

Experimental and seasonal exposure to birch pollen in allergic rhinitis and allergic asthma with regard to the inflammatory response

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Abstract

Background and Aims: Seasonal allergy is an interesting model to study the pathophysiological mechanisms involved in allergic inflammation. However, experimental allergen exposure is easier to perform and standardise. The primary aim of this study was to compare the inflammatory responses to high-dose bronchial challenge and natural exposure during birch pollen season. The second aim was to compare the responses of patients with allergic rhinitis and allergic asthma, respectively to both types of allergen exposure.

Methods: Fifteen birch pollen-allergic patients (seven with asthma and eight with rhinitis) and five healthy individuals were studied during pollen season and after challenge with birch allergen. Symptoms, medication and peak expiratory flow rate (PEFR) were recorded, and blood samples, spirometry and induced sputum were analysed during season and after challenge.

Results: Patients with allergic asthma demonstrated a greater bronchial responsiveness to bronchial provocation with birch allergen than patients with rhinitis ($P = 0.04$) whereas no difference was found regarding nasal challenge. No significant association was found between the level of responsiveness and the inflammatory response after seasonal exposure. Seasonal exposure was related to a more marked systemic inflammatory blood–eosinophil increase than bronchial challenge [(median) (0.25 vs $0.11 \times 10^9/L$, $P = 0.03$)] and after nasal challenge, respectively [(median) (0.25 vs $0.04 \times 10^9/L$, $P = 0.003$)]. A significant correlation in eosinophil cationic protein in induced sputum was found between the experimental and seasonal exposure ($\rho = 0.62$, $P = 0.02$).

Conclusions: Bronchial allergen challenge with inhalation of birch pollen gives a similar inflammatory response in the airway but less systemic inflammation than seasonal exposure in birch pollen allergic patients with asthma and rhinitis.

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Key words

eosinophils – ECP – allergic rhinitis – allergic asthma – pollen season – bronchial challenge

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Authorship

Kämpe M, Janson C, Stålenheim G and Carlson M have substantially contributed to the design, performance of the study and writing of the paper. Stolt I has contributed in performance of the study and analyzed the data.

Ethics

The study was performed with the approval of the ethics committee at the Medical Faculty at Uppsala University and informed consent was obtained from each subject.

Conflicts of interest

The authors have stated explicitly that there are no conflicts of interest in connection with this article

Introduction

Allergy is essentially an inflammatory disease involving the nasal, para nasal and bronchial airways (1). Recent investigations have demonstrated that inflammation affecting one site of the respiratory tract can extend to

other sites (2), but it is still unclear whether this depends on migration of inflammatory cells within the mucosa or if cells also migrate by a systemic–bone marrow route. The involvement of eosinophil granulocytes in the allergic inflammation is well established (3, 4), but mast cells and Th2-lymphocytes are also

Table 1. Characteristics at baseline (mean, range) and inflammatory markers at baseline (median and interquartile range). No significant differences were found between any of the groups in terms of gender, age, smoking, lung function and inflammatory markers or between the two allergic groups in terms of allergy variables

	Control group (n = 5)	Allergic rhinitis (n = 8)	Allergic asthma (n = 7)
Gender (male/female)	2/3	7/1	5/2
Age	38 (27–58)	43 (24–66)	44 (27–56)
Ex-smokers	0	2	1
SPT birch mm ²	0	42.9 (20–88)	43.6 (26–64)
*Number of positive SPT	0	3 (0–6)	5 (1–6)
FEV ₁ (% of predicted)	105 (88–125)	101 (75–139)	96 (83–108)
Inflammatory markers blood			
Eosinophils 10 ⁹ /L	0.14 (0.10–0.16)	0.12 (0.08–0.14)	0.14 (0.11–0.21)
S-ECP µg/L	7.5 (5.3–11.7)	8.6 (4.6–11.7)	6.1 (4.4–11.3)
S-HNL µg/L	64.6 (55.5–88.9)	89.0 (53.6–112)	99.4 (65.3–118)
Induced sputum			
ECP µg/L	162 (81–162)	149 (65.6–296)	146 (54–350)
HNL µg/L	848 (494–1900)	1724 (510–2850)	1600 (480–2360)
Nasal lavage			
ECP µg/L	2.0 (2.0–2.0)	5.0 (2.0–21.0)	2.0 (2.0–5.0)
HNL µg/L	30 (26–33)	111 (47–247)	83 (40–202)

*In addition to birch.

ECP, eosinophil cationic protein; HNL, human neutrophil lipocalin; FEV₁, forced expiratory volume in one second; SPT, skin prick test.

important in the inflammatory process (5). Jatakanon *et al.* reported for more than a decade ago that neutrophil granulocytes have an important role in severe asthma (6), but still the involvement of the neutrophils in asthma and allergy is under debate.

Seasonal allergies to birch pollen, for example, are characterised by increased levels of inflammatory cells and markers during the pollen season (7, 8). Several studies have utilised this seasonal variation in order to study therapeutic interventions (9, 10) and to broaden the understanding of the pathophysiological mechanisms underlying allergic inflammation (11–13).

Studies that depend on the natural variation of allergens are, however, time consuming and involve a level of uncertainty as the concentration of allergens may vary from 1 year to another. In contrast, studies employing experimental allergen exposure such as bronchial allergen challenge (14–16) are easier to perform. On the other hand, the result of such studies may be more difficult to interpret as the time and dose of exposure is fundamentally different from what is seen during natural exposure.

The aim of the present investigation is to compare the inflammatory response of high-dose bronchial challenge and natural exposure during birch pollen in patients with allergic rhinitis or allergic asthma to birch pollen.

Materials and methods

Patients

Fifteen birch pollen-allergic patients were selected for the study (Table 1). All these patients were skin prick test positive to birch pollen. None of the patients had symptoms during the winter or were on regular treatment for rhinitis or asthma outside the birch pollen season. Seven of the patients that had a diagnosis of allergic asthma and reported having respiratory symptoms during the pollen season were categorised as having asthma as the predominant symptom (allergic asthma). Eight patients with a diagnosis of allergic rhinitis but not allergic asthma and having mainly eye and nose symptoms were categorised as having rhinitis as the predominant symptom (allergic rhinitis). All the patients had doctor-diagnosed seasonal allergic rhinitis or allergic asthma, as diagnosed by a lung physician and allergologist at the allergy outpatient clinic. The patients with birch pollen-induced asthma had a positive history of wheezing and dyspnea during pollen season, whereas patients with allergic rhinitis all denied symptoms from the airways.

Inhaled steroids or nasal steroids were not allowed out of season nor during the birch pollen season, and none of the patients was on any other regular medica-

tion during season. None of the patients had smoked for the past 10 years. Forced expiratory volume in one second (FEV₁) out of season was more than 75% of predicted and FEV₁/forced vital capacity (FVC) was more than 70% in all patients.

Control group

The control group consisted of five healthy, non-atopic, never-smoking subjects (Table 1). These individuals had no allergic symptoms either outside or during the birch pollen season. They were skin prick test negative to nine standard allergens including birch pollen, had no immunoglobulin E (IgE) antibodies to birch and had normal lung function with an FEV₁ of more than 80% of predicted.

Study design

The design of the present study has been described in a previous report of Kämpe *et al.* (17).

The study included five visits to our outpatient clinic. Both the inclusion visit and the baseline visit were performed out of season between November and January. Bronchial challenge was performed in January and February. The season visit was made 2 to 3 weeks after the pollen counts had reached 4000 grains/m³ of air (17). The study was performed during the birch pollen seasons in 2000 and 2002, because of the low pollen counts in 2001. Patients were included consecutively, thus all patients were studied pre-season and during season in the same year. The subjects were told to avoid short-acting bronchodilators and antihistamines for 24 h before the visit and nasal decongestants for 4 h before the visit.

Bronchial allergen challenge test

The experimental bronchial challenge test was performed using a DeVilbiss-40 nebulizer (particle size 0.5 to 5.5 µm, output 0.175 ± 0.3 mL/min, mean ± standard deviation) (Devilbiss Co, Somerset, PA, USA) (18). Bronchial challenge with birch pollen extract (Aquagen® SQ, ALK-Abelló, Hørsholm, Denmark) was performed in three steps with the doses 1000 standardized quality units (SQE), 10 000 SQE and 100 000 SQE, starting with inhalation of a diluent. A positive response was defined as a reduction of FEV₁ with at least 20% from the FEV₁-value after inhalation of diluent. The response to the allergen provocation was calculated as the cumulative dose that caused at least 20% decrease in FEV₁ (allergen PD₂₀). In case that no significant fall in FEV₁ occurred, allergen PD₂₀ was

arbitrary given the value of 150 000 SQE. Blood and sputum samples were taken approximately after 18 h.

Nasal challenge test

The experimental nasal challenge test was performed by instillation of 0.3 mL birch pollen extract (Aquagen® SQ) in three steps with the doses 1000 SQE, 10 000 SQE and 100 000 SQE, starting with a diluent, and applied in the same nostril every 15 min. The symptom score was estimated and if pronounced local symptoms and sneezing occurred, the challenge test was stopped. The response to the allergen provocation was categorised into four groups: no response, response to 100 000 SQE, and response to 10 000 SQE or 1000 SQE. Blood samples and nasal lavage were taken approximately 18 h after the challenge test was completed.

Skin prick tests

Skin prick tests were performed with nine standard aeroallergen extracts (birch, timothy, mugwort, cat dander, dog dander, horse dander, *Dermatophagoides pteronyssinus*, *Cladosporium herbarum* and *Alternaria*), using Soluprick SQ ALK (Hørsholm, Denmark). The results were read after 15 min, measuring the largest diameter of the weal and its perpendicular diameter, and the product was expressed in mm². Skin reactions were considered positive when larger than 9 mm².

Spirometry

Lung function tests were performed with a Vitalograph-Compact spirometer (Vitalograph Ltd., Buckingham, UK). FEV₁, FVC, FEV₁/FVC% and PEF were recorded. The reference values were those from the European Community for Coal and Steel (19).

Blood sampling

Four millilitres of EDTA blood was collected for routine laboratory tests of eosinophil counts (Cell-Dyn 4000, Abbott Laboratories, Abbot Park, IL, USA). Serum for analyses of eosinophil cationic protein (ECP) and human neutrophil lipocalin (HNL) was collected from the 4 mL of blood in SST tubes (Becton Dickinson AB, Franklin Lakes, NJ, USA), kept for 60 min in room temperature and centrifuged for 10 min at 3600 rpm. The serum was frozen to -70°C.

Induced sputum

Sputum samples were obtained by hypertonic saline inhalation according to Pizzichini *et al.* (20), except that the subjects were not pre-treated with inhaled bronchodilators. An ultrasonic nebuliser (OMRON U 1, Sonesta Tamro no 28 36 06, Stockholm, Sweden) was used for the inhalations. After completing the inhalation, the subjects were instructed to 'huff' and cough into the container. The mucus clods were aspirated with a 2-mL syringe and collected. The entire sputum clod was weighed and immediately transported to the laboratory. The sputum sample was kept on ice and an equal amount of 0.2% dithiothreitol in phosphate buffer, sputolysin (CalbioChem, Sputolysin Reagent, art no 56 000; San Diego, CA, USA), was added before incubation at 22°C for 15 min. The sample was then centrifuged and the supernatant frozen at -70°C for the subsequent analysis of ECP and HNL.

Nasal lavage

Lavage of the nasal mucosa was performed according to Wählinder *et al.* (21), with a 20-mL syringe attached to a nose olive. The subjects were standing with their heads flexed about 30° forward. At room temperature (20°C–22°C), sterile 0.9% saline was introduced into the nasal cavity. Each nostril was lavaged with 5 mL of the solution, which was flushed back and forth five times via the syringe, at intervals of a few seconds. The recovered fluid was weighed and the amounts obtained were comparable in all subjects. The fluid was transferred into 10-mL polypropylene centrifuge tubes. It was kept on ice and, within 30 min, the solution was centrifuged at 800 g for 5 min. The supernatant was then centrifuged at 1400 g for 5 min and immediately frozen in small aliquots at -70°C for analyses of ECP and HNL.

Inflammatory markers

Blood eosinophil counts (normal range 0.0–0.5 × 10⁹/L) were determined using routine methods at the Department of Clinical Chemistry, Uppsala University Hospital. Differential cell counts were obtained using a cytopsin preparation (Cytospin, Shandon, Southern Instruments, Sewickley, PA, USA) stained with May Grünwald and Giemsa, and examined under light microscope. ECP was analysed by Unicap, Pharmacia. HNL was assayed by a double-antibody radioimmunoassay described in detail elsewhere (22). The inter- and intra-assay coefficients of variations were <10% for all tests. Specific IgE was determined with a Radio-

AllergoSorbent Test at the Department of Clinical Immunology, Uppsala University Hospital (normal < 0.35 kU/L).

Statistical evaluation

The chi-squared test and Mann–Whitney U test were used to evaluate statistical differences between the groups. For paired analyses, Wilcoxon's matched pairs test was used. Correlations were investigated with Spearman's test (ρ). A *P* value of < 0.05 was considered significant. Both patient groups were combined when analysing the inflammatory response as previous analysis showed a similar seasonal inflammatory response in both groups (17). The results are expressed as median and interquartile range unless otherwise stated.

All the calculations were performed using the Statistica statistical software package (Statsoft Inc, Tulsa, OK, USA).

Results

Provocation and bronchial allergen responsiveness

Patients with allergic asthma were more responsive, measured by FEV₁ decline, to bronchial challenge than patients with rhinitis (Fig. 1A). This was also found when responsiveness was expressed as PD₂₀ [PD₂₀ = 3700 (2450–7700) vs 34 500 (3850–150 000) SQE, *P* = 0.04] (Fig. 1B). None of the non-allergic subjects reacted to the provocation. No difference was found between asthmatics and patients with rhinitis regarding the response to nasal challenge.

Inflammatory response during season in relation to bronchial reactivity

No significant association was found between the level of bronchial responsiveness to the allergen provocation and the inflammatory response to seasonal allergen exposure (Table 2).

Inflammatory response after experimental and seasonal exposure

Seasonal exposure was related to a more marked inflammatory response in the blood than bronchial or nasal provocation when combining the patient groups with allergic asthma and rhinitis (Table 3, Fig. 2A,B). No difference was found between bronchial and seasonal exposure concerning inflammatory response in

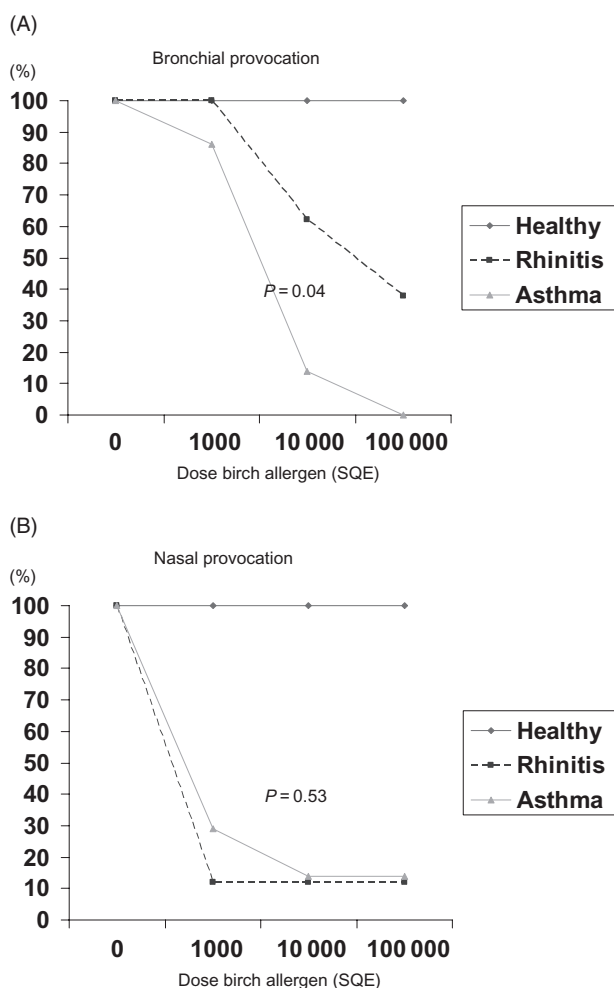


Figure 1. Proportion of patients and controls that responds to bronchial (A) and nasal (B) challenge. SQE, standardized quality units.

sputum (Table 3). Seasonal exposure was associated with a significant increase of ECP in nasal lavage ($P = 0.04$) whereas no significant increase was seen after nasal challenge. The difference in change in

Table 2. Correlation between change in inflammatory markers during the birch pollen season and bronchial provocation response expressed as allergen PD_{20}

	ρ	P value
Blood		
Δ Eosinophils $10^9/L$	0.06	0.84
Δ S-ECP $\mu g/L$	0.04	0.88
Δ S-HNL $\mu g/L$	-0.19	0.47
Induced sputum		
Δ ECP $\mu g/L$	0.04	0.89
Δ HNL $\mu g/L$	0.17	0.60

ECP, eosinophil cationic protein; HNL, human neutrophil lipocalin.

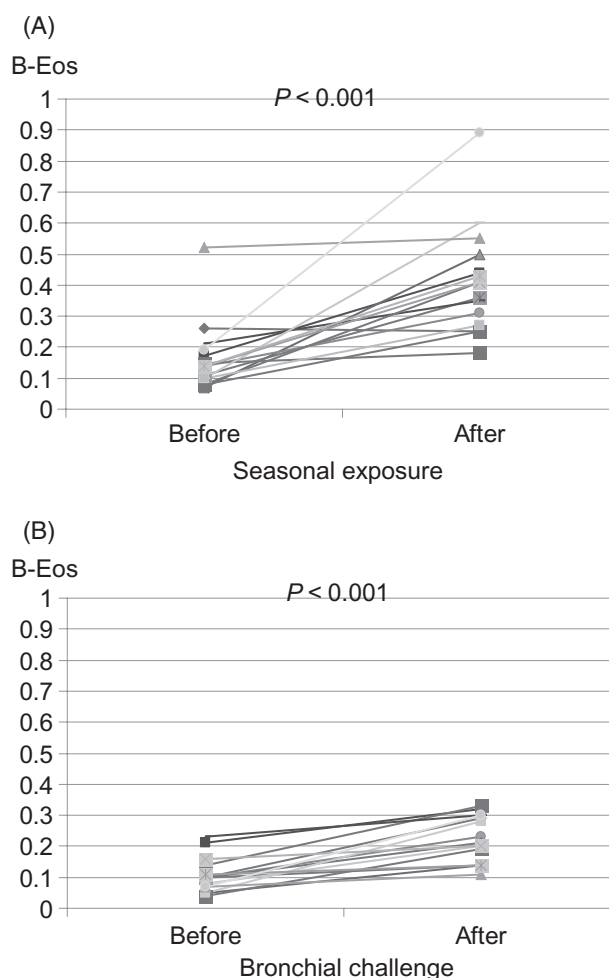


Figure 2. Change in blood eosinophils (B-Eos) after seasonal exposure (A) and bronchial provocation (B).

inflammatory markers between seasonal and nasal provocation was, however, not statistically significant (Table 3).

Correlation between seasonal and experimental exposure

A significant correlation was found between the experimental challenge and seasonal exposure in the change in sputum (SP)-ECP in induced sputum (Table 4).

Discussion

The main result of the present study is that bronchial allergen challenge gives a similar inflammatory response in the airways but less systemic inflammation than seasonal exposure in birch pollen allergic patients with asthma and rhinitis. The patients with predomi-

Table 3. Change in inflammatory markers (median and interquartile range) after seasonal and experimental exposure combining both allergic groups

	Seasonal exposure	Bronchial provocation	<i>P</i> value	Nasal provocation	<i>P</i> value
Blood					
ΔEosinophils 10 ⁹ /L	0.25 (0.14 to 0.33)	0.11 (0.04 to 0.19)	0.03	0.04 (0.02 to 0.1)	0.003
ΔS-ECP μg/L	8.6 (1.0 to 13.3)	4.3 (1.7 to 7.8)	0.16	1.3 (−0.8 to 3.9)	0.06
ΔS-HNL μg/L	1.4 (−3.5 to 5.3)	−2.0 (−8.6 to 9.6)	0.93	−4.4 (−11.4 to 2.8)	0.46
Induced sputum					
ΔECP μg/L	834 (11 to 2580)	734 (−7 to 5000)	0.93		
ΔHNL μg/L	−103 (−626 to 191)	188 (−94 to 474)	0.14		
Nasal lavage					
ΔECP μg/L	2.1 (0 to 9.1)			0 (−3.5 to 7.4)	0.18
ΔHNL μg/L	−10 (−42.6)			0 (−57 to 29)	0.98

ECP, eosinophil cationic protein; HNL, human neutrophil lipocalin.

nant asthma were more responsive to allergens than those with allergic rhinitis, whereas no difference was found between the level of allergen bronchial responsiveness and the seasonal inflammatory response.

The correlation between the inflammatory response in the airways after bronchial challenge and seasonal exposure indicates that bronchial challenge is useful when studying allergic inflammation in the airways. This may be an advantage as models using seasonal exposure to pollen are more time consuming and have many difficulties in standardisation. There are, however, several issues related to bronchial challenge methods such as getting the right lung deposition compared with natural exposure (23). In the present study, we used the most common model for bronchial challenge, e.g. high-dose provocation. Other models such as repeated low-dose regimens for 7 days are more time consuming but may simulate natural exposure to a larger degree (24, 25). Arvidsson *et al.* also recently reported a consistency between the early bron-

chial response to inhaled allergen, when comparing experimental and environmental challenge (26).

The lack of a systemic inflammatory response after bronchial challenge in the present study is in accordance with the results of Roquet *et al.*, though a significant increase in bronchial hyperresponsiveness was demonstrated (27). In that study, no significant systemic inflammatory response measured by eosinophils or ECP in blood could be demonstrated after bronchial challenge with allergen. Keen *et al.* also recently demonstrated an increase in Bet v1-specific IgA in nasal lavage during pollen season, but not after a single allergen challenge (28), indicating that allergen provocation gives rise to less systemic inflammatory changes than seasonal long-term exposure. Thus, both single challenge and environmental setting models (approximately 3–4 h daily) give a too short exposure time to activate systemic inflammation. In contrast, repetitive provocation or exposure during pollen season may cause this kind of systemic response. The lack of systemic effects after bronchial allergy challenging may be an advantage from a safety point but also a disadvantage if the purpose of the investigation is to study systemic allergic inflammation.

In our study, we showed that patients with allergic asthma were more responsive to the bronchial allergy challenge than patients with allergic rhinitis, but no difference in seasonal inflammatory response was found between subjects with a higher and lower allergen responsiveness. These results are in line with previous results from the same population where we found that allergic asthmatics were more responsive to hypertonic saline than those with allergic rhinitis, whereas no difference in seasonal inflammatory changes could be found between the groups (17). This is also in line with what Marcucci *et al.* recently

Table 4. Correlation between experimental and seasonal allergen exposure regarding change in inflammatory markers

	rho	<i>P</i> value
Blood		
ΔEosinophils 10 ⁹ /L	−0.08	0.74
ΔS-ECP μg/L	0.09	0.73
ΔS-HNL μg/L	0.06	0.82
Induced sputum		
ΔECP μg/L	0.62	0.02
ΔHNL μg/L	−0.30	0.28
Nasal lavage		
ΔECP μg/L	0.24	0.36
ΔHNL μg/L	0.03	0.90

ECP, eosinophil cationic protein; HNL, human neutrophil lipocalin.

demonstrated where children with allergic asthma and allergic rhinitis to house dust mite had the same degree of local inflammation in the airways and the nose, measured by nasal-ECP and exhaled nitric oxide (NO), during seasonal exposure in winter time (29). Thus, the difference in bronchial response between patients with allergic asthma and rhinitis is not only just the matter of ongoing inflammation, but also probably related to other mechanisms such as remodelling. Data in favour of this hypothesis point out that the remodelling occurring after chronic exposure to house dust mite in animal models (30, 31) leads to chronic inflammation and remodelling in the airway wall, subepithelial layer and extra cellular matrix with deposition of fibronectin and tensacin (32). This could interconnect the acute allergic inflammation which is dominated by eosinophils with the more neutrophilic inflammation in chronic and severe asthma (33). Recent studies have emphasised the role of eosinophils in remodelling and increase in airway smooth muscle mass after allergen challenge (34, 15).

In conclusion, bronchial allergen challenge gives a similar inflammatory response in the airways but less systemic inflammation than seasonal exposure in birch pollen-allergic patients with asthma and rhinitis. This indicates that bronchial challenge can replace models using seasonal exposure when studying allergic inflammation in the airways but are of limited value when investigating systemic allergic inflammation.

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