

Eosinophils are a feature of upper and lower airway pathology in non-atopic asthma, irrespective of the presence of rhinitis

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Summary

Background Asthma and rhinitis often co-exist and there are data to suggest that they may be two ends of the same disease spectrum. Immunohistochemical studies have shown that eosinophilia in the airways is a feature of rhinitic patients without asthma.

Objective The aim of our study was to examine whether cellular infiltration exists in the nasal mucosa of asthmatics even in the absence of symptoms and signs of rhinitis.

Methods Nasal mucosa biopsies were taken from 27 non-atopic subjects and comprised nine asthmatic rhinitic patients (AR), eight asthmatic non-rhinitic patients (ANR) and 10 healthy control subjects (N). Bronchial mucosa biopsies were also taken simultaneously from some of the patients ($n = 10$) to determine whether there was an association between cellular infiltration in the nose and the lungs. The alkaline phosphatase-anti-alkaline phosphatase (APAAP) method was used on 6 μ m thick cryostat sections using monoclonal antibodies against T cells (CD4, CD8), eosinophils (EG2) and mast cells (mast cell tryptase). Slides were counted blind and results expressed as cells per field.

Results The results showed that eosinophil counts were higher in both asthma groups compared with control nasal biopsies (median values AR 8.3, ANR 9.2, N 2.1 cells per field, $P < 0.01$). Furthermore, there was a significant correlation between eosinophil cell counts in the nose and the airways ($r = 0.851$ $P < 0.001$). No differences in eosinophil numbers were detected between the two groups of asthmatics. Also, no differences were noted for any other cell type (i.e. CD4, CD8, tryptase) among the three study groups.

Conclusions These results show that eosinophil infiltration was present in the nasal mucosa of asthmatic patients even in the absence of rhinitis, and add further support to the hypothesis that asthma and rhinitis are clinical expressions of the same disease entity.

Keywords: asthma, rhinitis, eosinophils, non-atopic

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Introduction

Asthma and rhinitis are common diseases that frequently co-exist [1–3] and share many clinical features. Both show intermittent symptoms or fluctuations in severity, are characterized by inflammation and obstruction, and a specific allergen elicits early and late phase reactions [4–6]. This

association is not surprising as the upper and lower airways are lined by the same pseudostratified columnar epithelium and the mucosal surface is contiguous throughout the respiratory tract. Therefore, both regions of the airway share a common susceptibility to various agents such as allergens, infectious agents, occupational sensitizers and drugs. Although several studies have shown that rhinitis and asthma are characterized by a similar inflammatory process [7–12], few have examined the association between the two diseases and most of the data are from atopic patients. Previous investigators showed that eosinophilia,

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a hallmark of asthmatic inflammation, is present in the bronchi of rhinitic patients without asthma [13,14]. Furthermore, it has been shown that patients with rhinitis and no history of asthma develop bronchoconstriction and demonstrate inflammatory changes in their bronchi when challenged with inhaled allergens [15–20], or when exposed to high allergen load such as during the pollen season. These data indicate that there is a pathogenetic link between rhinitis and asthma, and that the condition of the upper airways influences the lower airways. However, it is not clear whether the state of the lower airways influences the upper airway.

The aim of this study was to examine whether nasal mucosal inflammation, with particular reference to eosinophils, exists in asthma, irrespective of symptoms and clinical signs of rhinitis. Non-atopic patients and non-atopic normal control subjects were examined so that atopy was not a confounding factor in the study. The hypothesis was that the two conditions are part of the same disease spectrum. To test this hypothesis further, it was also examined whether a correlation exists between cell counts in the nose and the airways in asthma.

Materials and methods

Subjects

A total of 27 subjects participated in the study: nine patients with asthma and rhinitis, eight patients with asthma without rhinitis, and 10 healthy, non-atopic control subjects.

Asthma was defined as clear clinical history with current symptoms, based on diary card symptoms scores plus 15% reversibility in FEV₁ after two puffs of b₂ agonist, and/or positive methacholine challenge. Methacholine challenge was considered positive when a 20% drop in FEV₁ was induced at a cumulative dose of ≤1 mg. The challenge was performed with the equipment and the protocol described in the European Community Respiratory Health Survey (ECRHS) [21].

A diagnosis of rhinitis was based on clinical history and symptoms, direct examination of the nasal mucosa and Youtlen inspiratory peak flow readings. All patients answered a questionnaire on nasal symptoms, including itch, sneeze, discharge and blocking, and were excluded if they had recurrent symptoms of rhinitis. Patients with nasal polyps or significant deviation of the septum were also excluded. It should be mentioned that out of the 66 asthmatic patients screened, only 10 had no symptoms or signs of rhinitis (of which only two were atopic).

All subjects were non-atopic. Atopy was examined by skin prick tests to a panel of extracts of common aero-allergens, including house dust mite, grass, parietaria and olive tree pollen, cat dander, dog hair, cladosporium,

Table 1. Patient clinical characteristics

	Asthma–rhinitis	Asthma–no rhinitis	Normal control
<i>n</i>	9	8	10
Gender	2M:7F	3M:5F	6M:4F
Age	42 (22–61)	47 (24–64)	33 (20–66)
FEV ₁ % pred	81.2 (58–98)	80 (72–89)	94.3 (86–102)
Nasal PIFR	140 (70–170)	215 (170–275)	230 (180–345)

Values given as mean (range). M = male, F = female.

alternaria and aspergillus (Allergopharma, Germany), and/or raised total and specific IgE levels (Pharmacia, Sweden).

All patients were non-smokers, were receiving no oral, inhaled or topical treatment other than inhaled b₂ agonists prn, and had no evidence of respiratory infection in the previous 4 weeks. The study was approved by the Ethics Committee of Sotiria Hospital and was performed with the informed consent of the patients. Clinical data for the patients are shown in Table 1.

Study design

Asthmatic patients and healthy control subjects filled in a questionnaire on symptoms and duration of asthma and rhinitis, and had baseline and post-dilatation spirometry and inspiratory nasal peak flow measurement [22]. Within a week from lung function tests, nasal biopsies were taken between 09.00 and 23.00 h. A small subgroup of asthmatic patients (five asthmatic–rhinitic and five asthmatic non-rhinitic) underwent simultaneous nasal and bronchial biopsy. In this case, bronchoscopy was performed between 08.00 and 10.00 h, and nasal biopsy was taken immediately after bronchoscopy from the contralateral nostril of the one used at bronchoscopy.

Nasal biopsy

Local anaesthesia was achieved by applying a small cotton wool plug soaked in 3% cocaine and 0.025% epinephrine immediately below the inferior turbinate for 10 min. A 2.5 mm biopsy was taken using Gerritsma forceps [23]. Any local bleeding was controlled by cautery using a silver nitrate stick. Patients were observed for 1 h and discharged.

Fibreoptic bronchoscopy and biopsies

Bronchoscopy was performed using an Olympus model BFP20 bronchoscope with a 2.2 mm channel (Olympus Corporation, Tokyo, Japan). All subjects underwent bronchoscopy following pre-medication with 2.5 mg salbutamol by nebulizer, and 0.6 mg of atropine and 5–10 mg midazolam administered intravenously. Local anaesthesia of the vocal cords, trachea and bronchial tree was induced with 2% lidocaine. After inspection of the airways, biopsies were taken from the right middle, lower and upper lobe bronchi using Olympus forceps (FP-15C).

Immunohistology

Nasal biopsies were immediately placed in phosphate-buffered saline and within 15 min, transferred to a drop of OCT embedding medium (BDH) on a small piece of card. The biopsy was snap-frozen in isopentane pre-cooled in liquid nitrogen and stored at -80°C for a maximum of 1 month. Cryostat sections 6- μm thick were cut, air-dried for 1 h, and then fixed by immersion in a mixture of acetone and methanol (60:40) for 7 min. After a further 1 h drying period, slides were stored at -20°C until staining. Immunostaining was performed using a modified alkaline phosphatase anti-alkaline phosphatase (APAAP) method described in detail in previous publications [24,25]. Monoclonal antibodies against CD4+ T cells (Leu-3a, Becton-Dickinson, San Jose, CA USA) CD8+ T cells (CD8, Clone DK25, Dako A/S, Denmark) and eosinophils (EG2, Pharmacia Diagnostics, Sweden).

Counting method

Slides were counted blind in coded random order. An Olympus BH-2 microscope (Olympus Optical Co. Ltd, Tokyo, Japan) was used with an eyepiece graticule (0.202 mm²) at $\times 200$. At least two sections and a median of six fields were counted for each monoclonal antibody. The graticule was orientated along the epithelial basement membrane, and sub-epithelial counts were expressed as mean counts per high-power field. The mean intraobserver coefficient of variation for repeat counts was 8%.

Statistical analysis

Data were analysed using a statistical package (Sigma stat 2.0, Jandel Scientific, San Rafael, CA, USA). Three groups of patients were used in the analysis: (a) normal control subjects, (b) asthmatic non-rhinitic patients and (c) asthmatic rhinitic patients. For each group of patients, data are expressed as median and IQ range. When comparisons were made between groups, significant between-group variability

was first established using the Kruskal–Wallis ANOVA on ranks test. The Mann–Whitney *U*-test was then used for between-group comparison. Correlation coefficients between nasal and bronchial cell counts were obtained by Spearman's rank method. Correlation was examined for each asthma group separately and for all patients together. *P*-values less than 0.05 were considered significant.

Results

T-cells were quite numerous in the nose in all groups examined, but Kruskal–Wallis ANOVA on ranks failed to show significant differences in CD4⁺ cell counts between the groups (Fig. 1a). The median cell counts (IQ range) for CD4⁺ cells were 30.4 (18.7–78.8) in asthmatic rhinitic patients, 22.8 (3.2–47.8) in asthmatic non-rhinitic patients and 7.1 (0–22.6) in the normal control group ($P=0.066$). Median cell counts (IQ range) for CD8⁺ cells were also non-significant between the groups (Fig. 1g). These were 6 (0–28.2) in the asthma rhinitis group, 14.5 (3.7–44.5) in the asthma non-rhinitis group and 11 (2–14.4) in the control group ($P=0.118$).

Eosinophils were observed in the nose in all asthmatic patients, and Kruskal–Wallis ANOVA on ranks showed significant differences between the asthma groups and control biopsies ($P<0.01$) (Fig. 1c). Median cell counts (IQ range) were 8.3 (4.9–13.5) in the asthma rhinitis group, 9.2 (5.1–11.5) in the asthma non-rhinitis group and 2.1 (0.8–2.4) in the non-atopic control group ($P<0.01$). When using Mann–Whitney between-groups analysis, both asthma groups (rhinitic and non-rhinitic) were significantly different from the control ($P<0.001$), and there was no difference between them.

Although there was a trend for higher mast cell counts in rhinitis, this did not reach statistical significance and therefore, no differences were noted in mast cell infiltration in the nasal mucosa (Fig. 1d). Median cell counts were 12.3 (6.9–15.9) in the asthma–rhinitis group, 6.9 (3.7–13.9) in the asthma non-rhinitis group and 6.5 (0.9–13.5) in the control group ($P=0.315$). Results are shown in Fig. 1.

In the bronchial mucosa, cell count values were similar to those in the nose. Median values (IQ range) of bronchial cell counts were in AR: CD4 25.6 (19.8–69.4), CD8 15.2 (7.4–26.8), EG2 8.3 (6.2–14) and mast cells 17.6 (14.3–22.5), and in ANR: CD4 19.2 (5.4–56.8), CD8 19.5 (7.8–48.3), EG2 11.6 (7.3–19.1) and mast cells 16 (14.7–18.5). Mann–Whitney analysis was performed between the two asthma groups (with and without rhinitis) and no differences were noted. Analysis for correlation between cell counts in the nasal and bronchial mucosa was then performed for all asthmatic patients. No correlation was found for CD4, CD8 or mast cell counts but a highly

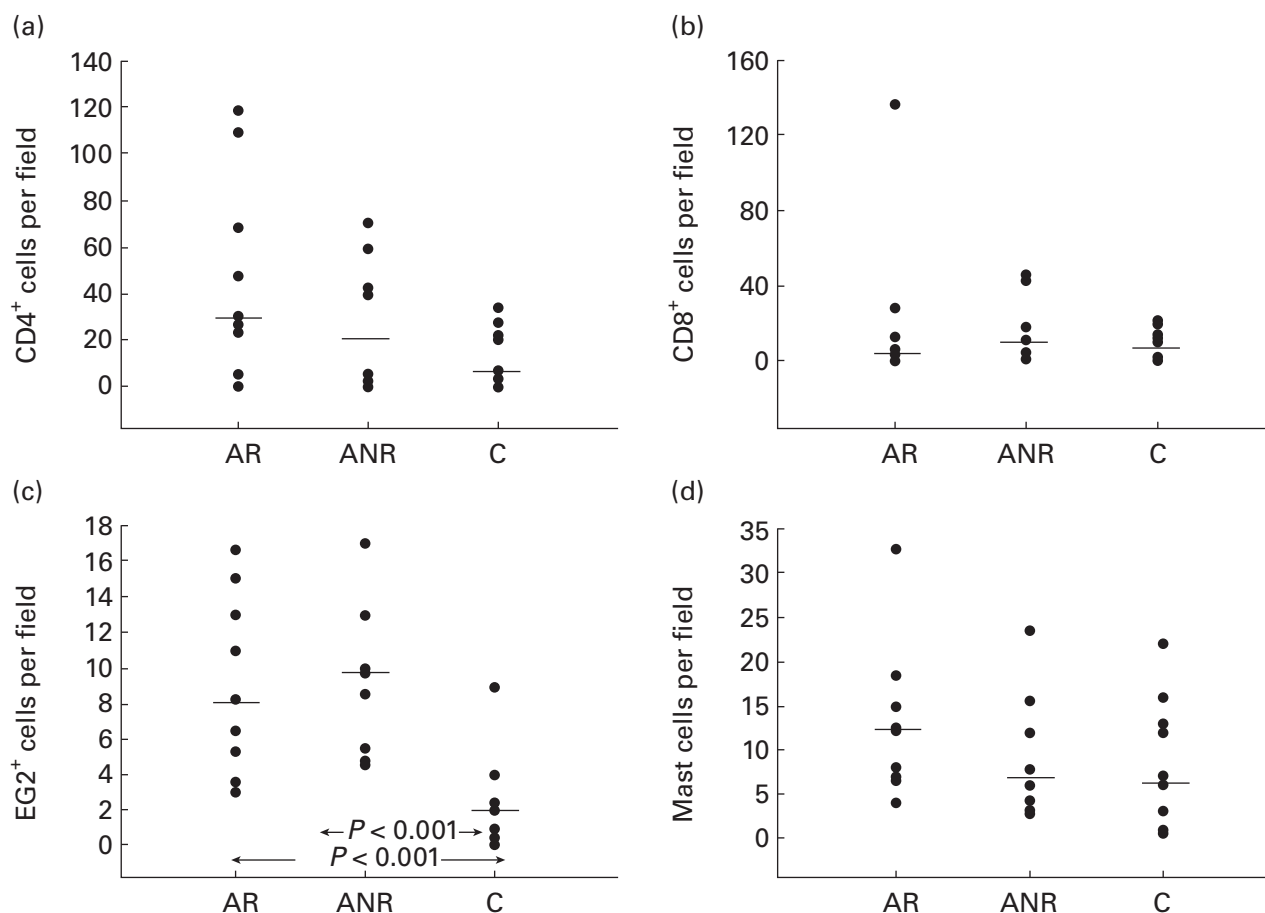


Fig. 1. Cell counts per high power field ($\times 200$). (a) CD4⁺ cells; (b) CD8⁺ cells; (c) EG2⁺ cells; (d) tryptase⁺ cells (mast cells). Bars represent median values. AR: asthma-rhinitis; ANR: asthma non-rhinitis; C: normal control subjects. *P*-values shown where significant.

significant correlation was observed with eosinophils ($r=0.851$, $P<0.001$) (Fig. 2). No correlation was found between the numbers of eosinophils in the nose and FEV₁.

Discussion

In this study, asthmatic patients were shown to have high numbers of EG2 positive cells in the nasal mucosa. The results show that nasal mucosal eosinophilia is present in asthma, irrespective of the presence of symptoms of rhinitis and in the absence of atopy. A trend for higher CD4 values was noted in both asthma groups, but differences did not reach statistical significance and no differences were noted in CD8⁺ cells or mast cells.

The clinical characterization of asthma and rhinitis is often a matter of debate. In this study, we carefully characterized asthmatic patients on the basis of history, symptoms, spirometry and methacholine challenge. Rhinitis symptoms were thoroughly checked and nasal patency was examined by respiratory nasal peak-flow measurements. The patient groups were matched for age and as all patients were

non-atopic, eosinophilia in the nasal and bronchial mucosa was a feature of asthma and not related to atopy.

The characteristic pathology of asthma is a chronic desquamating eosinophilic inflammation [8,13]. Blood and tissue eosinophilia in asthma is present in both atopic and non-atopic patients and indeed, eosinophils may be more numerous in non-atopic asthma where they show increased propensity for granule protein release [26]. Furthermore, it has recently been shown that IL-4 and IL-5 are equally prominent in the bronchial mucosa of atopic and non-atopic asthmatics [27], indicating that not only eosinophilia but probably also local B cell stimulation are features of asthma and not related to atopy. The pathology of rhinitis is similarly eosinophilic; allergic rhinitis [28], polypoid rhinosinusitis [29] and non-allergic rhinitis with eosinophilia (NARES) are associated with eosinophilia. Sinus mucosal eosinophilia in non-atopic asthmatic patients with chronic sinusitis has also been described [30]. IL-4 is present locally in the nose of atopic rhinitic patients [31,32] and so is IL-13 [33], an interleukin involved in eosinophil survival. Inflammation,

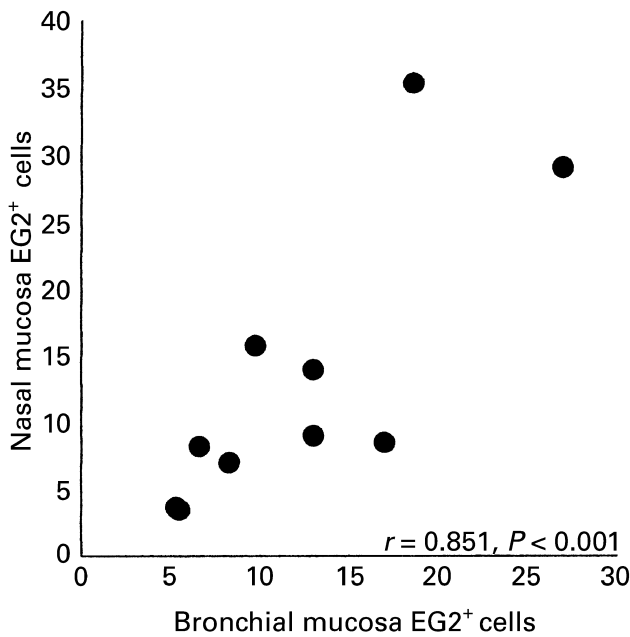


Fig. 2. Correlation between EG2⁺ cells in the bronchial and the nasal mucosa.

therefore, in rhinitis and in asthma is quite similar. On the other hand, it seems that there is a link between rhinitis and asthma, and this link is the subject of some excellent reviews [34,35]. Previous studies reported eosinophilia and loss of mast cell granular content in the bronchi of rhinitic patients without asthma [13,14], and elevated histamine levels in the bronchoalveolar lavage of allergic rhinitic patients with levels intermediate between atopic asthmatics and normal controls [36]. Furthermore, bronchial challenge with methacholine or allergen often results in bronchoconstriction in rhinitis patients without asthma [37], and treatment of rhinitis with topical steroids reduces bronchial hyperactivity [38,39]. A possible explanation for these phenomena might be the fact that when the nose is by-passed due to obstruction, as is often the case in rhinitis, air is inhaled through the mouth and reaches the bronchi colder, less humid and unfiltered. These changes in inhaled air may lead to hyperactivity and inflammation. Therefore, rhinitis may be linked to asthma through increased load of sensitizing agents. This should not be the case in the reverse situation.

Our study shows eosinophilia in the nose of asthmatic patients in the absence of clinical rhinitis. In addition, we found a significant correlation between eosinophil counts in the nose and the lung in asthma, as indeed between large and small airways in asthma [13]. These results show that the airway epithelium is affected all along its length in asthma, and further support the hypothesis that asthma and rhinitis are indeed parts of the same spectrum. Eosino-

phils are effector cells causing bronchial tissue damage and leading to remodelling in asthma. In rhinitis, there is also eosinophil granule protein release, and ECP and EPO are found in nasal biopsies and lavage fluid [40]. LTC₄ is also formed by eosinophils [41] and probably contributes to blocking and rhinorrhea. However, the presence of eosinophils in the nose is not always related to clinical disease, which is similar to findings reported in the bronchi [14]. The reason for this is unclear, but it may be that other mechanisms, such as T cell or neurogenic inflammation, are absent or that the threshold of patients differs. Furthermore, eosinophils do not seem to damage the nasal mucosa in rhinitis [42] although they are associated with epithelial damage in nasal polyps [43]. In a recent paper, increased basement membrane thickness was shown in the nasal epithelium of asthmatic-rhinitic patients with perennial rhinitis [44] but without epithelium shedding. In our study, measurement of basement membrane thickness would not have been accurate as we used frozen tissue but again, no epithelium shedding was noted, so although eosinophils are present in both asthma and rhinitis, it seems there may be differences in the nasal and bronchial tissue reaction to them. Although common mechanisms are implicated in the pathogenesis of both rhinitis and asthma, the end organs differ. The nose is the first contact of the respiratory system with the environment and acts as a barrier filtering inhaled air. It also has a very quick clearance rate so that noxious substances do not remain in contact with the mucosa for a long time. Perhaps, then, the nasal mucosa may withstand more assault without damage.

CD4⁺T cells are believed to be important in the pathogenesis of chronic asthma, but studies in mild asthma did not show significantly increased numbers of CD4⁺ cells compared with controls, similar to our results. Mast cells play an important role in allergen-induced asthma and rhinitis, and it has been shown that seasonal pollen exposure results in epithelial migration of mast cells in the nose [9,27,45]. However, in both atopic asthma and rhinitis, mast cell numbers in the submucosa of the upper and lower airways are not different from controls [46] and this is in accordance with our data.

The most important finding in this study was the presence of eosinophils in the nasal mucosa of asthmatic patients without symptoms of rhinitis, an eosinophilia that correlates well with eosinophilia in the airways of the same patients. These findings further support the hypothesis that asthma and rhinitis are clinical expressions of the same disease entity.

Guidelines exist for the classification and management in both rhinitis and asthma [47,48]. However, the terms of classification differ (e.g. intrinsic asthma, idiopathic rhinitis) and so does the approach to management. Perhaps those guidelines should be reconsidered and the upper and

lower respiratory tract treated as one system using the same approach to classification and management.

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