

Topical steroid treatment of allergic rhinitis decreases nasal fluid T_H2 cytokines, eosinophils, eosinophil cationic protein, and IgE but has no significant effect on IFN- γ , IL-1 β , TNF- α , or neutrophils

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Background: Topical treatment with glucocorticoids (GCs) is known to decrease eosinophils but not neutrophils in patients with allergic rhinitis.

Objective: We sought to examine whether the differential effects of GC treatment on eosinophils and neutrophils are mirrored by differential effects on T_H1/T_H2 cytokines and the neutrophil-associated cytokines IL-1 β and TNF- α .

Methods: Differential counts of eosinophils and neutrophils in nasal fluids from 60 children with seasonal allergic rhinitis treated with a topical GC were examined after staining with May-Grünwald-Giemsa stain. Nasal fluid levels of IFN- γ , IL-4, IL-6, IL-10, IL-1 β , and TNF- α were examined with ELISA, and IgE and eosinophil cationic protein (ECP) levels were examined with RIA.

Results: After GC treatment, there was a statistically significant decrease of the T_H2 cytokines IL-4, IL-6, and IL-10, as well as ECP and IgE. By contrast, there were no significant changes of the levels of IFN- γ , IL-1 β , TNF- α , or neutrophils. In the GC-treated patients IL-1 β and TNF- α levels correlated with neutrophils and ECP, and IL-1 β correlated with eosinophils. Furthermore, ECP correlated with both eosinophils and neutrophils. Neither IL-1 β nor TNF- α correlated with IgE. Patients with high neutrophil counts after GC treatment were found to have significantly higher eosinophil counts and ECP than patients with low counts.

Conclusions: The beneficial effects of topical treatment with GC in patients with allergic rhinitis could be attributed to downregulation of T_H2 cytokines, with an ensuing decrease of eosinophils, ECP, and IgE. It is possible that neutrophils could counteract the beneficial effects of GCs by releasing the proinflammatory cytokines IL-1 β and TNF- α . (*J Allergy Clin Immunol* 2000;106:307-12.)

Key words: Cytokines, IgE, eosinophil cationic protein, nasal fluids, glucocorticoids, allergic rhinitis

Allergic inflammation is associated with a shift in the balance between cytokines produced by T_H1 and T_H2 cells toward a T_H2 predominance. T_H2 cytokines, such as IL-4, IL-5, and IL-6, induce production of IgE, eosinophilia, and release of eosinophil cationic protein (ECP).^{1,2} The beneficial effects of treatment with glucocorticoids (GCs) in allergic diseases are attributable to downregulation of inflammatory cells and their products.³ Furthermore, GCs inhibit apoptosis in neutrophils, which, unlike eosinophils, do not decrease in nasal fluids from patients with GC-treated allergic rhinitis.⁴⁻⁶ Neutrophils are abundant in nasal fluids from patients with allergic rhinitis, and experimental work shows that inhibition of neutrophil influx prevents bronchial obstruction in animal models.⁷ This could be explained by the fact that neutrophils release proinflammatory cytokines like IL-1 β , IL-8, and TNF- α ,⁸ which may contribute to allergic inflammation by inducing the influx and degranulation of eosinophils.⁹⁻¹³ Moreover, neutrophils have been shown to carry ECP,¹⁴ which has toxic effects on bronchial epithelial cells.¹⁵ In patients with severe GC-treated asthma, increased levels of neutrophils, IL-8, and ECP have been found in bronchial lavage fluid or sputum.^{16,17} In a previous study we found that eosinophils and IL-5, but neither neutrophils nor IL-8, decreased in nasal fluids from children with allergic rhinitis after treatment with GCs.¹⁸ Taken together, these findings suggested the possibility that, although GCs reduce IL-5, eosinophils, ECP, and IgE, neutrophils could counteract this by producing other proinflammatory cytokines, such as IL-1 β , IL-8, and TNF- α , or directly by releasing toxins, such as ECP. The aim of this study was to examine the nasal fluids from our GC-treated patients to determine whether the differential effects of GC treatment on eosinophils and neutrophils were mirrored by differential effects on T_H1/T_H2 cytokines and the neutrophil-associated cytokines IL-1 β and TNF- α .

METHODS

Subjects

The material has been described in detail previously.¹⁸ In summary, it consisted of nasal lavage fluids from 60 schoolchildren with

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Abbreviations used

ECP: Eosinophil cationic protein
GC: Glucocorticoid

seasonal allergic rhinitis and 36 nonatopic schoolchildren. Allergic rhinitis was defined by a positive seasonal history and a positive skin prick test response to birch pollen, timothy pollen, or both. Patients with a history of perennial rhinitis were excluded from the study. Nasal lavage samples from the patients were obtained before the pollen season, after the start of symptoms during the pollen season, and after 1 week of treatment with two doses of 50 µg per dose budesonide nasal spray in each nostril twice daily. Nasal fluids from the nonatopic control subjects were obtained before and during the pollen season. All subjects were asked to mark their symptoms (sneezing, rhinorrhea, congestion, and itching) on a visual analogue scale of 10 cm. These values were added to the total symptom score. The maximum obtainable score was 40. The mean $\pm$ SEM scores were 0.2 ± 0.1 before treatment, 16 ± 0.9 after the start of symptoms, and 6 ± 0.8 after treatment.

Collection of nasal lavage fluids

Nasal lavage fluids were obtained as previously described.¹⁸ In summary, normal saline solution at normal room temperature was administered as an aerosol spray into each nostril, and the nasal washings were allowed to return passively and collected in a graded test tube, until 3 mL was recovered. This corresponded to $78\% \pm 1\%$ recovered fluid in the untreated patients and $77\% \pm 0.0\%$ in the treated patients. The samples were centrifuged, and the supernatant was separated from the pellet and stored at -70°C . Slides were stained according to the May-Grünwald-Giemsa method, and the percentages of epithelial cells, eosinophils, neutrophils, and mononuclear cells of a total of 100 cells per slide were then computed.

Mediator assays

All cytokines were measured with specific ELISA kits with improved sensitivity (Quantikine HS) obtained from R&D Systems (Abingdon, Oxon, UK). The ECP concentrations were determined by using a competitive radioimmunoassay (Pharmacia ECP RIA kit; Pharmacia & Upjohn Diagnostics AB, Uppsala, Sweden). The concentrations of total IgE were determined by using an RIA kit obtained from Pharmacia & Upjohn Diagnostics AB. The protein concentrations in all supernatants were determined by using a modified Lowry method (Protein assay; BIO-RAD, Hercules, Calif). The mean $\pm$ SEM protein contents of the samples in the patients was 0.31 ± 0.03 mg/mL before the pollen season, 0.33 ± 0.02 mg/mL during the pollen season, and 0.45 ± 0.03 mg/mL after treatment.

Statistics

Results are expressed as means $\pm$ SEM. The sign test was used to analyze the differences in mediators in the paired patients seen before and after treatment. This nonparametric test compares the number of patients with increasing levels of the analyzed variable with the number of patients with decreasing levels. Patients with unchanged levels are excluded from the analysis. The Kruskal-Wallis test was used to analyze differences in levels of mediators between GC-treated patients with high and low neutrophil counts. The Spearman rank correlation test was used to analyze correlations. Multiple inference with a P value of less than .05 was performed with the step-down Bonferroni method.

RESULTS

Eosinophils, neutrophils, IgE, and ECP in nasal fluids from patients with allergic rhinitis and control subjects before and during the pollen season

Results concerning eosinophils, neutrophils, IgE, and ECP in nasal fluids from patients and control subjects before and during the pollen season have been reported elsewhere.^{18,19} Before the pollen season, neutrophils, IgE, and ECP, but not eosinophils, were higher in patients compared with control subjects. In the patients, eosinophils and IgE, but not neutrophils and ECP, increased significantly during the pollen season compared with before the pollen season. During the pollen season, eosinophils, IgE, and ECP, but not neutrophils, were all higher in the patients than in the control subjects.

During GC treatment, the differential counts of eosinophils in the nasal fluids decreased from $10.6\% \pm 2.0\%$ to $2.7\% \pm 1.1\%$. The counts of neutrophils were $30\% \pm 3.9\%$ before treatment and $28\% \pm 4.5\%$ after 1 week of treatment.¹⁸

Treatment with budesonide resulted in a decrease in IgE levels in 43 patients and an increase in none ($P < .0001$). ECP decreased in 40 patients and increased in 19 patients ($P < .01$). The IgE levels were 2.7 ± 0.4 kU/L before treatment and 0.15 ± 0.12 kU/L after 1 week of treatment ($P < .0001$), and the corresponding ECP levels were 37.9 ± 5.9 µg/L and 22.7 ± 4.8 µg/L ($P < .01$, Fig 1). The symptom score before treatment was 16 ± 0.9 , and it was 6 ± 0.8 after treatment ($P < .0001$). The improvement in symptom score after treatment was associated with a reduction in eosinophil count ($r = 0.34$, $P < .001$) and IgE ($r = 0.42$, $P < .0001$).

IL-4, IL-6, IL-10, IFN- γ , IL-1 β , and TNF- α levels and IL-4/IFN- γ ratios in nasal fluids from patients with allergic rhinitis before and after treatment with budesonide

As earlier described,¹⁹ although there was only a small difference in mean values of IL-4 in specimens obtained from patients before the pollen season compared with those during the pollen season, analysis by sign test showed that the rise of IL-4 levels was statistically significant ($P < .05$), as was the rise of IL-10 ($P < .05$). During the pollen season, the IL-4/IFN- γ ratios were higher in patients than in control subjects during the pollen season. Furthermore, during the pollen season, the proportion of subjects with detectable IFN- γ was lower in patients than in control subjects. In contrast, the proportion of subjects with detectable IL-10 was higher in patients than in control subjects.

The mean $\pm$ SEM levels of IL-4, IL-6, IL-10, IFN- γ , IL-1 β , and TNF- α are given in Table I. After treatment with budesonide nasal fluid, IL-4 decreased in 15 patients and increased in one ($P < .001$). IL-6 decreased in 43 patients and increased in 17 patients ($P < .001$). IL-10 decreased in

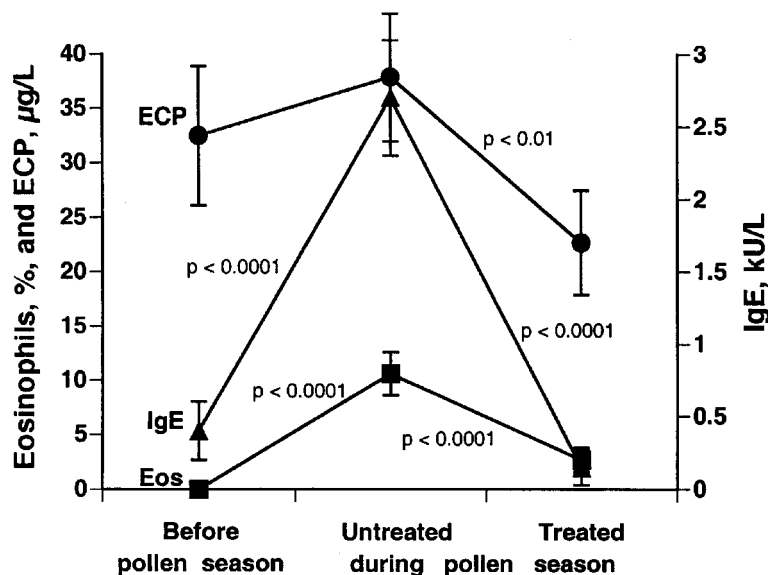


FIG 1. The mean $\pm$ SEM counts of eosinophils and levels of IgE and ECP in nasal fluids from before pollen season and during season in untreated and budesonide-treated patients with allergic rhinitis. Vertical bars indicate SEM. *P* values are based on the sign test.

TABLE I. IL-4, IL-6, IL-10, IFN- γ , IL-1 β , and TNF- α in nasal fluids from patients with allergic rhinitis before the pollen season and during the pollen season before and after treatment with budesonide

	IL-4 (pg/mL)	IL-6 (pg/mL)	IL-10 (pg/mL)	IFN- γ (pg/mL)	IL-1 β (pg/mL)	TNF- α (pg/mL)
Before season						
Mean $\pm$ SEM	0.16 $\pm$ 0.10	4.9 $\pm$ 0.7	0.23 $\pm$ 0.10	16.7 $\pm$ 3.3	34.9 $\pm$ 6.9	0.38 $\pm$ 0.10
Median	0	3.7	0	11	15.7	0
Range	0-3.6	0.7-25.5	0-2.5	0-100	4.1-175.5	0-2.6
Percent > detection limit	15	100	32	74	100	33
n	39	39	38	39	39	39
During season, before treatment						
Mean $\pm$ SEM	0.17 $\pm$ 0.05*	14.8 $\pm$ 4.8	0.43 $\pm$ 0.10*	15.5 $\pm$ 3.2	35.1 $\pm$ 6.0	0.39 $\pm$ 0.09
Median	0	3.9	0	8	17.3	0
Range	0-2.5	0.5-183	0-2.4	0-116	0-240	0-2.5
Percent > detection limit	27	100	46	55	97	31
n	59	60	41	60	60	59
During season, after treatment						
Mean $\pm$ SEM	0.05 $\pm$ 0.02†	9.2 $\pm$ 3.0‡	0.05 $\pm$ 0.03†	9.7 $\pm$ 2.5	31.4 $\pm$ 5.1	0.51 $\pm$ 0.09
Median	0	3.1	0	0	22.5	0
Range	0-0.75	0.3-161	0-1.15	0-100	0-240	0-4
Percent > detection limit	10	100	10	40	95	47
n	58	60	51	60	60	60

**P* < .05 compared with patients before the pollen season (sign test).

†*P* < .01 and ‡*P* < .001 compared with untreated patients during the pollen season (sign test).

17 patients and increased in 3 patients (*P* < .01). IFN- γ decreased in 23 patients and increased in 14 patients (not significant). IL-1 β in nasal fluids decreased in 30 patients and increased in 29 patients. TNF- α decreased in 15 patients and increased in 15 patients. The IL-4/IFN- γ quotients decreased in 17 patients and increased in 22.

The previously reported¹⁸ significant reduction of IL-5 after treatment correlated with eosinophils (*r* = 0.34, *P* < .001) and with ECP (*r* = 0.26, *P* < .01). The correlation between IL-4 and IgE did not reach statistical significance (*r* = 0.18, *P* = .05.)

Differential counts of eosinophils and levels of ECP and IgE in GC-treated patients with high and low differential counts of neutrophils

To examine the possibility that neutrophils may affect allergic inflammation in the GC-treated patients, the patients were divided into two groups, one with high ($\geq 8\%$; $\geq$ the median level) and one with low (<8%) differential counts of neutrophils.

In patients with high neutrophil counts, the mean $\pm$ SEM differential counts of eosinophils were 4.9% $\pm$

2.1% versus $0.22\% \pm 0.22\%$ in the patients with low neutrophil counts ($P < .0001$). The corresponding levels of ECP were $35.5 \pm 8.6 \mu\text{g/L}$ compared with $10.0 \pm 2.7 \mu\text{g/L}$ ($P < .001$). The mean IgE level in patients with high neutrophil counts was $0.30 \pm 0.23 \text{ kU/mL}$. No IgE was detectable in any of the samples from patients with low neutrophil counts (not significant).

Correlation of IL-1 β and TNF- α with neutrophils, eosinophils, ECP, and IgE in the GC-treated patients

In previous investigations of the present material, significant correlations among IL-4, IL-5, IL-6, IL-10, eosinophils, ECP, and IgE were observed in untreated patients.^{18,19} In the present study involving nasal fluid specimens from patients who were treated for a mean of 1 week with GC, IL-1 β and TNF- α correlated with differential counts of neutrophils ($r = 0.4$, $P < .01$ and $r = 0.5$, $P < .001$, respectively). IL-1 β correlated with differential counts of eosinophils ($r = 0.4$, $P < .01$). IL-1 β and TNF- α correlated with ECP ($r = 0.5$, $P < .0001$ and $r = 0.5$, $P < .001$, respectively). Both eosinophils and neutrophils correlated with ECP ($r = 0.5$, $P < .001$ and $r = 0.4$, $P < .001$, respectively). None of the cytokines correlated significantly with IgE.

DISCUSSION

GCs have profound inhibitory effects on allergic inflammation. T_H2 cytokines, eosinophils, ECP, and IgE have been reported to decrease after treatment with GCs.^{3,20,21} In contrast, neutrophils have not been found to decrease after treatment with GCs.^{4,18} A probable explanation for the differential effects of GCs on the two cell types is that GCs induce apoptosis in eosinophils but protect neutrophils from apoptosis.⁴ Neutrophils are abundant in nasal fluids from patients with allergic rhinitis and asthma and are capable of releasing proinflammatory cytokines and possibly ECP.^{8,14} We have previously found that eosinophils and the T_H2 cytokine IL-5 decreased in nasal fluids from GC-treated patients with allergic rhinitis, whereas neutrophils and IL-8 did not.¹⁸ Furthermore, IL-8 was found to correlate with eosinophils in the GC-treated patients. These findings are consistent with earlier suggestions that IL-8 is not only released from and chemotactic for neutrophils but is also a chemoattractant for eosinophils.⁹

Our findings of decreases of eosinophils, ECP, IgE, and the T_H2 cytokines after GC treatment are in accord with several other studies, as well as the positive clinical effects of treating allergic diseases with topical GCs.^{3,20-26} Indeed, in the present study symptom scores decreased significantly in the treated patients, and the reduction correlated with the reduction of eosinophils, as well as IgE, in nasal fluids. The decrease of IL-5, eosinophils, and ECP could be causally related, as indicated by a significant correlation among these variables. No strong correlation between IgE and IL-4 was found, despite the

importance of this cytokine in regulating synthesis of IgE. This could possibly be explained by earlier findings that IL-4 has no effect on committed B cells.²⁶ It should be noted, however, that in many of the samples the cytokines were not detectable, and the results should therefore be interpreted with caution. Interestingly, in a previous study of nasal fluids from patients with allergic rhinitis, performed during a year when air pollen levels were twice as high as in the present study, IL-4 levels and differential counts of eosinophils were 3 to 4 times higher, despite the use of the same sampling method, handling of samples, and assays.²⁷ Thus IL-4 levels could be influenced by the degree of allergen exposure, a possibility that is supported by in vitro studies.²⁸

From a statistical perspective, the finding that T_H2 cytokines were not detectable in many samples means that the distributions of these cytokines were skewed. Therefore the statistical analyses were performed with the sign test. This is a nonparametric test, which only compares the numbers of patients with increasing values with the numbers of patients with decreasing values. It is not affected by the magnitude of changes between individual patients and therefore is recommended when assumptions of symmetry cannot be made.²⁹

In contrast to the T_H2 cytokines, the T_H1 cytokine IFN- γ did not decrease significantly during treatment with GCs. This is in agreement with our previous study²⁷ and the understanding that GCs may have differential effects on the immune response. Indeed, in one study GCs have even been reported to increase IFN- γ messenger RNA in bronchoalveolar lavage cells.³⁰ From a clinical point of view, this may be beneficial because IFN- γ opposes the release of T_H2 cytokines.

The lack of effect of GCs on neutrophils in this and other studies leads to the question of whether neutrophils could counteract the beneficial effects of GC treatment on allergic inflammation. Neutrophils are abundant in nasal and bronchial fluids from patients with allergic rhinitis and asthma and predominate in bronchial fluids from patients with severe GC-treated asthma. In the samples taken before the pollen season, neutrophil counts were significantly higher in the patients than in the control subjects, as were total IgE and ECP levels. This could depend on cross-reactivity to hazel pollen, which is present in the air in February, when the samples were taken. Because skin prick tests were only performed against birch and timothy pollen, it is also possible that some patients were allergic to perennial allergens. It should be noted, however, that none of the patients had a clinical history of this, and all were asymptomatic at the time of sampling. Moreover, eosinophils did not significantly differ compared with levels found in the control subjects before the pollen season.

To assess the possibility that neutrophils may in some way affect allergic inflammation in the GC-treated patients, the differential counts of eosinophils and the levels of ECP and IgE were compared in patients with low and high differential counts of neutrophils. Both eosinophils and ECP were significantly higher in patients

with high neutrophil counts than in those with low neutrophil counts, and IgE was not detectable in any patient in the latter group.

These findings could be explained by neutrophils producing cytokines that may affect eosinophils in patients treated with GCs. IL-8 is, for example, a powerful chemoattractant for eosinophils. In a study of bronchial fluids from patients with severe asthma, high neutrophil counts were associated with high IL-8 and ECP levels, despite relatively low eosinophil counts.¹⁶ We have previously found that IL-8 correlated with both neutrophils and eosinophils in nasal fluids from GC-treated patients with allergic rhinitis.¹⁸ This suggested that neutrophils, through the release of IL-8, could exert proeosinophilic effects, despite treatment with GCs. This lead us to examine IL-1 β and TNF- α , which also may be released from neutrophils and induce influx and activation of eosinophils.¹⁰⁻¹³ Like the differential counts of neutrophils and IL-8, these cytokines did not decrease during GC treatment. This is in accord with a recent study showing that expression of IL-1 β and TNF- α , unlike IL-4, did not decrease in nasal polyps after treatment with a topical steroid.³¹ Both IL-1 β and TNF- α correlated with neutrophils in our GC-treated patients, suggesting that they were indeed derived from neutrophils. IL-1 β correlated with eosinophils, and IL-1 β and TNF- α correlated with ECP. These findings agree with the possibility that neutrophils, through the release of IL-1 β and TNF- α , could induce eosinophil degranulation during treatment with GCs.

An additional possible proallergenic activity of neutrophils could be the release of ECP. Although ECP is generally considered an eosinophil-specific protein, the neutrophil has recently been identified as a carrier of ECP.¹⁴ In the present study we found a positive correlation between ECP and neutrophils, as well as eosinophils. A positive correlation between neutrophils and ECP is compatible with the possibility that neutrophils can release ECP, but simple covariation appears to be a more likely explanation for the finding.

We also examined whether the number of neutrophils present in nasal fluids before treatment could predict the clinical response to GC in terms of symptom scores, eosinophils, IgE, and ECP, but we found no such association (data not shown).

In summary, our findings have shown that treatment with GCs lowers the levels of the T_H2 cytokines IL-4, IL-6, and IL-10, as well as those of ECP and IgE, in nasal fluids from patients with allergic rhinitis. By contrast, no significant decreases of the T_H1 cytokine IFN- γ or the neutrophil-associated cytokines IL-1 β and TNF- α were found. Neutrophils, IL-1 β , and TNF- α correlated with ECP. It is possible that neutrophils, by inducing release of ECP from eosinophils through IL-1 β and TNF- α or by directly releasing ECP, could counteract the anti-inflammatory effect of GCs.

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