Local treatment with IL-12 is an effective inhibitor of airway hyperresponsiveness and lung eosinophilia after airway challenge in sensitized mice

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Background: Systemic administration of IL-12 can prevent airway hyperresponsiveness (AHR) in mice after sensitization and repeated allergen challenge. However, systemic IL-12 has been associated with severe adverse effects.
Objective: We determined whether IL-12 administration to the airways in a dose sufficiently low so as not to result in systemic effects can modify allergic inflammation and AHR after allergen challenge.
Methods: Mice were sensitized to ovalbumin by intraperitoneal injection and challenged with ovalbumin aerosol on 3 consecutive days. During the period of challenge, IL-12 was administered intranasally following 2 regimens, designated high (500 ng) or low (50 ng). We monitored airway responsiveness to inhaled methacholine by barometric body plethysmography, lung inflammatory cells, local cytokine production, and, to assess systemic effects of IL-12 treatment, spleen weights and numbers of eosinophils in the bone marrow.
Results: Allergen challenge resulted in increases in airway responsiveness and in numbers of lung eosinophils. These increases were prevented by both high- and low-dose IL-12. Additionally, IL-12 administration resulted in enhanced local interferon-γ production and prevented the increases in local IL-4 and IL-5 production after airway challenge. A high dose, but not a low dose, of IL-l2 resulted in increased spleen weights and prevented the increase in numbers of bone marrow eosinophils after allergen challenge.
Conclusion: These data indicate that local administration of IL-12 can prevent AHR and reduce lung eosinophilia after allergen challenge in sensitized mice without eliciting systemic adverse effects. IL-12 exerts these effects by inducing local T_{H1}-type responses in the airways in a setting that is normally dominated by T_{H2}-type responses. (J Allergy Clin Immunol 1998;102:86-93.)

Key words: *IL-12, respiratory hypersensitivity, airway inflammation, allergen, body plethysmography, adverse effects, mice*

Abbreviations used

AHR:	Airway hyperresponsiveness
EU:	ELISA units
IFN:	Interferon
MCh:	Methacholine
MBP:	Eosinophilic major basic protein
OVA:	Ovalbumin
PBLN:	Peribronchial lymph node
PBS:	Phosphate-buffered saline
Penh:	Enhanced pause
P/I:	Phorbol 12,13 dibutyrate/ionomycin

Inflammation of the airways plays a major role in the pathogenesis of allergic asthma. This inflammatory response is characterized by increases in numbers of eosinophils and mast cells[1] and increased production of T_{H2}-type cytokines (IL-4, IL-5, IL-6, and IL-10)[2,3] by $CD4^+$ and $CD8^+$[4,5] T cells. It is also associated with airway hyperresponsiveness (AHR). Inhibitors of this inflammatory response, such as interferon-γ (IFN-γ),[6] also inhibit the development of AHR.[7,8] IL-12 is a heterodimeric cytokine[9,10] that is mainly produced by monocytes and macrophages[11] in response to infection. It upregulates the production of T_{H1}-type cytokines, especially IFN-γ, in human natural killer and T cells[9,12] promotes the development of T_{H1} cells,[13,14] and inhibits the expression of T_{H2} cytokines in vivo.[15] Considering these properties, IL-12 should serve as a potent modulator of the T_{H2} cytokine–driven inflammatory response seen after allergic sensitization. The ability of IL-12 to inhibit the allergic inflammatory reaction in the airways has been studied by several investigators in murine models of allergic sensitization and airway challenge. Administered during the sensitization phase, systemic IL-12 suppresses allergic airway inflammation as indicated by reduced numbers of eosinophils in bronchoalveolar lavage fluid, normalization of lung histology, and reduced AHR.[16,17] Allergen-specific IgE levels are also decreased.[16,17] More importantly, systemic IL-12 inhibits allergic airway inflammation and AHR in sensitized mice if given before and during the period of allergen challenge.[17-19]

Administration of IL-12 has been associated with severe adverse effects. Most notably, extramedullary

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hematopoiesis causing hepatomegaly and splenomegaly and bone marrow suppression resulting in anemia, leucopenia, and neutropenia have been observed in monkeys[20] and mice.[21,22] In addition, pulmonary edema,[23] hepatotoxicity, and skeletal muscle degeneration[24] have been reported in mice. In humans, IL-12 can induce the formation of mixed erythroid and myeloid colonies from peripheral blood in vitro,[25] which could lead to extramedullary hematopoiesis in vivo. Recently, clinical trials of IL-12 as an antitumor agent were discontinued because of unexpected deaths.[26] For all of these reasons, systemic IL-12 is probably not an option for the treatment of diseases such as asthma.

We previously demonstrated the potency of the local administration of IFN-γ in preventing allergic inflammation and AHR after both primary and secondary allergen challenge.[7,8] Because of the potential potency of IL-12 in reversing allergic sensitization and the necessity to avoid systemic effects, we determined whether the exclusive administration of IL-12 to the airways at a concentration sufficiently low so as not to result in systemic effects could prevent or modify the development of allergic airway inflammation and AHR in sensitized animals. To this end, we administered IL-12 to the respiratory tract in a murine model of airway sensitization, which enabled us to assess airway responsiveness to provocation with methacholine by barometric whole body plethysmography, pulmonary inflammation, local cytokine production, serum immunoglobulin levels, and, as a measure of systemic effects, bone marrow cellularity and spleen size.

METHODS

Animals

Female BALB/c mice, 8 to 12 weeks of age and free of specific pathogens, were obtained from Jackson Laboratories (Bar Harbor, Me). The mice were maintained on ovalbumin (OVA)-free diets. All experimental animals used in this study were under a protocol approved by the Institutional Animal Care and Use Committee of the National Jewish Medical and Research Center.

Experimental protocols

Mice were sensitized by intraperitoneal injection of 20 μg of OVA (Sigma, St. Louis, Mo) without adjuvant on days 1 and 10. They were challenged with nebulized OVA solution (1% in phosphate-buffered saline [PBS], 7 mL) or with PBS as a control by using an AeroSonic ultrasonic nebulizer (DeVilbiss, Sommerset, Pa) for 20 minutes daily on days 22, 23, and 24. On days 20, 22, and 24, recombinant murine IL-12 (kindly provided by Dr Stanley Wolf, Genetics Institute, Andover, Mass) dissolved in PBS was administered by using 2 dosing schedules, 500 ng (high) or 50 ng (low). Sensitized and challenged controls were treated with PBS. Each solution (50 μL) was administered intranasally after light anesthesia had been achieved. On days 22 and 24, administration was 2 hours before challenge with OVA. In separate experiments, sensitized mice were treated for 20 minutes with an aerosolized IL-12 solution (45 μg/7 mL PBS) on days 20, 21, 22, 23, and 24. On day 26, airway responsiveness was assessed, and animals were killed the following day for the collection of blood and the removal of peribronchial lymph nodes (PBLNs), lungs, spleen, and right femur.

Determination of airway responsiveness

Airway responsiveness was assessed with a single-chamber, whole-body plethysmograph (Buxco, Troy, NY). In this system an unrestrained, spontaneously breathing mouse is placed into the main chamber of the plethysmograph, and pressure differences between this chamber and a reference chamber are recorded. The resulting box pressure signal is caused by volume and resultant pressure changes during the respiratory cycle of the animal. A low pass filter in the wall of the main chamber allows thermal compensation. From these box pressure signals the phases of the respiratory cycle, tidal volumes, and the enhanced pause (Penh) can be calculated. Penh is a dimensionless value that represents a function of the proportion of maximal expiratory to maximal inspiratory box pressure signals and of the timing of expiration. It correlates closely with pulmonary resistance measured by conventional two-chamber plethysmography in ventilated animals.[27] Penh was used as the measure of airway responsiveness in this study. In the plethysmograph, mice were exposed for 3 minutes to nebulized PBS and subsequently to increasing concentrations of nebulized methacholine (MCh) (Sigma) in PBS with an AeroSonic ultrasonic nebulizer (DeVilbiss). After each nebulization, recordings were taken for 3 minutes. The Penh values measured during each 3-minute sequence were averaged and are expressed for each MCh concentration as the percentage of baseline Penh values after PBS exposure.[27]

Isolation of lung cells and bone marrow

Lung cells were isolated by collagenase digestion as previously described.[28,29] Bone marrow was rinsed out of the right femur, washed, and resuspended in Hank's balanced salt solution. Cells were counted with a hemocytometer, and cytospin slides were prepared and stained with Leukostat (Fisher Diagnostics, Pittsburgh, Pa). Differential cell counts were performed in a blinded fashion, counting at least 300 cells under light microscopy.

Immunohistochemistry studies

After perfusion through the right ventricle, lungs were inflated through the trachea with 2 mL of 10% formalin and then fixed in the same solution by immersion. Blocks of the left lung tissue were cut from around the main bronchus and embedded in paraffin blocks, and 5-μm tissue sections were affixed to microscope slides and deparaffinized. The slides were then stained immunohistochemically for cells containing eosinophilic major basic protein (MBP) as previously described by using a rabbit anti-mouse MBP antibody (kindly provided by Dr G. Gleich and Dr J. Lee, Mayo Clinic, Rochester, NY and Scottsdale, Ariz).[30] Numbers of eosinophils in the peribronchial, perivascular, and parenchymal tissue were analyzed separately by using the IPLab2 software (Signal Analytics, Vienna, Va) for Macintosh, counting 3 different sections per animal.[30]

Cell preparation

PBNLs were harvested, and mononuclear cells were purified by passing the tissue through a stainless steel mesh followed by density gradient centrifugation (Organon Teknika, Durham, NC). Cells were washed 3 times with PBS and resuspended in RPMI 1640 medium (Gibco, Gaithersburg, Md).

In vitro cytokine production

Mononuclear cells were cultured for 48 hours in 96-well round-bottom plates at a concentration of 4×10^5 cells/well in the presence or absence of the combination of phorbol 12,13-dibutyrate (10 ng/ml; Sigma) and ionomycin (0.5 μmol/L; Calbiochem, La Jolla, Calif) (P/I). Culture supernates were harvested and frozen at –20° C. The concentrations of IFN-γ, IL-4, and IL-5 in the supernates were assessed by ELISA as described.[31] Briefly, Immulon 2 plates

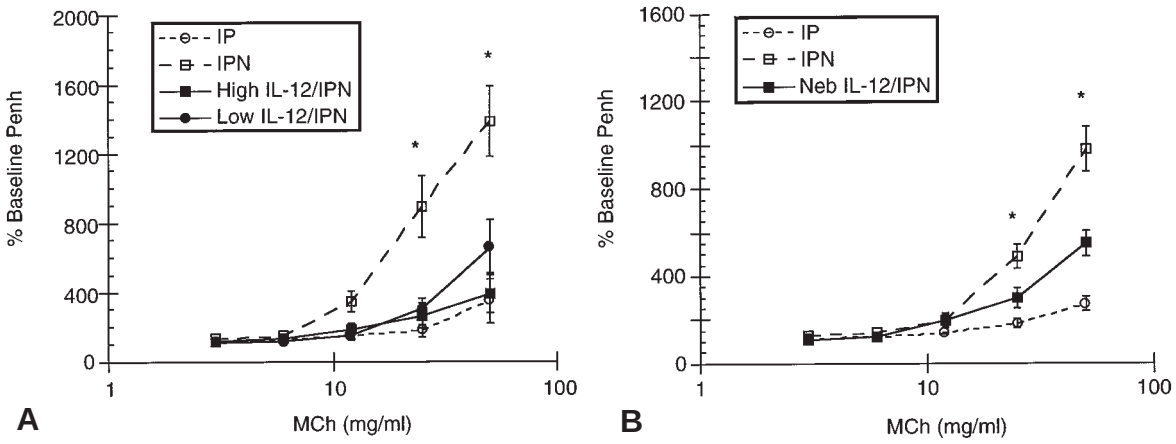

FIG. 1. Local administration of IL-12 before challenge prevents AHR. Mice were sensitized to OVA by 2 intraperitoneal injections (*IP, n* = 12) and subsequently challenged with nebulized OVA through the airways (*IPN, n* = 12). Two days before challenge and 2 hours before first and third of three challenges, IL-12 in a high (*High IL-12/IPN, n* = 12) or a low dose (*Low IL12/IPN, n* = 12) was administered intranasally under light anesthesia (**A**). In a different set of experiments, mice were exposed to aerosolized IL-12 (*Neb IL12/IPN, n* = 10) for 20 minutes daily 2 days before challenge and before each challenge (**B**). Fourty-eight hours after the last challenge, airway responsiveness to increasing concentrations of nebulized MCh (0 to 50 mg/mL) was assessed by barometric whole-body plethysmography, and Penh values were calculated. Means ± SEM of Penh values from 3 independent experiments are expressed as percentage of baseline Penh values observed after PBS exposure. Significant differences: *IPN versus all other groups; $P < .05$.

(Dynatech, Chantilly, Va) were coated with anti-IFN-γ (R4-6A2), anti-IL-4 (11B11) (both from Pharmingen, San Diego, Calif), or anti-IL-5 antibodies (TRFK-5) (kindly provided by Dr R. Coffman, DNAX, Palo Alto, Calif) and blocked with PBS/10% fetal calf serum overnight. Samples were added; biotinylated anti-IFN-γ (XMG 1.2), anti-IL-4 (BVD6-24G2), or anti-IL-5 antibodies (TRFK-4) (all from Pharmingen) were used as detecting antibodies; and the reactions were amplified with avidin–horseradish peroxidase (Sigma). Cytokine levels were calculated by comparison with known cytokine standards from Pharmingen. The limits of detection in the assays were 4 pg/mL for each cytokine.

Measurement of OVA-specific antibody levels

Total IgE levels and OVA-specific IgE, IgG1, and IgG2a antibody levels in the serum were measured by ELISA as previously described.[29] Briefly, Immulon 2 plates were coated with 5 μg/mL OVA. After addition of serum samples, a biotinylated anti-IgE antibody (02122D, Pharmingen) was used as the detecting antibody, and the reaction was amplified with avidin–horseradish peroxidase (Sigma). To detect IgG1 and IgG2a, alkaline phosphatase–labeled antibodies (02003 E and 02013 E, Pharmingen) were used. The OVA-specific antibody titers of samples were related to an internal pooled standard, which was arbitrarily assigned to be 100 ELISA units (EU).[7] The total IgE level was calculated by comparison with a known mouse IgE standard (Pharmingen). The limit of detection was 100 pg/mL for IgE.

Statistical analysis

Single pairs of groups were compared by Student's *t* test, and comparison of more than 2 groups was performed by the Tukey-Kramer HSD test. Probability *(P)* values for significance were set at 0.05. Values for all measurements are expressed as the mean ± standard deviation, except for values of airway responsiveness (Penh) and immunoglobulin levels, which are expressed as the mean ± SEM.

RESULTS

Local administration of IL-12 before allergen challenge prevents AHR

Sensitization and challenge with OVA resulted in altered airway responsiveness. Airway responsiveness to MCh was significantly increased in mice after sensitization and airway challenge; a 13.8 ± 2.1-fold increase in Penh values over the response to PBS was detected after inhalation of 50 mg/mL MCh (Fig. 1, *A*). Mice sensitized without challenge or challenged without sensitization (data not shown) showed little increase in Penh in response to MCh over the response to PBS. Administration of IL-12 with either a high or low dose prevented significant increases in airway responsiveness caused by challenge with OVA in sensitized mice. Similar treatment with PBS did not alter the response to MCh. In a separate set of experiments, treatment of sensitized mice with nebulized IL-12 before challenge significantly reduced the increases in airway responsiveness after allergen challenge (Fig. 1, *B*).

Local IL-12 treatment reduces eosinophil influx into the lung

Allergen challenge through the airways in sensitized mice resulted in a 3-fold increase in the number of lung eosinophils compared with those found in mice sensitized without challenge (Fig. 2, *A*). This increase was observed for both peribronchial and perivascular eosinophils; numbers of parenchymal eosinophils were increased as well but to a lesser degree (Fig. 2, *B* and *C*). IL-12 treatment at both concentrations significantly

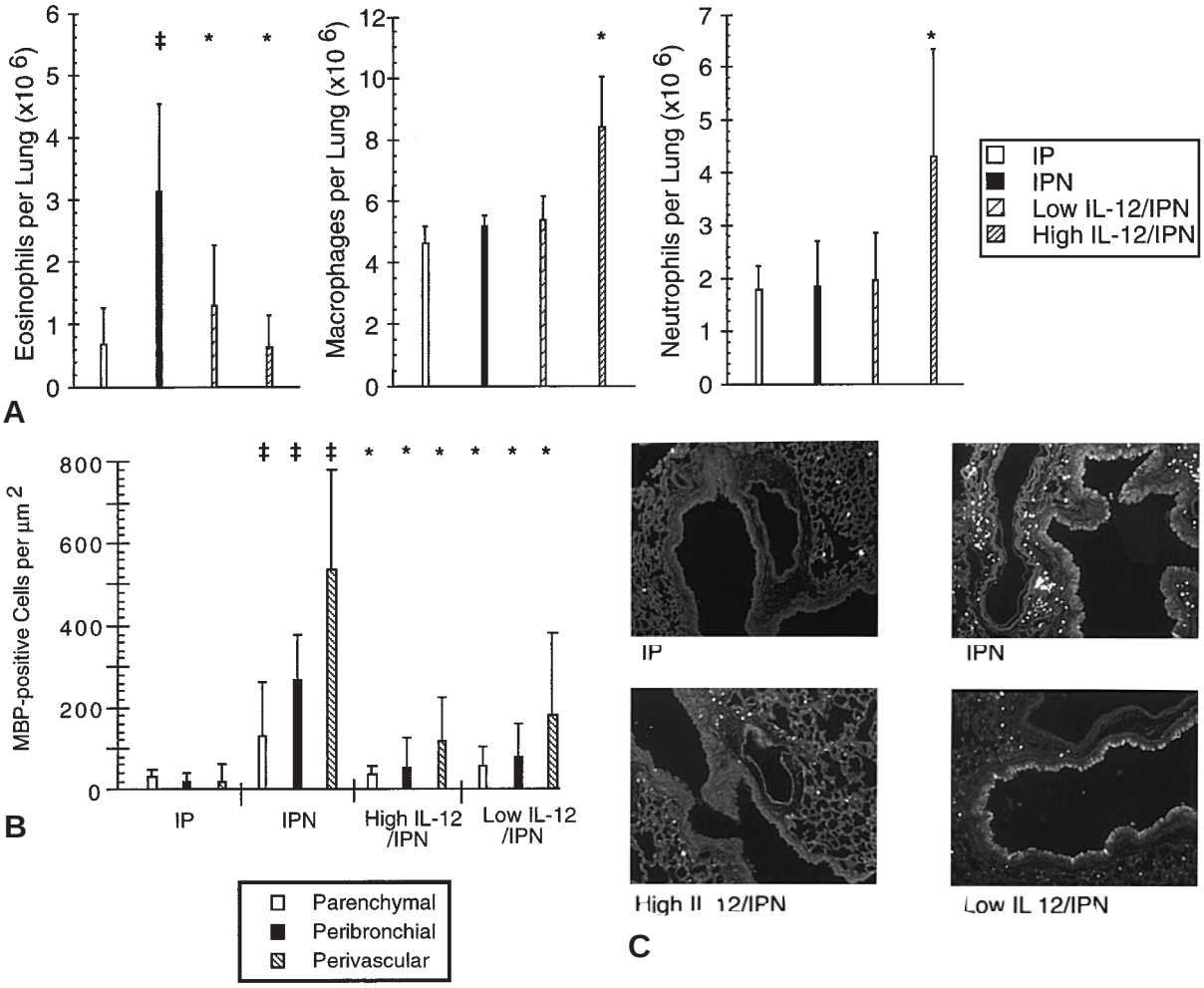

FIG. 2. Local IL-12 treatment reduces eosinophil influx into lungs. Lung cells were isolated from same mice described in Fig. 1, which were sensitized intraperitoneally to OVA alone (*IP, n* = 9), sensitized and challenged through the airways (*IPN, n* = 9), or sensitized and treated intranasally with high-dose (*High IL-12/IPN, n* = 9) or low-dose (*Low IL-12/IPN, n* = 9) IL-I2 before challenges. Numbers of eosinophils, neutrophils, and macrophages were determined (**A**). Remaining lungs (*n* = 3 per group) were fixed in formalin, and sections were stained with fluorescence-labeled anti-mouse MBP antibody. Numbers of eosinophils (MBP-positive) were analyzed separately in peribronchial, perivascular, and parenchymal tissue by using IPLab2 software. Three different sections were counted per animal (**B**). Representative images were photographed (**C**). Means ± SD of cell numbers from 3 independent experiments are shown. Significant differences: *IL-12/IPN versus IPN, ‡IPN versus IP; $P < .05$.

reduced eosinophil infiltration in each of these three compartments, accounting for the reduction in total number of lung eosinophils. In mice treated with PBS, no such effect was observed. The numbers of macrophages and neutrophils in the lungs increased significantly after treatment with high-dose IL-12, but this effect was not observed with the lower dose of IL-12. Treatment of mice with aerosolized IL-12 by inhalation also resulted in a significant reduction in lung eosinophil numbers from 4.5 ± 0.4 to 2.3 ± 0.5 × 10^6 per lung ($P < .05$, $n = 10$). Numbers of lung macrophages were significantly increased to 9.0 ± 1.0 from 6.0 ± 0.6 × 10^6 per lung ($P < .05$), whereas the number of neutrophils was unchanged.

High-dose IL-12 results in increased OVA-specific IgG2a levels

Intraperitoneal sensitization with OVA and subsequent challenge through the airways resulted in increased serum levels of total IgE and triggered the production of OVA-specific IgE and IgG1 antibodies but not of allergen-specific IgG2a. Administration of low-dose IL-12 or nebulized IL-12 to the airways did not significantly alter the serum levels of the immunoglobulins measured. In con-

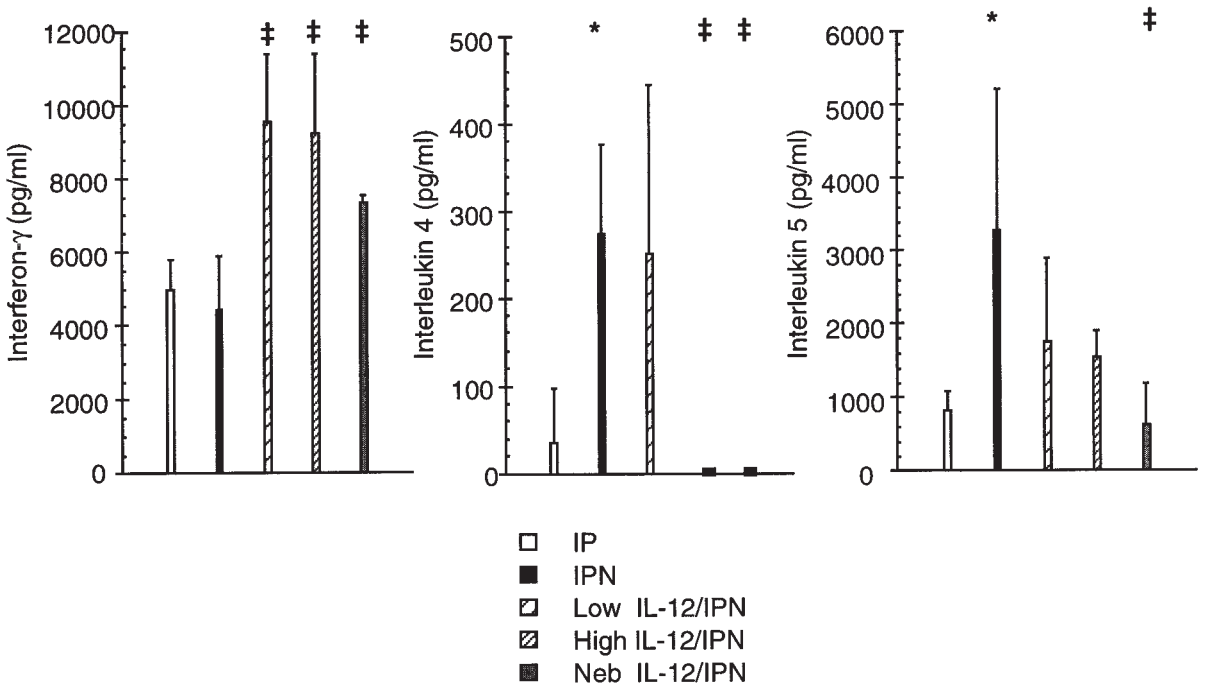

FIG. 3. IL-12 prevents increases in local T_{H2} cytokine production. PBLNs were harvested from the same mice described in Fig. 1, which were sensitized intraperitoneally to OVA alone (*IP, n* = 12); sensitized and challenged through the airways (*IPN, n* = 12); or sensitized and treated intranasally with high-dose (*High IL-12/IPN, n* = 12), low-dose (*Low IL-12/IPN, n* = 12), or nebulized IL-12 (*Neb IL-12/IPN, n* = 6) before challenge. Mononuclear cells were isolated and cultured in the presence of P/I for 48 hours. Concentrations of IFN-γ, IL-4, and IL-5 were assessed in culture supernatants by ELISA. Means ± SD of these concentrations from 3 independent experiments are shown (2 for Neb IL-12/IPN). Significant differences: *IP versus IPN, ‡IL-12/IPN versus IPN; $P < .05$.

trast, treatment with high-dose IL-12 resulted in lower OVA-specific IgE levels and a significant increase in OVA-specific IgG2a levels (Table I).

IL-12 prevents increases in T_{H2}-type cytokine production

Cytokine production was assessed in cultures of PBLN cells stimulated with the combination of P/I for 48 hours. After airway challenge with OVA in sensitized mice, a shift to the production of T_{H2}-type cytokines was observed (Fig. 3). The levels of IL-4 and IL-5 in supernates of P/I-stimulated cultures were significantly increased, and IFN-γ levels were unchanged when compared with cultures of PBLN cells obtained from mice that were sensitized but not challenged. Treatment of the airways with both high and low-dose IL-12 and with nebulized IL-12 resulted in a significant increase in IFN-γ production, and IL-5 production was decreased. IL-4 production was unchanged by low-dose IL-12 but was abolished by high-dose IL-12 and nebulized IL-12.

High-dose, but not low-dose, IL-12 results in systemic effects

Allergen challenge in sensitized mice resulted in a 2-fold increase in the number of bone marrow eosinophils. Airway administration of low-dose IL-l2 did not have a significant effect on this increase, whereas high-dose IL-12 prevented the increase in numbers of bone marrow eosinophils after allergen challenge (Table II).

Extramedullary hematopoiesis with splenomegaly is a well recognized side effect of systemic IL-12 treatment. Splenomegaly was observed only after high-dose IL-12 treatment. Spleen weights after this treatment were increased significantly compared with sensitized and challenged mice. In contrast, low-dose IL-12 did not alter spleen weights (Table II). After treatment with nebulized IL-12, spleen weights were also unchanged (data not shown).

DISCUSSION

In this study we monitored airway responsiveness in mice sensitized and challenged by repeated exposure to aerosolized allergen. This approach was used to assess the influence of local (airway) IL-12 administration on allergic airway inflammation and AHR after antigen challenge. Mice sensitized to OVA were treated intranasally with either low or high-dose IL-12 2 days before challenge and 2 hours before the first and third of three airway challenges with OVA. In a second approach mice were exposed to aerosolized IL-12 2 days before allergen challenge and before each challenge. Airway responsiveness to aerosolized MCh was assessed by using barometric whole-body plethysmography in unrestrained, spontaneously breathing animals. Effects of IL-12 on pulmonary inflammatory changes, cytokine production in the local draining lymph nodes of the lung, the PBLNs, and serum levels of allergen-specific immunoglobulins were also monitored. In addition, numbers of

TABLE I. High-dose IL-12 results in increased OVA-specific IgG2a levels

Group	Total IgE (ng/mL)	OVA-specific IgE (EU/mL)	OVA-specific IgG1 (EU/mL)	OVA-specific IgG2a (EU/mL)
NS	13.9 ± 1.8	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
IP	15.4 ± 1.5	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
IPN	28.1 ± 1.9*	98.1 ± 35.7*	51.1 ± 10.7*	3.9 ± 2.7
High IL-12/IPN	34.7 ± 3.4	28.3 ± 11.5	63.2 ± 17.4	43.1 ± 27.8†
Low IL-12/IPN	27.6 ± 3.8	82.9 ± 47.1	79.3 ± 25.1	2.9 ± 2.9
Neb IL-12/IPN	27.9 ± 3.5	29.1 ± 14.3	75.4 ± 19.4	0.0 ± 0.0

Serum was collected 72 hours after the last challenge from nonsensitized mice (*NS*, $n = 12$) or mice sensitized intraperitoneally to OVA alone (*IP*, $n = 12$); sensitized and challenged through the airways (*IPN*, $n = 12$); or sensitized and treated intranasally with high-dose (*High IL-12/IPN*, $n = 12$), low-dose (*Low IL-12/IPN*, $n = 12$), or nebulized (*Neb IL-12/IPN*, $n = 10$) IL-12 before the challenges. Concentrations of total IgE and OVA-specific IgE, IgG1, and IgG2a were assessed by ELISA. Expressed are the means ± SEM of immunoglobulin levels from 3 independent experiments.
*Significant differences ($P < .05$) between IP and IPN.
†Significant differences ($P < .05$) between High IL-12/IPN and all other groups.

TABLE II. High-dose, but not low-dose, IL-12 results in systemic side effects

Group	Bone marrow eosinophils (× 10⁶ per femur)	Spleen weights (mg)
IP	0.45 ± 0.04	143.5 ± 16.2
IPN	1.1 ± 0.13*	144.5 ± 8.2
High IL-12/IPN	0.32 ± 0.06†	288.8 + 46.6†
Low IL-12/IPN	0.95 ± 0.1	146.3 ± 11.2

Bone marrow from the right femur and spleen were collected 72 hours after the last challenge from mice sensitized intraperitoneally to OVA alone (*IP*, $n = 6$), sensitized and challenged through the airways (*IPN*, $n = 6$), or sensitized and treated intranasally with high-dose (*High IL-12/IPN*, $n = 6$) or low-dose (*Low IL-12/IPN*, $n = 6$) IL-12 before the challenges. Spleens were weighed. Bone marrow was resuspended, cell numbers were counted, and eosinophil numbers were enumerated under light microscopy. Expressed are the means ± SD of numbers of bone marrow eosinophils ($\times 10^6$) per femur and of spleen weights from 3 independent experiments.
*Significant differences ($P < .05$) between IP and IPN.
†Significant differences ($P < .05$) between IPN and High IL12/IPN.

bone marrow eosinophils and spleen weights were recorded to assess systemic effects of the IL-12 treatment.

Airway challenge with OVA in sensitized mice resulted in increased responsiveness to MCh and the influx of eosinophils into the lung, with predominant accumulation in peribronchial and perivascular sites accompanied by an increase in numbers of eosinophils in the bone marrow. These changes were associated with increased levels of IL-4 and IL-5 production by cultured PBLN cells. Furthermore, substantial levels of OVA-specific IgE and IgG1 antibodies in the serum were also detected. These responses to airway challenge of sensitized animals parallel those reported in similar models of systemic sensitization to OVA and subsequent airway challenge.[27,32]

Intranasal administration of both low and high-dose IL-12 before and during the period of airway challenge prevented the development of AHR to MCh in this model and significantly reduced the number of eosinophils detected in the lungs, with the reduction being complete after high-dose IL-12 treatment. Administration of high-dose IL-12 also resulted in an increase in the numbers of lung macrophages and neutrophils as has been reported after systemic IL-12 treatment.[33] No such effect was observed after low-dose IL-12 treatment. Treatment of mice with nebulized IL-12 before and during challenge yielded parallel results; AHR to MCh and lung eosinophilia were significantly reduced and numbers of lung macrophages were increased. The amount of IL-12 absorbed in the lungs by aerosol inhalation is probably in the same range as the amount of IL-12 administered intranasally because generally only 1% of nebulized solutions is absorbed in the lungs.[34] Additionally, local IL-12 treatment resulted in increased IFN-γ production, and high-dose and nebulized IL-12 prevented the increases in IL-4 production. A reduction in IL-5 levels was also observed. These findings indicate that locally administered IL-12 can induce T_{H1} cytokine production (and as a consequence, increased antigen-specific IgG2a antibody levels) and, in turn, interfere with the production of T_{H2} cytokines after allergen challenge of sensitized mice. The incomplete attenuation of IL-4 and IL-5 increases in these mice in vitro may not reflect the more dramatic effects in vivo because we only studied the response of PBLNs to stimulation in vitro with the potent combination of P/I. The observations on cytokine production are similar to those made in studies of systemic treatment with IL-12. Gavett et al.[33] demonstrated upregulation of IFN-γ mRNA and downregulation of IL-4 and IL-5 mRNA in the lung, with parallel changes in protein levels in bronchoalveolar lavage fluid in mice that were treated with IL-12 during the period of allergen challenge. An upregulation of IL-10 mRNA, in addition to the upregulation of IFN-γ mRNA and the downregulation of IL-5 mRNA after IL-12 treatment during allergen challenge, has been reported by Sur et al.[17]

The mechanism by which IL-12 administered to the respiratory tract prevents AHR and reduces eosinophil

influx after allergen challenge is not fully understood. IFN-γ, particularly in the local environment in contrast to systemic administration, has been shown to reduce allergic inflammation, specific IgE antibodies, and AHR in allergen-challenged mice.[7,8] It is not surprising then that local IL-12 has similar effects because it results, as we show, in increased IFN-γ production accompanied by increases in IgG2a, a direct effect of IFN-γ.[35] IL-12 promotes and facilitates T_{H1} responses by stimulating the differentiation of naive T_{H0} cells into T_{H1} cells[13,14] and serves as a strong costimulator of activated T_{H1} cells for maximum IFN-γ secretion.[9,12] Allergen challenge of sensitized mice triggers a predominant T_{H2} response as demonstrated here and elsewhere.[17,33,36,37] The mechanism whereby IL-12 shifts the balance to a T_{H1} response is slowly being unraveled. The IL-12 receptor is not present on naive T cells, but low levels of expression are induced after antigen stimulation.[38] The β2 chain of the IL-l2 receptor is the signal transducing component,[39] inducing phosphorylation of the transcription factor Stat 4 in T_{H1}, but not T_{H2}, cells.[39] Even under conditions that promote a predominantly T_{H2}-type response, IL-12 can induce β2 chain expression,[40] thus favoring a T_{H1} over a T_{H2} response. Presumably, manipulation of IL-12 receptor β2 expression by IL-12 in the local milieu of the lung in our studies was sufficient to induce or permit the emergence of the predominance of T_{H1} responses as demonstrated by the increases in IFN-γ and decreases in IL-4 and IL-5. The consequence of this shift was a decrease in eosinophil numbers and normalization of airway function.

Because of the toxicity associated with systemic administration of IL-12,[20-24] we determined whether there were systemic effects associated with local administration of IL-12 to the airways. To this end, spleen weights as an indicator of extramedullary hematopoiesis and numbers of eosinophils in the bone marrow were monitored. High-dose IL-12 resulted in significant increases in spleen weights and prevented the increase in numbers of eosinophils in the bone marrow after allergen challenge. In contrast, such changes were not observed after administration of low-dose IL-12. Treatment with nebulized IL-12 did not result in increased spleen weights either. These findings suggest that low-dose IL-12 administered nasally or by means of nebulization does in effect exhibit only local effects, whereas administration of high-dose IL-12 to the respiratory tract elicits a combination of local and systemic effects. Low-dose IL-12 in the airways nevertheless was still effective in preventing the development of AHR and suppressing eosinophil influx into the lung after allergen challenge of sensitized mice.

In summary, we present a murine model of airway sensitization to allergen in which local treatment of the airways with IL-12 before and during the period of allergen challenges prevents the development of AHR, reduces pulmonary eosinophilic inflammation, and increases local T_{H1} cytokine production. These findings demonstrate that local IL-12 treatment without the adverse effects associated with systemic IL-12 can modify the respiratory and immunologic consequences of airway challenge in sensitized mice. Because of its critical role in balancing T_{H1} responses in situations normally dominated by T_{H2} responses, local administration of IL-12 should prove useful in modulating allergic responses in the airways and could find application in the prevention and treatment of asthma.

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