

# Interleukin-10 Inhibits Antigen-induced Cellular Recruitment into the Airways of Sensitized Mice

Claudia Zuany-Amorim,\* Solomon Hailé,† Dominique Leduc,\* Claude Dumarey,\* Michel Huerre,‡ B. Boris Vargaftig,\* and Marina Pretolani\*

\*Unité de Pharmacologie Cellulaire, Unité Associée Institut Pasteur/Institut National de la Santé et de la Recherche Médicale No. 285; and †Unité d'Histopathologie, Institut Pasteur, 75015 Paris, France

## Abstract

This report examines the effect of recombinant murine (rm)IL-10 on antigen-induced cellular recruitment into the airways of sensitized Balb/c mice. The intranasal instillation of 10 µg ovalbumin induced an early (6–24 h) increase in the number of neutrophils, and a late rise (24–96 h) in that of eosinophils in the bronchoalveolar lavage (BAL) fluid and bronchial tissue. A single intranasal instillation of 0.01–0.1 µg of rmIL-10, administered concurrently with ovalbumin, but not 1 or 3 h thereafter, dose-dependently inhibited both airway neutrophilia and eosinophilia. This phenomenon was suppressed by treating the sensitized mice with 1 mg/mouse of a neutralizing anti-IL-10 mAb, which increased significantly ovalbumin-induced neutrophil and eosinophil accumulation in the BAL fluid. These results suggest that antigen stimulation may trigger the *in vivo* generation of IL-10, which, in turn, participates in the leukocyte infiltration into the airways.

rmIL-10 also reduced TNF-α release in the BAL fluid observed 1 and 3 h after antigen challenge. Furthermore, the intranasal instillation of an anti-TNF-α antiserum to sensitized mice markedly reduced ovalbumin-induced neutrophil and eosinophil accumulation in the BAL fluid. These findings indicate that leukocyte infiltration into the airways of antigen-challenged mice is regulated by IL-10. Furthermore, inhibition of TNF-α production by rmIL-10 suggests that allergic airway inflammation and TNF-α formation are parallel events in this model. (*J. Clin. Invest.* 1995. 95:2644–2651.) Key words: bronchial inflammation • eosinophils • neutrophils • tumor necrosis factor

## Introduction

Airway infiltration by inflammatory cells, particularly of eosinophils, is one of the characteristic features of asthma (1). Several mechanisms responsible for the attraction and localization of inflammatory cells at site of allergic reactions have been proposed. Among them, attention is presently focused on the hy-

pothesized switching of CD4<sup>+</sup> T lymphocytes for the preferential production of Th2-derived cytokines (2–4). These cytokines include IL-5, which promotes the growth, differentiation, survival, and activation of eosinophils (5–7) and IL-4, which induces IgE production by B cells (8). In murine models of allergic inflammation, infiltration of the airways by eosinophils is regulated by IL-5 and IL-4, since sensitized mice treated with anti-IL-5 (9) or anti-IL-4 (10) antibodies display reduced eosinophils in their bronchoalveolar lavage (BAL)<sup>1</sup> fluid and bronchial tissue after antigen challenge.

IL-10 was initially characterized as a product of CD4<sup>+</sup> T lymphocytes of the Th2 subtype (11) and shown to inhibit IFN-γ production by Th1 clones (12). Several properties of IL-10 on various cell types have been described since then, including growth stimulation of thymocytes, mast cells, and B cells, and inhibition of cytokine production by activated monocytes, macrophages (reviewed in reference 13) and, more recently, by neutrophils (14, 15) and eosinophils (16). Only a few studies, however, have focused on the *in vivo* modulatory properties of IL-10 during inflammatory reactions. In particular, Howard et al. (17) and Gerard et al. (18), recently demonstrated that the administration of IL-10 protects mice from LPS-induced death and the accompanying rise in the levels of circulating TNF-α, suggesting an involvement of IL-10 in inflammatory diseases such as bacterial sepsis. At this time, however, the ability of IL-10 to influence *in vivo* allergic airway inflammation has not been investigated.

In this report, we show that the intranasal administration of ovalbumin to sensitized Balb/c mice induces an early generation of TNF-α in the BAL fluid, which is followed by airway neutrophilia and eosinophilia. Treatment of the sensitized mice with recombinant murine (rm)IL-10, concurrently administered with ovalbumin, abrogates antigen-induced airway inflammation and TNF-α generation in the BAL fluid, suggesting that IL-10 plays an important role in regulating allergic inflammatory processes in the lung.

## Methods

**Mice and immunization.** Male Balb/c mice aged 8 wk, weighing ~25–30 g (Iffa-Credo, Saint-Germain sur l'Arbresle, France) were sensitized by two subcutaneous injections, 1 wk apart, of 0.4 ml 0.9% wt/vol NaCl (saline) containing 100 µg ovalbumin (ICN Biomedicals, Inc., Costa Mesa, CA), adsorbed in 1.6 mg aluminium hydroxide (Merck, Darmstadt, Germany). 7–10 d after the second immunization, sensitized mice were challenged by an intranasal administration of ovalbumin, as described below. This immunization procedure favors the production of

Address correspondence to Dr. Marina Pretolani, Unité de Pharmacologie Cellulaire, Unité Associée Institut Pasteur/Institut National de la Santé et de la Recherche Médicale No. 285, 25, rue du Dr. Roux, 75015 Paris, France. Phone: 33-1-40-61-32-02; FAX: 33-1-45-68-87-03.

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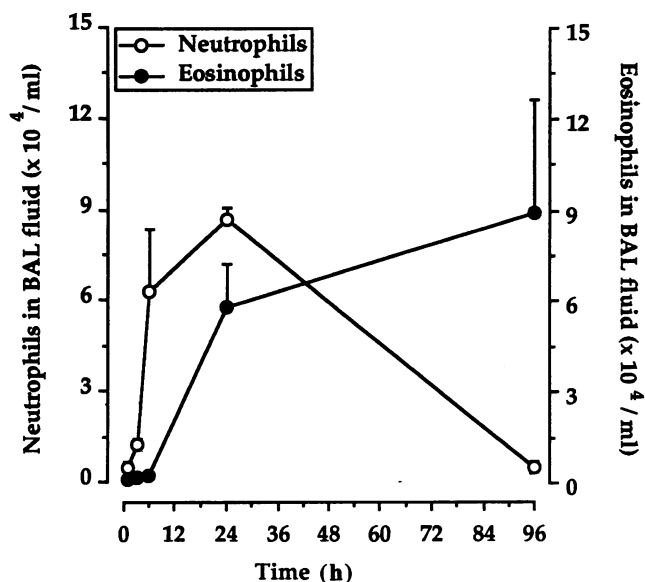
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1. Abbreviations used in this paper: BAL, bronchoalveolar lavage; EPO, eosinophil peroxidase; PBS-T, PBS-Tween 20; PBS-T-BSA, PBS-Tween 20-BSA; rm, recombinant murine.



**Figure 1.** Time course of antigen-induced cellular accumulation in the BAL fluid from sensitized Balb/c mice. Mice were challenged by an intranasal instillation of 10  $\mu\text{g}$  ovalbumin, killed at different time intervals after the challenge, and neutrophils (○) and eosinophils (●) were counted in the BAL fluid after cyto centrifugation and staining with Diff-Quik stain. Results are expressed as means  $\pm$  SEM of six to nine experiments at each time point.

IgE-like homocytotropic antibodies, as measured by passive cutaneous anaphylaxis (19).

**Bronchoalveolar cell counts and differentiation.** Cellular accumulation into the BAL fluid from sensitized mice was induced by the intranasal instillation of 50  $\mu\text{l}$  of a solution containing 200  $\mu\text{g}/\text{ml}$  ovalbumin diluted in sterile saline (10  $\mu\text{g}$  ovalbumin, as final dose administered). Control animals received the same volume of sterile saline. At various time intervals after challenge, mice were anesthetized by an intraperitoneal injection of 4 mg/kg sodium pentobarbital (Clin-Midy, Montpellier, France) and bronchoalveolar cells were collected in four successive lavages using 1-ml aliquots of sterile saline at room temperature, injected, and recovered through a polyethylene tracheal cannula. Total cells were counted using a Coulter Counter ZM (Coultronics, Margency, France). Differential cell counts were performed after cyto centrifugation (Hettich Universal, Tuttlingen, Germany) and staining with Diff-

Quik stain (Baxter Dade AG, Duedingen, Switzerland). Results are expressed as the number of each population per milliliter BAL fluid. Aliquots of 0.5 ml of the remaining lavage fluid were centrifuged at 200 g for 15 min, at 4°C and the supernatant was collected and stored at -80°C until the determination of TNF- $\alpha$ , as described below.

**Histology.** After the BAL collection was performed as described above, mice were exsanguinated via the abdominal aorta and the lungs were inflated by injecting into the trachea 1 ml solution of optimum cutter temperature compound (BDH, Poole, United Kingdom) in distilled water (vol/vol). The lobes were dissected and mounted over cork disks, covered by optimum cutter temperature compound and snap frozen in isopentane (Prolabo, Paris, France) cooled by liquid nitrogen. The frozen blocks were kept at -80°C before use. Sections alongside the main intrapulmonary bronchus were cut in a cryostat kept at -21°C and collected on glass slides previously coated with  $\gamma$ -methacryloxypropyltrimethoxysilane (Sigma Chemical Co., St. Louis, MO), fixed in acetone (Merck) for 10 min, wrapped in a plastic film, and kept at -20°C before use. Cyanide-resistant eosinophil peroxidase (EPO) activity, using potassium cyanide, diaminobenzidine, and hydrogen peroxide (Merck), was used to stain the eosinophils (20).

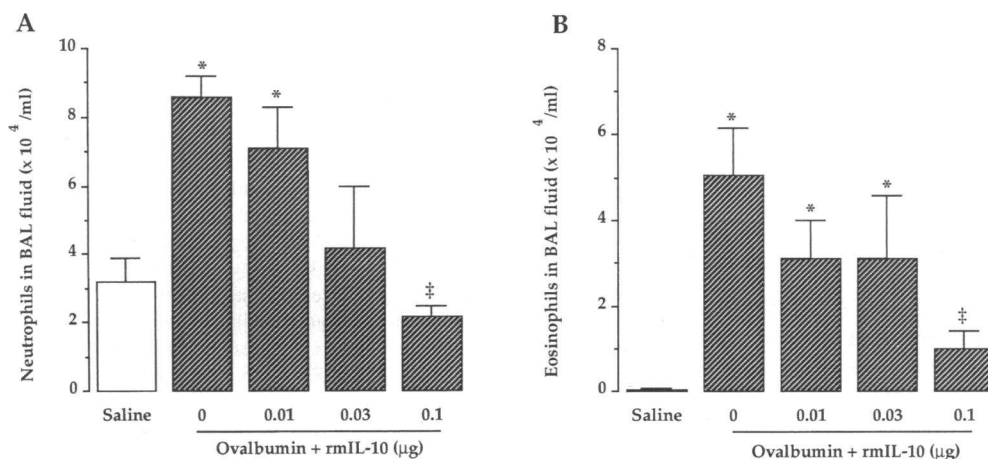
Sections were coded and read in a blind fashion. Positive cells were enumerated in the bronchial submucosa by means of a micrometer. The results of each stained slide were expressed as the number of EPO-positive cells per millimeter of basal lamina.

**Effect of murine rmIL-10.** To determine whether IL-10 regulates antigen-induced cellular infiltration into the airways, sensitized mice received a single intranasal instillation of 0.01, 0.03, 0.1, or 0.5  $\mu\text{g}$  rmIL-10 (Immugenex, Los Angeles, CA), diluted in 25  $\mu\text{l}$  sterile saline, and mixed with an equal volume of a solution containing 400  $\mu\text{g}/\text{ml}$  ovalbumin (10  $\mu\text{g}$  as final dose instilled). Cellular infiltration and TNF- $\alpha$  release in the BAL fluid were evaluated 1, 3, 6, 24, or 96 h after antigen challenge.

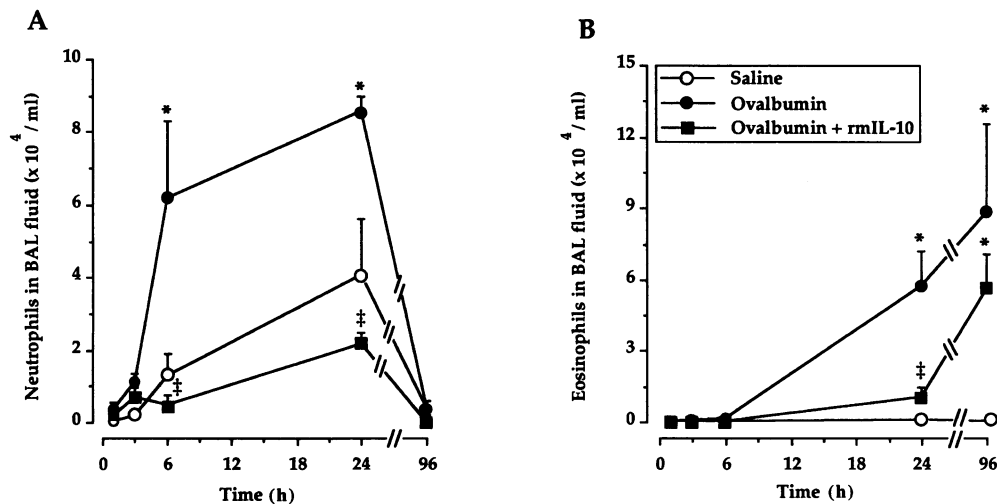
In preliminary experiments, the subcutaneous administration of 0.1–0.5  $\mu\text{g}$  rmIL-10, 1 h before antigen challenge, failed to modify ovalbumin-induced neutrophil and eosinophil accumulation in the BAL fluid (data not shown).

**Effect of anti-murine IL-10 mAb and of anti-TNF- $\alpha$  antiserum.** Sensitized mice were injected intravenously with 1 mg of a rat IgG1 anti-mouse IL-10 mAb, termed 2A5, or with its matched isotype control mAb, GL113 (17), (kindly provided by Dr. R. L. Coffman, DNAX Research Institute, Palo Alto, CA; and by Dr. H. F. J. Savelkoul, Erasmus University, Rotterdam, The Netherlands), 1 h before the intranasal administration of ovalbumin alone or with 0.1  $\mu\text{g}$  rmIL-10.

In separate experiments, 50  $\mu\text{l}$  of a sheep anti-mouse TNF- $\alpha$  antiserum (21), or its control preimmune serum (a gift from Drs. S. Poole and A. Meager, National Institute for Biological Standards and Control, Potters Bar, United Kingdom) were administered via the intranasal route, concomitantly with ovalbumin.



**Figure 2.** Effect of rmIL-10 on antigen-induced cellular accumulation in the BAL fluid from sensitized Balb/c mice. Mice were challenged by intranasal route either with saline, or with 10  $\mu\text{g}$  ovalbumin alone or concurrently administered with 0.01, 0.03, or 0.1  $\mu\text{g}$  rmIL-10. Neutrophil (A) and eosinophil (B) infiltration in the BAL fluid was evaluated 6 or 24 h after antigen challenge, respectively. Results are expressed as means  $\pm$  SEM of six to nine experiments for each group. \*  $P < 0.05$  and <sup>†</sup> $P < 0.05$ , as compared to saline- or ovalbumin-challenged untreated mice, respectively.



**Figure 3.** Kinetics of inhibition by rmIL-10 of antigen-induced neutrophil (A) and eosinophil (B) accumulation in the BAL fluid from sensitized Balb/c mice. Mice were challenged by intranasal route with saline (○), or with 10 μg ovalbumin alone (●), or concurrently administered with 0.1 μg rmIL-10 (■), and they were killed at different time intervals after the challenge. Results are expressed as means ± SEM of six to nine experiments for each group. \*  $P < 0.05$  and <sup>†</sup> $P < 0.05$ , as compared to saline- or ovalbumin-challenged untreated mice, respectively.

In both series of experiments, cell counts in the BAL fluid were evaluated 6 and 24 h after antigen challenge.

**TNF-α determination.** The levels of TNF-α in the supernatant of BAL fluid from sensitized saline- or ovalbumin-challenged untreated or rmIL-10 (0.1 or 0.5 μg)-treated mice were determined by a specific double sandwich ELISA. Briefly, ELISA plates (96-well-Immuno Plate MaxiSorp; Nunc, Roskilde, Denmark) were coated with 100 μl of a rat anti-mTNF-α mAb (Clinisciences-Endogen, Montrouge, France), at 5 μg/ml in PBS, pH 7.4, and incubated overnight at room temperature in the dark. The day after, the wells were washed three times with PBS containing 0.1% Tween 20 (PBS-T) and 200 μl of PBS-T containing 0.1% BSA (PBS-T-BSA) were added to each well.

After 1 h at 37°C followed by three washes with PBS-T-BSA, 100 μl of the samples or rmTNF-α standards (kindly provided by Dr. G. R. Adolf, Boehringer Institut für Arzneimittel-Forschung, Vienna, Austria), at concentrations ranging between 50 and 3,200 pg/ml in PBS-T-BSA were added to each well and incubated at 37°C for 1 h. The wells were washed three times with PBS-T and incubated for 1 h at 37°C with 100 μl of a solution of polyclonal rabbit anti-mTNF-α (Clinisciences-Endogen), at 5 μg/ml in PBS-T-BSA. After the wells were washed three times with PBS-T, 100 μl of horseradish peroxidase-conjugated goat anti-rabbit IgG F(ab')<sub>2</sub> (Biosis, Compiègne, France), at a dilution of 1/2,000 in PBS-T-BSA, was added for 1 h at 37°C. Then, 100 μl of freshly prepared substrate solution (orthophenylenediamine; Sigma Chemical Co.), at 2 mg/ml and 3% H<sub>2</sub>O<sub>2</sub> (Merck) was added to each well. After 7 min incubation in the dark, at room temperature, the reaction was stopped by the addition to each well of 50 μl of a 0.5% solution of Na<sub>2</sub>SO<sub>3</sub> (Merck). Absorbance was read at 490 nm with an automatic microplate-reader (Dinatech MR 5000; Dinatech Laboratories, Saint-Cloud, France). The lower limit of sensitivity of the assay was 50 pg TNF-α/ml sample.

**Data analysis.** Results are expressed as means ± SEM of the indicated number of experiments. One-way ANOVA was used to determine significance among the groups. If a significant variance was found, an unpaired Student's *t* test was used to assess comparability between means. A value of  $P \leq 0.05$  was considered significant.

## Results

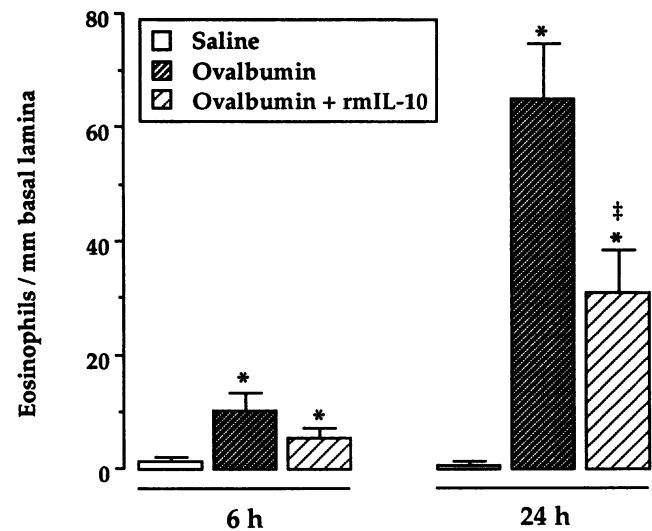
**Kinetics of antigen-induced cellular infiltration in the BAL fluid from sensitized mice.** The intranasal instillation of 10 μg ovalbumin to sensitized Balb/c mice induced a rise in the number of neutrophils in the BAL fluid, which reached a maximum between 6 and 24 h and resolved thereafter (Fig. 1). Intense eosinophil accumulation was observed at 24 h and progressively

increased until 96 h (Fig. 1). The number of eosinophils returned to background levels 14 d after antigen challenge (data not shown).

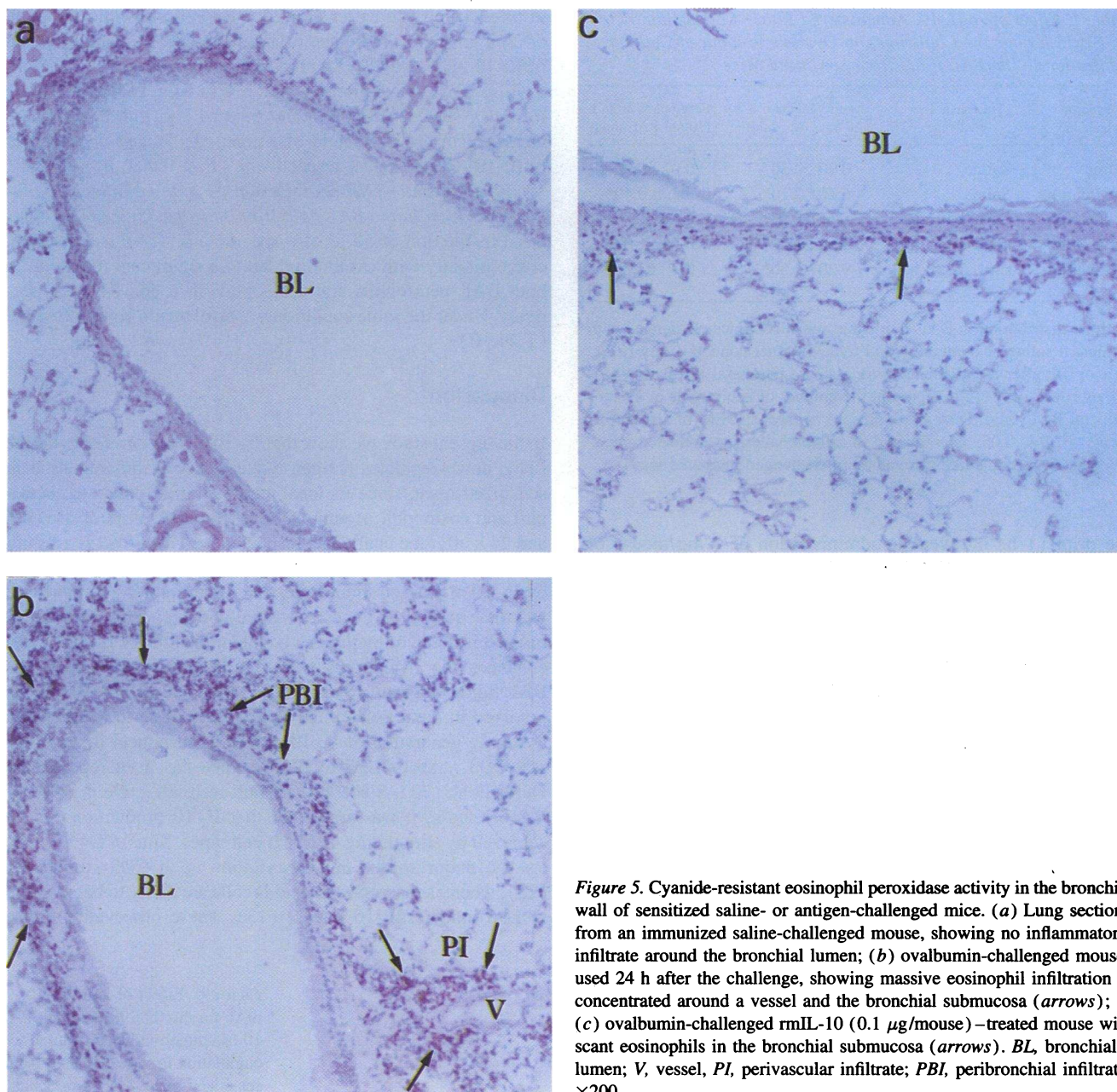
No changes in alveolar macrophage counts were observed, at any time point after antigen administration (data not shown).

Nonimmunized mice challenged with ovalbumin show a number of neutrophils in the BAL fluid similar to that found in sensitized saline-challenged animals. No eosinophils were enumerated at 24 h (data not shown).

The times of 6 and 24 h were selected to study the effect



**Figure 4.** Effect of rmIL-10 on eosinophil infiltration in the bronchial tissue of antigen-challenged mice. Sensitized Balb/c mice were challenged with intranasal saline (□), or with 10 μg ovalbumin, administered either alone (■), or with 0.1 μg rmIL-10 (▨), and they were killed 6 (left) or 24 h (right) after the challenge. The number of infiltrating eosinophils was evaluated in the bronchial submucosa by cyanide-resistant EPO activity technique. Data are expressed as the number of EPO-positive cells/mm of basal lamina. Results represent the means ± SEM of seven to nine experiments for each group. \*  $P < 0.05$  and <sup>†</sup> $P < 0.05$ , as compared to saline- or ovalbumin-challenged untreated mice, respectively.



**Figure 5.** Cyanide-resistant eosinophil peroxidase activity in the bronchial wall of sensitized saline- or antigen-challenged mice. (a) Lung section from an immunized saline-challenged mouse, showing no inflammatory infiltrate around the bronchial lumen; (b) ovalbumin-challenged mouse used 24 h after the challenge, showing massive eosinophil infiltration concentrated around a vessel and the bronchial submucosa (arrows); (c) ovalbumin-challenged rmIL-10 (0.1  $\mu\text{g}/\text{mouse}$ )-treated mouse with scant eosinophils in the bronchial submucosa (arrows). BL, bronchial lumen; V, vessel, PI, perivascular infiltrate; PBI, peribronchial infiltrate.  $\times 200$ .

of rmIL-10 and of anti-IL-10 mAb of antigen-induced neutrophilia and eosinophilia in the BAL fluid, respectively.

**Effect of rmIL-10 on antigen-induced cellular infiltration in the BAL fluid and bronchial tissue from sensitized mice.** A single intranasal administration of 0.01, 0.03, or 0.1  $\mu\text{g}$  rmIL-10 to sensitized mice, instilled concurrently with ovalbumin, inhibited dose-dependently neutrophil and eosinophil infiltration in the BAL fluid observed 6 and 24 h after the challenge, respectively (Fig. 2). At the highest dose (0.1  $\mu\text{g}/\text{mouse}$ ), rmIL-10 suppressed allergic neutrophilia and decreased by  $\sim 75\%$  eosinophilia ( $P < 0.001$ ).

A kinetics study showed significant protection of both BAL neutrophilia and eosinophilia until 24 h after ovalbumin and rmIL-10 (0.1  $\mu\text{g}$ ) administration (Fig. 3). In particular, neutrophil numbers in ovalbumin-challenged rmIL-10-treated mice were below those found in control saline-stimulated animals

(Fig. 3). At 96 h, however, only a slight and nonsignificant decrease in the numbers of eosinophils was noted in antigen-challenged rmIL-10 (0.1  $\mu\text{g}$ )-treated mice, as opposed to controls (Fig. 3). Antigen challenge also induced a time-dependent rise in the number of eosinophils in the bronchial tissue, mainly at 24 h (Figs. 4 and 5). Treatment with 0.1  $\mu\text{g}$  rmIL-10, markedly decreased ovalbumin-induced eosinophil infiltration in the bronchial tissue and significant inhibition was observed at 24 h (Figs. 4 and 5).

The intranasal administration of 0.1  $\mu\text{g}$  rmIL-10, 1 or 3 h after antigen challenge, failed to protect sensitized mice from antigen-induced cellular accumulation in the BAL fluid (Table I).

**Effect of anti-IL-10 mAb on antigen-induced cellular infiltration in the BAL fluid from sensitized mice.** IL-10-mediated inhibition of allergic airway inflammation was suppressed by

**Table I.** Effect of rmIL-10, Administered Either Concomitantly, or 1 or 3 h after the Challenge, on Ovalbumin-induced Cellular Infiltration in the BAL Fluid from Sensitized Mice

Type of challenge	Treatment after challenge	Neutrophils at 6 h (10 <sup>4</sup> /ml BAL fluid)	Eosinophils at 24 h (10 <sup>4</sup> /ml BAL fluid)
Saline	Saline	0.90±0.30	0.02±0.02
Ovalbumin	Saline	6.20±2.10*	3.20±0.40*
Ovalbumin	rmIL-10 (0 h)	0.50±0.25 <sup>†</sup>	1.04±0.40 <sup>†</sup>
Ovalbumin	rmIL-10 (1 h)	4.90±0.20*	3.60±1.00*
Ovalbumin	rmIL-10 (3 h)	9.20±2.60*	2.70±1.20*

Cellular distribution in BAL fluid from sensitized mice challenged with intranasal saline or ovalbumin and treated, either concomitantly (0 h), or 1 or 3 h after the challenge with a single intranasal instillation of 0.1 µg rmIL-10. Results represent the number of neutrophils or eosinophils/ml BAL fluid. Data are expressed as means±SEM of six experiments. \* *P* < 0.05, as compared to saline-challenged untreated mice. <sup>†</sup> *P* < 0.05, as compared to ovalbumin-challenged untreated mice.

the prior (1 h) intravenous administration of 1 mg/mouse of the rat anti-mouse IL-10 mAb, 2A5, but not of its matched isotype, GL113 (Fig. 6), indicating that the effect of rmIL-10 was due exclusively to its biological activity.

In separate experiments, the *in vivo* neutralization of IL-10 led to a significant (*P* < 0.05, *n* = 4–6) increase in the number of neutrophils and eosinophils in the BAL fluid 6 and 24 h after the challenge, respectively, (data not shown). This effect was not an artifact, since the intravenous injection of 1 mg GL113 or 2A5 to sensitized unchallenged mice did not modify the number of the different cell types in BAL fluid (data not shown).

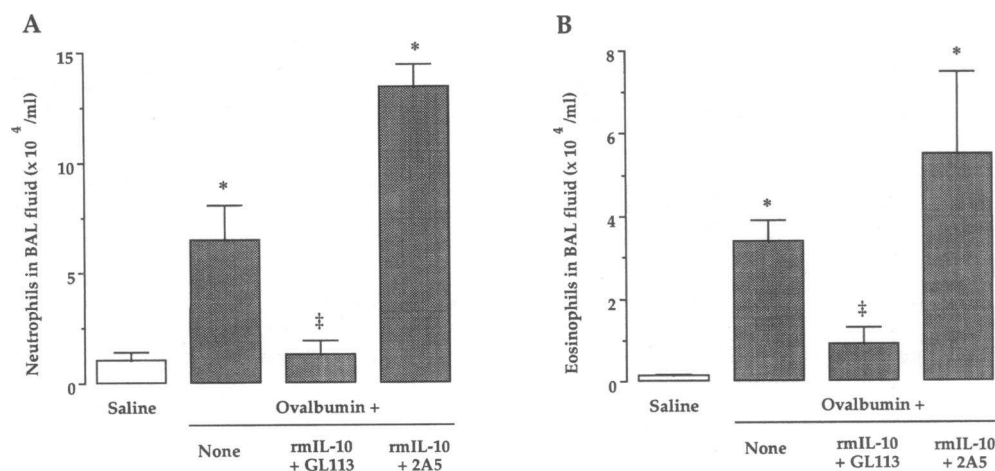
**Effect of rmIL-10 on antigen-induced TNF-α generation in the BAL fluid from sensitized mice.** Antigen challenge by sensitized mice was also followed by a time-dependent generation of TNF-α in the BAL fluid, which peaked at 1 h and returned

to basal levels thereafter (Fig. 7). BAL fluids collected 1, 3, or 24 h after the simultaneous administration of ovalbumin and rmIL-10 indicated a substantial reduction in the levels of TNF-α (Fig. 7). Total inhibition at 1 h was achieved with 0.5 µg rmIL-10, since 241.0±46.2 and 69.0±4.8 pg/ml TNF-α were measured in the BAL fluid of antigen-challenged untreated or rmIL-10-treated mice, respectively, (*P* < 0.001, *n* = 5–9).

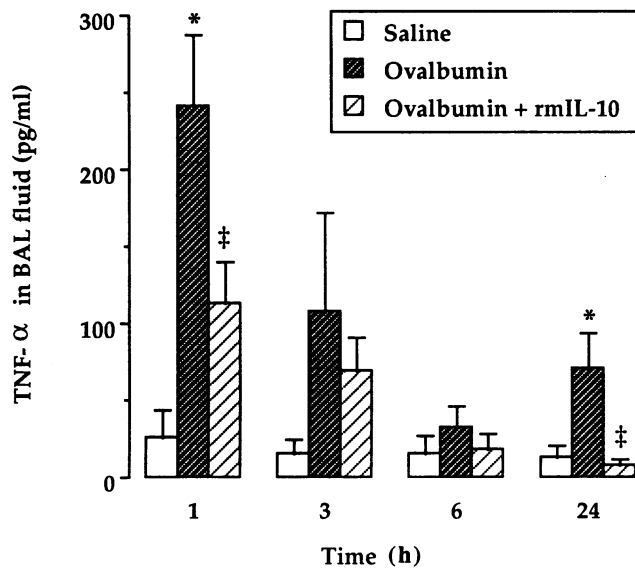
**Effect of anti-TNF-α antiserum on antigen-induced cellular infiltration in the BAL fluid from sensitized mice.** The intranasal instillation of 50 µl of a specific anti-TNF-α antiserum, concomitantly with ovalbumin, led to a significant reduction in both BAL neutrophils and eosinophils at 6 and 24 h, respectively. Under the same conditions, control serum was ineffective (Table II).

## Discussion

In the present study we show that IL-10, a Th2-derived cytokine (11), down-regulates antigen-induced airway inflammation in sensitized mice, since its local administration prevents neutrophil and eosinophil accumulation in the BAL fluid observed 6 and 24 h after the challenge, respectively. This effect is attributable to the biological activity of the cytokine, since inhibition by IL-10 of antigen-induced airway inflammation is suppressed when the sensitized mice are treated with a specific anti-IL-10 mAb. It is noteworthy that *in vivo* neutralization of IL-10 further enhances the ovalbumin-induced cellular recruitment in the BAL fluid, suggesting that endogenously released IL-10 may be involved in the regulation of leukocyte homing to the airways. Systemic generation of IL-10 after intraperitoneal injection of anti-CD3 mAb or LPS to Balb/c mice has been reported by Durez et al. (22). Since both stimuli were effective in eliciting IL-10 release, it was suggested that IL-10 production can be achieved by stimulating different cell types. Thus, CD4<sup>+</sup> T cells are the major source of this cytokine upon CD3 stimulation (23), whereas macrophages and B cells are likely to be involved in LPS-induced IL-10 release (13). These observations raise



**Figure 6.** Effect of anti-IL-10 mAb on the inhibition by rmIL-10 of antigen-induced cellular accumulation in the BAL fluid from sensitized mice. Mice were challenged by intranasal route either with saline, or with 10 µg ovalbumin alone or concurrently administered with 0.1 µg rmIL-10. Antigen-challenged animals were treated intravenously with 1 mg/mouse of a specific anti-IL-10 mAb, 2A5, or with its matched isotype, GL113, 1 h before ovalbumin administration. Neutrophil (A) and eosinophil (B) infiltration into the BAL fluid were evaluated 6 or 24 h after antigen challenge, respectively. Results are expressed as means±SEM of five to six experiments for each group. \* *P* < 0.05 and <sup>†</sup>*P* < 0.05, as compared to saline- or ovalbumin-challenged untreated mice, respectively.



**Figure 7.** Inhibition by rmIL-10 of antigen-induced TNF- $\alpha$  generation in the BAL fluid from sensitized mice. Sensitized Balb/c mice were challenged with intranasal saline ( $\square$ ), or with 10  $\mu$ g ovalbumin, administered either alone ( $\blacksquare$ ) or with 0.1 ( $\boxplus$ )  $\mu$ g rmIL-10 and they were killed at various time intervals after the challenge. The levels of TNF- $\alpha$  in the BAL fluid were evaluated by a double sandwich-specific ELISA. Results are expressed as means  $\pm$  SEM of five to nine experiments for each group. \*  $P < 0.05$  and ‡  $P < 0.05$ , as compared to saline- or ovalbumin-challenged untreated mice, respectively.

the question of the sources of IL-10 in ovalbumin-challenged mouse lung. Indeed, besides CD4<sup>+</sup> T lymphocytes, which are present in increased numbers in the bronchial tissue of sensitized mice 24 h after antigen challenge (reference 9 and our unpublished results), alveolar macrophages may also respond to antigen stimulation with IL-10 generation. Accordingly, Hamid et al. (24) described recently increased mRNA for IL-10 in BAL cells from asthmatics. In situ hybridization coupled with immunohistochemistry, revealed that IL-10 secreting cells were mostly T lymphocytes and macrophages (24).

Contrary to the inhibition of allergic airway inflammation observed when rmIL-10 was given by intranasal route, its subcutaneous injection did not modify antigen-induced cellular accumulation in the BAL fluid (data not shown). These results suggest that the target(s) for IL-10 are located in the airways and that the amounts of rmIL-10 reaching the bronchial compartment after its systemic administration are probably not sufficient to display an effect. Among the various bronchopulmonary targets for IL-10, the macrophage is a widely described cell type sensitive to this cytokine (13). Indeed, incubation of human or murine macrophages in the presence of IL-10 results in a marked down-regulation of their reactivity, particularly in terms of cytokine production and mediator release (25–27). On the other hand, IL-10 was first identified as a cytokine acting on Th1 clones and capable of inhibiting their IFN- $\gamma$  production (11, 12). However, protection by IL-10 of antigen-induced airway inflammation presently described seems unrelated to a blockade of IFN- $\gamma$  generation from lung T cells. Indeed, systemic (28) or bronchopulmonary (29) administration of IFN- $\gamma$  to mice sensitized and challenged under conditions similar to those used in this study, prevents allergic eosinophil infiltration

**Table II.** Effect of the Anti-TNF- $\alpha$  Antiserum, or Its Control Preimmune Serum, on Antigen-induced Cellular Accumulation in the BAL Fluid from Sensitized Mice

Challenge intranasal	Treatment intranasal	Neutrophils at 6 h (10 <sup>4</sup> /ml BAL fluid)	Eosinophils at 24 h (10 <sup>4</sup> /ml BAL fluid)
Saline	None	1.31 $\pm$ 0.35	0.04 $\pm$ 0.03
Ovalbumin	None	4.54 $\pm$ 1.10*	3.70 $\pm$ 1.09*
Ovalbumin	Preimmune serum	6.32 $\pm$ 2.11*	4.93 $\pm$ 1.50*
Ovalbumin	anti-TNF- $\alpha$ antiserum	0.76 $\pm$ 0.27**	0.46 $\pm$ 0.19**§

Cellular distribution in the BAL fluid from sensitized mice challenged with intranasal saline or ovalbumin and treated concomitantly, via the intranasal route, with 50  $\mu$ l/mouse of a sheep anti-murine TNF- $\alpha$  antiserum or with its control preimmune serum. Results represent the number of neutrophils or eosinophils  $\times 10^4$  ml BAL fluid. Data are expressed as means  $\pm$  SEM of four to nine experiments. \*  $P < 0.05$ , as compared to saline-challenged untreated mice. †  $P < 0.05$ , as compared to ovalbumin-challenged untreated mice. §  $P < 0.05$ , as compared to ovalbumin-challenged control serum-treated mice.

into the tracheal tissue. This effect is compatible with the inhibition by IFN- $\gamma$  of Th2 clone proliferation (30), and subsequent production of cytokines, such as IL-5, which is selectively involved in eosinophil mobilization (5–7).

The data presented herein show that rmIL-10 is equally effective in preventing both neutrophil and eosinophil infiltration into the airways of antigen-challenged mice. This suggests that IL-10 may regulate the expression and/or the release of factors involved in the migration of these two cell types to the lung. Interestingly enough, when administered 1 h after the challenge, rmIL-10 failed to protect sensitized mice from antigen-induced cellular accumulation in the BAL fluid. This observation led us to hypothesize that IL-10 may modulate the local release of preformed and thus rapidly released cytokines, which are potentially involved in allergic leukocyte recruitment.

Several lines of evidence designate TNF- $\alpha$  as a cytokine generated by numerous cell types during inflammatory reactions and shock states (reviewed in reference 31). The participation of TNF- $\alpha$  in allergic reactions is linked to different observations, including its release by mast cells, basophils, and alveolar macrophages upon IgE-dependent mechanisms (32–35). High levels of TNF- $\alpha$  have been shown in monocytes and epithelial cells from asthmatics (36) and BAL fluid from antigen-challenged guinea pigs (37). Finally, treatment of sensitized guinea pigs with an IL-1 receptor antagonist prevents antigen-induced eosinophil accumulation and TNF- $\alpha$  generation in the BAL fluid (37), indicating that TNF- $\alpha$  may modulate allergic airway inflammation. We thus investigated whether antigen challenge induced the release of this cytokine in the BAL fluid of sensitized mice and verified the potential modulatory activity of IL-10 in this process. The intranasal administration of ovalbumin was followed by a marked rise in the levels of TNF- $\alpha$  in the BAL fluid, which reached a peak at 1 h to resolve between 3 and 6 h after the challenge. rmIL-10 dose-dependently inhibited this release, a result extending previous observations showing reduction by IL-10 of LPS-induced in vivo TNF- $\alpha$  generation (16, 17). Thus, the observation that TNF- $\alpha$  released upon antigenic stimulation is also susceptible of inhibition by IL-10 may have important consequences for the subsequent invasion of the bronchial wall by inflammatory cells. Accordingly, failure by

IL-10 to modify antigen-induced cellular infiltration when administered 1 h after the challenge, i.e., the time of the peak release of TNF- $\alpha$  in the BAL fluid, support the hypothesis that blockade of local TNF- $\alpha$  generation and inhibition of leukocyte recruitment are related phenomena. In confirmation, we demonstrate that the local administration of an antiserum to TNF- $\alpha$  markedly reduced antigen-induced neutrophil and eosinophil accumulation in the BAL fluid. These results extend those from previous studies showing the participation of TNF- $\alpha$  in leukocyte infiltration associated with IgE-dependent cutaneous inflammation in sensitized mice (38).

As whole rat lung tissue passively sensitized with monoclonal IgE antibody was shown to produce TNF- $\alpha$  after antigen stimulation (39), the presence of bronchopulmonary source(s) for this cytokine, particularly macrophages and mast cells, has been suggested. Indeed, macrophages, which are directly involved in allergic responses through the presence of Fc $\epsilon$ RII receptors at their surface (40), produce and express TNF- $\alpha$  upon IgE-dependent stimulation (35). Similarly, IgE-mediated activation of mast cells triggers the release of TNF- $\alpha$ , which, in this cell type, is stored in the cytoplasmic granules (41) and rapidly secreted upon stimulation. However, while macrophage deactivation by IL-10 is well documented (13, 25–27), modulation by IL-10 of IgE-dependent responses on mast cells, including TNF- $\alpha$  generation, has not been described yet.

Once released in the airways, TNF- $\alpha$  may display a broad spectrum of biological activities, including activation and chemotaxis of eosinophils (42, 43) and neutrophils (44, 45). Interestingly enough, TNF- $\alpha$  directly stimulates the expression of adhesion molecules on cultured endothelial cells (46, 47), and, in vivo, in the baboon's skin (48). These molecules, which include intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1), are responsible for the adhesion of leukocytes to the endothelium. This is followed by their transendothelial migration and subsequent infiltration into the inflamed tissues. Accordingly, treatment of sensitized Balb/c mice with anti-ICAM-1 or anti-VCAM-1 mAbs, suppressed antigen-induced cellular recruitment into the tracheal tissue (49). Whether adhesion molecule expression in the allergic lung is also directly or indirectly regulated by IL-10 is an area for a further investigation.

In conclusion, our findings demonstrate that, when compared to other Th2-derived cytokines, such as IL-4 and IL-5, IL-10 is unique in down-regulating antigen-induced airway inflammation in the mouse. This evidence suggests that IL-10 production during allergic responses, may represent an important regulatory mechanism in the control of tissue inflammation in which leukocyte infiltration is a prominent feature. Consequently, therapeutic agents aimed to positively regulate IL-10 generation and/or its receptor interaction may have a promising future as inhibitors of allergic bronchopulmonary inflammation.

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