

Nasal lavage fluid concentrations of eotaxin-1 (CCL11) in naturally occurring allergic rhinitis: relationship to disease activity, nasal luminal eosinophil influx, and plasma protein exudation

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Summary

Background Eotaxin-1 (CCL11) is a CC chemokine whose nasal eosinophilic chemotactic activity *in vivo* and *in vitro* has been demonstrated primarily using nasal allergen challenge models. The extension of these challenge findings to the *in vivo* setting has been limited.

Objective To obtain nasal lavage fluid from volunteers with perennial and seasonal (in- and out-of-season) allergic rhinitis (AR) and non-atopic non-rhinitic controls for the measurement of eotaxin-1 concentrations and to relate these findings to the symptomatic disease severity, the percentage of lavage eosinophils, and to α_2 -macroglobulin (α_2 -MG) lavage concentrations, as a marker of vascular permeability and an index of airway inflammation.

Methods Thirty-seven volunteers with AR (16 seasonal and 21 perennial) and 20 non-atopic non-rhinitic volunteers were recruited and phenotyped. Nasal lavage fluid was obtained by standardized protocol. The nasal lavage fluid concentrations of eotaxin and α_2 -MG were measured by ELISA, and differential cell counts performed on cytopins.

Results Eotaxin-1 nasal lavage fluid concentrations were significantly higher in both the perennial and seasonal (in-season) AR groups compared with the controls, and significantly related to the severity of symptom expression and to the percentage of lavage eosinophils. The lavage eosinophil counts were significantly higher in both the symptomatic rhinitis groups compared with the control groups and correlated with the lavage concentrations of α_2 -MG. α_2 -MG levels were significantly increased in seasonal (in-season) rhinitis compared with both non-atopic controls and seasonal (out-of-season) rhinitis. A significant correlation was observed between the levels of α_2 -MG and levels of eotaxin in the symptomatic allergic rhinitic groups.

Conclusions This study clearly demonstrates the relevance of eotaxin-1 to the pathogenesis of naturally occurring AR.

Keywords Allergic rhinitis, α_2 macroglobulin, cytopins, ELISA, eosinophil, eotaxin, nasal lavage, naturally occurring disease

Submitted 28 September 2004; revised 9 February 2005; accepted 21 February 2005

Introduction

Allergic rhinitis (AR) is an inflammatory nasal disorder that is typified by plasma protein exudation and the tissue recruitment of eosinophils along with their directed migration into the epithelium and nasal airway lumen [1]. This directed tissue recruitment is considered to be under the influence of chemokines, with the CC chemokine eotaxin-1 (CC chemokine ligand 11, CCL11) being important in this respect [2]. Eotaxin binds with high affinity to CC chemokine receptor 3 (CCR3), which is expressed on cells central to the allergic inflammatory response, including eosinophils, mast

cells, basophils, and T helper type 2 (Th2) lymphocytes [3–6]. Eotaxin has been demonstrated to have eosinophil chemotactic activity both *in vitro* and *in vivo* [2, 7, 8]. Consistent with this, sensitized mice made genetically deficient in eotaxin or pre-treated with an anti-eotaxin antibody have diminished airway eosinophil recruitment after allergen challenge, in comparison with sensitized mice challenged in the absence of any modification of the biological activity of eotaxin [9–12]. Furthermore, intranasal challenge with eotaxin has been shown to induce nasal luminal eosinophil recruitment in volunteers with AR, particularly if these subjects are first subjected to an intranasal allergen challenge [13, 14]. Nasal allergen challenge in allergic rhinitis has itself been shown to increase lavage eotaxin concentrations and to induce tissue eosinophil recruitment [15, 16]. Significant correlations have been reported between the level of eotaxin in lavage and the magnitude of the lavage eosinophil count under such circumstances [15]. Information is, however, limited as to

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the relevance of eotaxin to nasal airway eosinophil recruitment in naturally occurring disease [17].

We have therefore extended our previous immunohistochemical studies in AR, which have found enhanced epithelial expression of eotaxin [18], to investigate the presence and concentrations of eotaxin in nasal lavage recovered from patients with perennial and seasonal (in- and out-of-season) AR and from non-atopic non-rhinitic controls for comparison purposes. Measures of α_2 -macroglobulin (α_2 -MG) as a marker of plasma leakage, and of eosinophil differential cell counts have also been made in the recovered lavage fluid to relate to the eotaxin findings.

Materials and methods

Subjects

Twenty-one house dust mite-sensitive subjects with perennial AR, 16 grass pollen-sensitive subjects with symptomatic seasonal AR and 20 matched healthy non-atopic non-rhinitic, participated in the study. Thirteen of the grass pollen-sensitive subjects were also studied out of the season when asymptomatic. The atopic patients were defined by a weal response of at least 3 mm greater than the negative control on skin prick testing to the aeroallergen *Dermatophagoides pteronyssinus* (ALK-Abello, Horsholm, Denmark) for perennial rhinitis, and mixed grass pollen (Hollister-Stier, Spokane, WA, USA) for seasonal rhinitis. The normal subjects were not on any medication, and skin prick tests (SPTs) to *D. pteronyssinus*, grass pollen, and eight other common aeroallergens (Allergopharma, Reinbek, Germany) were negative. The patients were each asked to fill in a rhinoconjunctivitis questionnaire relating to their specific symptoms, each scored on a standard 4-point rating scale of severity from 0 to 3 [19], in which 0 equals no symptoms and three equals severe symptoms interfering with activities of daily living, on the day of the nasal lavage in order to establish the activity of the allergic condition in the rhinitis groups as well as exclude any symptoms in the control groups. The subject demographics, including a summary of their atopic status and rhinitis symptom scores, are shown in Table 1. The inclusion criteria for the rhinitics included a relevant positive SPT along with a 2-year history of seasonally related rhinitic symptoms in the grass pollen-sensitive subjects, and a history of perennial rhinitis symptoms of at least 1 year and a history compatible with house dust mite-related AR in those sensitized to mite

allergens. All subjects were aged 18–50 and healthy controls were SPT negative and asymptomatic. None of the subjects were on current therapy for their rhinitis or had received immunotherapy. The study was approved by the Joint Research Ethics Committee of Southampton & South West Hampshire Health Authority and conformed to the Declaration of Helsinki. All participants gave written informed consent.

Nasal lavage fluid collection and processing

Nasal lavage fluid was obtained from subjects by the method of Naclerio [20], with the subject in the seated, head back position using 5 mL of normal saline, pre-warmed to 35 °C, with 2.5 mL instilled into each nostril and held there for 10 s using the Valsalva manoeuvre. The fluid was then expelled anteriorly and collected by asking the subject to lean forward and blow the nasal contents gently into a funnel connected to a 30 mL Universal container. The lavage fluid was initially filtered by passing it through a sieve, with a mesh size of 0.9 mm, to remove any nasal mucus and the filtrate was then collected in a pre-cooled 10 mL (polypropylene) graduated tube and the volume of the lavage effluent noted. The lavage fluid was kept on ice until centrifugation at 759 *g* for 10 min at 4 °C. The supernatant was then aliquoted into 0.5 mL samples and stored in pre-cooled 2 mL Eppendorf tubes, and then finally stored at –80 °C for future assays.

Cytospin preparation and analysis

After centrifugation of the lavage samples, the cell pellet was resuspended in 200 μ L of modified Eagle's medium (MEM) containing 2% fetal calf serum and two cytospin slides prepared from each sample using a Shandon cytocentrifuge (Cytospin2) (Thermo Shandon Ltd, Cheshire, UK), with centrifugation at 190 *g* for 8 min. The cytopspins were air dried and stored in aluminium foil at –20 °C until required.

For cell count analysis, the 'Rapi-Diff' (Bios Europe, Lancashire, UK) staining method was used. The slide cytopspins were fixed in methanol (solution A) for 30 s and then drained. Then they were stained with 'Rapi-Diff-II' solution B (Acid dye) for 30 s followed by drainage. Next they were stained with 'Rapi-Diff II' solution C (Basic dye) for 1 min and then rinsed thoroughly under tap water. The slides were then mounted with p-xylene-bis-pyridium bromide and cover slipped. Finally a differential cell count of epithelial cells, neutrophils, eosinophils, and macrophages was performed on 400 cells on each of the two separate cytopspins and the mean count used for analysis.

Table 1. Subjects demographics

	Seasonal (out)	Seasonal (in)	Perennial	Normal
N	13	16	21	20
Median age, (range)	39 (23–54)	33 (22–54)	32 (19–57)	35 (22–55)
Sex (M/F)	5 M/8 F	6 M/10 F	12 M/9 F	11 M/9 F
Median rhinitis Symptom score, (range)	1 (0–5)	13 (9–17)	12 (5–17)	0.5 (0–3)
Patients with +ve skin prick test responses	13	16	21	0
House dust mite	4	4	21	0
Animal danders	3	3	13	0
Seasonal pollen allergens	13	16	13	0

Enzyme-linked immunosorbent assays

Eotaxin-1 was assayed by ELISA according to the manufacturer's instructions (CytoSet, BioSource Europe, Belgium: Catalogue Number-CHC2234). This assay employed a sandwich technique using a murine anti-human monoclonal antibody specific for eotaxin-1. The minimum detectable level of eotaxin-1 was 0.3 pg/mL.

An 'in house' ELISA was used for α_2 -MG. Microtitre plates (Fisher Scientific, Leicester, UK) were coated with sheep anti- α_2 -MG (The Binding Site Ltd, Birmingham, UK) using 100 μ L per well at a dilution of 1:500 in 50 mM sodium carbonate (pH 9.6) and incubated overnight at 4 °C. They were then washed ($\times 3$) in phosphate-buffered saline (PBS) containing 0.05% polyoxyethylenesorbitan monolaurate (Tween-20, Sigma, Dorset, UK). All samples and standards were diluted in PBS containing 1% Tween-20 for blocking of non-specific protein sites. Duplicates of 100 μ L of diluted nasal lavage or α_2 -MG standard (0, 2–1000 ng/mL, Sigma) were applied to wells for 90 min at 37 °C. After washes ($\times 3$), 100 μ L of peroxidase-conjugated sheep anti- α_2 -MG (The Binding Site Ltd.) at a dilution of 1:1000 was added for 90 min at 37 °C. The plates were washed ($\times 3$) and then the colour reaction developed with 100 μ L of 0.4 mg/mL *o*-phenylene diamine (Sigma) dissolved in phosphate buffer (pH 7) containing 0.01% hydrogen peroxide. The reaction was stopped with 50 μ L of 2 M H_2SO_4 at 10 min. Absorbances were read at 490 nm and compared with those of the standard curve. The concentration was determined in ng/mL. For diluted samples, the value from the standard curve was multiplied by the dilution factor to calculate the sample value. The lower limit of detection was 2 ng/mL.

Statistical analysis

All statistical analyses were performed using SPSS for Windows (SPSS Inc., IL, USA). The biological data was not normally distributed and The Kruskal–Wallis test was used to perform non-parametric independent group comparisons. When appropriate the Mann–Whitney *U*-test was used when analysing differences between non-paired groups, and the Wilcoxon's signed ranks test used for related sample comparison, such as between the in- and out-of-season groups. Where indicated, the strength of correlations was explored using the Spearman's rank correlation test. The level of significance was accepted as $P < 0.05$.

Results

The rhinitic patients did not differ from the non-atopic controls in age or gender distribution. All rhinitic subjects were symptomatic, with both the perennial and seasonal groups significantly more so ($P < 0.001$) than the non-atopic healthy control group, and the in-season group more so than when evaluated out-of-season ($P < 0.001$).

Eotaxin

The Kruskal–Wallis test identified significant inter-group differences ($P < 0.001$).

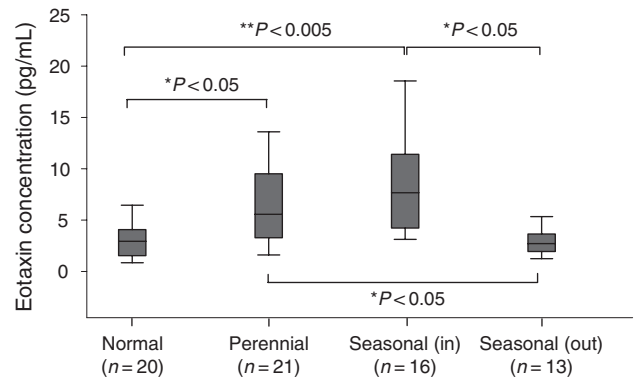


Fig. 1. Eotaxin concentration (pg/mL) in nasal lavage from non-atopic controls, and perennial, seasonal (in season), and seasonal (out of season) rhinitics. The box plots show the median and interquartile range, and the error bars show the 5th and 95th percentiles.

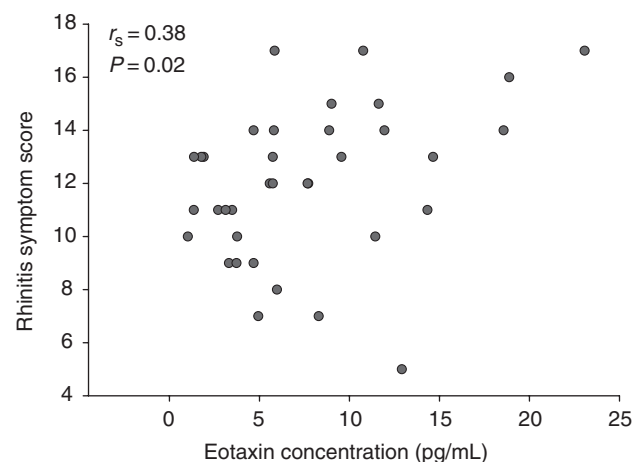


Fig. 2. Correlation between nasal lavage fluid eotaxin concentration (pg/mL) and rhinitis symptom scores in the symptomatic allergic rhinitis groups ($n = 37$). Spearman's rank correlation coefficient shown.

Eotaxin levels [median (range)] were significantly increased in the perennial [5.6 (1–23.1) pg/mL] ($P = 0.01$, $P = 0.01$), and seasonal (in-season) AR [7.7 (1.4–18.9) pg/mL] ($P = 0.002$, $P = 0.008$) groups compared with both non-atopic controls [2.9 (0.5–10.8) pg/mL] and seasonal (out-of-season) group [2.71 (1.2–6.6) pg/mL], respectively. There were no significant differences in eotaxin levels between the non-atopic and seasonal (out-of-season) rhinitis groups ($P = 0.77$) (Fig. 1). A significant correlation was found between eotaxin concentrations and severity of rhinitis symptoms ($n = 37$, $r_s = 0.38$, $P = 0.02$) (Fig. 2).

α_2 -macroglobulin

The Kruskal–Wallis test identified significant inter-group differences ($P < 0.02$).

α_2 -MG levels [median (range)] were significantly increased in seasonal (in-season) rhinitics [621.9 (35.2–7012) ng/mL] ($P = 0.009$, $P = 0.002$) compared with non-atopic controls [188.8 (12.9–2054) ng/mL] and seasonal (out-of-season) rhinitics [116.5 (13.4–870.2) ng/mL], respectively (Fig. 3). Although α_2 -MG levels were higher in the perennial AR group [267.4 (11.99–3842) ng/mL] than in the non-atopic controls, this difference was not statistically significant.

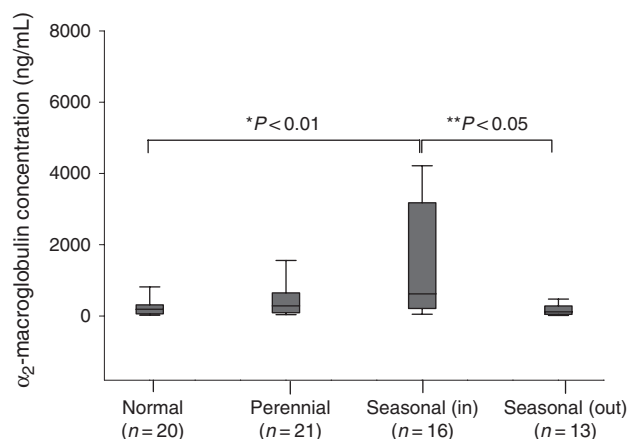


Fig. 3. α_2 -macroglobulin concentration (ng/mL) in nasal lavage from non-atopic controls, and perennial, seasonal (in-season), and seasonal (out-of-season) rhinitics. The box plots show the median and interquartile range, and the error bars show the 5th and 95th percentiles.

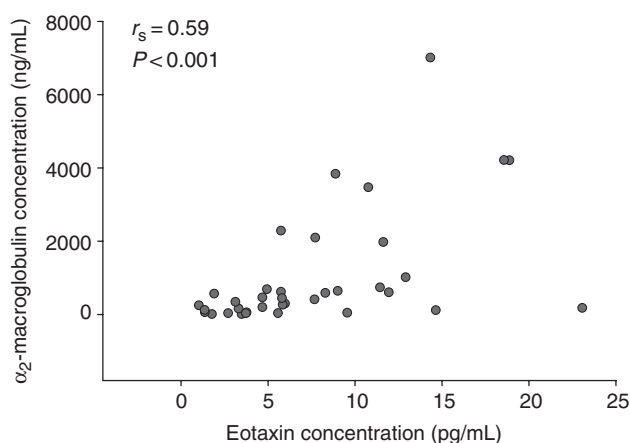


Fig. 4. Correlation between nasal lavage fluid eotaxin (pg/mL) and α_2 -macroglobulin (ng/mL) levels in the symptomatic allergic rhinitis groups ($n = 37$). Spearman's rank correlation coefficient shown.

In the seasonal and perennial rhinitis groups, a significant correlation was observed between the nasal lavage concentrations of α_2 -MG and those of eotaxin ($n = 37$, $r_s = 0.59$, $P < 0.001$) (Fig. 4).

Cytospins

Adequate cytospins for analysis were obtained in 11 seasonal rhinitis (in-season), 9 seasonal rhinitis (out-of-season), 13 perennial rhinitis, and 17 non-atopic controls.

Eosinophils

The percentage of eosinophils [median (range)] was significantly increased in seasonal (in-season) ($n = 11$) [2.7 (0–65)] ($P < 0.001$) and perennial AR ($n = 13$) groups [0 (0–51)] ($P = 0.008$), compared with non-atopic controls ($n = 17$) [0 (0–0.7)]. There was a significant increase in the percentage of eosinophils in the seasonal (in-season) group compared with the seasonal (out-of-season) group [0 (0–1)] ($P = 0.007$). There were no significant differences in eosinophil numbers

between non-atopic controls and seasonal (out-of-season) rhinitics ($n = 9$), or between perennial and seasonal (in-season) rhinitics (Fig. 5).

In the rhinitis group, a significant correlation was observed between the number of eosinophils and both the levels of eotaxin ($n = 24$, $r_s = 0.53$, $P = 0.008$) (Fig. 6) and α_2 -MG ($n = 24$, $r_s = 0.50$, $P = 0.012$) (Fig. 7).

Neutrophils

There appeared to be an increase in the percentage of neutrophils [median (range)] in the seasonal (in-season) [17 (0–76.3)] and perennial AR groups [29 (0–88.7)] when compared with non-atopic controls [7 (0–54.7)]. However, these changes did not reach statistical significance. There were no significant differences between non-atopic controls and seasonal (out-of-season) rhinitics [6 (0–82.7)] (Fig. 5).

No significant correlations were found between neutrophil numbers and the levels of eotaxin or α_2 -MG.

Macrophages

There were no significant differences in the percentage of macrophages [median (range)] between seasonal (in-season) [0 (0–3.7)], seasonal (out-of-season) [0 (0–1)] and perennial rhinitis [0 (0–6.7)] (Fig. 5).

No significant correlations were found between macrophage numbers and the levels of eotaxin or α_2 -MG.

Discussion

This is the first demonstration of elevated eotaxin concentrations in nasal lavage in naturally occurring AR and evaluation of its relationship to airway luminal eosinophil recruitment. This study identifies that eotaxin levels in nasal lavage fluid are significantly increased in both perennial and seasonal (in-season) AR as compared with both non-atopic controls and individuals with atopic rhinitis out-of-season. The concentration of eotaxin in the nasal lavage samples correlated significantly with nasal lavage eosinophil counts. Previous studies have predominantly focused on nasal allergen challenge models, in which allergen exposure in sensitized individuals leads to both early and late increases in eotaxin recovery from the nasal lumen [15, 16]. Correlations under such circumstances have suggested the relevance of eotaxin to airway eosinophil recruitment and consistent with this intranasal eotaxin challenge has been shown to promote nasal airway eosinophil influx [13, 14]. Elevated levels of eotaxin in nasal lavage have previously been found in seasonal AR in children [17], but these changes did not reach statistical significance, and no previous studies have addressed perennial allergic disease. Our findings of a significant correlation between nasal lavage eotaxin concentrations and the percentage of eosinophils present within the lavage in the present study in naturally occurring disease is likely to reflect the relevance of eotaxin to this airway cell recruitment. Consistent with such a tenure, we have found that a human anti-eotaxin monoclonal antibody, CAT-213, whether administered intranasally or intravenously, modifies the number of eosinophils within the nasal tissue after allergen challenge in

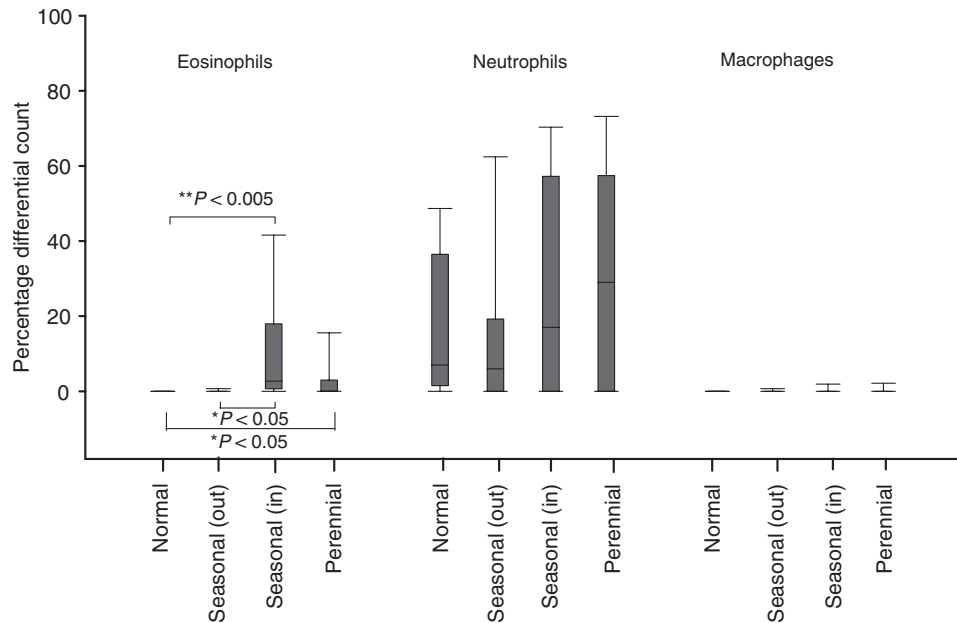


Fig. 5. Differential counts of eosinophils, neutrophils and macrophages expressed as a percentage of the total number of cells in cytospin samples from non-atopic controls ($n = 20$), and perennial ($n = 21$), seasonal (in-season) ($n = 16$), and seasonal (out-of-season) ($n = 13$) rhinitics. The box plots show the median and interquartile range, and the error bars show the 5th and 95th percentiles.

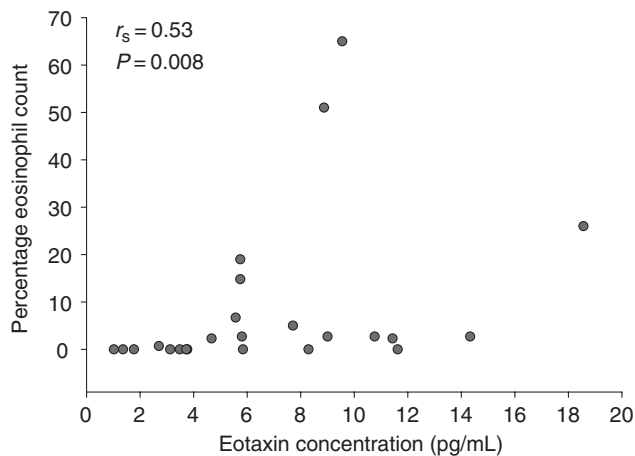


Fig. 6. Correlation between nasal lavage fluid eotaxin levels (pg/mL) and percentage eosinophil counts in the symptomatic allergic rhinitis groups ($n = 24$). Spearman's rank correlation coefficient shown.

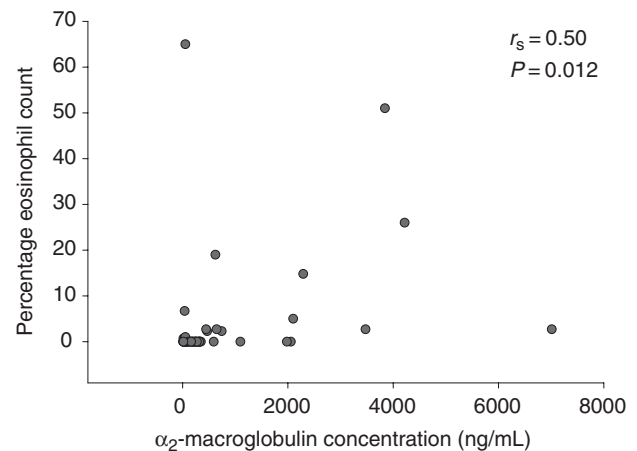


Fig. 7. Correlation between nasal lavage fluid α_2 -macroglobulin levels (ng/mL) and percentage eosinophil counts in the symptomatic allergic rhinitis groups ($n = 24$). Spearman's rank correlation coefficient shown.

AR [21, 22]. In animal models of allergic airways disease, the intranasal administration of either anti-eotaxin 1 or anti-eotaxin 2 has been found to significantly reduce the increase in bronchoalveolar lavage eosinophils induced by ovalbumin challenge in sensitized mice [23]. This effect was related to an influence on the tissue recruitment and not to bone marrow production, suggestive of the importance of eotaxin locally within the airways in tissue eosinophil recruitment.

There was considerable variability between subjects in the extent of nasal lavage eosinophilia. All samples were handled similarly and these differences are thus unlikely to be methodological and more likely to reflect heterogeneity in airway luminal eosinophil recruitment in AR. This is

consistent with findings after both nasal allergen challenge and in naturally occurring disease [24], and is also reflected in nasal biopsies [24, 25]. These findings suggest that there is heterogeneity in tissue and luminal eosinophil recruitment in AR. Within our study, there was no correlation between the extent of luminal eosinophil recruitment and severity of symptoms. It has to be appreciated that while eotaxin is linked to eosinophil recruitment, it also has other effects of relevance to AR.

Eotaxin is a ligand for the CCR-3 receptor. While attention has focused on the relevance of CCR-3 receptor to eosinophil chemotaxis it is now appreciated that CCR-3 receptors are also expressed on basophils, mast cells, antigen-presenting dendritic cells, and T cells [3, 5, 6, 26]. The increased

concentrations of eotaxin within the nasal airways would thus also be of relevance to the luminal accumulation of these cells, and this chemokine thus assumes a broader perspective in relationship to allergic inflammation in rhinitis. We have not specifically addressed this issue within the present study as metachromatic cells, such as mast cells and basophils, are sparse within lavage, being more prominent within the nasal tissue, and despite using specific cell surface receptor staining to delineate differing T cell subsets with analysis by flow cytometry, we have previously also found that there are insufficient T cells within nasal lavage fluid to provide reliable results by this approach. Thus we have focused on eosinophils which are reliably counted in nasal lavage cytopins.

In the upper airways, eotaxin mRNA has been localized to epithelial and inflammatory cells in nasal polyposis [27, 28], and in epithelial cells and macrophages in seasonal AR [29]. Our previous immunohistochemical study of eotaxin expression in nasal biopsies in naturally occurring AR [18], and that of Pullerits et al. [30], identified the nasal airway epithelium as a prominent cellular source of eotaxin and one that would be both relevant to luminal concentrations of eotaxin and directed luminal cell chemotaxis. The airway epithelium may also be an important target cell for eotaxin as intranasal eotaxin administration has been shown to increase the levels of nitric oxide (NO) in nasally exhaled air [13]. In naturally occurring AR there are increased NO concentrations in exhaled nasal air [31, 32], and the nasal epithelium has been shown to have enhanced expression of the inducible form of the enzyme nitric oxide synthase [33, 34]. This enzyme is up-regulated in allergic inflammation and can synthesize NO.

Eotaxin has been shown to be regulated by the Th2 cytokines, interleukin (IL)-4 and IL-13, and also by tumor necrosis factor- α (TNF- α), and transforming growth factor- β (TGF- β) [35–38]. Mast cell activation in AR will be associated with the release of IL-4, IL-13 and TNF- α , as these cytokines are stored preformed within the mast cell granules [39–42], and *ex vivo* allergen stimulation of nasal tissue from subjects with AR leads to an increase in IL-4 and IL-13 mRNA, predominantly colocalized to T-lymphocytes and mast cells [43]. Epithelial cells in rhinitis have been shown to express TGF- β [18], and thus, activation of mast cells, T cells and epithelial cells, in association with natural allergen exposure, is likely to contribute to the local tissue generation of eotaxin.

We have used nasal lavage to sample the airways in this study. It has previously been shown that the recovered fluid can be analysed both for mediator and cytokine levels as well as cell profiles [44–46]. The use of nasal lavage to sample the nasal mucosa has several advantages over the more invasive techniques of biopsy or scraping. It samples a larger surface area, allows analysis of both cell activation and cell recruitment and is well tolerated. Nasal lavage can be used repeatedly over various time points, which is why it has been particularly applied to studies of experimental allergen challenge. To date, compared with allergen challenge models, fewer studies have been undertaken in naturally occurring disease using this technique, although it is being increasingly applied as an outcome measure in AR [17, 47–53]. In addition to eotaxin we have measured the concentration of α_2 -MG in nasal lavage fluid, as a marker of plasma leakage and vascular permeability, in these samples. α_2 -MG levels were shown to be significantly higher in seasonal (in-season) AR compared

with the out-of-season group. This is consistent with previous results examining indices of plasma leakage [47, 54, 55] and more specifically studies evaluating levels of α_2 -MG in naturally occurring seasonal AR [17, 53, 56, 57]. There was a significant correlation between α_2 -MG and eotaxin levels.

The role of the eosinophil in allergic airway disease expression has been put into question following the studies within the lower airways with monoclonal antibodies against IL-5, which significantly reduce peripheral blood, tissue and airway luminal eosinophils but have little clinical impact either on markers of disease activity in naturally occurring disease or on early and late asthmatic reactions [58, 59]. In our present study, we have found a correlation between α_2 -MG concentrations in lavage and lavage eosinophil numbers. The relevance of eosinophils to this airway exudative process and the mechanisms of nasal airway eosinophil recruitment are not clearly defined. Vascular changes underlie both these processes, with evidence in nasal biopsies from subjects with AR of up-regulated expression of the leucocyte endothelial cell adhesion molecule, vascular cell adhesion molecule 1 (VCAM-1), an adhesion molecule relevant to eosinophil recruitment through attachment to the eosinophil expressed ligand, and very late antigen 4 (VLA-4) [1, 60, 61]. Contraction of the endothelial smooth muscle to increase inter-endothelial pore size and facilitate plasma exudation arises in response to the local release of mediators of inflammation, such as the sulphidopeptide leukotrienes (LT), LTC₄ and LTD₄, generated and released from mast cells, basophils, and eosinophils [1, 62]. The correlation between α_2 -MG concentrations in lavage and lavage eosinophil numbers may thus be a reflection of the relevance of eosinophil products to plasma protein exudation. While eosinophil-derived mediators, such as leukotrienes, have relevance to changes in vascular permeability [63], it has also been shown in an animal model that the eosinophil granule components, major basic protein, eosinophil cationic protein, eosinophil peroxidase, and eosinophil-derived neurotoxin can all increase vascular permeability [64]. Thus, activated eosinophils may alter vascular permeability by a number of mechanisms, and the present study is consistent with the interaction between eosinophils and vascular permeability alterations within the upper airways.

This study thus extends previous work as to the relevance of eotaxin as a chemokine of pertinence to allergic inflammation and clearly identifies its relevance to AR in the natural setting. In view of its relationship to symptom severity, luminal eosinophil recruitment and vascular permeability change, this study would also suggest that eotaxin represents a valid therapeutic target worth exploring in the treatment of AR.

Acknowledgements

This work was supported by the Louis Alexander and Augustus Newman Foundation Research Fellowships from the Royal College of Surgeons of England, and an equipment grant from the Section of Laryngology and Rhinology at the Royal Society of Medicine. We would also like to thank Dr John Powell of Cambridge Antibody Technology, Cambridge, UK for supplying the human eotaxin antibody CAT-213. The authors would like to thank Ms Lorraine Hewitt for

her help with subject recruitment and phenotyping, and nasal lavage recovery. We would also like to acknowledge Mr Jon Ward for his help with the cytospins.

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