

Nasal Obstruction in Patients with Seasonal Allergic Rhinitis: Relationships between Allergic Inflammation and Nasal Airflow

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Key Words

Seasonal allergic rhinitis · Nasal obstruction ·
Nasal airflow · Eosinophils · Cytokines

Abstract

Background: Nasal obstruction is a typical symptom of allergic rhinitis. Allergic rhinitis is characterized by a Th2-dependent inflammation. The aim of this study was to evaluate the possible relationships between allergic inflammation, including inflammatory cells and cytokine pattern, and nasal airflow in patients with nasal obstruction due to seasonal allergic rhinitis. **Methods:** Fifty patients (31 males and 19 females, mean age 31.9 ± 4.8 years) with seasonal allergic rhinitis were evaluated during the pollen season. All of them had moderate to severe nasal obstruction. Total symptom score, rhinomanometry, nasal lavage, and nasal scraping were assessed in all subjects. Inflammatory cells were counted by conventional staining; IL-4, IL-5, IL-8, and IFN γ were measured by immunoassay on fluids recovered from nasal lavage. **Results:** Significant positive relationships were demonstrated between eosinophil infiltration and IL-4 levels ($p < 0.0001$), eosinophils and IL-5 levels ($p <$

0.0001), and eosinophils and IL-8 levels ($p < 0.0001$). Significant negative relationships were demonstrated between eosinophil infiltration and IFN γ levels ($p < 0.0001$) and eosinophil infiltration and nasal airflow ($p < 0.001$). **Conclusions:** This study demonstrates the close connection between Th2 cytokines and eosinophil infiltration. In addition, there is clear evidence concerning the relationship between eosinophil infiltration and nasal airflow. These findings constitute first evidence of the relationship between nasal airflow impairment and Th2-related inflammation in seasonal allergic rhinitis.

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Introduction

Allergic rhinitis is the most frequent IgE-mediated disease and its prevalence is progressively increasing. The late phase of allergic rhinitis is characterized by an inflammatory process. Experimental evidence supports a direct relationship between the IgE-mediated reaction and the resulting inflammatory process [1]. Allergen exposure activates mast cells with consequent release of mediators and cytokines capable of inducing inflamma-

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tory cell recruitment (including eosinophils, neutrophils, and Th2 cells) and their activation at the target organ level [2].

The immunopathologic features of allergic rhinitis have been related to the effects of Th2-derived cytokines [3]. Increased levels of Th2-derived cytokines, including IL-3, IL-4, IL-5, IL-13, and GM-CSF, have been broadly described in allergic rhinitis [3]. On the other hand, IL-8 has been little investigated in seasonal allergic rhinitis.

The hallmark of allergic inflammation is eosinophilic infiltration, as its presence in the nasal smear is suggestive of allergic etiology [3]. Nasal obstruction constitutes a leading symptom in allergic rhinitis and may be objectively measured. Allergic inflammation, mucosal congestion, and mucus hypersecretion contribute to cause nasal obstruction [4]. Allergic inflammation induces a mucosal swelling. Vasodilation causes engorgement of sinusoidal capacitance vessels. Rhinorrhea and increased mucus production contribute to impair nasal airflow.

The aim of this study was to investigate the possible relationships among nasal airflow, inflammatory cells, and cytokines in patients with nasal obstruction due to seasonal allergic rhinitis.

Materials and Methods

Patients

Fifty patients with seasonal allergic rhinitis were consecutively evaluated: 31 males and 19 females (mean age 31.9 ± 4.8 years). A detailed clinical history was taken and a complete physical examination was carried out for each patient.

The diagnosis of seasonal allergic rhinitis was made, according to international guidelines [5], on the basis of history and skin prick test positivity for pollen allergens. In our geographic area, the most important pollen allergens include grasses and *Parietaria officinalis* [6]. Ten normal subjects served as controls.

Study Design

The study was conducted during the pollen season, when patients were symptomatic. Only patients who met all the following criteria were eligible for inclusion into the study: men and women aged >18 years, history of seasonal allergic rhinitis due to seasonal allergen exposure during the previous 2 years, rhinitis symptoms during the 2 previous weeks, total symptom score (TSS) minimum value >6 at baseline, particularly with moderate to severe nasal obstruction (see below); informed consent obtained prior to study entry. Exclusion criteria were: sensitization to perennial allergens, diagnosis of asthma, acute and chronic upper respiratory infections within 30 days before the study, anatomic nasal disorders (i.e. septum deviation), nasal polyps, use of antibiotics, nasal or oral corticosteroids within the previous 4 weeks, and use of antihistamines within the previous week.

Symptom assessment, rhinomanometry, nasal scraping, and nasal lavage were performed in all subjects. The study was approved by the Review Board.

Skin Prick Test

Allergy was assessed by the presence of sensitization to the most common classes of aeroallergens by performing a skin prick test. It was performed as stated by the Italian Society of Allergy and Clinical Immunology [7]. The allergen panel consisted of the following: house dust mites (*Dermatophagoides farinae* and *pteronyssinus*), cat, dog, grasses mix, *Composita* mix, *Parietaria officinalis*, birch, hazel tree, olive tree, *Alternaria tenuis*, *Cladosporium*, and *Aspergillus* mix; the concentration of allergen extracts was 100 IR/ml (Stallergenes, Milan, Italy). A histamine solution in distilled water (10 mg/ml) was used as positive control and the glycerol-buffer diluent of the allergen preparations as negative control. Each patient was skin tested on the volar surface of the forearm using 1-mm prick lancets (Stallergenes). The skin reaction was recorded after 15 min by evaluating the skin response to the inoculation of each allergen extract in comparison with the wheal produced by the positive and the negative control. A wheal diameter equal to or greater than 3 mm was considered a positive reaction. Antihistamines were stopped 1 week before the skin prick test.

Nasal Symptoms

The following symptoms were assessed by the patient, answering the investigator's questions: nasal obstruction, sneezing, rhinorrhea, and itchy nose. Each symptom was evaluated using the following scale: 0 = absent, 1 = mild (symptom was present but was not annoying or troublesome), 2 = moderate (symptom was frequently troublesome but did not interfere with either normal daily activity or sleep), and 3 = severe (symptom was sufficiently troublesome to interfere with normal daily activity or sleep). TSS, i.e. the sum of scores on individual symptoms was considered.

Rhinomanometry

Nasal airflow was measured by active anterior rhinomanometry. Patients wore a tight-fitting facemask, and with the mouth closed, breathed through one nostril. A sensor, placed in the contralateral nostril, recorded data on pre- and postnasal pressures via airflow and pressure transducers. The instrument (ZAN 100 Rhino, ZAN, Messgeräte GmbH, Germany) was connected to a personal computer. The signals of transnasal airflow and pressure were amplified, digitized, and saved for statistical analysis.

Nasal airflow was reported as the sum of recorded airflow through the right and left nostrils in milliliters per second at a pressure difference of 150 Pa across the nasal passage.

Four or more airflow measurements were performed for each patient and the mean was recorded when reproducible values were achieved.

Nasal Cytology

Nasal cytologic specimens were obtained by scraping the head of the inferior turbinate with a cotton swab, as described in previous reports [6, 8]. Briefly, after the nasal scraping, the cotton tip of the swab was immersed in a plastic tray with phosphate-buffered saline (PBS) and transferred to a 10-ml polypropylene tube. The recovered fluids were centrifuged at 220 g per minute for 10 min, and each pellet was resuspended in PBS (2 ml). Cell suspensions were filtered

to reduce the quantity of mucus, and cytospin slides were prepared by using standard techniques.

Smears were stained with Diff Quik stain and were analyzed with a light microscope (Olympus U-SPT). The number of inflammatory cells was expressed as a mean of 10 optical fields at $\times 100$ magnification. Samples were examined in a blinded fashion.

Nasal Lavage

A lavage of the nasal cavity was performed using 5 ml of physiologic saline, according to standard methods described elsewhere [9]. This was performed with the patient's head bent backwards during closure of the soft palate. The recovered fluid was comparable in all groups; it was collected to evaluate cytokine levels and stored at -20°C [9].

Cytokine Assessment

The cytokines were measured with an ELISA method (R&D Systems, USA). This assay employed the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for each cytokine was precoated onto a microplate. Study samples and standard dilutions of each specific cytokine were assayed separately in duplicate according to the ELISA kit manufacturer's instructions. Standards and samples were pipetted into the wells and each cytokine was bound by the immobilized antibody. After removing unbound substances, an enzyme-linked polyclonal antibody specific for each cytokine was added to the wells. After removing unbound antibody-enzyme reagent, a substrate solution was added to the wells and color developed in proportion to the amount of cytokine bound in the initial step. Then, color development was stopped and the intensity of the color measured. The color intensity was read at a wavelength of 490 nm using a spectrophotometer (Molecular Device, USA). The average net optical density of the standards was plotted, and the amount of each cytokine in each sample was evaluated by interpolation from the standard curve. Results were the means of duplicate samples. The inter- and intra-assay coefficients of reproducibility for each cytokine were as follows: IL-4, 2–5%, and 3–6%; IL-5, 2–4%, and 2–7%; IFN γ , 1–3%, and 3–5%; IL-8, 4–8%, and 1–4%. The sensitivity of the commercially prepared ELISA was less than 1 pg/ml for IL-4, IL-5 and IFN γ , and less than 10 pg/ml for IL-8.

Statistical Analysis

Analysis of correlation coefficients was calculated using Pearson's test. Nasal eosinophil numbers, cytokine levels, and nasal airflow measurements were presented as mean $\pm$ SD. p values <0.05 were considered statistically significant.

Results

Subjects

Fifty allergic patients with seasonal allergic rhinitis were studied. All of them were sensitized to grasses and/or *P. officinalis* only.

There was a large amount of these pollens in the environment during the study period as it was performed during the spring (data not shown). General physical exami-

Table 1. Nasal symptoms, inflammatory cells, cytokines and nasal airflow in patients with seasonal allergic rhinitis (SAR) and in normal subjects

Symptoms	SAR patients	Normal subjects
Total symptom score	8.2 ± 1.4	0.2 ± 0.1
Inflammatory cells		
Neutrophils	21.3 ± 5.8	1.2 ± 0.3
Eosinophils	10.5 ± 3.9	0.2 ± 0.1
Cytokines		
IL-4, pg/ml	10.2 ± 3.1	0.8 ± 0.4
IL-5, pg/ml	6.5 ± 1.7	1.3 ± 0.6
IL-8, pg/ml	662.2 ± 118.6	247.8 ± 44.8
IFN γ , pg/ml	1.6 ± 0.6	32.4 ± 4.6
Total nasal airflow		
Flow, cm^3/s	482.5 ± 110.3	742.8 ± 128.4

nation was normal, apart from rhinitis symptoms, in all patients.

Symptoms

All patients were symptomatic, with moderate to severe nasal obstruction, as all of them were untreated (table 1).

Rhinomanometry

It was performed in all patients. A clear decrease in nasal airflow was present in all subjects in comparison with normal subjects (table 1).

Nasal Cytology

Both neutrophils and eosinophils were present in nasal smears in all patients (table 1).

Cytokine Levels

All investigated cytokines were detectable in all patients (table 1). A clear Th2 polarization was present in all patients with seasonal allergic rhinitis levels of IFN γ and high levels of IL-4 and IL-5. IL-8 levels were increased in comparison with normal subjects.

Correlations

Significant positive correlations were detected between IL-4 levels and eosinophils ($p < 0.0001$) (fig. 1), IL-5 levels and eosinophils ($p < 0.0001$) (fig. 2), and IL-8 values and eosinophils ($p < 0.0001$) (fig. 3). Significant negative relationships were demonstrated between IFN γ and eosinophils ($p < 0.0001$) (fig. 4), and nasal airflow and eosinophils ($p < 0.001$) (fig. 5).

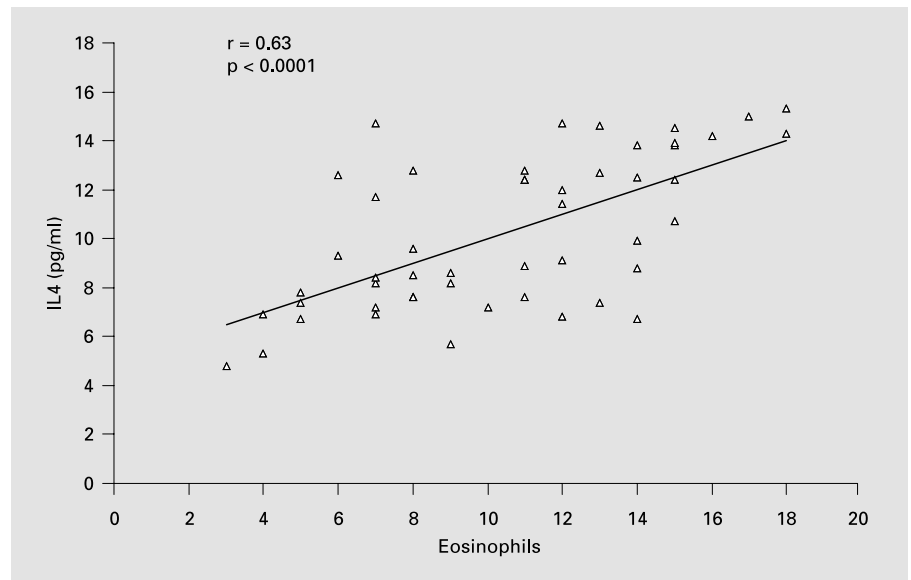


Fig. 1. Eosinophil number as a function of the concentration of IL-4 in nasal lavages of patients with seasonal allergic rhinitis.

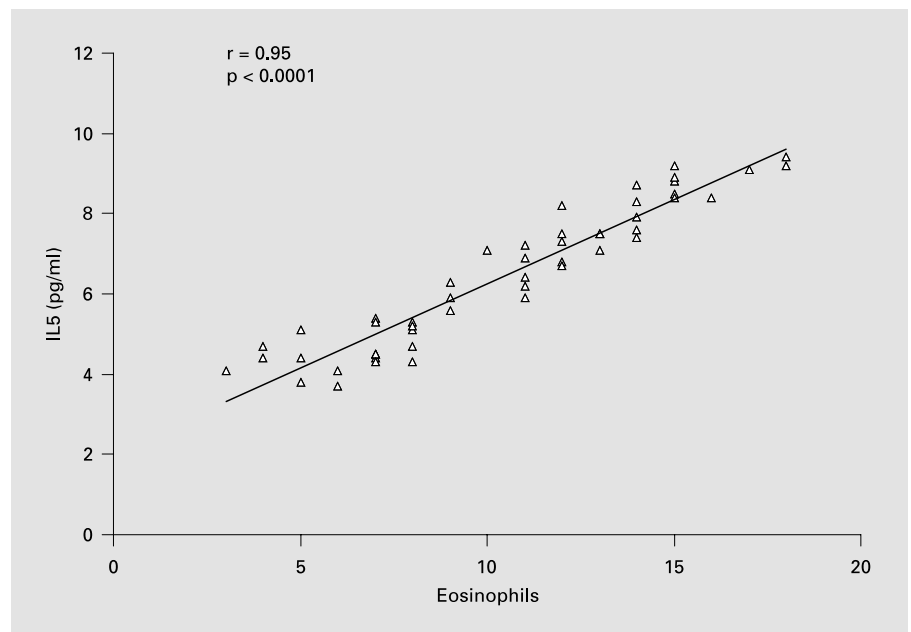


Fig. 2. Eosinophil number as a function of the concentration of IL-5 in nasal lavages of patients with seasonal allergic rhinitis.

Discussion

The present study aimed at investigating the possible relationships between allergic inflammation, including inflammatory cells and cytokine pattern, and nasal airflow in patients with nasal obstruction due to seasonal allergic rhinitis.

Allergic rhinitis is characterized by a Th2-polarized immune response. Th2-derived cytokines have been previously investigated in patients with seasonal allergic

rhinitis, particularly concerning the role of IL-4 and IL-5 [10].

IL-4 is a pivotal cytokine that orchestrates allergic inflammation as it is the most important signal to induce Th2 polarization in allergic subjects [11]. IL-4 is produced by a variety of cells, including Th2 lymphocytes, eosinophils, and mast cells. IL-4 promotes IgE synthesis, upregulates adhesion molecules selective for eosinophil recruitment, and causes increased mucus production [11]. Thus, there is agreement about the role exerted by IL-4 as a

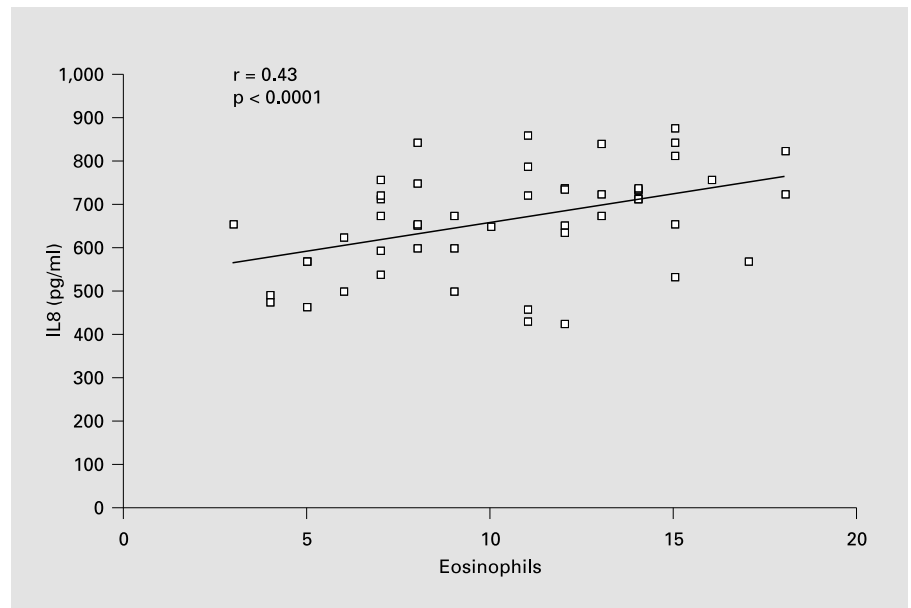


Fig. 3. Eosinophil number as a function of the concentration of IL-8 in nasal lavages of patients with seasonal allergic rhinitis.

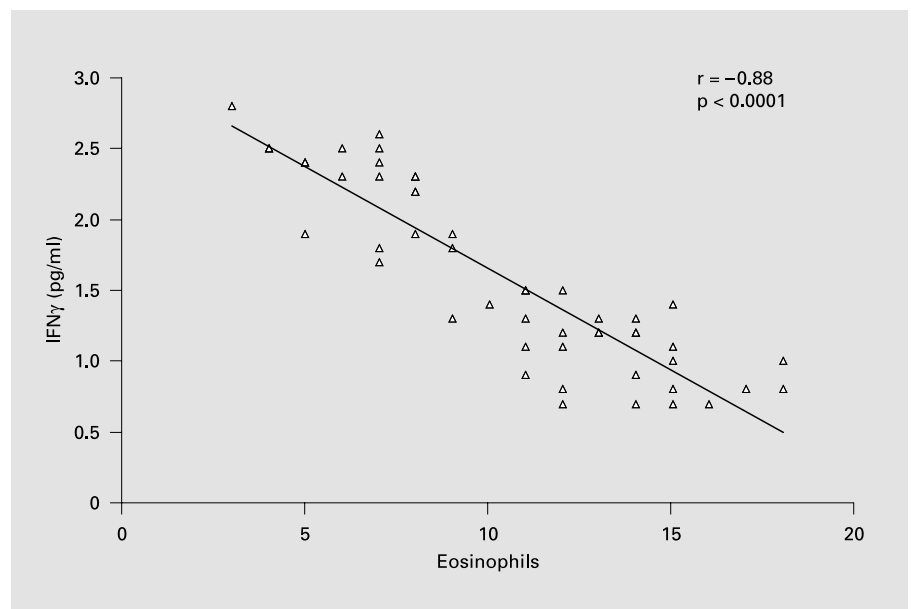


Fig. 4. Eosinophil number as a function of the concentration of IFN γ in nasal lavages of patients with seasonal allergic rhinitis.

main pathogenetic factor in inducing, maintaining, and amplifying inflammatory events in allergic rhinitis.

IL-5 plays a critical role in inducing recruitment of eosinophils from blood stream into nasal mucosa, activating eosinophils, and prolonging their survival in inflamed tissues, thus causing eosinophil accumulation at mucosal level [11].

On the other hand, neutrophils are recruited early after allergen exposure following chemotactic signals exerted by IL-8, which is released by activated epithelium and

mast cells and in turn activates neutrophils [12]. IL-8 is also a chemotactic agent both for eosinophils and lymphocytes.

Otherwise, there is accumulating evidence that Th1-related cytokines, such as IFN γ and IL-12, suppress and counteract this Th2 response and vice versa [13]. The suppressive effects of IFN γ have been shown to be mediated by various mechanisms, such as inhibiting Th2 cytokine production and skewing the differentiation of naive T cells toward Th1 subtype preference [14]. In addition,

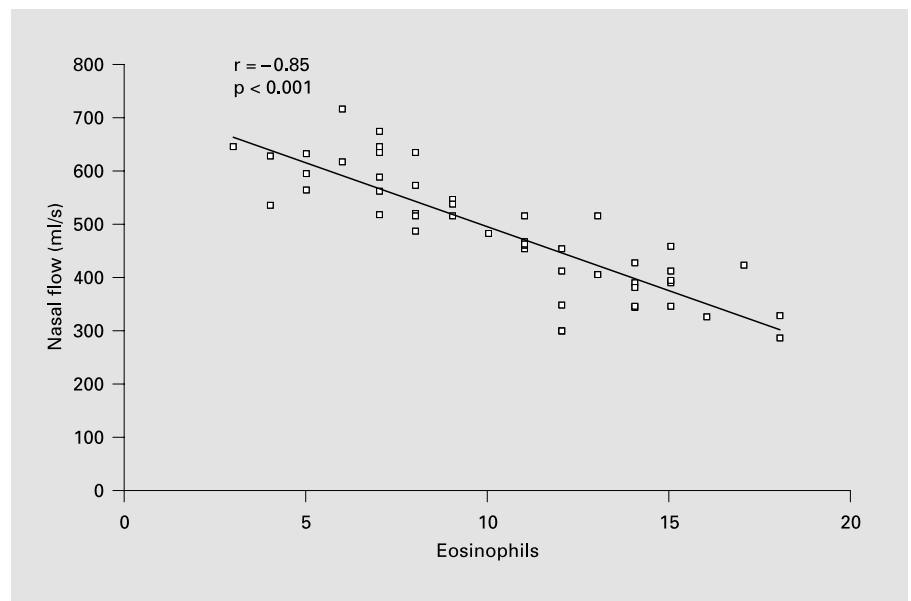


Fig. 5. Eosinophil number as a function of nasal airflow in patients with seasonal allergic rhinitis.

exogenous IFN γ protein could reverse the ongoing reactions [15]. Thus, IFN γ may play a regulatory role in the extent of airway responses to allergen. Some of the mechanisms that may be relevant include: suppressing the release of Th2-type cytokines from activated T cells [14], facilitating apoptosis of T cells and of eosinophils [16], and inhibiting local recruitment of eosinophils [17]. Therefore, IFN γ may clearly exert a crucial role to reverse ongoing allergic reaction.

Thus, Th1- and Th2-derived cytokines play an important role in modulating allergic inflammation, mainly concerning the recruitment and activation of eosinophils. In this regard, allergic inflammation accounts for nasal symptoms, especially nasal obstruction. Nasal obstruction constitutes a crucial symptom as it may affect quality of life, bronchial hyperreactivity, and ostiomeatal complex and eustachian tube patency, thus allowing the occurrence of several sequelae, including rhinosinusitis, otitis media, and asthma [18].

The pathogenesis of nasal obstruction is very complex and consists of three main events: inflammatory mucosal edema, vascular congestion, and mucus hypersecretion [18].

Activated inflammatory cells release several mediators, including histamine and leukotrienes, and Th2-derived cytokines that cause the occurrence of nasal obstruction. In this regard, nasal obstruction constitutes the nasal symptom most representative of allergic inflammation.

In this study, we have demonstrated that there is a significant positive relationship between eosinophils and Th2-derived cytokines in patients with seasonal allergic rhinitis, confirming previous findings [10]. This aspect highlights the pathogenetic role exerted by eosinophil accumulation and Th2-cytokine polarization in allergic rhinitis.

Moreover, there is a negative relationship between eosinophils and IFN γ levels: this finding is consistent with the 'antiallergic' activity of Th1-cytokines.

Finally, the most important result of the present study is the proof concerning the positive relationship among eosinophil infiltration, cytokine concentrations, and nasal obstruction. This issue underlines the close connection between allergic inflammation and nasal airflow impairment in patients with nasal obstruction due to seasonal allergic rhinitis. In other words, a clear structure-function relationship is detectable at nasal level during allergic inflammation.

In conclusion, the present study provides the first evidence of the relationships among nasal obstruction, eosinophil infiltration, Th2-derived cytokines, and nasal airflow in patients with seasonal allergic rhinitis. Therefore, we retain that, taken together, these data may be considered convincing proof of the role exerted by eosinophils and Th2 cytokines in causing nasal obstruction. Further studies should address this issue when investigating the effects of treatments.

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