

Administration of Interleukin-12 Prevents Mite Der p 1 Allergen-IgE Antibody Production and Airway Eosinophil Infiltration in an Animal Model of Airway Inflammation

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The aim of the present study was to examine the *in vivo* effect of interleukin (IL)-12 on a murine model of asthma induced by *Dermatophagoides pteronyssinus*-derived Der p 1 allergen. C57BL/6 mice immunized with Der p 1 allergen adsorbed to alum/pertussis toxin developed a T-helper type 2 (Th2)-dominant immune response characterized by the presence of IgE antibody, airway eosinophil infiltration and increased production of Th2 cytokine. Intraperitoneal injection of IL-12 (1 or 0.1 µg per day) for 5 days (day – 1 to + 3) simultaneously with each immunization, inhibited the production of IgE and IgG₁ antigen-specific antibodies, whereas production of IgG_{2a} was strongly enhanced. In addition, mice receiving both doses of IL-12 showed a strong inhibition of IL-5 but up-regulation of IFN-γ production by spleen cells stimulated with antigen. Administration of IL-12 also prevented antigen-induced eosinophil infiltration into the bronchoalveolar area in a dose-dependent manner and the primary inflammatory mediator serotonin in bronchoalveolar lavage (BAL) fluids was also reduced significantly. Taken together, the data indicate that IL-12 has a potent immunomodulatory effect on house-dust-mite-induced allergic disorders and may be used as an efficient agent for immunotherapy.

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INTRODUCTION

The house dust mite is one of the most important inhalant allergens in respiratory disorders such as bronchial asthma and allergic rhinitis [1]. Among many species of mites in house dust, *Dermatophagoides pteronyssinus* (Dp) is reported to be dominant, as many basic and clinical studies have indicated. However, mite extracts are heterogeneous and contain various proteins of different molecular weights, although most of the IgE antibody to Dp is directed against Der p 1 in the sera of mite-allergic subjects. Der p 1, a 25-kDa glycoprotein found in mite faeces, has been purified and shown to be the predominant antigen [2]. Patients allergic to mite antigen have been demonstrated to have elevated serum levels of allergen-specific IgE and local infiltration of inflammatory cells in which the presence of eosinophils is striking. It has been recognized that T-helper type 2 (Th2) cells and their cytokines are responsible for the initiation and

maintenance of allergic disorders [3]. Allergen-specific T cells isolated from atopic patients produce higher levels of the type-2 cytokines interleukin (IL)-4 and IL-5 and a lower level of type-1 cytokine interferon (IFN)-γ [4]. Furthermore, Th1 and Th2 cells have been found to show reciprocal regulation. Thus, agents that decrease IgE levels or Th2 cytokine production, or increase Th1 cytokine production may inhibit allergen-induced disorders.

It has been well documented that activation of naive T cells in the presence of IL-12 promotes differentiation to Th1 cells [5, 6]; conversely, IL-4 promotes Th2 development [7, 8]. IL-12 is a characterized heterodimeric cytokine that plays a primary role in the induction of cell-mediated immune functions. Previous studies have shown that treatment with IL-12 inhibits Th2 cytokines and related antibody production both *in vitro* and *in vivo* [9, 10]. Taken together, the apparent ability of exogenous IL-12, given as a biological adjuvant co-incident with antigen exposure, to promote a Th1-dominant response suggests a

potentially valuable therapeutic strategy that might reduce morbidity and mortality in asthmatic patients.

It has been demonstrated that CBA and C57BL/6 strains of mice are high IgE responders to Der p 1 antigen [11]. Therefore, to determine the potential role of IL-12 in the pathogenesis of allergic diseases, a murine model of airway inflammation induced by Der p 1 allergen has been developed. This model resembles human asthma in that it is characterized by an increase in serum level of antigen-specific IgE, the presence of peribronchial inflammatory infiltrates and an increased level of Th2 cytokine. In the present study, IL-12 treatment during allergic sensitization not only promotes Th1 immune responses but also inhibits airway inflammation. These results indicated that IL-12 might be useful as a therapeutic agent to reduce the duration of immunotherapy or even to prevent the attack of severe allergic diseases.

MATERIALS AND METHODS

Animals. Female, BALB/c and C57BL/6 mice between 6 and 8 weeks of age were obtained from and maintained in the Animal Center of the College of Medicine, National Taiwan University.

Preparation of antigens. The allergen Der p 1 was isolated by affinity column from spent mite media, kindly provided by Dr KY Chua (The National University of Singapore). Firstly, 5 g of the spent mite media was mixed with 100 ml of 0.1 M Tris-HCl (pH 7.6) and stirred overnight at 4°C. The mite extract was collected after centrifugation at 19000 g for 30 min at 4°C and passed through the anti-Der p 1 affinity column. The column was washed with phosphate-buffered saline (PBSA) and then Der p 1 protein was eluted with NH₄OH (pH 11) at 4°C. Immediately, 0.1 M Tris-HCl (pH 6.8) was added to neutralize the eluted fractions. The pooled fractions were dialysed against PBSA and further concentrated. Finally, the concentrated product was monitored at OD 562 nm and stored at -20°C before use.

The lyophilized house dust mite, Dp, was purchased from Allergon (Angelholm, Sweden). The allergen was prepared as described previously [12]. Briefly, 1 g of lyophilized mite body was defatted with 100 ml ether, homogenized and stirred continuously in 25 ml PBSA for 48 h at 4°C. After centrifugation (12000 g, 30 min), the crude extract was dialysed with PBSA and the mite extract dissolved in PBSA and stored at -20°C.

Immunizations and inhalation exposure of mice. To establish an animal model of asthma, BALB/c and C57BL/6 mice were immunized by an intraperitoneal injection of Der p 1 at doses of 1, 5 and 10 µg with either alum alone or alum plus 400 ng pertussis toxin (List Biological Lab. Inc., Campbell, CA, USA) as the adjuvant. Every 2 weeks the mice were boosted with the same dose of Der p 1 and adjuvant. On days 0, 14, 28 and 42, mice were bled from the retro-orbital venous plexus. Sera were collected and tested for the presence of Der p 1-specific antibodies using isotype-specific enzyme-linked immunosorbent assay (ELISA).

To further examine the *in vivo* effects of mouse rIL-12 (R&D, Minneapolis, MN, USA) on this murine model of asthma induced by Der p 1, C57BL/6 mice were immunized by an intraperitoneal injection of 10 µg Der p 1 with 2 mg alum plus 400 ng pertussis toxin as the adjuvant. On days 14 and 28, the mice were boosted with the same dose of Der p 1 and adjuvant. In addition, two groups of mice were treated with different doses IL-12 for 5 days (day -1 to +3) simultaneously with each immunization. IL-12 was administered at dose of 1 µg/mouse/

day or 0.1 µg/mouse/day. Control-sensitized mice received PBSA instead of IL-12. Each group is composed of 8–12 mice. On days 0, 14 and 28, the mice were bled from the retro-orbital venous plexus. Sera were collected and stored at -20°C until analysis. On day 42, all groups of mice were exposed to aerosolized crude mite extract (*Dermatophagoides pteronyssinus*) over a 20-min period, by placing them in a chamber which could hold 6–8 mice concurrently. The aerosols were generated in the chamber using an ultrasonic nebulizer (DeVilbiss, Somerset, PA). The output of the nebulizer was 0.3 ml/min and the produced particles had a size range of 0.5–5 µm. The concentration of crude mite extract in the nebulizer was 0.1% (wt/vol).

Mite-allergen-specific antibody assay. Sera anti-Der p 1 IgE, IgG₁ and IgG_{2a} antibody titres were determined by ELISA. Briefly, 96-well microtitre plates were coated with 4 µg/ml Der p 1 diluted in NaHCO₃ buffer, pH 8.2. After overnight incubation at 4°C, plates were washed and blocked with 3% bovine serum albumin (BSA) in PBSA for 2 h at 37°C. Serum samples were diluted and added to each well overnight at 4°C. Plates were then washed. Either biotin-conjugated antimouse IgE (0.4 µg/ml, Serotec, Raleigh, NC, USA) or IgG₁ (1 : 500, Pharmingen, San Diego, CA, USA) or IgG_{2a} (1 : 500, Pharmingen), diluted in 3% BSA-PBSA buffer, was added for 1 h at 37°C. Streptavidin-conjugated alkaline phosphatase (1 : 2000, Sigma, St Louis, MO, USA) was then added for an additional 2 h at room temperature. Finally, the reaction was developed by phosphatase substrate pNPP and the absorbance determined at 405 nm in a microplate reader. The levels of antibody were compared with IgG₁, IgE and IgG_{2a} standards with predetermined concentrations (immunoglobulin concentrations: IgG₁ = 28.2 µg/ml, IgE = 1.1 µg/ml, IgG_{2a} = 16.7 µg/ml). The concentration of standard serum was arbitrarily assigned 1 ELISA unit (1 EU).

Antigen-specific proliferative assay. To measure the Der p 1-specific T-cell proliferative response, Der p 1-immunized mice were sacrificed and 3×10^5 /well spleen cells were cultured with Der p 1 (10 µg/ml) or phytohaemagglutinin (PHA, 10 µg/ml) or medium only in RPMI-1640 medium containing 2% TCM (mouse serum replacement, Celox Corp., Hopkins, MN) plus 2% fetal calf serum (FCS). Cells were cultured in round bottom 96-well microculture plates at 37°C in 5% CO₂. After 2 days in culture, the cells were pulsed with 1 µCi/well of [³H]TdR for 15–17 h. Specific incorporation of TdR was analysed using a β-counter (Packard Instrument Co., Meriden, CT) and results were expressed as stimulation index (SI).

Cytokines assay. To measure the levels of cytokines, splenocytes (1×10^7 /well) of immunized mice treated with or without IL-12 after inhalation were cultured in 0.5 ml AIM-V medium supplemented with 2% TCM in the presence of 10 µg/ml Der p 1 or PHA (10 µg/ml) in 48-well microtitre plate at 37°C for 48 h. The culture supernatants were collected and centrifuged at 400 g at 4°C. The cell-free supernatants were stored at -20°C until they were used for the cytokine assay. The levels of IL-5 and IFN-γ in the culture supernatants were evaluated by sandwich-ELISA. Briefly, ELISA plates were coated with 2 µg/ml of monoclonal rat antimouse IL-5 (Pharmingen) or rat antimouse IFN-γ (Pharmingen) in NaHCO₃ buffer, pH 9.6 at 4°C overnight and blocked with 2% BSA-PBSA at room temperature for 2 h. The diluted supernatants were added and incubated for 1 h at 37°C. Biotinylated antimouse IL-5 (1 µg/ml, Pharmingen) or IFN-γ (1 µg/ml, Pharmingen) was applied to the plates and incubated at 37°C for 1 h. Avidin-horseradish peroxidase (1 : 1000, Immunopure, Pierce, Rockford, IL, USA) was then added for 30 min at 37°C. After the wells were washed, colour was generated with the addition of substrate 2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid). The absorbance at 420 nm was measured on an ELISA reader. The concentration of cytokines was measured by

converting the OD values of the samples to pg from the standard curve. The levels of sensitivity for the IL-5 and IFN- γ assay were 60 pg/ml and 150 pg/ml, respectively.

Bronchoalveolar lavage and histopathological study. Twenty-four hours after the aerosol exposure, all groups of mice were bled from the retro-orbital venous plexus and then sacrificed. In addition, the naive mice exposed to aerosolized allergen were used as the negative controls. The lung was immediately lavaged via the trachea cannula with 3×1 ml of Hank's balanced salt solution (HBSS), free of ionized calcium and magnesium. The lavage fluid was centrifuged at 400g for 10 min at 4°C. After washing, the cells were resuspended in 1 ml HBSS and total-cell counts were determined by counting in a haemocytometer. Cyto-centrifuged preparations were stained with Liu's stain for differential cell counts. A minimum of 200 cells was counted and classified as macrophages, lymphocytes, neutrophils and eosinophils, based on standard morphologic criteria.

To evaluate the effects of IL-12 on allergen-induced lung inflammation, each group of animals was sacrificed for histopathological examination. After the lavage, the lungs were immediately removed and fixed in sublimite formalin solution. The tissues were subsequently embedded in paraffin and cut into 5- μ m-thick sections. These frozen sections were stained with haematoxylin-eosin and examined for histological changes using light-microscopy changes.

The levels of serotonin in bronchoalveolar lavage fluids. Bronchoalveolar lavage (BAL) fluids were assayed 24 h after the antigen challenge. Animals were sacrificed, and BAL fluids were collected and centrifuged at 400g for 10 min at 4°C. The supernatants were collected and stored at -20°C until assay. Concentrations of serotonin in lavage fluids were measured by serotonin ELISA kit (IBL, Hamburg, Germany) according to the manufacturer's recommendations. Absorbance values, read at 405 nm were converted to concentration in BAL fluids by comparison with a standard curve. The level of sensitivity for serotonin assay was 0.03 ng/ml. Because the sample preparation leads to a 207.25-fold dilution, the values read from the standard curve have to be corrected by multiplying by 207.25.

Statistical analysis. Unpaired Student's *t*-test was used to analyse the data throughout the study. A *P*-value of <0.05 was considered to be significant.

RESULTS

Antibody responses of different mouse strains against Der p 1

To establish an animal model of airway inflammation, many factors involved in the regulation of Th1 and Th2 responses have been taken into consideration, including the route of antigen entry, the dose of antigen, the type of adjuvant and the genetics of mouse strains. Table 1 illustrates the immune responses to the allergen Der p 1 in BALB/c and C57BL/6 mouse strains. The IgG₁ antibody response against Der p 1 allergen was observed in all groups. In general, 10 μ g of antigen without pertussis toxin PT immunization was enough to induce persistent anti-Der p 1 IgG₁ antibody response in both mouse strains. The anti-Der p 1 IgG₁ antibody response was further enhanced significantly in BALB/c mice receiving Der p 1 immunization plus PT. In contrast, the anti-Der p 1 IgG_{2a} responses were poor and there was no difference among groups. Notably, the anti-Der p 1 IgE antibody titre was low except C57BL/6 strain mice in which IgE anti-Der p 1 antibody was significantly enhanced after immunization in the presence of PT. In general, C57BL/6 tended towards a relatively higher and more persistent IgE response to Der p 1 allergen than did BALB/c. Consequently, C57BL/6 strain mice were used in the following experiments.

IL-12 suppresses the synthesis of IgG₁ and IgE and enhances the production of IgG_{2a}

To examine the influence of IL-12 on Der p 1-specific immune responses *in vivo*, two different doses of IL-12 treatment on the murine model of airway inflammation were evaluated. C57BL/6 mice immunized with 10 μ g of the allergen Der p 1 adsorbed to 2 mg alum plus 400 ng PT induced a strong IgG₁ response (Fig. 1A). Furthermore, substantial amounts of IgE

Table 1. The titers of anti-Der p 1 antibodies from mice immunized with different doses of antigen

Strain	Immunization		IgE		IgG ₁		IgG _{2a}	
	Der p 1 (μ g)	PT	Day 0	Day 42	Day 0	Day 42	Day 0	Day 42
BALB/c	1	—	0.02 $\pm$ 0.04	0.08 $\pm$ 0.09	0	0.35 $\pm$ 0.12	0.01 $\pm$ 0.01	0.03 $\pm$ 0.02
	5	—	0	0.09 $\pm$ 0.05	0	1.27 $\pm$ 0.29	0.01 $\pm$ 0.00	0.12 $\pm$ 0.05
	10	—	0	0.04 $\pm$ 0.03	0	1.20 $\pm$ 0.15	0	0.14 $\pm$ 0.08
	10	+	0.01 $\pm$ 0.01	0.11 $\pm$ 0.06	0.01 $\pm$ 0.01	1.88 $\pm$ 0.32**	0.08 $\pm$ 0.02	0.20 $\pm$ 0.05
C57BL/6	1	—	0	0.02 $\pm$ 0.02	0	0.64 $\pm$ 0.42	0	0.09 $\pm$ 0.06
	5	—	0	0.02 $\pm$ 0.01	0	0.70 $\pm$ 0.32	0	0.05 $\pm$ 0.07
	10	—	0	0.02 $\pm$ 0.02	0	1.13 $\pm$ 0.23	0	0
	10	+	0.01 $\pm$ 0.02	0.40 $\pm$ 0.28*	0	1.38 $\pm$ 0.44	0	0.03 $\pm$ 0.03

Eight groups of mice were immunized by intraperitoneal injections of Der p 1 at doses of 1, 5 and 10 μ g adsorbed to either 2 mg alum alone or plus 400 ng pertussis toxin (PT) as the adjuvant. Mice of each group were boosted biweekly with the same doses as priming doses. The mice were bled at days 0, 14, 28 and 42 and serum Ab levels were determined by ELISA. The values represent the mean $\pm$ SD of 5–6 mice per group. **P* < 0.05 and ***P* < 0.005 compared with that of 10 μ g Der p 1 + alum immunized mice.

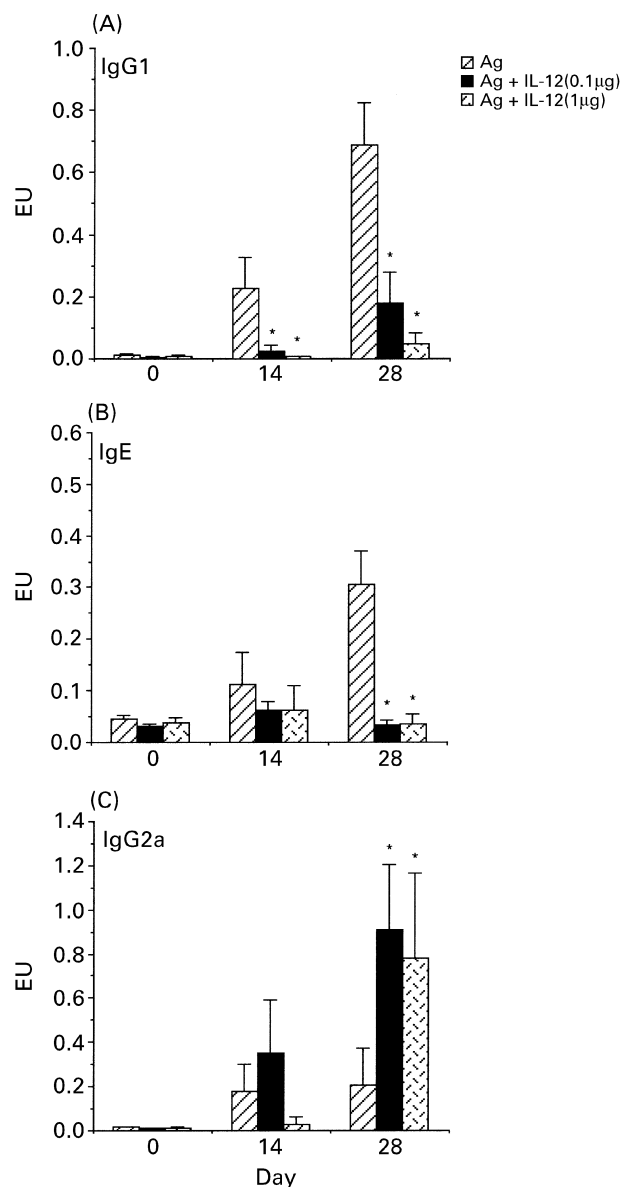


Fig. 1. Antigen-specific antibody isotype pattern in the sera of mice immunized with Der p 1 adsorbed to alum/PT with or without IL-12 treatment. C57BL/6 mice were immunized intraperitoneally three times with 10 µg/mouse Der p 1 adsorbed to alum plus PT every 2 weeks. IL-12 was administered to mice intraperitoneally at 0.1 µg/day or 1 µg/day for 5 days (day -1 to +3) simultaneously with each immunization. Blood was collected on days 0, 14 and 28, and sera levels of anti-Der p 1 antibodies IgG₁ (A), IgE (B) and IgG_{2a} (C) were assayed by using ELISA. Data are shown as mean ± SD for 10 mice per group. Significant differences (**P* < 0.05) from the immunized IL-12-untreated group are indicated.

were produced (Fig. 1B), whereas synthesis of IgG_{2a} was low (Fig. 1C). The data suggested that the Der p 1-specific IgG₁ response on day 28 was suppressed 14-fold or 3.8-fold by a daily dose of 1 µg or 0.1 µg of IL-12. However, the most striking effect of both doses of IL-12 on humoral immune responses is a significant inhibition (8–9-fold) of IgE level on day 28 (Fig. 1B).

In contrast, either dose of IL-12 administration during immunization up-regulated synthesis of IgG_{2a} about four-fold on day 28. Thus, IL-12 clearly promotes the development of characteristic Th1-associated pattern of antibody response in Der p 1-sensitized mice that normally develops in a Th2 manner.

IL-12 inhibits antigen-induced Th2-like cytokine production and increases Th1-like cytokine expression in sensitized mice

Twenty-four hours after the inhalation, splenocytes isolated from immunized mice treated with or without IL-12 were examined for Th-cell proliferative responses by stimulation with Der p 1 *in vitro*. Spleen cells from mite-exposed control mice were used as negative controls. The results are shown in Fig. 2. A dose response was observed when cells from Der p 1-sensitized mice were incubated with increasing amounts (0, 5, 10 and 20 µg/ml) of Der p 1 *in vitro* (data not shown). Der p 1-specific proliferative responses were reduced modestly by IL-12 treatment. In some experiments, McKinght *et al.* [6] observed a 20–30% reduction of T-cell proliferative response after IL-12 administration. However, the reason for this phenomenon is yet to be defined.

To determine whether the *in vivo* administration of rIL-12 affects antigen-specific T-cell function, the *in vitro* production of IFN-γ and IL-5 of spleen cells in IL-12-treated mice was examined. Both doses of IL-12 suppressed IL-5 production

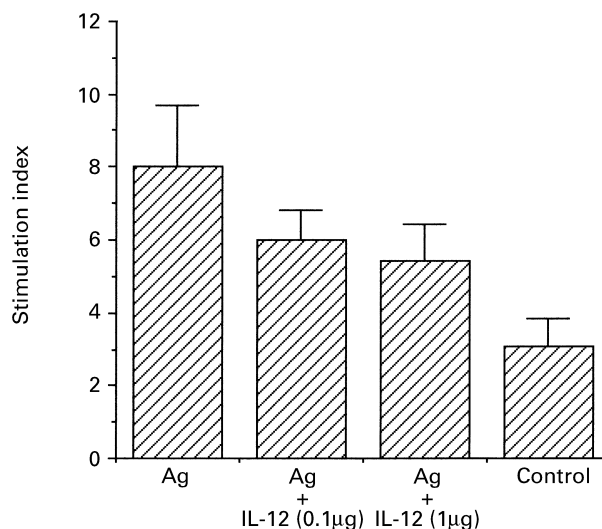


Fig. 2. The effect of IL-12 on Der p 1-antigen-specific T-cell proliferative response. C57BL/6 mice were treated as described in Fig. 1. On day 42, each group of sensitized mice was challenged with inhaled *D. pteronyssinus* and nonsensitized mice were also treated with the same protocol as the control group. Spleen cells were taken from these allergen-challenged mice after 24 h and restimulated with 10 µg/ml Der p 1 *in vitro*. Proliferation was measured by [³H]thymidine incorporation on day 3. The background values of spleen cells without Der p 1 stimulation were between 734 and 895 cpm in this assay. The results are expressed as SI and shown as mean ± SD for 8–10 mice per group.

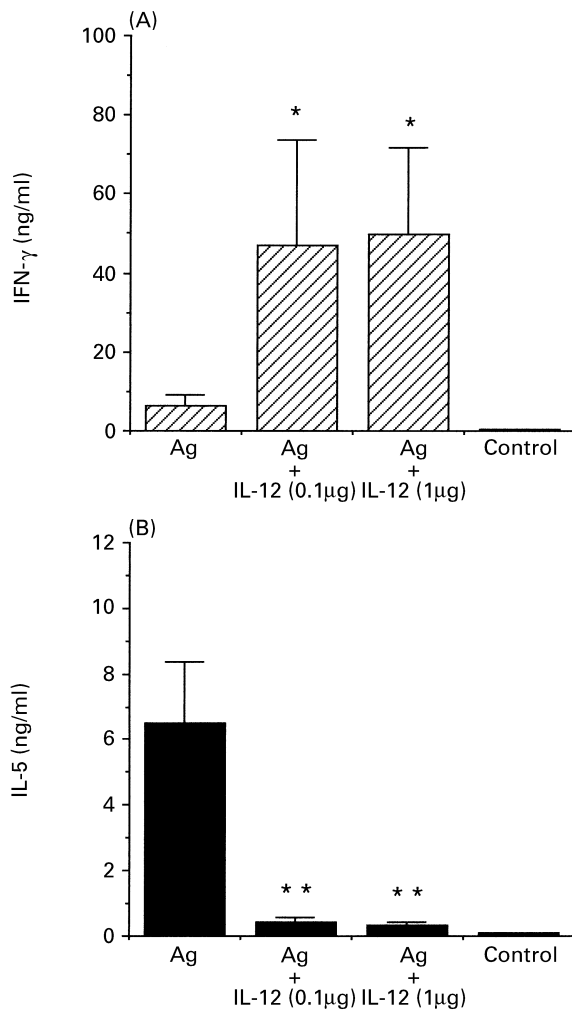


Fig. 3. The effect of IL-12 on IFN- γ , IL-5 production by splenocytes of mice after a single antigen challenge. Splenocytes were stimulated with 10 μ g/ml Der p 1 *in vitro* and culture supernatants were collected after 48 h, and the levels of cytokine production IFN- γ (A) and IL-5 (B) were measured by ELISA. Values shown are mean $\pm$ SD of 8–12 mice per group. Significant differences (* P < 0.05, ** P < 0.01) compared with those of mice without IL-12 treatment.

almost completely and enhanced IFN- γ secretion dramatically in allergen-challenged mice to a similar degree (Fig. 3A, B). These recall responses cannot be explained by residual IL-12 due to the *in vivo* IL-12 treatment because the spleens were removed 12 days later after the last injection of IL-12 and the serum half-life of IL-12 is only 2–5 h in rodents [13]. These results indicated that *in vivo* IL-12 treatment enhanced the differentiation of Der p 1-specific Th1 cells and suppressed the development of Th2 cells in response to immunization.

IL-12 inhibits airway inflammation induced by a single antigen challenge

To examine whether IL-12 could alleviate allergen-induced airway inflammation, both the cellular composition and inflammatory mediators in BAL fluids were analysed after aerosol exposure. In actively sensitized mice, a single exposure to aerosolized Dp induced a marked increase in the percentage of neutrophils, lymphocytes and eosinophils in BAL fluids, compared with those of the nonsensitized control mice exposed to aerosolized allergen (Table 2). The ratio of neutrophils and lymphocytes was increased slightly in mite-exposed control mice following inhalation. The mice receiving 1 μ g/day of IL-12 treatment showed a significant decrease in neutrophils and lymphocytes, whereas the percentage of these cells was not significantly affected by treatment with 0.1 μ g/day of IL-12. It seemed that IL-12 exerted a dose-dependent inhibition of antigen-induced airway inflammation. Both low and high doses of IL-12 significantly inhibited the eosinophil recruitment.

Histopathological examination of lung taken from sensitized mice after inhalation demonstrated inflammatory infiltrates in peribronchial and peribronchiolar regions (Fig. 4A). The infiltrates consisted of admixtures of predominantly eosinophils, neutrophils and lymphocytes. In contrast, the IL-12-treated animals were not noted to show significant airway inflammation (Fig. 4B). The serotonin levels in BAL fluids from allergen-challenged mice were measured and are shown in Fig. 5. The level of serotonin was increased markedly in sensitized mice

Table 2. Change in cellular composition in BAL of sensitized mice exposed to aerosolized allergen

Group	Treatment	BAL			
		Monocytes (%)	Neutrophils (%)	Lymphocytes (%)	Eosinophils (%)
1	Der p 1	28.8 $\pm$ 5.9	20.0 $\pm$ 3.4	16.6 $\pm$ 4.6	34.6 $\pm$ 8.1
2	Der p 1/IL-12(0.1 μ g)	68.0 $\pm$ 14.1	15.1 $\pm$ 9.6	14.9 $\pm$ 6.3	2.0 $\pm$ 1.6*
3	Der p 1/IL-12 (1 μ g)	94.4 $\pm$ 2.2*	3.1 $\pm$ 1.4*	2.0 $\pm$ 1.3*	0.5 $\pm$ 0.6*
4	None	85.6 $\pm$ 4.1	5.0 $\pm$ 2.9	8.9 $\pm$ 3.1	0.5 $\pm$ 0.5

BAL was performed 24 h after *Dermatophagoides pteronyssinus* inhalation in control and IL-12-treated mice. The differential counts of BAL were determined and data are mean $\pm$ SEM for 8–12 mice in each group.

* P < 0.001 for difference from the mean value of the corresponding response in Der p 1 sensitized mice.

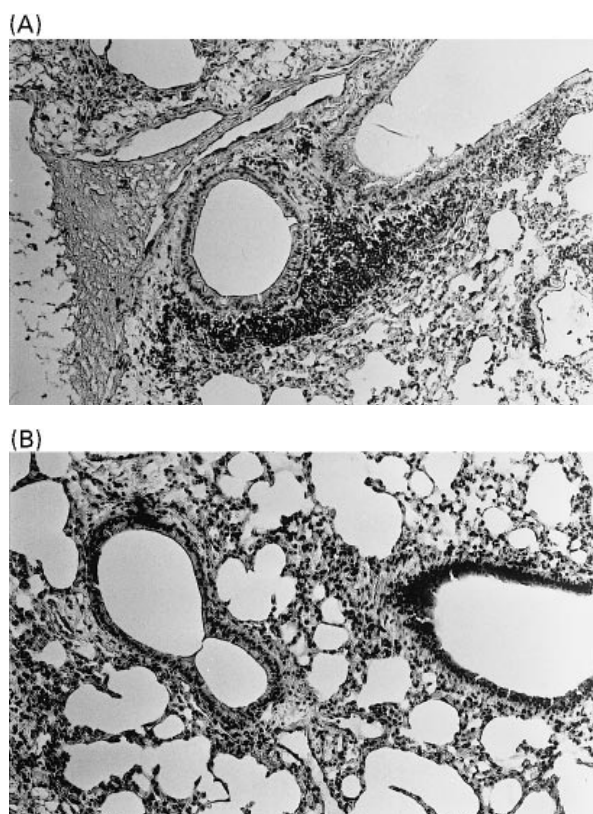


Fig. 4. Histological study of the lungs of immunized mice with or without IL-12 treatment (haematoxylin–eosin stain). Lung tissue from mice without IL-12 treatment (A) demonstrates extensive cellular infiltration of the periairway region ($\times 200$). In contrast, lung tissue from IL-12-treated mice (B) demonstrates a complete absence of histologically significant inflammation ($\times 200$).

without IL-12 treatment and was reduced significantly with either dose of IL-12 treatment.

DISCUSSION

Dermatophagoides pteronyssinus is dominant among the many species of mite found in house dust, and many studies have been devoted to the characterization of the Der p 1 involved in the pathogenesis of allergic diseases [14–16]. However, only a few studies have described an animal model of airway inflammation induced by Der p 1 allergen. Attention has therefore been directed at setting up a Der p 1-sensitized murine model of airway inflammation and investigating the application of IL-12 to modulate such an allergic inflammatory response. In studies designed to optimize the production of anti-Der p 1 IgE in mice, a range of doses of both antigen and pertussis toxin as the IgE adjuvant was investigated. The data showed that a significant anti-Der p 1 IgE antibody response can be detected in C57BL/6 mice immunized with 10 μ g of Der p 1 plus 2 mg of alum and 400 ng of PT intraperitoneally. It was recognized that different kinds of adjuvants were able to selectively activate one of the two CD4⁺ T-cell subpopulations, Th1 or Th2. For

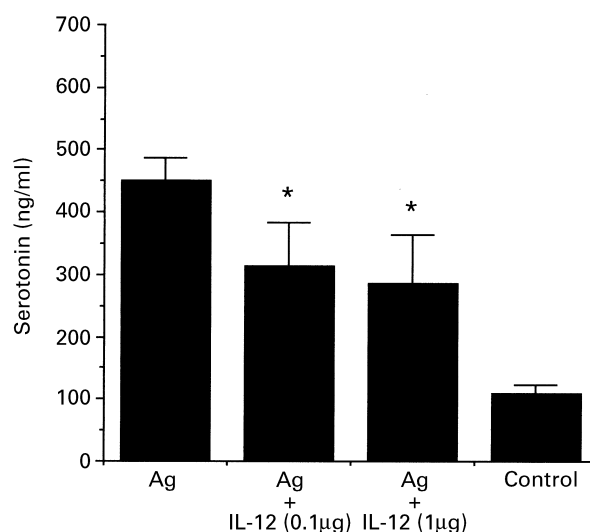


Fig. 5. The effect of IL-12 on serotonin production in BAL from mice after a single antigen challenge. C57BL/6 mice were treated as described in Fig. 1. On day 42, mice were challenged with inhaled *D. pteronyssinus* and BAL samples were taken from the mice with allergen challenged after 24 h. BAL fluids from nonsensitized mice exposed to aerosolized allergen were measured by using a specific ELISA. Data represent the mean $\pm$ SEM of 8–12 samples per group. * $P < 0.05$ compared with the level of immunized mice without IL-12 treatment.

example, in conjunction with the same antigen, alum activated Th2 cells whereas Freund's complete adjuvant (FCA) activated Th1 cells [17, 18]. PT, a protein toxin of *Bordetella pertussis*, was also used as the IgE adjuvant and the stimulation of IgE production by PT in response to protein antigens has been well documented [19–21]. In our studies, C57BL/6 mouse strain immunized with Der p 1 allergen + alum + PT may be used as a murine model of airway inflammation and as a prelude to further studies designed to examine and manipulate the immune response to the Der p 1 allergen at the molecular level, which may allow exploration of possible agents for immunotherapy.

IL-12 is a heterodimeric cytokine that is produced primarily by antigen-presenting cells, and plays an important role in the regulation of both innate and adaptive immune responses. One of the most striking effects of IL-12 is its ability to promote the development of Th1-type immune responses. Exogenous rIL-12, added to cultured bulk lymphocytes from atopic donors during *in vitro* stimulation with Der p 1, favoured the differentiation of antigen-specific T-cell lines and clones producing high concentrations of IFN- γ and low concentrations of IL-4 [5]. Following *in vivo* challenge with various intracellular pathogens, IL-12 has been shown to promote Th1 responses while inhibiting Th2 responses. In a murine leishmaniasis model, it has been demonstrated that IL-12 had to be given within the first week after infection to be effective in providing immunity, before the establishment of a Th2 response [22, 23]. In addition, Nabors *et al.* [24] have found that IL-12 plus the leishmanicidal drug given 3 weeks after infection cured 70% of the mice. Recently, a

IL-12 gene transfer approach for nucleic acid vaccination or for gene therapy has been used in various models [25, 26]. Therefore, the potential use of IL-12 plasmid in future as an application to the treatment of Der p 1-induced airway inflammation should be investigated further.

Consistent with the role of IL-12 in promoting Th1- and suppressing Th2-type responses, IL-12 also exerts regulatory effects on humoral immune responses. In CBA/J mice immunized with protein antigens adsorbed to alum, administration of IL-12 induced strong increases in antigen-specific IgG_{2a}, IgG_{2b} and IgG₃ production [27]. Kiniwa *et al.* [28] demonstrated that IL-12 dramatically inhibited IL-4-induced IgE synthesis by peripheral blood mononuclear cells (PBMC) *in vitro*. Since the distinguishing feature between an atopic and a nonatopic individual is their ability to produce a sustained high-level IgE response to allergens, IgE antibody has been defined to be the marker for sensitization. Recent studies have disclosed a correlation between serum IgE and airway responsiveness in asthmatic patients as compared with that of normal controls [29]. In the murine model of Der p 1-induced asthma, it has been demonstrated that administration of IL-12 at 1 µg/day inhibited the Der p 1-specific IgE response by 88% and the Der p 1-specific IgG₁ response by 93%. In contrast, the production of Der p 1-specific IgG_{2a} was enhanced strongly by either dose of IL-12. Likewise, high dose of 1 µg IL-12/day treatment given to allergen phospholipase A₂ (PLA₂)-sensitized CBA/J mice could reduce the IgE response. Nevertheless, the suppression phenomenon was not permanent, because IgE synthesis was up-regulated in mice after repeated antigen challenge without IL-12 [30].

Airway inflammation is the major pathological change of bronchial asthma [31]. It has been proposed that mast cells and T cells are the key effector cells involved in triggering inflammatory responses in allergic diseases. Mast cells present in the airway mucosa, stimulated by inhaled allergens, and releasing mediators such as histamine and serotonin are responsible for the acute phase reactions. Eosinophils are prominent inflammatory cells in the airways of asthmatic subjects and have been proposed to mediate tissue injury and airway hyper-responsiveness [32]. Recently, it has been observed that IL-12 has a profound effect on antigen-induced airway inflammation. IL-12 was able to abolish antigen-induced airway hyper-responsiveness and pulmonary eosinophilia in sensitized mice after an intratracheal (IT) challenge with sheep red blood cell antigen [33]. Similarly, the administration of IL-12 during active immunization decreased ragweed-specific serum IgE and BAL eosinophilia following IT allergen challenges [34]. In the animal model of ovalbumin-induced lung inflammation, the treatment of mice with IL-12 either before or during the aerosolized antigen challenge abrogated airway eosinophilia, whereas administration of IL-12 during the aerosolized antigen challenge did not decrease the level of specific IgE [35, 36]. In our study, it has been demonstrated that administration of IL-12 during active immunization strikingly decreased airway inflammatory cells and the levels of serotonin in BAL fluids after an inhaled challenge. Collectively, these studies demonstrated the ability of IL-12 given early during

the immune response to redirect a Th2-mediated immune response to inhaled antigens.

There was a discrepancy between our findings regarding the levels of IFN-γ secretion and those regarding serotonin production in IL-12-treated mice. The data demonstrated that IL-12 treatment strongly augmented the production of IFN-γ and markedly reduced IL-5 secretion by antigen-stimulated spleen cells. However, IL-12 treatment only modestly decreased serotonin production in BAL fluids. Firstly, it is presumed that exogenous IL-12 might potentially enhance IFN-γ production not only by steering Der p 1-specific CD4⁺ T cells to Th1-associated activation patterns but also by transient activation of natural killer (NK) cells. Secondly, the lack of a dramatic effect of IL-12 on serotonin production may be due to the long interval between the last dose of IL-12 and the assay of BAL in IL-12-treated mice. Indeed, it has been reported that up-regulating effects of IL-12 on BAL cell IFN-γ expression last for up to 2 days but faded out by 8 days [34]. Furthermore, the endogenous release of IFN-γ was not enough to completely inhibit aspects of mast cell function such as the release of serotonin.

In this study, the effects of IL-12-treatment protocols on a murine model of Der p 1-induced asthma have been evaluated. It has been demonstrated that the intraperitoneal administration of IL-12 during active immunization prevented the production of Der p 1-specific serum IgE levels, airway eosinophilia provoked by aerosol challenge and Th2-type cytokines production. In addition, allergen-induced eosinophils recruitment was also strikingly inhibited in the group treated with a dose of 1 µg/day of IL-12. Thus, IL-12 may be useful as a single immunotherapeutic agent, in combination with chemotherapeutic agents, or as a vaccine adjuvant for treatment of atopic diseases.

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