

IL-2 enhances allergic airway responses in rats by increased inflammation but not through increased synthesis of cysteinyl leukotrienes

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Background: IL-2 has been shown to increase allergic airway responses in rats.

Objective: The purpose of this study was to investigate whether induction of inflammation and enhancement of cysteinyl-leukotriene (cys-LT) synthesis were involved in the augmentation of airway responses caused by IL-2.

Methods: Brown Norway rats received human recombinant IL-2 or saline subcutaneously twice a day from day 9 to day 14 after sensitization to ovalbumin (OVA). On day 14, rats underwent either lung lavage or were challenged with an aerosol spray of OVA, the airway responses and biliary excretion of cys-LTs were measured for a period of 8 hours after challenge, and the lung leukocyte numbers were determined after enzymatic digestion of lung tissues.

Results: The early response after OVA increased from $184.2\% \pm 13.5\%$ in the animals receiving saline ($n = 10$) to $309\% \pm 51\%$ (baseline lung resistance) in IL-2-pretreated animals ($n = 17$; $P < .05$). The late response also increased from 19.6 ± 4.5 (area under the curve of baseline lung resistance vs time) in the animals receiving saline to 37 ± 5.4 after administration of IL-2 ($P < .05$). However, IL-2-treated animals had lower levels of biliary cys-LTs during the late response than saline-treated animals but similar levels during the early response. This difference could not be attributed to an increase in LT metabolism, which we assessed by the recovery of $^3\text{H-LTC}_4$ instilled intratracheally in challenged or unchallenged rats. When compared with control animals, pretreatment with IL-2 increased all cell types retrieved from lung lavage fluid before OVA challenge ($P < .05$). After OVA challenge, the total cell yield from lung lavage fluid was also increased, mostly because of an increase in neutrophils ($P < .05$). Eosinophils and lymphocytes were greater in the lungs of IL-2-treated than vehicle-treated and OVA-challenged rats ($P < .01$), and IL-2-treated rats had a lower $\text{CD4}^+/\text{CD8}^+$ ratio in the blood after challenge ($P < .001$).

Conclusion: In conclusion, IL-2 increases early and late responses in rats, and it induces lung inflammation. Altered

airway responses are not attributable to an increase in cys-LT production. (*J Allergy Clin Immunol* 1999;104:145-52.)

Key words: IL-2, Brown Norway rat, leukotrienes, antigen challenge

The sensitized Brown Norway (BN) rat demonstrates early and late airway responses to allergen challenge.¹ These reactions are accompanied by airway inflammation involving neutrophils in the first few hours after exposure and subsequently involving eosinophils and lymphocytes.^{2,3} Although the relationship between airway leukocytes and allergen-induced airway narrowing is unclear, several lines of evidence suggest that T lymphocytes are involved in the regulation of both airway inflammation and the associated airway narrowing. First, CD4^+ T cells from sensitized donors can transfer late allergic responses to naive recipients,⁴ suggesting an important role for this subset of T lymphocytes in modulating airway inflammation. Second, the administration of IL-2, a lymphokine released by CD4^+ T lymphocytes when they respond to antigen, increased the airway responsiveness to methacholine in rats^{5,6} and to acetylcholine in guinea pigs.⁷ In addition, in doses that did not affect airway responsiveness to methacholine, IL-2 also caused an increase in early and late airway responses to antigen challenge with ovalbumin (OVA) in the BN rat.⁸ Lung lavage showed an increase in lymphocytes and eosinophils, and lung histology showed an increase in mast cells.⁸ These results suggested that the effects of IL-2 on allergic airway narrowing were likely mediated by its immunomodulatory properties.

Cysteinyl leukotrienes (cys-LTs) are important biochemical mediators of the airway responses to antigen in the sensitized BN rat,^{9,10} sheep,¹¹ and humans.¹² Because pretreatment with a leukotriene (LT) D_4 receptor antagonist inhibits the airway responses after antigen challenge of BN rats,⁹ we postulated that the augmentation of allergic airway narrowing by IL-2 in BN rats was caused by an increase in LT production rather than an enhanced sensitivity to the LTs released. To examine this question, we studied the effects of IL-2 administration on allergen-induced bronchoconstriction, leukocyte numbers in the lungs, and cys-LT synthesis. The latter was evaluated by measuring the different LT metabolites in bile by RIA after purification by HPLC. Bile has been previously shown^{11,13,14} to be the major route of excre-

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Supported by the J. T. Costello Memorial Research Fund, the Research Center of the University of Montreal affiliated Hospitals, and the Medical Research Council of Canada (grant number MA 10381).

Received for publication Jan 14, 1998; revised Dec 16, 1998; accepted for publication Mar 5, 1999.

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0091-6749/99 \$8.00 + 0 1/1/98670

Abbreviations used

BN:	Brown Norway
cys-LT:	Cysteinyl leukotriene
LT:	Leukotriene
OVA:	Ovalbumin
R _L :	Lung resistance
S/P:	Small airways and parenchyma

tion of cys-LTs, and total LTs are reasonably well correlated with the magnitude of the late response.¹⁰

METHODS**Animals and sensitization**

Fifty-nine male highly inbred BN rats (Harlan Sprague Dawley) that were 7 to 9 weeks old were studied. All rats were sensitized with OVA by subcutaneous injection of 1 mL of sterile solution containing 1 mg of OVA and 3.5 mg of Al(OH)₃ gel in 1 mL of saline. On the same occasion, 0.5 mL of *Bordetella pertussis* vaccine containing 6×10^9 heat-killed bacilli was injected intraperitoneally into all of the rats except the 18 that underwent studies with lung lavage.

Drugs and chemicals

IL-2 was graciously provided by Hoffman-LaRoche Inc (Nutley, NJ). LTC₄, LTD₄, LTE₄, N-acetyl-LTE₄, and mAbs cross-reacting with cys-LTs were kindly provided by Dr I. Rodger of Merck-Frosst (Pointe Claire, Quebec, Canada). OVA, collagenase, Wright-Giemsa stain, and propidium iodide were purchased from Sigma (St Louis, Mo). *B pertussis* vaccine was purchased from the Armand-Frappier Institute (Laval-Des-Rapides, Quebec, Canada). W3/25, OX8, and OX33 mAbs were purchased from Dimension Labs (Mississauga, Ontario, Canada). FITC-conjugated rat anti-mouse mAb was obtained from Kappel, Organon Tecnica (Scarborough, Ontario, Canada). FBS, penicillin, streptomycin, L-glutamine, nonessential amino acids, McCoy's medium, HBSS, and trypan blue were obtained from Gibco Labs. Ficoll-Hypaque was obtained from Pharmacia (Montreal, Quebec, Canada).

Study protocol

IL-2 and OVA challenge. Two groups of rats received different doses of IL-2 to explore the dose-response relationship for allergic responses and cys-LT production. The doses of IL-2 that were chosen were less than those used in previous experiments, and this dosing regimen had no effect on airway response to methacholine.⁸ In group 1, 9 rats were given 8500 U of IL-2 in 0.2 mL of saline (low-dose IL-2) subcutaneously twice a day from day 9 to day 14 after sensitization. In group 2, 8 rats received 30,000 U of IL-2 in 0.2 mL of saline (high-dose IL-2) subcutaneously twice a day for 4.5 days as above. The IL-2 used in our experiments contained less than 1 ng/mL endotoxin before dilution, and we have previously shown that 8 ng/mL endotoxin does not affect the airway responses to methacholine or antigen.^{5,6,8} Because we found no significant difference between high- and low-dose IL-2-treated rats, the results were pooled for statistical analysis. In group 3, 10 rats were given only saline (0.2 mL) and otherwise treated as above. On day 14 after sensitization, all rats were challenged with an aerosol spray of OVA for 5 minutes (5% wt/vol in 4 mL of sterile saline), and subsequently lung resistance (R_L) was recorded for 8 hours.

IL-2 and cys-LT metabolism. In separate animals we assessed whether systemic administration of IL-2 affected cys-LT metabolism. Fourteen sensitized rats received either 30,000 U of IL-2 or

vehicle in 0.2 mL of saline subcutaneously twice a day for 4.5 days. The rats were anesthetized and intubated, and their bile ducts were cannulated. Tritiated LTC₄ was then injected intratracheally in challenged or unchallenged animals, and bile was collected for the measurement of ³H-LTs over 4 hours.

IL-2 and cellular infiltration in lung lavage fluid. Animals (n = 18) were anesthetized on day 14 after sensitization. Lung lavage was performed in 6 saline-pretreated rats and 6 IL-2-pretreated rats immediately after endotracheal intubation and in another 6 IL-2-pretreated rats at 8 hours after OVA challenge.

Animal preparation

The care and the use of animals was performed according to the Principles of Laboratory Animal Care formulated by the National Society for Medical Research. Rats were anesthetized with urethane (1 g/kg administered intraperitoneally). A heating pad was used to maintain constant body temperature during the experiment, and rectal temperature was monitored continuously with an electronic thermometer. After orotracheal intubation with 6 cm of PE-240 polyethylene catheter, the common bile duct was exposed through a small abdominal incision and cannulated (PE-20, 15 cm) after ligation of the duodenal end. All bile samples were collected on ice in 1.5-mL Eppendorf tubes under a stream of argon. Bile samples were collected for measurement of cys-LTs 1 hour before challenge (baseline) and for 8 hours after challenge. All samples were stored at -70°C before analysis.

Measurement of pulmonary responses

R_L was measured during spontaneous tidal breathing, with the animals in the supine position. Flow was measured by placing the tip of the tracheal tube inside a small Plexiglas box (265 mL in volume). A Fleisch pneumotachograph coupled to a piezoresistive differential pressure transducer (Migro-Switch 163PCOVID36; Honeywell, Scarborough, Ontario, Canada) was attached to the other end of the box to measure airflow. Transpulmonary pressure was measured by using a water-filled catheter placed in the lower third of the esophagus connected to one port of a differential pressure transducer (Transpac II, Abbott, Ill); the other port was connected to the Plexiglas box. The esophageal catheter consisted of a polyethylene tube (PE-240, 10 cm) with a terminal tip (6 cm) of a smaller bore tube (PE-160).

The pressure and flow signals were amplified and passed through 8-pole Bessel filters (9 model 902LPF; Frequency Devices, Haverhill, Mass) with their cut-off frequencies set at 100 Hz. The data were stored on a computer. R_L was calculated by multiple linear regression by fitting the equation of motion: $P_{tp} = E_L \cdot \dot{V} + R_L \cdot \dot{V} + K$, where P_{tp} is transpulmonary pressure, E_L is lung elastance, $\dot{V}$ is volume, and K is a constant value. Commercial software was used (RHT Infodat Inc, Montreal, Quebec, Canada).

After instrumentation, the rats were allowed to stabilize for a period of 1 hour. Rats were then challenged with an aerosol spray of OVA for 5 minutes. R_L was measured 5, 10, 15, 20, and 30 minutes after challenge and subsequently every 15 minutes for a total time of 8 hours.

Intratracheal administration of ³H-LTC₄

After general anesthesia, endotracheal intubation, and cannulation of the common bile duct, 1 μ Ci of ³H-LTC₄ was instilled intratracheally in 200 μ L of saline into animals that were either sensitized and unchallenged or that had been challenged with OVA 4 hours previously. The bile was recovered 30, 60, 120, 180, and 240 minutes later for the measurement of radioactivity corresponding to LTC₄ and its metabolites (LTD₄, LTE₄, N-acetyl-LTE₄) by using a liquid scintillation counter (model LS 6000K; Beckman Instruments, Irvine, Calif).

HPLC and RIA analysis

HPLC separation of the cys-LT metabolites was achieved and assessed as previously described¹⁰ by using an adsorbosphere HS C18 reversed-phase column (15 cm × 4.6 mm; Waters Associates, Milford, Mass) with a mobile phase of 64% methanol in aqueous buffer (0.1% acetic acid NH₄OH plus 1 mmol/L EDTA, pH 5.4) premixed and run isocratically at 0.7 mL/min. UV absorbance was monitored with a variable wavelength UV detector (model 481 Millipore; Waters, Bedford, Mass). Column eluate fractions were collected every minute and analyzed for cys-LTs by RIA.¹⁰ An mAb that was directed against LTC₄ but that cross-reacted with LTD₄ (64%), LTE₄ (50%), and N-acetyl-LTE₄ (60%) was used. The retention times of authentic standards were as follows: LTC₄, 9 minutes; LTD₄, 20 minutes; LTE₄, 25 minutes; and N-acetyl-LTE₄, 15 minutes.

Airway inflammation assessed by lavage

The lungs were lavaged through the tracheal tube by instillation of 5 × 5 mL of saline at room temperature followed by gentle aspiration. The volume retrieved averaged 81% of the 25 mL that was instilled and did not differ between the groups. The total cell count was determined by using a hemacytometer. Differential cell counts were assessed on cytologic preparations. Slides were prepared with a cytospin model III (Shandon, Cheshire, England) and stained with Wright-Giemsa stain. Two hundred cells were counted under oil-immersion microscopy.

Lung inflammation assessed by enzymatic digestion

Mincing and digestion were performed on lungs removed 8 hours after OVA challenge, as previously described.^{2,3} These animals did not undergo lung lavage before mincing and digestion. The chest wall was opened, and animals were exsanguinated by cardiac puncture. Blood was kept in heparinized tubes for analysis of lymphocyte subsets by flow cytometry. The pulmonary vasculature was flushed with a balanced salt solution, and the lungs were separated into the large airways (trachea and large bronchi until approximately the fifth generation) and small airways and parenchyma (S/P). Tissues were washed, cut into 1-mm sections, and incubated separately for tissue digestion. Tissue digestion was performed by three 20-minute incubations in 12 mL of enriched McCoy's solution containing 20 U/mL collagenase. After each incubation, cells were separated from tissue by filtration through a #60 sieve (Sigma) and pooled for counting with a hemacytometer. Slides were prepared by centrifuging 500,000 cells in enriched McCoy's solution for 5 minutes at 700 rpm. Cellular differentials were assessed on Wright Giemsa-stained slides as described above.

Isolation and staining of blood lymphocyte subsets

Mononuclear cells were isolated from fresh heparinized peripheral blood by standard Ficoll-Hypaque methods¹⁵ and prepared for flow cytometry. The isolated cells were washed with HBSS and stained indirectly by incubating with the monoclonal W3/25 antibodies (CD4 cells),¹⁶ OX8 (CD8 cells),¹⁷ and OX33 (B cells)¹⁸ for 30 minutes. After washing in HBSS, a second incubation was performed for 30 minutes with an FITC-conjugated rabbit anti-mouse IgG antibody. Cells were studied immediately or fixed in 1% paraformaldehyde/0.85% saline. The fixed cells were stored at 4°C in the dark until analysis. Flow cytometry was performed with an FACSscan analyzer (Becton Dickinson FACS Division, Sunnyvale, Calif), focusing on the lymphocyte cluster. Viability was determined by adding 1 µg/mL propidium iodide to unfixed specimens and averaged 96% ± 3%. Controls consisted of cells stained with an

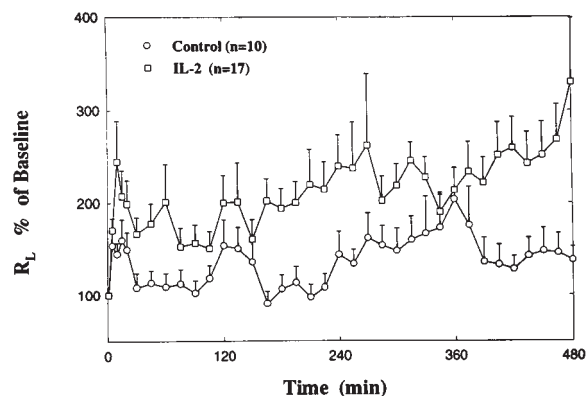


FIG 1. Effect of IL-2 on the airway response to OVA. BN rats received either IL-2 (8500 or 30,000 U) or saline (control) subcutaneously twice a day from day 9 to day 14 after sensitization and were challenged with OVA. R_L is presented as a percentage of baseline over time in minutes.

IgG₁ mAb obtained from control ascite fluid from BALB/C mice and averaged 1.9% ± 0.4%.

Large airways and S/P lymphocyte subsets

Large airway and S/P cells obtained from tissue digestion were washed in HBSS and stained as described above for OX33⁺ (B), W3/25⁺ (CD4), and OX8⁺ (CD8) cells. Staining and flow cytometry were performed concurrently for the large airways, S/P, and blood for each animal.

Data analysis

The early response to OVA instillation was calculated as the largest value of R_L within the first hour after challenge. The late response was calculated as the area under the curve of R_L above the baseline value over the 3- to 8-hour period after challenge.

The total amounts of cys-LTs in bile were calculated by adding the amounts (in picomoles per time period) for LTC₄, LTD₄, LTE₄, and N-acetyl-LTE₄. On the basis of previous observations,¹⁰ the increased levels of cys-LTs appearing in bile during the first and second hour after challenge were considered to have arisen principally as a result of the early response, whereas the amounts of cys-LTs in bile collected between 3 and 8 hours after challenge were considered to be related to the late response.

Data were analyzed by using a 1-way parametric ANOVA and/or subsequent paired and unpaired *t* tests or a Dunnett *t* test as appropriate. All results are expressed as means ± SEM. Differences were considered to be statistically significant when *P* values were less than .05.

RESULTS

Effect of IL-2 on airway responses to OVA

IL-2 caused an increase in the airway responses to OVA (Fig 1). The pattern of change in R_L was similar in the control and IL-2-treated animals, with the exception that recovery from the early response was incomplete, and R_L never returned to baseline values. There was a dose-dependent increase in the early and late responses for different doses of IL-2. The early responses to OVA were 280% ± 57.5% for low-dose IL-2 and 339% ± 54% for high-dose IL-2. The late responses for the 2 IL-2-treated groups of rats were 35.3 ± 6.7 and 43.2 ± 11.2 (area under the curve vs time), respectively. The effects

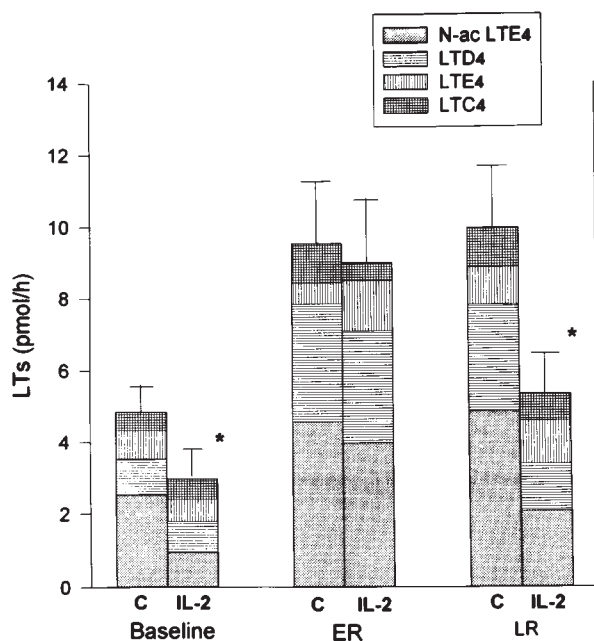


FIG 2. Effect of IL-2 on cys-LT production. Bile cys-LTs were collected for 1 hour before challenge and expressed in picomoles per hour (baseline). The total bile cys-LTs (and their metabolites) after antigen challenge are presented during the early response (ER; first 2 hours) and the late response (LR; between 3 and 8 hours). * $P < .05$ for IL-2-treated rats (IL-2) versus vehicle-treated controls (C).

of dose were not significant, however, for either the early or late responses, and therefore data from both groups were pooled.

During the early response, peak R_L was $184\% \pm 13.5\%$ of baseline for controls and $309.2\% \pm 51\%$ for IL-2-treated rats ($P < .05$). A significant late response to OVA was present in 6 of 10 vehicle-treated control rats and in 14 of 17 IL-2-treated rats. The late response was 19.6 ± 4.5 for controls and 36.9 ± 5.4 (area under the curve vs time) for IL-2-treated rats. These differences were statistically significant.

Effects of IL-2 on biliary cys-LT production after allergen challenge

Biliary cys-LTs were measured in 6 controls and 13 IL-2-pretreated rats that had bile cannulas inserted before OVA challenge. Among the controls, 3 had an early response, and 4 had a late response. In the IL-2-pretreated rats, 10 had an early response, and 11 had a late response. Baseline cys-LT level for the 4 rats that did not have a late response was 4.1 ± 1.8 pmol/h, and baseline cys-LT level for the 15 rats that had a late response was 3.5 ± 1.1 pmol/h ($P = .78$). When cys-LT levels were compared between the IL-2-pretreated rats and controls, they were lower in the IL-2-pretreated rats (Fig 2). Cys-LT levels increased in IL-2-treated animals during the early response but returned to levels that were not significantly different from baseline during the late response. Cys-LT levels were significantly elevated in control ani-

mals during the late response. When cys-LT levels were compared in the rats with late responses only, baseline cys-LT levels were 5.0 ± 0.9 and 3.7 ± 0.4 pmol/h for controls and IL-2-pretreated rats, respectively ($P > .05$); during the late response, cys-LT production was 9.8 ± 0.9 and 5.7 ± 0.3 pmol/h for controls and IL-2-treated rats, respectively ($P < .05$).

Effects of IL-2 on cys-LT metabolism

To assess the possibility that changes in cys-LT levels after IL-2 treatment reflect an alteration in metabolism/excretion by the liver, we evaluated cys-LT metabolism by instilling $^3\text{H-LTC}_4$ intratracheally and increasing its biliary excretion in controls and in IL-2-treated rats that were either challenged or unchallenged with OVA (Fig 3). Pretreatment with IL-2 tended to increase the biliary excretion of N-acetyl-LTE₄, and numbers of total cys-LTs compared with those found in controls (Fig 3, A and B). The total recovery was $39.5\% \pm 5\%$ and $33\% \pm 4\%$ ($P > .05$) in IL-2-treated and control rats, respectively. We found a significant decrease in radioactive cys-LT recovery when $^3\text{H-LTC}_4$ was instilled 4 hours after antigen challenge in IL-2-treated and control rats. The recovery of $^3\text{H-LTs}$ was $36\% \pm 4\%$ versus $23.5\% \pm 1.5\%$ of the amount instilled in rats that were unchallenged versus those examined 4 hours after challenge, respectively ($P < .05$). However, there was no difference between IL-2-pretreated rats and controls in total $^3\text{H-LT}$ recovery when instilled 4 hours after challenge (Fig 3, C and D). The total recovery was $22\% \pm 1\%$ and $25\% \pm 1.5\%$ for IL-2-treated and control rats, respectively. There was no alteration in the proportion of polar products of LTs that ran with short retention times on the HPLC column.

Effects of IL-2 on the cellular yield from lung lavage fluid

IL-2-treated rats had an increased yield of total cells, macrophages, neutrophils, lymphocytes, and eosinophils from lung lavage fluid before antigen challenge when compared with saline-treated rats ($P < .05$; Table I). The total cell yield also increased significantly in IL-2-treated animals 8 hours after antigen challenge when compared with IL-2-treated animals before antigen challenge, mostly because of a significant increase in the yield of neutrophils ($P < .05$; Table I).

Effects of IL-2 on the cellular yield from the S/P and large airways after antigen challenge

IL-2-treated rats had an increased yield of total cells from the S/P (Fig 4). The cellular return was $23.3\% \pm 3.2\%$, $32.9\% \pm 2.0\%$, and $38.2\% \pm 0.4\%$ ($\times 10^6$ cells) for controls and low- and high-dose IL-2-treated rats, respectively. Total eosinophils and lymphocytes were also significantly increased in the S/P in IL-2-treated rats. Eosinophils were 0.38 ± 0.12 , 3.76 ± 0.51 , and 5.57 ± 1.28 million cells for controls and low- and high-dose IL-2-treated rats, respectively. Lymphocytes were 1.29 ± 0.25 , 2.02 ± 0.29 , and 3.71 ± 0.49 million cells for con-

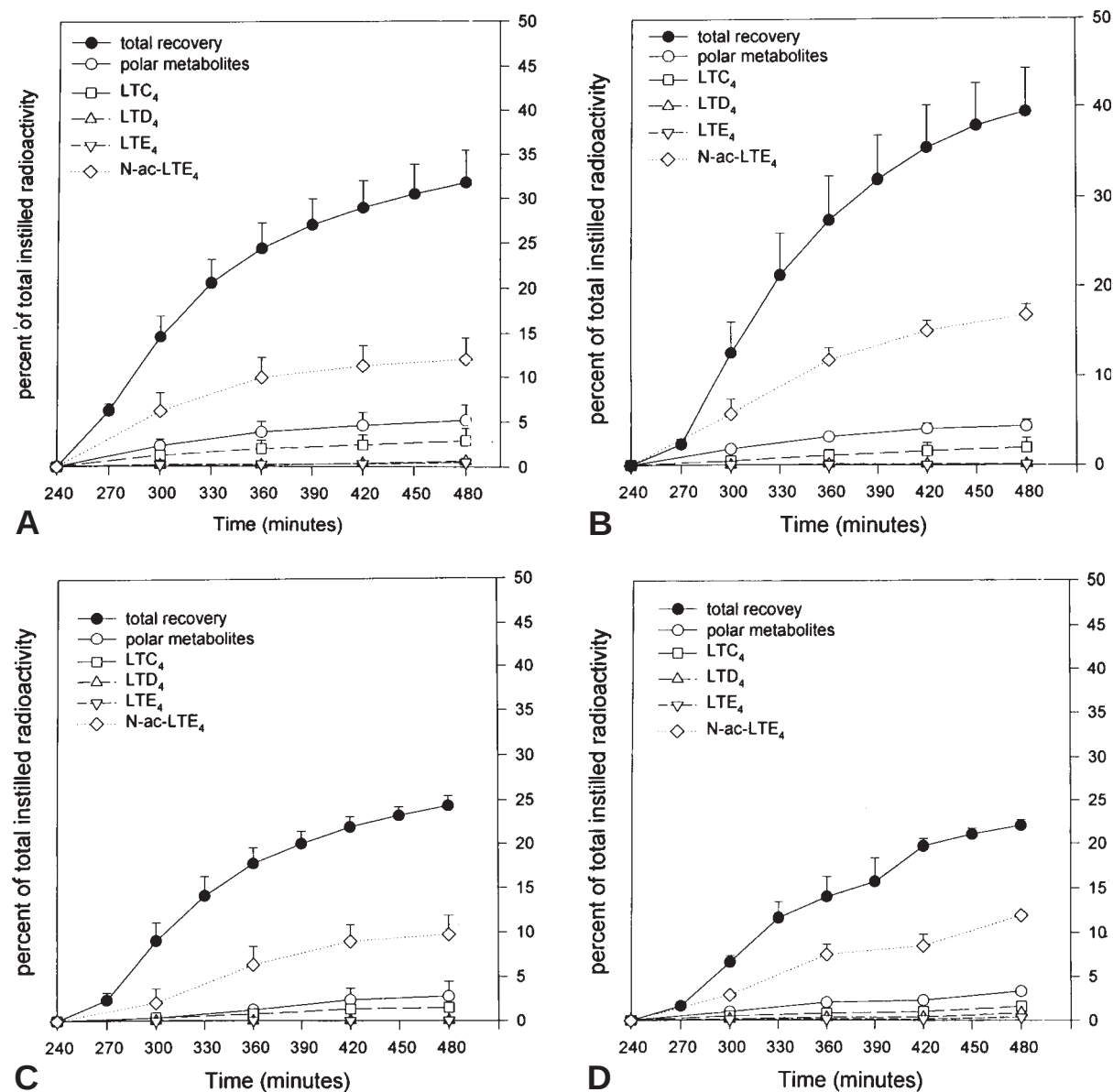


FIG 3. Effect of IL-2 on the metabolism of ^3H -LTC₄. One microcurie of ^3H -LTC₄ was instilled intratracheally into sensitized and unchallenged anesthetized rats 4 hours after anesthesia was obtained. Rats received either saline (**A**; $n = 4$) or IL-2 (**B**; $n = 3$) for 4 days, and the percentage of recovery of the different cys-LTs metabolites in the bile was measured over a 4-hour period. In other experiments sensitized rats receiving either saline (**C**; $n = 3$) or IL-2 (**D**; $n = 4$) were challenged with OVA, and ^3H -LTC₄ was instilled intratracheally 4 hours after challenge followed by the measurement of the different metabolites of LTC₄ in the bile as a percentage of the total radioactivity instilled. *N-ac-LTE₄*, N-acetyl-LTE₄.

TABLE I. Effect of IL-2 on the cellular return in lung lavage fluid

Experiment	Total cells (millions)	Macrophages (millions)	Neutrophils (millions)	Eosinophils (millions)	Lymphocytes (millions)
Control	0.52 ± 0.1	0.468 ± 0.09	0.0052 ± 0.002	0.0052 ± 0.003	0.042 ± 0.008
IL-2 unchallenged*	1.5 ± 0.2	0.87 ± 0.16	0.075 ± 0.016	0.225 ± 0.026	0.33 ± 0.022
IL-2 challenged*	$2.9 \pm 0.4^\dagger$	1.5 ± 0.25	$0.88 \pm 0.092^\dagger$	0.26 ± 0.032	0.26 ± 0.029

IL-2 unchallenged, Rats that received IL-2, with lavage performed immediately after anesthesia; *IL-2 challenged*, rats that received IL-2, with lavage performed 8 hours after antigen challenge.

* $P < .05$ for all values versus controls.

$^\dagger P < .05$ IL-2 challenged versus IL-2 unchallenged.

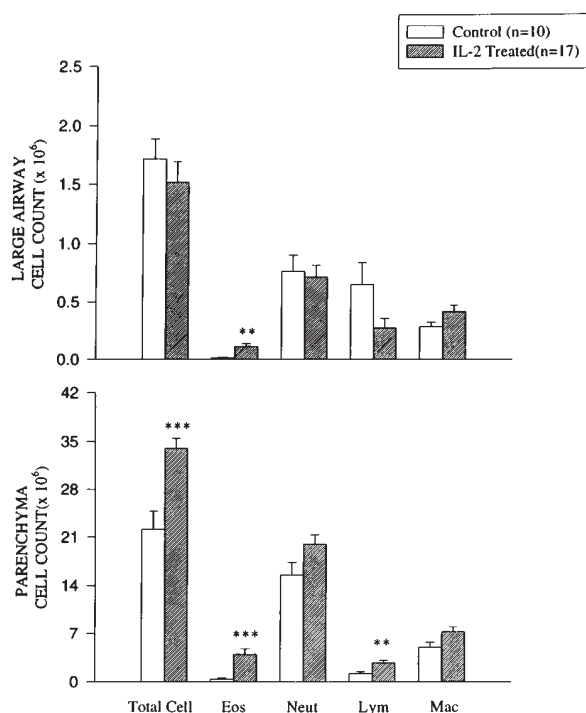


FIG 4. Effect of IL-2 on the cellular yield from the lungs 8 hours after OVA challenge. Sensitized BN rats received either saline (control) or IL-2 for 4.5 days and were challenged with OVA. Eight hours later, the rats were killed, and the large airway and S/P were digested for the total cell count and differential. *Eos*, Eosinophils; *Neut*, neutrophils; *Lym*, lymphocytes; *Mac*, macrophages. ** $P < .01$ versus controls; *** $P < .001$ versus controls.

controls and low- and high-dose IL-2-treated rats, respectively. Neutrophils and macrophages were not significantly different between controls and IL-2-treated rats.

In the large airways no difference was found in the total cellular return. However, eosinophils were significantly increased in IL-2-treated rats (Fig 4). Eosinophils were 0.014 ± 0.009 , 0.127 ± 0.037 , and 0.12 ± 0.027 ($\times 10^6$ cells) in controls and low- and high-dose IL-2-treated rats, respectively. Lymphocytes, neutrophils, and macrophages were not significantly different in the large airways between controls and IL-2-treated rats.

Effects of IL-2 on lymphocyte subsets

IL-2-treated rats had a higher percentage of CD4⁺ cells in the S/P and a lower percentage of CD4⁺ cells in the large airways and in the blood when compared with controls (Fig 5). In the blood the CD4⁺/CD8⁺ ratio was significantly lower in the IL-2-treated rats (low-dose IL-2 vs control, $P < .01$; high-dose IL-2 vs control, $P < .001$). No differences were found in the percentages of B cells in the S/P, large airways, or blood.

DISCUSSION

Pretreatment of BN rats with IL-2 for 4.5 days increased the early and late airway responses after OVA challenge. IL-2 enhanced airway inflammation, as evi-

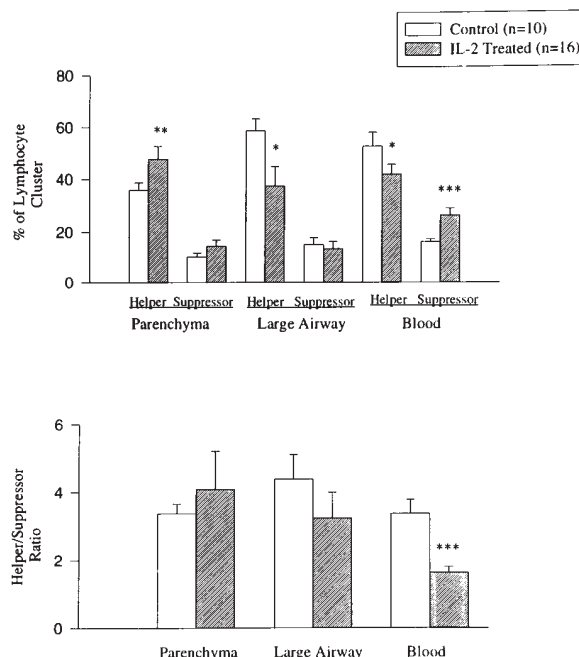


FIG 5. Effects of IL-2 on lymphocyte subsets in the lungs and blood after OVA challenge. Rats were killed 8 hours after challenge, blood was retrieved, the lungs were digested, and lymphocyte subsets, as well as the CD4⁺/CD8⁺ ratio (*helper/suppressor ratio*), were determined by flow cytometry. * $P < .05$ versus control; ** $P < .01$ versus control; *** $P < .001$ versus control.

denced by an increased cellular return from lung lavage fluid that was characterized by a higher yield of eosinophils and lymphocytes from the S/P after antigen challenge. Although IL-2 did not significantly increase the total cellular return from the tissues of the large airways, it did increase the yield of eosinophils. The mechanism of the increase in the airway responses to OVA could not be attributed to increased cys-LT production. In fact, cys-LTs were lower in IL-2-treated rats at baseline and during the late response, and this effect could not be explained by alterations in cys-LT metabolism.

The role of the T lymphocyte and cell-mediated immunity in allergic airway responses is still incompletely understood. In previous studies we have found that functionally normal CD4⁺ lymphocytes are necessary for the occurrence of late airway responses in the BN rat¹⁹ and that adoptive transfer of sensitized lymphocytes can lead to late airway responses after OVA challenge.^{4,20} Although the evidence is compelling that late responses can be modulated by T lymphocytes, it is unclear whether the modulation is exerted by the usual pathways that are involved in late responses. In this study we have focused on IL-2, an important T-cell growth factor. IL-2 is released by T lymphocytes in response to antigen presentation and leads to lymphocyte proliferation and cytokine production. Although the predominant lymphokines expressed by CD4⁺ T cells in the lungs of asthmatic subjects are of the CD4⁺ type 2 (T_{H2}) subset (expressing predominantly IL-4 and IL-5), IL-2 and its receptor are nonetheless increased in the lungs of asth-

matic subjects.^{21,22} IL-2 is capable of inducing the proliferation of T_{H2} lymphocytes,²³ and its administration to human subjects causes eosinophilia through the production of IL-5.²⁴ IL-2 also stimulates T lymphocytes from asthmatic subjects to release mediators that increase eosinophil proliferation and survival.²⁵

In previous studies performed in the BN rat, we found that pretreatment with 45,000 U of IL-2 for 4.5 days increased airway responses to OVA and increased the number of inflammatory cells present in lung lavage fluid.⁸ This report shows that much smaller doses (8000 or 30,000 U of IL-2 for 4.5 days) are sufficient to increase the early and late airway responses to OVA, confirming that stimulation of cell-mediated immunity through the administration of IL-2 can thus amplify the physiologic changes following allergen challenge. It seems likely that altered airway leukocytes, either in numbers or activation state, are causally related to the enhanced airway responses. Macrophages, neutrophils, lymphocytes, and eosinophils were all increased in lung lavage fluid in IL-2-treated rats before antigen challenge, indicating the capacity for this cytokine to induce a pleomorphic inflammation. After antigen challenge, lymphocytes and eosinophils were increased in the S/P, and eosinophils were increased in the large airways when compared with controls. Presumably, the lung eosinophilia is caused by IL-5-secreting T lymphocytes.²⁴

The increased yield of inflammatory cells from lung tissue of IL-2-treated rats appears to be explained in part by the presence of more inflammatory cells in the lungs before antigen challenge. However, analysis of lymphocyte subsets in lung tissue and the blood would suggest that some changes also occurred after antigen challenge. Such changes have also been observed in human asthmatic subjects, in whom a decrease in CD4⁺ cells in the blood is found after antigen challenge. It has been postulated that the egress of CD4⁺ cells from the blood into antigen-challenged tissues takes place.²⁶ In sensitized BN rats the CD4⁺/CD8⁺ ratio decreased in the blood after antigen challenge, which was also true of T cells in the large airways and the S/P in animals in which late airway responses occur.² In the experiments reported here, IL-2-pretreated rats had a decrease in the CD4⁺/CD8⁺ ratio only in the blood after antigen challenge. This change is consistent with results obtained from human asthmatic subjects showing migration of CD4⁺ cells into the airways after antigen challenge.

Studies in humans, primates, and sheep that have looked at the release of mediators from inflammatory cells have shown that cys-LT retrieval from the bile or lungs increases during the late response and that selective antagonists of cys-LTs block the late response.⁹⁻¹² In the BN rat the excretion rate of cys-LTs more than doubles in the bile during early and late airway responses, and LTD₄ receptor antagonists block the late response.^{9,10} This raised the possibility that the IL-2-induced increases in early and late allergic airway responses were caused by increased cys-LT production. However, we were unable to detect an increase in the *in vivo* production of cys-LTs

in IL-2-treated rats. Cys-LT levels were actually lower at baseline in IL-2-treated animals, and although cys-LT levels were comparable in IL-2-treated and control rats during the early response, they were lower in IL-2-treated rats during the late response.

We were concerned that systemic administration of IL-2 could alter cys-LT metabolism and excretion in the bile. We reasoned that an enhanced inflammatory airway response might lead to oxidation of cys-LTs, causing a shift in excreted products to more polar species for which our analysis was not optimized. To determine whether the effects of IL-2 could be mediated through alterations in the metabolism of cys-LTs, we examined the *in vivo* metabolism of ³H-LTC₄ instilled into the trachea in challenged or unchallenged animals that were pretreated with either IL-2 or vehicle. Tritiated LTC₄ was instilled intratracheally 4 hours after initiation of anesthesia and instrumentation (corresponding to the time when a late response occurs in allergen-challenged rats).

In the IL-2-treated rats that were unchallenged, 39% of the instilled radioactivity was retrieved in the bile as LTC₄ or its metabolites in a 4-hour period, which was comparable with the recovery in saline-treated rats (33% of the instilled radioactivity retrieved). However, after OVA challenge, sensitized rats excreted lower fractions of radioactivity in the bile whether treated with IL-2 or saline. In IL-2-treated rats only 22% of the instilled radioactivity was retrieved in the bile as LTC₄ or its metabolites in a 4-hour period; in saline-treated rats 25% of the instilled radioactivity was retrieved in the bile as LTC₄ or its metabolites in a 4-hour period. These results showed that the combination of IL-2 and OVA challenge did not affect cys-LT metabolism, although the excretion rate of radioactivity was reduced in all OVA-challenged animals, raising the possibility of an underestimate of cys-LT synthesis by analysis of bile collected during the late airway response.

Our results clearly demonstrate that IL-2 does not increase cys-LT production after antigen challenge. The late response is a complex reaction that seems to be influenced by several mediators. Although we cannot rule out increased sensitivity to cys-LTs in rats pretreated with IL-2, other mechanisms could explain the effects of IL-2 on the late response. These include increased production or enhanced sensitivity to bradykinin, platelet-activating factor, or IL-5, mediators that have been shown to be involved in the late airway response in different animal models.²⁷⁻²⁹

In summary, pretreatment with IL-2 increased the cellular yield from lung lavage fluid before antigen challenge, increased the early and late airway responses with an increased yield of eosinophils and lymphocytes in lung tissue, and increased changes in lymphocyte subsets in the blood 8 hours after antigen challenge. The enhanced airway narrowing was not caused by increased cys-LT production.

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