

Nasal eosinophilia and IL-5 mRNA expression in seasonal allergic rhinitis induced by natural allergen exposure: Effect of topical corticosteroids

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Background: Nasal allergen provocation in patients with allergic rhinitis leads to expression of the proeosinophilic cytokines IL-5 and GM-CSF and tissue eosinophilia.

Objective: We sought to examine the effect of natural seasonal allergen exposure on IL-5 and GM-CSF mRNA expression and nasal eosinophilia and to evaluate the effects of topical corticosteroid therapy on these responses.

Methods: Nasal biopsy specimens were collected from 46 grass pollen-sensitive patients with seasonal rhinitis before the grass pollen season. A second biopsy specimen was collected during the pollen season, by which time patients had received 6 weeks treatment with either fluticasone propionate (200 µg twice daily) or placebo nasal spray.

Results: Fluticasone treatment was clinically effective ($P < .005$). Patients receiving placebo, but not fluticasone, showed increased numbers of epithelial and submucosal EG2+ eosinophils ($P < .005$) and IL-5 and GM-CSF mRNA-expressing cells ($P < .0001$) during the pollen season. Colocalization experiments showed that greater than 80% of IL-5 mRNA-expressing cells were submucosal CD3+ T cells in both groups. The numbers of submucosal CD3+ T cells did not increase during the pollen season or decrease with fluticasone treatment. Fluticasone also inhibited IL-5 secretion by grass pollen-stimulated peripheral blood T cells from patients with seasonal rhinitis ($n = 5$, inhibitory concentration of 50% = 10^{-9} to 10^{-10} mol/L).

Conclusions: These results suggest that topical corticosteroids may reduce eosinophilia in seasonal rhinitis by inhibiting T cell IL-5 production. (*J Allergy Clin Immunol* 1998;102:610-7.)

Key words: T cells, eosinophils, cytokines, IL-5, allergy, rhinitis, corticosteroids

A prominent feature of allergic rhinitis is infiltration of the nasal mucosa by activated T cells and eosinophils.¹ Although mast cells are presumed to play a role in the early (0 to 1 hour) nasal response to allergen provocation, eosinophil recruitment and activation, with release of toxic granule proteins and lipid mediators, is thought to make a significant contribution to the late (2 to 24 hours) response.

We previously showed that late-phase nasal responses induced by allergen challenge are accompanied by local expression of the T_H2-type cytokines IL-5, GM-CSF, IL-4,² and IL-13.³ Although IL-4 and IL-13 are required for IgE synthesis by B cells and may play a role in local heavy chain switching within the nasal mucosa⁴ and upregulation of vascular cell adhesion molecule-1 (VCAM-1) expression on endothelial cells,⁵ IL-5 and GM-CSF have pleiotropic proeosinophilic properties. IL-5 is of particular interest because many of its actions are specific for eosinophils. It promotes eosinophil maturation and endothelial adhesion, activation, and survival⁶⁻⁸; contributes to eosinophil release from bone marrow⁹; and primes these cells for enhanced chemotactic response to C-C chemokines such as RANTES¹⁰ and eotaxin.¹¹ Previous studies have demonstrated that the majority of nasal mucosal cells expressing IL-5 mRNA after allergen provocation are CD3+ T cells.¹²

The aims of this study were 2-fold. First, we sought to confirm that allergic rhinitis symptoms arising from natural exposure to grass pollen are associated with nasal mucosal eosinophilia and IL-5 and GM-CSF mRNA expression. Second, we sought to test the hypothesis that topical corticosteroid therapy is clinically effective at least partly through a suppressive effect on IL-5 and GM-CSF mRNA expression by T cells, reducing local eosinophil recruitment and activation. Nasal biopsy specimens were collected from asymptomatic patients with allergic rhinitis before the start of the summer grass pollen season. Under double-blind conditions, patients were then randomized to receive either a prophylactic course of topical corticosteroid (fluticasone propionate) treatment or placebo. Further biopsy specimens were subsequently collected during the peak of the grass pollen season. The numbers of activated eosinophils and T cells in nasal mucosal biopsy specimens were assessed

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TABLE I. Clinical details of study patients

	Treatment	
	Placebo	Fluticasone
No of patients	23	23
Sex (M:F) ratio	16:7	14:9
Age (y; mean $\pm$ SD)	33.1 $\pm$ 10.7	32 $\pm$ 8.9
RAST (Timothy grass; score 0-5; mean $\pm$ SD)	3.2 $\pm$ 0.8	2.9 $\pm$ 0.8
SPT (Timothy grass; mean $\pm$ SD)	3.6 $\pm$ 0.8	3.3 $\pm$ 0.8

TABLE II. Colocalization of IL-5 mRNA in situ hybridization signals with cellular phenotypic markers

	Treatment	
	Placebo (n = 4)	Fluticasone (n = 3)
IL-5/CD3 (%)	82.5 ($\pm$ 9.6)	84.7 ($\pm$ 4.2)
IL-5/MBP (%)	7.8 ($\pm$ 3.1)	2.0 ($\pm$ 3.5)
IL-5/Tryptase (%)	6.5 ($\pm$ 4.4)	9.7 ($\pm$ 8.4)
IL-5/CD68 (%)	0	0

Values are means $\pm$ SD. Data shown is for biopsy specimens collected during the pollen season.

MBP, Major basic protein.

by immunocytochemistry, and the numbers of IL-5 and GM-CSF mRNA-expressing cells was determined by in situ hybridization with specific riboprobes. Finally, to seek evidence in support of our hypothesis that topical corticosteroids may act by locally suppressing T-cell cytokine expression, IL-5 mRNA signals were colocalized to CD3+ T cells within biopsy specimens, and the effects of fluticasone propionate on in vitro IL-5 secretion by allergen-stimulated T cells was examined.

METHODS

Subject populations

Forty-six nonsmoking Timothy grass pollen-sensitive patients were recruited from the outpatient clinic of the Asthma and Allergy Center, Department of Medicine, Sahlgrenska Hospital, Göteborg, Sweden. Patients were selected on the basis of (1) a history of moderate-to-severe seasonal allergic rhinitis for at least 2 years and (2) a positive skin prick test response (>5 -mm wheal diameter on skin testing in the presence of positive histamine and negative diluent controls) to Timothy grass pollen extract (*Phleum pratense*; ALK, Hørsholm, Denmark). Venous blood was also collected for measurement of total and allergen-specific IgE concentrations by ELISA. Patients were excluded if they gave a history of perennial allergy or birch pollen allergy, had received immunotherapy within the last 5 years, or had taken oral or topical glucocorticosteroids within the preceding 6 months. This study was performed with the approval of the hospital ethics committee, and the written informed consent of all participants was obtained.

Study design and nasal biopsy

The 46 patients were stratified into either "moderate" or "severe" groups according to previous history of disease. Patients were placed in the severe group if they had symptoms for greater than 10 weeks per year and topical antiallergic drugs (either corticosteroids or cromolyn sodium) had previously failed to control symptoms. All

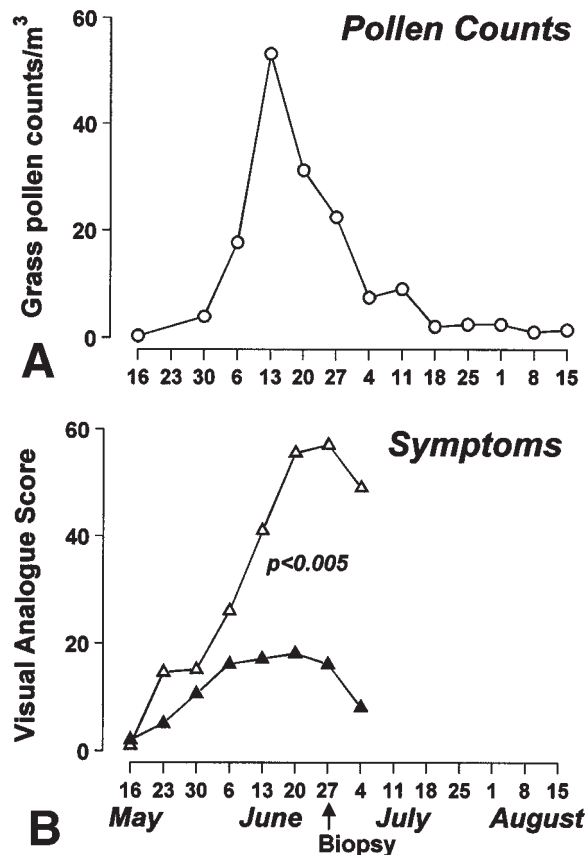


FIG 1. A, Timothy grass pollen (*Phleum pratense*) counts in Göttingen during the summer when this study was conducted. **B,** Median visual analogue symptoms scores over the same period in placebo- (open triangles) and fluticasone-treated (closed triangles) patients with allergic rhinitis. Peak season nasal biopsy specimens were collected at time point indicated.

other patients were placed in the moderate group. Both groups were then randomized to receive either a 6-week course of fluticasone propionate aqueous spray (Flixonase, Glaxo), 2 sprays (200 μ g) twice daily, or a placebo spray containing diluent. Nasal biopsies were performed before the grass pollen season, when all patients were asymptomatic, and during the peak season. Local anaesthesia of the inferior nasal turbinate was achieved with 3% cocaine and 0.025% adrenaline, and a 2.5-mm biopsy specimen was taken 10 minutes later with Gerritsma forceps.¹³ Biopsy specimens were immediately cut in half and processed separately for subsequent in situ hybridization and immunohistochemistry.

Immunohistochemistry

Biopsy specimens were snap-frozen and stored at -80°C pending analysis. Immunohistochemistry was performed on 6-mm cryostat sections (fixed for 7 minutes in 60:40 acetone:methanol) by using the modified alkaline phosphatase-antialkaline phosphatase method as previously described.¹ The EG2 mAb (Kabia Pharmacia, Milton Keynes, UK) was used to quantify the numbers of activated eosinophils within the nasal submucosa. The mAb recognizing T cells (CD3) was obtained from Dako Ltd (High Wycombe, UK).

In situ hybridization

Riboprobes, both antisense (complementary to mRNA) and sense (identical sequence to mRNA), were prepared from cDNA

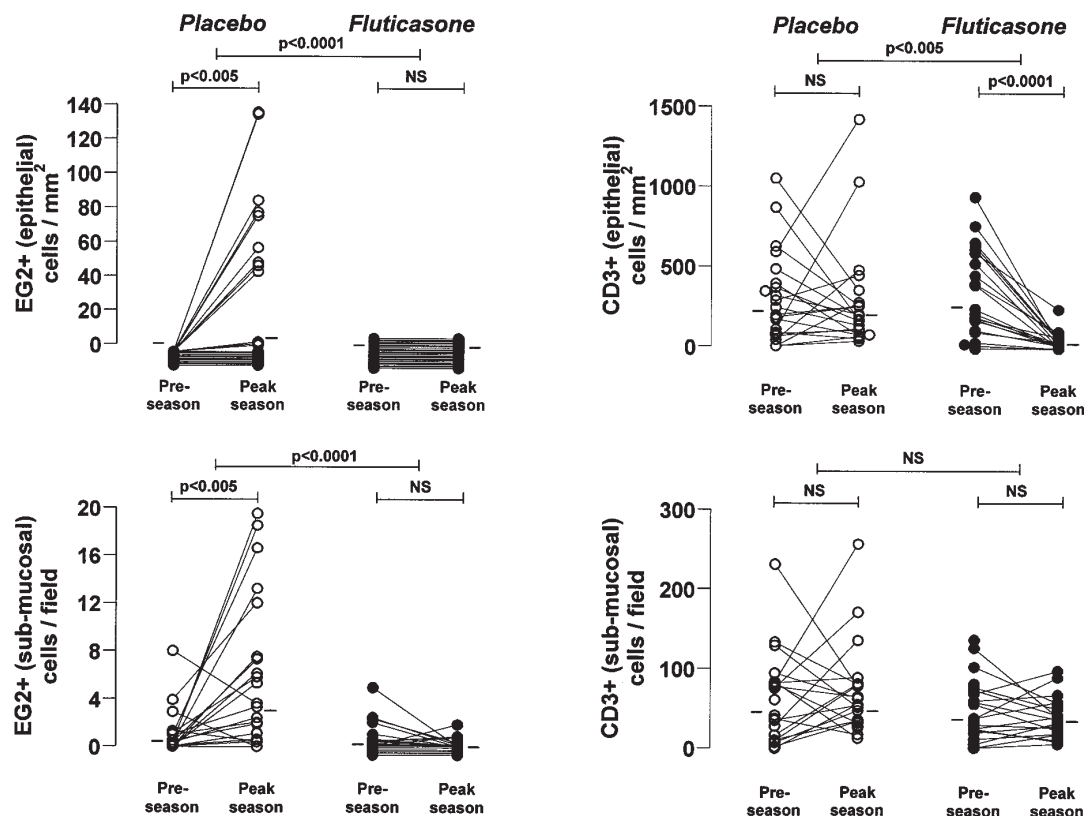


FIG 2. Effect of fluticasone on numbers of EG2+ eosinophils and CD3+ T cells in nasal epithelium and submucosa. Data shown are for baseline (*preseason*) and during natural grass pollen allergen exposure (*peak season*), in placebo- (*open circles*) and fluticasone-treated (*closed circles*) patients with allergic rhinitis. Lines represent median values. Within group preseason and peak-season counts were compared by the Wilcoxon test, and differences (peak season minus preseason) between fluticasone- and placebo-treated patients were compared by using the Mann-Whitney U test.

encoding IL-5 and GM-CSF. cDNAs were inserted into different pGEM vectors and linearized with restriction enzymes before transcription. Transcription was performed in the presence of ³⁵S-uridine triphosphate and the appropriate T7 or SP6 RNA polymerase. In situ hybridization on 10-μm cryostat sections was performed as previously described.² To minimize nonspecific binding of ³⁵S, preparations were treated with 10 mmol/L iodoacetamide and 10 mmol/L N-ethylmaleimide for 30 minutes at 37°C and then in 0.5% acetic anhydride in 0.1 mol/L triethanolamine for 10 minutes at 37°C. Positive controls for IL-5 and GM-CSF mRNA expression were cytospin preparations prepared by using a peripheral blood T-cell clone propagated from a patient with hyper-IgE syndrome. For negative controls, nasal biopsy sections were (1) hybridized with sense riboprobes for IL-5 and GM-CSF and (2) pretreated with RNAase A solution before hybridization with antisense riboprobes. Specific hybridization was recognized as clear dense deposits of silver grains in the photographic emulsion overlaying tissue sections or cytopins.

Simultaneous in situ hybridization and immunohistochemistry

Initial immunohistochemistry was performed with mAbs (unless stated all from Dako Ltd) recognizing T cells (CD3), mast cells (tryptase), total eosinophils (MBP; gift of Dr R. Moqbel), and macrophages (CD68). Positive signals were visualized by diaminobenzidine brown staining, and in situ hybridization was

then performed with a ³⁵S-labeled antisense IL-5 riboprobe. Double-positive cells were identified as brown cells within a clump of black silver grains. Specimens characterized by high numbers of IL-5 mRNA-expressing cells were selected for colocalization. These specimens also showed correspondingly high numbers of eosinophils.

Cell culture

Peripheral venous blood was collected from symptomatic atopic subjects and PBMCs isolated by density gradient centrifugation over Histopaque (Sigma, Poole, UK). PBMCs were cultured at 5×10^6 cells/mL for 6 days with 20 μg/mL *P pratense* extract (ALK, Hørsholm, Denmark). Cultures were supplemented with various doses of fluticasone 17α-propionate (Glaxo Wellcome, UK). Fluticasone 17α-propionate was dissolved in dimethyl sulphoxide to give a stock concentration of 10^{-2} mol/L. Serial dilutions were then prepared in culture medium (RPMI-1640 supplemented with 5% human AB serum, L-glutamine, and penicillin/streptomycin), and a vehicle control was prepared in parallel. IL-5 concentrations in culture supernatants were determined in duplicate by ELISA (Pharmingen, UK) and quantified above 62.5 pg/mL.

Statistical analysis

Within-group paired comparisons were performed by using the Wilcoxon test. Between-group comparisons were made by using the

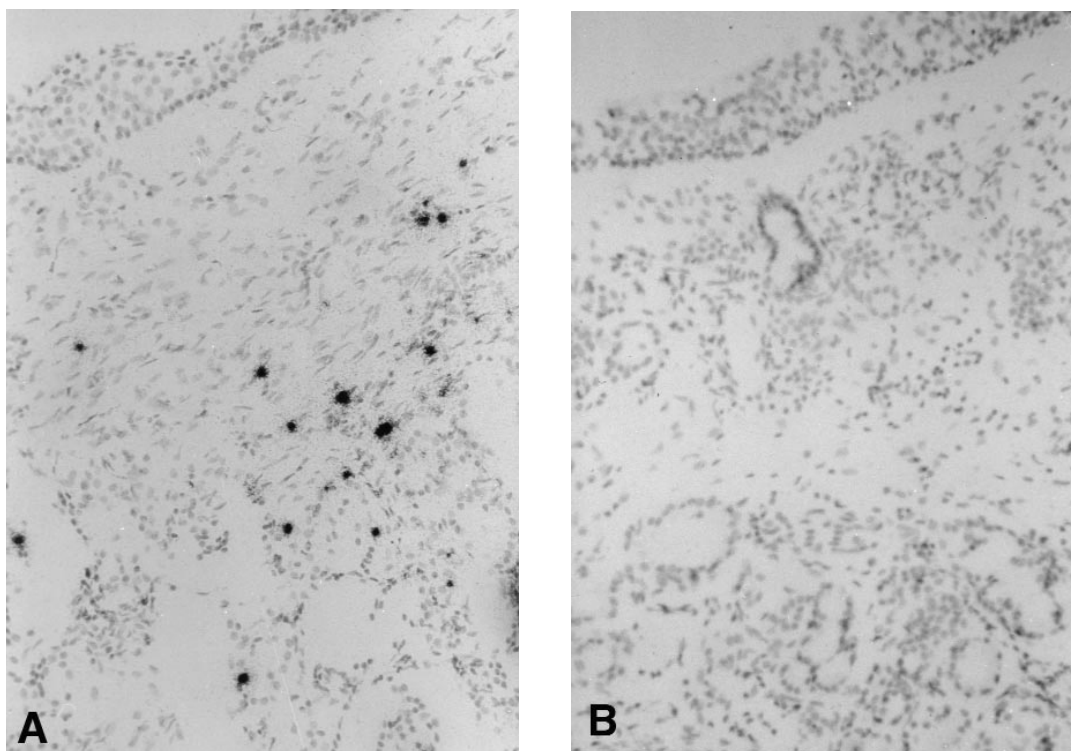


FIG 3. Autoradiographs of cryostat sections of nasal biopsy specimens collected from a patient receiving placebo during the pollen season. Sections were hybridized with a ^{35}S -labeled antisense IL-5 riboprobe (**A**) and a ^{35}S -labeled sense IL-5 riboprobe (negative control) (**B**).

Mann-Whitney U test. Correlations were performed by using Spearman's rank method. All analyses were performed with the aid of a commercial software package (Minitab Inc), and *P* values less than .05 were considered significant.

RESULTS

Randomization resulted in 23 patients receiving fluticasone treatment and 23 receiving a placebo nasal spray. The groups were well matched for age, gender, disease severity, and pollen sensitivity as assessed by responses to skin testing and serum concentrations of Timothy grass pollen-specific IgE antibodies (Table I). In both fluticasone- and placebo-treated patients, increased symptoms of allergic rhinitis (as assessed by a weekly nasal visual analogue score) were observed to coincide with the increase in Timothy grass pollen counts over the months of May to August (Fig 1). Fluticasone-treated patients showed markedly reduced symptoms over this observation period as compared with placebo-treated control subjects (*P* = .003).

Numbers of activated eosinophils and CD3+ T cells in the nasal mucosa before and during the pollen season

In patients receiving placebo treatment, the numbers of intraepithelial and submucosal eosinophils (EG2+) in biopsy specimens obtained during the grass pollen season (medians = 4.0 cells/mm² and 3.0 cells/field,

respectively) were significantly increased compared with biopsy specimens obtained before the pollen season (medians = 0 cells/mm² and 0.30 cells/field, respectively; *P* < .005) (Fig 2). In contrast, the numbers of epithelial EG2+ eosinophils (preseasonal and peak seasonal medians = 0 cells/mm²) and submucosal EG2+ eosinophils (preseasonal and peak seasonal medians = 0.20 cells/field and 0 cells/field, respectively) did not increase during the pollen season in the fluticasone-treated patients. The seasonal increase in the numbers of EG2+ eosinophils was significantly greater in the placebo-treated group than in the fluticasone-treated group (*P* < .0001 for both intraepithelial and submucosal EG2+ eosinophils).

In the placebo-treated patients the numbers of epithelial CD3+ T cells did not change with natural exposure to grass pollen (preseasonal and peak seasonal medians = 218 and 190 cells/mm², respectively). In the fluticasone-treated patients, however, peak pollen season biopsy specimens showed significantly reduced numbers of epithelial CD3+ T cells as compared with preseasonal biopsy specimens taken before commencement of therapy (preseasonal and peak seasonal medians = 223 and 27 cells/mm², respectively; *P* < .0001), and these changes were significantly different between the 2 study groups (*P* < .005). The numbers of submucosal CD3+ T cells did not change during the pollen season in the placebo-treated group (preseasonal and peak

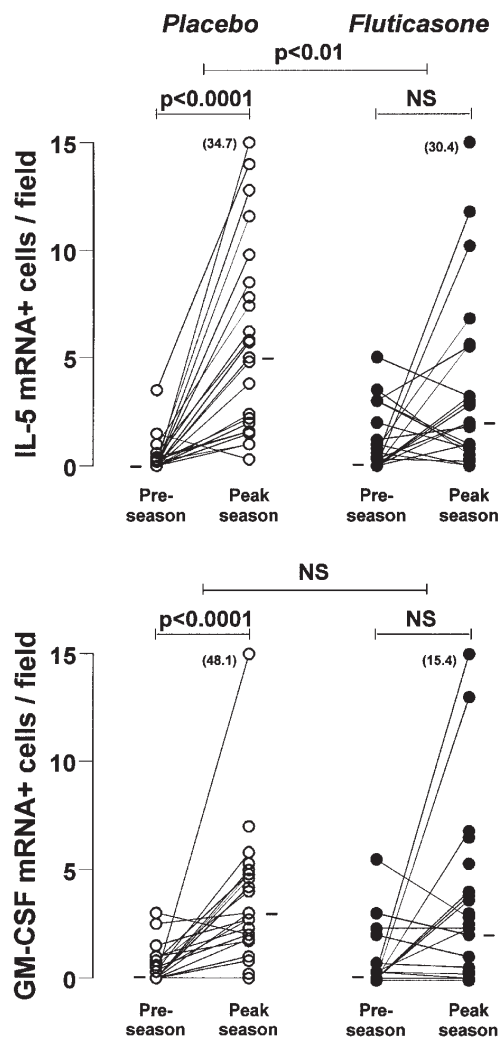


FIG 4. Effect of fluticasone on numbers of IL-5 and GM-CSF mRNA-expressing cells in nasal submucosa. Data shown are for baseline (*preseason*) and during natural grass pollen allergen exposure (*peak season*), in fluticasone- (*open circles*) and placebo-treated (*closed circles*) patients with allergic rhinitis. Lines represent median values. Differences (peak-season minus preseason) between fluticasone- and placebo-treated patients were compared by using the Mann-Whitney U test.

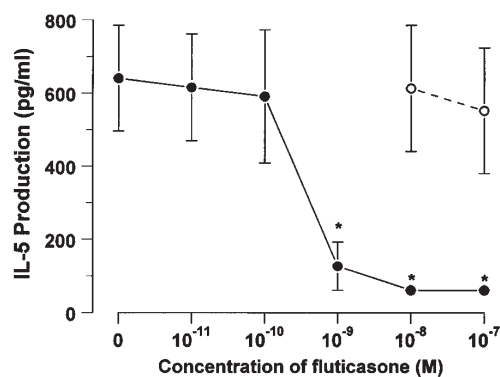


FIG 5. Effects of fluticasone on in vitro secretion of IL-5 protein by allergen-stimulated peripheral blood T cells. PBMCs were stimulated with *Phleum pratense* at 5×10^6 cells/mL, and supernatants were collected after 6 days for measurement of IL-5 by ELISA. Data points represent mean values (+ SE) of 5 atopic subjects (*closed circles*). *Open circles* represent IL-5 production in presence of drug vehicle controls. * $P < .05$ versus control culture (no fluticasone).

seasonal medians = 51 cells/field and 54 cells/field, respectively; Fig 2). Furthermore, unlike for epithelial CD3+ T cells, fluticasone treatment did not show any effect on the baseline numbers of T cells in the submucosal region (preseasonal and peak seasonal medians = 37 cells/field and 33 cells/field, respectively).

Expression of mRNA encoding IL-5 and GM-CSF during the pollen season

The location of IL-5 and GM-CSF mRNA-expressing cells was almost entirely submucosal (Fig 3). In patients receiving placebo treatment, natural allergen exposure was associated with a significant increase in the numbers of nasal submucosal cells expressing mRNA encoding IL-5 (preseasonal and peak seasonal medians = 0 cells/field and 5.0 cells/field, respectively; $P < .001$) (Fig 4). In patients receiving treatment with fluticasone, however, no increase was observed in the numbers of IL-5 mRNA+ cells during the pollen season (preseasonal and peak seasonal medians = 0.5 cells/field and 2.0 cells/field, respectively; Fig 4), and the seasonal increase in numbers of IL-5 mRNA-expressing cells was significantly greater in the placebo-treated group than in the fluticasone-treated group ($P < .01$). Although in placebo-treated patients grass pollen exposure was associated with increased numbers of GM-CSF mRNA-expressing cells (preseasonal and peak seasonal medians = 0.2 cells/field and 3.0 cells/field, respectively; $P < .0001$), fluticasone-treated patients did not show significantly increased GM-CSF mRNA expression during the pollen season (preseasonal and peak seasonal medians = 0.3 cells/field and 2.0 cells/field, respectively; Fig 4). Seasonal changes in the numbers of GM-CSF mRNA-expressing cells were not, however, significantly different in placebo- and fluticasone-treated groups.

Phenotypic identification of IL-5 mRNA-expressing cells by in situ hybridization and immunocytochemistry

In peak-season biopsy specimens obtained from 4 fluticasone-treated patients and 3 subjects in the placebo group, IL-5 mRNA signals identified by in situ hybridization were colocalized with phenotypic markers for T cells (CD3), eosinophils (MBP), mast cells (tryptase) and macrophages (CD68). These experiments (Table II) demonstrated that the majority of nasal mucosal cells expressing IL-5 mRNA during the grass pollen season were CD3+ T cells. Moreover, although fluticasone treatment attenuated the increase in IL-5 expression during the pollen season, IL-5 mRNA-expressing cells continued to be predominantly CD3+ T cells in these patients.

Correlations between numbers of submucosal eosinophils and numbers of IL-5 and GM-CSF mRNA-expressing cells

No significant associations were observed between the numbers of peak-seasonal submucosal eosinophils and IL-5 or GM-CSF mRNA-expressing cells in either

group. A weak correlation was observed within the placebo-treated group between the peak seasonal numbers of epithelial EG2+ eosinophils and IL-5 mRNA-expressing cells ($r = 0.461$, $P = .05$).

Effects of fluticasone on in vitro IL-5 secretion by allergen-stimulated T cells

PBMCs isolated from atopic subjects secreted IL-5 protein when cultured with *P pratense* allergen extract (Fig 5). Addition of fluticasone to these cultures resulted in a dose-dependent inhibition of IL-5 production. The inhibitory concentration of 50% for this effect was between 10^{-9} mol/L and 10^{-10} mol/L. GM-CSF concentrations in the same supernatant samples were below the limit of detection (<32.5 pg/mL) in 4 of the 5 patients tested.

DISCUSSION

In this study we have shown that allergic rhinitis associated with natural exposure to grass pollen is associated with both nasal mucosal eosinophilia and increases in the numbers of cells expressing mRNA encoding the proeosinophilic cytokines IL-5 and GM-CSF. In contrast, comparison of nasal biopsy specimens collected before and during the pollen season suggested that natural allergen exposure was not associated with an influx of CD3+ T cells into either the nasal epithelium or submucosa. In a double-blind, placebo-controlled trial, we next examined the effects of a 6-week prophylactic course of the corticosteroid fluticasone propionate on these changes. Fluticasone therapy was associated with reduced symptoms, inhibition of nasal eosinophilia during the pollen season, and a reduction in the numbers of epithelial CD3+ T cells. Because the numbers of CD3+ T cells did not increase during the pollen season, this reduction most probably reflected an inhibition of the T-cell migratory processes that function under basal conditions. In contrast to placebo-treated subjects, patients receiving fluticasone failed to show significant increases in the numbers of cells expressing IL-5 and GM-CSF mRNA during the summer pollen season. We next examined the phenotype of IL-5 mRNA-expressing cells by double immunohistochemistry/in situ hybridization in biopsy specimens obtained during the peak of the pollen season. In both fluticasone- and placebo-treated groups, more than 80% of IL-5 mRNA-expressing cells were CD3+ T cells. Finally, to test our hypothesis that fluticasone acts directly on T cells to inhibit cytokine production, we cultured PBMCs from atopic subjects with allergen and demonstrated that fluticasone inhibited IL-5 secretion under these conditions.

The association between allergic diseases and local tissue eosinophilia (with expression of T_H2 -like cytokines) is a well-established concept. The eosinophil, as well as the mast cell, is considered to be an effector cell in allergic inflammation. It is a potential source of proinflammatory lipid mediators, proallergic cytokines such as IL-4,¹⁴ and toxic granule proteins, all

of which have the potential to contribute to upper airways pathology in allergic rhinitis. It has previously been demonstrated that eosinophil cationic protein is released into nasal lavage fluid during the allergen-induced late reaction and that this process can be suppressed by topical fluticasone propionate treatment.¹⁵ This is consistent with the data shown in this study and the hypothesis that degranulation of activated eosinophils takes place within the nasal mucosa of patients with allergic rhinitis.

The significance of the reduction in the numbers of epithelial CD3+ T cells in the actively treated group is uncertain. We believe that it is unlikely that this reduction was directly related to clinical improvement in fluticasone-treated patients because cell numbers did not increase with worsening of symptoms (ie, during the pollen season) in the control group. More importantly, the majority of IL-5 and GM-CSF mRNA-expressing cells during the peak of the pollen season were located in the submucosa. On the other hand, the numbers of submucosal CD3+ T cells, which accounted for greater than 80% of IL-5 mRNA signals in patients receiving either fluticasone or placebo, did not increase during the peak season, nor were they obviously affected by fluticasone treatment. Collectively, these data suggest either that during the pollen season there is a preferential recruitment of a subpopulation of IL-5-expressing T cells to the submucosa, or that IL-5 expression by T cells is induced locally in response to natural physiologic allergen exposure. This latter conclusion is supported by a recent study showing that the propensity of T cells to secrete IL-5 is markedly upregulated at mucosal surfaces exposed to allergen.¹⁶ Because we were unable to find any evidence that fluticasone inhibited overall T-cell recruitment to the nasal submucosa, we hypothesized that reduced IL-5 mRNA expression in treated patients resulted from a local inhibition of allergen-induced IL-5 mRNA expression by CD3+ T cells. To seek further evidence in support of this hypothesis, we examined the effects of fluticasone on IL-5 production by PBMCs obtained from atopic subjects with rhinitis stimulated with grass pollen allergen *in vitro*. We previously showed that IL-5 secretion in this model is dependent on CD4+ T cells and elevated in subjects with allergic rhinitis relative to control subjects.¹⁷ These data confirmed that fluticasone is a potent inhibitor of allergen-driven IL-5 secretion by T cells *in vitro* (inhibitory concentration of 50% of between 10^{-9} and 10^{-10} mol/L), either through an effect on T cells themselves, an indirect effect on antigen-presenting cells, or both.

Reduced expression of IL-5 mRNA by bronchoalveolar lavage T cells after a course of oral prednisolone treatment has been described,¹⁸ and we have previously reported inhibition by topical corticosteroids of allergen-induced increases in nasal mucosal IL-4¹⁹ and IgE ϵ heavy chain⁴ mRNA expression. We have also confirmed that topical corticosteroids inhibit IL-4 and IgE ϵ heavy chain mRNA expression during natural season

exposure.²⁰ This study was novel in that we examined the effects of a topical corticosteroid on exposure on nasal eosinophilia and expression of proeosinophilic cytokines occurring naturally during the grass pollen season. Bradding et al²¹ demonstrated a seasonal increase in the numbers of eosinophils in the nasal mucosa and inhibition of this response by corticosteroid (fluticasone) therapy. In that study, however, the numbers of nasal mucosal cells immunostaining positive for IL-4 or IL-5 protein did not increase significantly during the peak pollen season in the patients receiving placebo. In the group of patients treated with corticosteroids, the numbers of IL-4 cells in the nasal mucosa during the pollen season decreased below levels observed both before the pollen season and before treatment. In contrast to this study, Bradding et al²¹ did not report any reduction in the numbers of IL-5-positive cells in patients receiving corticosteroid therapy. This most probably reflects the fact that immunostaining techniques preferentially identify preformed and stored IL-5 protein stored in granular cells^{22,23} in contrast to *in situ* hybridization, which identifies all cells actively expressing IL-5 mRNA, including T cells.

In this study the numbers of GM-CSF mRNA-expressing cells were also increased in biopsy specimens collected during the peak pollen season compared with those collected before the pollen season. Although this increase was demonstrable only in placebo-treated, and not fluticasone-treated, patients, unlike with IL-5 the difference between the groups was not statistically significant. This may have reflected the fact that in the placebo group the absolute numbers of GM-CSF mRNA-expressing cells during the peak season were smaller than for IL-5, and therefore the effects of reduced expression would have had less of an impact.

In summary, we have shown that natural exposure to grass pollen allergens in subjects with seasonal rhinitis coincides with nasal mucosal eosinophilia and expression of mRNA encoding the proeosinophilic cytokines IL-5 and GM-CSF. Eosinophilia was suppressed in patients receiving corticosteroid therapy. The decreased numbers of IL-5 mRNA-expressing cells in these patients raises the possibility that the efficacy of corticosteroids may arise from their ability to suppress IL-5 secretion by T cells *in vivo*.

REFERENCES

1. Varney VA, Jacobson MR, Sudderick RM, Robinson DS, Irani AM, Schwartz LB, et al. Immunohistology of the nasal mucosa following allergen-induced rhinitis. Identification of activated T lymphocytes, eosinophils, and neutrophils. *Am Rev Respir Dis* 1992;146:170-6.
2. Durham SR, Ying S, Varney VA, Jacobson MR, Sudderick RM, Mackay IS, et al. Cytokine messenger RNA expression for IL-3, IL-4, IL-5, and granulocyte/macrophage-colony-stimulating factor in the nasal mucosa after local allergen provocation: relationship to tissue eosinophilia. *J Immunol* 1992;148:2390-4.
3. Ghaffar O, Laberge S, Jacobson MR, Lowhagen O, Rak S, Durham SR, et al. IL-13 mRNA and immunoreactivity in allergen-induced rhinitis: comparison with IL-4 expression and modulation by topical glucocorticoid therapy. *Am J Respir Cell Mol Biol* 1997;17:17-24.
4. Durham SR, Gould HJ, Thienes CP, Jacobson MR, Masuyama K, Rak S, et al. Expression of ϵ germ-line gene transcripts and mRNA for the ϵ

- heavy chain of IgE in nasal B cells and the effects of topical corticosteroid. *Eur J Immunol* 1997;27:2899-906.
5. Ying S, Meng Q, Barata LT, Robinson DS, Durham SR, Kay AB. Associations between IL-13 and IL-4 (mRNA and protein), vascular cell adhesion molecule-1 expression, and the infiltration of eosinophils, macrophages, and T cells in allergen-induced late-phase cutaneous reactions in atopic subjects. *J Immunol* 1997;158:5050-7.
6. Lopez AF, Sanderson CJ, Gamble JR, Campbell HD, Young IG, Vadas MA. Recombinant human interleukin 5 is a selective activator of human eosinophil function. *J Exp Med* 1988;167:219-24.
7. Rothenberg ME, Petersen J, Stevens RL, Silberstein DS, McKenzie DT, Austen KF, 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;143:2311-6.
8. Walsh GM, Hartnell A, Wardlaw AJ, Kurihara K, Sanderson CJ, Kay AB. IL-5 enhances the in vitro adhesion of human eosinophils, but not neutrophils, in a leucocyte integrin (CD11/18)-dependent manner. *Immunology* 1990;71:258-65.
9. Collins PD, Marleau S, Griffiths Johnson DA, Jose PJ, Williams TJ. Cooperation between interleukin-5 and the chemokine eotaxin to induce eosinophil accumulation in vivo. *J Exp Med* 1995;182:1169-74.
10. Schweizer RC, Welmers BA, Raaijmakers JA, Zanen P, Lammers JW, Koenderman L. RANTES- and interleukin-8-induced responses in normal human eosinophils: effects of priming with interleukin-5. *Blood* 1994;83:3697-704.
11. Mould AW, Matthaei KI, Young IG, Foster PS. Relationship between interleukin-5 and eotaxin in regulating blood and tissue eosinophilia in mice. *J Clin Invest* 1997;99:1064-71.
12. Ying S, Durham SR, Barkans J, Masuyama K, Jacobson M, Rak S, et al. T cells are the principal source of interleukin-5 mRNA in allergen-induced rhinitis. *Am J Respir Cell Mol Biol* 1993;9:356-60.
13. Fokkens WJ, Vroom TM, Gerritsma V, Rijntjes E. A biopsy method to obtain high quality specimens of nasal mucosa. *Rhinology* 1988;26:293-5.
14. Moqbel R, Ying S, Barkans J, Newman TM, Kimmitt P, Wakelin M, et al. Identification of messenger RNA for IL-4 in human eosinophils with granule localization and release of the translated product. *J Immunol* 1995;155:4939-47.
15. Garredts IM, De Graaf in 't Veld T, Nahori MA, Vargaftig BB, Gerth van Wijk R, Zijlstra FJ. Interleukin-5 and eosinophil cationic protein in nasal lavages of rhinitis patients. *Eur J Pharmacol* 1995;275:295-300.
16. Kelly EAB, Rodriguez RR, Busse WW, Jarjour NN. The effect of segmental bronchoprovocation with allergen on airway lymphocyte function. *Am J Respir Crit Care Med* 1997;156:1421-8.
17. Till S, Dickason R, Huston D, Humbert M, Robinson D, Larche M, et al. IL-5 secretion by allergen-stimulated CD4+ T cells in primary culture: relationship to expression of allergic disease. *J Allergy Clin Immunol* 1997;99:563-9.
18. Robinson DS, Hamid Q, Ying S, Bentley A, Assoufi B, Durham SR, et al. Prednisolone treatment in asthma is associated with modulation of bronchoalveolar lavage interleukin-4, interleukin-5, and interferon-gamma cytokine gene expression. *Am Rev Respir Dis* 1993;148:401-6.
19. Masuyama K, Jacobson MR, Rak S, Meng Q, Sudderick RM, Kay AB, et al. Topical glucocorticosteroid (fluticasone propionate) inhibits cells expressing cytokine mRNA for interleukin-4 in the nasal mucosa in allergen-induced rhinitis. *Immunology* 1994;82:192-9.
20. Cameron LA, Durham SR, Jacobson MR, Masuyama K, Juliusson S, Gould HJ, et al. Expression of IL-4, Cε RNA, and Iε RNA in the nasal mucosa of patients with seasonal rhinitis: effect of topical corticosteroids. *J Allergy Clin Immunol* 1998;101:330-6.
21. Bradding P, Feather IH, Wilson S, Holgate ST, Howarth PH. Cytokine immunoreactivity in seasonal rhinitis: regulation by a topical corticosteroid. *Am J Respir Crit Care Med* 1995;151:1900-6.
22. Bradding P, Roberts JA, Britten KM, Montefort S, Djukanovic R, Mueller R, et al. Interleukin-4, -5, and -6 and tumor necrosis factor-α in normal and asthmatic airways: evidence for the human mast cell as a source of these cytokines. *Am J Respir Cell Mol Biol* 1994;10:471-80.
23. Ying S, Humbert M, Barkans J, Corrigan CJ, Pfister R, Menz G, et al. Expression of IL-4 and IL-5 mRNA and protein product by CD4+ and CD8+ T cells, eosinophils, and mast cells in bronchial biopsies obtained from atopic and nonatopic (intrinsic) asthmatics. *J Immunol* 1997;158:3539-44.