Inhibition of nasal mucosal eosinophils after immunotherapy is associated with a decrease in interleukin-13 mRNA and vascular cell adhesion molecule-1 expression

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ABSTRACT

Background: Grass pollen immunotherapy is highly effective in reducing seasonal hay fever symptoms and medication requirements. Clinical improvement is accompanied by a reduction in nasal mucosal eosinophils, although the mechanism is unknown.

Methods: Nasal biopsies were taken from 37 adults before immunotherapy and during the peak pollen season following 2 years treatment. Biopsies were processed for immunohistochemistry for CCR3, adhesion molecules (intercellular adhesion molecule (ICAM)-1 and vascular cell adhesion molecule (VCAM)-1) and apoptotic cells (terminal deoxynucleotidyl transferase-mediated dUTP–digoxigenin nick end-labeling; TUNEL), as well as for interleukin (IL)-4 and IL-13 mRNA-positive cells. Results were compared with eosinophil numbers in the nasal mucosa.

Results: Analysis of the clinical data confirmed that the proportion of patients who showed greater than 60% improvement in symptoms (47 and 15%) and in rescue medication (79 and 10%) were significant for the immunotherapy group compared with placebo group (P < 0.03 and P < 0.02, respectively). Seasonal increases were observed for VCAM-1 expression (P = 0.05) and IL-13 mRNA-expressing cells (P < 0.05) in the placebo group, but not in the immunotherapy group. The differences for VCAM-1 expression achieved significance between groups (P = 0.05). There was no significant difference in either ICAM-1 expression or in the number of CCR3+ cells, TUNEL+ apoptotic cells and IL-4 mRNA-expressing cells.

Conclusion: Successful grass pollen immunotherapy was associated with inhibition of seasonal increases in nasal eosinophils, IL-13 mRNA-expressing cells and VCAM-1 expression, but no change in CCR3 expression or in the number of apoptotic cells. The reduction in eosinophils after immunotherapy may be due to suppression of eosinophil recruitment to the nasal mucosa rather than enhanced apoptosis.

Key words: adhesion molecule, allergic rhinitis, apoptosis, immunotherapy, interleukin-13.

INTRODUCTION

Tissue eosinophilia is a characteristic feature of seasonal allergic rhinitis. A balance between recruitment and clearance of these cells determines the number of infiltrating inflammatory cells in the airways.

Adhesion-related events are important in leukocyte recruitment. Leukocyte adhesion to venular endothelium is an early and obligatory step in leukocyte migration into tissue and is mediated through binding between membrane receptors on the surface of leukocytes and ligands on the endothelium. Intercellular adhesion molecule (ICAM-)1 and vascular cell adhesion molecule (VCAM)-1
are known endothelial adhesion molecules. Intercellular adhesion molecule-1 mediates the attachment of all classes of leukocytes to endothelial cells, whereas VCAM-1 interacts with very late antigen (VLA)-4, which is expressed on lymphocytes, eosinophils and basophils, but not on neutrophils.\textsuperscript{3,4} Increased expression of ICAM-1 and VCAM-1 in the nasal mucosa has been shown to occur following local nasal allergen challenge.\textsuperscript{5–7}

Cellular recruitment is modulated not only by cell adhesion systems, but also by chemotactic signals elicited through chemokine and chemoattractant receptors. The C-C chemokine receptor (CCR) 3 is strongly expressed on eosinophils and is responsible for both migration and degranulation.\textsuperscript{8–10} Although the C-C chemokines generally act on several receptors, eotaxin and eotaxin-2 act uniquely through CCR3.\textsuperscript{11} Increased expression of eotaxin in the nasal mucosa has been shown in patients with seasonal allergic rhinitis after allergen challenge\textsuperscript{12} and during the pollen season.\textsuperscript{13}

The ‘Th2-type’ T lymphocyte responses play an important role in human allergic responses. Interleukin (IL)-4 and IL-13 are Th2 T lymphocyte-derived cytokines involved in the generation of \( \varepsilon \) germline gene transcripts, the critical first step in inducing B cell class switching towards IgE synthesis.\textsuperscript{14,15} In addition, both IL-4 and IL-13 selectively induce VCAM-1 expression on human endothelial cells.\textsuperscript{16,17} Interleukin-13 also upregulates CD69 expression on blood eosinophils and promotes eosinophil viability.\textsuperscript{18} Increased expression of both IL-13 mRNA and protein has been shown in the nasal mucosa of patients with perennial allergic rhinitis\textsuperscript{19} and in seasonal hay fever sufferers after allergen challenge.\textsuperscript{20}

Eosinophil accumulation in tissues is dependent not only on the recruitment and migration of eosinophils, but also on the duration of survival of eosinophils in tissues.\textsuperscript{21} Apoptosis is a form of programmed cell death distinct from necrosis. Although necrosis may promote inflammation by releasing the cell contents, apoptosis results in the ‘silent’ clearance of inflammatory cells.\textsuperscript{22} Thus, apoptosis may be vital for the resolution of inflammation. Conversely, the inhibition of eosinophilic apoptosis may result in persistent tissue eosinophilia, as observed in patients with nasal polyposis.\textsuperscript{23} In contrast with nasal polyposis, the presence/absence of apoptosis in the nasal mucosa with seasonal allergic rhinitis, to our knowledge, has not been studied.

We recently reported that grass pollen immunotherapy was effective in patients with severe summer hay fever unresponsive to usual anti-allergic drugs.\textsuperscript{24} We observed that immunotherapy inhibited seasonal increases in major basic protein (MBP)\textsuperscript{+} and eosinophil granule-2 (EG2)\textsuperscript{+} eosinophils.\textsuperscript{25,26} We also observed that reductions in both EG2\textsuperscript{+} eosinophils and IL-5 correlated with the degree of symptomatic improvement after 2 years immunotherapy.\textsuperscript{26} In the same study, we have now explored possible mechanisms for the inhibition of tissue eosinophilia following immunotherapy. Thus, we studied the expression of the eosinophil-selective molecule VCAM-1 and those cytokines responsible for VCAM-1 upregulation, namely IL-4 and IL-13. We also examined nasal biopsies before and after immunotherapy for eosinophil apoptosis.

**METHODS**

**Patients**

The clinical features of the 44 patients in the present study have been reported previously.\textsuperscript{24} All subjects had a history of severe summer hay fever that was not controlled by anti-allergic drugs and a positive skin test reaction (wheat > 5 mm) to Phleum pratense (Timothy grass pollen). Patients were excluded if they had multiple allergies or had received immunotherapy within the preceding 5 years.

The mean age of immunotherapy and placebo-treated patients was 32 and 32 years, respectively. The gender ratio (M : F) in the two groups was 10 : 12 and 13 : 9, respectively, results of median skin prick tests (in mm\textsuperscript{2}) were 49.5 and 42.8, respectively, and baseline hayfever severity (mean weekly visual analog symptom scores during the baseline pollen season) was 4.4 and 4.5 cm, respectively, on a scale of 0–10 cm. The clinical data have been reported previously in terms of symptoms and rescue medication use.\textsuperscript{24} We now report the clinical response as a percentage of patients responding by a given change (worse, improved < 30%, improved 30–59% and improved > 60%) in diary symptom and medication scores compared with the baseline season before treatment.

**Study design**

The present study was randomized, double blind and placebo controlled. The active treatment comprised an aluminum hydroxide adsorbed grass pollen (\textit{P. pratense}) extract for subcutaneous injection.\textsuperscript{24} The placebo appeared identical and was composed of the allergen diluent in histamine acid phosphate and induced local itching and whealing at injection sites. Patients were
monitored for one summer prior to randomization to immunotherapy or placebo treatment. Nasal biopsies were taken at baseline and during the peak pollen season following 2 years treatment. Paired nasal biopsies were obtained from 37 of 44 patients who completed the clinical study (20 immunotherapy and 17 placebo-treated patients). The study was approved by the Ethics Committee of the Royal Brompton and Harefield Hospitals NHS Trust and was performed with the patients’ written informed consent.

Nasal biopsy

Nasal biopsies (2–5 mm) were taken under local anesthesia from the under surface of the inferior turbinate, as described previously. For immunohistochemistry, half of each biopsy was mounted in OCT compound (VWR, Lutterworth, Leics, UK), snap-frozen in isopentane precooled in liquid nitrogen and stored at –80°C. For in situ detection of apoptotic cells and in situ hybridization, the other half of each biopsy was placed in 4% paraformaldehyde for 2 h and then two changes of 15% paraformaldehyde for 2 h and then two changes of 15% sucrose before being mounted in OCT and snap-frozen as described above.

Immunohistochemistry

Immunohistochemistry was performed on 6 µm cryostat sections fixed in acetone : methanol (60 : 40) by using the alkaline phosphatase anti-alkaline phosphatase method, as described previously. Briefly, sections were incubated for 30 min at room temperature with the primary antibody, followed by 30 min incubation each with rabbit antimouse antibody and alkaline phosphatase anti-alkaline phosphatase complex (both from DAKO, Cambridge, UK). The reaction was developed with Fast Red, giving positive cells a red appearance. Monoclonal antibodies used were BMK13 (a gift from J Barkans/R Moqbel, Allergy and Clinical Immunology, Imperial College London at National Heart & Lung Institute, London, UK) for eosinophils against MBP, EG2 (Pharmacia and Upjohn Diagnostics, Uppsala, Sweden) for detecting the actively secreted form of eosinophil cationic protein (ECP), CCR3 (7B11; Millennium Pharmaceuticals, Boston, MA, USA), ICAM-1 (DAKO) and VCAM-1 (DAKO).

Terminal deoxyribonucleotidyl transferase-mediated dUTP–digoxigenin nick end-labeling (TUNEL) method with TACSXL in situ apoptosis detection kit (R&D Systems, Minneapolis, MN, USA). The labeling targets are the multitude of new 3’-OH DNA ends generated by DNA fragmentation and are typically localized in morphologically identifiable nuclei of apoptotic cells. The assay was performed as recommended by the manufacturer. Slides were pretreated with 3% hydrogen peroxide in methanol. Apoptotic cells were visualized with dianmonobenzidine (DAB) solution in 0.03% H2O2 and counterstained with hematoxylin. As a positive control, the permeabilized sections were treated with TACS-nuclease and Buffer (R&D Systems) for 20 min at 37°C and were then stained by the TUNEL procedure. This resulted in virtually all individual cell nuclei staining with DAB. In addition, Cell Culture Control Slides (R&D Systems) were used as an apoptotic positive control for TUNEL. Negative controls were also performed with omission of terminal deoxynucleotidyl transferase.

In situ hybridization

Riboprobes (antisense and sense) were prepared from cDNA for IL-4 and IL-13. The cDNA was inserted into different pGEM vectors and linearized with appropriate enzymes before transcription. Transcription was performed in the presence of [35S]-labeled uridine triphosphate and the appropriate T7 or SP6 RNA polymerizes. In situ hybridization was performed on 6 µm cryostat sections on Polysine™ slides (VWR). Sections were permeabilized with Triton X-100 in phosphate-buffered saline (PBS) followed by proteinase K digestion. Sections were treated with iodoacetamide and N-ethylmaleimide and then acetic anhydride–triethanolamine to inhibit non-specific binding of [35S]. As a negative control, sections were either hybridized with the sense probe or treated with ribonuclease A solution before the prehydration step with antisense probes.

Quantitation

Biopsy specimens were coded and sections were assessed in a blinded fashion. Immunoreactivity of ICAM-1 and VCAM-1 was expressed according to the modified visual analog scale described by Bentley et al. Intensity of staining was scored as follows: 0, none; 1, weak; 2, moderate; 3, strong; and 4, very strong. The extent of staining was scored as follows: 0, none; 0.1, < 1% of section area; 0.2, < 3% of section
area; 0.5, < 5% of section area; 1, < 10% of section area; 2, 10–50% of section area; 3, 50–75% of section area. Cell counts for MBP (BMK13), EG2, CCR3, IL-4 and IL-13 were performed by using an Olympus BH2 microscope (Olympus Optical Company, Tokyo, Japan) with an eyepiece graticule. Sections were counted to one grid depth beneath the epithelium and results are expressed as the number of positive cells per mm².

Statistical analysis
Statistical analysis was performed using a commercial software package (Minitab release 7; Minitab, State College, PA, USA). Comparisons between measurements obtained before and after treatment in the same subject were analyzed using the Wilcoxon matched-pairs signed-ranks test. Comparisons between immunotherapy and placebo-treated patients were performed using the Mann–Whitney U-test. Correlations were performed using the Spearman rank correlation method. P < 0.05 was considered significant.

RESULTS
Clinical response to immunotherapy
Following 2 years of immunotherapy, the percentages of patients who showed reductions in symptom scores and decreases in medication scores during the pollen season for the immunotherapy were compared with those for the placebo-treated group (Fig. 1). After 2 years treatment, the percentage of patients who showed a greater than 60% reduction in symptoms during the pollen season was significantly higher for the immunotherapy treated patients compared with placebo patients (47 vs 15%, respectively; P < 0.03). Similarly, the percentage of patients who showed a greater than 60% reduction in rescue medication requirements during the pollen season was higher in

![Graphs](https://via.placeholder.com/150)

**Fig. 1** Percentage reductions in (a,c) symptom and (b,d) medication scores obtained from immunotherapy (a,b) and placebo-treated (c,d) patients. There was a significant difference between immunotherapy and placebo-treated patients in symptom (P < 0.03) and medication (P < 0.02) scores.
the immunotherapy group (79 vs 10%, respectively; 
P < 0.02).

Eosinophils, CCR3, apoptotic cells, IL-4 and IL-13

In placebo-treated patients, as reported previously,25,26 there was a highly significant, greater than five- to sevenfold increase in the number of MBP+ or EG2+ eosinophils in the nasal mucosa during the pollen season compared with a one- to 2.5-fold increase in the immunotherapy treated group. The peak seasonal values were significantly different between groups (Table 1).

There were no significant increases in the number of CCR3-positive cells in the nasal mucosa (Fig. 2) for either group and no significant between-group differences (Table 1).

The TUNEL-positive apoptotic cells (8.3–10.5/mm²) were detected within the nasal mucosa of both placebo- and immunotherapy treated subjects (Fig. 2). During the pollen season, there was no significant difference in the number of apoptotic cells between the two groups (Table 1).

The number of cells expressing IL-4 mRNA was low before treatment and at peak season in both groups. In contrast, a significant increase was observed in the number of cells expressing IL-13 mRNA (P < 0.05) during the pollen season in placebo-treated subjects, whereas this was not observed in the immunotherapy treated group (Figs 2,3; Table 1).

Expression of VCAM-1 and ICAM-1

Significant increases in both the extent (P < 0.05) and the intensity (P < 0.05) of VCAM-1 staining were observed in placebo-treated subjects during the pollen season. These increases were not observed in the immunotherapy treated group. The differences between before season and peak season were significantly different between groups in the extent of VCAM-1 staining (P < 0.05) and a trend for a difference in the intensity of VCAM-1 staining (P = 0.08) between the groups was also observed (Figs 2,3; Table 2). In contrast, no differences were observed in the expression of ICAM-1 either at baseline or following 2 years treatment between the immunotherapy and placebo-treated groups (Table 2).

In patients who had received immunotherapy, there was no correlation between peak seasonal symptoms and VCAM-1 (extent) expression (r = 0.08; P = 0.7) or the number of IL-13 mRNA+ cells (r = 0.3; P = 0.16). Similarly, there was no significant correlation between MBP+ cells and VCAM-1 (extent) expression (r = 0.3; P = 0.2) or IL-13 mRNA+ cells (r = 0.4; P = 0.08).

DISCUSSION

We have shown that grass pollen immunotherapy for hay fever inhibited seasonal increases in nasal mucosal eosinophils and that this was accompanied by a decrease in IL-13 mRNA+ cells and a decrease in both the extent and intensity of VCAM-1, but not ICAM-1, staining within the nasal mucosa. In contrast, no significant differences were observed in the number of

### Table 1

<table>
<thead>
<tr>
<th>Immunohistochemistry and in situ hybridization of the nasal mucosa</th>
<th>Immunotherapy</th>
<th>Placebo</th>
<th>( P )†</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Immunohistochemistry</strong></td>
<td><strong>Before treatment</strong></td>
<td><strong>Peak season</strong></td>
<td><strong>Before treatment</strong></td>
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<tr>
<td>MBP</td>
<td>5.9 (2.7, 11.2)</td>
<td>16.4 (6.8, 75)*</td>
<td>11.3 (2.7, 21)</td>
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<td>EG2</td>
<td>12 (3.2, 21)</td>
<td>14.2 (9.4, 27)*</td>
<td>7.5 (2.8, 28.8)</td>
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<tr>
<td>CCR3</td>
<td>29 (18, 44.9)</td>
<td>34.3 (20, 47.4)</td>
<td>32.5 (20, 43.5)</td>
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<tr>
<td>TUNEL</td>
<td>ND</td>
<td>8.3 (4.5, 26.3)</td>
<td>ND</td>
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<td><strong>In situ hybridization</strong></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>IL-4</td>
<td>0 (0, 1.1)</td>
<td>0 (0, 1.1)</td>
<td>0 (0, 0.6)</td>
</tr>
<tr>
<td>IL-13</td>
<td>0 (0, 4.6)</td>
<td>0 (0, 6.3)</td>
<td>0 (0, 2)</td>
</tr>
</tbody>
</table>

Data (cells per mm²) are the median with the interquartile range given in parentheses.

†Between-group comparisons (peak season) were performed using the Mann–Whitney U-test.

*P < 0.05,**P < 0.01 (within-group comparisons were performed using the Wilcoxon matched-pairs signed-rank test).

MBP, major basic protein; TUNEL, terminal deoxyribonucleotidyl transferase-mediated dUTP–digoxigenin nick end-labeling; IL, interleukin; CI, confidence interval.
apoptotic cells between the groups. There were also no differences in the number of CCR3-positive cells between the groups. The present data suggest that the effect of immunotherapy on nasal mucosal eosinophils may be mediated, at least in part, by inhibiting IL-13-induced VCAM-1 expression rather than via enhanced apoptosis.

The present study was a double-blind and placebo-controlled trial in which immunological parameters were correlated with the clinical response to treatment. For example, there was a correlation between immunotherapy in duced decreases in eosinophils and symptomatic improvement following immunotherapy. However, there are some limitations. For example, the trend (not significant) for an elevated level of VCAM-1 expression (extent) at baseline in the immunotherapy patients must have occurred by chance. However, this does not invalidate the observed differences for VCAM-1 between the groups because: (i) the analysis was based on the differences between the baseline and the peak seasonal values between the groups, an analysis that corrects for the baseline differences between groups; and (ii) comparable

Table 2  Intercellular adhesion molecule-1 and vascular cell adhesion molecule-1 staining of the nasal mucosa

<table>
<thead>
<tr>
<th></th>
<th>Immunothe</th>
<th>Placebo</th>
<th>Point estimate (95%CI)</th>
<th>P†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before treat</td>
<td>Peak season</td>
<td>Before treat</td>
<td>Peak season</td>
</tr>
<tr>
<td>Intercellular adhesion molecule-1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intensity</td>
<td>3 (2, 3)</td>
<td>2.5 (2, 3)</td>
<td>3 (2, 3)</td>
<td>2 (2, 3)</td>
</tr>
<tr>
<td>Extent</td>
<td>1 (1, 2)</td>
<td>2 (1, 2)</td>
<td>1 (1, 2)</td>
<td>2 (1, 2)</td>
</tr>
<tr>
<td>Vascular cell adhesion molecule-1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intensity</td>
<td>3 (2, 3)</td>
<td>2.5 (2, 3)</td>
<td>2 (2, 2)</td>
<td>3 (2, 3)*</td>
</tr>
<tr>
<td>Extent</td>
<td>0.5 (0.2, 0.5)</td>
<td>0.2 (0.1, 0.5)</td>
<td>0.1 (0.1, 0.2)</td>
<td>0.2 (0.1, 0.75)*</td>
</tr>
</tbody>
</table>

Data (visual analog scores) are the median with the interquartile range given in parentheses.

Between-group comparisons (differences between before treatment and peak season) were performed using the Mann–Whitney U-test.

P < 0.05 (within-group comparisons were performed using the Wilcoxon matched-pairs signed-rank test).

CI, confidence interval.
results were found for the intensity of VCAM-1 staining where the baseline values for both groups were comparable. Second, there was no correlation between IL-13 and VCAM-1 expression and the clinical response within the immunotherapy group. However, other factors are involved in the clinical expression of symptoms, including the IgE-dependent release of mast cell mediators, sensorineural reflexes and, possibly, the psychological state of the patients. Finally, no significant correlation was observed between the number of eosinophils in the nasal mucosa and the level of IL-13 or VCAM-1 expression. However, this does not invalidate the association, because other factors may be involved in determining the number of tissue eosinophils, such as the degree of degranulation of eosinophils (with a possible reduction in the number of eosinophils that can be immunostained) and the extent to which transepithelial migration of eosinophils into the nasal lumen may result in loss of tissue eosinophils.

In patients with allergic rhinitis, the nasal mucosa contains increased numbers of inflammatory cells, particularly eosinophils, both after allergen challenge and during natural seasonal pollen exposure. The mechanism of eosinophil accumulation in the nasal mucosa and how immunotherapy suppresses eosinophilia remain unclear. We have reported previously that immunotherapy inhibits local IL-5 mRNA+ cells and that this correlated with both the number of eosinophils and symptomatic improvement during the pollen season. Interleukin-5 has a number of properties that effect recruitment and survival of eosinophils. These include activation, adhesion and survival. It is well established that IL-5 is one important factor for tissue eosinophilia.
Eosinophil numbers in the airways can be related to a balance between incoming recruited cells and apoptotic cells, which undergo phagocytic clearance. The interaction between these different but interrelated processes represents a fine balance that determines the tissue load of eosinophils. Selective recruitment of eosinophils is influenced by adhesion molecules, chemokines (e.g. eotaxin) and their respective receptors. It has been demonstrated that eotaxin and CCR3 expression are increased and related to eosinophil infiltration into the airways of patients with asthma, suggesting a critical role for this pathway in airway eosinophilia. Large numbers of CCR3-positive cells in the nasal mucosa were detectable at baseline and we were unable to show any increase in the number of CCR3-positive cells in either group. One explanation may be that most CCR3-expressing cells were neither eosinophils nor T lymphocytes, but that CCR3 was expressed by other cells, such as mast cells. Alternatively, CCR3 may be internalized following chemokine receptor binding in the tissues.

Leukocyte adhesion to venular endothelium is an early and obligatory step in leukocyte migration into tissue and is mediated through binding between membrane adhesion molecules on the surface of leukocytes and ligands on the vascular endothelium. The endothelial ligands include ICAM-1 and VCAM-1. Intercellular adhesion molecule-1 recognizes the β2 leukocyte integrin leukocyte function antigen-1 (LFA-1) and Mac-1, whereas VCAM-1 binds the β1 integrin VLA-4. Both ligands are involved in eosinophil recruitment. However, VCAM-1 is more selective for eosinophil recruitment, because its counter adhesion molecule VLA-4 is expressed by eosinophils and lymphocytes, but not by neutrophils, whereas LFA-1 and Mac-1 are also expressed by neutrophil leukocytes. It has been reported that the expression of ICAM-1 and VCAM-1 is increased in the nasal mucosa in subjects with perennial allergic rhinitis. The expression of ICAM-1 and VCAM-1 in the nasal mucosa increased following allergen challenge.

Expression of VCAM-1 is known to be selectively enhanced by both IL-4 and IL-13. The effect of IL-13 on VCAM-1 is more potent than that of IL-4 in human mucosal microvascular endothelial cells. Similarly, in allergen-induced late-phase cutaneous responses, mRNA expression and protein product for IL-13 but not for IL-4 significantly correlated with VCAM-1 immunoreactivity and the number of tissue eosinophils. Significantly elevated expression of IL-13 has also been observed in the nasal mucosa of patients with perennial allergic rhinitis and with seasonal hay fever after antigen challenge. This expression of IL-13 was greater than that of IL-4. The seasonal increase in the expression of both VCAM-1 and IL-13 mRNA that we have observed in placebo-treated patients is consistent with the hypothesis that IL-13 and VCAM-1 cooperatively take part in eosinophil recruitment.

In nasal polyp tissue, neutralizing concentrations of anti-IL-5 have been shown to increase eosinophil apoptosis and reduce tissue eosinophilia. In addition, IL-13 has been shown to enhance eosinophil survival. Glucocorticoids, such as dexamethasone, can cause a striking reduction in eosinophil numbers in vivo. One way in which glucocorticoids may exert their effects on eosinophils may be through inducing their apoptosis, either directly or by inhibiting the production of survival-enhancing cytokines. One recent study in patients with rhinosinusitis demonstrated that oral administration of prednisolone induced eosinophilic apoptosis accompanied by a significant decrease in the number of EG2- and IL-5-positive cells in the sinus mucosa. To our knowledge, there are no previously reported measurements of apoptotic cells in the nasal mucosa in allergic rhinitis, nor any studies examining the effects of immunotherapy. We were unable to show any relationship between apoptotic cells and eosinophil accumulation and no apparent influence of immunotherapy. One recent study has shown that lymphocytes derived from immunotherapy treated patients demonstrated increased allergen-induced apoptosis in vitro. These increases in apoptotic cells occurred mainly in IL-4+/CD4+ T lymphocytes.

In conclusion, in patients with hay fever, we have shown that increased IL-13 and VCAM-1 expression that accompanied eosinophil accumulation in the nasal mucosa during the pollen season was inhibited following successful immunotherapy, whereas no differences in local numbers of apoptotic cells were observed. These data suggest that immunotherapy leads to changes in the local cytokine milieu that reduce eosinophil infiltration. We suggest that the mechanism of reduction in eosinophil numbers after immunotherapy may result from suppression of eosinophil adhesion/recruitment rather than enhanced apoptosis. This finding highlights the IL-13–VCAM-1 axis as potential target for therapeutic intervention in seasonal allergic rhinitis.

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REFERENCES


10 Banderia-Melo C, Herbst A, Weller PF. Eotaxins. Contrib-


33 Rothenberg ME, Petersen J, Stevens RL et al. IL-5-dependent conversion of normodense human eosinophils to the hypodense phenotype uses 3T3 fibroblasts for enhanced viability, accelerated hypodensity, and sustained antibody-dependent cytotoxicity. J. Immunol. 1989; 143: 2311–16.


40 Ying S, Meng Q, Zeibecoglou K et al. Eosinophil chemo-tactic chemokines (eotaxin, eotaxin-2, RANTES, monocyte chemoattractant protein-3 (MCP-3), and MCP-4), and C-C chemokine receptor 3 expression in bronchial biopsies from atopic and nonatopic (intrinsic) asthmatics. J. Immunol. 1999; 163: 6321–9.


