

IL-5 synthesis is upregulated in human nasal polyp tissue

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Background: In most nasal polyps, tissue eosinophilia is a striking finding, the pathologic mechanism of which is not understood.

Objective: This study was performed to investigate a possibly distinct cytokine and chemokine pattern that could explain the characteristic tissue eosinophilia in nasal polyps.

Methods: Polyps from 23 patients and turbinate tissue from 18 control subjects were investigated. The cytokine protein content (IL-1 β , IL-3, IL-4, IL-5, IL-6, IL-8, IL-10, tumor necrosis factor- α , granulocyte-macrophage colony-stimulating factor, IL-1RA, RANTES, GRO- α) of tissue homogenates was measured by ELISA. Immunohistochemistry was performed in selected samples to detect IL-5⁺, major basic protein-positive, and EG2⁺ cells.

Results: IL-5 was detectable in only one sample of tissue from 18 control subjects but was found in 18 of 23 nasal polyps. Immunohistochemistry revealed an abundant number of IL-5⁺ cells, of which 69.5% could be identified as eosinophils by morphology. IL-6, IL-8, IL-10, tumor necrosis factor- α , GRO- α , and RANTES were detected in all specimens, without significant differences between groups ($p \geq 0.05$), whereas significantly higher concentrations of IL-1 β and IL-1RA were found in turbinate mucosa ($p \leq 0.05$). IL-3 was not detectable; granulocyte-macrophage colony-stimulating factor could only occasionally be found.

Conclusion: This study indicates that IL-5 plays a key role in the pathophysiology of eosinophilic nasal polyps and may be produced by eosinophils. (*J Allergy Clin Immunol* 1997;99:837-42.)

Key words: Nasal polyps, aspirin sensitivity, asthma, allergy, cytokines, chemokines, eosinophils, ELISA, immunohistochemistry

Nasal polyps are transparent, pale grey protrusions of the nasal and paranasal sinus mucosa, which prolapse into the nasal cavity. The prevalence of this disease has been estimated to be about 4% in the general population.¹ Nasal polyposis is believed to be a multifactorial disease that is frequently associated with asthma and other respiratory diseases such as cystic fibrosis, primary ciliary dyskinesia, and aspirin sensitivity. The cause and pathologic mechanism of this disease are so far ill-defined.² Numerous hypotheses concerning the patho-

Abbreviations used

GM-CSF:	Granulocyte-macrophage colony-stimulating factor
RA:	Receptor antagonist
TNF- α :	Tumor necrosis factor- α

genesis of eosinophilic nasal polyps range from chronic infections, inhalant or food allergies, and T-cell disturbances to aerodynamic factors.³

Histologic studies to elicit type and morphology of the infiltrating cells have shown that about 80% to 90% of the polyps are characterized by abundant eosinophils, which are partially EG2⁺ (activated).⁴ Other cells, such as CD8⁺ T cells and mast cells, are also regularly found in the polyp tissue and may contribute to the pathophysiology.

Recently, *in situ* hybridization techniques allowed the identification and localization of messenger RNA of cytokines, chemokines, and growth factors, which could be involved in the formation of nasal polyps. These studies have shown that mRNA for granulocyte-macrophage colony-stimulating factor (GM-CSF), IL-3, tumor necrosis factor- α (TNF- α), macrophage inflammatory protein-1 α , IL-1, and transforming growth factor- β can be detected in nasal polyps. IL-5 mRNA was only found in low quantities.⁵⁻⁹

After performing a pilot study,¹⁰ we investigated the protein concentrations of various cytokines and chemokines in homogenized nasal polyps and compared them with turbinate nasal mucosa of control subjects. Furthermore, we used immunohistochemistry to detect and identify IL-5 protein-positive cells.

METHODS

Subjects

Twenty-three subjects (7 women and 16 men; mean age, 43.9 years) with polyps and 18 control subjects (9 women and 9 men; mean age, 36.0 years) were studied. Nasal polyp samples were obtained during routine endonasal surgery. Nasal mucosal biopsy specimens from the inferior turbinates of 18 patients, who underwent surgical procedures because of septal deviations, were used as controls. The distribution of the underlying diseases (allergy, bronchial asthma, and aspirin sensitivity) is shown in Fig. 1. Subjects were identified as allergic by means of history, skin prick test results, and specific IgE levels. Aspirin sensitivity and asthmatic disease were diagnosed from patients' history and results of prior diagnostic procedures.

None of the subjects had received systemic or topical steroids for at least 4 weeks. Informed consent was obtained from all the

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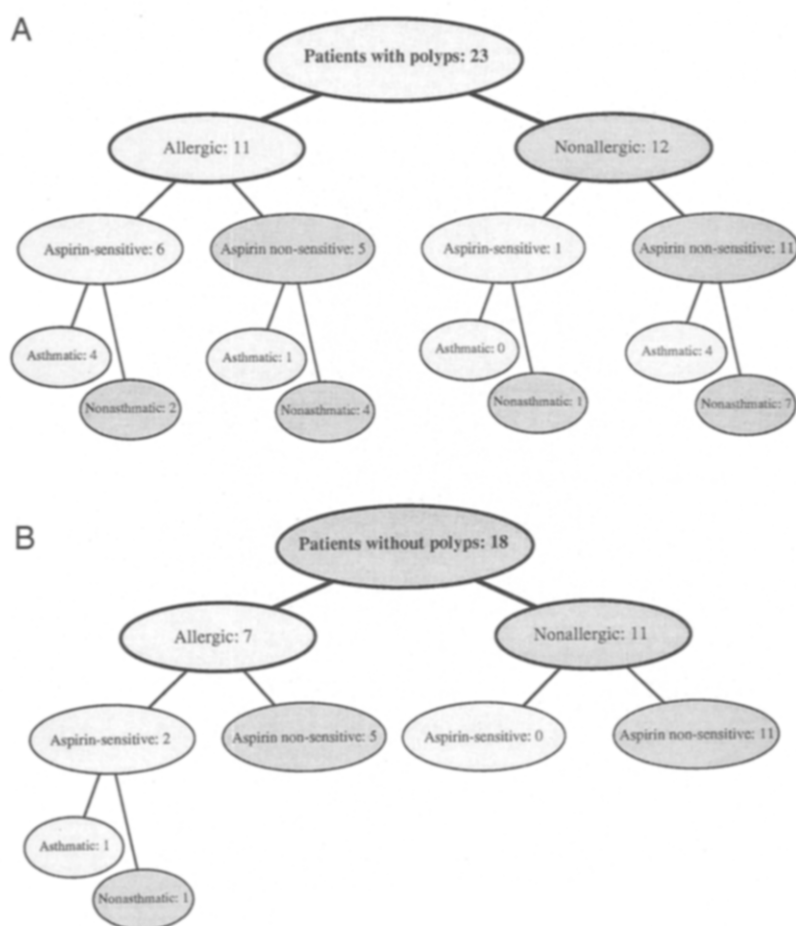


FIG. 1. Distribution of underlying diseases in patients with polyps (A) and control subjects (B).

subjects, and the study was approved by the ethics committee of the University of Düsseldorf.

Histology and immunohistochemistry

A part of each sample was fixed in 10% buffered neutral formalin, processed routinely, and embedded in paraffin wax. Fresh sections were cut at 5 μ m and stained with Papanheim's stain to detect eosinophils. Selected slides were stained with the monoclonal antibodies EG2 (Pharmacia, Uppsala, Sweden; clone EG2; concentration: 0.2 μ g/ml), major basic protein (Cell Systems, Remagen, Germany; clone MON 6008; concentration: 1 μ g/ml), and IL-5 (gift from Glaxo, UK; clone MAB7; concentration: 28.5 μ g/ml) by using the alkaline phosphatase-anti-alkaline phosphatase technique as detailed elsewhere.^{11, 12}

Preparation of fluids for ELISA

Biopsy specimens were weighed, chopped into small pieces, transferred into 5 ml of a 0.9% sodium chloride solution, and homogenized for 5 minutes at 1000 rpm on ice. Suspensions were centrifuged at 4°C at 3000 rpm for 10 minutes, and supernatants were stored in aliquots of 250 μ l at -80°C for later experiments.

The total protein concentration was measured by the method of Biuret in a Protein Assay Reagent Kit (Pierce Chemical Co., Rockford, Ill.). Mean total protein concentrations (per 0.1 gm of tissue) were 514.62 ± 55.27 μ g/ml in polyp tissue and 669.43 ± 35.25 μ g/ml in nasal mucosa. Because there was a

significant difference between these groups ($p = 0.049$), reflecting the edematous nature of nasal polyps, we decided to normalize cytokine concentrations for this parameter. The cytokine concentrations are given in picograms per milligram of total protein.

The cytokine concentrations were measured by using commercially available ELISA kits (R&D Systems, Inc., Minneapolis, Minn.). Standard curves were prepared according to the manufacturer's instructions. The specific sensitivities of the ELISA kits were IL-1 β : 0.125 pg/ml; IL-6: 0.156 pg/ml; TNF- α : 0.5 pg/ml; IL-4: 1.5 pg/ml; IL-5, GM-CSF: 7.8 pg/ml; IL-10: 10.0 pg/ml; IL-1RA, IL-3, IL-8, RANTES, GRO- α : 31.3 pg/ml.

Statistics

The data in the text and figures are expressed as medians and ranges. All data were analyzed nonparametrically with the Mann-Whitney U test. Significance was accepted for $p < 0.05$ (two-tailed).

RESULTS

IL-5 protein concentrations

IL-5 was detected in 18 of 23 nasal polyps but in only one of 18 turbinate samples. The median concentration in the polyp group was 11.45 pg/mg (Fig. 2, A).

Within the group of polyp samples, we tested for

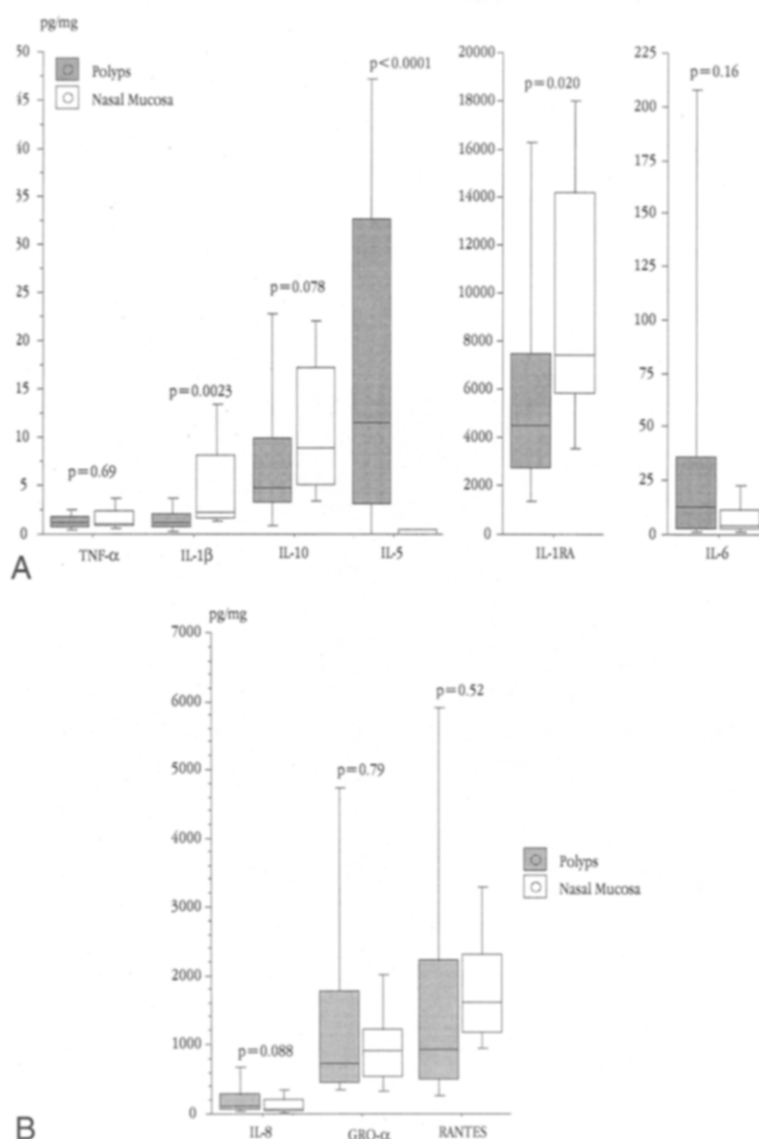


FIG. 2. Comparison of concentration of cytokines (**A**) and chemokines (**B**) from nasal polyps ($n = 23$) and turbinates nasal mucosa ($n = 18$) measured by ELISA. Values are expressed as medians and 10th, 25th, 50th, 75th, and 90th percentiles. Stated p values are based on Mann-Whitney U test.

differences of IL-5 protein concentrations in patients with different underlying diseases. We found no significant difference between polyps from allergic (11.96 pg/mg) and nonallergic patients (10.73 pg/mg, $p = 0.54$) (Fig. 3). Although they did not reach statistical significance, IL-5 concentrations were higher in aspirin-sensitive patients (19.24 pg/mg) than in nonsensitive patients (7.57 pg/mg, $p = 0.10$). However, IL-5 concentrations in polyps from patients with asthma (19.23 pg/mg) were significantly higher than those from nonasthmatic subjects (5.26 pg/mg, $p = 0.037$).

Histology and immunohistochemistry

In polyp tissue samples we found moderate to severe eosinophilia, whereas in normal nasal mucosa samples,

eosinophils were only occasionally observed (Papanicolaou's stain).

Three selected polyp specimens showed abundant numbers of eosinophils and major basic protein-positive and EG2⁺ cells. Fifty-four percent of the eosinophils (identified by morphologic criteria) were IL-5⁺, and 46% were negative for IL-5 as determined by staining (Fig. 4).

Protein concentrations of cytokines and chemokines

The concentrations of IL-1 β , IL-1RA, IL-5, IL-6, IL-8, IL-10, TNF- α , GRO- α , and RANTES are shown in Fig. 2. IL-3 was not detectable in both groups; GM-CSF could only be found in one mucosa sample and three

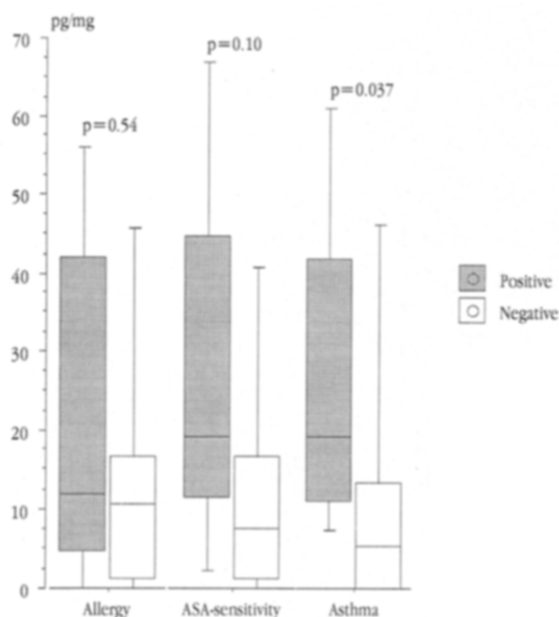


FIG. 3. Comparison of IL-5 concentrations in nasal polyps between allergic ($n = 11$) and nonallergic patients ($n = 12$), aspirin-sensitive ($n = 7$) and nonsensitive patients ($n = 16$), and asthmatic ($n = 9$) and nonasthmatic patients ($n = 14$). Values are expressed as medians and 10th, 25th, 50th, 75th, and 90th percentiles. Stated p values are based on Mann-Whitney U test. ASA, Acetyl salicylic acid.

polyp samples in concentrations close to the limit of detection. IL-4 was not found in either turbinate nasal mucosa or nasal polyps.

Compared with turbinate nasal mucosa, the concentrations of IL-1 β and IL-1RA in nasal polyps were significantly lower. No significant differences were observed for TNF- α , IL-6, IL-8, IL-10, RANTES, and GRO- α .

DISCUSSION

In this study we demonstrated that IL-5 protein can be detected in most of the eosinophilic polyp samples but not in turbinate nasal mucosa. The IL-5 concentration was significantly increased in polyp tissue compared with turbinate mucosa, whereas IL-3 and GM-CSF were not or only occasionally detectable in both groups. Immunohistochemically, a large proportion of eosinophils were IL-5⁺ cells.

For IL-6, TNF- α , IL-8, IL-10, and the chemokines RANTES and GRO- α , we could not find significant differences between the groups. IL-1 β and IL-1RA were significantly decreased in polyps in comparison with turbinate mucosa.

From clinical experience, nasal polyps are associated with different airway diseases such as cystic fibrosis, primary ciliary dyskinesia, asthma, aspirin sensitivity, and allergic fungal sinusitis.^{2,3} Tissue neutrophilia is found in polyps from patients with cystic fibrosis and

antrochoanal polyps, whereas eosinophilia is characteristic for polyps in patients with asthma and aspirin sensitivity. These associations suggest heterogeneous pathologic mechanisms, even in the subgroup of eosinophilic polyps that represent 80% to 90% of all polyps, which could become clarified by investigating cytokine patterns. In bronchial asthma, a disease that is frequently associated with nasal polyps, it is now widely accepted that eosinophils play an important role in the pathogenesis.^{13,14} Interestingly, IL-5 protein concentrations were significantly higher in nasal polyps of patients with asthma compared with nonasthmatic subjects and tended to be higher in aspirin-sensitive subjects than in nonsensitive subjects. These correlations represent another link between asthma, nasal polyps, and aspirin sensitivity, as observed clinically in the Samter triad.^{2,3}

In nasal polyps, eosinophils could contribute to the tissue damage and inflammation through the release of vasoactive substances and chemotactic factors. The abundance of eosinophils in nasal polyps can be explained in three different ways: by an increased migration of eosinophils into the tissue, by a prolonged survival of these cells,¹⁵ or by a combination of both. Several studies have demonstrated the importance of cytokines in mediating the migration of inflammatory cells into the tissue. IL-4, IL-5, and RANTES have been shown to deliver signals that cause selective influx of eosinophils.¹⁶⁻¹⁸ In our study IL-4 could not be detected in either nasal polyps or turbinate nasal mucosa specimens, which does not provide support for an important role for IL-4 in this disease. RANTES protein concentrations did not differ between the groups, bringing into question the importance of RANTES as a major recruitment factor for eosinophils in nasal polyps.

The proinflammatory cytokines IL-1 β and TNF- α can also lead to increased transendothelial migration of eosinophils, an effect that is nevertheless not specific for this cell type.¹⁹ Hamaguchi et al.^{20,21} have detected IL-1 β and IL-1 α in nasal polyps and suggested their significance in the pathogenesis. In our study, however, IL-1 β concentrations were significantly lower in polyps than in nasal mucosa, contradicting this hypothesis. Similar to IL-1 β , the protein concentrations of the naturally occurring antagonist IL-1RA were significantly lower in polyps. As we have previously shown for nasal secretions,²² IL-1RA was detected in concentrations about 1000-fold higher than IL-1 β in both polyp and turbinate mucosa samples. TNF- α mRNA has been demonstrated in eosinophils from nasal polyps in contrast to nasal mucosal samples.^{6,8} Comparison of the protein concentrations of this cytokine did not reveal significant differences in our study.

The accumulation of eosinophils in the tissue could also be due to prolonged survival of these cells in the polyps. IL-3, GM-CSF, and IL-5 have been demonstrated to be the main survival factors for human eosinophils.²³ Ohno et al.²⁴ have detected protein of GM-CSF in polyp fluids and have also shown that about 30% of the eosinophils in nasal polyp tissue produce GM-CSF

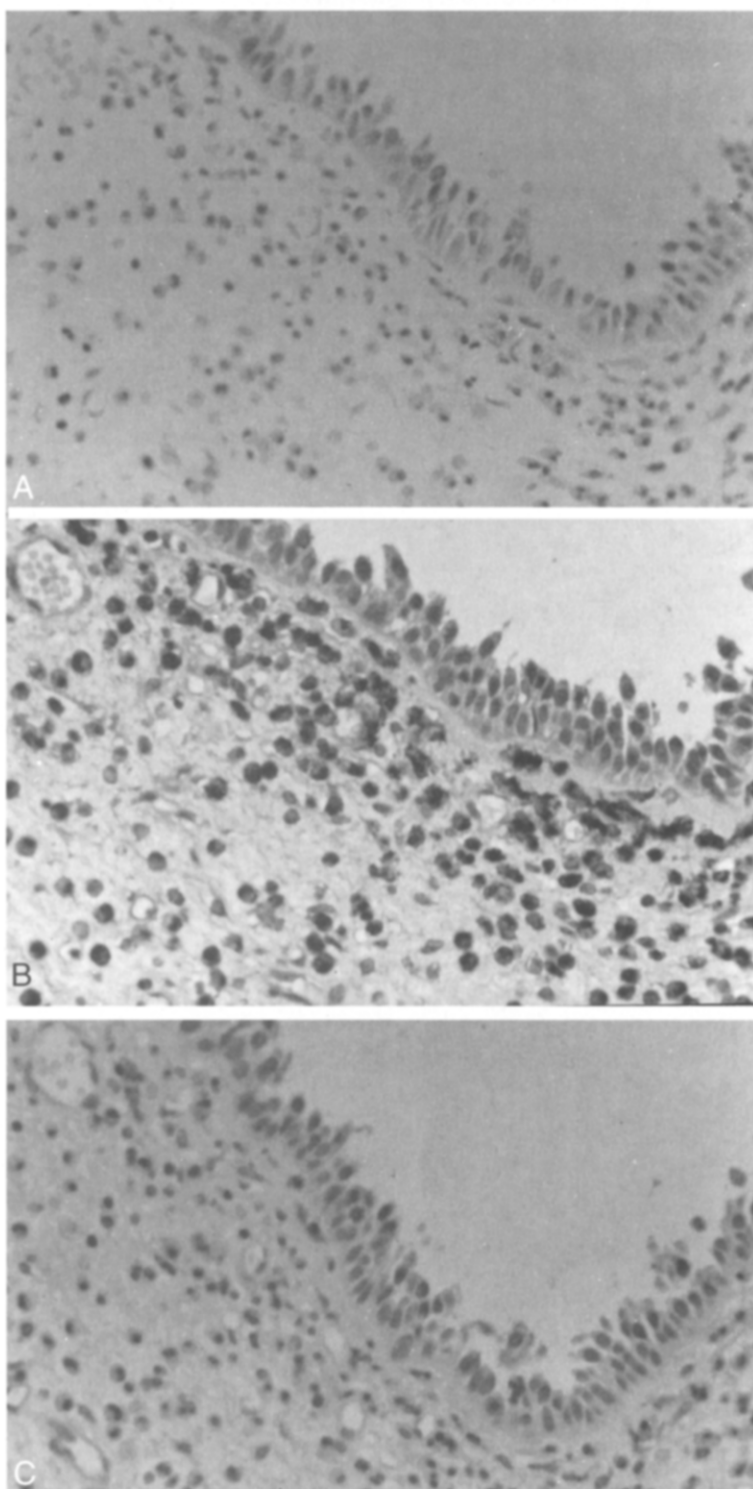


FIG. 4. Serial sections from a polyp stained with Papanheim's stain for eosinophils (**A**), anti-major basic protein (**B**), and anti-IL-5 (**C**).

mRNA. It has further been demonstrated that polyp tissue expresses more GM-CSF mRNA than turbinate nasal mucosa⁵ and that there is a correlation between the number of activated EG2⁺ eosinophils and IL-3

mRNA and the amount of GM-CSF mRNA. Protein concentrations of cytokines were not measured in these experiments. Hamilos et al.⁵ suggest a main role of IL-3 and GM-CSF in the pathogenesis of polyps. However,

the results of our examination of the protein concentration do not support this hypothesis but point to IL-5 as the key protein for eosinophil activation and survival in this disease.

To determine the cellular source of IL-5, we used immunohistochemistry to investigate selected samples with abundant numbers of eosinophils. As was shown before for eosinophilic heart disease by Capron et al.,²⁵ immunohistochemistry revealed that eosinophils represent a major source for IL-5. Accordingly, in our study 69.5% of IL-5⁺ cells were recognized as eosinophils by their typical morphology. Further investigations to precisely identify the cellular source of IL-5 are currently in preparation. Our findings do not rule out that there is a substantial synthesis of IL-5 by T lymphocytes, because these cells do not store the protein and therefore are not expected to be positive in immunohistochemical investigations.

Our findings suggest an autocrine stimulation of eosinophils by IL-5, thus enhancing their activation and survival. However, we must bear in mind that we investigated the disease at a later, chronic stage of development and that eosinophils might have been T-cell-dependent in the beginning of disease.

In summary, our data point to IL-5 as a key protein in the pathologic mechanism of tissue eosinophilia in nasal polyposis. As shown by immunohistochemistry, eosinophils may be a major source of IL-5, thus creating a possible autocrine loop for activation and survival. IL-5 therefore represents a main target for treatment of polyps, and nasal polyposis may serve as a good model for other eosinophil-related diseases such as asthma. The investigation of cytokine patterns may help to differentiate between polyp subgroups and to link diseases of the upper airways with those of the lower respiratory tract.

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