

Effects of post-inhalation treatment with interleukin-12 on airway hyper-reactivity, eosinophilia and interleukin-18 receptor expression in a mouse model of asthma

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Summary

Background Correcting Th1/Th2 imbalance with administration of IL-12 before and during antigen challenge holds therapeutic promise in asthma. However, the effects of IL-12 on the established asthmatic responses have not fully been examined.

Objective We investigated whether IL-12 administered after antigen challenge could diminish airway hyper-reactivity (AHR) and eosinophilia in mice actively sensitized to ovalbumin. We also have investigated the ability of administered IL-12 to induce IL-18 receptor (IL-18R) expression that may lead possible synergic action of IL-12 with endogenous IL-18.

Methods C57BL/6 mice immunized to ovalbumin (OVA) by intraperitoneal (i.p.) injection, were challenged three times with an aerosol of OVA every second day for 8 days. Recombinant IL-12 (500 ng) was intravenously administered on a single occasion 1 h after the final challenge of mice. Mice were analysed for effects of IL-12 on AHR, inflammatory cell infiltration and cytokine levels in lung tissue as well as serum immunoglobulin (Ig) E levels. Immunohistochemistry for IL-18R was performed using rat monoclonal antibody specific for murine IL-18R α (IL-1 receptor related protein; IL-1R ρ).

Results An intravenous IL-12 administration diminished AHR, pulmonary eosinophilia and T lymphocyte infiltration, serum IgE, IL-4 and IL-13 in lung tissue. Expression of IL-18R was induced in the mononuclear cells in the lung of mice exposed to OVA. IL-12 administration enhanced the IL-18R expression compared with the control.

Conclusion These data indicate that IL-12 can attenuate established antigen-induced AHR and inflammation. In this mechanism it would be interpreted as follows: IL-12 administration in OVA-challenged mice decreased IL-4 production and IgE production thereafter through direct effect on inhibiting the activation of established Th2 cells response and also combined effect with up-regulation of IL-18R expression by inflammatory cells in the lung.

Keywords bronchial asthma, cytokines, interferon- γ interleukin-12, interleukin-18 receptor, Th1/Th2 balance

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Introduction

Allergic asthma is characterized clinically by chronic mucosal airway inflammation, airway hyper-reactivity (AHR) and elevated serum IgE. Various studies have demonstrated that agents decreasing Th2 cytokines or increasing production of Th1 cytokines can prevent airway inflammation and development of AHR [1,2]. IL-12, produced by macrophages, is a Key cytokine promoting Th1-type cell-mediated immunity [3]. IL-12 upregulates production of Th1-type cytokines, such as IFN- γ by human natural killer and T cells, promotes development of Th1 cells and inhibits expression of Th2 cytokines *in vivo*. The ability of IL-12 to inhibit allergic inflammatory reactions in the airways has been studied by several investigators in murine

models of asthma. Administration of recombinant murine IL-12 before and during the period of allergen challenge prevented antigen-induced changes such as airway eosinophilia, AHR and production of ovalbumin-specific IgE [4–7]. The studies mentioned above focused on the ability of IL-12 to prevent development of Th2-dominated immune responses, which may lead to improved allergen immunotherapy. However, whether this cytokine is effective in reversing established allergen-induced changes when given to mice after challenge has not fully been investigated. In addition, multiple injections of IL-12 given either before or during the period of antigen challenge in mice [5–7] may cause such severe adverse effects as hepatomegaly, splenomegaly, leukopenia, pulmonary oedema and/or skeletal muscle degeneration in mice [8]. In this study, we assessed the effects in mice sensitized to OVA of a single post-OVA-challenge administration of IL-12 on AHR, airway eosinophilia and production of IgE and cytokines. Since an *in vitro* study has reported that IL-12 up-regulates IL-18 receptor expression on T cells, Th1 cells and B cells [9], we also examined whether the

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treatment with IL-12 also could enhance IL-18R expression in the lung in view of attenuating pulmonary inflammation.

Materials and methods

Animals

Specific pathogen-free male C57BL/6 mice, 6 to 8 weeks old, were purchased from Charles River Laboratories (Atsugi, Japan). They were kept in standard animal housing facilities and given free access to tap water and OVA-free rodent chow.

Immunization and challenge exposure of mice

Immunization and subsequent challenge exposures in mice were carried out according to the method of Foster et al. [10]. C57BL/6 mice were immunized to OVA (Sigma Chemicals, St Louis, MO, USA) by intraperitoneal (i.p.) injection of 50 µg of OVA adsorbed to 1 mg of alum on days 0 and 12. On day 24, mice were exposed three times to an aerosol of OVA (10 mg/mL) in 0.9% saline for 30 min with an ultrasonic nebulizer, and then every second day thereafter for 6 days. Control mice were injected with 0.5 mL of sterile saline and then exposed to aerosolized sterile saline using similar equipment and schedules.

Study design

OVA-sensitized C57B/6 mice were divided in two groups: mice receiving an OVA challenge and mice receiving a saline challenge. One hour after the last challenge with OVA (on day 30) animals were treated with a single injection via the tail vein of murine recombinant IL-12 (rIL-12; 5, 50 and 500 ng in 0.25 mL of saline based on the experiments by Hofstra et al. [11]; IL-12-treated group). Control animals were treated with 0.25 mL of saline (vehicle-treated group). Mice receiving a saline challenge were treated with vehicle or rIL-12. On day 31, at 24 h after the last OVA or saline inhalation, numbers of inflammatory cells in bronchoalveolar lavage (BAL) fluid were analysed (eight animals per group). Airway responsiveness was determined in animals treated with 500 ng of rIL-12, animals treated with vehicle after OVA challenge, and vehicle-treated animals after saline challenge (eight animals per group). Similarly, in the animals treated with 500 ng of IL-12 and with vehicle after OVA challenge and the vehicle-treated, saline-challenged animals (eight animals per group), the blood was collected in a tube to measure serum IgE, after which lungs were removed for histological and cytokine analyses.

Each experimental group consisted of at least eight mice.

Airway responsiveness measurements

Mice were anaesthetized with pentobarbital (50 mg/kg i.p.) and a small polyethylene catheter (PE10) was inserted into the jugular vein. The trachea was cannulated and connected to a rodent ventilator (number 683; Harvard Apparatus, South Natick, MA, USA) with an in-line pressure transducer (DP45-28; Validyne Engineering, Northridge, CA, USA) that was coupled to a pulmonary mechanics analyser (number 6; Buxco Electronics, Sharon, CT, USA). Mice were ventilated at 120 strokes/min with a stroke volume of 0.3 mL after neuromuscular blockade with gallamine triethiodide (10 mg/kg i.v.). After establishment of a stable baseline in airway resistance,

acetylcholine (ACh) (Daiichi Pharmaceutical, Tokyo, Japan) was administered (62.5–2000 µg/kg) via the jugular vein and changes in airway resistance were monitored.

Bronchoalveolar lavage

Lungs were lavaged six times with 0.5-mL aliquot of phosphate buffered saline (PBS) supplemented with 0.05 mM sodium EDTA (Sigma Chemical) and 0.1% bovine serum albumin (Sigma Chemical) per wash. The lavage fluid was centrifuged (55.32 g for 10 min at 4 °C), and the cell pellet was resuspended in normal saline after a brief hypotonic exposure to lyse red blood cells. A total cell count was performed in a fresh BAL fluid specimen using a haemocytometer. Differential cell counts were obtained from cytologic preparations of BAL fluid. The slides were prepared using a cytospin (Cytospin2; Cytospin, Shandon, UK) and stained with Diff-Quik (Baxter, McGraw Park, IL, USA). Percentages of cell types were determined based on light microscopic evaluation of at least 500 cells/slide.

Lung histology

After the lungs were excized and frozen quickly using dry ice, 4-µm-thick sections were cut on a cryostat and thaw-mounted on gelatinized glass slides. Lung sections were fixed in 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4) and then were subjected to Giemsa staining. For immunohistochemical analyses of CD4⁺ T cells that had migrated into the lung, sections were fixed with acetone at room temperature (20–25 °C) for 5 min. All sections were treated with 0.3% H₂O₂ in methanol overnight at room temperature to quench the endogenous peroxidase. Immunostaining was performed overnight at 4 °C with rat antimouse CD4 antibody (L3T4; Pharmingen, San Diego, CA, USA) diluted 1 : 200 with 1% NGS in PBS. After incubation with secondary biotinylated antibody (goat anti-rat IgG; Tago, Camarillo, CA, USA) diluted 1 : 500 with 1% NGS in PBS, immunoreactivity was detected by an avidin-biotin peroxidase complex technique (Vectastain ABC Kit, Vector, Burlingame, CA, USA). Specificity of each antibody was confirmed by replacement of primary antibodies with non-immunized rat IgG2a,k (Clone, G155-178). Eosinophils and CD4⁺ T cells were counted in all medium-sized airways present in each section using a light microscope, and image analysis was performed (Interaktive Build-Analyse System, Kontron-Bildanalyse, Karl Zeiss, Germany). These counts reflected the number of cells per length of the bronchial basement membrane (B.M.) in medium-sized airways.

Detection of IL-18 receptor

Immunohistochemistry for IL-18 receptor (IL-18R) was performed using rat monoclonal antibody specific for murine IL-18Rα (IL-1 receptor related protein; IL-1Rrp) [9,12,13]. After fixation of lung sections in acetone, immunoreactivity was detected by an avidin-biotin peroxidase complex technique as described above. To confirm specificity of staining for IL-18R in lung, immunohistochemistry for IL-18R was performed in IL-18R-deficient mice recently produced in our laboratory [14]. Thymocytes from C57BL/6 mice stained positively but thymic cells from IL-18R-deficient mice did not stain. Specificity of the antibody also was confirmed by replacement of primary antibodies with non-immunized rat IgG2a,k. No immunostaining was observed in controls.

Quantification of cytokines in lung tissue supernatants

After blood sampling, lungs were removed, snap-frozen in liquid nitrogen and stored at -80°C . The lungs then were homogenized with hypotonic lysis buffer. In lysis buffer the lung tissue was disrupted with 10 strokes of a Wheaton Dounce homogenizer. Homogenates were centrifuged at $15000g$ for 10 min at 4°C . Supernatants were stored at -80°C until each measurement. Protein concentrations of the extracts were adjusted to 1 mg/mL after measurement by Bradford's method (Protein Assay Kit; Bio-Rad Laboratories, Richmond, VA, USA). Tissue supernatant was assayed for protein levels of IL-4, IL-5, IL-13 and IFN- γ by commercially available ELISA kits (Amersham, Buckinghamshire, UK; except IL-13, R & D Systems, Minneapolis, MN, USA). Secondary antibodies were conjugated with horseradish peroxidase. Subtractive readings at 550 nm and 450 nm were converted to pg/mL using values for known concentrations of recombinant IL-4, IL-5, IL-13 and IFN- γ . Limits of detection were less than 5 pg/mL for all cytokine assays except IFN- γ assay, where the limit was 15 pg/mL .

Measurement of serum IgE

Blood was collected in a tube to measure serum IgE. Serum IgE was detected by ELISA as previously reported [12,15]. Sera were obtained from mice 24 h after challenge with either saline or OVA.

Statistical analysis

The data are presented as the mean $\pm$ SE. Statistical comparisons were performed using one-way ANOVA followed by Fischer's least significant difference test. Statistical significance was accepted at the 5% level of confidence.

Results

BAL fluid

In the C57BL/6 mouse model of asthma studied here, the major cellular components of BAL fluid are macrophages and eosinophils, with very few cells of other types. Numbers of eosinophils in BAL fluid increased significantly in the vehicle-treated group in OVA-challenged mice compared with the saline-challenged, vehicle-treated group. Treatment with IL-12 resulted in a decrease in eosinophils in OVA-challenged mice in a dose-dependent manner. The number of eosinophils was significantly decreased when 500 ng of IL-12 were administered after OVA challenge (Fig. 1). Therefore, the following experiment was carried out in mice treated with 500 ng of IL-12. Numbers of other inflammatory cells were not significantly affected by the treatment in OVA-challenged mice, except that IL-12 treatment slightly increased the number of macrophages.

Airway hyper-reactivity

No significant differences in airway response were observed between saline-challenged mice in different treatment groups. In OVA-challenged animals, a single administration of IL-12 caused a significant inhibition of the antigen-induced AHR compared with vehicle administration. However, antigen-induced AHR in mice treated with IL-12 still was significantly higher than that in saline-challenged controls treated with vehicle (Fig. 2).

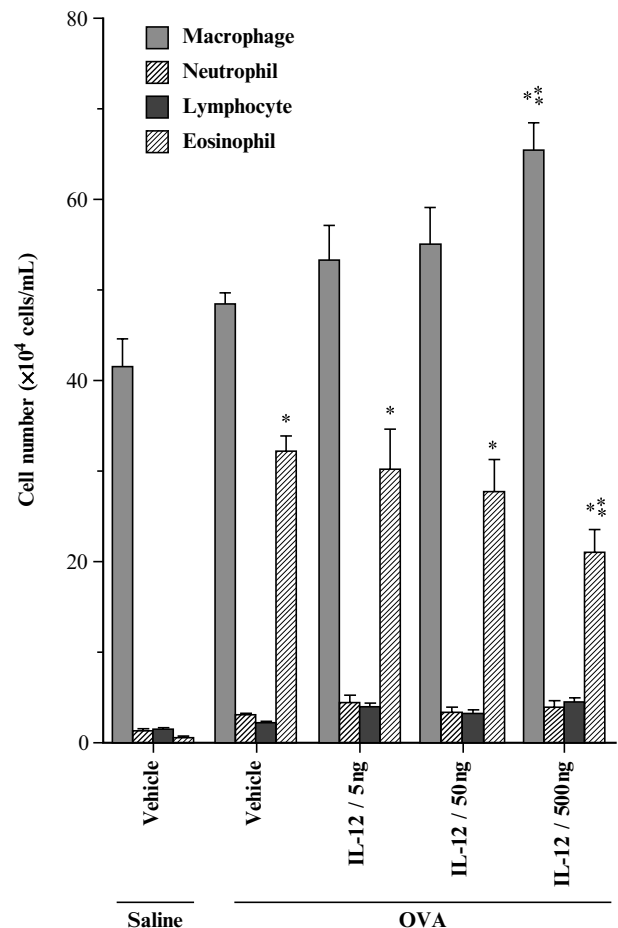


Fig. 1 Effect of recombinant IL-12 on numbers of BAL inflammatory cells of mice. Mice received 5, 50 or 500 ng of IL-12 1 day after the final challenge with antigen. Values shown are mean and SE of eight mice per group. Saline-challenged mice were injected with vehicle, and antigen-challenged mice were injected with vehicle or IL-12. * $P < 0.05$ compared with saline-challenged, vehicle-treated group. † $P < 0.05$ compared with antigen-challenged, vehicle-treated group.

Lung histology

We examined histology of the lungs (Fig. 3) and analysed cells infiltrating into lung tissue on the same specimen (Figs 4 and 5). Lung sections from the vehicle-treated group are characterized by gross alteration in the structural integrity of the airway walls, epithelial cell shedding and extensive mucosal oedema, and the infiltration of inflammatory cells (Fig. 3b). Lung sections from the IL-12-treated group showed a decrease in inflammatory cells (Fig. 3c). The OVA-challenged vehicle-treated group showed a marked increase in eosinophils (Fig. 4b) and a slight increase in CD4^+ T cells in infiltrates (Fig. 4e). A single administration of IL-12 caused a significant inhibition of antigen-induced eosinophilia (Figs 4c and 5a) ($P < 0.01$) and CD4^+ T lymphocytes infiltrate (Figs 4f and 5b) ($P < 0.01$).

IL-18 receptor expression

Immunohistochemistry for IL-18R revealed that only a few IL-18R-staining cells were present in the lungs of the control group (Fig. 4g). In the OVA-challenged, vehicle-treated group, IL-18R immunoreactivity was observed in some mononuclear

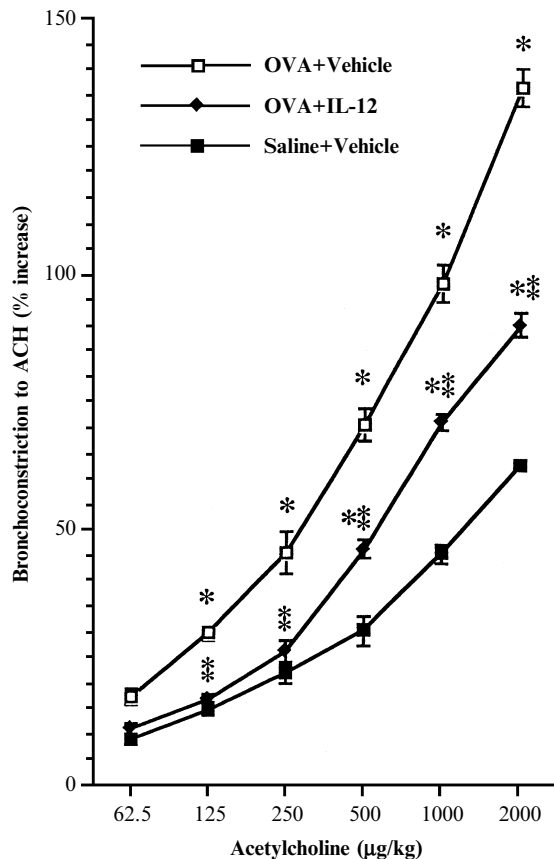


Fig. 2. Effect of recombinant IL-12 on airway responsiveness to i.v. acetylcholine in mice 1 day after the final challenge with antigen. Values shown are mean and SE of eight mice per group. Saline-challenged mice were injected with vehicle, and antigen-challenged mice were injected with vehicle or IL-12 (500 ng). * $P < 0.05$ compared with saline-challenged, vehicle-treated group. ** $P < 0.05$ compared with antigen-challenged, vehicle-treated group.

cells infiltrating around airways and vessels (Fig. 4h). The number of IL-18R-expressing mononuclear cells in lung tissues was significantly increased (fivefold) in the IL-12-treated group (Figs 4i and 5c).

Cytokine expression

Administration of IL-12 to saline-challenged mice caused a slight but significant increase in IFN- γ in the lung. IL-12 reduced IL-4 and IL-13 but these changes in saline-challenged mice were not significant compared with vehicle-treated animals. IL-5 did not change notably in different treatment groups in saline-challenged mice.

After exposure to OVA, in parallel with eosinophil infiltration, lung tissues obtained from vehicle-treated mice contained significantly increased amounts of Th2-type cytokines IL-4, IL-5 and IL-13, compared with corresponding measurements in saline-challenged mice. The amount of IFN- γ was not altered in mice even after exposure to OVA, suggesting a selective induction of a Th2-like cytokine profile in the lungs of OVA-challenged mice. In antigen-challenged mice, a single dose of IL-12 completely suppressed IL-4 and IL-13 ($P < 0.01$, Figs 6a and c). IL-12 increased IFN- γ significantly, but did not affect IL-5 in antigen-challenged mice (Figs 6b and d).

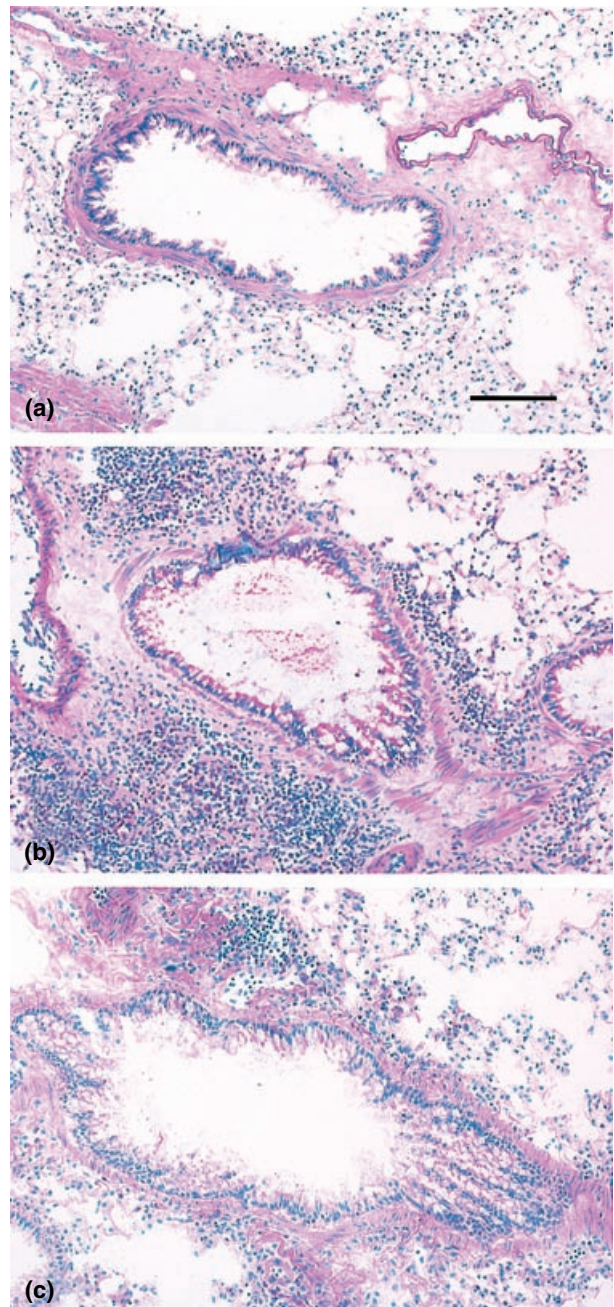


Fig. 3. Histology of lung sections from antigen-challenged mice. Control saline-challenged mice were injected with vehicle(a), and antigen-challenged mice were injected with vehicle (b) or IL-12 (500 ng) (c) 1 h after the final challenge. Lung sections were subjected to Giemsa staining. Lung sections from the IL-12-treated group still show morphologic changes in pulmonary structure. The degree of peribronchial-perivascular inflammatory infiltrate, however, is significantly less in the IL-12-treated mouse than in the vehicle-treated mouse. Scale bar = 100 μ m.

Serum IgE levels

Only a very small amount of IgE was detected in saline-challenged mice of the different treatment groups. In antigen-sensitized and antigen-challenged animals, a single administration of IL-12 caused a significant inhibition of the

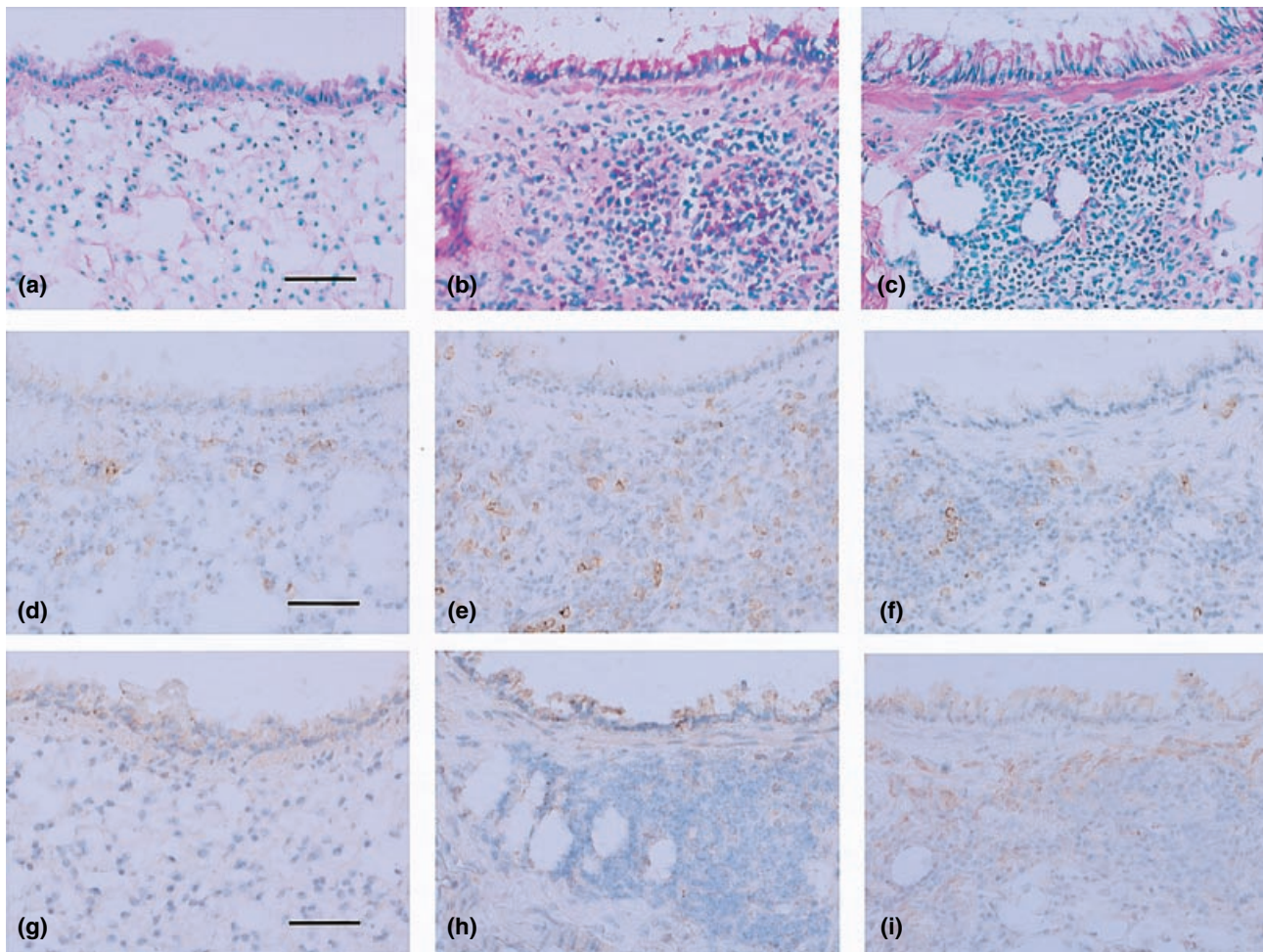


Fig. 4. Histology of lung sections from antigen-challenged mice. Control saline-challenged mice were injected with vehicle (a, d and g), and antigen-challenged mice were injected with vehicle (b, e and h) or IL-12 (500 ng) (c, f and i) 1 h after the final challenge. Lung sections were subjected to Giemsa staining (a, b and c), immunostaining for CD4⁺ T cells (d, e and f) and immunostaining for IL-18 receptor (g, h and i). Degree of eosinophilia and T cell infiltrate is less in the IL-12-treated group than in the vehicle-treated group. The IL-12-treated group showed the increased number of IL-18R-expressing mononuclear cells (i) compared with the vehicle-treated group (h). Scale bar = 50 μ m.

antigen-induced increase in serum IgE (Fig. 7) compared with vehicle administration. However, IgE still was significantly higher ($P < 0.05$) in the IL-12-treated group than in saline-challenged, vehicle treated animals.

Discussion

In this study, repeated OVA inhalation induced IgE production, AHR and infiltration of eosinophils and CD4⁺ T cells into the lung, accompanied by a Th2 pattern of cytokine expression. Our previous studies have shown that such an inflammatory state has been already observed at 3 h and persists for at least 7 days after the last challenge [12,15,16]. Therefore, it is supposed that allergen-induced inflammation has been established in this asthma model before the administration of the recombinant IL-12 or vehicle. Most previous studies in which cytokines were administered during active immunization or during antigen challenge have focused on the prevention effects of allergic responses [1,2,4–7]. However, asthmatic patients whose allergic responses have fully developed then might be treated with these agents. Thus, we investigated whether exogenous recombinant

IL-12 was effective when given after a presumably established inflammatory state. Preliminary studies have shown that exogenous recombinant IL-12 can increase in amount of IL-12 in lung tissue 1 day after administration (data not shown), so that the cytokine would be able to affect inflammatory cells located in the airways as well as circulating immune cells.

In this study we demonstrated that a single intravenous administration of IL-12 1 h after the final antigen challenge partially blocked airway symptoms concurrent with suppression of local IL-4 and IL-13 expression as well as serum IgE. Although the current study did not confirm the beneficial effects of IL-12, especially at the later time-point of the treatment, these results suggest that IL-12 can reverse asthmatic responses by inhibiting production of Th2-type cytokines such as IL-4 and IL-13. These data also showed that amelioration of AHR and eosinophilia by IL-12 was not in conjunction with suppression of increases in IL-5 production. Previous investigations have shown that administration of IL-12 can inhibit both IL-4 and IL-5 production in antigen-challenged mice [4]. *In vitro* antigen-induced production of IL-4 and IL-5 by splenic cells from antigen-challenged mice was also blocked by pre-treatment with IL-12 [7]. However, production of IL-5 by thoracic lymph node cells

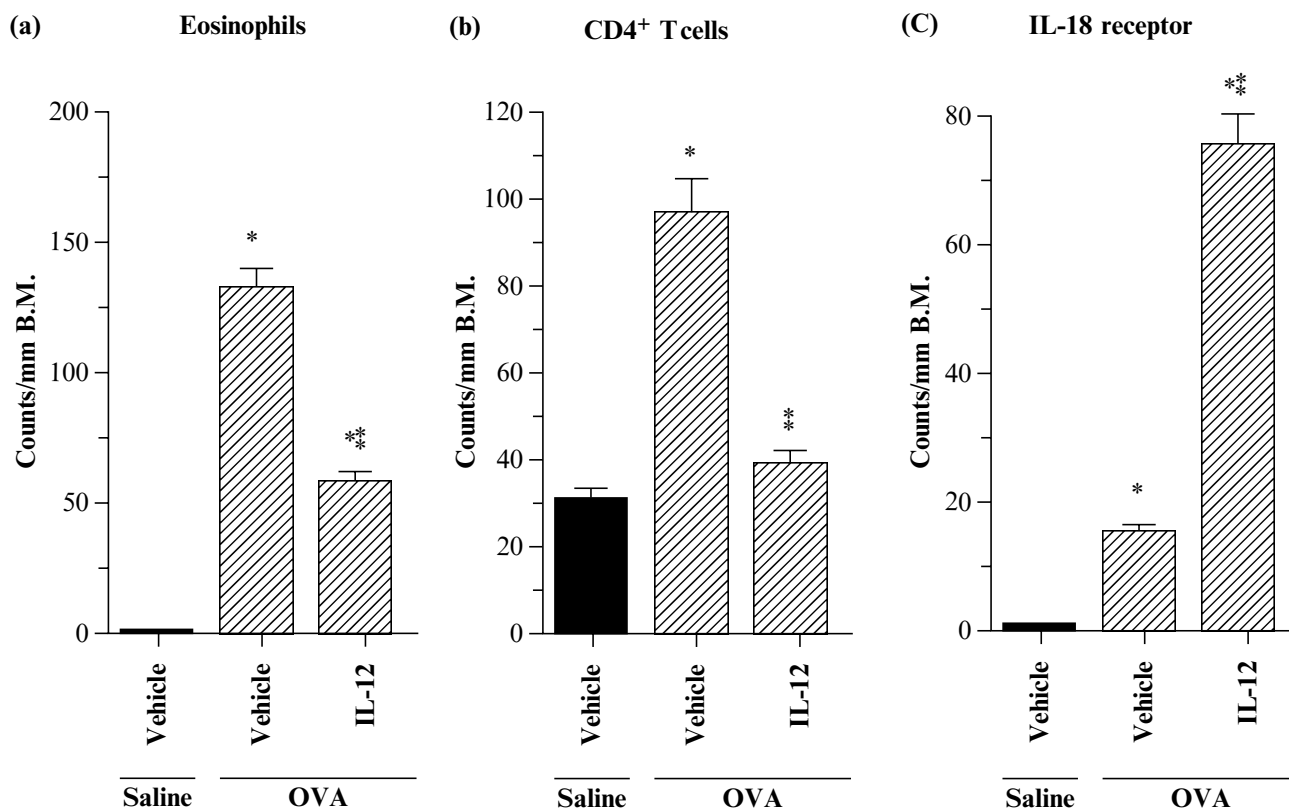


Fig. 5. Effect of recombinant IL-12 (500 ng) on numbers of (a) eosinophils, (b) CD4⁺ T cells and (c) IL-18 receptor expressing cells infiltrating in lung tissue from mice 1 day after the final challenge with antigen. The number of cells per length of the bronchial basement membrane (B.M.) in medium-sized airways was shown. Values shown are mean and SE of eight mice per group. * $P < 0.01$ compared with saline-challenged, vehicle-treated group. ** $P < 0.01$ compared with antigen-challenged, vehicle-treated group.

from antigen-challenged mice was increased by IL-12 [11]. The amount of IL-5 in lung extract of this study showed that IL-12 administration did not suppress the IL-5 production in OVA-challenged mice. Thus, the effects of IL-12 on IL-5 production by lymphocytes are incompletely understood and possibly indirect. It is suggested that IL-5 is not absolutely required in a murine model of acute AHR and pulmonary eosinophilia.

In contrast to IL-5, suppression of IL-4 and IL-13 by IL-12 treatment is particularly important because IL-4 is a key regulator favouring development of a Th2 phenotype, while IL-13 is important for the induction of AHR [17,18]. As these two cytokines induce similar physiological responses through their shared use of Stat6-mediated signalling by the IL4R α subunit [18], the effects of depleting both IL-4 and IL-13 on allergic responses are likely to be similar to those of Stat6 deficiency [15, 19]. However, some distinct effects are mediated by the individual cytokines [20]. For example, IL-13 recently was found to modulate acute inflammatory responses by suppressing activation of NF- κ B [21], a transcription factor that is known to play a role in the expression of IL-5, eotaxin and eosinophilic inflammation in the lung independently of IL-4 [22]. These reports suggest the presence of IL-4- or IL-4R α -independent processes regulating production of IL-5. Our previous study demonstrated that Stat6-deficient mice still produced a substantial amount of IL-5 after antigen challenge, but they did not express IL-4 [15]. Taken in this context, the results of this study suggest that IL-12 can block AHR and eosinophilia by

suppressing IL-4 and IL-13 production without affecting IL-5 production. However, compared with the suppression of these cytokines, the suppressive effect of IL-12 on airway symptoms is less and only partially observed. AHR is associated with remodelling of the airway structure [23]. Accordingly the current data may suggest that airway symptoms such as AHR can occur independently of cytokine content in the tissue.

The mechanism for the suppression of IgE production by IL-12 has been poorly understood. *In vitro* studies have proposed both IFN- γ -dependent [23] and IFN- γ -independent mechanisms [24]. In mouse models of asthma conflicting data have been reported concerning the role of IL-12 in secondary IgE responses [25]. Treatment with IL-12 before the challenge period can suppress IgE production [5,11] but unaltered IgE concentrations have been reported after treatment with IL-12 during the challenge period [5,6,11]. Additional direct inhibitory effects of IL-12 on IgE-dependent activation of inflammatory cells, including mast cells, have been proposed [6]. The current study indicated that IL-12 administered after antigen challenge could inhibit up-regulation of IgE, probably through reduction of IL-4 expression, increased IFN- γ expression or an additional clearance mechanism.

IL-18 is widespread and is produced by many cell types, including macrophages [26]. It strongly augments IFN- γ production by T cells in combination with IL-12. However, recent reports have shown the pro-inflammatory aspect of IL-18, inducing production of IL-13, TNF- α or GM-CSF, especially

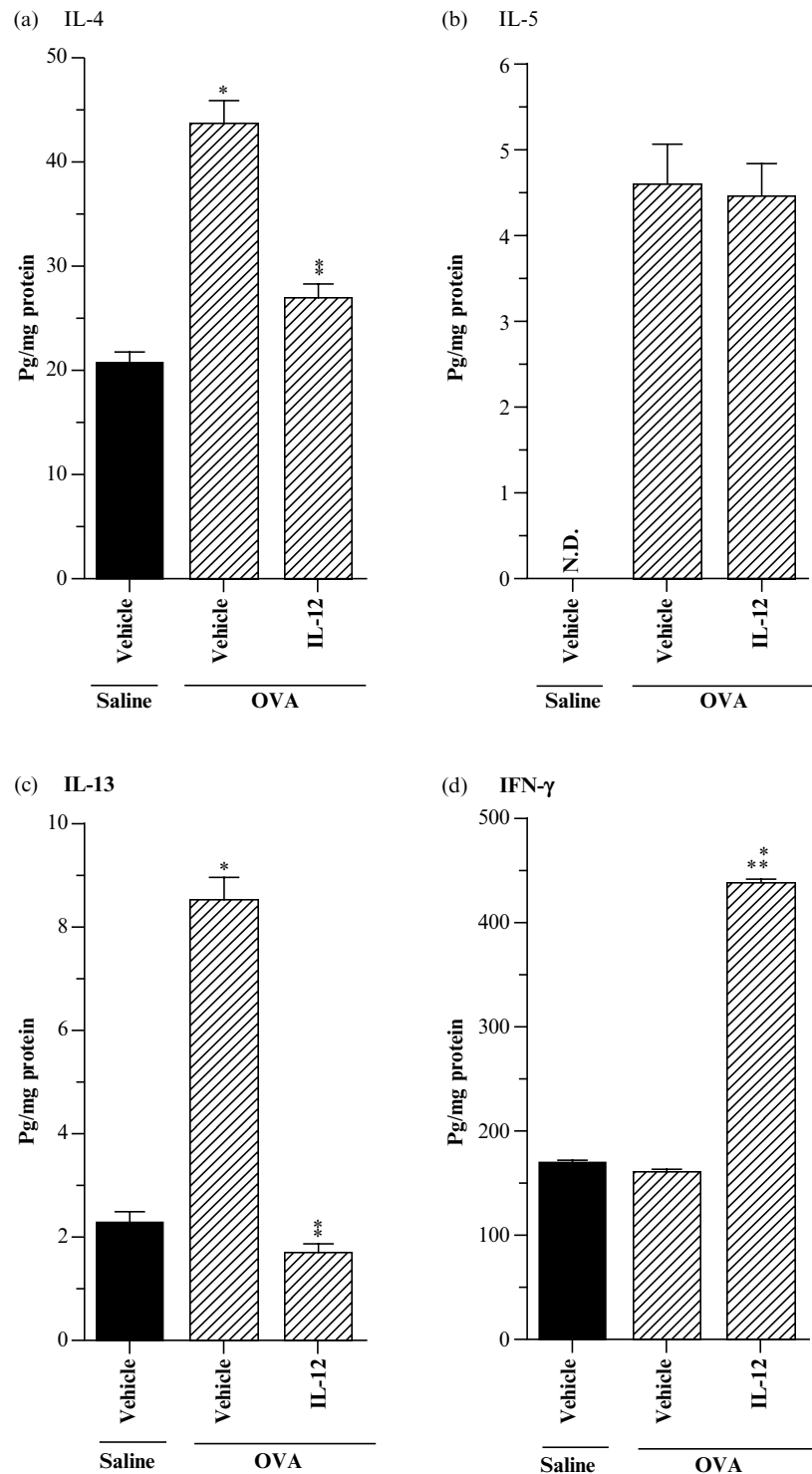


Fig. 6. Effects of recombinant IL-12 (500 ng) on IL-4 (a), IL-5 (b), IL-13 (c) and IFN- γ (d) in lung tissue supernatants of mice 1 day after the final challenge with antigen. Values shown are mean and SE of eight mice per group. Saline-challenged mice were injected with vehicle, and antigen-challenged mice were injected with vehicle and IL-12 (500 ng). * $P < 0.01$, compared with saline-challenged, vehicle-treated group. ** $P < 0.01$, compared with antigen-challenged, vehicle-treated group.

when it functions on the CD4⁺ T cells without IL-12 [27]. These findings suggest that the discrete and multiple functions of IL-18 probably depend on the IL-12 content in the tissue. A recent paper has suggested that IL-18 has an apoptotic effect on tumour cells through a Fas-dependent pathway [28]. The effects of exogenous IL-18 administration on pulmonary inflammation also are in controversy in the asthma model. Recombinant

murine IL-18 was reported to enhance antigen-induced eosinophil recruitment into airways when given before antigen challenge, while IL-18 decreased pulmonary eosinophilia when given during challenge or given in combination with IL-12 [11,29].

The immunostaining of lung tissue in this study identified IL-18R-positive lymphocytes and macrophages after antigen

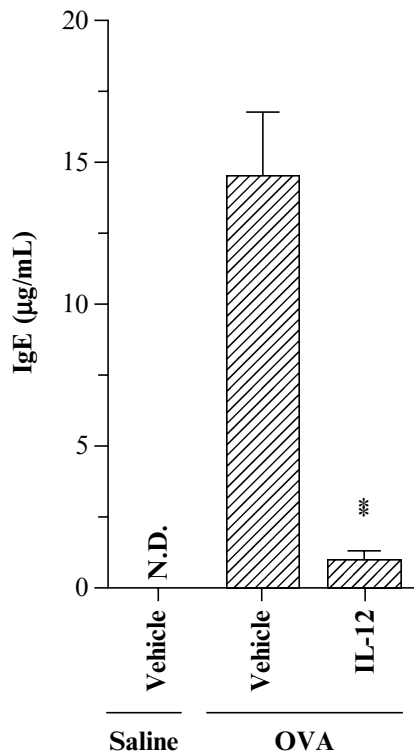


Fig. 7. Effects of recombinant IL-12 (500 ng) on serum IgE in mice 1 day after the final challenge with antigen. Saline-challenged mice were injected with vehicle, and antigen-challenged mice were injected with vehicle and IL-12. Values shown are mean and SE of eight mice per group. ** $P < 0.01$ compared with antigen challenged, vehicle-treated group.

challenge in wild-type mice. This finding indicates that after antigen challenge IL-18R was expressed in the airway, where it could regulate the antigen-induced lung inflammatory response. IL-18 has been reported to show no direct stimulation to Th2 cells [30,31] because Th2 cells do not express the IL-18R [32]. However, under some conditions, such as the presence of IL-12, it can stimulate T cells expressing the IL-18R to produce a Th2 cytokine [33,34]. NK cells also may mediate effects of IL-18, as these cells express IL-18R [35] and produce IL-13, IL-5 or IL-4 [34,36,37]. In addition, IL-18 administration to animals unexposed to antigen has been reported to increase total serum IgE, suggesting that IL-18 can stimulate IgE production in the absence of a protein immunogen [38]; however, IL-18 alone showed no effect on IgE production in purified splenic B cells [39]. These findings also suggest that when IL-12 was administered the endogenous IL-18 could affect migrated cells that express the IL-18R modulating cellular inflammatory responses.

Thus, the effects of IL-12 administration may include the synergism with endogenous IL-18; IL-12 can reverse asthmatic responses by inhibiting production of Th2-type cytokines such as IL-4 and IL-13, while enhancing the action of endogenous IL-18 on T or NK cells by upregulating expression of IL-18R [9,40] may modulate such inflammatory reaction. In conclusion, IL-12 administration is a clinically available therapeutic modality, while regulation of endogenous IL-18 function also is important. Further study is needed on the treatment of asthma with IL-12 to determine the dose and timing of administration.

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