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Airway Inflammation in Atopic Patients: A Comparison of the Upper and Lower Airways

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Abstract

Objective. The purpose of this study was to understand and assess the inflammatory response within the upper and lower airways in patients suffering from both asthma and allergic rhinitis.

Study Design. Cross-sectional study.

Setting. A laboratory-based study of patients with allergic rhinitis and asthma.

Subjects and Methods. Glycol methacrylate resin-embedded specimens from 10 patients with allergic rhinitis and asthma taken from the nose and bronchi were assessed by immunohistochemistry. Monoclonal antibodies directed against specific cell markers for mast cells (AA1), eosinophils (EG2), neutrophils (NOE), and lymphocytes (CD3⁺, CD4⁺, CD8⁺) were studied. Cells were counted blind (as cells/mm²) in the submucosal matrix. Mann-Whitney *U* test was used for analyses. *P* values of .05 or lower were considered statistically significant.

Results. There was a significant increase in CD4⁺ (*P* = .05) and CD8⁺ cell counts (*P* = .001) in the lower airway compared to the upper airway. There were no differences between the 2 groups in the number of neutrophils, mast cells, eosinophils, and the CD3⁺ cell counts.

Conclusion. The upper and lower airways have parallel inflammation with possible bidirectional extension of inflammation in patients suffering from asthma and allergic rhinitis. There is increased lymphocytic infiltration in the lower airway, suggesting a possible preponderance for development and maintenance of allergic disease in the lower airway.

Keywords

allergic rhinitis, asthma, mast cells, eosinophils, neutrophils, lymphocytes, nasal mucosa, bronchial mucosa

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Allergic rhinitis is an inflammatory disease of global prevalence with a parallel increase in the prevalence of asthma. Most patients with asthma also have a history or evidence of rhinitis, and up to 30% of patients with persistent rhinitis have or develop asthma. Individuals with preexisting allergic rhinitis (AR) have an increased risk for the development of asthma,¹ particularly in the presence of risk factors such as family history of asthma and atopy.²

Asymptomatic bronchial hyperresponsiveness (BHR) is generally caused by airway inflammation, a feature of asthma, and is also frequently present in allergic rhinitis.³ The inflammatory responses in the nasal mucosa and in the bronchial mucosa have not been fully elucidated in patients with clinical symptoms of allergic rhinitis and asthma. Very few studies have investigated upper and lower airways simultaneously in atopic patients.

Chanez et al⁴ compared nasal and bronchial inflammation in asthmatic patients with perennial rhinitis and found more eosinophilia and structural changes in the bronchial mucosa than in the nasal mucosa. Two other studies compared upper and lower airway inflammatory cell infiltration in asthma and allergic rhinitis but in individual groups of patients with asthma, rhinitis, and healthy controls.^{5,6} We studied the inflammatory cell infiltration in the upper and lower airways of patients with the clinical symptoms of allergic rhinitis and asthma simultaneously to assess any difference or preponderance of inflammation in the 2 airways.

Materials and Methods

Subjects

Ten subjects with perennial allergic rhinitis and mild to moderate asthma on short-acting β_2 -agonists on demand volunteered to

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participate in the study. The volunteers were patients referred by their family physicians to the specialist allergy clinic and represented a subgroup of patients from the general population with both asthma and allergic rhinitis.

At inclusion, all subjects underwent skin prick testing to a standard panel of aero-allergens, which included birch, timothy grass, mugwort, cat, horse, dog, *Dermatophagoides pteronyssinus* (house dust mite [HDM]), *Dermatophagoides farinae* (HDM), ragweed, *Cladosporium* (mold), and *Alternaria* (mold). A positive result was recorded if the skin prick test (SPT) resulted in a wheal reaction equal to or greater than 3 mm than the histamine control at 15 minutes.

Forced expiratory volume in the first second (FEV_1) was measured in all subjects using a computerized pneumotach spirometer (Vitalograph, Buckinghamshire, UK). To determine airway responsiveness, methacholine challenge with nebulized concentrations of methacholine ranging from 0.03 to 32 mg/mL was performed in the patients with allergic rhinitis and asthma. The provocative concentration (PC) of methacholine was calculated on the log-dose-response curve as the concentration of methacholine required to result in a decline in FEV_1 of 20% (PC20).

The inclusion criteria for asthma were based on the history of breathlessness, wheeze, cough, and/or chest tightness with signs of wheezing or evidence of hyperinflation; positive methacholine challenge; and treatment with short-acting β_2 -agonist for symptom relief. Asthma was classified as mild persistent asthma (symptoms lasting more than 1 week but presenting less than once a day, no disturbance of sleep or daily activities) and moderate persistent asthma (symptoms occur daily, symptoms affect sleep and daily activities, and regular use of short-acting β_2 -agonist is needed) according to the Global Initiative for Asthma (GINA) guidelines.⁷ The subjects also had AR diagnosed on the basis of a clinical history and clinical examination. All patients had positive skin hypersensitivity to HDM. The patients were classed as having mild or moderate-severe persistent AR (perennial) based on the Allergic Rhinitis and its Impact on Asthma (ARIA) guidelines depending on the clinical symptoms.⁸

The characteristics of the patients are detailed in **Table 1**. Exclusion criteria included any history of smoking and an airway infection within 6 weeks before or at the time of biopsies. Current uses of any medications were noted, and no patient was receiving corticosteroid therapy. The study took place outside the pollen season. Nasal and bronchial biopsies were taken simultaneously at the same time in the last week of November. The South West Local Research Ethics Committee (SWLREC) approved the study, and the subjects gave their written informed consent.

Nasal Biopsy

Subjects were given local anaesthesia spray (4% topical lignocaine and adrenaline). After 15 minutes, the nasal mucosa was checked for pain sensation. The nose was visualized using Thudichum's nasal speculum. The nasal biopsies were taken under direct vision from the inferior or inferomedial border of the inferior turbinate because of the ease of access and management of any bleeding after the procedure. The biopsies were

Table 1. Patient Demographics

Age	Sex	Atopy	Allergic Rhinitis (Persistent)	FEV_1 % of Predicted
20	Female	HDM	Moderate-severe	91
32	Female	HDM	Mild	99
28	Male	HDM	Moderate-severe	92
21	Female	HDM	Mild	90
23	Male	HDM	Moderate-severe	83
25	Female	HDM	Mild	91
22	Male	HDM	Moderate-severe	85
36	Male	HDM	Moderate-severe	88
20	Female	HDM	Mild	95
30	Female	HDM	Moderate-severe	75

Abbreviations: FEV_1 , forced expiratory volume in the first second; HDM, house dust mite.

taken by Hartmann's aural forceps, straight, round cup, 80 mm to shoulder (Medicon, Tuttlingen, Germany). Any bleeding was controlled using a local pressure pack.

Bronchoscopy and Biopsy

Research bronchoscopic procedures have not been standardized, and the reproducibility of reported findings often is not known.⁹ A validated protocol was used.¹⁰ The subjects were premedicated with 1.0 mg atropine given subcutaneously 30 minutes before the procedure. Topical local anesthetic was achieved using lidocaine. All subjects tolerated the procedure well. A flexible video bronchoscope (Olympus BFIT200, Tokyo, Japan) was inserted through the mouth via a mouthpiece with the subjects in the supine position. Four to 6 endobronchial mucosal biopsies were taken for immunohistochemistry. The biopsies were obtained either from the anterior aspect of the main carina and the subcarina of the second- to fourth-generation airways on the right side or from the posterior aspect of the main carina and the corresponding subcarina on the left side as per the local guidelines. The coded biopsy specimens were embedded in glycol methacrylate (GMA) resin.

Immunohistochemistry

The coded biopsy specimens were processed into GMA resin for immunohistochemistry as described previously.¹¹ Immunohistochemical staining was performed using the streptin-avidin-biotin-peroxidase technique. Monoclonal antibodies were directed against specific cell markers, including mast cells (AA1), eosinophils (EG2), neutrophil elastase (NOE), and lymphocytes (CD3⁺, CD4⁺, CD8⁺). Positively stained nucleated cells were counted separately in the epithelium and in the submucosa, excluding mucosal glands, blood vessels, and muscle, in 4 separate fields and were averaged. Using a microscope equipped with a graticule, 2 authors independently counted these cells, and the average of their final count was analyzed. The difference in counts between the 2 authors was within 5%. The area of the submucosa and the length of the epithelium counted were measured using computed-assisted image analysis. To be included in the study, the biopsies had to have a minimum area of 0.46 mm², as this has been previously shown to give representative data.¹² The results

Table 2. Median (Interquartile Range) of the Cells in the Bronchial and Nasal Mucosa

	Bronchial Mucosa	Nasal Mucosa	PValue
AA1	45 (35.5-46.8)	40 (35.8-42.8)	.63
EG2	11.5 (6.1-20.8)	15 (8.3-31.5)	.52
CD3 ⁺	186 (107-380)	256 (80-405)	.82
CD4 ⁺	91 (54.3-222.0)	24 (12.5-98.5)	.05
CD8 ⁺	91 (44.5-143.5)	14 (8.3-18.5)	.01
NOE	15 (7.5-33.3)	31(13.8-61.8)	.31

Abbreviations: AA1, mast cell; EG2, eosinophil. Lymphocytes: CD3⁺, T cell coreceptor; CD4⁺, T helper cell; CD8⁺, T suppressor cell; NOE, neutrophil elastase.

were expressed as cells/mm². The analysis of the biopsies was performed with the observer blinded to the biopsy source.

Three of the 10 biopsy specimens had poor epithelium, and only 5 patients had a matched pair of epithelium. Epithelial analysis of cellular infiltration could not be undertaken, but all 10 patients had intact submucosa and were analyzed.

Statistics

Data were not normally distributed and are presented as medians with 25th and 75th percentiles. Because of the small number of patients, a power calculation was not performed. Comparison of baseline immunohistochemistry indices was performed, and a Mann-Whitney *U* test was used for post hoc analyses if a significant difference was observed between the 2 groups. *P* values of .05 or lower were considered statistically significant.

Results

Analyzable biopsies were obtained from all patients. The biopsies had a mean submucosal area of 1.51 and 0.62 mm² in the nasal (rhinitic) and bronchial (asthmatic) mucosa, respectively. The nasal mucosa had a CD4⁺ count of 24 cells/mm² and a CD8⁺ count of 14 cells/mm². The bronchial mucosa had a CD4⁺ count of 91 cells/mm² and a CD8⁺ count of 91 cells/mm². The difference in the CD4⁺ and CD8⁺ cell counts between the bronchial and nasal mucosa was statistically significant with *P* = .05 and *P* = .01, respectively (**Figure 1** and **Figure 2**). The nasal mucosa also showed more CD3⁺ cell expression than the bronchial mucosa but was not statistically significant (*P* = .82). There was no difference in the number of mast cells (*P* = .63), eosinophils (*P* = .52), and neutrophils (*P* = .31) between the nasal and bronchial mucosa (see **Table 2**).

Discussion

In this study, we have demonstrated similar inflammatory changes in the upper and lower airways of patients with AR and asthma. The observation that the inflammation is parallel and extends either from the lower to the upper airway or from the upper to the lower airway is consistent with previous findings. Eosinophilia was shown in the bronchial mucosa of asthmatic, atopic nonasthmatic, and normal subjects.¹³ Similarly, allergic inflammation in the nasal mucosa was demonstrated in nonallergic asthmatic patients without rhinitis,⁵ although both studies had small sample sizes.

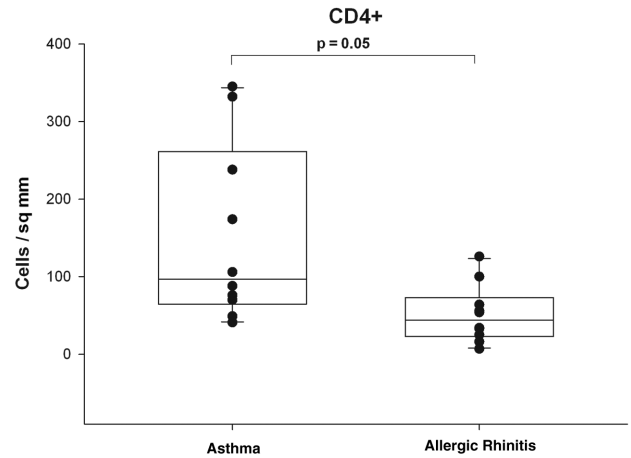


Figure 1. CD4⁺ infiltration in the lower airway (asthma) and upper airway (allergic rhinitis) with cells/mm² on the y-axis.

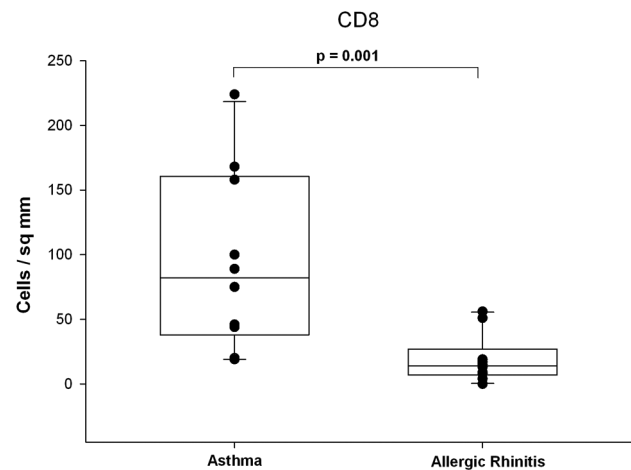


Figure 2. CD8⁺ infiltration in the lower airway (asthma) and upper airway (allergic rhinitis) with cells/mm² on the y-axis.

Use of the same patients with both upper and lower airway allergic manifestation reduces the variability of the inflammatory process in different patient groups compared previously. To assess the level of inflammation, the mast cells and eosinophil counts of our subjects were compared to the mast cells and eosinophil counts in the nasal mucosa of nonatopic, non-rhinitic subjects who were previously examined by our group in a different study. The study showed that nonatopic and non-rhinitic subjects had negligible or no mast cells and eosinophils in their submucosa. The literature on mast cells and eosinophil numbers in normal controls suggests a similar pattern with negligible infiltration of eosinophils. The actual numbers of inflammatory cells in individual studies differ because of variations in the methodologies, but the pattern can be compared. In one such study, the mast cells in control subjects were 2.1 cells/mm² in the nasal submucosa.⁵

The average mast cell and eosinophil counts were similar. The eosinophilic response in both the nasal and bronchial mucosa in our study was consistent with the findings of

Braunstahl and Hellings,¹⁴ who compared the upper and lower airways and showed similar nasal eosinophilia and bronchial eosinophilia in atopic patients. The mast cell, which activates and perpetuates the inflammatory response, was found to be similar in response to the eosinophils. This finding was consistent with previously published results by Brown et al,⁶ who observed similar submucosal mast cell numbers in subjects with AR and allergic asthma.

Although there is no statistical difference in the number of T cell receptors between the nasal and bronchial mucosa, a higher CD3⁺ cell count in the nasal mucosa was recorded in our study. This was in contradiction to a significant increase in CD4⁺ and CD8⁺ cells in the bronchial mucosa compared to nasal mucosa. This was an unusual finding, and we believe that the CD3⁺ cells probably represent a bigger group of T cells apart from CD4⁺ and CD8⁺ that was not included in our study, leading to a skewed result. This result may also be due to a small sample size.

Our results of T cells were also in contrast to a study by Brown et al,⁶ who showed increased CD3⁺ and CD8⁺ in the nasal mucosa of patients with allergic rhinitis compared to allergic asthma. The differences may be due to the study model, with use of bronchial and nasal mucosa from the same patients in our study compared to that of Brown et al,⁶ who compared 2 sets of subjects with allergic rhinitis and allergic asthma. The other factors that may have affected our results are subject selection, staining technique used, and methods of sampling.

The increased CD4⁺ counts in the bronchial mucosa may be a feature in patients who have both nasal and bronchial symptoms and may represent higher inflammation in the bronchial mucosa. The CD4⁺ cells are a known source of interleukin (IL)-5 and IL-6. These interleukins have shown to induce airway contractility and increase the risk of asthma. The CD8⁺ has a dual function; some are cytotoxic and others mediate T cell suppression. A depletion of CD8⁺ T cells leads to an enhanced Th2 response and bronchial hyperresponsiveness before sensitization in both rat and murine challenge models.¹⁵ It is thus possible that the increased number of CD8⁺ cells in the lower airways of these patients may represent a protective phenotype, preventing exacerbation of airway contraction and thus counteracting the effects of CD4⁺ cells.

Although similar numbers of inflammatory cells in the upper and lower airways suggest a parallel inflammatory response, it is possible that there may be a difference in the activation status of the cells that determine the manifestation of allergic airway disease; testing this needs a different methodology and cannot be assessed from our study. The triggers, which activate the inflammatory cells, may come from natural exposure to allergens, which have been shown to increase the number of eosinophils and mast cells.

In our study, subjects had clinical symptoms of mild to moderate asthma with predominantly moderate to severe rhinitis. It will be interesting to see the inflammatory response in subjects with severe asthma and rhinitis. As our study had a small number of subjects, a larger study representing the total spectrum of the disease with a normal control group may be

able to answer whether patients with a similar number of effector cells can have varying severity of symptoms or vice versa.

We propose a parallel inflammatory response with bidirectional extension of inflammation between the nasal and bronchial mucosa. We accept that the study is limited because of its small sample size. A comparison between the lower and upper airways across the whole spectrum of severity of both AR and asthma will help in understanding the relationship of the inflammatory changes within in the nasal and bronchial airways.

Author Contributions

Sanjiv K. Bhimrao, conduct of study, experiments, data collection, analysis; **Susan J. Wilson**, data collection and supervision; **Peter H. Howarth**, overall supervision, correction of manuscript.

Disclosures

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