

Eosinophil infiltration precedes development of airway hyperreactivity and mucosal exudation after intranasal administration of interleukin-5 to mice

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Recently, we demonstrated that antibody to interleukin-5 (IL-5) prevents the infiltration of eosinophils in the respiratory airways and the development of bronchial hyperreactivity in an animal model of allergic asthma. In this study we investigated the influence of long-term intranasal administration of IL-5 on airway responsiveness in vitro, the infiltration of inflammatory leukocytes, and mucosal exudation. Mice (BALB/c) received 1 µg of recombinant human IL-5 in 30 µl of saline solution or vehicle alone twice a day for 1, 3, and 7 days. At 3 and 7 days after IL-5 administration, the number of bronchoalveolar lavage eosinophils increased approximately fourfold and sixfold, respectively. Blood eosinophil numbers showed a similar increase. In addition, 7 days after IL-5 treatment, total lung eosinophil peroxidase activity was significantly increased by 170% as compared with controls. The maximal responsiveness of the trachea in vitro to methacholine was significantly increased by 34%, as compared with controls, only at 7 days after IL-5 administration. Furthermore, mucosal exudation was also only increased significantly at 7 days after IL-5 administration. It can be concluded that the IL-5-induced eosinophil infiltration precedes the development of airway hyperreactivity and mucosal exudation. (J ALLERGY CLIN IMMUNOL 1995;96:104-12.)

Key words: Hyperreactivity, respiratory airways, interleukin-5, eosinophil, asthma, mucosal exudation

In patients with allergic asthma, inhalation of allergen induces an IgE-mediated early asthmatic reaction, and in approximately 50% of the patients a late-phase bronchoconstriction is also observed. The late-phase bronchoconstriction is correlated with recruitment of inflammatory cells, in particular eosinophils, into the airways.^{1,2} Bronchial hyperreactivity is a characteristic feature of patients

Abbreviations used

AMT:	3-Amino-1, 2, 4-triazole
BAL:	Bronchoalveolar lavage
EPO:	Eosinophil peroxidase
IL:	Interleukin
OD:	Optical density

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with asthma and correlates well with the severity of the disease. Moreover, bronchial hyperreactivity is potentiated after allergen inhalation, especially after the development of a late-phase reaction, and is associated with infiltration of eosinophils.²⁻⁵

During the last decade, it has become increasingly clear that cytokines produced by T-helper lymphocytes regulate various aspects of the pathogenesis of allergic asthma. Allergen-specific T-helper lymphocyte clones derived from the blood of asthmatic patients produce predominantly interleukin (IL)-4 and IL-5, a pattern compatible with

the cytokine secretion profile of T_{H2} lymphocytes.⁶ In addition to the crucial role of T_{H2} cell-derived IL-4 in the initiation of allergic asthma through the regulation of IgE antibody production, these cells may also participate in the effector phase of allergic asthma.⁷ After allergen inhalation, recruitment of T-helper lymphocytes into the respiratory airways has been observed in asthmatic patients.⁸ It has been demonstrated that these infiltrating T-helper lymphocytes are activated and express mRNA for IL-5 and granulocyte-macrophage colony-stimulating factor.⁹ Furthermore, IL-5 has been shown to be the predominant eosinophil-active cytokine in antigen-induced pulmonary late-phase reactions in human beings.¹⁰ In contrast to other T-lymphocyte-derived cytokines that are regulators of eosinophil proliferation and effector function, such as IL-3 and granulocyte-macrophage colony-stimulating factor, the effects of IL-5 appear to be specific for eosinophils. These effects include the growth of eosinophils from bone marrow precursor cells, induction of chemotaxis, and expression of integrin-type adhesion molecules on eosinophils, which are involved in adhesion to endothelial cells.¹¹⁻¹⁴ IL-5 may therefore be involved in the preferential eosinophil recruitment observed in patients with asthma after allergen inhalation. When activated, eosinophils can release mediators capable of inducing bronchoconstriction and bronchial hyperreactivity.⁷ Thus local release of IL-5 in the airway tissue may be related to the development of bronchial hyperreactivity.

Recently, it has been demonstrated that monoclonal antibody to IL-5 inhibits airway eosinophilia after ovalbumin challenge in sensitized guinea pigs and mice.¹⁵⁻¹⁸ Additionally, antibody to IL-5 prevents the development of bronchial hyperreactivity after ovalbumin challenge of sensitized guinea pigs.^{15, 19} In this study the influence of long-term administration of IL-5 into the respiratory airways on airway responsiveness to methacholine, infiltration of inflammatory leukocytes, and mucosal exudation is examined.

METHODS

Administration of recombinant human IL-5

Animal care and use were performed in accordance with the guidelines of the Dutch Committee of Animal Experiments.

Male specific pathogen-free BALB/c mice, 6 to 8 weeks of age, were obtained from Harlan Olac (Zeist, The Netherlands). The animals received 1 μ g of recombinant human IL-5 (Glaxo, Greenford, U.K.) or saline solution in a volume of 30 μ l intranasally, while under

light halothane anesthesia, twice a day. After 1, 3, and 7 days, tracheal responsiveness to methacholine, mucosal exudation, blood and bronchoalveolar lavage (BAL) leukocytes, and lung eosinophil peroxidase (EPO) activity were determined.

Tracheal responsiveness in vitro

At 1, 3, and 7 days after IL-5 or vehicle treatment, mice were killed by an intraperitoneal injection of 0.25 ml of pentobarbital sodium (60 mg/ml). Subsequently, the trachea was carefully removed from each animal and transferred to a Petri dish containing a modified oxygenated Krebs bicarbonate buffer (118.1 mmol/L NaCl; 4.7 mmol/L KCl; 2.5 mmol/L $\text{CaCl}_2 \cdot 6 \text{H}_2\text{O}$; 0.5 mmol/L $\text{MgCl}_2 \cdot 6 \text{H}_2\text{O}$; 25.0 mmol/L NaHCO_3 ; 1.0 mmol/L $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$; 11.1 mmol/L glucose). The trachea was prepared free of excess tissue with the use of a binocular preparation microscope, and pieces of approximately 6 mm in length (9 tracheal rings directly beneath the larynx) were used. The tracheal segment preparations were mounted in an organ bath filled with 20 ml of the Krebs buffer and connected to a force displacement transducer (Harvard Bioscience, Boston, Mass.) for registration of the isometric tension on a two-channel recorder (Servogor type SE-120; Plato B.V., Diemen, The Netherlands). The optimal basic tension for the mouse tracheal preparations was 1 gm. The Krebs buffer was aerated with a mixture of oxygen (95%) and carbon dioxide (5%). Temperature was maintained at 37°C with a constant temperature circulating unit. Before drugs were applied to the organ bath, the preparations were allowed to equilibrate for at least 1 hour. During the equilibration, the bath fluid was exchanged every 15 minutes. Subsequently, a cumulative concentration response curve to methacholine was established.

BAL and blood leukocytes

At 1, 3, and 7 days after treatment, mice were killed by an intraperitoneal injection of 0.25 ml of pentobarbital sodium (60 mg/ml). Blood samples were taken by retro-orbital puncture for blood leukocyte counts. The trachea was trimmed free of connective tissue, and a small incision was made to insert a cannula into the trachea. The lungs were filled in situ with 1 ml of NaCl-ethylenediaminetetraacetic acid buffer (0.15 mol/L NaCl, 2.6 mmol/L ethylenediaminetetraacetic acid). Fluid was withdrawn from the lungs after gentle massage and collected in a plastic tube on ice. This procedure was repeated five times, and the cell suspensions recovered from one animal were pooled. The cells were sedimented by centrifugation at 400 g for 10 minutes at 4°C and washed twice with Krebs bicarbonate buffer. The cells were stained with Türk solution and counted. All cell preparations were analyzed morphologically after centrifugation on microscope slides. Air-dried preparations were fixed and stained with Diff-Quik (Merz & Dade A.G., Düringen, Switzerland). Differential counts were made under oil immersion microscopy.

TABLE I. Maximal contraction of tracheal segments in response to methacholine

Treatment day	Maximal contraction (mg)	
	Control	IL-5
1	1417 $\pm$ 250	1732 $\pm$ 154
3	1534 $\pm$ 147	1899 $\pm$ 140

Maximal contraction of methacholine concentration response curves of tracheal segments isolated from mice treated with IL-5 (1 μ g) or with saline solution in a volume of 30 μ l twice a day for the indicated time period. Results are expressed as mean $\pm$ SEM for six animals.

Histologic examination

At 3 and 7 days after treatment, mice were killed by an intraperitoneal injection of 0.25 ml of pentobarbital sodium (60 mg/ml). The lungs were filled in situ with a fixative of 3.8% paraformaldehyde solution in phosphate buffer (pH 7.4). The unfolded lungs were fixed for at least 24 hours in the fixative, dehydrated, and embedded in Paraplast (Sherwood Medical Company, St. Louis, Mo.). Five-micrometer thick sections were stained with hematoxylin and eosin. Slides were microscopically examined for the presence of eosinophils, and the results are presented in tabular form.

Lung eosinophil peroxidase activity

Lungs were flushed through the pulmonary artery with 10 ml of saline solution and subsequently stored at -70° C until analysis. Lungs were homogenized in 2 ml of ice-cold 0.05 mol/L Tris-HCl buffer (pH = 8) containing 0.1% Triton X-100 with a Polytron (Ystral, Dottingen, Germany). Lung homogenates were exposed to five freeze-thaw cycles and centrifuged at 1600 g for 5 minutes at 4° C. The EPO activity in the supernatant was measured according to the method of Strath and et al.,²⁰ which is based on the oxidation of o-phenylenediamine by EPO in the presence of hydrogen peroxide. The substrate solution consists of 10 mmol/L o-phenylenediamine (Sigma Chemical Co., St. Louis, Mo.) in 0.05 mol/L Tris-HCl buffer (pH = 8) and 4 mmol/L hydrogen peroxide (BDH, Poole, U.K.). Substrate solution (100 μ l) was added to lung supernatant samples (50 μ l) in a 96-well microplate and incubated at room temperature for 60 minutes before the reaction was stopped by the addition of 50 μ l of 4 mol/L sulfuric acid. The absorbance was then measured at 492 nm with a Titertek Multiskan (Flow Laboratories, Irvine, U.K.). Duplicate incubations were carried out in the absence or presence of the EPO inhibitor 3-amino-1,2,4-triazole (AMT) (2 mmol/L) (Sigma).²⁰ Blanks were lung supernatant samples (50 μ l) incubated with Tris-HCl buffer. Results are expressed as optical density (OD) at 492 nm.

Mucosal exudation

Two hours before the end of an experiment, Evans blue dye (1.25%, 50 μ l in sterile saline solution; Fluka

Chemie AG, Buchs, Switzerland) was injected intravenously into anesthetized mice. This allowed a 2-hour "window" of mucosal exudation to be determined. Shortly before the mice were killed, heparin was administered intravenously (10 U/ml blood), and a blood sample was taken. BAL and blood samples were taken as described above. Samples of lavage fluid, in a known volume, were used to evaluate mucosal exudation. The extravasation of Evans blue dye-labeled macromolecules from the microcirculation into bronchoalveolar spaces was quantified by measuring the OD of the lavage and plasma samples at a wavelength of 620 nm with a spectrophotometer. The amount of mucosal leakage in the lavage fluid (microliters per lung) was determined by dividing the Evans blue dye content in the lavage fluid by the Evans blue dye content in 1 ml of plasma.

Statistics

Student's *t* test was used for comparing BAL and blood leukocytes, lung EPO activity, maximal contractions of tracheal segments in response to methacholine, and mucosal exudation. An analysis of variance was used to compare methacholine concentration response curves of isolated tracheal segments. A *p* value of less than 0.05 was considered significant. Responses are presented as means $\pm$ SEM.

RESULTS

Tracheal responsiveness in vitro

At 1 day and 3 days after IL-5 treatment, the maximal responsiveness of tracheal segments to methacholine was slightly but not significantly enhanced as compared with saline-treated animals (Table I). At 7 days after IL-5 treatment, the maximal contraction of tracheal segments in response to methacholine was significantly increased by 34% $\pm$ 4% (*p* < 0.05) as compared with saline-treated animals (Fig. 1). Median effective concentration values did not differ between the saline- and the IL-5-treated mice at any time point.

BAL and blood leukocytes

One day after IL-5 treatment, the relative number of mononuclear cells in BAL was increased significantly (*p* < 0.05) to 186% $\pm$ 21% as compared with vehicle-treated animals (Fig. 2, A). The number of BAL mononuclear cells in IL-5-treated animals did not differ from that in control animals at 3 and 7 days after treatment. At 3 and 7 days after IL-5 treatment, the relative number of BAL eosinophils, but not the number of BAL neutrophils, was increased as compared with control animals; the increase of BAL eosinophils in IL-5-treated mice amounted to 450% $\pm$ 100% (*p* < 0.05) and 589% $\pm$ 100% (*p* < 0.01), respectively

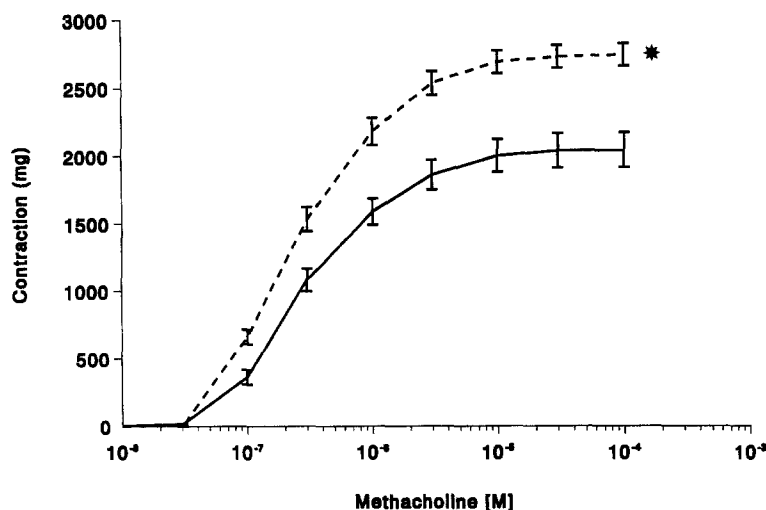


FIG. 1. Responsiveness to methacholine of tracheal segments isolated from mice treated with IL-5 (1 μ g) twice a day for 7 days (dotted line, $n = 5$) or saline solution (straight line, $n = 6$) in a volume of 30 μ l. * $p < 0.01$ compared with saline-treated animals as determined by analysis of variance.

(Fig. 2, A). In Table II the absolute numbers of the different leukocytes in the BAL of saline-treated animals are given.

Three days after IL-5 treatment, the relative numbers of blood lymphocytes, monocytes, neutrophils, and eosinophils were significantly increased to $163\% \pm 2\%$ ($p < 0.05$), $270\% \pm 42\%$ ($p < 0.01$), $183\% \pm 15\%$ ($p < 0.01$), and $320\% \pm 62\%$ ($p < 0.01$) as compared with saline-treated animals (Fig. 2, B). At 7 days after IL-5 treatment, only the relative number of blood eosinophils was significantly enhanced. The increase amounted to $590\% \pm 58\%$ ($p < 0.01$) as compared with control treated animals. In Table II the absolute numbers of the different leukocytes in the blood of saline-treated animals are given.

Histologic examination

The results of the microscopic examination of eosinophil presence in lung tissue are presented in Table III. Overall, lungs of animals treated with IL-5 for 3 or 7 days showed a mild increase in the numbers of interstitial and alveolar eosinophils. There was no obvious difference in peribronchial eosinophils between IL-5- and saline-treated animals.

Lung EPO activity

Total lung EPO activity increased significantly from 0.30 ± 0.06 OD units in control animals to 0.81 ± 0.14 OD units ($p < 0.01$) in animals treated for 7 days with IL-5 (Fig. 3). The EPO activity

could be almost completely inhibited by the EPO inhibitor AMT, which demonstrates that other peroxidases are not involved.

Mucosal exudation

One day and 3 days after IL-5 administration, no difference in mucosal exudation between saline- and IL-5-treated mice was observed as determined by Evans blue dye extravasation (Fig. 4). In contrast, at 7 days after IL-5 treatment, Evans blue dye extravasation into the lavage fluid was significantly increased by $78\% \pm 19\%$ ($p < 0.01$, Fig. 4) when compared with a time-matched saline-treated control.

DISCUSSION

IL-5 is a growth, differentiation, and survival factor for eosinophils and can induce adhesion to endothelial cells, transmigration, and chemotaxis of eosinophils.^{11-14, 21} In this study we have demonstrated that long-term intranasal treatment with IL-5 induces the selective infiltration of eosinophils in the BAL fluid. In addition, interstitial presence of eosinophils was observed in lung tissue. These data are supported by the increase in total lung EPO activity. The peroxidase activity was almost completely inhibited by AMT, which is a selective inhibitor of EPO.²⁰ In addition, the number of eosinophils in the blood increased after IL-5 treatment. This may be due to an effect of IL-5 on precursor cells in the bone marrow. Indeed, other investigators have demonstrated that

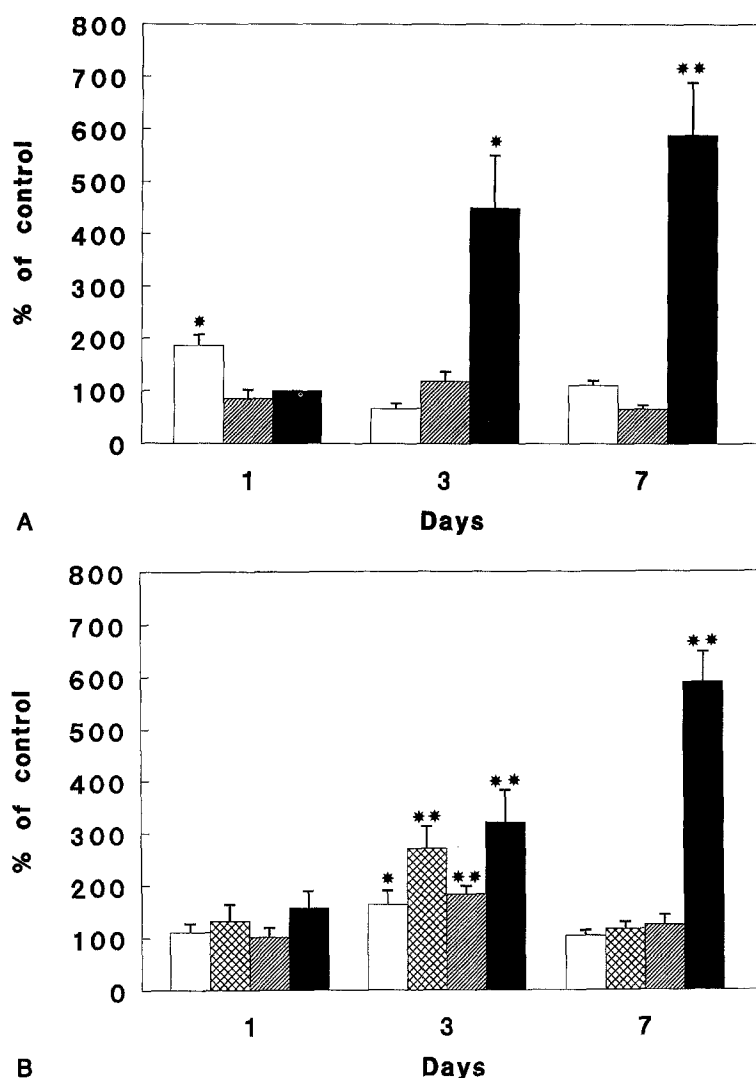


FIG. 2. **A**, Number of bronchoalveolar mononuclear cells (open bars), neutrophils (hatched bars), and eosinophils (black bars) in mice treated with IL-5 (1 μ g) twice a day for 1 ($n = 4$), 3 ($n = 4$), or 7 days ($n = 5$). **B**, Number of blood lymphocytes (open bars), monocytes (cross-hatched bars), neutrophils (hatched bars), and eosinophils (black bars) in mice treated with IL-5 (1 μ g) twice a day for 1 ($n = 8$), 3 ($n = 8$), or 7 days ($n = 7$). Results are expressed as percentage increase compared with the number of cells recovered from animals treated with saline solution for the respective time periods ($n = 6$). * $p < 0.05$ and ** $p < 0.01$ compared with saline-treated animals as determined by Student's t test.

intraperitoneal IL-5 treatment increases the number of eosinophils in the blood, bone marrow, and—in particular—in the peritoneal cavity.^{22, 23} However, despite the presence of increased numbers of eosinophils in the blood, these other investigators did not observe airway eosinophilia²³ (D. Fattah; oral communication, 1994). Thus in our experiments airway eosinophilia does not appear to be a passive consequence of an increase in the number of blood eosinophils but rather a result of other IL-5-mediated processes such as adhe-

sion, transmigration, and chemotaxis of circulating eosinophils or increased survival of resident eosinophils.^{11, 13, 14, 21} Similarly, in IL-5-transgenic mice with marked blood eosinophilia, tissue eosinophilia seems to be related to the expression of IL-5 in the tissue.²⁴ The reason that we did not observe airway eosinophilia at 1 day after IL-5 treatment may be that in naive mice the number of eosinophils in the blood is very low. Although we did not study higher doses of IL-5, recent studies by Dr. Fattah have revealed that 10 μ g of IL-5

TABLE II. Number of leukocytes in BAL fluid and blood

Leukocytes	Treatment time (days)		
	1	3	7
BAL			
Total number of cells $\times 10^4$	$N = 4$	$N = 4$	$N = 5$
Mononuclear cells	8.35 ± 1.27	14.53 ± 2.99	32.11 ± 2.34
Neutrophils	1.71 ± 0.71	0.93 ± 0.27	1.48 ± 0.22
Eosinophils	0.02 ± 0.02	0.06 ± 0.04	0.19 ± 0.08
Blood			
Number of cells/ml $\times 10^5$	$N = 8$	$N = 8$	$N = 7$
Lymphocytes	40.22 ± 3.47	37.90 ± 3.18	32.59 ± 3.24
Monocytes	0.55 ± 0.13	0.91 ± 0.13	2.11 ± 0.32
Neutrophils	4.96 ± 0.28	5.05 ± 0.68	4.53 ± 0.75
Eosinophils	1.12 ± 0.18	0.95 ± 0.18	1.16 ± 0.17

Number of leukocytes in BAL fluid and blood of mice treated with saline solution twice a day for 1, 3, or 7 days.

TABLE III. Histologic scores on lungs from mice treated with saline solution or IL-5

Treatment	Animal No.	Day 3		Day 7	
		Bronchial	Interstitial	Bronchial	Interstitial
Saline solution	1	2	2	1	2
Saline solution	2	2	2	2	2
Saline solution	3	2	2	F2	2
IL-5	1	2	3	2	3
IL-5	2	F3	3	2	3
IL-5	3	2	3	2	3

Histologic scores on lungs from mice treated with saline solution or IL-5 twice a day for 3 or 7 days.

1, No eosinophils; 2, occasional eosinophils present; 3, general eosinophil presence; F, focal, not general, presence over whole section.

induces a greater increase in lung eosinophil numbers than $1 \mu\text{g}$ (oral communication, December 1994).

The number of mononuclear cells in BAL and the number of several other leukocytes in the blood are also increased temporarily at 1 or 3 days after IL-5 treatment, respectively. Although IL-5 is regarded as relatively specific to eosinophils, other leukocytes also respond to IL-5 (e.g., basophils, B lymphocytes, and cytotoxic T lymphocytes),²⁵⁻²⁹ which may partially explain alterations in leukocytes other than eosinophils. Apart from the increase in eosinophils, the increases in other leukocytes in both BAL and blood are only temporary. This suggests that they may not be related to IL-5 administration itself but to a nonspecific phenomenon. At present we have no explanation for this phenomenon.

Repeated intranasal application of sterile saline solution increases the number of mononuclear cells and eosinophils in BAL and the number of

monocytes in blood. At present we have no precise explanation for this observation. Saline solution may induce an irritant reaction in the airways. It is also possible that these changes in cell numbers are due to the repeated halothane anesthesia.

In this study we have demonstrated that long-term treatment with IL-5 induces an increased maximal tension of the trachea in response to methacholine. The relevance of tracheal responsiveness in vitro to airway hyperresponsiveness in patients with asthma remains elusive. Few studies have examined the responsiveness of isolated human airway smooth muscle preparations to constrictor stimuli.³⁰ However, data exist that the in vitro responsiveness to contractile stimuli is increased.^{31, 32} In addition, it has been suggested that muscle mass is increased in asthma, which may lead to increased generation of smooth muscle tension.³³ Interestingly, in a murine model of allergic asthma in which ovalbumin-sensitized mice were exposed to ovalbumin aerosol, we have also

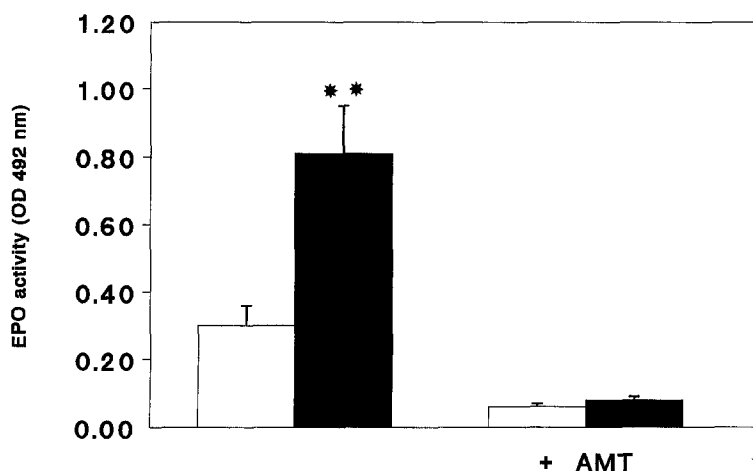


FIG. 3. Total lung EPO activity in the absence or presence of the EPO inhibitor 2 mmol/L AMT in animals treated with IL-5 (1 µg, black bars, $n = 5$) or sterile saline solution (open bars, $n = 5$) twice a day for 7 days in a volume of 30 µl. ** $p < 0.01$ compared with saline-treated animals as determined by Student's t test.

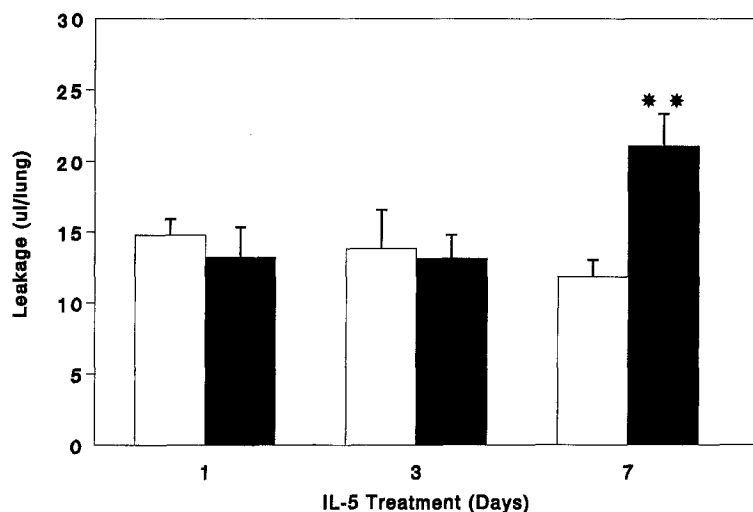


FIG. 4. Mucosal exudation (microliters per lung) as determined by Evans blue dye extravasation (leakage) into the lavage fluid in animals treated with IL-5 (1 µg) or sterile saline solution twice a day for 1 ($n = 6$), 3 ($n = 5$), or 7 days ($n = 7$) in a volume of 30 µl. ** $p < 0.01$ compared with saline-treated animals as determined by Student's t test.

observed an increased maximal tension of isolated tracheal segments in response to methacholine.³⁴ The increase in the maximal responsiveness of tracheal segments to methacholine after ovalbumin challenge or IL-5 treatment is comparable: 27% and 34%, respectively. Furthermore, the number of BAL eosinophils (5.5×10^3) in ovalbumin-challenged animals is comparable to the eosinophil number after 7 days of IL-5 treatment (11.2×10^3). Although there is strong circumstantial evidence that eosinophils are involved in the

development of hyperreactivity, a causal relationship remains to be proven.⁷ In our experiments, the IL-5-induced eosinophil infiltration preceded the development of hyperreactivity of airway smooth muscle in vitro to methacholine. The number of BAL eosinophils increased at 3 and 7 days after IL-5 treatment, whereas the hyperreactivity was only observed after 7 days. Histologic examination of lung tissue also revealed that at both 3 and 7 days after IL-5 treatment, the number of interstitial eosinophils was mildly increased. Although

there seems to be a dissociation between eosinophil infiltration into the BAL and the development of airway hyperreactivity, it remains possible that the eosinophils need to mature or differentiate before they are capable of inducing airway hyperreactivity. Long-term incubation of eosinophils with IL-5 leads to the release of eosinophil granule proteins.^{35, 36} These granule proteins have been demonstrated in sputum, lung tissue, and BAL fluid of patients with asthma^{1, 37-39} and are capable of inducing bronchial hyperreactivity when incubated with airway tissue in vitro or after in vivo administration.⁴⁰⁻⁴² Interestingly, Terada et al.⁴³ demonstrated that repeated application of IL-5 onto the nasal mucosa of patients with allergy induced an increased response to histamine together with an increased level of eosinophil cationic protein in the nasal lavage fluid.

In our experiments the presence of airway hyperreactivity coincided with increased mucosal exudation. Interestingly, eosinophil recruitment and activation have been implicated in increased vascular permeability and mucosal exudation in the lung.⁴⁴⁻⁴⁶ It is tempting to speculate that the IL-5-induced infiltration of eosinophils is involved in the increased mucosal exudation and development of airway hyperreactivity.

In addition to eosinophils, it has been demonstrated that IL-5 affects basophils, B lymphocytes, and cytotoxic T lymphocyte effector functions.²⁵⁻²⁹ These cells may also be involved in IL-5-induced hyperreactivity.

It can be concluded that IL-5 is a potentially important mediator in airway eosinophilia, mucosal exudation, and hyperreactivity in asthma. Other data also point to the involvement of IL-5 in asthma pathology. First, IL-5 mRNA has been demonstrated in bronchial biopsy specimens and BAL cells of patients with allergic asthma^{47, 48} or symptomatic nonallergic asthma.⁴⁹ Second, IL-5 mRNA expression is increased in T-helper lymphocytes and eosinophils in patients with allergic asthma after allergen inhalation.^{9, 50} Third, activation of T-helper lymphocytes in asthma is accompanied by increased serum levels of IL-5 and circulating eosinophils, which are both reduced by glucocorticoid therapy.⁵¹ Recently, we and others have shown that antibody to IL-5 inhibits antigen-induced airway eosinophil infiltration and bronchial hyperreactivity in animal models of allergic asthma.^{15, 19} To further establish the role of IL-5 in human asthma, the development of IL-5 antagonists is awaited.

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