

Mucosal output of eotaxin in allergic rhinitis and its attenuation by topical glucocorticosteroid treatment

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Summary

Background Eotaxin is a chemokine that attracts and activates eosinophils. The present study examines the occurrence of eotaxin in nasal mucosal surface liquids in patients with seasonal allergic rhinitis without allergen exposure and during repeat allergen challenge with and without topical glucocorticosteroid treatment. The number of subepithelial eosinophils and mucosal outputs of bulk plasma (α_2 -macroglobulin) and eosinophil cationic protein (ECP) are also examined.

Methods Twelve patients underwent daily allergen challenges for 6 days. Separately, 14 patients, who were receiving budesonide and placebo in a parallel group design, also underwent allergen challenge for 6 days. Nasal biopsies were obtained before and 24 h after the allergen challenge series, and lavages were carried out before and 15 min after selected allergen challenges.

Results At baseline nasal lavage fluid levels of eotaxin correlated to levels of α_2 -macroglobulin and ECP. After the first allergen challenge there was a correlation between nasal lavage fluid levels of eotaxin and ECP. Repeat allergen exposure increased the mucosal output of eotaxin ($P < 0.05$) and ECP ($P < 0.01$) as well as eosinophil numbers ($P < 0.01$), but no correlation was found between increased eosinophil numbers and eotaxin. Budesonide reduced eotaxin levels during repeat allergen challenge ($P < 0.05$).

Conclusions Repeat allergen exposure in allergic rhinitis is associated with increased mucosal output of eotaxin. Topical budesonide attenuates this effect, suggesting the possibility that inhibitory effects on mucosal eotaxin may contribute to anti-eosinophilic actions of topical glucocorticosteroids.

Keywords: airway, allergy, cytokines, eosinophils, inflammation

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Introduction

Infiltration and degranulation of eosinophil granulocytes is a prominent feature of airway inflammation in allergic rhinitis, nasal polyposis, and asthma. Eosinophils thus participate in allergic airway diseases [1] and may also be involved in common respiratory tract infections [2,3].

Eotaxin is a recently discovered chemokine that attracts

and activates eosinophils [4–7]. Intratracheal administration of eotaxin increases bronchoalveolar lavage (BAL) fluid numbers of eosinophils in guinea-pigs [8]. In bronchial airways of sensitized guinea-pigs, allergen challenge up-regulates eotaxin mRNA expression [5,9–13]. In human airways, eotaxin mRNA has been found in epithelial and inflammatory cells in nasal polyposis [7], in epithelial cells co-localized with macrophages in seasonal allergic rhinitis [14], and in epithelial and endothelial cells in asthma [15,16]. Increased levels of tissue eotaxin (eotaxin protein immunoreactivity) has been shown 24 h

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after allergen challenge in allergic rhinitis [14], and increased levels of free eotaxin have been demonstrated in asthma [16] and in atopic subjects developing common cold after rhinovirus inoculation [17]. Whether topical glucocorticosteroid treatment affects the mucosal production of eotaxin is unknown.

The observation by Lamkhoud *et al.* [16] that eotaxin may enter the bronchial lumen agrees with the possibility that the presence of eotaxin in airway mucosal surface liquids may reflect the local production of this chemokine. One further possibility is that plasma exudation, a characteristic of active eosinophilic airway disease [18], by its binding and transporting properties [18], may move eotaxin into the airway lumen.

In the present study, involving patients with seasonal allergic rhinitis out of season, we have examined nasal levels of eotaxin at repeated daily nasal allergen challenges. Nasal lavage fluid levels of eotaxin, eosinophil cationic protein (ECP) and α_2 -macroglobulin (reflecting microvascular-epithelial exudation of bulk plasma) were measured, and eosinophils were counted in nasal biopsies. In a first part, i.e. study group 1, we explored whether repeated daily allergen challenges were associated with nasal mucosal output of eotaxin. In a second part, i.e. study group 2, we examined effects of a topical glucocorticosteroid on nasal airway eotaxin levels in a placebo-controlled and parallel-group design.

Material and methods

Study design

Two separate groups of patients with birch and/or grass pollen allergic rhinitis were examined out of the pollen seasons. A nasal allergen challenge series (daily challenges for 6 days) was performed in both groups. Nasal biopsies were obtained before and 24 h after, and nasal lavages were obtained before, during and 24 h after, the allergen challenge series. One group of patients received topical budesonide treatment in a placebo-controlled, double-blind, and parallel-group design.

Patients

Twelve patients with seasonal allergic rhinitis were recruited to study group 1 (mean age 20 years, range 18–48 years, eight males) and 14 patients to study group 2 (mean age 25 years, range 22–32 years, nine males). The history of birch and/or grass pollen allergic rhinitis was verified by a positive skin prick test. Exclusion criteria were other nasal disease (including perennial allergic rhinitis and nasal polyposis), asthma, on-going drug

treatment, and pregnancy. The study was approved by the local ethics committee, and informed consent was obtained.

Allergen challenge procedure

Nasal challenges with birch or grass pollen allergen (ALK, Copenhagen, Denmark) started (study day 1) and proceeded for 6 days. The challenges were given in the morning of each day using a nasal spray device. In study group 1, all patients were given individual daily doses to maintain moderate levels of symptoms: after an initial moderate dose, i.e. 10 000 standard quantity units (SQ-U), subsequent doses were individually chosen that were believed to produce a score of about 2 on a scale from 0 to 3 of either sneezes, secretion, or blockage. The allergen doses (mean values per nasal cavity) given at study days 1–6 were 10 000 SQ-U, 1750 SQ-U, 1833 SQ-U, 1917 SQ-U, 3167 SQ-U, and 2083 SQ-U, respectively. In study group 2, all patients were given the same daily dose of allergen (in order to allow comparison between budesonide and placebo treatment). Based partly on experiences with study group 1, the allergen doses (absolute values per nasal cavity) were chosen to be 10 000 SQ-U at study days 1 and 4, and 3000 SQ-U at study days 2, 3, 5 and 6.

Drug treatment

The patients in study group 2 were randomized to treatment with either budesonide or placebo, administered using nasal spray devices (Rhinocort®; Aqua, Astra, Sweden; 64 µg/actuation, two actuations per nasal cavity once daily). The patients were given allocation numbers and the order of treatment proceeded according to a randomization scheme of the allocation numbers prepared in blocks. Blindness was maintained by the identical appearance of budesonide and placebo preparations. No medication other than the study drug was allowed during the study. Any previous nasal therapy was discontinued for at least 4 weeks before entry into the study. The treatment started 2 weeks prior to the first allergen challenge.

Symptom scores

Nasal symptoms, i.e. rhinorrhoea, blockage, and sneezes, were scored once every day (immediately before the daily allergen challenge) during the study period by the patients in both study groups: score 0, none; 1, mild; 2, moderate; and 3, severe symptoms.

Nasal lavages

Nasal lavages were carried out using the nasal pool device [19]. The device contained 15 mL isotonic saline and the

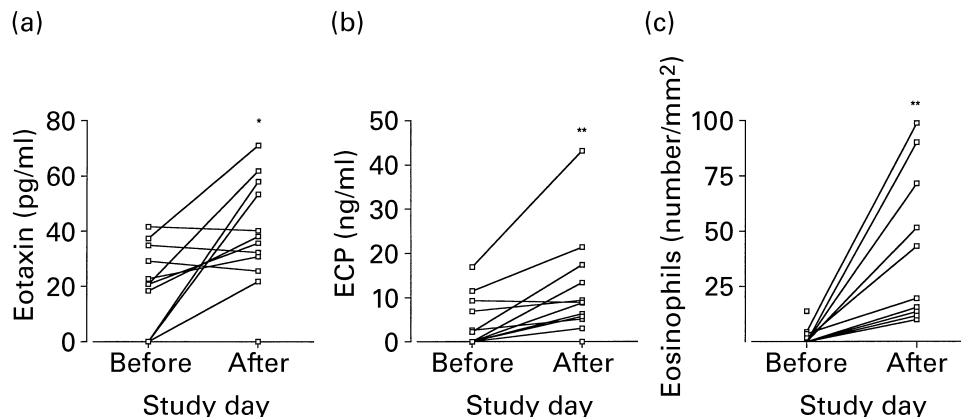


Fig. 1. Nasal lavage fluid levels of eotaxin (a) and eosinophil cationic protein (ECP) (b), as well as tissue (biopsy) numbers of eosinophils (c), in samples obtained before and after a 1-week series of daily allergen challenges. (One patient presented nasal biopsies with marked damage, and data from this patient were excluded from the analysis. One patient refused nasal biopsy after the allergen challenge series.)

lavage fluid was kept in the nasal cavity for 10 min. In study group 1, nasal lavages were carried out before the allergen challenge series and 24 h after the last allergen challenge. In study group 2, nasal lavages were similarly carried out before the allergen challenge series and 24 h after the last allergen challenge. In addition, in study group 2, nasal lavages were carried out before the daily allergen challenge on challenge days 4 and 6, and 15 min after the allergen exposure on challenge days 3, 4 and 5. The recovered lavage fluids were centrifuged (105 g, 10 min, 4 °C) and aliquots were prepared from the supernatants and frozen (−30 °C) for later analysis.

Nasal mucosal biopsies

In both study groups, nasal biopsies were obtained before the allergen challenge series and 24 h after the last allergen challenge. Topical anaesthesia was applied using a solution of tetracain (20 mg/mL) and adrenaline (0.1 mg/mL) delivered first by a nasal spray device and thereafter by cotton swabs. Nasal biopsies were taken from the inferior nasal turbinate about 0.5 cm from its anterior margin. A cutting forceps with a 2-mm punch was used for this purpose. Nasal biopsies at study day 1 were obtained from the left nasal cavity, whereas biopsies at study day 7 were obtained from the right nasal cavity.

Analysis

Eosinophils were visualized in conventional cryostat sections (10 µm) using an enzyme histochemical staining of cyanide-resistant eosinophil peroxidase. Immediately after excision, biopsies were placed in Stefanini fixative (PBS buffer, pH 7.6, supplemented with 4% formaldehyde

and picric acid) overnight at 4 °C. Thereafter, the specimens were rinsed thoroughly in PBS buffer and incubated during 8 min in PBS supplemented with 3,3-diaminobenzidine-tetrahydrochloride (75 µg/100 mL), H₂O₂ (0.3 mL/100 mL), and NaCN (50 mg/100 mL) at room temperature. The samples were rinsed in water, mounted in Kaiser's medium, and examined in a bright field microscope. Areas subjected to mechanical stress were excluded from evaluation. The total numbers of intact eosinophils were counted in two standardized sections from each biopsy. All quantifications were carried out in a blinded fashion.

The nasal lavage fluids were put in coded vials. The nasal lavage fluid levels of eotaxin were measured using a commercially available radioimmunoassay (RIA) (Pharmacia-Upjohn, Uppsala, Sweden). The detection level of the eotaxin assay is < 5 pg/mL. The nasal lavage fluid levels of ECP were measured using a commercially available fluoroimmunoassay (FEIA) (Pharmacia-Upjohn). The detection level of the ECP assay is < 2 ng/mL.

The lavage fluid levels of α₂-macroglobulin (study group 2) were measured using a RIA sensitive to 7.8 ng/mL. Rabbit anti-human α₂-macroglobulin (Dakopatts, Copenhagen, Denmark) was used as antiserum and human serum (Behringwerke Diagnostica, Marburg, Germany) as standard. Human α₂-macroglobulin (Capel-Organon Teknika, Turnhout, Belgium) was iodinated using the lactoperoxidase method. Tracer and standard (or sample) was mixed with antiserum before adding goat anti-rabbit antiserum (Astra Draco, Lund, Sweden). The bound fraction was measured using a gamma counter. The intra- and interassay coefficients of variation were between 3.8% and 6.0% and 3.1% and 7.2%, respectively.

Table 1. Levels of eotaxin (pg/mL), eosinophil cationic protein (ECP) (ng/mL), and α_2 -macroglobulin (μ g/mL) in nasal lavage samples obtained at study days 1, 4 and 6 immediately before the daily allergen challenge

Study day	Placebo			Budesonide			Mann–Whitney
	25th percentile	Median value	75th percentile	25th percentile	Median value	75th percentile	
Eotaxin (pg/mL)							
1	0	0	38.5	0	0	0	NS
4	0	20.2	28.9	0	0	0	<i>P</i> <0.05
6	0	8.5	25.1	0	0	0	NS
ECP (ng/mL)							
1	0	0	60.2	0	0	0	NS
4	0	6.0	11.6	0	1.1	16.1	NS
6	0	4.9	6.7	0	0	2.9	NS
α ₂ -macroglobulin (μg/mL)							
1	0	0.05	0.25	0.10	0.10	0.20	NS
4	0.20	0.35	0.75	0.10	0.10	0.80	NS
6	0.10	0.20	0.75	0.10	0.10	0.30	NS

Statistical analysis

Differences in nasal lavage fluid levels of eotaxin, ECP, and α_2 -macroglobulin as well as in mucosal eosinophil numbers were examined using the Friedman test (study group 2) and the Wilcoxon signed rank test. Differences between placebo and budesonide treatment were examined using the Mann–Whitney *U*-test. Correlations were examined using the Spearman rank correlation test. *P*-values of <0.05 were considered significant. Nasal symptoms are presented as mean (range). Otherwise, data are presented as

individual values or as medians with 25th and 75th percentiles.

Results*Study group 1—allergen challenges*

The allergen challenge series produced nasal symptoms of allergic rhinitis. The mean daily scores during the allergen challenge series for nasal blockage, secretion, and sneezes,

Table 2. Levels of eotaxin (pg/mL), eosinophil cationic protein (ECP) (ng/mL), and α_2 -macroglobulin (μ g/mL) in nasal lavage samples obtained at study days 1, 3, 4, and 5, 15 min after the daily allergen challenge

	Placebo			Budesonide			
Study day	25th percentile	Median value	75th percentile	25th percentile	Median value	75th percentile	Mann–Whitney
Eotaxin (pg/mL)							
1	0	0	0	0	0	0	NS
3	0	11.5	26.0	0	0	0	NS
4	7.8	24.2	54.6	0	9.8	26.2	NS
5	24.4	26.6	42.6	0	18.6	20.5	$P < 0.05$
ECP (ng/mL)							
1	0	0	2.9	0	0	0	NS
3	1.9	6.9	11.4	0	11.3	34.0	NS
4	4.2	7.2	24.7	0	14.9	104.0	NS
5	2.6	5.8	20.4	0	5.9	60.3	NS
α_2 -macroglobulin (μ g/mL)							
1	0.7	5.4	12.2	0.4	1.8	3.8	NS
3	0.8	1.1	4.1	0.3	0.5	2.3	NS
4	1.4	3.5	10.9	0.3	2.2	3.3	NS
5	0.6	2.0	4.1	0.3	0.7	1.2	NS

respectively, were 2.1 (1.5–2.8), 1.9 (1.4–2.3), and 2.2 (1.7–2.7). Nasal lavage fluid levels of eotaxin and ECP, as well as mucosal (biopsy) numbers of eosinophils, in samples obtained before and after the 1-week series of daily allergen challenges are demonstrated in Fig. 1. Eotaxin was detected in nasal lavage fluids obtained before the allergen challenge series in 8/12 patients. The levels of eotaxin ($P < 0.05$), ECP ($P < 0.01$), and eosinophils ($P < 0.01$) were increased 24 h after the completed allergen challenge series (cf. baseline). Nasal lavage fluid levels of eotaxin correlated significantly to nasal lavage fluid levels of ECP before ($r_s = 0.70$, $P < 0.01$) but not after the allergen challenge series. Nasal lavage fluid levels of eotaxin levels did not correlate significantly to mucosal eosinophil numbers after the completed allergen challenge series. (Eosinophils were scarce prior to allergen exposure.)

Study group 2—allergen challenges and budesonide treatment (Tables 1 and 2)

The allergen challenge series produced nasal symptoms of allergic rhinitis. The mean daily scores during the allergen challenge series for nasal blockage, secretion, and sneezes, respectively, were 1.2 (0.2–2.5), 1.2 (0.3–2.2), and 1.2 (0.5–2.5) in subjects receiving placebo, and 0.7 (0.0–2.3), 0.8 (0.2–2.0), and 1.0 (0.5–1.6) in patients receiving budesonide.

Study group 2—effects of topical budesonide treatment on mucosal eosinophils and nasal lavage fluid levels of eotaxin and ECP

Levels of eotaxin in lavages obtained before the allergen challenge series as well as during the allergen challenge series ('baseline' lavages before allergen challenges 1, 4, and 6 days into the challenge series, and lavages after allergen challenges 1, 3, 4, and 5 days into the challenge series) are shown in Tables 1 and 2. Topical budesonide treatment reduced the nasal lavage fluid levels of eotaxin on challenge days 4 and 5 ($P < 0.05$, cf. placebo). Significant effects of budesonide on numbers of eosinophils and nasal lavage fluid levels of ECP were not observed in the present study.

Study group 2—relationship between nasal lavage fluid levels of eotaxin and eosinophil activity

In patients receiving placebo treatment, there was a correlation between levels of eotaxin and ECP after the first allergen challenge ($r_s = 0.76$, $P < 0.05$), and, nearly, in the baseline lavage carried out before the allergen challenge series ($r_s = 0.69$, $P = 0.06$).

In patients receiving placebo treatment, there was no

significant correlation between baseline lavage fluid levels of eotaxin and numbers of tissue eosinophils 7 days into the allergen challenge series. Similarly, there was no significant correlation between cumulative levels of eotaxin (recorded during the allergen challenge series) and tissue eosinophils 7 days into the allergen challenge series. (Eosinophils were scarce in biopsies obtained prior to the allergen challenge series.)

Study group 2—relationship between allergen challenge-induced plasma exudation (α_2 -macroglobulin) and mucosal output of eotaxin

In patients receiving placebo treatment, there was a significant correlation between nasal lavage fluid levels of α_2 -macroglobulin and eotaxin in the first baseline lavage (before the allergen challenge series) ($r_s = 0.92$, $P < 0.05$). This correlation was lost following allergen challenge ($r_s = 0.56$). No significant correlations between α_2 -macroglobulin and eotaxin were then detected during the allergen challenge series.

Discussion

The present study, involving patients with seasonal allergic rhinitis (examined out of season), demonstrated that repeated allergen challenges, producing symptomatic eosinophilic nasal airway inflammation, were associated with increased levels of eotaxin in nasal mucosal surface liquids. The luminal eotaxin levels did not correlate to mucosal eosinophil numbers, but exhibited some correlation to nasal lavage fluid levels of ECP, i.e. before and early into the allergen challenge series. Nasal mucosal output of eotaxin during allergen exposures was reduced by topical budesonide treatment given for 2 weeks prior to the start of the allergen challenge series and continued throughout the allergen challenge series. This effect may be one mechanism by which topical glucocorticosteroid drugs reduce eosinophil activity in rhinitis and asthma.

Luminal eotaxin was detectable already at baseline in this study, i.e. before the allergen challenge series, when mucosal eosinophil numbers were low and ECP levels low. There was, further, a significant correlation at baseline between luminal eotaxin and ECP, suggesting the possibility of a role of eotaxin in maintaining a low grade of recruitment/activation of eosinophils in sensitized human airways. The present data also agree with a role of eotaxin in physiological homing of eosinophils to the airway mucosa, as suggested by studies of guinea-pig tracheal airways under baseline conditions [13]. A significant role in baseline homing of eosinophils to mucosae has been demonstrated in the gastrointestinal tract of mice [20].

In the present study, repeated allergen challenges

(creating sustained eosinophilic inflammation similar to that seen in seasonal allergic rhinitis at seasonal allergen exposure [21]) were associated with a sustained mucosal output of eotaxin. Both luminal eotaxin and tissue eosinophils were increased, but no correlation was detected between eotaxin levels and the numbers of eosinophils in the present day 7 biopsy. It is possible that airway recruitment of eosinophils in allergic nasal airway inflammation is not critically dependent on the mucosal production of eotaxin. Indeed, there are many eosinophil-active molecules in the airway mucosa that are actively involved in allergic airway disease: the plasma exudation process that occurs in these diseased airways contribute fibrinogen, fibronectin, and other proteins with capacities to cause migration, adhesion, and activation of granulocytes [18]. Furthermore, allergen challenge-induced nasal levels of eosinophil active chemokines/cytokines such as IL-5 [22], IL-8 [23], granulocyte-macrophage colony-stimulating factor (GM-CSF) [24], and RANTES [25] have been reported. Perhaps the contribution of eotaxin is particularly important for the early recruitment/activation of eosinophils, as suggested by significant correlations between luminal eotaxin and ECP at baseline and early into the allergen challenge series. This may also explain the loss of correlation between eotaxin and ECP experienced into the allergen challenge series in the present study.

Topical steroid treatment has been demonstrated to reduce airway recruitment of eosinophils and the levels of granulae proteins in rhinitis and asthma [26–28]. In the present study, we further demonstrate for the first time that baseline levels of eotaxin as well as nasal lavage fluid levels of eotaxin occurring at repeated allergen challenges are attenuated by topical budesonide given at a clinical dose. We suggest that glucocorticosteroid treatment reduces local eosinophil activity in allergic airway disease in part through inhibition of mucosal eotaxin levels. It has previously been demonstrated that dexamethasone, administered intraperitoneally, had no effect on allergen challenge-induced bronchial mucosal output of eotaxin in guinea-pigs in an experimental condition where the treatment reduced tissue and BAL fluid numbers of eosinophils [12]. Whether the differing results in the study by Humbles *et al.* and the present study may be explained by species differences, different administration routes, or other factors remains to be examined.

The present repeated allergen challenges produced the expected symptoms of allergic rhinitis in patients receiving placebo. It was further expected that topical budesonide would reduce nasal symptoms as well as plasma exudation (α_2 -macroglobulin) and eosinophil number and activity (ECP) [19]. However, probably because of low numbers of patients in combination with a parallel-group design, the statistical power of the present study was low and the lower

mean symptom scores recorded by patients receiving budesonide (compared with placebo) failed to reach statistical significance. Similarly, the lower mean levels of α_2 -macroglobulin and ECP determined in nasal lavages obtained before the daily allergen challenges in patients receiving budesonide (compared with placebo) failed to reach statistical significance. Nevertheless, the present study succeeded in demonstrating significant effects of budesonide on nasal mucosal output of eotaxin. This observation suggests the possibility that nasal mucosal output of eotaxin involves mechanisms that are particularly glucocorticosteroid-sensitive.

We conclude that seasonal allergic rhinitis is associated with a mucosal output of eotaxin, that this output is increased at allergen exposure, and that topical glucocorticosteroid treatment attenuates this effect both at baseline and at allergic nasal airway inflammation.

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