

Original article

Comparison of nasal immunohistology in patients with seasonal rhinoconjunctivitis treated with topical steroids or specific allergen immunotherapy

Background: Specific allergen immunotherapy (SIT) and nasal steroids (NS) are considered effective anti-inflammatory treatments for allergic rhinitis, although their mechanism of action differs.

Objective: The aim of this study was to examine the effect of treatment with NS and SIT on different populations of inflammatory cells in the nasal mucosa and to compare cell numbers before and during the birch pollen season in patients with seasonal allergic rhinitis.

Methods: In a randomized, double-blind, double dummy comparative study, 41 patients with seasonal rhinoconjunctivitis were treated with birch SIT or NS (budesonide 400 µg daily). Treatment with NS started before the birch pollen season and at the same time SIT-treated patients reached the maintenance dose. Nasal biopsies for immunohistochemistry were obtained before the season and start of the treatments and at the peak of the pollen season during treatment.

Results: Symptoms of rhinoconjunctivitis increased significantly in both groups during the pollen season but less in the NS-treated group and the difference between the treatment groups was significant at the end of the season ($P = 0.03$). Immunohistochemistry of nasal biopsies from NS-treated patients showed significantly fewer CD1a⁺, IgE⁺ and FcεRI⁺ cells during the season compared with pre-season ($P = 0.02$, $P = 0.001$ and $P = 0.0004$, respectively) and with seasonal values of the SIT-treated group ($P = 0.002$, $P = 0.002$ and $P = 0.0004$ respectively).

Conclusion: Treatment with NS but not SIT decreased the numbers of CD1a⁺, IgE⁺ and FcεRI⁺ cells during the birch pollen season. Our data indicate that treatment with NS has a broader anti-inflammatory range than SIT.

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Birch pollen is the main cause of Spring hay fever and asthma in Scandinavian countries (1). Most patients suffer mild seasonal symptoms of rhinoconjunctivitis that may be adequately treated with antihistamines systemically or locally because the main symptoms such as a runny nose and sneezing are caused by histamine release from mast cells. In patients with moderate or severe disease, nasal obstruction is the dominating symptom and the effect of antihistamines is not sufficient to alleviate this condition; thus anti-inflammatory treatment is required.

Topical steroids, mostly in combination with antihistamines, are commonly used and have been shown to be an effective treatment for seasonal rhinitis. In addition, the anti-inflammatory effect of steroid treatment has been proven in nasal lavage and biopsy studies (2). The other, evidently effective, treatment for pollen-induced rhinoconjunctivitis is specific allergen immunotherapy (SIT),

which also has the advantage of modifying immunological responses to the pertinent allergen (3, 4). The examination of nasal lavages and skin and nasal biopsies from treated patients revealed reductions in the influx of inflammatory cells, in mediator levels and in the production of proinflammatory cytokines, thus confirming the anti-inflammatory effect of SIT (5).

Only two previous clinical studies have attempted to compare the two treatment methods and their effects on symptoms of rhinitis. However, in neither study was the effect of the treatments on cellular markers of inflammation compared.

We have performed a double-blind trial comparing the effect of SIT and nasal steroid (NS) (budesonide) on clinical symptoms and medication in patients with seasonal rhinitis sensitized to birch pollen (6). Nasal biopsy specimens from participating patients were obtained before the season and the start of the treatment

and at around the peak of the birch pollen season with all patients on allocated treatments. Here, we report on the immunostaining of the biopsy specimens using antibodies against immunoglobulin (Ig)E and the high affinity receptor for IgE, and markers for lymphocytes, mast cells, eosinophils and Langerhans cells.

Patients and methods

Patients

Forty-one birch pollen-allergic patients participated in the study (6). They were recruited from among patients attending the Ear, Nose and Throat (ENT) department at Västerås County Hospital. The study was approved by the Ethics Committee of Uppsala University. The patient group contained 22 men and 19 women with a mean age of 29 years (range: 19–42). The diagnosis was based on a history of rhinoconjunctivitis during birch pollen season, a positive skin prick test (SPT) with birch pollen extract (ALK-Abelló, Hørsholm, Denmark), and the detection of birch pollen-specific serum IgE (CAP-FEIA, Pharmacia Diagnostics, Uppsala, Sweden). Thirty of 41 patients were also sensitized to grasses but birch and grass pollen seasons do not interfere with each other and are well separated. Also 30 of 41 patients were positive for cat dander but only those with no daily contact with animals were included in the study.

Patients with perennial symptoms or those who own the pets were excluded.

Clinical data

Diary cards were filled in daily by each patient for 1 week outside the pollen season, which served as baseline data, and again just before and throughout the birch pollen season (see Design, Fig. 1). Daily symptom scores for eyes and nose from 0 to 3 (0, no symptoms; 1, slight; 2, moderate; 3, severe) were recorded, as was self-administered medication for eye and nose symptoms: decongestant (Otrivin®, number of drops; Novartis Ophthalmics, Copenhagen, Denmark), local antihistamine for eyes and nose Livostin® (levocabastine, number of drops; Novartis Ophthalmics) and antihistamine tablets (Semprex®, number of tablets; GlaxoSmithKline, Mölndal, Sweden). The medication was scored according to the duration of the effect as recommended by manufacturer; 1 point for Semprex, 0.5 point for one drop of decongestant and 1.5 for one dose Livostine.

Design

Inclusion tests were performed during winter. After disease history was obtained, SPT and specific IgE to birch were performed. Patients filled in a diary card during 1 week out of season, which served as baseline. Nasal biopsies were obtained from a randomized nostril. The study had double-blind, double-dummy comparative

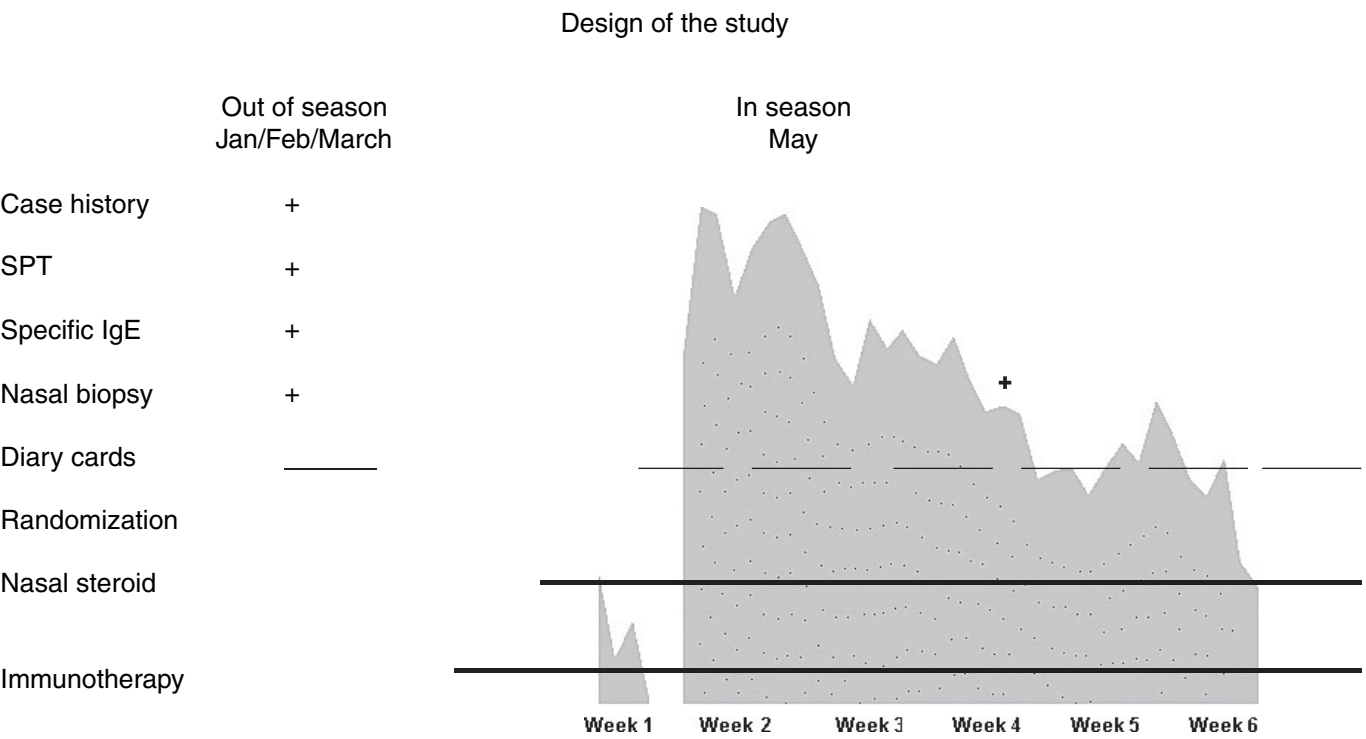


Figure 1. Design of the study. The grey area constitutes the birch pollen season (6 weeks). The first nasal biopsy was obtained during winter, outside the pollen season, once the case history had been confirmed by positive skin prick test (SPT) and the detection of specific birch serum immunoglobulin (IgE). After randomization the specific allergen immunotherapy (SIT) treatment was started and patients reached the maintenance dose before the season. Nasal steroid (NS) treatment started approximately 10 days prior to the expected start of the birch pollen season. The second nasal biopsy was obtained around the peak of the pollen season with treatments ongoing.

design. The patients were randomized to SIT with birch pollen extract Alutard® (ALK-Abelló) or placebo (diluent for Alutard® spiked with histamine dihydrochloride). All reached maintenance dose before the birch pollen season.

Two weeks before the predicted start of birch pollen season the treatment with nasal topical steroids was commenced in such a manner that the actively SIT-treated patients received topical steroid placebo and SIT-placebo-treated received Rhinocort Turbuhaler® 200 µg (Astra, Lund, Sweden) in each nostril once daily in the morning. Treatment with topical steroid continued throughout the entire birch pollen season, i.e. 6 weeks, starting from the middle of April and covering all of May. Around the peak of the season (second week of May) a second nasal biopsy was obtained (Fig. 1).

Skin prick test

The SPT was performed with a panel of highly standardized allergen extracts (Soluprick® SQ; ALK-Abelló). The panel contained the following antigens: birch, hazel, alder, timothy, mugwort, two mites, *Dermatophagoides pteronyssinus* and *D. farinae*, two molds, *Cladosporium herbarium* and *Alternaria alternata*, dog and cat dander. Glycerine solution served as a negative control and histamine dihydrochloride (10 mg/ml) as a positive control.

Immunotherapy

Specific immunotherapy with standardized depot preparations of birch pollen allergen extract (Alutard® SQ; ALK-Abelló) or placebo was performed. The patients reached the maintenance dose over a period of 7 weeks prior to the start of the season. All reached the maintenance dose of 1 ml of 100 000 SQ-U and continued with this dose over a period of 3 years.

Nasal biopsy specimens

Nasal biopsies (approximately 3 mm) were obtained using Gerritsma forceps (7). Local anaesthesia was achieved by inserting cotton swabs soaked in 3% cocaine and 0.025% adrenaline into the nasal cavity for 10 min. Specimens were embedded in Tissue-Tek medium (Miles Inc., Elkhart, IL, USA), placed in gelatine capsules then snap-frozen in chilled isopentane and stored at -70°C until processed.

Immunohistology

Immunohistochemical staining was performed on 6 µm thick acetone-fixed cryostat sections using a three-step monoclonal antibody (moAb) peroxidase-antiperoxidase (PAP) or alkaline phosphatase-antialkaline phosphatase (APAAP) technique. The following panel of mouse moAb was used: Leu-3a (CD4; 'T-helper/inducer cells', activated Langerhans cells, some macrophages, dilution 1 : 256), anti-interleukin (IL)-2 receptor (CD25; activated T-helper cells, 1 : 16), Leu-6 (CD1a; Langerhans cells, 1 : 100) all from Becton Dickinson (Mountain View, CA, USA), EG₂, binding to eosinophil cationic protein and eosinophil protein X (ECP and EPX respectively) staining activated/'secreting' eosinophils (Pharmacia Diagnostics, Uppsala, Sweden, 1 : 80), anti-IgE from Southern Biotechnology Associates (Birmingham, AL, USA; 1 : 1000), 22E7 against the high-affinity Fc-receptor for IgE, FcεRI, which recognizes the receptor whether IgE is bound or not (a gift from Dr R. Chizzonite, Hoffman-LaRoche, NJ, USA, 1 : 1600) (8) and finally an antitryptase antibody binding to mast cells and to a lower degree to basophils (Chemicon, Temecula, CA, USA, 1 : 1250). The PAP technique was used for staining with moAbs against CD4, CD25,

EG₂ and antitryptase and APAAP with moAbs for CD1a, IgE and FcεRI. The sections were counterstained with Mayer's haematoxylin. Examination of the sections was performed under coded conditions. At least two sections per moAb and biopsy specimen were evaluated using a semiquantitative scale from 0 to 3, where 0 = no, 1 = few, 2 = moderate and 3 = many positive cells. The scale was adjusted for each antibody so that score 3 referred to the maximum number of positive cells within all specimens.

Statistics

Daily symptom scores for the eyes and nose were added, and the mean values of daily sums were shown. If any data were missing during a day, whole day was omitted so the weekly mean values were calculated based on complete daily data.

For patient data, symptom scores and medication, weekly mean values were calculated and compared using the Student's *t*-test. The readings of positive cell numbers in biopsy specimens are shown as median values and range of scores or ±SEM and nonparametric statistical analysis was used: the Wilcoxon matched-pair test within the groups and the Mann-Whitney *U*-test for comparison between treatment groups. Correlations were analysed using the Spearman rank order correlation test. STATISTICA software (StatSoft, Tulsa, OK, USA) was used. A *P*-value of <0.05 was considered to be statistically significant.

Results

Clinical data

The patients in the SIT- and the NS-treatment groups were comparable with regards to age [30 (18–41) years mean and range and 29 (21–42) years] and sex (12/nine and seven/13, F/M) respectively. Also co-sensitization to grass and cat was evenly distributed between the groups. All patients reached maintenance dose before the start of the season and those on active treatment received between 120 and 150 µg of birch allergen protein.

Symptoms of rhinoconjunctivitis. Both treatment groups had comparable symptom scores before the birch pollen season 13.4 ± 6.5 (mean and SEM) and 8.3 ± 4.9 in the SIT- and the NS-treated groups respectively. Significant increases in rhinoconjunctivitis symptoms were noted within each of the treatment groups during all weeks of the season in comparison with before-season values ($P = 0.002$). When the symptoms in the two treatment groups were compared, significant differences were found during the 2 last weeks of the season, with less symptoms among the NS-treated patients [24.4 ± 4.6 and 11.4 ± 2 (mean ± SEM)] when compared with the SIT-treated patients (35.3 ± 5 and 21.6 ± 3.6) $P = 0.03$ and $P = 0.04$ at weeks 5 and 6, respectively.

Medication. No significant differences were found between the groups concerning the use of medication for rhinoconjunctivitis, although it increased significantly in both groups during the season compared with the baseline ($P < 0.05$).

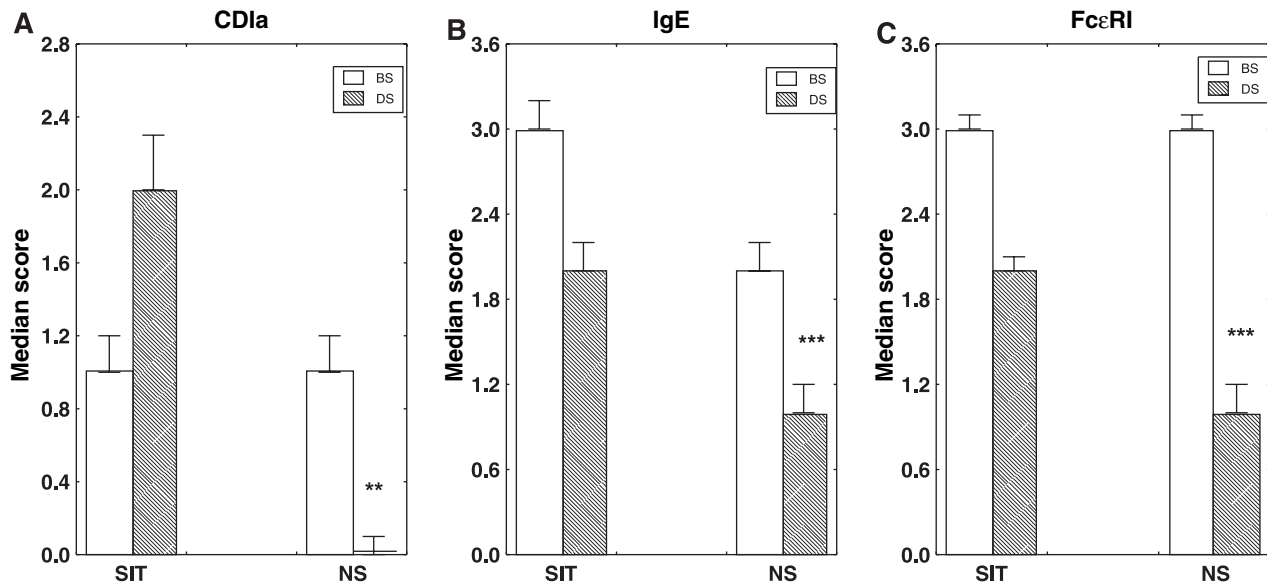


Figure 2. Cell numbers in specific allergen immunotherapy- (SIT) and nasal steroid (NS)-treated patients before (BS, empty bars) and during (DS, filled bars) the pollen season. All values are scores of points in medians and SEM. A significant decrease was found in $CD1a^{+}$ $^{**}P = 0.02$ (A), IgE^{+} $^{***}P = 0.001$ (B), $Fc\epsilon RI^{+}$ $^{***}P = 0.0004$ (C) cells in the NS-treated group during season.

Nasal biopsy specimens

In the cryostat section of nasal mucosa, the scores of $CD1a^{+}$ Langerhans cells did not significantly change in the SIT-treated patients, while in NS-treated patients a significant decrease in abundance of these cells was noted during the season ($P = 0.02$) (Fig. 2A). The difference between the two treatment groups in season was also highly significant ($P = 0.002$) (Table 1).

The scores of IgE^{+} cells did not change in the SIT-treated patients; however, in the NS-treated patients the decrease in scores of IgE^{+} cell numbers during the season was highly significant ($P = 0.001$) (Fig. 2B). A between-group comparison of seasonal values also showed significantly lower IgE^{+} cell scores in NT-treated patients ($P = 0.002$) (Table 1).

Unaltered scores of cells positively stained for $Fc\epsilon RI$ were observed in sections of nasal mucosa from

SIT-treated patients, whereas significantly lower scores were found in sections from seasonal compared with preseasonal biopsies ($P = 0.0004$) of the NS-treated group (Fig. 2C). Comparison between the groups in season showed significantly lower scores of $Fc\epsilon RI^{+}$ cells in nasal mucosa of NS-treated patients ($P = 0.0004$) (Table 1).

In the NS-treated group, a correlation was found between the scores of cells expressing $Fc\epsilon RI$ receptor and IgE ($r = 0.74$, $P = 0.00001$) while such a relationship was much less clear in the SIT-treated group ($r = 0.38$ and $P = 0.02$). During the season, the scores of $CD1a^{+}$ cells correlated positively with the scores of IgE^{+} cells ($r = 0.5$, $P = 0.002$) and slightly with $Fc\epsilon RI^{+}$ cell scores ($r = 0.4$, $P = 0.01$) in all patients.

The scores of $CD4^{+}$ cells were similar in both treatment groups in preseasonal biopsies (Table 1). Compared with preseason during the pollen season a

Table 1. Result of immunohistochemical staining of nasal mucosa

Treatment	Specific allergen immunotherapy (SIT)		Nasal steroid (NS)	
	Before season	During season	Before season	During season
CD1a	1 (0–2) ($n = 16$)	2 (0–3) ($n = 18$)	1 (0–3) ($n = 15$)	0 ^a (0–2) ($n = 20$)
IgE	3 (1–3) ($n = 19$)	2 (0–3) ($n = 20$)	2 (0–3) ($n = 20$)	1 ^b (0–2) ($n = 18$)
FcεRI	3 (1–3) ($n = 19$)	2 (2–3) ($n = 21$)	3 (2–3) ($n = 18$)	1 ^c (0–3) ($n = 20$)
CD4	2 (0–3) ($n = 21$)	1 (0–3) ($n = 21$)	2 (0–3) ($n = 20$)	1 (0–3) ($n = 19$)
CD25	0 (0–3) ($n = 21$)	0 (0–2) ($n = 21$)	1 (0–2) ($n = 20$)	0 (0–2) ($n = 20$)
EG ₂	0 (0–3) ($n = 21$)	0.5 (0–3) ($n = 21$)	0 (0–3) ($n = 20$)	1 (0–3) ($n = 20$)
Tryptase	1 (0–3) ($n = 10$)	1 (0–3) ($n = 8$)	1 (0–3) ($n = 13$)	1 (0–2) ($n = 8$)

All values are scores of points in medians and ranges. In the season, significantly less staining was demonstrated in the NS-treated group compared with the SIT-treated patients for $CD1a^{+}$ (^a $P = 0.002$), IgE^{+} (^b $P = 0.002$) and $Fc\epsilon RI^{+}$ (^c $P = 0.0004$) cells.

tendency to decrease in CD4⁺ cell scores was found in the SIT- ($P = 0.06$) and NS-treated patients ($P = 0.08$). When the two treatment groups were compared at each time-point, no significant differences were found in CD4⁺ cell number. The scores of CD25⁺ cells was unaltered in both groups (Table 1). EG2⁺ cell scores remained unchanged in seasonal compared with preseasonal biopsies of SIT- and NS-treated patients (Table 1). No changes were seen when the two treatment groups were compared with each other.

The scores of cells staining with the moAb against tryptase (characteristic of mast cells) remained unchanged in both groups throughout the study (Table 1).

Discussion

We have shown a significant decrease in the scores of CD1a⁺ Langerhans cells, IgE⁺ and FcεRI⁺ cells in nasal biopsies of NS- but not SIT-treated birch-allergic patients during the pollen season. T cell, eosinophil and mast cell scores were comparable in biopsies obtained from both treatment groups.

We have examined two sets of nasal biopsies obtained from each patient before the season and the start of treatment and during the peak of the season and the course of the treatment. The cells in the epithelium and lamina propria were assessed together using a scoring system that has been used in earlier studies (9). It could be questioned whether a comparison between locally applied steroid and a systemic treatment such as SIT is valid. There are, however, data indicating that the systemic effect of higher levels of circulating steroid inhibits haematopoietic processes in the bone marrow (10), thus affecting inflammatory changes in a distant organ. Local steroids may also exert an indirect effect by interfering at many levels, with the chain of events following antigen-IgE-antibody coupling. The effects of local steroid treatment on a variety of cell types: antigen-presenting cells (11), T cells (12), mast cells (13), eosinophils (14) and the functions such as production of mediators (15) and cytokines (16) in both the upper and lower airways has been well documented.

We observed that in contrast to SIT, treatment with NS effectively decreased the scores of CD1a⁺ Langerhans cells. Langerhans cells are potent specialized antigen-presenting cells in the mucosa of the respiratory tract and in the skin. Exposure to allergen following challenge or in pollen season gives rise in Langerhans cell numbers, while treatment with NS leads to their decrease (17, 18). Inhibition of the production of T cell-derived factors such as IL-4 (19) and granulocyte-macrophage colony-stimulating factor (GM-CSF) (20), necessary for maturation of Langerhans cells from their monocyte precursor, could be one possible mechanism while an inhibitory effect on migration and adhesion of Langerhans cells or

their precursors another (21). The reason for the lack of an observable effect of SIT on Langerhans cell score is not clear, but because of specificity of the treatment, the following inhibition of cytokine production could be just too weak, or the effect of the extremely heavy pollen season during this study (>3000 grains/m³ compared with the usual <1000 grains/m³) overwhelming.

Most IgE-bearing cells are tryptase⁺ mast cells as shown by co-localization studies (22). However, the significant correlation between CD1a⁺ cell scores and IgE⁺ and FcεRI⁺ cell scores found here indicates that it is rather NS-induced alteration in Langerhans cell and not mast cell populations that is behind the changes in IgE⁺ and FcεRI⁺ staining cell scores during the birch pollen season.

Both NS and SIT treatments have been shown to inhibit the seasonal rise in circulating allergen-specific IgE (5, 23). It is possible that the lack of increase in the score of IgE⁺ cells seen in our study is a result of the SIT treatment. However, NS treatment was more effective in suppressing seasonal changes than SIT.

High-affinity IgE receptors originally described on mast cells and basophils has also been demonstrated on Langerhans cells (24) and monocytes (25). We have demonstrated for the first time that local steroid treatment during a pollen season decreases the score of cells expressing the high affinity IgE receptor. The reason for that in the tissue is not clear. In blood, however, decrease in circulating IgE levels has been shown to down-regulate expression of FcεRI⁺ on basophils expression (26). The same mechanism could be valid in the tissue.

Both SIT and NS treatments have been shown to decrease the number of tryptase⁺ (13, 27) and CD4⁺ T cells (12, 28) in nasal biopsies. The clear absence of any decrease in the scores of mast cells and CD4⁺ T cells in our study could be a result of high allergen exposure.

This seems also to account for the lack of decrease in the scores of EG2⁺ cells in both treatment groups in the present study despite numerous earlier studies showing less eosinophils and their granule proteins in NS- and SIT-treated patients (12, 28).

The cellular picture of biopsies may not entirely reflect the difference in clinical effects of the treatments. It should however be kept in mind that the differences in clinical scores were seen late in the season while the biopsies were obtained earlier at the peak of birch pollination. Another reason could be that the short preseasonal SIT treatment was not fully adequate.

In conclusion, we have demonstrated that NS treatment attenuates clinical symptoms of allergic rhinoconjunctivitis in the late phase of the birch pollen season significantly more effectively than SIT. This clinical effect is associated with or caused by changes in the cellular components of inflammation such as the populations of antigen-presenting cells, Langerhans cells, and other cells expressing FcεRI and IgE.

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