

ORIGINAL ARTICLE

Different roles of interleukin-10 in onset and resolution of asthmatic responses in allergen-challenged mice

KOICHIRO MATSUMOTO, HIROMASA INOUE, MIYUKI TSUDA, YASUKO HONDA, ATSUKO KIBE,
KENTARO MACHIDA, YUKI YOSHIURA AND YOICHI NAKANISHI

*Research Institute for Diseases of the Chest, Graduate School of Medical Sciences, Kyushu University,
Fukuoka, Japan*

Different roles of interleukin-10 in onset and resolution of asthmatic responses in allergen-challenged mice

MATSUMOTO K, INOUE H, TSUDA M, HONDA Y, KIBE A, MACHIDA K, YOSHIURA Y, NAKANISHI Y.
Respirology 2005; **10**: 18–26

Objective: Although interleukin (IL)-10 is an immunoregulatory cytokine produced by various cells including T cells, its precise role in asthma remains uncertain. The aim of this study was to investigate the role of IL-10 in experimental asthma using ovalbumin (OVA)-sensitized mice.

Methodology: Mice were challenged with OVA aerosol, and airway responsiveness and inflammation were measured. OVA-specific IL-10-producing CD4⁺ T cells were counted from lung cells collected by enzymatic digestion and stimulated *ex vivo* with OVA. The effects of an anti-IL-10 antibody on airway responsiveness and inflammation were also evaluated.

Results: The OVA challenge caused airway hyperresponsiveness and eosinophilic inflammation. A significant increase in IL-10-producing CD4⁺ T cells was observed, mainly in the CD45RB^{low} subset, for several days after the OVA challenge. Anti-IL-10 antibody treatment before the OVA challenge did not affect eosinophilic inflammation but significantly inhibited airway hyperresponsiveness 24 h after the OVA challenge. However, anti-IL-10 antibody treatment just before the last OVA challenge significantly attenuated the resolution of eosinophilic inflammation without affecting airway responsiveness 2 weeks after the OVA challenge.

Conclusions: Intrinsic IL-10 may have a distinct role in the early and late phases of asthmatic responses. In the early phase, IL-10 induces airway hyperresponsiveness, while in the late phase IL-10 contributes to the resolution of eosinophilic inflammation.

Key words: airway hyperresponsiveness, asthma, eosinophil, interleukin-10, mouse.

INTRODUCTION

Asthma is characterized by chronic airway inflammation, mucus hypersecretion, and airway hyperresponsiveness. Although the precise mechanisms underlying these changes remain to be elucidated, accumulating evidence suggests that CD4⁺ T-helper type 2 cells (Th2 cells) have important roles in the development of asthma by excessive production of

interleukin (IL)-4, IL-5, IL-9, and IL-13.^{1–3} Because the cellular responses mediated by these Th2-type cytokines are effectively attenuated by the Th1-type cytokine, IFN- γ , experimental efforts have focused on augmenting intrinsic Th1-type responses as a therapeutic approach to asthmatic responses.^{4–9} However, several studies have reported that the passive transfer of allergen-specific Th1-skewed CD4⁺ T cells into allergen-sensitized mice not only failed to attenuate but, rather, exacerbated airway responses after an allergen challenge.^{10,11} Thus, it might be difficult to understand asthma on the basis of the simple counterbalance paradigm of Th1 versus Th2.

During the last decade, several investigators have identified a unique population of T cells showing the capacity to regulate a variety of Th1- and Th2-induced inflammation.^{12–15} Some of those T cells are reported to elicit their effects via the selective production of

Correspondence: Hiromasa Inoue, Research Institute for Diseases of the Chest, Graduate School of Medical Sciences, Kyushu University, 3-1-1 Maidashi, Higashi-ku, Fukuoka 812-8582, Japan.
Email: inoue@kokyu.med.kyushu-u.ac.jp

Received 12 January 2004; revised 05 August 2004; accepted for publication 5 August 2004.

IL-10. IL-10 is a pleiotropic cytokine produced by inflammatory cells, including dendritic cells, the macrophage/monocyte lineage, granulocytes, B cells, and T cells.^{16–18} IL-10 markedly inhibits the expression of MHC class II molecules and co-stimulatory molecules on antigen-presenting cells (APC), the proliferation of activated T cells by reducing IL-2 production, and the release of inflammatory cytokines and nitric oxide from the activated macrophage/monocyte lineage. Lines of *in vitro* evidence have led to the hypothesis that endogenous IL-10 may act as one of the anti-inflammatory cytokines that regulate ongoing inflammatory responses *in vivo*. Indeed, mice deficient in IL-10 (IL-10^{-/-} mice) spontaneously develop chronic enterocolitis^{12,14,19,20} and show a hyper-inflammatory intracerebral immune response in Listeria-induced meningoencephalitis.²¹

The regulatory role of endogenous IL-10 in asthma, however, remains unknown. Previous studies have reported that IL-10 production in response to lipopolysaccharide (LPS) was impaired in the monocyte/macrophage cell lineage of asthmatics,^{22,23} whereas other studies have reported an increase of IL-10-producing T cells and macrophages in asthmatic airways and a further increase of those cells after an allergen challenge.^{24–26} Furthermore, experimental asthma studies using IL-10^{-/-} mice have provided results that are inconsistent with those of other similar experiments.^{27–31} A major problem with using IL-10^{-/-} mice for asthma models is that IL-10 deficiency may affect the entire process of allergic reactions, from the sensitization phase to the effector phase, as a result of recall challenge with an allergen. To elucidate the role of IL-10 in the effector phase of asthma, we examined the effect of the temporary blockade of endogenous IL-10 by treatment with anti-IL-10 monoclonal antibody (mAb).

MATERIALS AND METHODS

Animals

Male BALB/c mice, 6–7 weeks of age, were purchased from Charles River Japan Inc. (Kanagawa, Japan) and housed in environmentally-controlled pathogen-free conditions. All animals were maintained in-house for 1 week prior to their use in this study. All procedures and protocols were approved by the institutional ethics committee for animal research.

Allergen sensitization and challenge

Mice were sensitized by intraperitoneal injection of 10 µg of chicken ovalbumin (OVA; Sigma-Aldrich, St. Louis, MO, USA) and 0.3 mg of aluminium potassium sulphate (SERVA Electrophoresis, Heidelberg, Germany) on days 1 and 11, and were then challenged with 5% OVA in saline mist for 20 min on 5 consecutive days (from day 19 to day 23). The OVA mist was generated using an ultrasonic nebulizer (Omron NE-U17, Mie, Japan). Control mice were subjected to 0.9% saline sensitization and challenge and are

referred to as naive mice. Subgroups of animals received intravenous injections (0.5 mg/animal) of anti-mouse IL-10 monoclonal antibody (mAb; JES5-2A5, rat IgG1, BD PharMingen, San Diego, CA, USA) or control rat IgG (Sigma-Aldrich), 3 h before the initial OVA challenge on day 19. Other subgroups received the same anti-mouse IL-10 mAb or control rat IgG 3 h before the last OVA challenge on day 23.

Measurement of airway hyperresponsiveness

Measurement of airway hyperreactivity (AHR) was performed according to our previously described protocol.³² Briefly, animals were anaesthetized by intraperitoneal injection with a mixture of ketamine and sodium pentobarbital, and their tracheas were cannulated via tracheostomy. The animals were ventilated mechanically (Harvard Apparatus Model 687, South Natick, MA, USA) with a tidal volume of 0.3 mL and at a frequency of 120 breaths/min. The airway-opening pressure was measured with a differential pressure transducer (Nihon Kohden Model TP-603T, Tokyo, Japan) and recorded continuously with a pen recorder (Nihon Kohden RJG-4124). Stepwise increases in acetylcholine (diluted in 0.9% saline; Sigma-Aldrich) concentrations (0.6–10 mg/mL per 120 breaths) were administered with an ultrasonic nebulizer (Omron NE-U07). The data were expressed as the provocative concentration 200 (PC₂₀₀), the concentration at which airway pressure was 200% of its baseline value, as calculated by log-linear interpolation for individual animals. Values of PC₂₀₀ were expressed as log (PC₂₀₀ × 100).

Bronchoalveolar lavage

Mice were exsanguinated following a lethal dose of pentobarbital, and their lungs were gently lavaged three times with 1 mL of 0.9% saline via a tracheal cannula. Total and differential bronchoalveolar lavage (BAL) cell counts were performed as previously described.³²

Collection of lung cells

To determine the time course of IL-10-producing CD4⁺ T cells in allergen-challenged lung tissues, we carried out intracellular cytokine measurements using flow cytometry. Additionally, we focused on CD45RB^{low}CD4⁺ T cells, as previous studies have shown that these cells may play a regulatory role in an experimental model of inflammatory bowel disease, presumably by dominant production of IL-10.^{12,14,20} Enzymatic digestion of the lungs was performed as previously described.³³ Briefly, mice were exsanguinated after a lethal dose of pentobarbital, and the lungs were carefully removed, cut, and minced. A total of 2 mL of complete Dulbecco's phosphate-buffered saline (PBS) containing 0.4 mg/mL collagenase type 1 A, 330 U/mL hyaluronidase, 50 U/mL DNase, 10% FBS (all additives: Sigma-Aldrich),

100 U/mL penicillin and 100 mg/mL streptomycin (Gibco BRL, Grand Island, NY, USA) was added to the minced lungs, and the suspension was incubated for 60 min at 37°C. The samples were then filtered through a 52- μ m nylon mesh. The single-cell suspensions were washed once with complete Dulbecco's PBS, and the erythrocytes were removed by lysis with NH_4Cl -Tris buffer. Cells were suspended in complete RPMI 1640 medium (Sigma-Aldrich) and adjusted to a concentration of 2×10^6 cells/mL.

Ex vivo cell stimulation and intracellular cytokine staining

All monoclonal antibodies were purchased from BD PharMingen. Whole lung cells (2×10^6 cells/well in complete RPMI 1640) were stimulated with OVA (0.4 mg/mL) plus soluble anti-CD28 mAb (1 μ g/mL) for 6 h. Monensin (0.7 μ L/well, GolgiStop, BD PharMingen) was added for the final 4 h of stimulation. Cells were then washed and incubated with unlabelled anti-CD16/32 mAb (2.4G2, rat IgG2b) for 20 min to prevent non-specific binding via Fc γ R. Cells were incubated with FITC-labelled anti-CD4 mAb (RM4-5, rat IgG2a), PE-labelled anti-CD45RB mAb (16 A, rat IgG2a), and PerCP-labelled anti-CD3 mAb (145-2C11, hamster IgG) for 30 min. After fixation with 10% paraformaldehyde (Medium A, Caltag Laboratories, An Der Grub, Austria), cells were permeabilized with a saponin-containing solution (Medium B, Caltag Laboratories) for 10 min and stained with APC-labelled anti-IL-10 mAb (JES5-16E3, rat IgG2b) or isotype control IgG2b for 30 min. Paired samples were also stained with APC-labelled anti IL-5 mAb (TRFK-5, rat IgG1) or isotype control IgG1. After several washes, the cells were suspended in PBS containing 1% paraformaldehyde and stored at 4°C until analysed.

Flow cytometric analysis

A FACScalibur instrument with CELLQuest software (BD Biosciences, San Jose, CA, USA) was used for detecting the intracellular cytokine-positive T-cell subset. A total of 20 000 cells were acquired and an electronic gate was set on lymphocytes using the forward and side scatter plots. $\text{CD}3^+\text{CD}4^+\text{CD}45\text{RB}^{\text{low}}$ and $\text{CD}3^+\text{CD}4^+\text{CD}45\text{RB}^{\text{high}}$ cells falling within the gated area were then identified. Cells positive for intracellular IL-10 or IL-5 were expressed as percentages of each $\text{CD}4^+$ T-cell subset, and the numbers of positive cells were calculated by multiplying the total cell count obtained after lung digestion.

Data analysis

Values were expressed as the mean $\pm$ SEM. Differences among groups were analysed using unpaired *t*-tests or an analysis of variance followed by posthoc Bonferroni analysis. Non-parametric data were analysed using the Kruskal-Wallis test followed by the

Mann-Whitney *U*-test. *P*-values less than 0.05 were considered significant.

RESULTS

Time course of ovalbumin-induced airway inflammation and hyperresponsiveness

To examine the kinetics of allergen-induced asthmatic responses, airway responsiveness and differential cell counts in BAL fluid were performed 24 h, 48 h, 72 h, 96 h, and 14 days after the last OVA challenge. Airway responsiveness to inhaled acetylcholine was significantly higher from 24 h to 48 h, suggesting the induction of airway hyperresponsiveness (Fig. 1a). Airway hyperresponsiveness was not observed thereafter. From 24 h to 96 h after the OVA challenge, the absolute numbers of macrophages, eosinophils, and lymphocytes were significantly higher than those of time-matched naive mice (Fig. 1b). The numbers of neutrophils were significantly higher than those of naive mice at 24 h and 48 h and rapidly returned to the baseline level. Although these inflammatory cells tended to decrease 14 days after the OVA challenge, the numbers of eosinophils and lymphocytes remained higher than those of naive mice.

Time course of interleukin-10- and interleukin-5-producing $\text{CD}4^+$ T cells after the ovalbumin challenge

Typical dot plots of IL-10- and IL-5-producing $\text{CD}4^+$ T cells from samples obtained 24 h after the last OVA challenge are shown in Fig. 2a. The absolute numbers of those cells were further assessed with regard to their $\text{CD}45\text{RB}^{\text{low}}$ and $\text{CD}45\text{RB}^{\text{high}}$ populations (Fig. 2b). The numbers of IL-10-producing $\text{CD}45\text{RB}^{\text{low}}\text{CD}4^+$ T cells were significantly higher than those of time-matched naive mice from 24 h to 72 h after OVA challenge. Although there was a statistically significant increase in IL-10-producing $\text{CD}45\text{RB}^{\text{high}}\text{CD}4^+$ T cells from 24 h to 48 h, their absolute numbers were lower than those of $\text{CD}45\text{RB}^{\text{low}}\text{CD}4^+$ T cells. The numbers of IL-5-producing $\text{CD}45\text{RB}^{\text{low}}\text{CD}4^+$ T cells, but not those of $\text{CD}45\text{RB}^{\text{high}}\text{CD}4^+$ T cells, were significantly higher than those of naive mice from 24 h to 72 h after OVA challenge.

Effects of anti-interleukin-10 antibody treatment on asthmatic responses

To elucidate the role of intrinsic IL-10 in allergen-induced asthmatic responses, the effects of anti-IL-10 mAb treatment were examined using two different protocols. In the first protocol, anti-IL-10 mAb was administered at 3 h before the initial OVA challenge, and measurements of the outcomes were performed 24 h after the OVA challenge to examine the role of IL-10 in the development of asthmatic responses (Fig. 3a). OVA-induced airway hyperresponsiveness

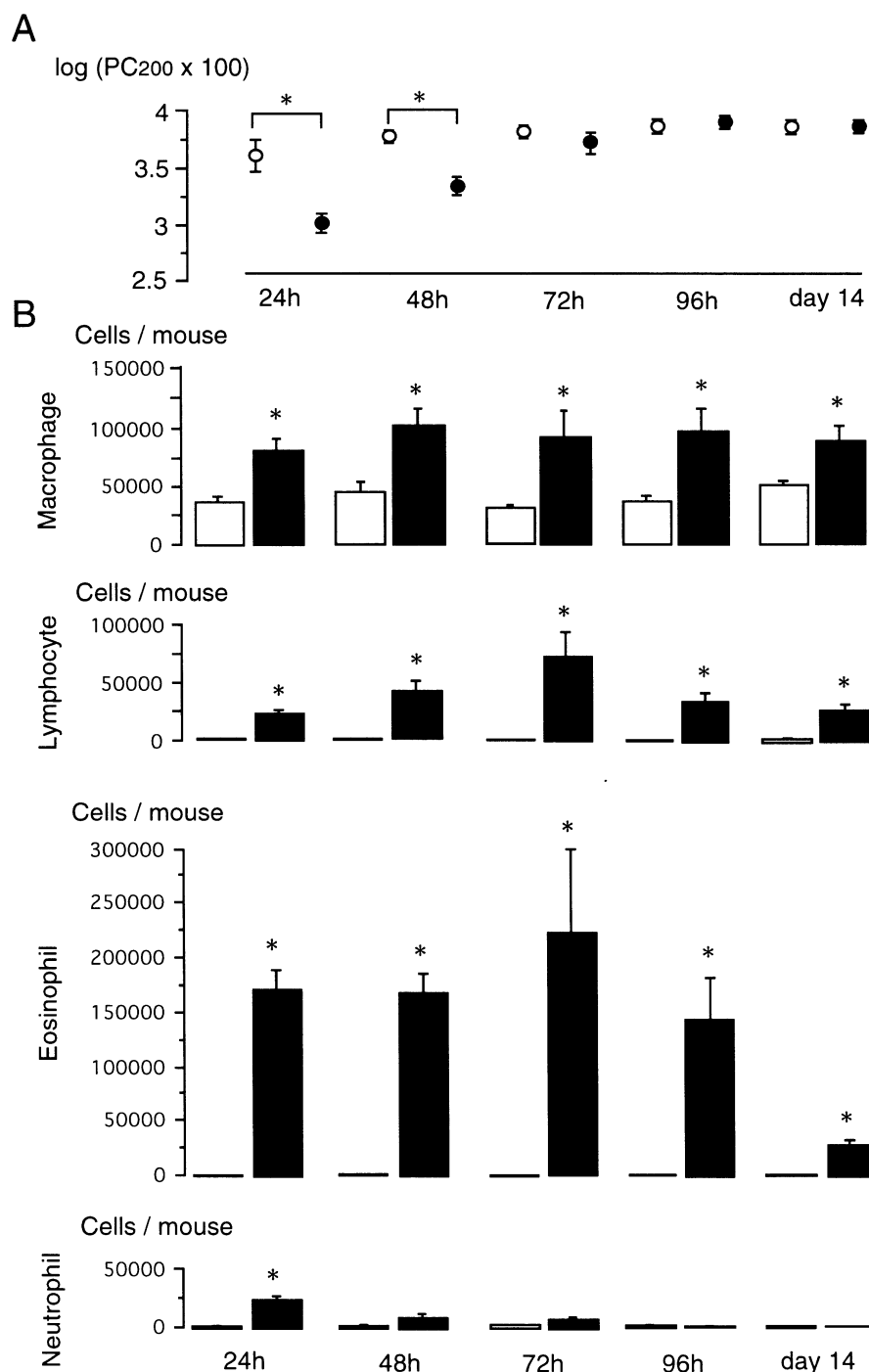


Figure 1 (a) Time courses of airway responsiveness and (b) inflammatory cells in broncho-alveolar lavage fluid (BALF), in allergen-challenged mice (black circle/bar) and time-matched naive mice (white circle/bar). Mice were sensitized with intraperitoneal ovalbumin (OVA) on days 1 and 11 and then challenged by OVA inhalation on days 19–23. Measurements of the outcomes were performed 24 h, 48 h, 72 h, 96 h, and 14 days after the last OVA challenge. Values are expressed as means with SEM. Mouse numbers per group were 4–7. * $P < 0.05$ compared with naive mice.

was significantly attenuated by this anti-IL-10 mAb treatment, while the numbers of macrophages, eosinophils, neutrophils and lymphocytes were not affected by anti-IL-10 mAb treatment (Fig. 3b).

In the second protocol, anti-IL-10 mAb was administered 3 h before the last OVA challenge and the outcomes were measured 14 days later in order to examine the role of IL-10 in the resolution of asthmatic responses (Fig. 4a). Although the numbers of macrophages, neutrophils, and lymphocytes were not affected by the anti-IL-10 mAb treatment, there were significantly higher numbers of eosinophils in the

BAL fluid than after the control IgG treatment (Fig. 4b). Airway responsiveness was similar among anti-IL-10 mAb-treated mice, control IgG-treated mice, and time-matched naive mice.

DISCUSSION

In order to clarify the role of IL-10 in asthmatic responses, it is important to understand the kinetics of IL-10 production. We focused on CD4⁺ T cells because allergen-specific CD4⁺ T cells are known to

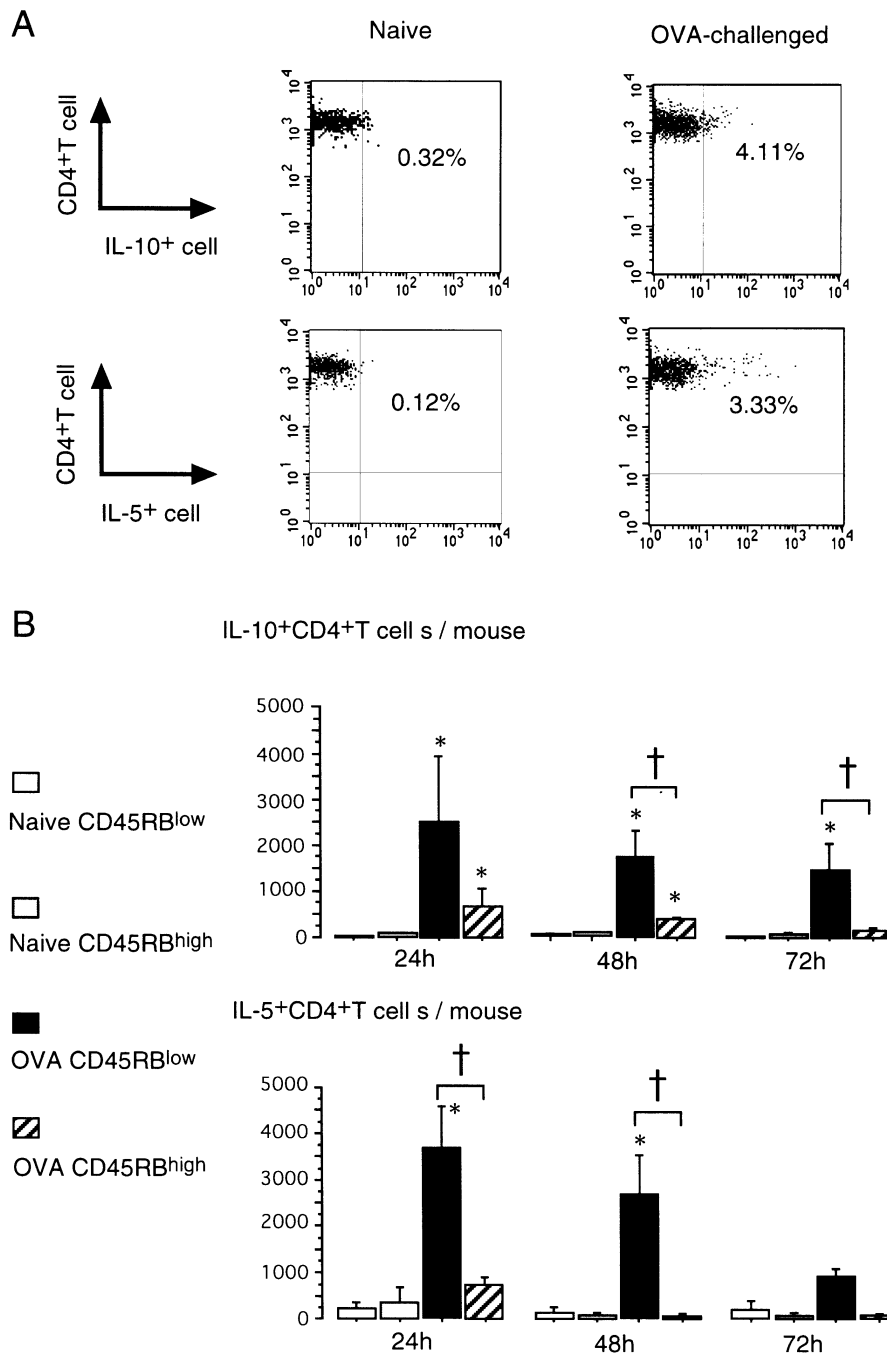


Figure 2 Allergen-induced increases of interleukin (IL)-10-producing CD4⁺ T cells and IL-5-producing CD4⁺ T cells. Flow cytometry was performed 24 h, 48 h and 72 h after the last OVA challenge. (a) Typical plots of cytokine-producing cells from samples obtained 24 h after the last OVA challenge. An electronic flow cytometry gate was set on the CD45RB^{low}CD4⁺ T cell subset and the frequency of cells positive for the indicated cytokine is shown as dot plots. The numbers indicate the mean $\pm$ SEM percentages of CD45RB^{low}CD4⁺ T cells that were IL-10-positive or IL-5-positive. (b) Time courses of IL-10- or IL-5-producing CD4⁺ T cells in CD45RB^{low} or CD45RB^{high} subsets. Values are expressed as means with SEM. Mouse numbers per group were 4–7. * P < 0.05 compared with naive mice. ** P < 0.05 compared with CD45RB^{high} subset.

orchestrate allergic inflammation and are potent producers of IL-10. Additionally, we aimed to identify a population responsible for IL-10 production, using the surface marker, CD45RB, as previous studies had shown that CD45RB^{low} CD4⁺ T cells may play a regulatory role in an experimental model of Th1-skewed inflammatory bowel disease, presumably via dominant production of Th2 cytokines, including IL-10.^{12,14,20} Our experiment showed a more profound increase of IL-10-producing CD4⁺ T cells and IL-5-producing CD4⁺ T cells in the CD45RB^{low} population than in the CD45RB^{high} population, which is consistent with the previous studies. In humans, it is well known that alveolar macrophages are another potent

source of IL-10.^{22,23,25,26} However, we did not examine the IL-10 production in macrophages because murine resident alveolar macrophages are reportedly deficient at producing IL-10 protein.³⁴

Despite the accumulating knowledge from experimental asthma models, the precise roles of intrinsic IL-10 in asthma remain controversial. Administration of recombinant IL-10 during allergen challenges of BALB/c mice reportedly suppressed eosinophilic airway inflammation but reciprocally enhanced airway hyperresponsiveness.^{35,36} In contrast, airway IL-10 gene-transfer during allergen sensitization was reported to suppress both eosinophilic inflammation and hyperresponsiveness.³⁷ These studies at least

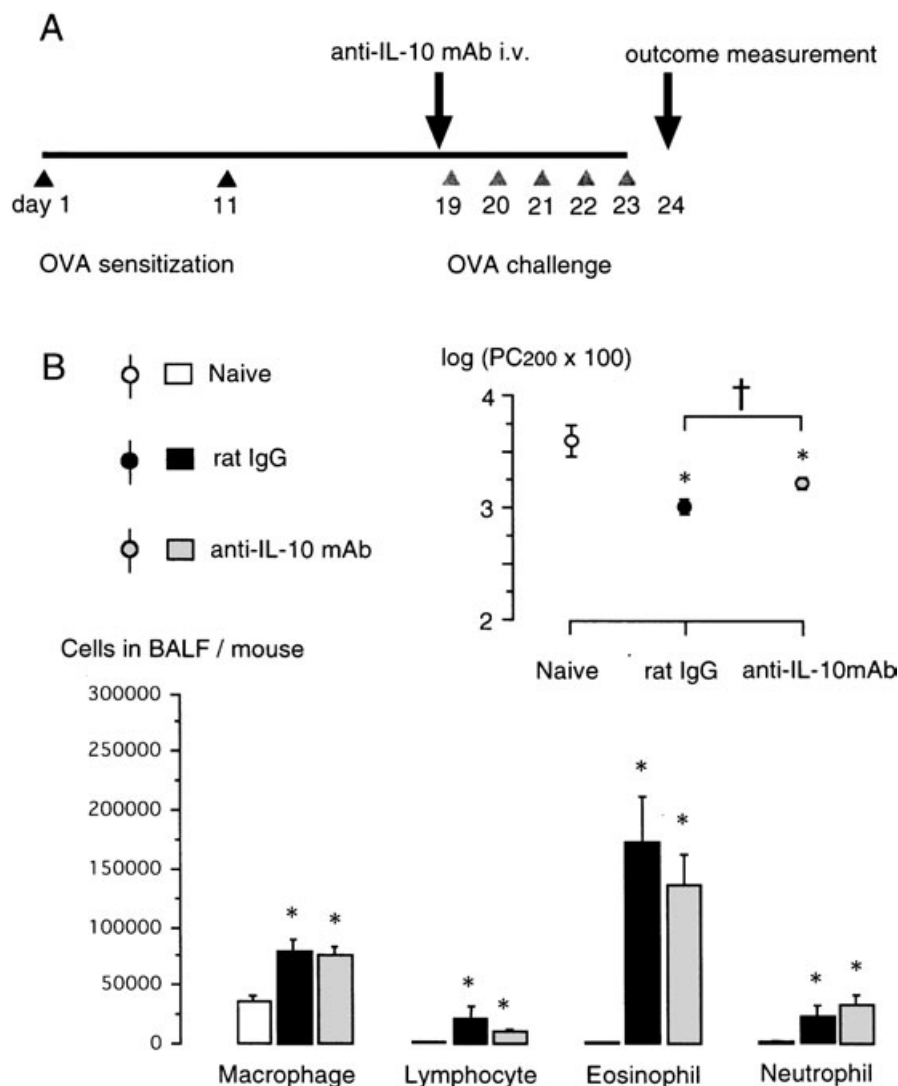


Figure 3 Effect of anti-interleukin (IL)-10 monoclonal antibody (mAb) on the development of airway hyperresponsiveness and airway inflammation. (a) Protocol of anti-IL-10 mAb treatment, in which an anti-IL-10 mAb (0.5 mg/animal) or a control rat IgG was administered at 3 h before the initial OVA challenge. (b) Measurements of the outcomes were performed 24 h after the last OVA challenge. Values are expressed as means with SEM. Mouse numbers per group were 4–7. * $P < 0.05$ compared with naive mice. ** $P < 0.05$ compared with rat IgG-treated and OVA-challenged mice.

suggest that supra-physiological IL-10 may work as a strong antiallergic cytokine to prevent eosinophilic inflammation. There have been several reports of studies using IL-10^{-/-} mice on a C57BL/6 background.^{27–31} One report showed the attenuation of IL-5 production and eosinophilic inflammation after an allergen challenge.³¹ However, other studies reported that allergen-induced eosinophilic inflammation was enhanced in IL-10^{-/-} mice.^{27–30} One of the critical issues is that IL-10, produced by antigen-presenting cells themselves or other bystander cells during allergen sensitization, may have a considerable effect on the interaction between antigen-presenting cells and allergen-specific T cells.³⁸ Indeed, several studies have reported that IL-10 is important for preventing Th1 commitment, which may lead to the development of Th2-skewed airway inflammation.^{31,39} Once the sensitization is established, however, IL-10 may work as a regulator of inflammation following recall challenge with an allergen. The latter possibility is supported by our results, which showed that anti-IL-10 mAb inhibited the resolution of eosinophilic inflammation.

Previous studies have shown that an allergen challenge did not augment airway hyperresponsiveness in IL-10^{-/-} mice, although the baseline responsiveness in naive IL-10^{-/-} mice was different in these studies.^{27–30} In the present study, anti-IL-10 mAb treatment just before the OVA challenge inhibited airway hyperresponsiveness, which was measured 24 h after the last OVA challenge. Thus, endogenous IL-10 appears to contribute to the development of allergen-induced airway hyperresponsiveness. Of note, is a previous report that the contractility of isolated rabbit airway smooth muscle is augmented by passive sensitization with serum from atopic asthmatics and that augmented responsiveness is attributable to the autocrine release of IL-10 and IL-5 from sensitized muscle itself.⁴⁰ Importantly, the present study has demonstrated concurrent increases of IL-10-producing CD4⁺ T cells and IL-5-producing CD4⁺ T cells in allergen-challenged lung tissues. Thus, endogenous IL-10 might not always counteract IL-5-mediated eosinophilic responses but may simultaneously cooperate with IL-5 to induce airway hyperresponsiveness. However, a

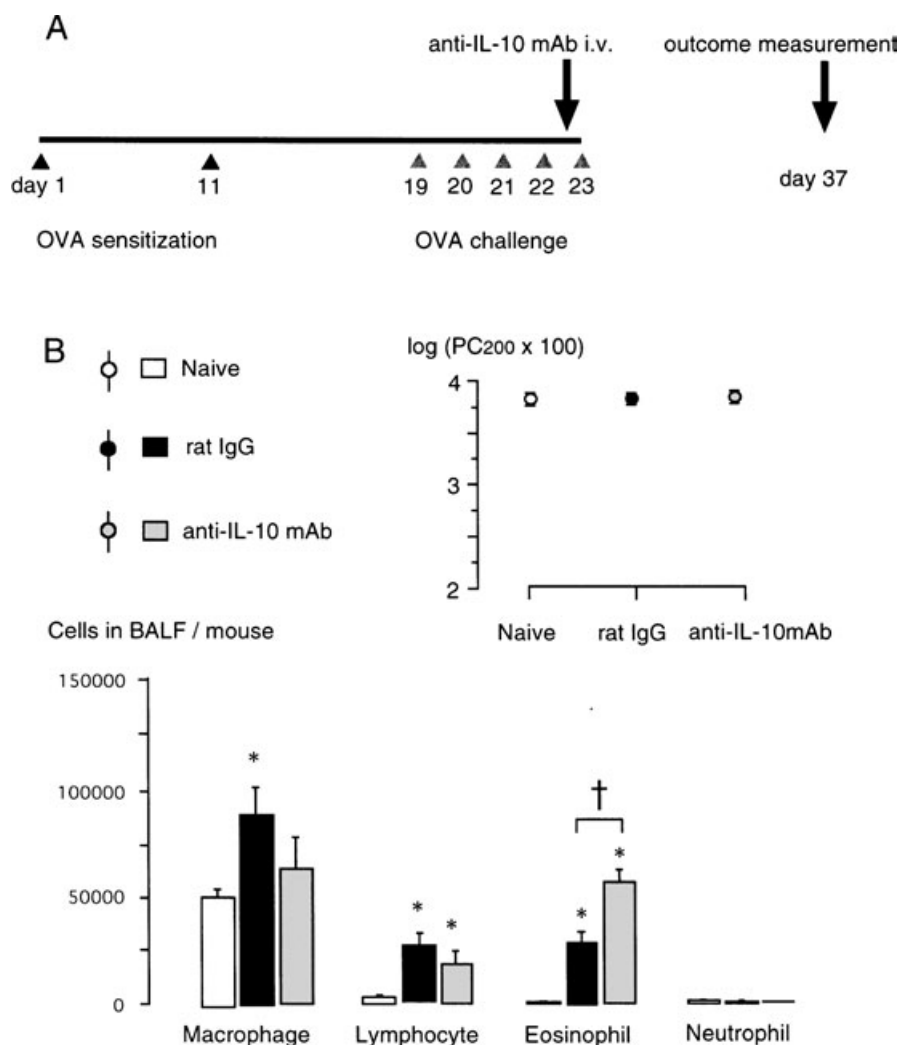


Figure 4 Effect of anti-interleukin (IL)-10 mAb on the resolution of airway hyperresponsiveness and airway inflammation. (a) Protocol of anti-IL-10 mAb treatment, in which an anti-IL-10 mAb (0.5 mg/animal) or a control rat IgG was administered at 3 h before the last OVA challenge. (b) Measurements of the outcomes were performed 14 days after the last OVA challenge. Values are expressed as means with SEM. Mouse numbers per group were 4–7. * $P < 0.05$ compared with naive mice. ** $P < 0.05$ compared with rat IgG-treated and OVA-challenged mice.

recent study using the same model of OVA-sensitized BALB/c mice, showed that anti-IL-10 mAb treatment before an OVA challenge resulted in the enhancement of airway hyperresponsiveness.⁴¹ That study used a short protocol in which animals were sensitized once (day 0), followed by three consecutive days of allergen challenge (days 7, 8, and 9). Such a difference in the experimental protocol might explain the contrasting effects of anti-IL-10 mAb between the studies.

It remains to be elucidated why the anti-IL-10 mAb treatment just before the last OVA challenge failed to affect airway responsiveness 14 days later. Allergen-induced airway hyperresponsiveness partly depends on eosinophilic airway inflammation.^{42,43} The restoration of airway hyperresponsiveness by prolonged eosinophilic inflammation might be overlaid by the attenuated contractility of airway smooth muscle resulting from treatment with anti-IL-10 mAb.

In summary, the OVA challenge caused eosinophilic airway inflammation, airway hyperresponsiveness, and an increase in IL-10-producing CD45RB^{low}CD4⁺ T cells in the lung of OVA-sensitized mice. Anti-IL-10 mAb treatment before the OVA challenge did not affect eosinophilic airway inflammation

but attenuated airway hyperresponsiveness 24 h after the OVA challenge. In contrast, anti-IL-10 mAb treatment just before the last OVA challenge significantly attenuated the resolution of eosinophilic airway inflammation without affecting airway responsiveness 2 weeks after the OVA challenge. Thus, endogenous IL-10 may have a distinct role in the early and late phases of asthmatic responses.

REFERENCES

- Chung KF, Barnes PJ. Cytokines in asthma. *Thorax* 1999; **54**: 825–57.
- Kirby JG, Hargreave FE, Gleich GJ, O'Byrne PM. Bronchoalveolar cell profiles of asthmatic and nonasthmatic subjects. *Am. Rev. Respir. Dis.* 1987; **136**: 379–83.
- Umetsu DT, DeKruyff RH. TH1 and TH2 CD4⁺ cells in human allergic diseases. *J. Allergy Clin. Immunol.* 1997; **100**: 1–6.
- Gavett SH, O'Hearn DJ, Li X, Huang SK, Finkelman FD, Wills-Karp M. Interleukin 12 inhibits antigen-induced airway hyperresponsiveness, inflammation, and Th2 cytokine expression in mice. *J. Exp. Med.* 1995; **182**: 1527–36.

- 5 Herz U, Gerhold K, Gruber C *et al.* BCG infection suppresses allergic sensitisation and development of increased airway reactivity in an animal model. *J. Allergy Clin. Immunol.* 1998; **102**: 867–74.
- 6 Hofstra CL, Van Ark I, Hofman G, Nijkamp FP, Jardieu PM, Van Oosterhout AJ. Differential effects of endogenous and exogenous interferon-gamma on immunoglobulin E, cellular infiltration, and airway responsiveness in a murine model of allergic asthma. *Am. J. Respir. Cell Mol. Biol.* 1998; **19**: 826–35.
- 7 Kim TS, DeKruyff RH, Rupper R, Maecker HT, Levy S, Umetsu DT. An ovalbumin-IL-12 fusion protein is more effective than ovalbumin plus free recombinant IL-12 in inducing a T helper cell type 1-dominated immune response and inhibiting antigen-specific IgE production. *J. Immunol.* 1997; **158**: 4137–44.
- 8 Lack G, Bradley KL, Hamelmann E *et al.* Nebulized IFN-gamma inhibits the development of secondary allergic responses in mice. *J. Immunol.* 1996; **157**: 1432–9.
- 9 Li XM, Chopra RK, Chou TY, Schofield BH, Wills-Karp M, Huang SK. Mucosal IFN-gamma gene transfer inhibits pulmonary allergic responses in mice. *J. Immunol.* 1996; **157**: 3216–19.
- 10 Hansen G, Berry G, DeKruyff RH, Umetsu DT. Allergen-specific Th1 cells fail to counterbalance Th2 cell-induced airway hyperreactivity but cause severe airway inflammation. *J. Clin. Invest.* 1999; **103**: 175–83.
- 11 Randolph DA, Stephens R, Carruthers CJ, Chaplin DD. Cooperation between Th1 and Th2 cells in a murine model of eosinophilic airway inflammation. *J. Clin. Invest.* 1999; **104**: 1021–9.
- 12 Asseman C, Mauze S, Leach MW, Coffman RL, Powrie F. An essential role for interleukin 10 in the function of regulatory T cells that inhibit intestinal inflammation. *J. Exp. Med.* 1999; **190**: 995–1004.
- 13 Chen Y, Kuchroo VK, Inobe J, Hafler DA, Weiner HL. Regulatory T cell clones induced by oral tolerance: suppression of autoimmune encephalomyelitis. *Science* 1994; **265**: 1237–40.
- 14 Groux H, O'Garra A, Bigler M *et al.* A CD4⁺ T-cell subset inhibits antigen-specific T-cell responses and prevents colitis. *Nature* 1997; **389**: 737–42.
- 15 Shevach EM. Certified professionals: CD4(+)CD25(+) suppressor T cells. *J. Exp. Med.* 2001; **193**: F41–6.
- 16 Borish L. IL-10: evolving concepts. *J. Allergy Clin. Immunol.* 1998; **101**: 293–7.
- 17 Pretolani M. Interleukin-10: an anti-inflammatory cytokine with therapeutic potential. *Clin. Exp. Allergy* 1999; **29**: 1164–71.
- 18 Umetsu DT, DeKruyff RH. Interleukin-10: the missing link in asthma regulation? *Am. J. Respir. Cell Mol. Biol.* 1999; **21**: 562–3.
- 19 Kuhn R, Lohler J, Rennick D, Rajewsky K, Muller W. Interleukin-10-deficient mice develop chronic enterocolitis. *Cell* 1993; **75**: 263–74.
- 20 Leach MW, Davidson NJ, Fort MM, Powrie F, Rennick DM. The role of IL-10 in inflammatory bowel disease: 'of mice and men'. *Toxicol. Pathol.* 1999; **27**: 123–33.
- 21 Deckert M, Soltek S, Geginat G *et al.* Endogenous interleukin-10 is required for prevention of a hyper-inflammatory intracerebral immune response in *Listeria monocytogenes* meningoencephalitis. *Infect. Immun.* 2001; **69**: 4561–71.
- 22 Borish L, Aarons A, Rumblyrt J, Cvietusa P, Negri J, Wenzel S. Interleukin-10 regulation in normal subjects and patients with asthma. *J. Allergy Clin. Immunol.* 1996; **97**: 1288–96.
- 23 John M, Lim S, Seybold J *et al.* Inhaled corticosteroids increase interleukin-10 but reduce macrophage inflammatory protein-1alpha, granulocyte-macrophage colony-stimulating factor, and interferon-gamma release from alveolar macrophages in asthma. *Am. J. Respir. Crit. Care Med.* 1998; **157**: 256–62.
- 24 Colavita AM, Hastie AT, Musani AI *et al.* Kinetics of IL-10 production after segmental antigen challenge of atopic asthmatic subjects. *J. Allergy Clin. Immunol.* 2000; **106**: 880–6.
- 25 Magnan A, van Pee D, Bongrand P, Vervloet D. Alveolar macrophage interleukin (IL) -10 and IL-12 production in atopic asthma. *Allergy* 1998; **53**: 1092–5.
- 26 Robinson DS, Tsicopoulos A, Meng Q, Durham S, Kay AB, Hamid Q. Increased interleukin-10 messenger RNA expression in atopic allergy and asthma. *Am. J. Respir. Cell Mol. Biol.* 1996; **14**: 113–17.
- 27 Grunig G, Corry DB, Leach MW, Seymour BW, Kurup VP, Rennick DM. Interleukin-10 is a natural suppressor of cytokine production and inflammation in a murine model of allergic bronchopulmonary aspergillosis. *J. Exp. Med.* 1997; **185**: 1089–99.
- 28 Justice JP, Shibata Y, Sur S, Mustafa J, Fan M, Van Scott MR. IL-10 gene knockout attenuates allergen-induced airway hyperresponsiveness in C57BL/6 mice. *Am. J. Physiol. Lung Cell. Mol. Physiol.* 2001; **280**: L363–8.
- 29 Makela MJ, Kanehiro A, Borish L *et al.* IL-10 is necessary for the expression of airway hyperresponsiveness but not pulmonary inflammation after allergic sensitisation. *Proc. Natl. Acad. Sci. USA* 2000; **97**: 6007–12.
- 30 Tournoy KG, Kips JC, Pauwels RA. Endogenous interleukin-10 suppresses allergen-induced airway inflammation and nonspecific airway responsiveness. *Clin. Exp. Allergy* 2000; **30**: 775–83.
- 31 Yang X, Wang S, Fan Y, Han X. IL-10 deficiency prevents IL-5 overproduction and eosinophilic inflammation in a murine model of asthma-like reaction. *Eur. J. Immunol.* 2000; **30**: 382–91.
- 32 Kibe A, Inoue H, Fukuyama S *et al.* Differential regulation by glucocorticoid of interleukin-13-induced eosinophilia, hyperresponsiveness, and goblet cell hyperplasia in mouse airways. *Am. J. Respir. Crit. Care Med.* 2003; **167**: 50–6.
- 33 Winterrowd GE, Chin JE. Flow cytometric detection of antigen-specific cytokine responses in lung T cells in a murine model of pulmonary inflammation. *J. Immunol. Meth.* 1999; **226**: 105–18.
- 34 Salez L, Singer M, Balloy V, Creminon C, Chignard M. Lack of IL-10 synthesis by murine alveolar macrophages upon lipopolysaccharide exposure. Comparison with peritoneal macrophages. *J. Leukoc. Biol.* 2000; **67**: 545–52.
- 35 Zuany-Amorim C, Haile S, Leduc DB *et al.* Interleukin-10 inhibits antigen-induced cellular recruitment into the airways of sensitized mice. *J. Clin. Invest.* 1995; **95**: 2644–51.
- 36 van Scott MR, Justice JP, Bradfield JF, Enright E, Sigounas A, Sur S. IL-10 reduces Th2 cytokine production and eosinophilia but augments airway reactivity in allergic mice. *Am. J. Physiol. Lung Cell. Mol. Physiol.* 2000; **278**: L667–74.

- 37 Stampfli MR, Cwiartka M, Gajewska BU *et al.* Interleukin-10 gene transfer to the airway regulates allergic mucosal sensitization in mice. *Am. J. Respir. Cell Mol. Biol.* 1999; **21**: 586–96.
- 38 Kalinski P, Hilkens CM, Wierenga EA, Kapsenberg ML. T-cell priming by type-1 and type-2 polarized dendritic cells: the concept of a third signal. *Immunol. Today* 1999; **20**: 561–7.
- 39 Laouini D, Alenius H, Bryce P, Oettgen H, Tsitsikov E, Geha RS. IL-10 is critical for Th2 responses in a murine model of allergic dermatitis. *J. Clin. Invest.* 2003; **112**: 1058–66.
- 40 Grunstein MM, Hakonarson H, Leiter J *et al.* Autocrine signaling by IL-10 mediates altered responsiveness of atopic sensitized airway smooth muscle. *Am. J. Physiol. Lung Cell. Mol. Physiol.* 2001; **281**: L1130–7.
- 41 Oh JW, Seroogy CM, Meyer EH *et al.* CD4 T-helper cells engineered to produce IL-10 prevent allergen-induced airway hyperreactivity and inflammation. *J. Allergy Clin. Immunol.* 2002; **110**: 460–8.
- 42 Justice JP, Borchers MT, Crosby JR *et al.* Ablation of eosinophils leads to a reduction of allergen-induced pulmonary pathology. *Am. J. Physiol. Lung Cell Mol. Physiol.* 2003; **284**: L169–78.
- 43 Shen HH, Ochkur SI, McGarry MP *et al.* A causative relationship exists between eosinophils and the development of allergic pulmonary pathologies in the mouse. *J. Immunol.* 2003; **170**: 3296–305.