donaldson, Kathleen J. Haley, and Craig M. Lilly. Effects of IL-13 on airway responses in the guinea pig. Am J Physiol Lung Cell Mol Physiol 282: L44–L49, 2002; 10.1152/ajplung.00296.2001.—Levels of interleukin (IL)-13 are increased in asthmatic airways. IL-13 has been shown to be necessary and sufficient for allergen-induced airway hyperresponsiveness and increased inflammatory cell counts in bronchoalveolar lavage (BAL) fluid in a murine model of asthma but is thought to protect against airway inflammation when low doses are provided to the guinea pig lung. To determine the role of IL-13 in the guinea pig, we studied the effects of a 360-μg/kg dose of nebulized IL-13 in naive animals and of IL-13 abrogation after airway challenge of sensitized animals. Nebulized IL-13 significantly decreased the dose of histamine required to double baseline respiratory system resistance (ED100, 22 ± 3 vs. 15 ± 2 nmol/kg; P < 0.05) and was associated with recovery of significantly greater numbers of macrophages, lymphocytes, eosinophils, and neutrophils in BAL fluid. Guinea pigs pretreated with a fusion protein that binds IL-13 [soluble IL-13 receptor (sIL-13Rα2)] were protected from developing antigen-induced airway hyperresponsiveness (ED100, 210 ± 50 vs. 20 ± 10 nmol/kg; P <0.01). sIL-13Rα2 (2 doses of 20 mg/kg) significantly reduced the histological grade of allergen-induced lung eosinophil accumulation, whereas the effects of two doses of 10 mg/kg were not significant. These findings demonstrate that the tissue levels of IL-13 induced by allergen challenge of sensitized animals induce airway hyperresponsiveness and inflammation and that IL-13 is required for the expression of allergen-induced airway hyperresponsiveness in the guinea pig ovalbumin model.

interleukin-13; eosinophil; inflammation; airway hyperresponsiveness; ovalbumin

IN THE PAST DECADE, ASTHMA has been increasing in prevalence and is now thought to affect 15 million Americans (13). Advances have been made in our understanding of the immunobiology of sensitization (1); the role of interleukin (IL)-4, IL-5, and IL-13 in the development of airway hyperresponsiveness; the recruitment of inflammatory cells to the airway; and the augmented mucus production that follows allergen challenge in a murine system. Strong circumstantial evidence indicates that Th2 lymphocytes and the cytokines they produce are important in the pathogenesis of asthma (3, 5, 6, 10–12, 20, 26).

In the mouse, Th2 lymphocytes selectively develop and expand in a process that depends on IL-4 (1, 5). Studies comparing allergen-induced airway hyperresponsiveness in genetically altered receptor- or ligand-deficient mice have demonstrated a greater dependence on the IL-4 receptor α-chain than on IL-4 (2, 14). In addition to the IL-4 avid form of the heterodimeric receptor (IL-4 receptor α-chain/common γ-chain), the IL-4 receptor α-chain can combine with IL-13 receptor α1 and efficiently bind IL-13 (9, 12, 24); this realization in murine biology led to speculation that IL-13 was the mediator responsible for allergen-induced pulmonary inflammatory cell recruitment and airway hyperresponsiveness in the mouse model (2). The availability of the fusion protein soluble sIL-13 receptor α2 (sIL-13Rα2), which selectively limits the ability of murine IL-13 to reach its receptor (8), allowed for differentiation of the role of IL-13 from that of IL-4. In the murine system, IL-4 appears to be critical for the selection and development of Th2 lymphocytes, but its abrogation after sensitization has little effect on the development of airway hyperresponsiveness and cellular recruitment after allergen challenge (5, 7). In contrast, IL-13 appears to be necessary for the induction of airway hyperresponsiveness after allergen challenge and sufficient to induce it in the absence of sensitization (14, 29). Although it is increasingly clear that Th2 lymphocytes and their products play a pivotal role in allergy immunogenesis in the mouse (1), a dominant role is less well established in other species such as the guinea pig, where IL-13 has been reported to have a protective role in allergic inflammation. In this study, we explore the effects of murine IL-13 and sIL-13Rα2 on airway hyperresponsiveness and cellular recruitment in allergen-challenged sensitized guinea pigs.

METHODS

Tracheal Administration of Murine IL-13

Twenty-one guinea pigs (414–428 g) were anesthetized by intraperitoneal injection of ketamine (60 mg/kg) and xylazine...
(7 mg/kg) and were placed in the supine position. The trachea was cannulated with PE-240 polyethylene tubing (Intramedic, Becton-Dickinson, Parsippany, NJ), and 50 μl (690 μg/kg) of recombinant murine IL-13 or its diluent was nebulized into the trachea via a syringe nebulizer by a method similar to that which we used to study the effects of IL-5 (18) (Penn-Century, Philadelphia, PA); after injection, the tracheal cannula was removed, and the guinea pig was returned to the animal facility after recovery. Twenty-four hours later, contractile agonist responsiveness was measured by body plethysmography, and cells in bronchoalveolar lavage (BAL) fluid were enumerated as described below. The concentration of endotoxin in the preparation of IL-13 was measured at <2 EU/mg with a commercially available kit used according to the manufacturer’s instructions (Cape Cod, LAL assay).

Administration of sIL-13Ra2

Airway contractile responses to histamine and the presence of lung eosinophils were measured in unsensitized guinea pigs and in ovalbumin-sensitized animals treated with sIL-13Ra2 or nonspecific immunoglobulin IgG1 administered by intraperitoneal injection. The sIL-13Ra2 employed was modified to prevent the activation of complement (4) and was shown to bind guinea pig IL-13 in stimulated splenocytes. The sensitization and treatment status of the various groups studied are given in Table 1. The volume given by intraperitoneal injection was 0.5 ml for each 10-mg/kg sIL-13Ra2 dose, 1.0 ml for each 20-mg/kg IgG dose, and 3.8 ml for each 20-mg/kg sIL-13Ra2 dose.

Sensitization Protocol

Antigen sensitization was accomplished with minor modifications of the repetitive ovalbumin exposure protocol that we have described previously (19, 22, 23, 27). Thirty male Hartley guinea pigs (300–350 g) were pretreated with 10 mg of pyrilamine maleate/kg ip and sensitized by exposure to aerosolized antigen [7% (wt/vol) ovalbumin in 0.9% sterile PBS, pH 7.4]; the aerosol exposure chamber exposed six animals simultaneously to a single aerosolized stream. Each animal had its snout fixed ~15 cm from the point of aerosol entry in a chamber with a volume of 33 l. For nebulization, a Devilbiss Ultra-99 large-volume nebulizer was used (Sunrise Medical, Somerset, PA). After an 8-min exposure period, the chamber was cleared of aerosol by vacuum suction through a filter apparatus, and room air was provided to the animals. Animals were sensitized by aerosol exposure on two occasions at 7-day intervals, challenged with nebulated allergen 14 days after the initial sensitization, and prepared for study 24 h after challenge (day 15). We previously demonstrated that exposure to the antigen-diluent has no significant effect on physiological responses or cell recovery in this model (19).

Measurement of Histamine-Induced Contractile Responses

Animal preparation. Guinea pigs were prepared for study by body plethysmography in accordance with our previously described methods (18, 22, 23). In brief, guinea pigs were anesthetized by intraperitoneal injection of ketamine (60 mg/kg) and xylazine (7 mg/kg); a constant level of anesthesia was maintained, with additional doses of ketamine (20 mg/kg) administered at ~30-min intervals. When surgical anesthesia had been achieved, the anterior neck of the animal was shaved and dissected. Vascular access was established via the internal jugular vein, with 0.25/0.12-mm outer diameter/inner diameter silicone tubing (Technical Products, Decatur, GA). The common carotid artery was catheterized with a 24G Teflon catheter (Becton-Dickinson; Parsippany, NJ). Finally, the subglottic trachea was cannulated by anterior tracheotomy with PE-240 polyethylene tubing (Intramedic).

Body plethysmography. Guinea pigs were placed into a constant-mass body plethysmograph connected to a 4-l isothermal reservoir. The animals were ventilated in the supine position with room air by a constant-volume ventilator (Harvard Apparatus, South Natick, MA) at a tidal volume of 10 ml/kg, 60 breaths/min, a positive end expiratory pressure of 2.5 cm H2O, and a fixed inspiratory-to-expiratory time of 1:1. Plethysmograph pressures were measured relative to the pressure in a similar 4-l reservoir across a differential pressure transducer (Celesco, Canoga Park, CA), blood pressure was measured with a pressure transducer (P23Db; Statham Instruments, Oxnard, CA), and transrespiratory pressure was measured with a differential pressure transducer (Celesco) placed between a side tap of the tracheal cannula and the animal chamber of the plethysmograph. Tidal volume, mean arterial pressure (blood pressure), and transpulmonary pressure were monitored and recorded with a data acquisition program (Dataq Instruments, Akron, OH), whereas pulmonary resistance and dynamic compliance and conductance were calculated with GRC4 software (Andrew Jackson, Boston University, Boston, MA). Baseline values for respiratory system resistance (Rbaseline) were measured as the mean over 10 s, and this measurement was repeated after administration of increasing doses of histamine, as described below.

Contractile agonist administration. Baseline measurements of Rbas were recorded 5 and 10 min after the initiation of body plethysmography and 5 min before contractile agonist administration. A large-volume breath (three stacked tidal volumes) was given 30 s before each measurement to standardize volume history. Geometrically increasing doses of histamine (1–100 nmol/kg) were administered through the jugular venous catheter at 5-min intervals. The airway contractile responses were assessed by recording changes in Rbas for the subsequent 3 min (22, 23). The peak increase in resistance after challenge (Rmax) was divided by the baseline resistance (Rbaseline), multiplied by 100, and recorded as Rmax/Rbaseline. Injection of 100–500 μl of agonist diluent had no effect on the measured physiological parameters.

Histological identification of eosinophils. A carbol chromotrope stain was used to identify guinea pig eosinophils, with an adaptation of the method described by Lendrum (17). This stain is concentrated in the granules of eosinophils due to their avidity for acid dyes and has been shown by several groups to stain eosinophils specifically.

Table 1. Sensitization and treatment status of guinea pigs in a study of the effects of IL-13 on airway responses

<table>
<thead>
<tr>
<th>Group</th>
<th>Exposure</th>
<th>Agent and Route</th>
<th>Time of Administration, h before challenge</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unexposed</td>
<td>No exposure</td>
<td>No treatment</td>
<td></td>
</tr>
<tr>
<td>Unsensitized</td>
<td>PBS, pH 7.4</td>
<td>No treatment</td>
<td></td>
</tr>
<tr>
<td>Sensitized/</td>
<td>No treatment</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Untreated</td>
<td>Ovalbumin</td>
<td>No treatment</td>
<td></td>
</tr>
<tr>
<td>IgG control</td>
<td>Ovalbumin</td>
<td>IgG, 10 mg/kg ip</td>
<td>2 and 24</td>
</tr>
<tr>
<td>IgG control</td>
<td>Ovalbumin</td>
<td>IgG, 20 mg/kg ip</td>
<td>2 and 24</td>
</tr>
<tr>
<td>sIL-13Ra2-Fc</td>
<td>Ovalbumin</td>
<td>sIL-13Ra2, 10 mg/kg ip</td>
<td>2 and 24</td>
</tr>
<tr>
<td>sIL-13Ra2-Fc</td>
<td>Ovalbumin</td>
<td>sIL-13Ra2, 20 mg/kg ip</td>
<td>2 and 24</td>
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sIL-13Ra2-Fc, Soluble interleukin-13 receptor α2 modified to prevent complement activation.
investigators to be specific for eosinophils (15–17, 25). Briefly, paraffin slides were dewaxed in xylenes, rehydrated through graded alcohols, and washed in PBS. The slides were transferred to Mayer’s hematoxylin solution for 4 min and then washed in deionized water. Next, they were incubated in acidified alcohol (1% HCL, 70% ETOH) for 2 min, washed in deionized water, incubated in an aqueous solution of 1% wt/vol chromotrope 2R (Sigma, St. Louis, MO) with 5% wt/vol phenol (Sigma) for 20 min, and washed again in deionized water. The slides were then dehydrated, and a coverslip was applied.

Morphometric analysis. The density of eosinophilic infiltration was evaluated by a semiquantitative scale, 0–5+: 0 was defined as the absence of any visible eosinophil staining; 1+ as the presence of a few scattered eosinophils around airways and blood vessels; 2+ as the presence of frequent eosinophil infiltration, with nearly all the eosinophils being identified in a single cell layer surrounding the airways and/or the blood vessels; 3+ as eosinophil infiltration of a moderate density within a band at least 10 μm wide surrounding at least one-third of the airways and blood vessels; 4+ as abundant eosinophil infiltration of most of the airways and blood vessels, but with clear separation of the infiltrates between most of the airways and blood vessels; and 5+ as abundant eosinophil infiltration with lack of separation between the airway and perivascular infiltrates in at least one-quarter of the airways. The individual who determined the histological score was unaware of the treatment status of the animals from which the tissues were derived.

Statistical Methods

Results are expressed as the group mean followed by the SE, unless otherwise specified. The data were tested for normalcy and compared by one-way ANOVA, ANOVA based on ranks, t-test, or Mann-Whitney’s rank sum test, as appropriate. Data that met normalcy assumptions are presented as the mean and SE, and those that did not are presented as the median and interquartile range. A P value < 0.05 was considered significant.

RESULTS

Effects of Exogenous IL-13

Tracheal injection of recombinant murine IL-13 (360 μg/kg) was associated with significant increases in the contractile effects of histamine at doses of 33 nmol/kg (580 ± 80 vs. 320 ± 35% increase in baseline R_{resp}, P = 0.003) and 100 nmol/kg (1,200 ± 170 vs. 770 ± 110% increase in baseline R_{resp} P = 0.04, Fig. 1). The dose of histamine required to double baseline R_{RS} (ED_{100}) was significantly greater in control animals than in IL-13-treated animals (22 ± 3 vs. 13 ± 2 nmol/kg; n = 8 and 13, respectively; P < 0.05). IL-13-induced airway hyperresponsiveness was accompanied by the recovery of significantly more cells from BAL fluid (total cells 120 (56–170) vs. 10 (7–19), P < 0.0001; macrophages 41 (18–62) vs. 8 (7–15), P = 0.004; eosinophils 9 (5–20) vs. 2 (1–3), P < 0.0001; lymphocytes 3 (1–8) vs. 0.08 (0–0.3), P = 0.01; neutrophils 42 (32–110) vs. 0.05 (0.01–0.1), P < 0.0001; millions of cells, median and interquartile range (Fig. 2)].

Effects of sIL-13Ra2 on Antigen-Induced Airway Hyperresponsiveness

Treatment with both the 10- and 20-mg/kg doses of sIL-13Ra2 prevented allergen-induced airway hyperresponsiveness when administered 2 and 24 h before allergen challenge. Significantly more histamine was required to double R_{baseline} (histamine ED_{100}) in both of the sIL-13Ra2 groups (sIL-13Ra2, two doses of 20 mg/kg: 210 ± 50 nmol histamine/kg, n = 6; sIL-13Ra2, two doses of 10 mg/kg: 120 ± 22 nmol histamine/kg, n = 5) than in sensitized/untreated animals (23 ± 4 nmol histamine/kg, n = 7; P < 0.05) and those treated with nonspecific IgG (IgG two doses of 20 mg/kg: 20 ± 10 nmol histamine/kg, n = 7; IgG two doses of 10 mg/kg: 40 ± 10 nmol histamine/kg, n = 6; P < 0.05). The protective effects of sIL-13Ra2 were consistent for all of the relevant doses of histamine studied (Fig. 3). When sIL-13Ra2 was given as a single 20-mg/kg dose 2 h before allergen challenge, plasma IL-13 binding capacity was increased, but there were no effects on airway hyperresponsiveness (63 ± 9 nmol/kg sIL-13Ra2 group and 58 ± 6 IgG control).

Effects of sIL-13Ra2 on Lung Tissue Eosinophil Recruitment

We found that treatment with sIL-13Ra2 (20 mg/kg) significantly reduced allergen-induced accumulation of eosinophils in the guinea pig lung. Lung tissue eosinophil levels, on a 0 (absent) to 5+ (abundant) scale, were significantly lower in the higher-dose sIL-13Ra2-treated group (sIL-13Ra2 two doses of 20 mg/kg: 2.2 ± 0.4, n = 6, P < 0.05) than in the sensitized/untreated control group (sensitized/untreated: 3.6 ± 0.2, n = 7, P < 0.05, Fig. 4). The effects of the 10-mg/kg dose for reducing lung tissue eosinophilia were not significant (sIL-13Ra2 two doses of 10 mg/kg: 2.8 ± 0.4, n = 5). The histological grades for the control groups were unexposed: 0.8 ± 0.1, n = 6; unsensitized: 1.0 ± 0.2, n = 6; IgG two doses of 20 mg/kg: 3.5 ± 0.3, n = 6 (Fig. 5).

Fig. 1. Contractile effects of geometrically increasing doses of histamine after tracheal nebulization of a 360-μg/kg dose of interleukin (IL)-13 (○) or its diluent (●).
DISCUSSION

Our findings that IL-13 induces airway hyperresponsiveness and is required for its expression after allergen challenge in the guinea pig are consistent with the findings of other investigators in the mouse model. We found that a single exposure to nebulized murine IL-13 induced a significant increase in airway hyperresponsiveness and was associated with greater numbers of eosinophils, lymphocytes, neutrophils, and monocytes in BAL fluid 24 h after exposure. Whereas the tracheal nebulization procedure employed to deliver IL-13 to the lower airways itself increases airway hyperresponsiveness, IL-13 significantly increased responsiveness beyond that of the instillation procedure alone. In the A/J strain of mice, eosinophils were strikingly increased 24 h after IL-13 exposure and returned to normal levels by 96 h, whereas airway responsiveness was normal at 24 h and increased at 96 h after IL-13 exposure (29). BALB/c mice repeatedly exposed to IL-13 and studied 12–15 h after a third dose demonstrated both airway hyperresponsiveness and in-

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**Fig. 2.** Recovery of cells from bronchoalveolar lavage (BAL) fluid after tracheal administration of a 360-μg/kg dose of IL-13 or its diluent (control).

**Fig. 3.** Airway hyperresponsiveness measured as %increase in baseline respiratory resistance ($R_{\text{resp}} = R_{\text{max}}/R_{\text{baseline}} \times 100$) in sensitized untreated (positive control) animals (■); IgG control animals, 2 doses of 10 mg/kg (○); unsensitized (negative control) animals (□); soluble IL-13 receptor α2 (sIL-13Rα2), 2 doses of 10 mg/kg (∆); and sIL-13Rα2, 2 doses of 20 mg/kg (▲).

**Fig. 4.** Morphometric analysis of groups of lungs prepared for eosinophil detection by carbol chromotrope staining. Lungs from animals treated with the higher dose of sIL-13Rα2 had significantly lower histological scores than control lungs.

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increased recovery of inflammatory cells (14). Our findings from microgram doses of nebulized murine IL-13 contrast with those of Watson et al. (28), who reported the effects of nanogram quantities of tracheally instilled human IL-13. Watson et al. reported that tracheal instillation of IL-13 had little effect on BAL eosinophil recovery and was associated with small increases in BAL neutrophil recovery in some experiments. We found that tracheal nebulization of microgram quantities of IL-13 was associated with a significant increase in BAL eosinophils and a robust increase in neutrophils. It is not likely that endotoxin contamination could account for these findings because the levels in our preparations were less than 2 eU/mg. Watson et al. also reported that nanogram quantities of IL-13, instilled into the trachea before allergen challenge, limited BAL eosinophil recovery in sensitized guinea pigs in a dose-dependent manner. This observation leads to the interesting possibility that endogenously released IL-13 may protect against allergen-induced increases in BAL eosinophil recovery. We studied a higher dose of IL-13 and found both increased BAL inflammatory cell recovery and airway hyperresponsiveness. Although the dose of IL-13 studied was significantly greater in our study, there are other differences that could also be important. Different homologues of IL-13 were studied, and the alternative modes of tracheal delivery could have resulted in different distributions of IL-13 within the lung. To more directly determine whether IL-13 is promoting or limiting airway inflammation and hyperresponsiveness in sensitized and challenged guinea pigs, we studied lung eosinophil accumulation in the presence of an agent that binds guinea pig IL-13 and limits its availability at the receptor. The 20-mg/kg dose of sIL-13Ra2 significantly reduced pulmonary eosinophil accumulation as judged by direct histological examination of the lung. This finding suggests that endogenously mobilized IL-13 contributes to pulmonary eosinophilia after allergen challenge in the guinea pig. IL-13 also appears to be required for the induction of airway hyperresponsiveness in this model.

The demonstration that higher doses of sIL-13Ra2 are required to reduce airway eosinophilia than to eliminate allergen-induced hyperresponsiveness is another indication that the mechanisms responsible for these phenomena are distinct. It is also possible that the lower dose of sIL-13Ra2 may have affected the presence or distribution of eosinophils in a manner that we were not able to detect with the semiquantitative method employed. Although our findings demonstrate that endogenously mobilized IL-13 promotes airway eosinophilia after allergen challenge in the guinea pig, they do not exclude a protective role for exogenous IL-13 in some circumstances.

We found that both doses of sIL-13Ra2 prevented allergen-induced airway hyperresponsiveness when given 2 and 24 h before challenge. Pretreatment at 2 and 24 h before challenge appears to be required; treatment 2 h before challenge did not prevent allergen-induced hyperresponsiveness. This requirement for pretreatment may reflect a long time constant for penetration into airway smooth muscle, but this seems unlikely based on the known ability of therapeutic immunoglobulins to rapidly penetrate the guinea pig airway (21). Reasonable explanations for the necessity of pretreatment include a direct time-dependent “priming” effect of IL-13 or indirect effects that are depen-
dent on the synthesis of other mediators with prolonged effects. These findings are similar to those reported in murine models where sIL-13Ra2 is dosed before allergen challenge (14, 29). The main finding of our studies is that abrogation of IL-13 activity with sIL-13Ra2 is a highly effective strategy for limiting allergen-induced airway hyperresponsiveness in the guinea pig.

Our data indicate that IL-13 can induce airway hyperresponsiveness and inflammation in the guinea pig. It is required for the expression of allergen-induced airway hyperresponsiveness and contributes to, rather than inhibits, airway eosinophilia in allergen-challenged guinea pigs. Our findings are consistent with those obtained in murine models and suggest that effective treatment with agents that abrogate IL-13 receptor activation should be administered in advance of allergen challenge.

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REFERENCES