Counterbalancing of T_H2-driven allergic airway inflammation by IL-12 does not require IL-10

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Background: Asthma is characterized by allergen-induced airway inflammation orchestrated by T_H2 cells. The T_H1-promoting cytokine IL-12 is capable of inhibiting the T_H2-driven allergen-induced airway changes in mice and is therefore regarded as an interesting strategy for treating asthma.
Objective: The antiallergic effects of IL-12 are only partially dependent of IFN-γ. Because IL-12 is a potent inducer of the anti-inflammatory cytokine IL-10, the aim of the present study was to investigate in vivo whether the antiallergic effects of IL-12 are mediated through IL-10.
Methods: C57BL/6J-IL-10 knock-out (IL-10$^{-/-}$) mice were sensitized intraperitoneally to ovalbumin (OVA) and subsequently exposed from day 14 to day 21 to aerosolized OVA (1%). IL-12 was administered intraperitoneally during sensitization, subsequent OVA exposure, or both.
Results: IL-12 inhibited the OVA-induced airway eosinophilia, despite the absence of IL-10. Moreover, a shift from a T_H2 inflammatory pattern toward a T_H1 reaction was observed, with concomitant pronounced mononuclear peribronchial inflammation after IL-12 treatment. Allergen-specific IgE synthesis was completely suppressed only when IL-12 was administered along with the allergen sensitization. Furthermore, treating the animals with IL-12 at the time of the secondary allergen challenge resulted not only in a significant suppression of the airway responsiveness but also in an important IFN-γ–associated toxicity.
Conclusions: These results indicate that IL-12 is able to inhibit allergen-induced airway changes, even in the absence of IL-10. In addition, our results raise concerns regarding the redirection of T_H2 inflammation by T_H1-inducing therapies because treatment with IL-12 resulted not only in a disappearance of the T_H2 inflammation but also in a T_H1-driven inflammatory pulmonary pathology. (J Allergy Clin Immunol 2001;107:483-91.)

Key words: *IL-10, IL-12, allergen, airway inflammation, airway responsiveness, T_H1, T_H2, eosinophil, IgE, asthma*

Abbreviations used
BALF: Bronchoalveolar lavage fluid
OVA: Ovalbumin
WT: Wild type

Airway inflammation in allergic asthma is orchestrated by activated CD4+ T cells that produce T_H2 cytokines. The release of IL-4, IL-5, and IL-13 induces the activation of inflammatory cells, such as eosinophils, mast cells, and basophils. These cells secrete a plethora of inflammatory mediators important in airway narrowing and hyperresponsiveness.[1,2] Inhaled glucocorticosteroids are very effective for controlling the symptoms of asthma and associated airway inflammation. When stopped, the symptoms of asthma reappear, and therefore inhaled steroids cannot be considered as a cure. The development of treatments acting at an earlier level of the pathogenesis is therefore necessary. The idea emerged that a shift in polarization of the cytokine production from a T_H2 toward a T_H1 profile could allow a more specific therapy. The T_H1-inducing immunoregulatory cytokine IL-12 is considered an especially good candidate for this role.[3,4] Murine in vivo models of asthma have shown that IL-12 inhibits T_H2-mediated immune responses to inhaled antigens with a suppression of airway eosinophilia and hyperresponsiveness.[5-9] The exact mechanisms of IL-12's inhibitory effects are, however, not well understood, and it is currently believed that IL-12 has direct and indirect effects. The latter implicate an effect through a differential regulation of cytokines affecting the allergic inflammation. Because IL-12 is capable of inducing IL-10,[10,11] and because IL-10 is a natural dampener of the immune responses,[12] we tested whether the antiallergic effects of IL-12 were mediated through IL-10. Using IL-10 knock-out mice (IL-10$^{-/-}$), we here demonstrate that IL-12 remains capable of abolishing T_H2-driven inflammatory processes in the absence of IL-10. Moreover, we report on the possible adverse effects of this immunoregulatory cytokine that go along with the switching of T_H2 toward T_H1 inflammation.

METHODS

Animals

Specific pathogen-free male C57BL/6 wild-type (WT) mice and IL-10–deficient mice (IL-10$^{-/-}$, C57BL/6-IL-10(tm1Cgn); The

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TABLE I. Overview of the experimental groups

	IL-12 treatment, d 0-5	IL-12 treatment, d 14-21	Aerosol challenge, d 14-21
A			
Group I-a	Placebo	Placebo	PBS
Group II-a	1.0 μg of IL-12	1.0 μg of IL-12	PBS
Group III-a	Placebo	Placebo	OVA
Group IV-a	1.0 μg of IL-12	Placebo	OVA
Group V-a	1.0 μg of IL-12	1.0 μg of IL-12	OVA
Group VI-a	Placebo	1.0 μg of IL-12	OVA
B			
Group I-b	Placebo	Placebo	PBS
Group II-b	Placebo	Placebo	OVA
Group III-b	0.1 μg of IL-12	0.1 μg of IL-12	OVA
Group IV-b	0.3 μg of IL-12	0.3 μg of IL-12	OVA
Group V-b	1.0 μg of IL-12	1.0 μg of IL-12	OVA

A, IL-10$^{-/-}$ mice were challenged with either PBS or OVA after immunization with OVA. IL-12 treatment (1 μg/d per mouse) was administered intraperitoneally either at the time of sensitization, at the time of secondary allergen challenge, or both. **B,** Dose-response curve for the effects of IL-12 on allergen-induced airway changes.

Jackson Laboratory, Bar Harbor, Me) 6 to 8 weeks of age and weighing 25 ± 1 g were housed in sterilized cages and fed with sterile food and water ad libitum.

Immunization and exposure

On the first day (day 0) of the experiments, all mice (n = 8-18 per group) were immunized by means of intraperitoneal injection of 10 μg of ovalbumin (OVA; Sigma, St Louis, Mo) adsorbed to 1 mg of aluminum hydroxide. From day 14 to day 21, mice were exposed daily to PBS or OVA aerosol sprays (1%) for 30 minutes by using an ultrasonic nebulizer (Ultraschallvernebler Sirius Nova; Heyer Medizintechnologie, Bad Ems, Germany). The output of the nebulizer was 3 mL/min, with a mean particle size of 3.2 μm.

IL-12 treatment

Murine recombinant IL-12 was a gift from Dr M. Gately (Roche, Nutley, NJ). One microgram of IL-12 dissolved in 100 μL of PBS (with 10% mouse serum albumin) was injected daily as a purified protein intraperitoneally from day 0 to day 5 (ie, during active sensitization), from day 14 to day 21 (ie, during secondary allergen challenge), or both. The different treatment regimens are shown in Table I. In view of the very pronounced effects, we also performed a dose-response curve for IL-12 (0.1 vs 0.3 vs 1.0 μg per mouse per day; Table I).

Airway responsiveness

Airway responsiveness was measured 24 hours after the final allergen exposure.[6] The mice were anesthetized with pentobarbital (100 mg/kg intraperitoneally), and a tracheal cannula was inserted. The femoral artery and the jugular vein were cannulated. A pressure catheter was inserted in the pleural space. The animals, placed on a 37°C heated blanket, were ventilated with a Palmer respirator (Bioscience, Sheerness, United Kingdom) at 145 strokes per minute (stroke volume, 0.5 mL). Neuromuscular blockade was induced by injecting pancuronium bromide (1 mg/kg) intravenously (Organon, Teknika N.V., Turnhout, Belgium). Airway resistance was calculated from the differential pressure between the airways and the pleural cavity, tidal volume, and flow. These parameters were measured with a computerized pulmonary mechanics analyzer (Mumed PMS800 system; Mumed Ltd, London, United Kingdom). Increasing doses of carbachol were administered (microinfusion pump: 40, 120, 400, and 1200 μg/kg intravenously). Between each dose, the airway resistance returned to the baseline value. The provocative dose of carbachol causing a 50% increase in resistance was calculated from the linear interpolation on a semilogarithmic dose-response curve.

Bronchoalveolar lavage

After the assessment of airway responsiveness, 1 mL of HBSS supplemented with 0.05 mmol/L EDTA and 5% BSA was instilled 4 times through the trachea and recovered by means of manual aspiration. The recovered bronchoalveolar lavage fluid (BALF) was centrifuged (1800 rpm for 10 minutes at 4°C), whereas the supernatants of the first fraction were stored for cytokine determination. The cell pellets of the first fraction and of the 3 other fractions were resuspended in 1 mL of HBSS. A total cell count was done in a Bürker chamber, and differential cell counts were performed on cytocentrifuged preparations (Cytospin 2; Shandon Ltd, Runcorn, Cheshire, United Kingdom) after May-Grünwald-Giemsa staining.

Cytokine measurements in BALF supernatant

The cytokines IL-4, IL-5, IL-13, and IFN-γ were measured with commercially available ELISAs (Biosource Europe, Fleurus, Belgium for IL-4 and IFN-γ; R&D Systems Europe, Abingdon, United Kingdom for IL-13; and Biotrak, Amersham Pharmacia Biotech Benelux, Lenaarts, Belgium for IL-5). Sensitivity of the kits was 1, 1.5, 5, and 5 pg/mL for IFN-γ, IL-13, IL-4, and IL-5, respectively.

Allergen-specific serum IgE

At the end of the experiment, blood was drawn from the heart for measurement of OVA-specific serum IgE. Microtiter plates were coated with OVA. Serum was added, followed by a biotinylated polyclonal rabbit anti-mouse IgE (S Florquin, ULB, Brussels, Belgium). A serum pool from OVA-sensitized animals was used as the internal laboratory standard; 1 unit was arbitrarily defined as a 1:100 dilution of this pool.

Histologic analysis and scoring of pulmonary inflammation

The lungs were infused with 4% paraformaldehyde. Sections of 2.5-μm thickness from all lobes were stained with hematoxylin and eosin stain or with Congo-Red and hematoxylin stain (for the photomicrographs). The extent of peribronchial infiltrates was estimated by counting the cells in the peribronchial area relative to the length of the basement membrane (total bronchial inflammation

TABLE II. Effect of IL-12 on the total leukocyte count and on the cellular composition of the BALF in IL-10$^{-/-}$ mice

Group	Total cell count ($\times 10^5$)	Macrophages (%)	Lymphocytes (%)	Neutrophils (%)	Eosinophils (%)
I-a	1.5 ± 0.1	98.6 ± 0.3	1.2 ± 0.3	0.1 ± 0.1	0.0 ± 0.0
II-a	2.9 ± 0.9	79.9 ± 11.6*	9.7 ± 3.4*	0.4 ± 0.3	0.0 ± 0.0
III-a	22.8 ± 3.8*†‡§	29.0 ± 3.4$^{\|}$	9.3 ± 1.0*	3.0 ± 0.6*†	58.7 ± 4.0$^{\|}$
IV-a	21.0 ± 2.0*†‡§	65.2 ± 2.1*§	27.5 ± 2.5$^{\|}$	7.1 ± 1.5$^{\|}$	0.3 ± 0.3
V-a	9.3 ± 1.6*†	74.0 ± 3.8*§	5.7 ± 0.7*	20.4 ± 4.1$^{\|}$	0.0 ± 0.0
VI-a	5.6 ± 1.0*†	92.5 ± 2.9	5.2 ± 2.0*	2.2 ± 1.0	0.2 ± 0.1

$^{*}P < .05$ versus group I-a.
$^{\dagger}P < .05$ versus group II-a.
$^{\ddagger}P < .05$ versus group V-a.
$^{\S}P < .05$ versus group VI-a.
$^{\|}P < .05$ versus all other groups.

index in number of inflammatory cells per micrometer of basement membrane). The cellular composition of the infiltrates was analyzed and expressed as a percentage. By multiplying the total bronchial inflammation index with the cellular composition, an inflammatory score was obtained. Because 15 to 20 peribronchial infiltrates per experimental group were scored, inflammation scores were expressed as means for comparison.

Statistical analysis

Data were analyzed with the statistical package SPSS 6.1.2 (SPSS Inc, Chicago, Ill). All results are expressed as means ± SEM. Kruskal-Wallis H tests were used for screening significant differences between the groups. When P values were less than .05, Mann-Whitney U tests were applied for comparing the individual groups. The significance levels were adapted with the Bonferroni conservative correction. The dose-response curves obtained from the pulmonary function tests were analyzed with the GLM Univariate procedure, providing regression analysis and ANOVA for one dependent variable by 2 factors, variables, or both (the groups and the carbachol concentration). Differences were considered significant when P values were less than .05 after the Bonferroni conservative correction.

RESULTS

Summary of the effects of IL-12 on allergen-induced airway changes in WT mice

Previous experiments showed that treatment of OVA-sensitized and OVA-challenged WT mice with IL-12 (1 μg/d) at the time of sensitization resulted in a complete inhibition of the BALF eosinophilia (from 20.7% ± 7.0% to 0.2% ± 0.0%), allergenspecific IgE, and airway hyperresponsiveness (data not shown). Administration of IL-12 (1 μg/d) during the secondary allergen challenge equally resulted in an abrogation of airway eosinophilia (0.3% ± 0.2%) and hyperresponsiveness but not of IgE. Under IL-12 treatment, the total lavage cell counts and the BALF lymphocytosis of the OVA-challenged mice remained comparable with those in the untreated OVA-challenged mice (data not shown).[6]

Effect of IL-12 on composition of the BALF in IL-10$^{-/-}$ mice

The different challenge and treatment regimens in the IL-10$^{-/-}$ mice are represented in Table I. IL-12 administration (1 μg/d) in OVA-sensitized IL-10$^{-/-}$ mice in the absence of a secondary allergen challenge (group II-a) did not affect the total BALF cell count (Table II). However, a significant increase in the BALF lymphocytosis was observed. Repeated exposure to aerosolized OVA (group III-a) induced a significant increase in the total cell count, as well as in the numbers of eosinophils, neutrophils, and lymphocytes, when compared with the untreated PBS-exposed mice (Table II). In the OVA-challenged mice different effects on the BALF were observed depending on the time of IL-12 administration. When IL-12 was exclusively given at the sensitization (group IV-a), an increase in total cell count and lymphocytosis was observed up to similar levels as those observed in the allergen-challenged untreated mice. Combining the IL-12 treatment during sensitization with IL-12 treatment during the allergen exposure (group V-a) partially suppressed total BALF cell count and the associated lymphocytosis. A similar effect was found when treating the animals with IL-12 only at the time of allergen exposure (group VI-a). Regardless of the IL-12 treatment regimen (groups IV-a, V-a, or VI-a), a significant suppression of the BALF eosinophilia was noted when compared with the untreated but OVA-challenged mice (group III-a, Table II).

Effect of IL-12 on allergen-specific serum IgE in IL-10$^{-/-}$ mice

A significant production of allergen-specific IgE was observed in the IL-10$^{-/-}$ mice when exposed to OVA aerosol sprays (Table III). Treatment with IL-12 in the absence of a secondary allergen challenge had no effect on IgE levels. Treating the OVA-challenged IL-10$^{-/-}$ mice with IL-12 during the sensitization (groups IV-a or V-a) completely abrogated this IgE production. In contrast, injecting IL-12 only at the time of secondary allergen challenge (group VI-a) only partially reduced the IgE synthesis.

Effect of IL-12 on the histopathology of the lungs in IL-10$^{-/-}$ mice

Sham-exposed IL-10$^{-/-}$ mice (group I-a) displayed normal pulmonary histology (Fig 1). Injecting IL-12 in these sham-challenged mice (group II-a) induced a mild infiltration of mononuclear cells into the perivascular and peribronchial areas. Challenge with OVA (group III-a) induced mixed lymphocytic and eosinophilic peribronchial and perivascular pulmonary infiltrates. Treat-

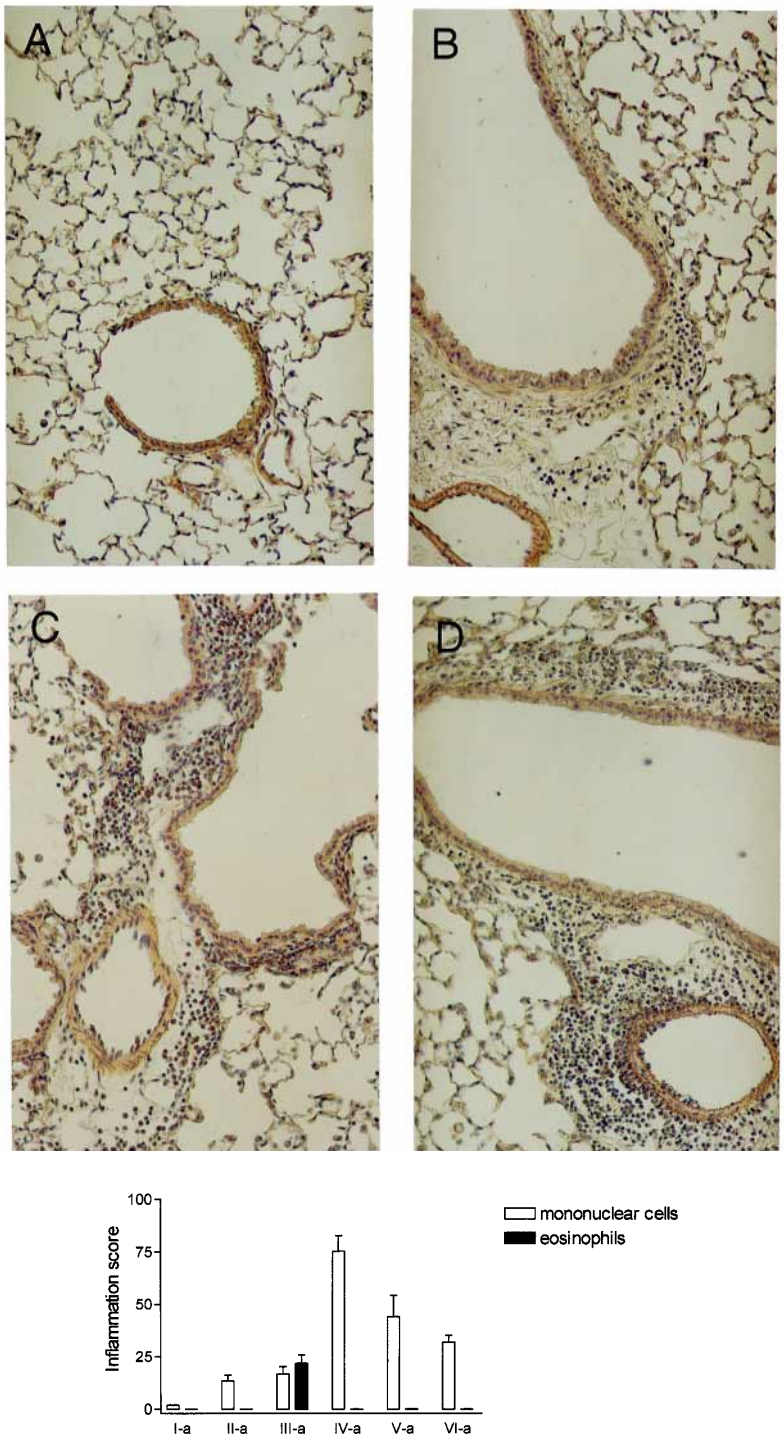

FIG 1. *Upper panel,* Photomicrographs depicting the airways of placebo-treated, PBS-challenged IL-10$^{-/-}$ mice **(A)**; mild mononuclear cell infiltration in the IL-12–treated, PBS-challenged IL-10$^{-/-}$ mice **(B)**; eosinophil-rich airway inflammation in the OVA-challenged IL-10$^{-/-}$ mice **(C)**; and macrophage-lymphocyte infiltration observed in the allergen-challenged mice treated with IL-12 at the time of sensitization **(D)**. (Original magnification, 200×.) *Lower panel,* Effect of IL-12 on the severity and type of airway inflammation in IL-10$^{-/-}$ mice (for experimental groups, see Table I). The number of inflammatory cells per unit of length of basement membrane was multiplied by the differential cell counts of the peribronchial infiltrates, giving a detailed peribronchial inflammation score. A mild mononuclear cell infiltration was observed after IL-12 treatment in the sham-challenged mice. Allergen exposure induced an eosinophilic airway inflammation. IL-12 treatment abolished the eosinophilic airway inflammation but induced a mononuclear cell inflammation in the peribronchial areas. This experiment was repeated 3 times.

ing the OVA-challenged mice with IL-12 exclusively at sensitization (group IV-a) completely abolished the eosinophilic inflammation but induced an overwhelming mononuclear cell infiltration, with macrophages and lymphocytes cuffing the peribronchial and perivascular areas. Combining the IL-12 treatment at the time of sensitization with an IL-12 treatment at the time of allergen exposure (group V-a) equally abolished the eosinophilic infiltration but also reduced to some extent the mononuclear cell infiltration observed in the challenged mice exclusively treated at sensitization. A similar picture was observed when IL-12 was given only at the time of the secondary allergen challenge (group VI-a). Importantly, treating the animals with IL-12 at the time of the allergen challenge (groups V-a and VI-a) induced a significant wasting (weight, 20.1 ± 0.7 and 21.4 ± 0.9 g), whereas IL-12 treatment in the absence of allergen challenge (group II-a: 26.6 ± 0.4 g or exclusively at the time of sensitization in the challenged mice; group IV-a: 24.1 ± 0.3 g) did not induce weight loss compared with that found in the untreated mice (groups I and III: 25.9 ± 0.5 and 26.6 ± 0.4 g).

Effect of IL-12 on airway responsiveness in IL-10$^{-/-}$ mice

Fig 2 shows the dose-response curves of the airway responsiveness in the different groups. As earlier demonstrated, secondary allergen challenges in sensitized IL-10$^{-/-}$ mice (group III-a) are unable to increase the airway responsiveness when compared with PBS-challenged mice (group I-a). This is related with the higher baseline airway responsiveness of the IL-10$^{-/-}$ mice, as earlier described.[13] This is also reflected by similar provocative dose of carbachol causing a 50% increase in resistance values in both groups (data not shown). Treating the PBS-exposed mice with IL-12 (group II-a) did not affect the baseline airway responsiveness. Accordingly, IL-12 treatment exclusively at the time of sensitization (group IV-a) was unable to significantly suppress the airway responsiveness. In contrast, administration of IL-12 during allergen challenges (groups V-a or VI-a) suppressed the airway hyperresponsiveness to a level that was significantly lower than the baseline responsiveness of the sham-challenged mice.

Effects of lower doses of IL-12 on allergen-induced airway changes in IL-10$^{-/-}$ mice

Because IL-12 treatment is associated with severe toxicity in IL-10$^{-/-}$ mice, we studied whether the same immunomodulatory effects could be achieved without severe wasting by constructing a dose-response curve with lower doses of IL-12 (see Table I for experimental groups). The different challenge and treatment regimens are shown in Table I. Administration of IL-12 was associated with a comparable reduction in the total BALF cell counts for the 3 doses tested when compared with the untreated OVA-challenged mice (group II-b); however, the reduction only reached statistical significance for group V-b treated with 1 μg of IL-12 per day. However,

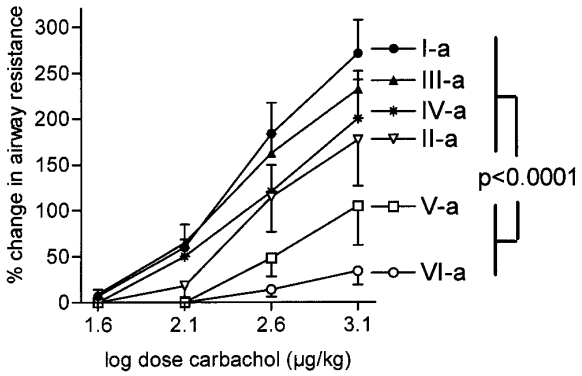

FIG 2. Effect of IL-12 on the airway responsiveness to carbachol in IL-10$^{-/-}$ mice (for experimental groups, see Table I). Allergen challenge does not cause a significant increase in the lung resistance in IL-10$^{-/-}$ mice. IL-12 treatment did not influence the airway responsiveness in either the sham-challenged mice or in the mice treated with IL-12 exclusively during the sensitization period. In contrast, IL-12 treatment from day 14 to day 21 significantly inhibited the airway responsiveness in the OVA-exposed animals. This experiment was repeated twice.

TABLE III. Effect of IL-12 treatment on allergen-specific IgE production in IL-10$^{-/-}$ mice

Group	Serum OVA-specific IgE (U/mL)
I-a	1.9 ± 0.2
II-a	0.7 ± 0.2
III-a	57.3 ± 3.1†
IV-a	5.4 ± 1.2
V-a	3.3 ± 0.8
VI-a	33.6 ± 9.1*

IL-12 significantly inhibits the synthesis of allergen-specific IgE.
$^{*}P < .05$ versus PBS-challenged and OVA-challenged mice that were treated with IL-12 at the time of primary allergen contact.
$^{\dagger}P < .05$ versus all other groups.

these total BALF cell counts were still significantly increased when compared with the PBS-challenged mice (group I-b; Fig 3, *A*). The percentages of BALF lymphocytes were significantly and to the same extent suppressed in the OVA-challenged IL-12–treated mice (groups III-b, IV-b, and V-b) when compared with the OVA-challenged untreated mice (group II-b). At the same time, they were still significantly elevated when compared with the PBS-challenged mice (1.2% ± 0.3% for the PBS-challenged mice [group I-b] vs 9.3% ± 1.0% for the OVA-challenged mice [group II-b] vs 4.9% ± 0.6% for the IL-12–treated mice). In contrast, the neutrophils were increased after IL-12 treatment, regardless of the dose (0.1% ± 0.1% for the PBS-challenged mice vs 3.0% ± 0.6% for the OVA-challenged mice vs 21.9% ± 2.8% for the IL-12–treated mice). The eosinophilic infiltration was significantly suppressed for all doses of IL-12 (Fig 3, *B*). Moreover, the eosinophils differed for the 3 different IL-12 doses, with significantly less eosinophils in the group treated with 1 μg of IL-12 (group V-b: 0.0% ± 0.0%) compared with the groups treated with 0.3 or 0.1 μg of IL-12 (group IV-b: 0.8% ± 0.6% and group III-b: 2.2% ± 1.1%). Allergen-

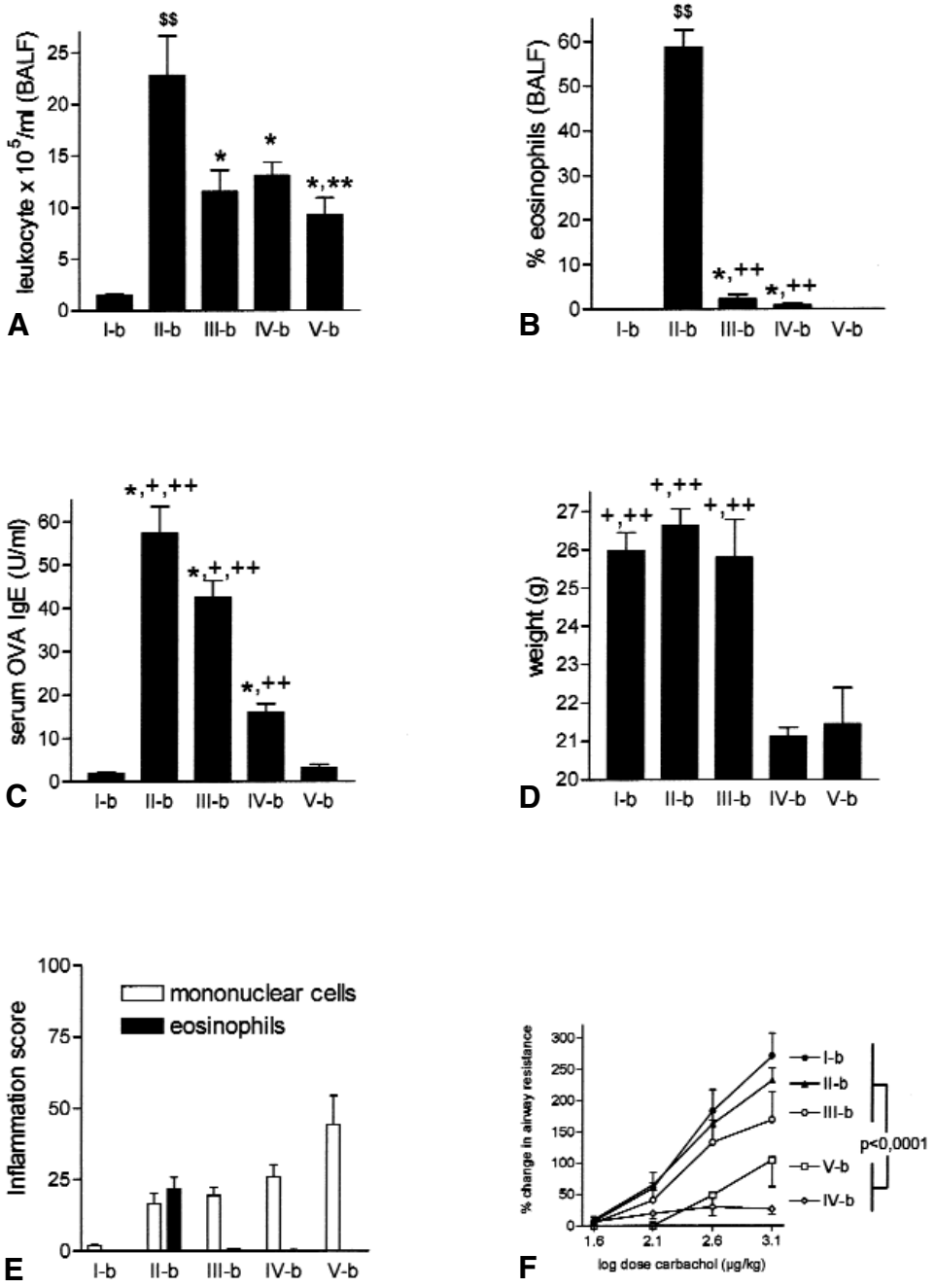

FIG 3. Effect of lower doses of IL-12 on the allergen-induced airway changes in IL-10$^{-/-}$ mice (for experimental groups, see Table I). The total BALF cell counts **(A)** and the eosinophilic infiltration **(B)** are significantly influenced on IL-12 treatment. The differential effects of increasing doses of IL-12 on serum OVA IgE **(C)** and on the weight of the animals **(D)** are also shown. *$P < .05$ versus group I-b, **$P < .05$ versus group II-b, +$P < .05$ versus group IV-b, ++$P < .05$ versus group V-b, and \$\$$P < .05$ versus all other groups. Lung histopathology and pulmonary function test results are represented in **E** and **F**, respectively.

specific IgE was significantly suppressed in the groups treated with 0.3 or 1.0 µg of IL-12 but not in the group treated with only 0.1 µg of IL-12. Only the group treated with 1.0 µg of IL-12 per day had IgE levels not differing from those of the PBS-challenged mice (Fig 3, *C*). The pulmonary histopathology revealed, in accordance with the findings on the BALF eosinophils, that the eosinophilic inflammation was abrogated after IL-12 treatment, regardless of the dose of IL-12. However, a dose-response curve arose, with more infiltration of mononuclear cells when treated with increasing doses of IL-12 (Fig 3, *E*). Importantly, evaluation of the animal

weight revealed that there was a significant wasting in the groups receiving 0.3 and 1.0 μg of IL-12 per day but not in the group treated with only 0.1 μg of IL-12 per day (Fig 3, *D*). Fig 3, *F*, shows the dose-response curves of the airway responsiveness in the different groups. Treatment of the animals with only 0.1 μg of IL-12 per day (group III-b) was not able to reduce the airway responsiveness when compared with levels in the untreated animals (groups I-b and II-b). This contrasts with the significantly suppressed airway responsiveness in animals treated with 0.3 (group IV-b) or 1.0 μg (group V-b) of IL-12 per day (Fig 3, *F*).

Effect of IL-12 on the BALF cytokine profile in IL-10$^{-/-}$ mice

Allergen challenge induced a significant increase in the BALF concentrations of IL-13. Although there was a small increase in IL-4, neither IL-4 nor IFN-γ levels were significantly different from those in the PBS-challenged mice (Fig 4). On IL-12 treatment, there was a significant increase in IFN-γ detected. Moreover, there was a dose-response curve correlating the amount of IFN-γ with the dose of IL-12 given (Fig 4, *A*). Although IL-12 treatment suppressed to some extent the allergen-induced IL-4 secretion, this suppression did not reach statistical significance (Fig 4, *B*). IL-13 was not affected at all on IL-12 treatment, regardless of the dose of IL-12 (Fig 4, *C*). IL-5 was below detection limit in all samples.

DISCUSSION

In this study we demonstrate that IL-12 reduces allergen-induced T_H2 immune responses, even in the absence of the immunomodulatory cytokine IL-10.

It is well documented that IL-12 not only promotes the differentiation of naive T_H0 cells toward a T_H1 profile but also inhibits allergen-induced T_H2 cell development both in vitro and in vivo.[5-9,14-16] In A/J and BALB/c, as well as in C57BL/6, mouse asthma models, it was repeatedly shown that administration of IL-12 during immunization not only prevented allergen-induced airway eosinophilia and hyperresponsiveness but also prevented the synthesis of allergen-specific IgE.[5-7,9] Conflicting data exist on the effects of IL-12 during secondary allergen challenges. We and others[5,6,8] found an inhibition of airway eosinophilia and hyperresponsiveness but not of IgE production. In contrast, other groups showed only a moderate effect on eosinophilia and no effect on airway responsiveness during the secondary challenge.[9,17] In vivo data showed that in BALB/c, but not in C57BL/6, mice a T_H2 development induces a rapid burst of IL-4.[18] IL-4, together with the absence of IFN-γ, induces a full suppression of IL-12Rβ2 expression.[19] The latter is critical for the maintenance of IL-12 responsiveness during secondary allergen challenges.[20] Thus differences in mouse strains, including differences in the regulation of the IL-12Rβ2 chain, and in doses of IL-12 could at least partially explain these differences.[21]

Whether IL-12 exerts its effects directly or requires the production of other cytokines to mediate a redirection

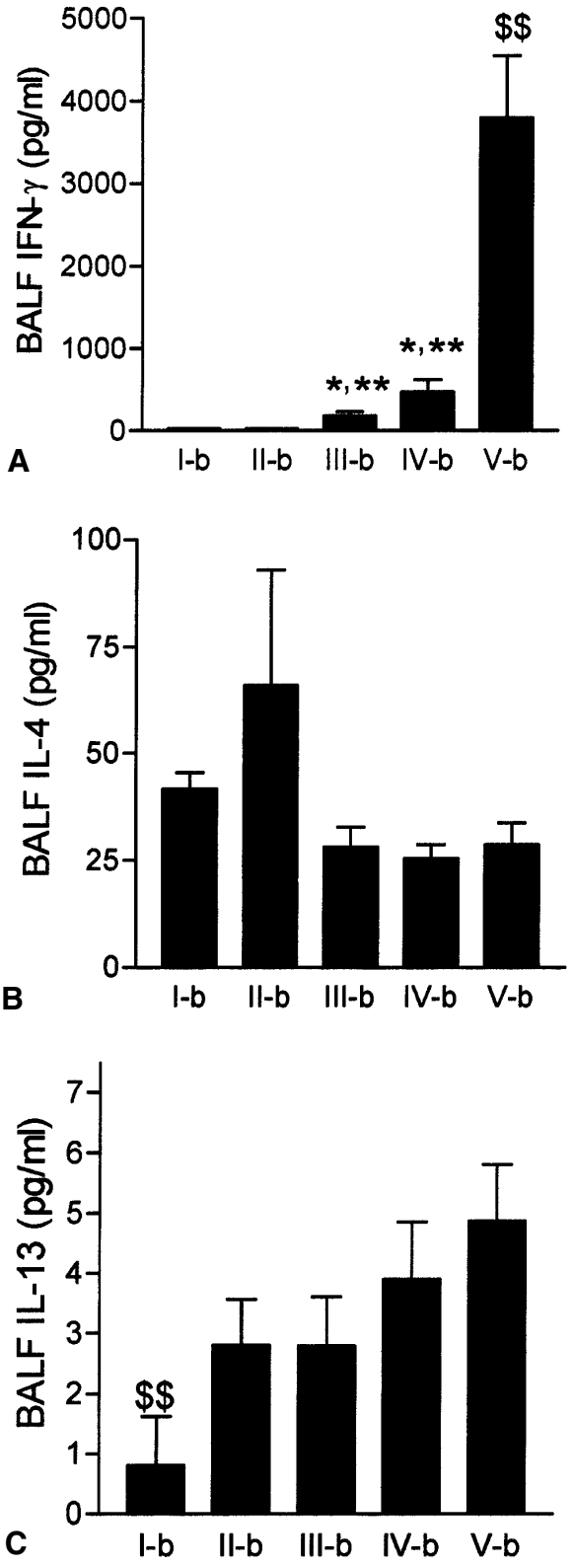

FIG 4. Effect of IL-12 treatment on BALF cytokine profiles. Treating allergen-challenged mice with IL-12 shifts the T_H2 BALF cytokine profile toward a T_H1 profile, with a significant upregulation of the IFN-γ production. *$P < .05$ versus group I-b, **$P < .05$ versus group II-b, and $$$P < .05$ versus all other groups.

of the T_H2 inflammatory pattern remains unclear.[6] IL-12 is a potent inducer of a range of cytokines, such as IFN-γ and IL-10.[10,11,22] It was shown that the effects of IL-12 are partially mediated through IFN-γ because the inhibition of eosinophil influx by IL-12 is IFN-γ dependent during a primary contact but not during secondary allergen exposures.[23] Because IL-10 is an important endogenous downregulator of T_H1 and T_H2 cytokine synthesis,[12] this cytokine should be considered as a possible mediator of the antiallergic effects observed after IL-12 treatment.[24] The suppressive activity of IL-10 in allergic disease is based on several lines of evidence,[25] including in vitro experiments[26] and in vivo experiments in which exogenous IL-10 was administered in murine asthma models[27,28] or in which an enhanced eosinophilic airway inflammation and baseline airway responsiveness was observed in IL-$10^{-/-}$ mice.[13] In addition, clinical data supported these findings because alveolar macrophages from asthmatic patients produce significantly lower amounts of IL-10 compared with those of healthy control subjects,[29] whereas a genotype associated with low IL-10 production was associated with severe asthma.[30]

In this study we therefore tested whether IL-12 needed IL-10 for achieving the T_H2 downregulation. Administration of IL-12 to allergen-challenged IL-$10^{-/-}$ mice completely inhibited eosinophil influx into the airways to the same extent as in the corresponding WT animals.[6] Moreover, a dramatic change of the BALF cytokines from a T_H2 toward a predominantly T_H1 profile, as demonstrated by the upregulation of IFN-γ, was observed.

This switch in inflammatory reaction was also documented with pulmonary histology. Even in the absence of an allergen challenge, a mild mononuclear cell infiltration was detectable in the peribronchial and perivascular areas after IL-12 treatment. In the allergen-challenged mice, however, an extreme inflammatory reaction was observed, especially when IL-12 was given at the time of sensitization. This effect of IL-12 was less pronounced but still impressive when given at the time of secondary allergen challenge. The fact that IL-12 can prime virtually all T cells for high IFN-γ production, provided IL-12 is administered early during clonal expansion, and that IL-12 is less efficient on established T_H2 clones probably explains these findings.[22,31] Nevertheless, there was, in these IL-$10^{-/-}$ mice, also a sustained IL-12 responsiveness (as assessed by pulmonary histology and BALF cytology) during the secondary immune responses, suggesting that the IL-12Rβ2 chain was not fully downregulated, despite the T_H2 cytokine environment. IL-10 has been described, just like IL-4, as a powerful downregulator of this IL-12Rβ2 chain.[20,32] The complete absence of IL-10 in these mice could therefore also contribute to the maintenance of the IL-12 responsiveness.

The combination of allergen exposure together with IL-12 treatment induced a toxic reaction with wasting, as evidenced by the weight of the animals. The severity of this toxic reaction was not paralleled with the severity of the IL-12–induced pulmonary inflammation. Although the dose of IL-12 was carefully chosen[33] and the sham-challenged mice treated with IL-12 or the allergen-challenged mice treated exclusively at the time of primary antigen contact displayed no wasting or toxic syndrome, we must conclude that the combination of allergen exposure and IL-12 treatment in sensitized subjects could potentially be dangerous. We also evaluated whether lower doses of IL-12 were capable of inhibiting the allergen-induced airway changes without inducing the severe toxicity. Although a 10-fold lower dose of IL-12 did not result in significant weight loss, there was still a very strong suppression of the airway eosinophilia. Unfortunately, even with this very low dose of IL-12, there was still a marked recruitment of other inflammatory cells (including neutrophils, lymphocytes, and macrophages) toward the lungs and the BALF. Thus we were not able to dissect the beneficial antieosinophilic effects of IL-12 while getting rid of the potentially dangerous immunomodulatory effects by lowering the dose. The increase in IFN-γ (produced by natural killer and T cells) has been thought to play an especially crucial role in the toxic effects (on liver, lungs, intestines, and the hematopoietic system) observed after IL-12 therapy.[34] Our findings support this hypothesis because we found more IFN-γ in the mice with IL-12–induced wasting.

For all these reasons, one could question the current strategy of developing new drugs for asthma aimed at the counterbalancing of the T_H2 inflammatory reaction. From our observations in IL$10^{-/-}$ mice, in which all inflammatory processes appear to be enhanced, it becomes clear that IL-12 treatment not simply downregulates the allergic T_H2 inflammation but also replaces it with another type of acute pulmonary inflammation. The treatment-induced inflammation might be less overt in the presence of IL-10 but does implicate that reversing T_H2-associated pathology with IL-12 does not necessarily result in beneficial effects, especially in asthmatic subjects with a low IL-10 profile. This is supported by animal experiments in which T_H2-driven allergic airway inflammation was counterbalanced by the transfer of IFN-γ–secreting T_H1 cells. The transfer of OVA-specific T_H1 cells, either before sensitization or after allergen challenge, resulted in an increase of pulmonary inflammation without attenuation of the airway responsiveness.[35,36]

IL-12 treatment also influenced the specific IgE synthesis in the IL-$10^{-/-}$ mice. No IgE was formed provided IL-12 treatment was given at the time of sensitization, which is similar to the data obtained in WT mice.[6,7] IgE was partially suppressed when IL-12 was given only at the time of the allergen challenge, a finding that contrasts with findings in WT mice. The absence of IL-10, associated with more IL-12Rβ, could be the underlying mechanism. A nice dose-response curve emerged when mice were treated with lower doses of IL-12. Only the mice treated with the highest doses (1 μg/d) presented with fully suppressed IgE, whereas the mice treated with 0.3 or 0.1 μg of IL-12 per day had IgE levels that were still significantly elevated when compared with those of the PBS-challenged mice.

In previous experiments we showed that nonexposed IL-$10^{-/-}$ mice had a higher baseline airway responsiveness when compared with WT animals and that allergen

challenges did not result in a further increase of the airway responsiveness.[13] This was confirmed in this study. Treating the IL-10$^{-/-}$ mice with IL-12 shortly before the pulmonary function test, however, markedly suppressed the airway responsiveness. Unfortunately, all the groups in which we found a significant suppression of the airway responsiveness were characterized by an IFN-γ–associated severe wasting syndrome. The latter could influence the normal airway responsiveness through alternative pathways. This implicates that our results are not conclusive about the potential involvement of IL-10 in the IL-12–induced reduction of allergen-induced airway hyperresponsiveness.

In summary, we demonstrated that treatment of allergic airway inflammation with IL-12 remains effective, even in the absence of IL-10. In addition, our results raise concerns regarding the redirection of T_H2 inflammation by IL-12 because IL-12 treatment not only resulted in a disappearance of the T_H2 inflammation but also induced a T_H1-driven acute pulmonary pathology.

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