

Fluticasone propionate aqueous nasal spray reduces inflammatory cells in unchallenged allergic nasal mucosa: Effects of single allergen challenge

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Background: Topical corticosteroid therapy reduces symptoms and nasal mucosal inflammatory cells in patients with allergic rhinitis. Usually patients are advised to start their medication (1 week) before the beginning of the pollen season. The effect of pretreatment with a topical corticosteroid on unchallenged nasal mucosa is not well documented.

Objectives: The purpose of this study was to investigate, in a double-blind, placebo-controlled study, the effect of 6 weeks' pretreatment with 200 µg twice daily fluticasone propionate on nasal symptoms and inflammatory cell numbers after nasal allergen provocation in patients with seasonal allergic rhinitis. **Methods:** Nineteen patients with grass pollen-induced allergic rhinitis were treated for a 6-week period out of the grass pollen season. After completing the treatment period, patients were challenged with grass pollen. Nasal mucosal biopsy specimens were taken 5 times in every patient. In nasal mucosa changes in numbers of T cells, B cells, mast cells, eosinophils, macrophages, and Langerhans' cells were investigated.

Results: After 4 weeks of treatment but before allergen provocation, significantly fewer epithelial Langerhans' cells, macrophages, mast cells, T cells, and eosinophils were found in the fluticasone propionate group compared with those found in the placebo group. In the lamina propria significantly fewer Langerhans' cells and eosinophils were found in the fluticasone propionate group. Cell influx in nasal mucosa after allergen provocation was significantly inhibited in the fluticasone propionate group compared with that in the placebo group for epithelial Langerhans' cells, mast cells, macrophages, and T cells and for lamina propria eosinophils, mast cells, Langerhans' cells, macrophages, and T cells.

Conclusions: Fluticasone propionate is effective in reducing early- and late-phase nasal symptoms. Topical corticosteroid treatment reduces inflammatory cells in unchallenged nasal mucosa. (*J Allergy Clin Immunol* 2001;107:627-33.)

Key words: Nasal challenge, fluticasone propionate, inflammatory cells, allergic rhinitis, allergic inflammation

The mucosal inflammation in allergic rhinitis is characterized by the presence of tissue infiltration of Langerhans' cells, T cells and activated T cells, mast cells, eosinophils, and neutrophils in the nasal mucosa after allergen challenge.¹⁻⁶ Topical steroids are effective in the clinical management of allergic rhinitis and inhibit both early- and late-phase responses after allergen challenge. Topical corticosteroids reduce the influx of eosinophils, neutrophils, Langerhans' cells, and mononuclear cells in nasal secretions and nasal biopsy specimens from patients with allergic rhinitis.⁷⁻¹⁰ Usually in allergen provocation studies investigating the effect of corticosteroids on nasal mucosal inflammatory cells, baseline nasal biopsy specimens are taken before the treatment period, and a second biopsy specimen is taken at some moment (maximum, 24 hours) after nasal allergen provocation.^{11,12} Lozewicz et al¹³ investigated the effect of fluticasone propionate on eosinophils in nasal mucosal biopsy specimens taken at several time points between 0 and 8 hours. However, there remains a shortage of knowledge concerning treatment effects on unchallenged nasal mucosa of patients with allergic rhinitis and how treatment interferes with events occurring directly and after an extended period after allergen provocation. With a repeated biopsy method, we tried to understand better the pathophysiologic aspects occurring before and after allergen provocation with corticosteroid protection. Topical corticosteroids are usually effective in reducing nasal symptoms after 1 or 2 weeks' use. Andersson et al⁷ found that even a short (1 day) pretreatment with topical steroids abolished the allergen-induced increase in nasal hyperresponsiveness. Allergic patients usually start taking their medication at the presentation of the first symptoms, but there are clues that it is advisable to start the steroid treatment before the grass pollen season begins.¹⁴ In this study we have examined the influence of a 6-week pretreatment with a topical corticosteroid, fluticasone propionate aqueous nasal spray, on nasal mucosal inflammatory cells during allergen provocation.

METHODS

Patients

Nineteen patients (11 women and 8 men) between 18 and 51 years of age participated in this study. They all had a history of seasonal allergic rhinitis induced by grass pollen for at least 2 years. None of

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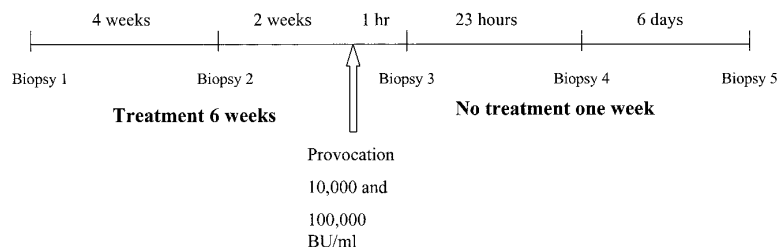


FIG 1. Schematic chart of the study design. After 6 weeks of fluticasone propionate or placebo treatment, an allergen challenge was performed. Each patient underwent 5 nasal biopsies: at baseline; after 4 weeks of treatment (2 weeks before allergen challenge); 1 hour after challenge with grass pollen; 24 hours after allergen challenge; and 1 week after allergen challenge.

TABLE I. Symptoms of sneezing, rhinorrhea, nasal blockage, itching, and total symptom score over time recorded on a 4-point scale (0-3)

Symptom	Time interval									
	Baseline	During treatment	Before provocation	15 min*	30 min†	1-3 h	3-6 h	6-9 h	9-12 h	12-24 h
Placebo										
Blockage	0 (0-0)	0 (0-0)	0 (0-0)	1 (1-2)	2 (2-3)	1 (0-3)	1 (0-2)	2 (0-2)	1 (0-3)	1 (0-2)
Discharge	0 (0-0)	0 (0-0)	0 (0-0)	1 (1-1)	1 (1-2)	1 (1-2)	1 (0-3)	1 (0-3)	1 (0-2)	0.5 (0-2)
Eye watering	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-1)	0 (0-1)	0 (0-1)	0 (0-1)	0 (0-1)
Itching	0 (0-0)	0 (0-0)	0 (0-0)	3 (1-3)	3 (1-3)	0 (0-1)	0 (0-1)	1 (0-1)	1 (0-2)	1 (0-2)
Sneezing	0 (0-0)	0 (0-0)	0 (0-0)	1 (0-3)	3 (0-3)	0 (0-1)	0 (0-1)	0 (0-1)	1 (0-2)	1 (0-2)
Total	0 (0-0)	0 (0-0)	0 (0-0)	5 (4-9)	9 (4-11)	4 (1-6)	3 (0-6)	4 (0-6)	2 (0-7)	2 (0-6)
Fluticasone propionate										
Blockage	0 (0-0)	0 (0-0)	0 (0-0)	1 (0-2)	2 (0-2)	1 (0-2)	0 (0-2)	0 (0-1)	0 (0-1)	0 (0-1)
Discharge	0 (0-0)	0 (0-0)	0 (0-0)	1 (0-2)	1 (0-2)	0.5 (0-2)	0 (0-1)	0 (0-2)	0 (0-1)	0 (0-1)
Eye watering	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)	0.5 (0-5)	0 (0-1)	0 (0-1)	0 (0-1)	0 (0-1)
Itching	0 (0-0)	0 (0-0)	0 (0-0)	3 (0-3)	3 (1-3)	0 (0-1)	0 (0-1)	1 (0-1)	1 (0-2)	0 (0-2)
Sneezing	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-3)	2 (0-3)	0.5 (0-2)	0.5 (0-1)	0 (0-1)	0 (0-1)	0 (0-1)
Total	0 (0-0)	0 (0-0)	0 (0-0)	3 (1-10)	5 (3-10)	3 (0-7)	1 (0-4)	0 (0-5)	0 (0-5)	0 (0-3)

Values are given as median (range).

*Immediate symptoms after allergen provocation with 10,000 BU/mL.

†Immediate symptoms after allergen provocation with 100,000 BU/mL.

the patients had a history of an allergy to other common allergens, such as house dust mite, cat, and dog. This was confirmed by a positive skin prick test response ($\geq 3+$) with Alutard Soluprick extract of 1 SQ/mL and no extensive skin prick reactions ($\leq 1+$) to other relevant allergens. The patients were not taking medication 6 weeks before the study start and had no other nasal complaints. They received no immunotherapy in the 3 years before this study. No abnormalities were seen at ear, nose, and throat examination. There were no significant differences between the fluticasone propionate and placebo groups concerning severity or duration of allergic rhinitis. There were no significant differences in demographic characteristics.

All patients gave their written informed consent, and the medical ethics committee of the hospital approved the study.

Study design

This double-blind, randomized, placebo-controlled, parallel-group study was conducted during autumn of 1995, more than 2 months after the hay fever season. After randomization, 10 patients used 200 μ g twice daily fluticasone propionate and 9 patients used placebo for 6 weeks. GlaxoWellcome, The Netherlands, supplied fluticasone propionate and placebo. The placebo drug was identical in taste, bottle type,

and volume of the delivered dose. After this treatment period, provocation took place, and the use of fluticasone propionate or placebo was stopped. Nasal mucosal biopsy specimens were taken from the inferior turbinate with a Gerritsma forceps.¹⁵ Each patient underwent 5 nasal biopsies: at baseline; after 4 weeks of treatment (2 weeks before provocation); 1 hour after provocation with grass pollen; 24 hours after provocation; and 1 week after provocation (Fig 1).

Provocation

The patient was acclimatized to the room for 15 minutes. After that, PBS, an inert water-like solution, was administered to rule out aspecific hyperreactivity. After 10 minutes, patients were provoked in each nostril with 50 μ L of an aqueous nasal spray containing 10,000 BU/mL grass pollen (ALK), and 10 minutes later, patients were again provoked with 100,000 BU/mL (ALK). After both provocations, nasal symptoms (nasal blockage, itching, and rhinorrhea) were recorded on a 4-point (0 = none, 1 = mild, 2 = moderate, and 3 = severe) scale. Sneezing was recorded as follows: 0, no sneezes; 1, 1 to 2 sneezes; 2, 3 to 4 sneezes; and 3, more than 5 sneezes. Patients were asked to rate their symptoms 3, 6, 9, 12, and 24 hours after provocation.

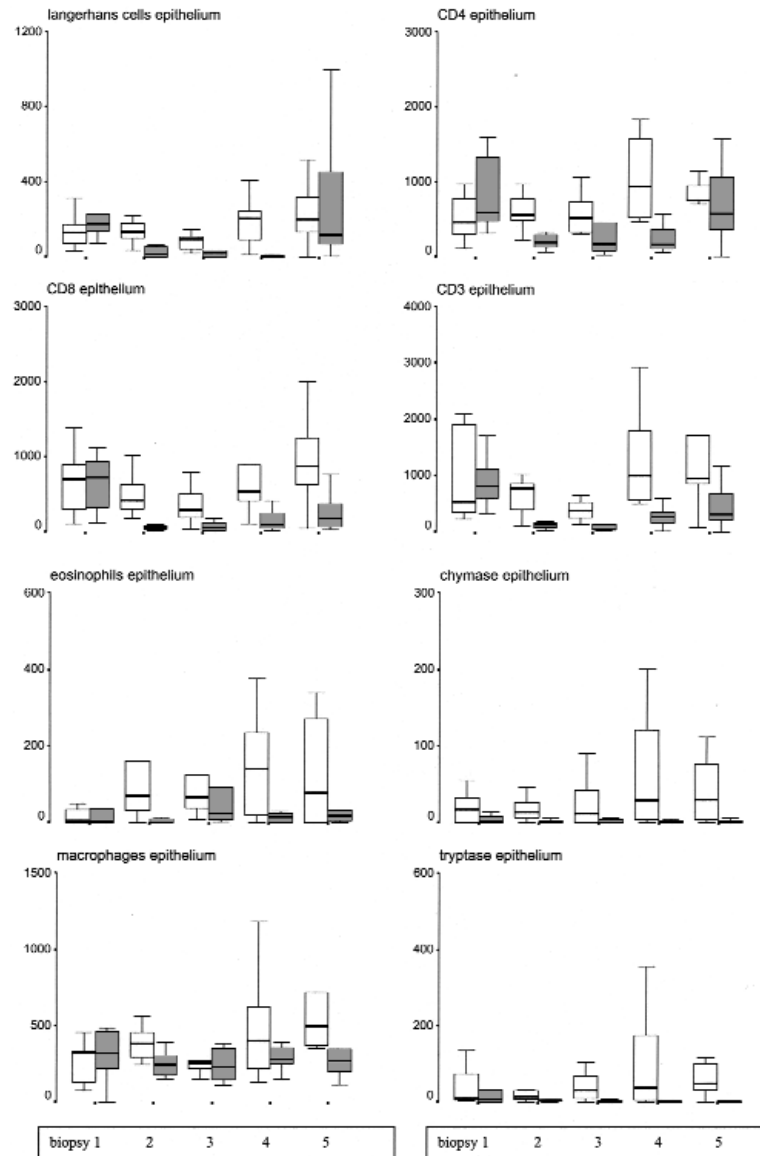


FIG 2. Cell counts per square millimeter in the nasal epithelium at the 5 biopsy moments (1, baseline; 2, after 4 weeks of treatment [2 weeks before provocation]; 3, 1 hour after provocation with grass pollen; 4, 24 hours after provocation; and 5, 1 week after provocation) during the study period (filled bars, fluticasone propionate; open bars, placebo). Results are expressed as medians, interquartile ranges, and the 2.5 and 97.5 percentiles.

Nasal biopsies

Five nasal biopsy specimens were obtained from each patient from both nostrils, each time from the alternating side. Local anesthesia was achieved by applying a cotton-wool carrier with 50 to 100 mg of cocaine and 3 drops of adrenaline (1:1000) under the inferior turbinate without touching the biopsy site for approximately 10 minutes. Biopsy specimens of nasal mucosa were taken from the lower edge of the inferior turbinate, about 2 cm posterior to the edge, by using a Gerritsma forceps with a cup diameter of 2.5 mm. The biopsy specimens were embedded in Tissue-Tek II OCT compound in a gelatin capsule and immediately frozen.

From each specimen, serial 6- μ m thick sections of nasal mucosa were cut on a Reichert-Jung 2800 Frigocut cryostat and transferred to gelatin-coated microscope slides. The sections were stained for cell surface and intracellular markers according to a previously described supersensitive immunoalkaline method.¹⁶ Langerhans' cells were stained with the mAb 66IIC7 (CD1a; Sanbio), T cells, and subsets with anti-CD4 (Central Laboratory of the Red Cross Blood Transfusion Service, Amsterdam, The Netherlands), anti-CD3 (Becton Dickinson), and anti-CD8 (Monosan, Sanbio). Macrophages were stained with anti-CD68, and eosinophils were stained with anti-BMK13. Mast cells were stained with the mAb anti-tryptase (G3 4 μ g/mL) and with anti-chymase biotin (B7 3.3 μ g/mL; Chemicon).

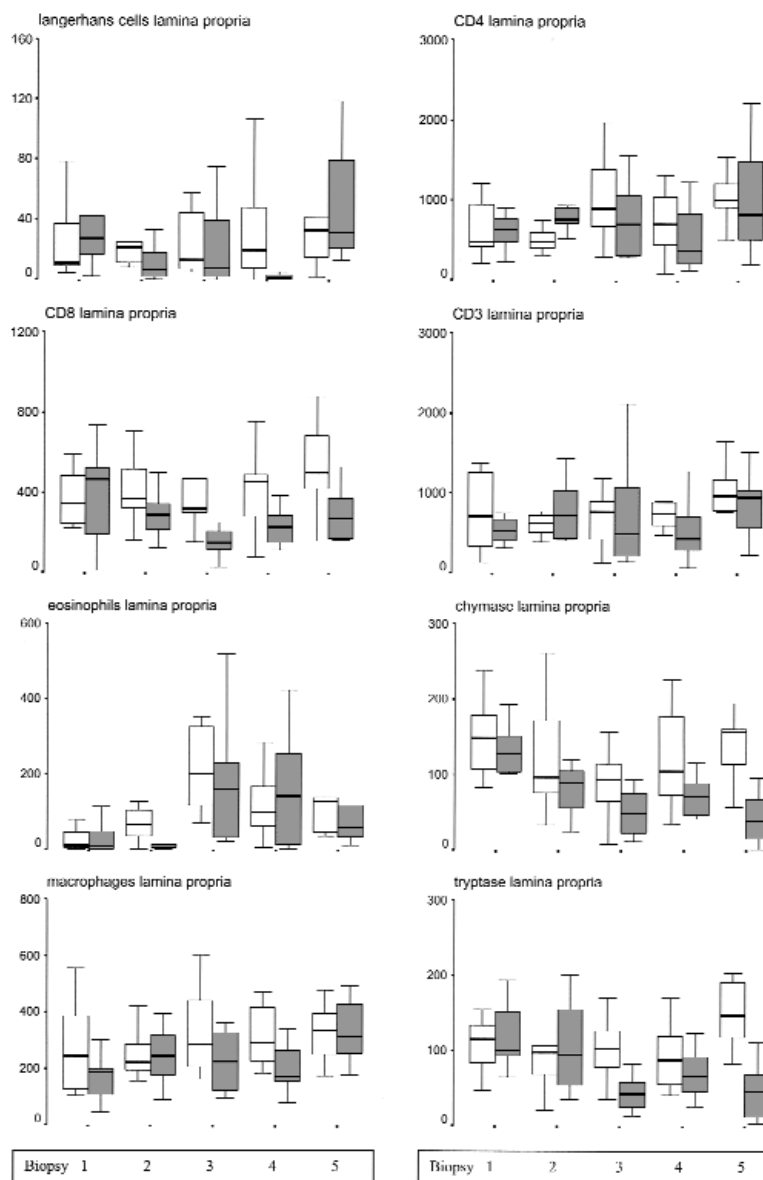


FIG 3. Cell counts per square millimeter in the nasal lamina propria at the 5 biopsy moments (1, baseline; 2, after 4 weeks of treatment [2 weeks before provocation]; 3, 1 hour after provocation with grass pollen; 4, 24 hours after provocation; and 5, 1 week after provocation) during the study period (filled bars, fluticasone propionate; open bars, placebo). Results are expressed as medians, interquartile ranges, and the 2.5 and 97.5 percentiles.

Cell quantification

The surface area of 2 sections in total and of the epithelium and lamina propria separately was estimated by means of the image analysis system Videoplan Kontron 2.1. Cells binding the mAb used had bright red surface membranes, red-stained cytoplasm, or both, depending on the cell type or mAb evaluated. Cells were counted if they stained red and contained a nucleus. When groups of positive cells lay very close together or overlapped each other, only the definitely positive cells were counted. The cell numbers in epithelium and lamina propria were divided by the area and expressed as the number of cells per square millimeter.

Statistical analysis

Efficacy was analyzed by using a mixed-model ANOVA. All outcome variables (y) showed a positive skew and were therefore log transformed as $\ln(y + 1)$. The baseline measurement of the transformed outcome variable was included as a covariate in the statistical model. The other explanatory factors included in the model were treatment (2 between-subject levels), biopsy moment (3 within-subject levels), and the interaction of these 2 factors. The covariance structure of the residuals was supposed to be of the compound symmetry type (constant variances across biopsy moments and constant covariances between biopsy moments).

TABLE II. Correlations between nasal symptoms and cell numbers in the placebo-treated group

Early-phase sneezing	Eosinophils, epithelium, biopsy 3	$r = -0.82, P = .007$
	Chymase-positive cells, lamina propria, biopsy 3	$r = -0.80, P = .009$
Sneezing 12 h after provocation	Tryptase-positive cells, lamina propria, biopsy 2	$r = 0.96, P = .003$

TABLE III. Correlations between nasal symptoms and number of cells for the fluticasone propionate-treated group

Eosinophil, epithelium, biopsy 3	Rhinorrhea 9 h	$r = -0.84, P = .005$
	Rhinorrhea 12 h	$r = -0.87, P = .005$
Chymase, epithelium, biopsy 4	Duration of symptoms	$r = 0.88, P = .002$

A P value of less than .05 was considered to indicate a significant difference. Correlations were performed by using the Spearman rank method. Because of multiple testing, a P value of less than .01 was considered to indicate a significant difference.

RESULTS

Clinical response to grass pollen

Nasal challenge with grass pollen resulted in a significant increase in immediate and late nasal symptoms in both groups, with increases in number of sneezes, itching, nasal blockage, and rhinorrhea. A significant reduction in early-phase obstruction ($P = .006$), itching ($P = .015$), late-phase obstruction ($P = .024$), and rhinorrhea ($P = .04$) was found in the fluticasone propionate-treated group compared with the placebo group (Table I).

Immunohistochemistry

Effect of treatment (before provocation). At baseline, there were no significant differences in the numbers of cells between the 2 treatment groups, and therefore the randomization of the 2 groups was correct. After 4 weeks of treatment but before allergen provocation, in the epithelium fewer Langerhans' cells ($P = .0014$), macrophages ($P = .04$), eosinophils ($P = .007$), CD3⁺ cells ($P = .006$), CD4⁺ cells ($P = .007$), CD8⁺ cells ($P = .0006$), chymase-positive cells ($P = .01$), and tryptase-positive cells ($P = .03$) were counted in the fluticasone propionate group compared with those in the placebo group. In the lamina propria fewer Langerhans' cells ($P = .03$) and eosinophils ($P = .003$) were found in the fluticasone propionate group. Results of nasal epithelium and lamina propria are depicted separately in Figs 2 and 3.

Changes after allergen provocation. Cell influx in nasal mucosa after allergen provocation was significantly inhibited in the fluticasone propionate group compared with that found in the placebo group for epithelial Langerhans' cells ($P < .001$), chymase-positive cells ($P = .036$), macrophages ($P = .005$), CD3⁺ cells ($P = .0001$), and CD8⁺ cells ($P = .0001$) and for lamina propria eosinophils ($P = .012$), chymase-positive cells ($P = .04$), Langerhans' cells ($P = .0001$), macrophages ($P = .034$), CD3⁺ cells ($P = .0001$), CD8⁺ cells ($P = .042$), and CD4⁺ cells ($P = .04$). Because of the effect of corticosteroid treatment on cell numbers before allergen provocation (biopsy 2), medication-related differences between the 2 treatment groups were analyzed. This depicts only the

effect of treatment on cell numbers after allergen provocation and does not take into account changes that occur before allergen provocation. Significant medication-related differences between the 2 treatment groups were found in epithelial eosinophils ($P = .007$), chymase-positive cells ($P = .001$), tryptase-positive cells ($P = .0001$), Langerhans' cells ($P = .0001$), CD3⁺ cells ($P = .0001$), CD4⁺ cells ($P = .0001$), and CD8⁺ cells ($P = .0001$) and in lamina propria chymase-positive cells ($P = .001$), tryptase-positive cells ($P \leq .0001$), Langerhans' cells ($P = .04$), and CD8⁺ cells ($P = .0001$).

Associations were sought between the number of cells in nasal mucosa and the size of the early- and late-phase responses. Because of the repeated biopsies, it is possible to relate cell numbers before allergen provocation with the severity of the nasal complaints after allergen provocation and early- and late-phase responses with cell numbers after 1 and 24 hours. Results are shown in Table II for the placebo-treated group and in Table III for the fluticasone propionate-treated group.

DISCUSSION

In this study we were able to obtain 5 nasal mucosal biopsy specimens in each patient within a period of 7 weeks, with 3 biopsies in a period of approximately 24 hours. Although very demanding for the patients, the procedure was well tolerated and not considered unethical by the investigators and the local medical ethics committee. With this study design, it was possible not only to investigate the early- and late-phase reaction but also to study the events that occur after that period. In addition, the effect of pretreatment with a topical corticosteroid on unchallenged allergic nasal mucosa was investigated.

In this study inflammatory cells were seen throughout the mucosa in unchallenged asymptomatic patients allergic to grass pollen. Other investigators also found inflammatory cells in unchallenged nasal mucosa in patients with seasonal allergic rhinitis.¹⁷⁻¹⁹ However, the presence of these inflammatory cells is probably not an indication of inflammation because Igarashi et al²⁰ observed abundant inflammatory cells in normal nonallergic mucosa throughout the anterior three quarters of the inferior turbinate. An alternative explanation may be a persistent immunologic activation in the allergic nasal mucosa as a result of air pollution and exposure to bacterial and viral antigens. Julius-

son et al¹¹ found more intraepithelial mast cells in the pre-seasonal nasal mucosa of asymptomatic atopic patients compared with that found in normal nonallergic control subjects, which also may indicate immunologic activation.

In this study we were able to monitor the allergic response in nasal mucosa up to 1 week after a single allergen provocation. In the placebo group eosinophils in lamina propria and epithelial macrophages rose significantly during the whole observation period ($P = .015$ and $P = .003$, respectively). Furthermore, an increasing trend was noted in the number of epithelial CD3⁺ ($P = .1$) and tryptase-positive cells ($P = .07$) in the placebo group. This suggests that one single provocation can trigger the allergic response for several days. Mechanisms responsible for this sustained reaction are probably the released T_H2 cytokines.²¹⁻²³

In agreement with the hypothesis of persistent immunologic activation present in unchallenged nasal mucosa of asymptomatic patients with allergic rhinitis¹¹ is our finding that inflammatory cells decrease in number in nasal epithelium after corticosteroid treatment. This persistent immunologic activation is attenuated by local corticosteroid treatment. Konno et al²⁴ found a significant reduction of eosinophils and basophils in nasal lavage fluid after 2 weeks of fluticasone propionate pretreatment without allergen provocation. However, others found no changes in mast cell and eosinophil numbers after 1- or 2-week topical or systemic corticosteroid pretreatment in nasal mucosal biopsy specimens and nasal washings of unchallenged patients with seasonal allergic rhinitis.^{13,25,26} It is possible that the duration of pretreatment with topical steroids is of importance for the effects on cell numbers in epithelium. We treated the patients for 4 weeks with fluticasone propionate before taking nasal mucosa biopsy specimens, which is in contrast to the method of Lozewicz et al,¹³ who pretreated their patients for 2 weeks, and Bascom et al,²⁵ who pretreated their patients for 1 week with topical corticosteroids. Interestingly, also in patients with nonallergic, noninfectious, perennial rhinitis, a significant decrease in nasal mucosal inflammatory cells after treatment with topical corticosteroids was found.¹⁶ This decrease was more pronounced in nasal epithelium and after a longer treatment period (4 vs 8 weeks).

This pretreatment effect of fluticasone propionate with lowered numbers of several cells hampers interpretation of the changes that occur after allergen challenge. Mast cells disappear from the epithelium after active treatment and remain absent despite allergen provocation, indicating good sensitivity of mast cells to steroid treatment irrespective of mucosal condition. In the placebo-treated group the numbers of epithelial chymase-positive cells and lamina propria tryptase-positive cells increased significantly. Eosinophils already increase in the placebo-pretreated group in unchallenged nasal mucosa, with a further increase after allergen provocation. With only 2 biopsy moments, one baseline before treatment and one after provocation, it is not possible to distinguish between changes in cell counts caused by allergen provo-

cation, treatment, or other stimuli (eg, placebo spray). Epithelial T cells and Langerhans' cells showed a clear response to corticosteroid treatment, with significant decreases after the beginning of the active treatment, even without allergen challenge. In a previous study we have demonstrated in perennial allergic rhinitis that treatment with topical corticosteroids reduces numbers of nasal mucosal Langerhans' cells.¹⁰

The susceptibility of the several inflammatory cells to corticosteroids and the duration of the response to corticosteroids after allergen provocation differ. Mast cell and T-cell numbers decrease and remain low after allergen provocation for 1 week, even after having stopped the active treatment. Langerhans' cells decrease with corticosteroid treatment and remain low despite allergen provocation but return to baseline levels after 1 week. Eosinophils are very sensitive to corticosteroid treatment and were reduced in the nasal mucosa after local corticosteroid treatment during both artificial and natural allergen challenge.^{12,27-29} However, in the present study with a large allergen stimulus, a significant increase in nasal mucosal eosinophils in the following 24 hours was found despite treatment with 400 μ g of fluticasone propionate. This finding suggests a decreased sensitivity of eosinophils to corticosteroids. On the other hand, perhaps the stimulus given with the allergen provocation overrides the effect of corticosteroids on cells.

Twenty-four hours after allergen provocation, patients in either treatment group experienced symptoms, with no difference in clinical symptoms between the 2 treatment groups apart from sneezing, which was lower in the fluticasone propionate group. Sneezing is a result of mast cell degranulation. In the fluticasone propionate group no mast cells were seen in the nasal epithelium 24 hours after provocation, indicating a low to zero histamine release in this group. A very strong correlation was found between the number of lamina propria tryptase-positive cells 2 weeks before allergen provocation and sneezing 12 hours after allergen provocation ($r = 0.96$, $P = .003$), suggesting that the number of mast cells present in nasal mucosa before allergen provocation dictates part of the late-phase response. This is in contrast with the findings of Naclerio et al,³⁰ who demonstrated that basophils, and not mast cells, are partly responsible for the late-phase response.

Despite a 6-week active corticosteroid treatment, symptomatology 24 hours after a single allergen provocation is the same as in the placebo group. These findings suggest that the duration of the effects of the local corticosteroid is shorter than the duration of the allergic response. If this were the case, a second allergen provocation after 24 hours would have resulted in the comparable response in either treatment group. This, however, was not undertaken. A clinical consequence can be the importance of daily use of topical corticosteroids. If the medication is not properly used, symptoms may not be well controlled. This has also been found by Juniper et al³¹ during the grass pollen season in patients with seasonal allergic rhinitis. In their study Juniper et al described that a subgroup of patients using beclomethasone dipropionate on an as-needed basis experienced significantly more symptoms than the patient

group using the medication on a daily basis. Also, on the basis of this study, we cannot substantiate the need to commence with topical corticosteroid treatment 6 weeks before the start of the pollen season.

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