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Eotaxin protein levels and airway pathology in a mouse model for allergic asthma

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Abstract

Eotaxin is a chemokine implicated in eosinophil trafficking and may be involved in the development of airway hyperresponsiveness. The role of eotaxin in a mouse model for allergic asthma was investigated. Challenging ovalbumin-sensitised mice with ovalbumin aerosol leads to airway hyperresponsiveness and airway eosinophilia 24 h after the last challenge. Furthermore, eotaxin concentrations were markedly increased in lungs and broncho-alveolar lavage fluid of ovalbumin-challenged mice compared to vehicle treated mice. This could mean that eotaxin is implicated in the pathology of this model. To further investigate the role of eotaxin in this murine model for allergic asthma, the ovalbumin response was modulated by either treatment with eotaxin antibodies or additional eotaxin, to suppress or promote the development of airway hyperresponsiveness and inflammation. Administration of eotaxin antibodies or an additional intravenous eotaxin injection did not alter the development of ovalbumin-induced airway hyperresponsiveness and eosinophilia. In conclusion, eotaxin concentrations were increased in a murine model for allergic airway inflammation. However, anti-eotaxin antibodies or additive intravenous murine eotaxin did not influence airway inflammation and hyperresponsiveness in this mouse model for allergic asthma.

Keywords: Eotaxin; Allergic asthma; Eosinophils; Mouse model

1. Introduction

Airway inflammation is a key feature of allergic asthma (Busse, 1998). The late asthmatic phase reaction to an allergen often coincides with an increased number of eosinophils in the airways. In asthmatic patients increased numbers of these cells are detectable in the submucosa of bronchial biopsies, and in broncho-alveolar lavage fluid (Vignola et al., 1998). Whether the number of eosinophils is associated with disease severity is currently under debate. Some studies showed that there is a relationship between the number of eosinophils in the airways and the degree of airway hyperresponsiveness (another key feature of asthma). However, this association is weak, which might indicate that these phenomena might not be dependent on each other (De Bie et al., 1996; Oosterhout et al., 1995).

The mechanism underlying eosinophil migration to the airways remains intriguing. Chemokines are small inducible cytokines involved in trafficking and activation of leukocytes. Eotaxin (CCL11) is a chemokine which binds to the CC-chemokine receptor-3 (CCR3) (Kitaura et al., 1996). This receptor is present on eosinophils, Th2 cells, basophils and mast cells (Daugherty et al., 1996; Romagnani et al., 1999; Sallusto et al., 1997). Seeing that all these cells are important in asthma, it is believed that eotaxin is involved in the pathogenesis of this disease. Indeed, eotaxin and CCR3 mRNA and protein are up-regulated in epithelium, submucosa of asthmatic airways and also in eosinophils in sputum and eosinophil precursor cells in the bone marrow of asthmatic patients (Lamkioued et al., 1997; Yamada et al., 2000; Ying et al., 1997; Zeibecoglou et al., 1999). Eotaxin induces eosinophil migration in the direction of high eotaxin concentrations, both in vitro and in vivo (Griffiths-Johnson et al., 1993). Animal models indicated that eotaxin injection caused trafficking of these leukocytes to the site of injection. Interleukin-5 enhances the responsiveness of eosinophils to eotaxin (Collins et al., 1995).

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The objective of the present study was to assess whether eotaxin is implicated in allergen-induced airway inflammation and airway hyperresponsiveness. As a tool to investigate the development of these two phenomena, a murine model for allergic asthma was used. Mice were sensitised and challenged with ovalbumin, which leads to formation of asthma-like symptoms, e.g. increased levels of IgE, airway hyperreactivity to methacholine and serotonin, and influx of eosinophils to the airway lumen (Hessel et al., 1995). The first question to address was whether eotaxin protein levels are increased in the airways of mice sensitised and challenged with ovalbumin. Second, could treatment with anti-eotaxin antibodies prevent airway inflammation and hyperresponsiveness in ovalbumin treated mice? Third, does intravenous administration of eotaxin worsen asthma features in these mice?

2. Methods

2.1. Animals

In these experiments male specified pathogen-free BALB/c mice of 6–8 weeks of age were obtained from the Central Animal Laboratory at Utrecht University, The Netherlands. They were housed under controlled condition in macrolon cages containing 12 mice per cage. Water and standard chow was presented ad libitum. Dutch committee of animal experiments approved the experiments described in this paper.

2.2. Treatment

At 6–8 weeks of age, mice received intraperitoneal injection of 10-μg ovalbumin (grade V, Sigma, Zwijndrecht, The Netherlands) in 0.5-ml saline every other day, seven times. Four weeks after the last sensitisation, mice were challenged with ovalbumin aerosols (2 mg/ml, 5 min) for 8 consecutive days. Vehicle-treated animals were challenged with saline. Twenty-four hours after the last challenge, airway reactivity was measured, and blood and broncho-alveolar lavage was analysed.

In one set of experiments, mice were treated with rat neutralising anti-mouse eotaxin antibodies (MAB420, R&D, Abingdon, UK) during the challenge period 30 min before the challenge on days 0, 3 and 7 or days 5, 6 and 7 (Gonzalo et al., 1998). The antibodies were injected intravenously (20 μg/mouse/time). Rat Ig(immunoglobulin)G was used for control antibody treatment.

In another set of experiments additional recombinant murine eotaxin (500 ng/mouse, R&D) was injected intravenously 24 h after the last ovalbumin challenge. Thirty minutes, 5 h and 24 h after eotaxin injection airway responsiveness was measured. Twenty-four hours after eotaxin treatment, blood and broncho-alveolar lavage was taken.

Each group consisted of six mice, unless otherwise stated.

2.3. Eotaxin ELISA

Eotaxin was measured by enzyme-linked immunosorbent assay (ELISA) using matched-paired antibodies (R&D). A 96-well plate was coated with 4 μg/ml capture antibody (polyclonal goat IgG, 100 μl/well) in phosphate buffered saline (PBS) and incubated overnight. The next day the plate was washed three times with wash buffer (PBS, 0.05% Tween-20 (Sigma), pH 7.4). The wells were blocked for 1 h with 300-μl blocking buffer per well (PBS, 1% bovine serum albumin, 5% sucrose, 0.005% NaN_3). The samples were diluted once in assay diluent (tris-buffered saline, 0.1% bovine serum albumin, 0.05% Tween-20, pH 7.3). One hundred microliters of the diluted samples was pipetted into the wells after washing three times. Also, 100 μl of standard (1000–15.6 pg/ml murine eotaxin R&D in assay diluent) was added. After 2 h of incubation the plate was washed again followed by adding biotinylated detection antibody (polyclonal goat IgG, 100 μl/well, 180 ng/ml) to the wells. Again, after incubation for 2 h, the plates were washed with wash buffer. One hundred microliters of streptavidin–horseradish peroxidase (HRP) (0.125 mg/ml) was added to each well and incubated for 20 min. Following this, the plate was washed and 100 μl of substrate solution (TMB (3,3′,5,5′-tetramethylbenzidine 0.9 mg/ml) and hydrogen peroxide (0.003%); Pharmingen, Heidelberg, Germany) was added per well. After an incubation period of 20–30 min in the dark the reaction was stopped by adding 50 μl of H_2SO_4 in each well. The absorbency was read at 450 nm using a plate reader. The whole assay was performed at room temperature.

2.4. Airway responsiveness in vivo

Airway responsiveness was measured in mice using whole body plethysmography (Buxco, Sharon, CT, USA). In short, unrestrained, unanaesthetised mice were placed in a whole-body chamber. Airway responsiveness was measured by aerosolising increasing concentrations of methacholine (1.6 mg/ml saline to 50 mg/ml saline, Sigma) for 3 min in the chamber. Airway responsiveness was expressed as enhanced pause (Penh) (Hamelmann et al., 1997).

2.5. Blood

At several time points (2, 6, 18 or 24 h) after the last ovalbumin challenge, mice were killed with an overdose of pentobarbital (0.5 g/kg bodyweight). Blood was retrieved by heart punction. Blood (40 μl) was dissolved in 20-μl heparin (1000 U/ml). This was used for enumerating the number of leukocytes. One drop was smeared on a slide and stained with Diff Quick (Merz & Dale, Dudingen, Switzerland) for differential cell counts.

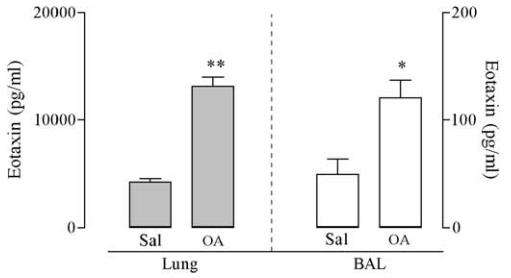

Fig. 1. Eotaxin concentrations in lung homogenates (grey bars, $n=5$) and broncho-alveolar lavage fluid (white bars, $n=6$) of mice sensitised with ovalbumin and challenged with saline (Sal) or ovalbumin (OA). Challenging mice with ovalbumin resulted in increased levels of eotaxin in both lung and broncho-alveolar lavage fluid. Data are represented as mean ± S.E.M. ($*P=0.0085$; $**P<0.0001$ compared to saline-challenged animals, Students *t*-test).

2.6. Broncho-alveolar lavage

A cannula was inserted in the trachea. The lungs were lavaged four times with 1-ml PBS (37 °C). The fractions were stored on ice. Thereafter, the fractions were centrifuged for 10 min at 300 × *g*. The supernatant of the first fraction was stored at − 70 °C for further analysis. The cells of all four fractions were pooled and resuspended in 100-μl PBS. These cells were enumerated and cytopsins were made. These cytospins were coloured with Diff Quick (Merz & Dale) for differential cell counts.

2.7. Lung isolation

Lungs were perfused by injection of 5-ml PBS into the right ventricle of the heart. Lungs were dissected and stored on ice. One milliliter of 1.5 M KCl was added per 0.10-g lung tissue. Thereafter, lungs were homogenised with a mixer and centrifuged at 580 × *g* for 10 min. The supernatant was stored at − 70 °C until further analysis.

2.8. Eosinophil peroxidase measurement

Samples were diluted once in 0.05 M Tris–HCl. In a 96-well plate 50-μl sample and 100-μl substrate solution (10 mM *o*-phenylenediamine dihydrochloride, 4 mM H_2O_2 in 0.05 Tris–HCl) were added. The plate was incubated at room temperature for 30 min in the dark. Thereafter, the reaction was stopped with 50-μl 4 M H_2SO_4. The absorbency was determined at 490 nm.

2.9. Statistical analysis

Data are represented as mean ± standard error of the mean (S.E.M.), unless stated otherwise. Differences in airway responsiveness were analysed using repetitive measures ANOVA (analysis of variance) followed by a least significant difference (LSD) post hoc test for multiple comparison. Data were analysed for normal distribution using Kolmogorov–Smirnov test. Normally distributed data were analysed with a one-way ANOVA using an LSD post hoc test; otherwise Kruskal–Wallis test was used. Correlation was demonstrated by way of linear regression. Differences were considered statistically significant if $P<0.05$.

3. Results

3.1. Eotaxin protein levels

In naïve mice, eotaxin protein was detected in broncho-alveolar lavage fluid and serum (403 ± 17.1 pg/ml eotaxin, 361 ± 13.6 pg/ml eotaxin, respectively).

Twenty-four hours after the last challenge, when ovalbumin-challenged mice displayed airway hyperresponsiveness and airway eosinophilia, eotaxin levels were significantly increased after ovalbumin challenge compared to saline challenge (Fig. 1).

As soon as 2 h after the last challenge, eotaxin levels were increased in ovalbumin-challenged mice compared to saline-challenged mice. This difference got larger in time at 6 and 18 h after the last challenge (Fig. 2).

Serum concentrations were comparable between saline- and ovalbumin-challenged mice. There was no correlation in ovalbumin-treated mice between eotaxin concentration in broncho-alveolar lavage fluid and the number of eosinophils in the airways ($r^2=0.017$, $P=0.804$) or airway responsiveness to methacholine ($r^2=0.051$, $P=0.67$).

3.2. Effect of anti-eotaxin antibodies

Challenging sensitised mice with ovalbumin resulted in increased airway responsiveness ($P<0.05$, Fig. 3) and

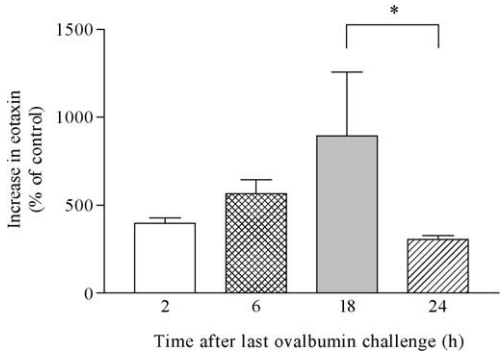

Fig. 2. Increase of eotaxin concentrations in lung homogenates at different time points (2 h ($n=5$), 6 h ($n=6$), 18 h ($n=4$), and 24 h ($n=6$)) after the last ovalbumin challenge compared to saline-challenged mice. As early as 2 h after the challenge, eotaxin levels are increased in ovalbumin-challenged mice compared to saline-challenged mice. Eotaxin levels in lung homogenates increased for up to 18 h after the challenge. At 24 h the increase in eotaxin levels decreased compared to 18 h ($*P<0.05$, ANOVA). Data are represented as mean ± S.E.M. All values were statistically significant compared to saline-challenged animals ($P<0.05$ Student's *t*-test).

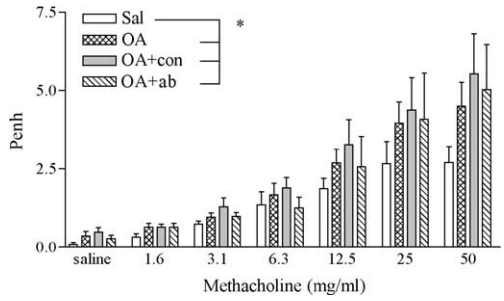

Fig. 3. Airway responsiveness to methacholine measured in mice sensitises with ovalbumin challenged with saline (Sal, white bars) or ovalbumin (OA, other bars) treated with rat IgG antibodies (Con, grey bars) or antibodies directed against eotaxin (ab, hatched bars). Challenge with ovalbumin resulted in airway hyperresponsiveness. This was not impeded with treatment of mice with anti-eotaxin antibodies. Data are represented as mean ± S.E.M., $n=6$ (* $P<0.05$ compared to ovalbumin-challenged mice, ANOVA).

increased numbers of eosinophils in broncho-alveolar lavage fluid compared to mice challenged with saline (Table 1). Treatment with antibodies directed to eotaxin did not result in a decrease in antigen-induced airway hyperresponsiveness (Fig. 3), nor did it result in a decreased number of eosinophils in the airways (Table 1). Furthermore, no difference in eosinophil peroxidase levels, which is a measure for eosinophil activation, could be demonstrated in the four different groups of mice (Table 1). As expected, an increase in total IgE (Table 1) and ovalbumin-specific IgE (data not shown) was increased in serum of mice challenged with ovalbumin compared to mice challenged with saline, which was not altered by treatment with anti-eotaxin antibody treatment.

3.3. Eotaxin administration

Since eotaxin can activate eosinophils and influence airway responsiveness, it was investigated whether an additional dose of eotaxin could worsen or enhance the asthma-like symptoms induced by ovalbumin. Intravenous injection of eotaxin 24 h after the last ovalbumin challenge did not result in increased airway responsiveness in saline-challenged mice at 30 min, 5 h and 24 h after eotaxin injection. In addition, airway hyperresponsiveness in ovalbumin-challenged mice was not enhanced by intravenous eotaxin administration (Table 2). Only the response to the highest dose of methacholine was increased in ovalbumin-treated animals 24 h after receiving eotaxin. Ovalbumin-challenged mice were hyperreactive compared to saline-challenged mice 30 min and 5 h after eotaxin administration. Airway hyperresponsiveness was not present at 48 h after the last challenge. This indicates that ovalbumin-induced airway hyperresponsiveness is time-dependent. Intravenous addition of eotaxin did not prolong airway hyperresponsiveness. Eotaxin injection did not affect the number of eosinophils in the blood or broncho-alveolar lavage fluid in saline- or ovalbumin-challenged animals. (Fig. 4).

Table 1
Effect of anti-eotaxin antibodies on the number of broncho-alveolar lavage cells, percentage of eosinophils, eosinophil activation and total IgE

Treatment	Total cells[a]	Eosinophils[b]	Eosinophil peroxidase[c]	Total IgE[d]
Sal	17.0 ± 2.30	0.10 ± 0.10	267 ± 96.0	163.8 ± 37.0
OA	55.7 ± 10.2[e]	49.4 ± 9.6[f]	309 ± 55.2	1736.0 ± 484.5[e]
OA + Con	45.0 ± 7.13	29.3 ± 2.9[f]	389 ± 81.7	1534.7 ± 479.0[e]
OA + Ab	63.3 ± 13.3[g]	52.3 ± 6.5[f]	361 ± 26.6	2405.5 ± 482.9[g]

Sal: saline-challenged animals; OA: ovalbumin-challenged animals; Con: control antibody (rat IgG); Ab: neutralizing antibody directed against eotaxin. Data are represented as mean ± S.E.M., $n=6$.

[a] $\times 10^4$ cells in broncho-alveolar lavage fluid.
[b] Percentage of eosinophils in broncho-alveolar lavage fluid.
[c] In lung homogenates (ng/ml).
[d] Total IgE in ng/ml.
[e] Statistical significance: $P<0.05$ (vs. saline challenge).
[f] Statistical significance $P<0.01$ (vs saline, Kruskal–Wallis).
[g] Statistical significance: $P<0.01$ (vs saline challenge, ANOVA).

Table 2
Maximal airway responsiveness to methacholine (E_{max}) of ovalbumin of saline-challenged mice after an additional dose of eotaxin

Treatment	0.5 h	6 h	24 h
Sal–vehicle	4.43 ± 0.88	4.57 ± 0.836	4.14 ± 0.24
Sal–eotaxin	4.52 ± 0.449	4.132 ± 0.831	3.17 ± 0.69
OA–vehicle	7.143 ± 1.640[a]	6.13 ± 0.99[a]	3.37 ± 0.23
OA–eotaxin	7.443 ± 1.54[a]	8.11 ± 1.27[a]	7.92 ± 1.92[b]

Sal: saline-challenged animals; OA: ovalbumin-challenged animals with additional treatment of intravenous injection of vehicle or eotaxin 24 h after the last challenge. Data are represented as mean ± S.E.M, $n=6$.

[a] Statistical significance: $P<0.01$ vs. saline-challenged animals (ANOVA on the whole methacholine curve).
[b] Statistical significance: $P<0.05$ vs. saline-challenged animals and ovalbumin receiving vehicle (ANOVA on maximal response to methacholine) (not significant if whole methacholine curve is considered).

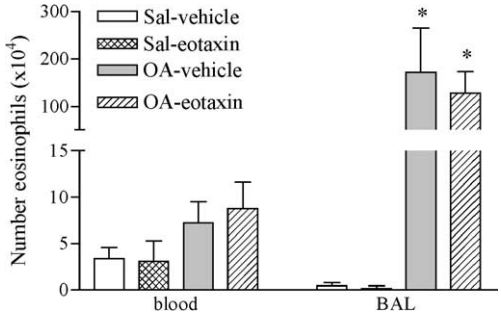

Fig. 4. Number of eosinophils in blood and broncho-alveolar lavage of ovalbumin-sensitised mice 24 h after eotaxin treatment, and 48 h after the last challenge with saline (Sal, white or crossed bars) or ovalbumin (grey or hatched bars) with an additional treatment with vehicle (white and grey bars) or eotaxin (500 ng/mouse, intravenously, crossed or hatched bars). Challenge with ovalbumin results in increased numbers of eosinophils in blood and broncho-alveolar lavage. However, intravenous injection with eotaxin did not increase the number of eosinophils in these compartments. Data are represented as mean ± S.E.M., $n=6$. (* $P<0.005$ compared to saline–vehicle and saline–eotaxin, ANOVA).

4. Discussion

The objective of this study was to investigate the role of eotaxin in airway inflammation and airway hyperresponsiveness. As a tool to investigate this, a mouse model for allergic asthma was used. Mice sensitised with ovalbumin were challenged with ovalbumin leading to the formation of asthma-like symptoms compared to saline challenged mice, e.g. airway hyperresponsiveness, increased number of eosinophils in the broncho-alveolar lavage and production of antigen specific IgE (Hessel et al., 1995). Using this model we demonstrated that (a) eotaxin levels were increased in the airways after ovalbumin challenge; (b) treatment with anti-eotaxin antibodies did not prevent ovalbumin-induced airway hyperresponsiveness and lung inflammation; (c) intravenous eotaxin instillation did not worsen or enhance airway pathology although there was a tendency for prolonged airway hyperresponsiveness in ovalbumin challenged mice after eotaxin administration.

Although eotaxin is produced under basal conditions, allergen challenge results in up-regulation of this protein production (Rothenberg et al., 1995). Our finding is consistent with other data that have shown before that allergen inhalation results in increased eotaxin mRNA and protein expression in mice, guinea pigs and humans (Gauvreau et al., 1999; Gonzalo et al., 1998; Humbles et al., 1997; Li et al., 1997; Minshall et al., 1997). The increase in eotaxin levels after allergen challenge is a rapid response, because we detected high eotaxin levels in ovalbumin-challenged mice 2 h after the last challenge. In our model, 24 h after allergen challenge, eotaxin concentrations in lung homogenates and broncho-alveolar lavage fluid were still more then doubled. This increase in protein coincides with an influx of eosinophils into the airway lumen. However, no correlation was observed between eotaxin level and the number of eosinophils in the broncho-alveolar lavage fluid or airway hyperresponsiveness, questioning the role of eotaxin in these two parameters.

The fact that we could not detect a direct correlation between airway responsiveness and eotaxin levels in the airways at 24 h after the last challenge might depend on the time of measurement. In humans, a correlation was found between eotaxin protein and the level of airflow obstruction at 4 h after allergen challenge (Brown et al., 1998). Possibly, at earlier time points eotaxin might have correlated with the degree of airway responsiveness in our animal model. For example, 18 h after the last challenge eotaxin levels were maximally increased.

From recent publications it appeared that eotaxin is involved in eosinophil recruitment early after allergen challenge and not in later stages. In an adoptive transfer model of Th cells, antibodies directed against eotaxin resulted in a decreased eosinophil accumulation in the airways at day 4 of repeated antigen challenge, but not at day 7 of ovalbumin challenge. In the same model blocking of monocyte-derived chemokine (MDC, a CCR4 agonist) resulted in a decreased airway inflammation at day 7 and not at day 4 (Lloyd et al., 2000). This indicates that different stages of eosinophil recruitment are regulated by distinct chemokines. Moreover, in a cutaneous model for allergic reactions, eotaxin concentrations correlated with the number of eosinophils at 6 h after allergen provocation, while at a later time point other chemokines were responsible for eosinophil migration (Ying et al., 1999). This illustrates that trafficking of eosinophils in response to antigen is a multistep process in which more chemokines are involved. In the present study, we looked at eosinophil recruitment after several allergen challenges. At this time point other chemokines might have attracted eosinophils to the airways. We did not measure eotaxin at earlier time points. Moreover, eotaxin knockout mice (ICR strain) have the same number of eosinophils in their airways after ovalbumin sensitisation and challenge compared to their normal littermates (Yang et al., 1998). This indicates that other factors contribute to eosinophil migration. For example, other chemokines can bind to CCR3 and eosinophils express other chemokine receptors. This redundancy and promiscuity of the chemokine system questions whether blocking one chemokine or one chemokine receptor is sufficient for abrogating migration of one cell type.

Although eotaxin can induce eosinophil activation in vitro (Griffiths-Johnson et al., 1993), antigen stimulation is required for eosinophil activation in vivo (Mould et al., 2000). In this study by Mould and colleagues, gene transfer of eotaxin and interleukin-5 into the airways of mice resulted in eosinophil accumulation in the airways. No eosinophil degranulation and induction of airway hyperresponsiveness were detectable unless the animals were treated with antigen. In our model, ovalbumin challenge did not result in increased levels of eosinophil peroxidase in the airways, indicating that eosinophils are not increasingly activated after ovalbumin challenge at this time point. Therefore, eotaxin was administered intravenously to see whether an additional effect on the airways could be obtained. However, this treatment did not result in increased airway responsiveness and inflammation. This could be another indication that eotaxin is not involved in inducing these parameters. However, these cells might have released their mediators at earlier stages prior to measurement. Importantly, in the investigation of eosinophil degranulation in mice, one has to keep in mind that mice eosinophils hardly degranulate in response to allergen, in vivo (Malm-Erjefält et al., 2001). Intranasal administration of eotaxin directly into the airways might have a different effect compared to intravenous administration. Intranasal injection of eotaxin might have resulted in a gradient of eotaxin with high concentrations in the airways which might have resulted in eosinophil migration.

Treatment with anti-eotaxin antibodies did not have any effect on eosinophil migration to the airway lumen, eosinophil peroxidase activity, airway hyperresponsiveness and IgE production induced by ovalbumin challenge. This might

suggest that eotaxin is not involved in the development of these characteristics in this model. In contrast, other researchers have shown that intravenous injection with anti-eotaxin antibodies reduced airway eosinophilia in ovalbumin-treated mice (Gonzalo et al., 1996, 1998). The ovalbumin model and time and way of antibody administration used by Gonzalo et al. were similar to ours. An explanation for this contradiction might be that they used a different strain of mice, C75/bl6. Presumably this strain is more dependent on eotaxin to induce eosinophil migration. A drawback of our study was that we were not able to check whether the concentration of antibodies used was sufficient to block all the eotaxin.

Previously, it was demonstrated that treatment of ovalbumin-challenged mice with anti-interleukin-5 antibodies completely inhibited airway eosinophilia in this model (Hessel et al., 1997). Interleukin-5 is a central cytokine regulating eosinophil function; this mediator primes eosinophils, promotes eosinophil survival and releases precursor cells from the bone marrow. Eotaxin and interleukin-5 synergistically act on eosinophils (Collins et al., 1995). Treatment with anti-interleukin-5 decreased airway eosinophilia in this model but did not influence airway responsiveness. In contrast, anti-interferon-γ did not affect the degree of airway inflammation, while airway hyperresponsiveness was completely inhibited (Hessel et al., 1997). This indicates that airway hyperresponsiveness and airway eosinophilia are two independent processes, which are differentially regulated.

In conclusion, eotaxin levels were increased in mice sensitised and challenged with ovalbumin compared to mice challenged with saline. However, this increase did not correlate with eosinophil migration to the lungs or airway hyperresponsiveness. Furthermore, blockade of eotaxin with anti-eotaxin antibodies did not have any effect on airway hyperresponsiveness or airway eosinophilia. Additional increase in eotaxin by installing this protein intravenously did not effect airway eosinophilia, eosinophil activation or airway responsiveness in both saline- and ovalbumin-challenged mice. From these experiments we conclude that although eotaxin levels are increased in the airways of mice sensitised and challenged with ovalbumin, this protein is not involved in airway eosinophilia or airway hyperresponsiveness.

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