

Original article

IL-12 regulates bone marrow eosinophilia and airway eotaxin levels induced by airway allergen exposure

Background: Airway allergen exposure causes local eosinophilic cell infiltration. This cellular inflammatory response is likely to involve the release of eosinophils from peripheral storage pools, and possibly also regeneration of eosinophils in the bone marrow. IL-12 is an inhibitory cytokine in allergic inflammation, shown to reduce eosinophilic cell infiltration. The aim of the present study was to determine whether airway allergen exposure increases bone marrow eosinophil production, and, if so, whether IL-12 modulates this effect.

Methods: Ovalbumin-sensitized C57BL/6 mice and IL-12 knockout (KO) mice were exposed to allergen via the airway route, and the inflammatory cell response was evaluated in bronchoalveolar lavage fluid, blood, and bone marrow.

Results: Allergen instillation intranasally produced a dose-dependent inflammatory response in the lower airways of sensitized mice. This inflammatory response was dominated by eosinophils, but there were also increases of both lymphocytes and neutrophils. Sensitization and airway allergen exposure also increased the IL-5-dependent growth of bone marrow cells *in vitro*. The enhanced bone marrow responsiveness *in vitro* was paralleled by an increased number of bone marrow eosinophils *in vivo*. After sensitization and repeated allergen exposure, IL-12 KO mice showed higher eosinophil levels in both BAL and bone marrow than parallel wild-type control mice. Furthermore, BAL-eotaxin levels were increased in IL-12 KO mice as opposed to parallel wild-type controls after allergen exposure.

Conclusions: Airway allergen exposure induced systemic immunologic responses, including increased eosinophil numbers in both airways and bone marrow, and also enhanced IL-5 responsiveness in bone marrow cells. IL-12 may regulate airway eosinophilia at both the level of eosinophilopoiesis and the level of local recruitment of eosinophils into the airways.

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Airway allergen exposure causes airway eosinophilia in allergic asthmatic patients, as well as in sensitized experimental animals (1–7). This airway inflammation is associated with an increased number of circulating eosinophils, presumably due to the release of eosinophils from peripheral storage pools of cells, and increased production of eosinophils in the bone marrow (3). However, the mechanisms behind these phenomena are poorly understood.

In contrast to the eosinophilia-promoting effects of Th2 cytokines, treatment of animals with IL-12 inhibits Th2 cytokine synthesis *in vitro* and *in vivo* (8–12). It has been proposed that IL-12 plays a critical role in determining the balance between Th1 and Th2 responses, thereby inhibiting allergic inflammation. Thus, in mice, recombinant IL-12 treatment during active sensitization reduces allergen-induced eosinophilic

inflammation, and inhibits the development of a Th2 response (13, 14). IL-12 production seems to be reduced in whole-blood cultures from patients with asthma compared with normal controls (15), and there is a reduction of expression of IL-12 mRNA in airway biopsies from patients with asthma compared with normal subjects (16).

Eotaxin is a highly selective mediator regulating eosinophil chemotaxis in tissues undergoing allergic reaction (5, 17). Eotaxin is expressed in many different tissues, and may therefore regulate allergen-induced homing of eosinophils to the site of inflammation.

The main aim of the current study was to determine whether repeated airway allergen exposure increases eosinophil production in the bone marrow in C57BL/6 mice, and, if so, whether this increase is associated with an enhanced *in vitro* bone marrow response to the

eosinophilopoietic cytokine IL-5. Furthermore, we used IL-12-deficient mice for similar experiments to determine whether IL-12 is involved in such regulation of bone marrow eosinophilia.

Material and methods

Animals

Male C57BL/6 mice 6–8 weeks of age (for animal numbers, see challenge protocol) were purchased from B&K Universal AB (Sollentuna, Sweden). IL-12 knockout (KO) mice (6–8 weeks of age) were originally kindly donated by Dr M. K. Gately (Department of Inflammation/Autoimmune Diseases, Hoffmann-La Roche, Inc., Nutley, NJ, USA). The animals were housed in a room with a 12-h light-dark cycle and were allowed food and water *ad libitum*. In the room, all cages, food, and bedding were autoclaved. The study was approved by the Animal Ethics Committee in Göteborg, Dnr 278/1996.

Sensitization

Mice were intraperitoneally sensitized twice (on days 0 and 7) with 10 µg chicken egg ovalbumin (OA) (Sigma Chemical Company, St Louis, MO, USA) adsorbed to 1 mg aluminum hydroxide Al(OH)₃, suspended in 0.5 ml PBS. Control mice were injected with 1 mg Al(OH)₃ mixed with 0.5 ml PBS.

Antigen exposure

The mice were slightly anesthetized with CO₂ (AGA Gas AB, Gothenburg, Sweden), and the antigen was instilled into the nostrils with a micropipette. This procedure leads to distribution of antigen into the trachea and bronchial tree, as proven by evaluation of distribution of instilled Evans blue dye.

Exposure protocol

Protocol 1. This experiment was designed to compare the effect of different allergen doses on allergen-induced inflammatory responses. C57BL/6 mice were divided into six groups ($n=9$ –15 per group). Five of these groups were sensitized to OA adsorbed to 1 mg Al(OH)₃ in a volume of 0.5 ml PBS per animal, and one group was injected with Al(OH)₃ suspended in 0.5 ml PBS only. Three groups of mice were on 5 consecutive days given different doses of intranasally instilled OA in 50 µl PBS (1, 10, and 100 µg). The fourth group of sensitized mice was exposed four times to PBS only, and once (day 5) to OA (100 µg). The fifth group of sensitized mice was exposed five times to PBS. Nonsensitized mice were exposed to intranasally instilled OA (100 µg) in the same manner.

Protocol 2. This experiment was designed to determine whether the effect of prolonged exposure to allergen could further enhance the allergen-induced inflammatory responses. Mice were divided into four groups ($n=5$ per group). Three of these groups were sensitized to OA, and one was given Al(OH)₃ suspended in 0.5 ml PBS only. The OA dose was 100 µg, and all exposures were given intranasally. The first group of sensitized mice was given PBS for 20 days (5 days per week), the second group was given PBS for 15 days and then OA for 5 days (the last week), and the third group was given OA for 20 days (5 days per week). The nonsensitized group of mice was given OA for 20 days (5 days per week). Instillation was performed as in protocol 1.

Protocol 3. This experiment was designed to determine whether IL-12 regulates allergen-induced bone marrow eosinophilia. IL-12 KO mice, and parallel wild-type control mice (C57BL/6) were each divided into three groups ($n=5$ –8 per group). All six groups of mice were sensitized to OA. One group of the IL-12 KO mice and one group of the wild-type mice were given 10 µg of OA intranasally in 50 µl PBS on 5 consecutive days. One group of the IL-12 KO mice and one group of the wild-type mice were given 100 µg of OA intranasally in 50 µl PBS on 5 consecutive days. One group of the IL-12 KO mice and one group of the control mice were exposed five times to PBS. Instillation was performed as in protocol 1.

All mice were killed 24 h after the last administration of OA or PBS. The mice were deeply anesthetized by an i.p. injection of ketamine 50 mg kg⁻¹ (Park-Davis Scandinavia, Stockholm, Sweden) and xylazine 5 mg kg⁻¹ (Bayer Sverige AB, Gothenburg, Sweden).

Bronchoalveolar lavage (BAL)

The trachea was carefully exposed surgically, and a thin polyethylene tracheal cannula (outer diameter 1.34 mm) was inserted into the trachea. Bronchoalveolar cells were collected in successive lavages using 4 × 1 ml aliquots of PBS at room temperature. Recovered BAL fluid was pooled, and kept on ice until cytocentrifugation. The BAL fluid was centrifuged at 1200 rpm for 10 min at 4°C. The total number of cells in the BAL was counted with a standard hemocytometer (Bürker Chamber, Germany). Cytospin was made from cell preparation with a cytospin-equipped centrifuge (Eppendorf Centrifuge 5403; Hamburg, Germany) and stained with May-Grünwald-Giemsa. Differential cell counts were performed with at least 300 leukocytes. All differential counts used the coded slides; thus, the investigator was blinded to group.

Blood eosinophils

Blood was taken from the right ventricle of the heart, and a smear was made from each animal. The slides were dried in air for 1 h, fixed with CAM (a combination of citrate buffer, acetone (Histo-Lab, Göteborg Sweden), and methanol (Histo-Lab, Göteborg Sweden) for 5 min, and then rinsed in distilled water. Eosinophils were stained with Luxol fast blue (LFB Sigma, MO, USA) for 30 min and chloroacetate esterase (CAE, Sigma) for 15 min at 37°C. The staining shows eosinophils as bright blue-green, and neutrophils as purple-red. Counterstain with Mayer's hematoxylin was used. All slides were evaluated at $\times 1000$ magnification with a Zeiss microscope (Jena, Germany).

Bone marrow cell preparation

The right femur from each mouse was opened at the ends. A 27-G needle was inserted, the bone marrow was collected by flushing the femur with 4 ml of room temperature PBS, and the cells were then kept on ice until further preparation. Bone marrow cytocentrifugation, staining, and counting were performed similarly to BAL. Eosinophils were exclusively defined as cells containing eosinophilic granules. Immature eosinophils were defined as cells with eosinophilic granules, but immature nucleus structure (large and nonsegmented nucleus).

Bone marrow cell culture

Cells from one femur were diluted 1:4 in PBS, carefully placed on top of Ficoll-Paque gradient, and centrifuged at 400 *g* for 30 min at room temperature to remove most granulocytes and erythrocytes. The cells of the interface (mainly mononuclear cells) were carefully transferred to a new tube and washed three times in PBS supplemented with 0.5% BSA.

The mononuclear cells were seeded in a concentration of 0.5×10^6 /ml in 0.9% methylcellulose-enriched Iscoves medium (IMDM; GibcoBRL, UK) supplemented with 20% FCS, 1% Pen-Strep, and 10 mM mercaptoethanol in 12-well plates. An amount of 10 ng/ml (as an optimal dose) of mouse recombinant IL-5 (R&D Systems Ltd, Oxford, UK) was added to the medium as a specific growth factor for supporting proliferation and maturation of eosinophil colony-forming units from progenitor cells in the bone marrow. After 7 days in culture at 37°C, 7% CO₂ in a humid incubator, the number of colonies was counted. Only colonies containing at least 50 cells were counted. Three wells/animal were cultured. These cultures used cells acquired from animals exposed according to protocol 1.

IL-5 and eotaxin assay

ELISA kits for mouse IL-5 and eotaxin were purchased

from R&D Systems (Abingdon, Oxon, UK). The analysis was performed according to the manufacturer's instructions. The sensitivity of detection was 7.8 pg/ml for both IL-5 and eotaxin.

Data evaluation

All differential counts were performed with the slides coded; thus, the investigator was blinded to the treatment group. Data are reported as mean $\pm$ SEM, and were statistically analyzed with nonparametric analysis of variance (Kruskal-Wallis test). If a significant variance among groups was found, the Mann-Whitney U-test was applied to determine significant differences between individual groups. Data were analyzed by standard statistical packages on a Macintosh computer. The Spearman rank *R_s* test was used to evaluate dose-dependent effects of allergen. A *P* value of less than 0.05 was considered to be significant.

Results

Dose-dependent allergen-mediated inflammation

In C57BL/6 mice, airway allergen exposure by intranasal instillation caused a significant increase in the total number of BAL cells, as well as BAL eosinophils, lymphocytes, and neutrophils (Fig. 1). For eosinophils, this increase was dose dependent (*R_s* = 0.78 for number of eosinophils in relation to dose of allergen; *P* < 0.01).

Airway allergen exposure by intranasal instillation also increased blood eosinophils (Fig. 2) in a dose-dependent way.

Intranasal allergen exposure caused a dose-dependent increase in the relative number of bone marrow eosinophils (Fig. 3A) (*R_s* = 0.44 for percent eosinophils in relation to dose of allergen; *P* = 0.038). An evaluation of the maturity of the bone marrow eosinophils showed that airway allergen exposure increased the relative numbers of both immature (Fig. 3B) and mature eosinophils (Fig. 3A and B). A representative photomicrograph of a bone marrow cytospin in an allergen-exposed, sensitized group is shown in Fig. 4.

When the airway exposure to allergen was extended from 5 days to 4 weeks (five exposures per week), the effect on airway eosinophils was maintained (50.3 ± 39.8 vs $72.8 \pm 44.5 \times 10^4$ per animal), but there was no further enhancement of bone marrow eosinophilia (Table 1).

Bone marrow IL-5 colony growth

IL-5 produced a significantly higher number of bone marrow colonies in sensitized mice exposed to allergen than in sensitized mice exposed to PBS (Fig. 5).

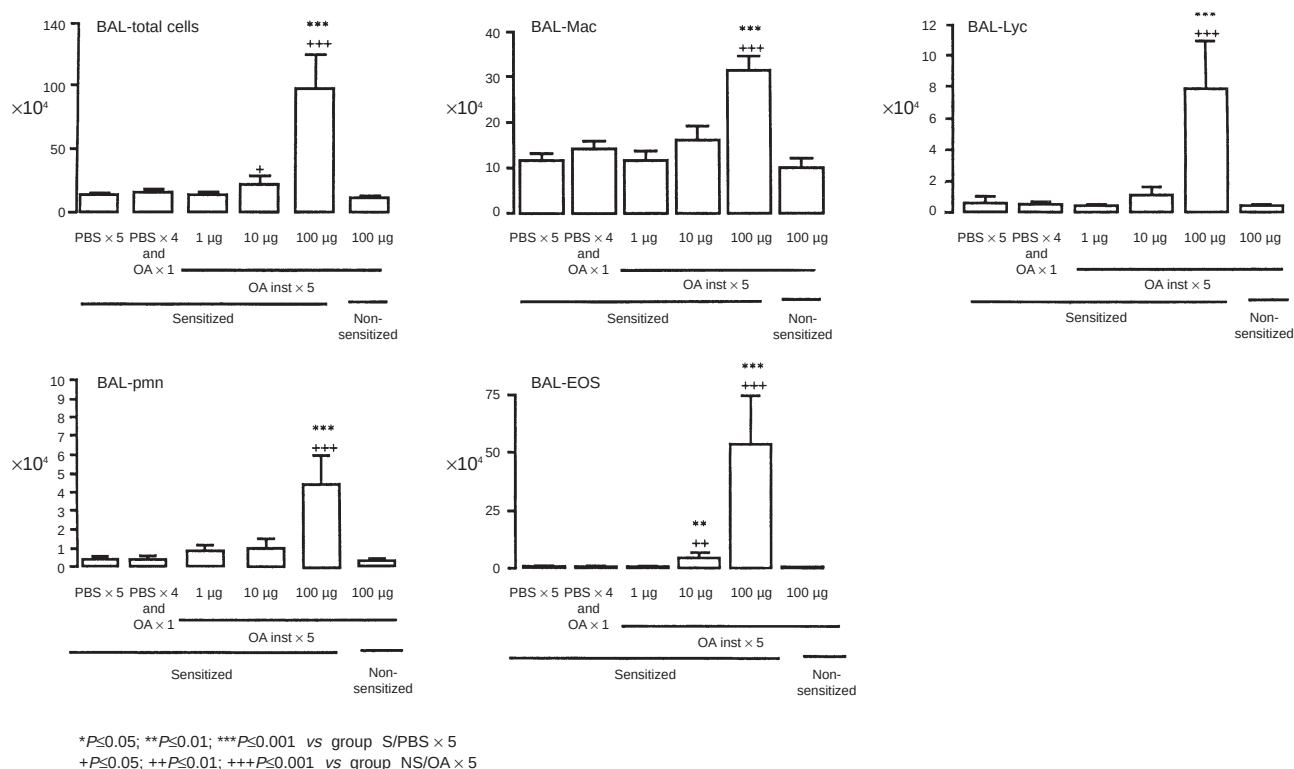


Figure 1. Total number of cells in BAL as well as total number of macrophages (Mac), lymphocytes (Lyc), neutrophils (pmn), and eosinophils (EOS) in OA-sensitized mice exposed on 5 consecutive days to either PBS or different doses of OA, instilled intranasally (1, 10, or 100 µg in 50 µl PBS). Data are shown as mean ± SEM (*n* = 9–15 per group). Kruskal-Wallis test showed significant variance among groups for BAL total cells, macrophages, lymphocytes, neutrophils, and eosinophils (*P* < 0.005); Mann-Whitney U-test, **P* = 0.05, ***P* = 0.01, ****P* = 0.001 vs sensitized PBS-exposed groups, and +*P* = 0.05, ++*P* = 0.01, +++*P* = 0.001 vs nonsensitized OA-exposed groups. inst: instilled.

IL-12 KO mice

In IL-12 KO mice, airway allergen exposure increased

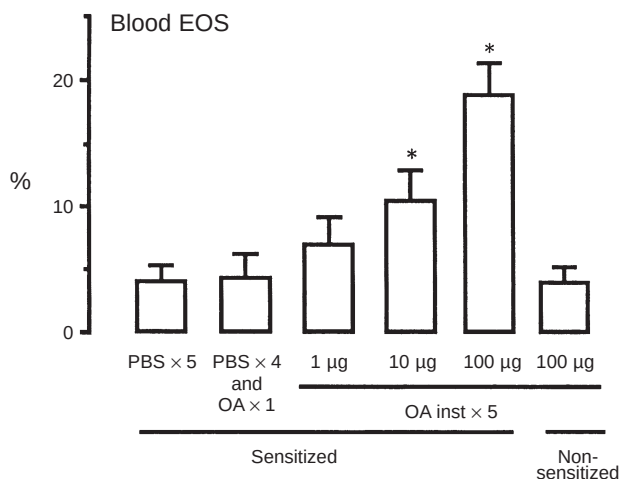


Figure 2. Percentage of blood eosinophils (EOS) in OA-sensitized mice exposed on 5 consecutive days to either PBS or different doses of OA instilled intranasally (1, 10, or 100 µg in 50 µl PBS). Data shown as mean ± SEM. Kruskal-Wallis test showed significant variance among groups (*P* < 0.05). Mann-Whitney U-test, **P* = 0.05 between individual groups. inst: instilled.

the number of eosinophils in airway (BAL), blood, and bone marrow in a more pronounced manner than in wild-type control mice (Fig. 6).

BAL eotaxin levels were significantly higher in IL-12 KO mice exposed to the lower dose of allergen (10 µg) than in parallel wild-type control mice (Fig. 7). However, serum and BAL levels of IL-5 were not significantly different between the two types of mice at either dose of allergen (Table 2).

Discussion

The present study demonstrates that repeated airway allergen exposure in sensitized mice caused a dose-dependent increase of several inflammatory cells in the airways, including macrophages, lymphocytes, neutrophils, and, most prominently, eosinophils. This allergen exposure caused a clear increase in the relative number of blood and bone marrow eosinophils. Furthermore, IL-12 KO mice displayed a more pronounced airway eosinophilia than parallel wild-type control mice, as well as a higher relative number of eosinophils in the bone marrow. In addition to this, IL-12 KO mice had higher levels of eotaxin in BAL after airway allergen exposure.

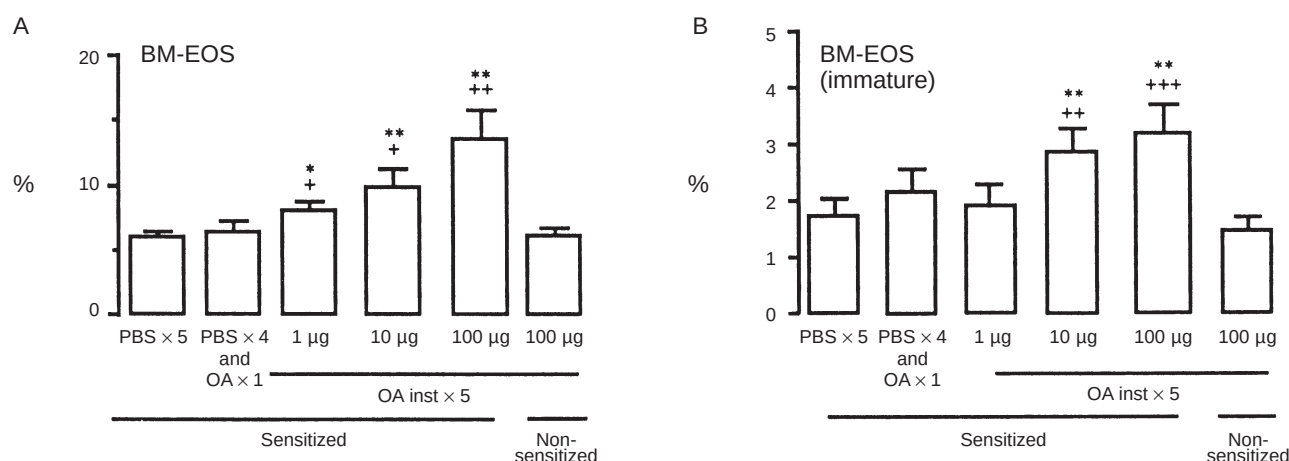


Figure 3. A) Relative number of bone marrow (BM) eosinophils (EOS), in OA-sensitized mice exposed on 5 consecutive days to either PBS or different doses of OA instilled intranasally (1, 10, or 100 µg in 50 µl PBS). Data are shown as mean ± SEM. Kruskal-Wallis test showed significant variance among groups ($P < 0.005$); Mann-Whitney U-test $*P = 0.05$, $**P = 0.01$, $***P = 0.001$ vs sensitized PBS-exposed groups, and $+P = 0.05$, $++P = 0.01$, $+++P = 0.001$ vs nonsensitized OA-exposed groups. B) Relative number of immature bone marrow eosinophils, in OA-sensitized mice exposed on 5 consecutive days to either PBS, or different doses of OA intranasally instilled (50 µl PBS or OA 1, 10, or 100 µg total dose). Data are shown as mean ± SEM. Kruskal-Wallis test showed significant variance among groups ($P < 0.01$). Mann-Whitney U-test $*P = 0.05$, $**P = 0.01$, $***P = 0.001$ vs sensitized PBS-exposed groups, and $+P = 0.05$, $++P = 0.01$, $+++P = 0.001$ vs nonsensitized OA-exposed groups. inst: instilled.

The parallel dose-dependent increase of eosinophils in airway, blood, and bone marrow after repeated airway allergen exposure suggests that the bone marrow is intricately involved in the inflammatory response of the airways; i.e., via enhanced eosinophilopoiesis. This conclusion, based on the higher number of eosinophils in bone marrow after allergen exposure, is supported by enhanced colony formation of cultured bone marrow mononuclear cells in response to IL-5. Thus, it is likely that the airway allergen exposure induces a chain of events, including enhanced bone marrow eosinophilopoiesis, release of these cells from bone marrow to blood, and subsequent traffic of these cells from the

blood into the lung tissue and airway lumen. This finding is supported by two previous studies that used allergen-exposed mice, showing

- 1) increased number of eosinophils in the bone marrow after airway allergen exposure (3)
- 2) increased colony formation of bone marrow cells *in vitro* after allergen exposure *in vivo* (4).

At a specific time, the number of eosinophils present in the bone marrow may depend on at least two factors: the release of cells into the blood and the local production of cells in the bone marrow (3). After single allergen exposure, a transient decrease in bone marrow eosinophils is seen for a very short time, suggesting a release of bone marrow eosinophils into the blood (3). However, in the current experiments

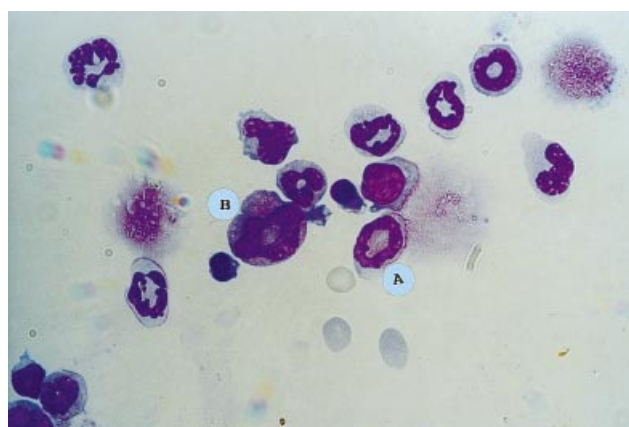


Figure 4. Representative photomicrograph of bone marrow cytocentrifuge preparations from OA-exposed animal (100 µg × 5) stained with May-Grünwald Giemsa (×1000). A) mature eosinophil; B) immature eosinophil.

Table 1. Total number of BAL macrophage (Mac), lymphocytes (LYM), neutrophils (pmn), and eosinophils (EOS), and relative number of bone marrow eosinophils in OA-sensitized mice challenged either 20 times with PBS, 15 times with PBS, and five times with OA, or 20 times with OA (100 µg, 50 µl per challenge)

	Sensitized			Nonsensitized OA × 20
	PBS × 20	PBS × 15 + OA × 5	OA × 20	
BAL total cells × 10 ⁴	17 ± 4	15 ± 4.3	142 ± 93*	23 ± 4
BAL EOS × 10 ⁴	0.11 ± 0.06	3.89 ± 3	72.8 ± 44.5**	2.9 ± 1.4
BAL LYM × 10 ⁴	0.11 ± 0.04	0.3 ± 0.1	8.19 ± 7.3	1.6 ± 0.75
BAL pmn × 10 ⁴	0.25 ± 0.07	0.42 ± 0.2	18.39 ± 16.7	1.28 ± 0.4
BAL Mac × 10 ⁴	16.18 ± 3.7	10.65 ± 1.2	41.55 ± 25.1	17.01 ± 2.1
BM EOS %	5.95 ± 1.2	9.31 ± 2.9	18 ± 4.4*##	5.95 ± 0.65

Data are shown as mean ± SEM.

* $P \leq 0.05$; ** $P \leq 0.01$; vs S/PBS × 20.

$P \leq 0.05$; ## $P \leq 0.05$; vs NS/OA × 20.

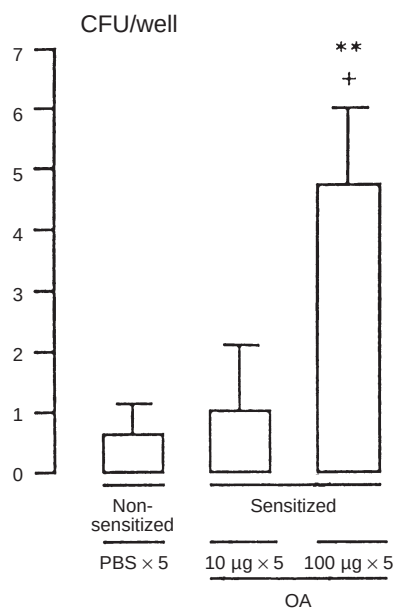


Figure 5. Number of colonies in semisolid bone marrow cultures from sensitized mice exposed on 5 consecutive days either to PBS or to two different doses of OA (10 or 100 µg). Data shown as mean ± SEM. Kruskal-Wallis test showed significant variance among groups ($P < 0.05$). Mann-Whitney U-test $*P = 0.05$ between individual groups. CFU: colony-forming units.

using repeated airway allergen exposure, it is likely that both eosinophilopoiesis and eosinophil release from the bone marrow are enhanced 24 h after the last allergen exposure. Thus, an increase of bone marrow eosinophils might mean that the production of cells is more rapid than the release of these cells into the blood. In support of this, we found an increased number of eosinophils in the blood after the highest dose of allergen. In one of the experiments, we extended the period of allergen exposure to 4 weeks, in order to determine whether any additional increase of bone marrow eosinophils would be induced. However, no further increase was found, suggesting a steady state between eosinophil production and eosinophil release into the blood already induced after allergen exposures on 5 consecutive days.

The finding that repeated allergen challenge pro-

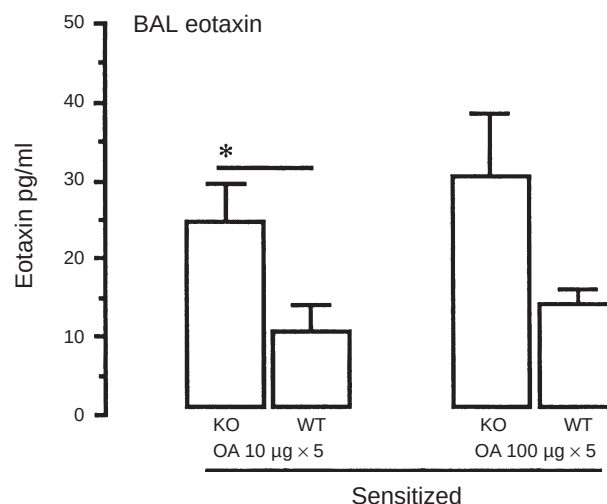


Figure 7. Eotaxin level in BAL from sensitized IL-12 KO mice and their control mice (WT) (C57BL/6) exposed on 5 consecutive days to different doses of OA intranasally instilled (10 and 100 µg daily dose). Data shown as mean ± SEM. Kruskal-Wallis test showed significant variance among groups ($P < 0.05$). Mann-Whitney U-test $*P = 0.05$ between individual groups.

duced a very clear increase in airway inflammatory cells, including eosinophils, lymphocytes, and neutrophils, is in line with previously published work (3, 18). According to our results, the dose-response curve for BAL eosinophils is steep, with a significant increase of different cell types at the two highest doses of allergen (10 and 100 µg × 5) only. However, bone marrow eosinophilia was increased already at the lowest dose of allergen (1 µg × 5), implying that the preceding bone marrow response to this dose of allergen was too weak to be reflected in the airway. Moreover, the mechanism that stimulates the bone marrow to produce eosinophils during repeated airway allergen exposure may be more sensitive than the mechanisms releasing eosinophils into blood and recruiting these cells to airways.

The previous studies have suggested that IL-12 regulates airway eosinophilia in response to airway allergen exposure. These conclusions are mainly based

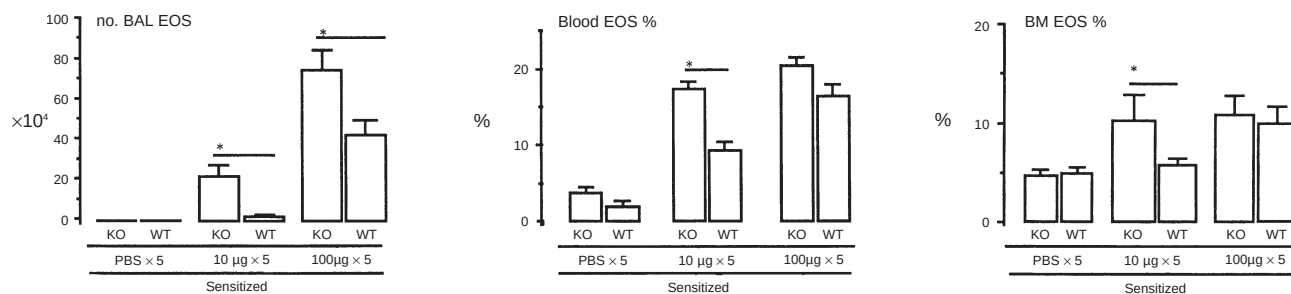


Figure 6. Number of eosinophils (EOS) in BAL, and percentage of eosinophils in blood and bone marrow from sensitized IL-12 KO mice and their control mice (WT) (C57BL/6) exposed on 5 consecutive days to different doses of OA intranasally instilled (10 or 100 µg total dose) and PBS. Data shown as mean ± SEM. Kruskal-Wallis test showed significant variance among groups ($P < 0.05$). Mann-Whitney U-test $*P = 0.05$ between individual groups.

Table 2. BAL and serum IL-5 levels in OA-sensitized IL-12 KO and control mice exposed on 5 consecutive days to OA instilled intranasally (10 or 100 μg per challenge)

	BAL		Serum	
	OA 100 $\mu\text{g} \times 5$	OA 10 $\mu\text{g} \times 5$	OA 100 $\mu\text{g} \times 5$	OA 10 $\mu\text{g} \times 5$
IL-12 KO	17.3 $\pm$ 5.1 pg/ml	13.0 $\pm$ 8.5 pg/ml	27.2 $\pm$ 13.9 pg/ml	17.0 $\pm$ 10.2 pg/ml
Wild-type	10.2 $\pm$ 4.5 pg/ml	0 $\pm$ 0 pg/ml	17.8 $\pm$ 7.5 pg/ml	10.8 $\pm$ 10.8 pg/ml

Data are shown as mean $\pm$ SEM, $n=5-8$ per group.

on experiments using sensitized mice. Firstly, i.p. administration of IL-12 abolishes the airway hyper-responsiveness and pulmonary eosinophilia induced by allergen, promotes an increase in interferon-gamma (IFN- γ) production, and decreases IL-4 and IL-5 expression (13, 19, 20). Secondly, allergen-induced airway eosinophilia is enhanced in IL-12 KO mice (21). Furthermore, airway administration of high doses of IL-12 prevents the increase in numbers of bone marrow eosinophils after allergen challenge (22).

Our study now confirms that IL-12 KO mice have increased number of eosinophils in airways after airway allergen exposure compared with wild-type control mice. This is evident at both doses of allergen (10 and 100 $\mu\text{g} \times 5$). In addition to the previous experiments, we now show that the number of eosinophils in the bone marrow is higher in IL-12 KO mice, a fact which is obvious with the lower dose of allergen. Thus, the bone marrow eosinophilopoiesis is enhanced *in vivo* in IL-12 KO mice compared with wild-type mice, further suggesting that IL-12 regulates eosinophilia at this level. This enhancement is in line with the regulatory effects of IL-12 on the Th2 subset (21), since T cells have been found to be involved in bone marrow eosinophilopoiesis (23, 24). In addition to an increased number of eosinophils in the bone marrow, IL-12 KO mice had higher levels of eotaxin in BAL fluid than wild-type mice given the same dose of allergen. This suggests that IL-12 regulates allergen-induced eosinophilia at several levels, including both bone marrow eosinophilopoiesis and local eosinophil chemotaxis in airway tissue.

According to previous studies, the local effects of IL-12 in the airways may also involve regulatory effects on IFN- γ , IL-4, and IL-5 (13, 18, 19). However, we have not been able to document any differences in IL-5 levels in either BAL or serum in the allergen-exposed IL-12 KO mice. This result is similar to that of another

recently published study, in which no difference in levels of IL-5 in the airways between IL-12 KO mice and wild-type mice could be detected (25).

IL-12 is produced predominantly by macrophages and dendritic cells. It promotes the proliferation of activated T cells and NK cells, and is considered to be the most potent inducer of IFN- γ production in these cells, and some effects of IL-12 are considered to be IFN- γ -dependent. Perhaps one of the most important regulatory effects of IL-12 is its ability to influence the balance between Th1 and Th2 cells. IL-12 selectively promotes the differentiation of Th1 cells and thus promotes cell-mediated immunity, but it suppresses Th2-dependent functions, including the production of IL-4, IL-10, and IgE antibodies. However, the mechanisms of the regulatory effects of IL-12 on IgE production and allergen-induced eosinophilia may be different (26). The exact cellular and tissue location of the regulatory effect of IL-12 on allergen-induced bone marrow eosinophilia and airway eotaxin production is difficult to determine in this experiment *in vivo*. As judged by our recent experiments and by previously presented data, it seems likely that IL-12 regulates these events at several tissue levels, including airways, local lymph nodes, and the bone marrow (25).

In conclusion, the present study shows that repeated airway allergen exposure by intranasal instillation increases the eosinophil number in bone marrow in a dose-dependent fashion, in parallel with an increased number of eosinophils in blood and in the airways. This eosinophilia is associated with an enhanced bone marrow response *in vitro* to the eosinophilopoietic cytokine IL-5. IL-12 seems to regulate allergen-induced airway eosinophilia at several levels, including eosinophil production in the bone marrow and local eotaxin release into the airways.

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