

The effects of IL-5 on airway physiology and inflammation in rats

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Background: There is evidence that the cytokine IL-5 is a prominent feature of airway inflammation in asthma. **Objective:** The aim of this study was to determine whether exogenous IL-5 could cause changes in lung physiology, the early and late airway response after antigen challenge, and airway inflammation in rats that do not have a propensity to develop these changes after sensitization and challenge. **Methods and Results:** Intratracheal administration of IL-5 to ovalbumin sensitized Brown Norway SSN rats increased the airway responsiveness to methacholine (AHR) 20 hours after administration of IL-5 at the same time as an increase in neutrophils occurred in the lung lavage. This effect was dose dependent and was not caused by endotoxin. Concurrent intratracheal administration of 50 ng of anti-IL-5 monoclonal antibody with 10 µg of recombinant human IL-5 decreased the AHR and neutrophil influx. Pretreatment with 3 µg of IL-5 had no effect on the early and late airway response or on AHR after ovalbumin challenge. However, IL-5 increased lung resistance 20 hours after antigen challenge. Although total lung cells and differential counts did not differ significantly 8 hours after antigen challenge, the blood lymphocyte CD4/CD8 ratio decreased in IL-5 pretreated rats ($P < .05$). In addition, in situ hybridization showed a significant increase in cells within the airway wall expressing IL-4 and IL-5 mRNA in IL-5 treated/challenged rats compared to controls ($P < .05$). **Conclusion:** The intratracheal administration of IL-5 causes only part of the physiologic changes that are associated with asthma. Other factors are necessary to obtain the complete asthma phenotype. (*J Allergy Clin Immunol* 2003;111:558-66.)

Key words: Interleukin-5, airway responsiveness, late airway response, lung inflammation

Asthma is an inflammatory disease of the airways with 2 characteristics, reversible airway obstruction and increased airway responsiveness (AHR).^{1,2} In recent years, it has become clear that infiltration of the airways of patients with asthma by chronic inflammatory cells, particularly lymphocytes and eosinophils, is likely to be

Abbreviations used

AHR:	Increased airway responsiveness
BAL:	Bronchoalveolar lavage
BN:	Brown Norway
CFU-Eo:	Eosinophil-type colony
EC ₂₀₀ R _L :	Effective concentration of methacholine required to double R _L
ER:	Early airway response
LR:	Late airway response
PBMNC:	Peripheral blood mononuclear cell progenitor
rh:	Recombinant human
R _L :	Lung resistance

of major importance in causing sustained changes in airway responsiveness.³ Activated lymphocytes are present and increased in the airways of patients with asthma.⁴ These cells seem to be involved in the physiologic response that occurs after antigen challenge.⁵ Indeed, adoptive transfer of antigen-specific CD4⁺ T lymphocytes induces airway bronchoconstriction and eosinophilia in challenged rats.⁵ The mechanism by which lymphocytes might be affecting the airway response to antigen is unclear, but it may be through one of its mediators, IL-5.

Several lines of evidence suggest that IL-5 may be important in asthma. IL-5 is an important growth factor for eosinophils⁶ that is increased in the lungs of asthmatic patients with and without allergy.⁷ IL-5 increases in the lungs and sputum of patients with allergic asthma 24 hours after antigen challenge,⁸ and a clear correlation exists between IL-5 expression and the presence of eosinophils in the airways of patients with asthma.⁹ Intratracheal administration of IL-5 to asthmatic patients or to sensitized animals with the characteristics of atopic asthmatic patients increases the airway response to antigen.^{10,11} There is compelling evidence for a role of IL-5 in patients with asthma and in animal models of asthma. However, there is currently no information on whether the sole administration of IL-5 is sufficient to induce the abnormal physiologic changes that occur after sensitization and antigen challenge in animals that do not develop all these changes.

In this study we assessed whether administration of recombinant human (rh) IL-5 to the SSN substrain of Brown Norway (BN) rats that do not develop early (ER) or late (LR) airway responses after sensitization and antigen challenge¹² will cause several of the physiologic changes that are characteristic of asthma. We first determined in vitro the functional activity of rhIL-5 in BN rats by demonstrating eosinophil differentiation in rat periph-

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eral blood mononuclear cell progenitors (PBMNCs). We then assessed in vivo the effects of intratracheal administration of IL-5 on AHR, the ER and LR, lung resistance (R_L), and airway inflammation in BN SSN rats. Our results show that although IL-5 can induce some changes, it does not cause all the physiologic changes that are found in asthma.

METHODS

Animals and sensitization

Ninety-three highly inbred male BN SSN rats, 7 to 8 weeks old and weighing 200 to 240 g, were obtained from Harlan Sprague-Dawley Inc (Walkerville, Md). Rats were maintained in conventional animal facilities at the Meakins-Christie Labs of McGill University or the CHUM Research Center. The Animal Care Committees of each institution approved all the experiments that were performed in this study.

We actively sensitized the rats by subcutaneous injection of 1 mL of saline containing 1 mg of ovalbumin and 200 mg of aluminum hydroxide (Sigma Chemicals, St Louis, Mo). At the same time 1 mL of *Bordetella pertussis* vaccine containing 5×10^9 heat-killed organisms was given intraperitoneally as an adjuvant.

Eosinophil colony proliferation from PBMNCs

These experiments were performed to assess whether rhIL-5 induced eosinophil proliferation from progenitors in rats as previously described in humans.¹³ We isolated PBMNCs from 10 BN rats by centrifugation over Ficoll-Hypaque (Pharmacia, Montreal, Quebec, Canada). The cells were plated at a concentration of 1×10^6 cells per mL in 35×10 mm tissue culture dishes (Falcon, Oakville, Ontario, Canada) with various supplements. After 14 days of culture at 37°C in a humidified atmosphere containing 5% CO₂ in air, colonies (defined as any aggregation of more than 40 cells) were counted by inverted microscopy, and eosinophil-type colonies (CFU-Eo) were counted as a function of their morphologic appearance after May-Grunwald Giemsa staining.

IgE determination

Specific IgE levels to ovalbumin in the blood were determined by ELISA as previously described.¹⁴ Briefly, assays were performed on 96-well microtiter plates (Immulon II; Fisher, Pittsburgh, Pa). The wells were washed 3 times with PBS-0.5% casein. Horseradish peroxidase-conjugated avidin (Sigma Chemicals, Montreal, Quebec, Canada) in PBS-0.5% casein (1:500 dilution) was added for 30 minutes. Plates were developed at room temperature for 15 minutes after addition of TMB substrate (Sigma Chemicals, Montreal, Quebec, Canada) diluted (1:10) in substrate buffer and were read with an ELISA plate reader (SLT Lab Instruments, Fisher Scientific Co, Pittsburgh, Pa) at 450 nm.

Endotoxin determination and challenge

Because recombinant human cytokines contain a small amount of endotoxin, the concentration of endotoxin in the administered rhIL-5 was quantified by using the Limulus Amebocyte Lysate (LAL) test (Associates of Cape Cod, Falmouth, Mass). The same concentration of endotoxin (lipopolysaccharide; Sigma, St Louis, Mo), diluted in 0.9% saline, was given intratracheally to animals, and airway responsiveness to methacholine and inflammatory cells in the bronchoalveolar lavage (BAL) were measured.

Lung BAL

These experiments were performed 14 days after sensitization. Forty-three rats were anesthetized with Pentothal (50 mg/kg; BDH

Pharmaceuticals, Montreal, Quebec, Canada), intratracheally intubated, and were awakened after intratracheal injection of BSA, rhIL-5 (1 to 10 µg) or 10 µg rhIL-5 and 50 ng TRFK5 (BD Biosciences, Oakville, Ontario, Canada). Twenty hours later the animals were again anesthetized with Pentothal, intubated, and BAL was performed after methacholine challenge. The lungs were lavaged through the tracheal tube by 5 instillations and immediate retrieval of 5 mL of saline at room temperature. Total cells were counted, and the differential cell count was assessed after centrifugation onto a cytospin slide that was prepared with a Cytospin model III (Shandon, Pittsburgh, Pa) and stained with Wright-Giemsa. At least 200 cells were counted under oil immersion microscopy.

Staining of BAL cells for major basic protein

The cells obtained from the BAL were fixed in acetone-methanol, endogenous peroxidase quenched and incubated with 60 µL of primary monoclonal major basic protein IgG antibody (diluted 1:30 in Tris borate solution) at 4°C in a humid chamber overnight. The slides were washed and then stained as previously described¹⁵ by using alkaline phosphatase-antialkaline phosphatase and alkaline phosphatase followed by counterstaining with Harris' hematoxylin. Cells were then viewed and counted under a light microscope.

Measurement of lung mechanics

General anesthesia was induced with either Pentothal (50 mg/kg) or urethane (1.1 g/kg) intraperitoneally. Intratracheal intubation was then performed by using a 6-cm length of PE-240 polyethylene catheter. A heating pad was used to maintain body temperature constant, and rectal temperature was monitored continuously with an electronic thermometer (Telethermometer; Yellow Springs Instrument Co, Yellow Springs, Ohio). Lung resistance (R_L) was measured during spontaneous tidal breathing with the animals in the lateral decubitus position. Flow was measured by placing the tip of the tracheal tube inside a small Plexiglas box (265 mL in volume). A Fleisch no. 0 pneumotachograph coupled to a differential pressure transducer (MP-45 ± 2 cm H₂O; Validyne Corp, Northridge, Calif) was attached to the other end of the box to measure airflow, and volume was obtained by numeric integration of the flow signal. Changes in esophageal pressure were measured by using a saline-filled catheter and a differential pressure transducer (Sanborn 267 BC; Hewlett-Packard, Waltham, Mass). The other port of the transducer was connected to the box. The esophageal catheter consisted of polyethylene tubing (PE 200) 20 cm long attached to a shorter length (6 cm) of tubing (PE 100). Transpulmonary pressure was computed as the difference between esophageal and box pressure. The airway response was evaluated from R_L , which was determined by fitting the equation of motion of the lung by multiple linear regression by using commercial software (RHT-Infodat Inc, Montreal, Quebec, Canada). Intratracheal tube resistance was 0.11 cm H₂O/mL/s at a flow of 25 mL/s. Tube resistance was subtracted from all R_L values.

Airway responsiveness to methacholine

Fourteen days after sensitization, rats were given rhIL-5 (1, 3, and 10 µg) or 10 µg of rhIL-5 and 50 ng TRFK5 or 60 endotoxin units of lipopolysaccharide. Thirty minutes, 20 hours, or 72 hours later rats were anesthetized with Pentothal (50 mg/kg), intubated, and baseline R_L was measured. One group of rats was challenged with a nebulized aerosol of ovalbumin (50 mg/mL in sterile water) for 30 seconds 20 hours after IL-5 administration. All rats were given an aerosol of PBS followed by progressively doubling concentrations of methacholine (from 0.0625 to 32 mg/mL in sterile saline), and R_L was measured after each aerosol until it increased to at least 200% of the PBS baseline value. Aerosols were generated

from 3 mL of solution by using a Hudson nebulizer (Model 1400; Hudson, Temecula, Calif) with an airflow of 10 L/min; each administration lasted for 30 seconds.

Measurement of airway responses to ovalbumin

Fourteen days after sensitization, rats were anesthetized with Pentothal (50 mg/kg), intratracheally intubated, and received either IL-5 (3 μ g) or the same weight of BSA intratracheally before being awakened. The following day, rats were anesthetized with urethane (1.1 g/kg), intubated, and baseline R_L was measured. The rats were then challenged with aerosolized ovalbumin (50 mg/mL in sterile water) by using a Hudson nebulizer at an airflow of 10 L/min for 5 minutes. R_L was measured before and at 5, 10, and 15 minutes after the ovalbumin challenge and at 15-minute intervals for a total period of 8 hours.

Lung mincing and digestion

These experiments were performed 8 hours after ovalbumin challenge as previously described¹³ in 16 rats pretreated with 3 μ g of IL-5 or 3 μ g of BSA. Briefly, lung tissue was dissected, and slides from the cell retrieval were prepared by centrifuging 500,000 cells in enriched RPMI 1640 for 5 minutes at 400 rpm in a cytocentrifuge. The cellular differential was assessed on a Wright-Giemsa stained slide by counting 200 cells under oil immersion microscopy.

Isolation and staining of blood lymphocyte subsets

PBMCs were isolated from fresh heparinized peripheral blood by standard Ficoll-Hypaque methods and prepared for flow cytometry as previously described.¹⁵ Briefly, the isolated cells were washed with Hank's balanced salt solution and stained directly by incubating with the monoclonal antibodies W3/25 (equivalent of the CD4 helper lymphocytes) or OX8 (CD8 suppressor/cytotoxic lymphocytes) for 30 minutes. Flow cytometry was performed with an argon laser, 488-nm FACScan analyzer (Becton Dickinson FACS Division, Sunnyvale, Calif) focusing on the lymphocyte cluster. Controls consisted of cells stained with polyclonal antibodies obtained from mouse ascites fluid.

Measurement of cytokine mRNA expression

These experiments were performed 20 hours after ovalbumin challenge as previously described¹³ in 16 rats pretreated with 3 μ g IL-5 or BSA. All the animals were killed by exsanguination, and lung airways were cut into 8- μ m thick sections and placed on poly-L-lysine coated slides and stored at -80°C .

In situ hybridization was performed as previously described.¹⁶ Labeling of RNA sense and antisense probes specific for IL-4, IL-5, and IFN- γ with digoxigenin-11-UTP was performed according to the manufacturer's recommendations (Boehringer Mannheim, Mannheim, Germany). The labeled probes were digested by alkaline hydrolysis to an average length of 100 to 200 bases before precipitation, and hybridization was performed at 42°C for 12 hours. As a negative control, preparations were hybridized with digoxigenin-UTP-labeled sense probes under the same conditions. Specimens were then stained for 1 minute with Hoechst 33258 dye (bisbenzimidazole; Sigma Chemical Co, St Louis, Mo) at a dilution of 1 μ g/mL in PBS for visualization under a Zeiss Axiophot fluorescence microscope (Carl Zeiss (Oberkochen), Ltd, Welwyn Garden City, UK). Positive cells were counted in a random coded order at $\times 200$ magnification. In the airway submucosa, positive cells were counted along the entire length of the epithelial basement membrane in a minimum

of 6 sections. The percentage of cytokine-positive cells was used during analysis.

Statistical analysis

The effective concentration of methacholine required to double R_L ($EC_{200}R_L$) was obtained by linear interpolation between the 2 concentrations bounding the point at which R_L reached 200% of the control value. Comparisons of airway responsiveness to methacholine between groups were performed with log transformed data. To compare the prevalence of ER among treatment groups, we defined a significant ER as an increase in R_L to at least 150% of baseline value within 1 hour after ovalbumin challenge. The late response was calculated as the area under the R_L versus time curve from 180 to 480 minutes after challenge. The differences between the tested and control group means were analyzed by using ANOVA when several groups were tested followed by Student t test or the Mann-Whitney U test, as appropriate, to compare magnitude, time to peak, ER and LR, inflammatory cells, and cytokines. Results are presented as mean $\pm$ SEM, except for values of $EC_{200}R_L$, which are reported as geometric means. To determine the statistical significance of the airway responsiveness to methacholine challenge, we used unpaired Student t test. Significance was accepted when the probability (P) value was less than .05.

Chemicals

Ovalbumin, BSA, alkaline phosphatase-antialkaline phosphatase, urethane, methacholine, paraformaldehyde, collagenase, and Wright-Giemsa stain were purchased from Sigma Chemicals (St Louis, Mo). *Bordetella pertussis* vaccine was purchased from the Armand Frappier Institute (Montreal, Quebec, Canada). Fetal calf serum, penicillin, streptomycin, L-glutamine, nonessential amino acids, RPMI 1640 medium, Iscove's modified Dulbecco's medium, Hank's balanced salt solution, and trypan blue were obtained from GIBCO Laboratories (Grand Island, NY). Ficoll-Hypaque was obtained from Pharmacia (Montreal, Quebec, Canada). rhIL-5 was donated by Merck Frosst (Montreal, Quebec, Canada). Pentothal was used to put rats to sleep and was obtained from BDH Pharmaceuticals (Montreal, Quebec, Canada). Harris' hematoxylin was purchased from Zymed Chemicals (San Francisco, Calif), and rompun was bought from MTC Pharmaceuticals (Cambridge, Ontario, Canada). W3/25 and OX8 were obtained from DAKO, Cedarlane (Carpinteria, Calif). Anti-MBP antibody was a gift from Dr Redwan Moqbel.

RESULTS

Effect of rhIL-5 on eosinophil colony formation from progenitors

Rat PBMNCs from 10 rats were incubated in 0, 0.25, 1, 2, and 5 ng/mL of rhIL-5 in enriched RPMI medium for 14 days. Concentrations of IL-5 above 1 ng/mL caused a significant increase in eosinophil/basophil colonies when compared to controls ($P < .05$), and the number of eosinophil/basophil colonies increased with the concentration of rhIL-5 (Fig 1).

Effect of ovalbumin sensitization on ovalbumin-specific IgE levels from serum

Serum from 2 actively sensitized rats was collected 0, 1, 2, and 3 weeks after sensitization. Ovalbumin-specific IgE levels were measured in duplicate and showed an increase in ovalbumin-specific IgE levels.

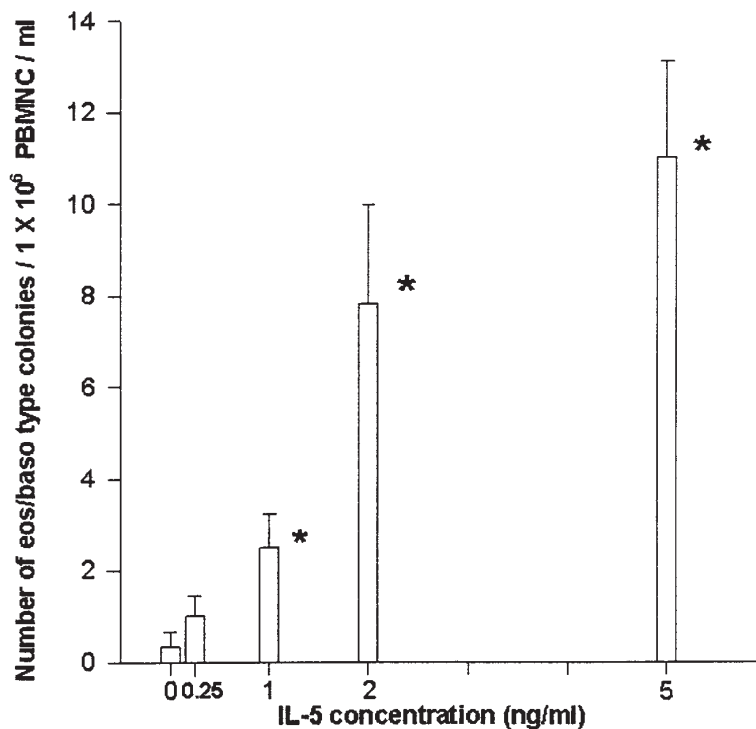


FIG 1. Effect of rhIL-5 on eosinophil (eos)/basophil (baso) type colony production in rat PBMNCs (n = 10). **P* < .05 between IL-5 groups (1, 2, and 5 ng/mL) and control.

Effect of intratracheal administration of rhIL-5 on airway responsiveness to methacholine

BSA, 1, 3, or 10 μ g of rhIL-5 were administered intratracheally, and airway responsiveness to methacholine was measured 20 hours later. The amount of methacholine that caused a doubling in R_L decreased significantly as doses of IL-5 increased from 1 to 10 μ g (Fig 2, A). The airway response to methacholine was significantly increased in the rats that received 3 μ g of IL-5 when compared to the group that received BSA (*P* < .05). The airway response to methacholine was also significantly increased in the group that received 10 μ g of rhIL-5 when compared to the BSA group (10 μ g IL-5: 1.3 ± 0.4 mg/mL vs BSA: 6.5 ± 0.9 mg/mL; *P* < .01) (Fig 2, A). Fifty micrograms of TRFK5 significantly reduced the effect of 10 μ g of rhIL-5 on the airway response to methacholine (4.75 ± 0.48 mg/mL; *P* < .05). Experiments testing the effect of lipopolysaccharide at a concentration equal to that measured in the rhIL-5 (60 endotoxin units) showed no significant difference in airway responsiveness to methacholine with control animals. Airway responsiveness to methacholine 30 minutes and 72 hours after administration of 10 μ g of IL-5 was not significantly affected (Fig 2, B).

Effect of dose of intratracheal rhIL-5 on the cellular return from lung lavage

Intratracheal administration of rhIL-5 increased the cellular return from BAL 20 hours later. The total cellular return was $6.65 \pm 2.08 \times 10^6$ cells for 1 μ g, $9.75 \pm 1.84 \times 10^6$ cells for 3 μ g, and $13.1 \pm 3.07 \times 10^6$ cells for 10 μ g (*P* < .05). Fifty nanograms of TRFK5 given in combination with 10 μ g of rhIL-5 significantly reduced the total cellular return from the BAL ($3.28 \pm 0.53 \times 10^6$ cells; *P* < .05). The number of neutrophils increased significantly in the rats that received 10 μ g of IL-5 when compared to the group that received no IL-5 (10 μ g IL-5: $8.01 \pm 2.21 \times 10^6$ cells vs no IL-5: $2.78 \pm 0.73 \times 10^6$ cells; *P* < .05). The effect of IL-5 on BAL neutrophils was significantly reduced by TRFK5 ($2.23 \pm 0.42 \times 10^6$ cells; *P* < .05). No difference was found in the total number of macrophages, lymphocytes, and basophils that were present in the BAL of each group (macrophages: 10 μ g IL-5: $4.53 \pm 0.92 \times 10^6$ cells vs controls: $3.95 \pm 0.27 \times 10^6$ cells; lymphocytes: 10 μ g IL-5: $0.252 \pm 0.067 \times 10^6$ cells vs controls $0.154 \pm 0.09 \times 10^6$ cells; basophils: 10 μ g IL-5: $0.0655 \pm 0.0403 \times 10^6$ cells vs controls: $0.05 \pm 0.03 \times 10^6$ cells). Also, rats given TRFK5 did not show a significant difference between controls or IL-5 alone (data not shown). There was no difference in total

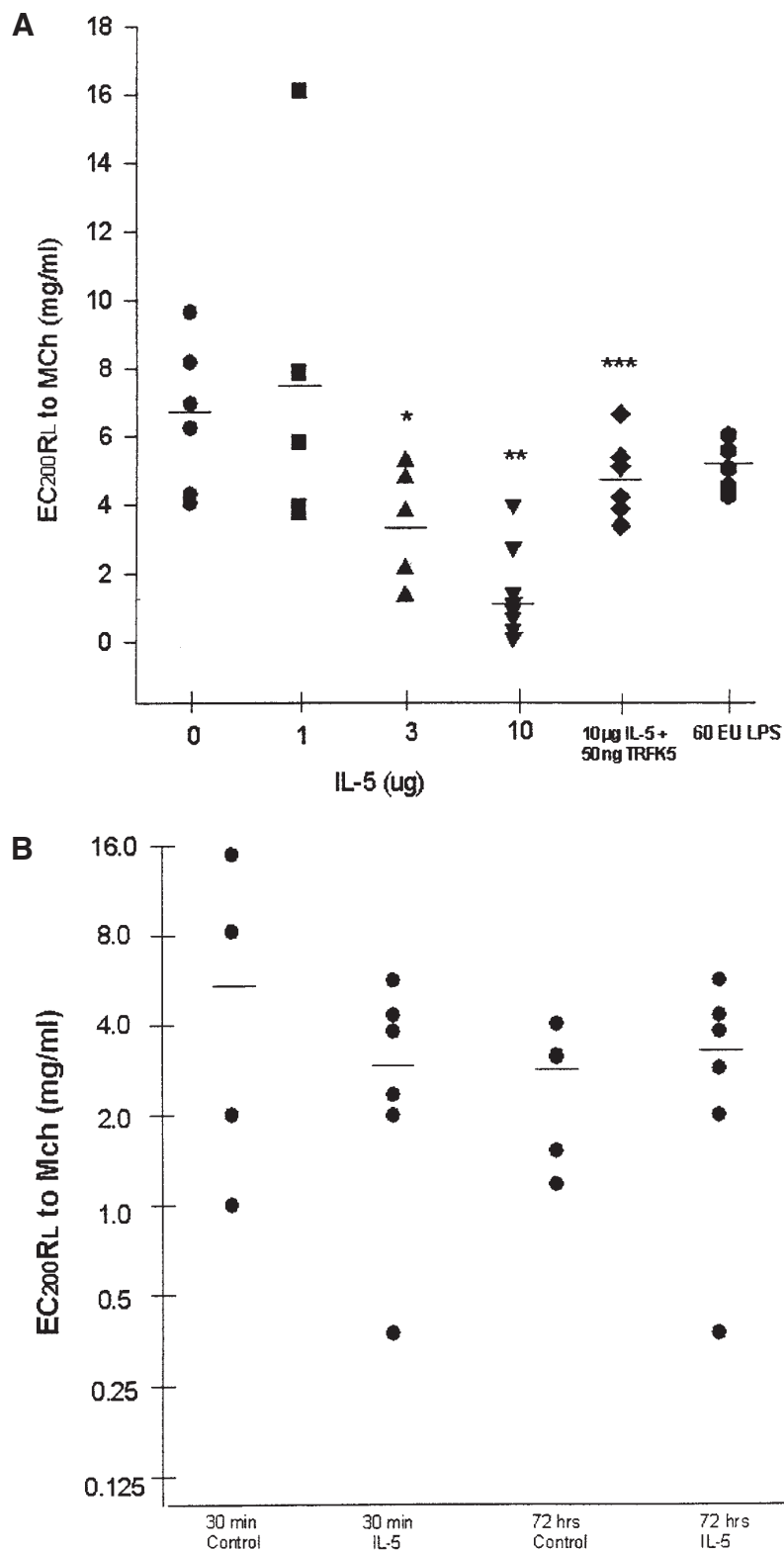


FIG 2. A, Effect of IL-5 on airway responsiveness to methacholine (*Mch*) 20 hours after administration. EC₂₀₀R_L was calculated as the amount of *Mch* necessary to double R_L. Bars represent the means of each group. *EU*, Endotoxin units; *LPS*, lipopolysaccharide. **P* < .05 between IL-5 treated (3 μg) and BSA treated (control). ***P* < .01 between IL-5 treated (10 μg) and BSA treated (control). ****P* < .05 between IL-5 treated (10 μg) and 10 μg IL-5 with 50 ng TRFK5. **B,** Effect of 10 μg of IL-5 on airway responsiveness to *Mch* 30 minutes and 72 hours after administration (*n* = 22). Bars represent the arithmetic means of each group.

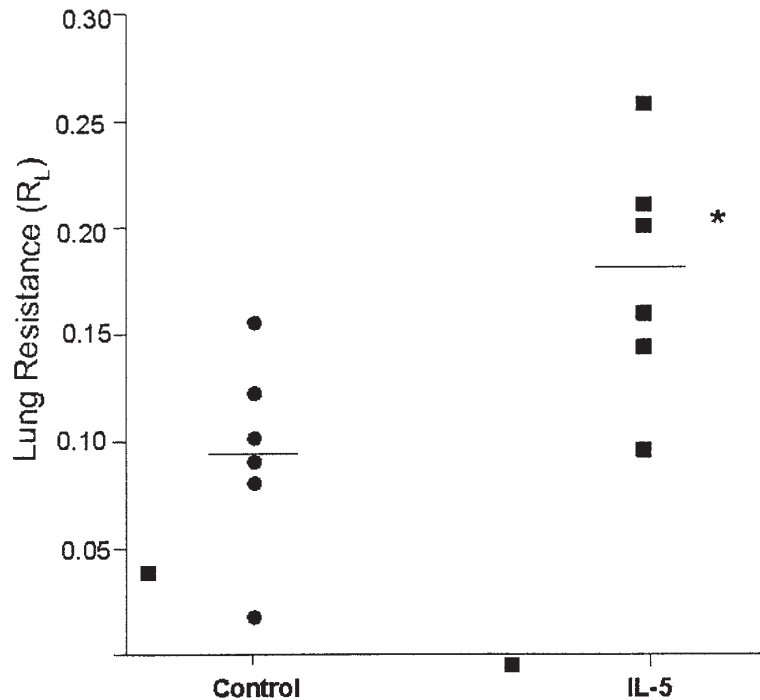


FIG 3. Effect of rhIL-5 on R_L 20 hours after antigen challenge. Rats ($n = 12$) were given $3 \mu\text{g}$ of rhIL-5 or BSA and challenged with ovalbumin 20 hours later. R_L was measured 20 hours after antigen challenge in both groups of rats. Bars represent the mean of each group. * $P < .05$ between IL-5 treated/challenged and control groups.

eosinophils between all groups when evaluated by Wright-Giemsa stain or with an anti-MBP stain (eosinophils: $10 \mu\text{g}$ IL-5: $0.243 \pm 0.0403 \times 10^6$ cells vs controls: $0.092 \pm 0.07 \times 10^6$ cells vs $10 \mu\text{g}$ IL-5 and 50 ng TRFK5: $0.0689 \pm 0.02 \times 10^6$ cells; $P > .05$).

Effect of rhIL-5 on the physiologic and inflammatory response after ovalbumin challenge

Pretreatment with $3 \mu\text{g}$ of rhIL-5 did not affect the ER (R_L for control $109\% \pm 17\%$ of baseline vs $98\% \pm 9\%$ for IL-5 treated rats) or LR (area under the curve 3.9 ± 1.5 for control vs 4.7 ± 2 for IL-5 treated rats) but significantly increased R_L 20 hours after challenge with ovalbumin (IL-5: $0.178 \pm 0.046 \text{ cm H}_2\text{O/mL/s}$ vs control: $0.094 \pm 0.057 \text{ cm H}_2\text{O/mL/s}$, $P = .01$, Fig 3). There was no significant difference in responsiveness to methacholine between the group that was saline-challenged and given IL-5 and the ovalbumin-challenged groups that were given BSA or IL-5 (EC₂₀₀ R_L : IL-5 + saline, $10.69 \pm 0.55 \text{ mg/mL}$ vs BSA + ovalbumin, $11.8 \pm 2.93 \text{ mg/mL}$ vs IL-5 + ovalbumin, $17.95 \pm 3.48 \text{ mg/mL}$; $P > .05$). When we calculated the amount of methacholine necessary to cause an increase in R_L of $0.1 \text{ cm H}_2\text{O/mL/s}$, there was also no difference between both groups (IL-5: $17.7 \pm 15.75 \text{ mg/mL}$ vs control: $10.64 \pm 6.20 \text{ mg/mL}$, $P = .33$). We assessed by lung mincing and digestion total cells, eosinophils, neutrophils, lymphocytes, and macrophages in rats treated with BSA or IL-5, 8 hours after ovalbumin

challenge. We found no difference in all the measures assessed between both groups (data not shown).

Effect of rhIL-5 on blood lymphocyte subsets 8 hours after ovalbumin challenge

When compared to controls, pretreatment with rhIL-5 caused significant differences in blood lymphocyte subsets 8 hours after ovalbumin challenge. We found a higher percentage of blood CD8⁺ cells in IL-5 pretreated and challenged rats (mean control, 20.6 ± 5.8 vs challenged, 30.6 ± 3.7 , $P < .05$), whereas the mean percentage of CD4⁺ cells was lower (control mean, 43.0 ± 6.4 vs challenged, 32.8 ± 5.2 , $P < .05$). The CD4/CD8 ratio in the blood of IL-5 pretreated and challenged rats was significantly lower when compared to BSA pretreated rats (IL-5, 1.18 ± 0.47 vs BSA, 2.92 ± 0.57 ; $P < .05$).

Effect of IL-5 on lung cytokine mRNA expression after ovalbumin challenge

More cells were expressing mRNA for IL-4 in the airways of IL-5 pretreated rats 8 hours after antigen challenge (IL-5 pretreated, 1.92 ± 0.28 positive cells/ 0.45 mm^2 basal membrane vs BSA control, 0.33 ± 0.04 positive cells/ 0.45 mm^2 basal membrane; $P < .05$). Similar results were found for cells expressing IL-5 (IL-5 pretreated, 1.19 ± 0.08 positive cells/ 0.45 mm^2 basal membrane vs BSA control, 0.25 ± 0.09 positive cells/ 0.45 mm^2 basal membrane; $P < .05$). However, we found no difference in interferon- γ mRNA expressing cells between both groups (IL-5 pretreated, 0.482 ± 0.042 positive cells/ 0.45 mm^2 basal

membrane vs BSA control, 0.394 ± 0.086 positive cells/0.45 mm² basal membrane; $P > .05$).

DISCUSSION

This study assessed whether in normal sensitized rats rhIL-5 can cause the principal changes seen in allergic asthma, namely, AHR, airway inflammation, and the ER and LR after antigen challenge. To elucidate the role that IL-5 plays, we administered various doses of rhIL-5 to BN SSN rats that do not develop ER and LR after antigen challenge. Intratracheal administration of rhIL-5 caused AHR 20 hours later but had no effects 30 minutes or 72 hours after administration. The AHR was accompanied by a significant increase in total cells as well as in neutrophils in the lung BAL. Pretreatment of rats with 3 μ g of rhIL-5 did not affect the ER or LR after ovalbumin challenge but decreased the blood CD4/CD8 ratio 8 hours after challenge and increased the number of cells expressing IL-4 and IL-5 mRNA in the airways 20 hours after challenge. In addition, baseline lung resistance increased significantly in IL-5 pretreated/challenged rats 20 hours after ovalbumin challenge, whereas methacholine responsiveness was unchanged.

Allergen challenge of certain sensitized animals causes the activation and recruitment into the airways of a variety of cells, including eosinophils, lymphocytes, mast cells, and neutrophils.^{9,16,17} These inflammatory cells are involved in the physiologic and pathologic changes that are present in atopic asthma.¹³ The mechanism by which these changes occur is not clear; however, recent studies suggest that IL-5 may be an important modulator of allergic inflammation. Studies to date have shown IL-5 to be increased in the blood and lungs of patients with asthma¹⁸ and that administration of IL-5 to patients with allergic bronchial asthma increases cholinergic responsiveness and the number of activated eosinophils in the airways.¹⁰ Studies in animal models of asthma have shown that administration of IL-5 to sensitized guinea pigs enhances antigen-induced hyperresponsiveness¹⁹ and that IL-5 neutralizing antibodies inhibit the LR after antigen challenge.²⁰ Although there is convincing evidence that IL-5 is important in asthma and in animal models of asthma, no studies have examined the effects of IL-5 in animals that do not respond to sensitization and antigen challenge with physiologic changes. We performed this study in the SSN substrain of BN rat that does not develop an LR after antigen challenge^{12,21} to assess whether IL-5 has the capacity to transform non-responsive BN (SSN) rats into those with the characteristics encountered in atopic asthma.

For the study reported here, we first determined whether rhIL-5 (which has 71% homology with rat IL-5²⁰) had effects on rat blood cells *in vitro*. Experiments performed on rat PBMCs showed that rhIL-5 caused a concentration-dependent proliferation of eosinophils from progenitors at a dose ranging from 1 to 5 ng/mL. These results would suggest, but not confirm, that rhIL-5 is having the same effects in rats as rat IL-5 would have.

We also determined whether the BN SSN rats were indeed sensitized to ovalbumin by measuring levels of ovalbumin-specific IgE for 1 to 3 weeks after sensitization. Rats showed a significant increase in serum ovalbumin-specific IgE for at least 3 weeks after ovalbumin sensitization. We then determined whether intratracheal administration of rhIL-5 had effects on AHR. An increase in airway responsiveness to methacholine occurred 20 hours after intratracheal administration of rhIL-5 at a dose exceeding 3 μ g. Recombinant human IL-5 did not affect airway responsiveness 30 minutes or 72 hours after intratracheal administration. The time course of these effects would suggest that IL-5 is acting indirectly. Results obtained from lung lavage would concur with this hypothesis. We measured the level of endotoxin in the rhIL-5 given to animals, and to be certain that this low level of endotoxin was not affecting the results we administered the same dose of lipopolysaccharide intratracheally to animals and measured their airway response to methacholine and inflammatory cells in the BAL 20 hours later. Data collected from animals given lipopolysaccharide did not differ significantly with controls; therefore we were confident that the effects of rhIL-5 were not due to endotoxin contamination. We also performed additional experiments confirming the direct effect of rhIL-5 by using TRFK5, an IL-5 neutralizing antibody. Intratracheal administration of 50 ng of TRFK5 at the same time as 10 μ g of rhIL-5 significantly reduced airway responsiveness and cell influx when compared to the groups that received only 10 μ g of rhIL-5.

We found that IL-5 caused an increase in BAL neutrophils but not in eosinophils as has been described in guinea pigs. This discrepancy may be due to the inherent differences between the 2 animal models. BN SSN rats may not have had an increased number of eosinophils in lung lavage because of technical considerations. Eosinophil degranulation and mediator release may have already taken place 20 hours after IL-5 administration, preventing us from measuring their presence by a regular Wright-Giemsa stain. However, major basic protein staining did not show evidence of eosinophil degranulation or mediator release (data not shown), which adds more probability to the conclusion that eosinophils were not increased in lung lavage 20 hours after IL-5 administration. Another reason for this discrepancy is that perhaps eosinophils are unavailable for recruitment into the lungs in this strain of rat. Until now, experiments assessing whether IL-5 is involved in asthma have used animal models that develop allergic responses after antigen challenge and have shown that the physiologic response can be increased by IL-5. It is possible that in "atopic" animal models, eosinophils or their progenitors are readily available to be recruited into the lungs, whereas in BN SSN rats these cells are not. However, neutrophils do release mediators that have the potential to alter airway function by causing tissue injury, namely, oxygen radicals, proteases, and cationic proteins.²² Therefore, although the SSN strain of BN rat may not respond to IL-5 with increased eosinophil accumulation into the air-

ways, increased responsiveness to methacholine may have still occurred through neutrophil degranulation.

We assessed the effects of pretreatment with rhIL-5 on the ER, LR, and AHR in BN SSN rats after antigen challenge. Intratracheal administration of 3 μ g of rhIL-5, 24 hours before ovalbumin challenge, did not affect the ER, LR, or cellular influx after challenge when compared to controls receiving the same amount of BSA. It may be that in the predisposed subject, intratracheal IL-5 is capable of increasing all the physiologic changes that occur after antigen challenge, but that several crucial factors that are necessary for an atopic airway response may be lacking in the normal rat.²³⁻²⁵ We found a significant decrease in the CD4⁺/CD8⁺ T-cell ratio in the blood between IL-5 pretreated/challenged and control animals 8 hours after antigen challenge. The difference is mainly due to an increase in CD8⁺ cytotoxic/suppressor T cells in the blood of challenged animals. A decrease in the helper/suppressor ratio in the blood has been described during the LR in humans.¹³ It has been postulated that CD4⁺ lymphocytes are recruited into the lungs after antigen challenge in the subjects that develop an LR. This hypothesis has been predicated on the finding of increased CD4⁺ cells in lung lavage after antigen challenge.²⁶ We have previously reported a decrease in the helper/suppressor ratio in the blood of BN rats that develop an LR.¹⁵ Interestingly, pretreatment with IL-5 induced the same changes in the CD4/CD8 ratio as those previously reported in BN rats with LR. The absence of an LR in the IL-5 pretreated rats would suggest that changes in blood lymphocyte subsets do not have an important role in mediating an LR.

In searching for other ways by which IL-5 could increase R_L 20 hours after ovalbumin challenge, we assessed lung T_H2 cytokine mRNA expression. We found increases in both IL-4 and IL-5 mRNA expression after antigen challenge but no effect on the T_H1 cytokine interferon- γ mRNA expression. Increases in T_H2 cytokines are associated with increases in R_L after antigen challenge in humans.⁷ It is thus possible that the effects in R_L encountered in ovalbumin-challenged BN rats are explained by changes in these cytokines.

IL-5 administration followed by allergen challenge has been shown to increase eosinophil numbers in lung tissue and increase AHR.¹⁰ However, in our study there was no observed increase in AHR after IL-5 treatment/ovalbumin challenge. A recent report has found that the treatment of humans with an anti-IL-5 antibody does not prevent the development of LR or improve their asthma.²⁷ These results concur with those that are reported here and suggest that despite the abundance of data showing that IL-5 has a crucial role in mediating the asthma phenotype, it may be only partially responsible for all the physiologic changes encountered in asthma.

In conclusion, we have found that intratracheal administration of rhIL-5 to rats that do not have all the characteristics of atopic asthma will cause some but not all of the physiologic changes that are found in atopic asthma. As suggested in a recent publication,²⁷ therapeutic strate-

gies directed against IL-5 alone may be insufficient to revert the airway response to normal in patients with atopic asthma.

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