

Short-term preseasonal birch pollen allergoid immunotherapy influences symptoms, specific nasal provocation and cytokine levels in nasal secretions, but not peripheral T-cell responses, in patients with allergic rhinitis

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Summary

Background Birch pollen allergic rhinitis can be sufficiently treated with specific subcutaneous allergoid immunotherapy (IT). However, little is known about the clinical and immunological effects of short-term therapy protocols.

Objective To investigate the clinical efficacy of a birch pollen allergoid IT using seven preseasonal injections and to evaluate immunological parameters that might explain clinical findings.

Methods Thirty-seven patients were included into the study and randomized to either a symptomatic treatment or allergoid IT plus symptomatic treatment. Patients were examined during the pre-IT season, at two extraseasonal visits both before and after IT and during the post-IT season. At each visit, nasal secretion samples were taken and analysed for levels of IL-4, IL-5 and IFN γ . In addition, short-term birch-specific T-cell lines (TCLs) were cultured from peripheral blood mononuclear cells of 10 patients of the IT group, both before and after IT, and the ratios of lymphocyte subpopulations were determined. Cytokine production by TCLs (IL-4, IL-5, IFN γ , IL-10) and proliferation of TCLs in response to stimulation with birch pollen allergen were measured.

Results It was possible to evaluate 27 patients in accordance with the study protocol. Clinical symptoms and medication intake were reduced as a result of the IT as were nasal secretion levels of IL-5 ($P=0.007$). IFN γ was increased in nasal secretions ($P=0.01$), while IL-4 was not measurable in most samples. No effect was found on proliferation of birch pollen-reactive TCLs, cytokine production by TCLs and the frequency and ratio of CD4 $^{+}$ and CD8 $^{\text{bright}}$ or CD45RA $^{+}$ and CD45RO $^{+}$ cells in peripheral blood (all $P>0.05$).

Conclusion Preseasonal IT with a birch pollen allergoid is clinically effective in allergic rhinitis and influences cytokine production in the nose, but does not modulate the measured responses of peripheral blood T cells.

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Introduction

Specific immunotherapy (IT) of human allergic disorders as introduced by Freeman and Noon [1] involves injection with

gradually increasing doses of extracts of the material to which the individual is sensitive. IT has been widely used in pollen allergic rhinitis, and the clinical efficacy demonstrated in several controlled clinical trials [2–6].

Various approaches have been undertaken to improve IT, either by modifying the antigens, the route of

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administration, or the dosage and schedule of application. Marsh and associates applied the concept of modified allergens (allergoids) to immunotherapy [3,7]. Allergoids have been described to reduce specific IgE antibody-binding activity by comparison with the native allergen and this is thought to enable a maximum dose to be achieved with a relatively short IT course of seven preseasonal injections at intervals of 1 week leading to effective relief of clinical symptoms of pollen allergic rhinitis [3]. The mechanisms of this treatment regime are at yet unclear. Induction and maintenance of type 1 allergic reactions are mainly dependent on T cells of TH2 type [8], and evidence is accumulating that successful IT targets the T-cell response resulting in a diversion of the allergen-specific responses towards a TH1-type response [9,10].

The functional importance of cytokines generated at sites of allergic reactions has been demonstrated by studies showing that cytokines activate inflammatory cells, induce cell surface adhesion glycoproteins, stimulate the further production and secretion of cytokines and regulate IgE synthesis [11]. The approach of measuring cytokines from the nasal mucosa in nasal fluid is well established by studies examining immune reactions induced by allergen exposure, treatment with corticosteroids or immunotherapy in allergic rhinitis [12–17].

It was of interest to determine whether or not such T-cell responses are influenced by a short preseasonal IT regime. Moreover, we hypothesized that modulation of T-cell responses might be detectable in the peripheral blood as well as in the nasal mucosa at the site of the allergic reaction. In order to detect local changes in the nasal mucosa, IL-4, IL-5 and IFN γ were measured in native nasal secretions (NS). The possible effects on peripheral T cells were assessed by measuring proliferation of birch pollen-specific T-cell lines (TCLs), cytokine production by TCLs, and the ratio of lymphocyte subpopulations. These data were complemented by measuring clinical symptoms, medication intake, and reactivity in the shock organ (titrated nasal provocation test [t-NPT]).

Materials and methods

Study design and ethical considerations

The study was performed as a randomized, controlled, open trial according to good clinical practice for trials on medical products in the European Community (EC-GCP-guidelines). The study protocol and informed consent were in agreement with the declaration of Helsinki in its revised version of Hong Kong. The study protocol was approved by the regional Ethics Committee. Written informed consent was given by all patients prior to the study.

Study population

A total of 37 patients were enrolled in the study and randomized to either a symptomatic drug treatment ($n=18$) or symptomatic drug treatment plus additional preseasonal allergoid IT ($n=19$). The indications for IT were in accordance with the proposals of the EAACI [18].

Females and males aged >18 years, <50 years with typical symptoms of seasonal allergic rhinitis during the birch pollen season, a positive skin prick test with a biologically standardized birch pollen extract (50 000 SBU/mL) (Allergopharma, Reinbek, Germany) (area of the weal response at least as big as that of the positive control, histamine 1 mg/mL), and positive specific IgE to birch pollen > class 2 (CAP-System, Pharmacia, Uppsala, Sweden) were included in the study.

Exclusion criteria were allergic bronchial asthma requiring anti-asthmatic treatment, allergen-specific IT (birch pollen) during the last 3 years, rhinitis of other origins including perennial allergic rhinitis, pregnancy, planned pregnancy or lactation, need for ACE-inhibitors or beta-blockers, chronic diseases such as active tuberculosis, autoimmune diseases, immune deficiency, chronic inflammatory diseases, malignancy, participation in another clinical trial during the preceding 4 months and other reasons that might interfere with the aim of the trial (e.g. insufficient compliance, alcohol abuse, etc.)

Treatments

Patients in the 'IT group' received seven preseasonal injections of a standardized birch-pollen allergoid (Allergovit[®], Allergopharma) (100, 200, 400, 800, 1500, 3000, 6000 therapeutic units [TU]) in addition to symptomatic drug treatment during the season. The birch pollen allergoid is produced from characterized and standardized extracts of *Betula alba*. Allergovit[®] is an aluminium hydroxide adsorbed preparation of the allergoid. Therapeutic units are assigned on the basis of data on tolerance and efficacy from clinical studies. The maximum dose of 6000 TU contained the equivalent of 25 μ g Bet v 1 as measured with a monoclonal antibody two-site binding assay prior to chemical modification of the allergen extract. Deviations from the proposed IT dosage scheme were allowed for medical indications according to the routine procedure of specific immunotherapy [18]. Patients with a modified dosage could be treated by additional injections in order to reach the maximum dose, but all injections had to be applied before the beginning of the birch pollen season.

The recommended drug treatment was identical for both IT and medication groups. The medication was not prescribed to be taken continuously during the season. Both groups received drugs at an on-demand basis according to

their individual complaints. Patients were offered a topical antihistamine (azelastine) nasal spray twice daily if they suffered from mild to moderate sneezing, itching and/or runny nose. If these symptoms were severe they should additionally take terfenadine tablets (60 mg) twice daily. If nasal blockage was the main symptom, patients were offered a topical corticosteroid (budesonide), that could be combined with terfenadine as well. The weekly medication score was calculated as percentage of the recommended standard dosage. The way of handling drug use in this manner according to an individual's symptoms was explained to the patients at the extraseasonal visit (E-pre-IT). At this visit, all patients received identical prescriptions of the aforementioned drugs. The drug regimens were adjusted by the patients as needed and the individual use of drugs was reported in the patients' diaries.

Study schedule

All patients were subjected to the following assessments/investigations in the clinic (Fig. 1):

The prestudy visit included: history, check for inclusion/exclusion criteria, skin prick test, and a general otorhinolaryngological examination including nasal endoscopy.

The intraseasonal visit before IT (S-pre-IT) included: symptom scoring, NS sampling, blood sampling.

The extraseasonal visit before IT (E-pre-IT) (1 week before first injection) included: rhinoscopy, symptom scoring, t-NPT, NS sampling, and blood sampling. After this visit, short-term immunotherapy with the birch pollen allergoid extract was performed in the IT-group.

The extraseasonal visit after IT (E-post-IT) (1 week after last injection) included: rhinoscopy, symptom scoring, t-NPT, NS sampling, blood sampling, check for adverse events. All patients received a diary and a prescription for a standardized antiallergic medication.

The intraseasonal visit after IT (S-post-IT) included: rhinoscopy, NS sampling, blood sampling, symptom scoring and check for adverse events.

Post-study visit: After the study, patients were contacted by telephone and asked to provide a global evaluation of treatment efficacy and drug safety.

Symptom scoring

Patients were required to complete a diary card, that included 92 mm visual analogue scales (VAS) for each of the following symptoms: itching of the eyes, eye redness and tears secretion; and nasal symptoms of sneezing, itching of the nose, nose obstruction and runny nose once daily in the evening. The daily ocular and nasal symptom score was composed from the individual nasal and ocular VAS readings. These symptom scores were added up with the medication scores (see above) and a weekly combined symptom/medication score was composed to describe the magnitude of efficacy.

Titrated intranasal provocation testing (t-NPT)

A specific nasal provocation test was performed according to Gonsior *et al.* [19] with the modification of titrated allergen application. Active anterior rhinomanometry was used as an objective measure of nasal congestion. It was performed using a nasal volume flow (cm^3/s) with a trans-nasal pressure difference of 150 Pascals (V150) as target parameter (Rhinomanometer 200, Atmos Inc., Lenzkirch, Germany). After adaptation (30 min) to the test surrounding, rhinoscopy and rhinomanometry were performed and symptom scores were evaluated to obtain basic values. Then the diluent solution of the commercial allergen extract (Allergopharma) was applied, followed by increasing doses of

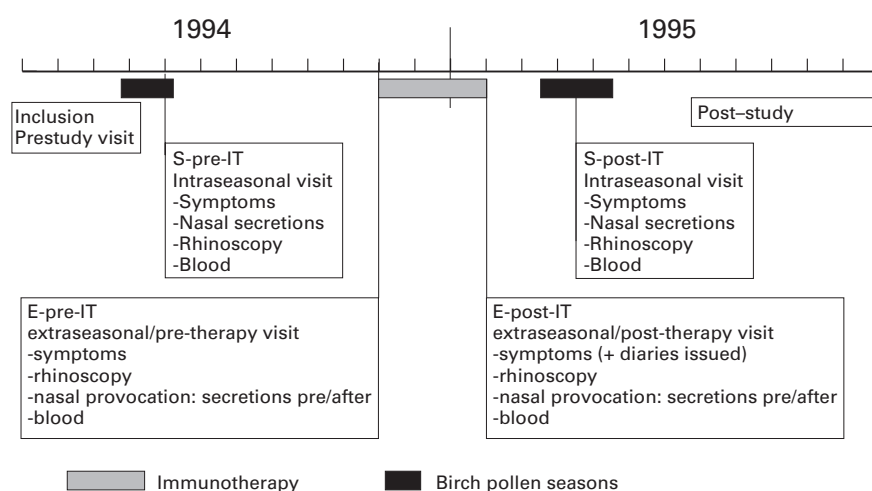


Fig. 1. Study design

allergen solution (5250 SBU/mL, 10 500 SBU/mL, 21 000 SBU/mL, 42 000 SBU/mL, 84 000 SBU/mL, 168 000 SBU/mL) until a test result was judged positive. Two puffs (0.1 mL) of solution were applied directed to the head of the inferior turbinate using a metered pump spray on the side of better nasal airflow. Fifteen minutes after each application, rhinomanometry and symptom score were evaluated. The t-NPT was judged to be positive (according to Gonsior) and discontinued, if the nasal volume flow decreased by 40% compared with the value after application of the control solution, and/or symptom score was >5 out of 11 score points [19]. The t-NPT result was expressed as the concentration of allergen extract producing a positive response.

Nasal secretions sampling

Nasal secretions were collected using absorbent rubber foam samplers by a technique well established for the determination of inflammatory mediators in NS [20]. In short, patients were advised to blow their nose before insertion. Samplers were placed between septum and head of the inferior turbinate on both sides under anterior rhinoscopic control using a bayonet pincer, and were left for 10 min. They were then removed and put into special centrifuge tubes with a sieve placed 1.5 cm above the bottom of the tube. The tubes were centrifuged at 1650 g for 10 min to squeeze out the NS. The clear sol phase was separated from mucus and cells and transferred to an Eppendorf tube. The amount of NS gained was documented for all samples. NS were immediately snap-frozen in liquid nitrogen and stored at -80°C .

IL-4, IL-5 and IFN γ were analysed in NS using commercial ELISA (QuantikineTM and QuantikineTM HS immunoassays) (R & D Systems, Minneapolis, MN, USA) according to the manufacturer's instructions. Results are expressed as means of duplicate measurements. The detection limit was 0.1 pg/mL (IL-4), 3.0 pg/mL (IL-5) and 3.0 pg/mL (IFN γ).

Assessment of adverse events

Potential allergic reactions and other adverse events were documented by the investigator after every injection and on all visits after IT on a separate data sheet. Time of appearance, duration and severity of allergic reactions as well as preventive measures and reversibility were recorded as were other adverse events. Local reactions were differentiated into weak or strong (erythema/swelling at the injection site >5 cm diameter). Systemic allergic reactions were graded according to EAACI guidelines [18], i.e. unspecific symptoms probably not IgE-mediated, e.g. discomfort, headache, arthralgia, etc.

(grade 1); mild reactions including rhinitis and asthma that respond adequately to medication (grade 2); non-life-threatening reactions, e.g. urticaria, angioedema, or severe asthma, which respond well to treatment (grade 3); and anaphylactic shock (grade 4).

Preparation of short-term cultured T-cell lines

Birch pollen-reactive TCLs were established from 10 patients out of the IT group, following the method described previously [21]. Shortly, peripheral blood mononuclear cells (PBMCs) were separated by density gradient centrifugation on Ficoll (Biochrom, Berlin, Germany) and resuspended in RPMI-1640 supplemented with 2 mmol/L L-glutamine, 100 IU penicillin, 100 $\mu\text{g/mL}$ streptomycin, 5×10^{-5} mol/L 2-mercaptoethanol and 5% pooled, heat-inactivated AB⁺ serum (Blood Transfusion Service, Mainz and Bad Kreuznach, Germany) (complete medium). PBMCs ($5 \times 10^5/\text{mL/well}$) were stimulated with 20 $\mu\text{g/mL}$ birch pollen (BP)-extract in 24-well culture plates (Costar, Cambridge, MA, USA) for 5 days. Subsequently, human recombinant IL-2 (20 U/mL, a kind gift by Cetus, Emeryville, CA, USA) was added, and the cells were cultured for an additional 8–10 days.

Proliferation assays

TCLs ($2 \times 10^4/\text{well}$) were cultured in triplicates in 96-well, flat-bottom microtitre plates (Costar) in the presence of irradiated (50 grey) autologous PBMCs ($10^5/\text{well}$), 20 $\mu\text{g/mL}$ BP extract or medium as a control for 48 h at 37°C in a 5% CO₂ humidified incubator. After pulsing for another 18 h with 0.5 $\mu\text{Ci/well}$ [³H]thymidine (Amersham Buchler, Braunschweig, Germany), cells were harvested and the radioactivity incorporated into cellular DNA was measured by liquid scintillation counting. The results are expressed as Δ counts per minute ($\Delta\text{c.p.m.} = \text{c.p.m. in the presence of } 20 \mu\text{g/mL BP extract} - \text{c.p.m. in the presence of medium}$).

Cytokine production by TCLs

TCLs were cultured at $1 \times 10^6/\text{mL}$ complete medium together with irradiated (50 grey) autologous PBMCs ($1 \times 10^6/\text{mL}$) in the presence or absence of 20 $\mu\text{g/mL}$ BP extract for 24 h. Thereafter, cells were spun down and cell-free supernatants were collected and stored at -80°C until used. Supernatants were analysed for IL-4, IL-5, IL-10 and IFN γ content by ELISA (PharMingen, San Diego, CA, USA) according to the manufacturer's instructions. Antibodies were used at 1 $\mu\text{g/mL}$ for coating and detection. The detection limit was 15 pg/mL (IL-4), 15 pg/mL (IL-5), 15 pg/mL (IL-10) and 60 pg/mL (IFN γ).

Flow cytometric determination of surface marker expression

PBMCs were prepared from 10 patients of the IT-group as described. Briefly, 2.5×10^5 PBMCs were incubated in the presence of 5 mg/mL IgG (Polyglobin N, Troponwerke, Cologne, Germany), in PBS/0.1% (w/v) NaN_3 /2% FCS for 10 min on ice, to block non-specific binding of antibodies. Thereafter, surface phenotyping was performed by staining each sample with marker-specific mAb for 30 min on ice. Antibodies used were fluorescein isothiocyanate (FITC)-conjugated anti-CD45RA mAb ALB11 (mouse IgG1, Immunotech, Marseille, France), FITC-conjugated anti-CD45RO mAb UCHL1 (mouse IgG2a, Immunotech), biotinylated anti-CD4 mAb 13B8.2 (mouse IgG1, Immunotech) and biotinylated anti-CD8 α mAb B9.11 (mouse IgG1, Immunotech). After washing, cell-bound biotinylated antibodies were visualized by the addition of Streptavidin-Phycoerythrin (Becton Dickinson, San Jose, CA, USA) diluted 1 : 20 in PBS/0.1% NaN_3 /2% FCS (w/v) for 20 min on ice. After two final washes, cells were fixed with PBS/0.7% paraformaldehyde and analysed using a FACScan (Becton Dickinson) equipped with FACScan Research Software. Ten thousand cells per sample were analysed. For analysis a gate was set on lymphocytes according to

scatter properties. Isotype-matched antibodies were used as a control.

Birch pollen counts

Birch pollen concentrations in air were measured using Burkhard volumetric spore traps (courtesy of the German Pollen Information Service, Bad Lippspringe). Pollen counts were expressed as the mean daily number of pollen grains per cubic meter of filtered air.

Statistics

Statistical evaluations were performed using SPSS[®] statistical software. Statistical analyses were performed using a non-parametric test (Wilcoxon Matched-Pairs Signed Rank test) to search for differences within the groups at the different study visits. The χ^2 -test for categorical variables was used to compare the t-NPT-results in both groups at the different visits. Fisher's exact test was used to search for differences in the allergen concentration producing a positive response in t-NPT at E-pre-IT vs E-post-IT. Spearman's Rank correlation coefficient (r_s) was determined to evaluate the correlation between disease severity (symptom scores)

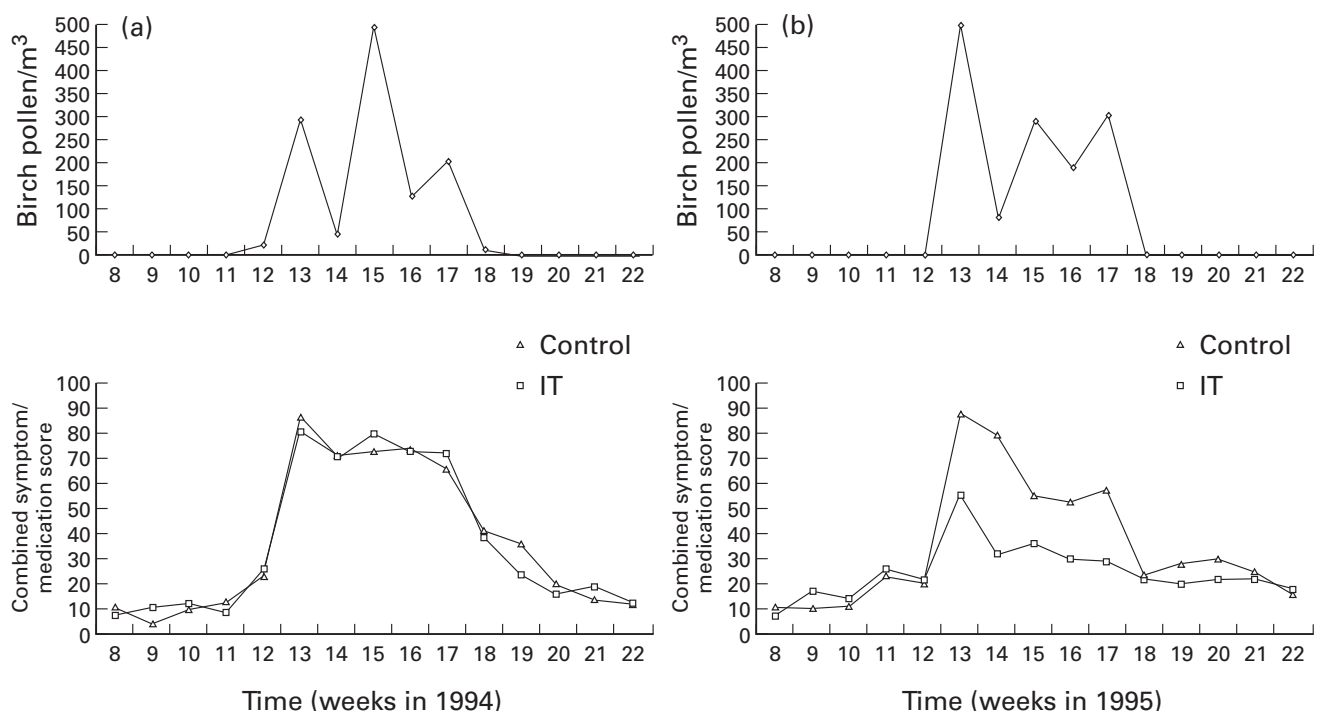


Fig. 2. Birch pollen count and combined symptom/medication score in the pre- (a) and post-IT (b) seasons. Symptoms/medications were significantly lower in the season after IT as compared with the season before IT ($P < 0.01$, AUC) in the IT-group, but not in the controls ($P > 0.05$, AUC).

and nasal cytokine levels. A P -value less than 0.05 was regarded to be statistically significant.

Results

From the 37 patients recruited for the study, 27 patients could be evaluated in accordance with the study protocol. In the control group, six patients were excluded for poor compliance and one patient because of an acute sinusitis, one patient because of a retraction of the informed consent. In the IT group, poor compliance was the reason two patients were excluded. In the IT group, 17 patients (10 females, seven males, mean age 28.6, range 19–48 years) were evaluated according to study protocol, and compared with 10 patients (five females, five males, mean age 25.2, range 18–37 years) in the control group.

Fifteen patients got the entire course of seven injections up to 6000 TU. In one patient, IT was stopped at 3000 TU due to systemic reactions, in one patient IT was stopped at 3000 TU due to beginning birch pollen symptoms.

Birch pollen counts were in the mean range of the middle-European distribution and no differences in allergen exposition were found between both study seasons for the region the patients were living in ($P > 0.05$, AUC) (Fig. 2a and b).

Adverse events

Local reactions at the injection site were observed in 79% of the patients. Systemic reactions occurred following 12% of injections.

Grade 1 reactions were observed in 9% of injections, grade 2 reactions in 3%. No grade 3 or 4 reactions occurred. All patients recovered completely without the need for drug therapy.

Symptom scoring and drug consumption

In the pre-IT season, there was no difference in the combined symptom/medication scores between both groups (Fig. 2a) ($P \geq 0.05$, AUC). However, there was a significantly lower total symptom score in the IT-group if compared with the control group in the post-IT-season (Fig. 2b) ($P \leq 0.0001$, AUC).

In the control group, there was no difference in total symptom scores between pre- and post-IT seasons ($P \geq 0.05$ AUC). In the IT-group, symptoms were significantly lower in the post-IT-season when compared with the pre-IT-season ($P \leq 0.01$, AUC) (Fig. 2a and b).

Titrated intranasal provocation test (t-NPT)

There was no difference in nasal reactivity to birch pollen between either groups at the extraseasonal visit before IT

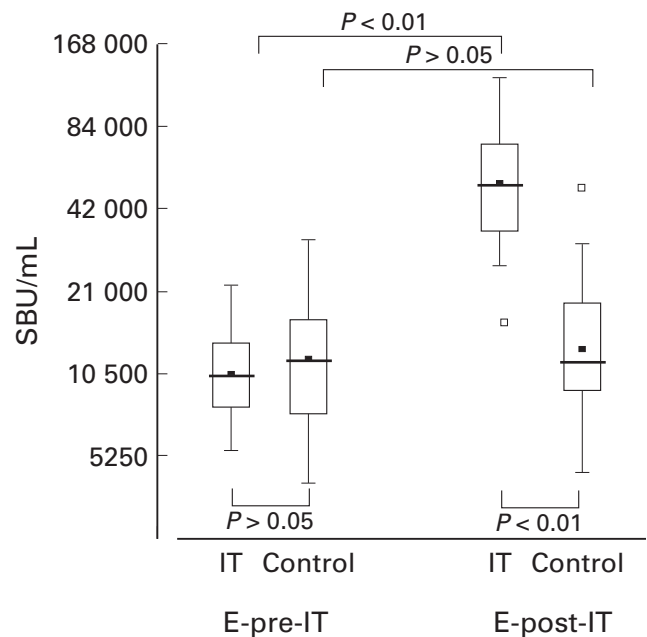


Fig. 3. Results of the titrated intranasal allergen provocation with birch allergen. There was no difference in nasal reactivity between both groups at the extraseasonal visit before IT (E-pre-IT), but a significant difference after IT (E-post-IT). Moreover, the concentration required to provoke a positive response in the IT-group was significantly higher at E-post-IT than at E-pre-IT, while there were no such changes in the control group (P -values after χ^2 -test for inter-group and Wilcoxon test for intragroup comparisons).

(E-pre-IT: Fig. 3) ($P > 0.05$, χ^2 -test). However, a significantly higher concentration of allergen extract was necessary in the IT-group compared with the control group to elicit a positive response in t-NPT at the extraseasonal visit after IT (E-post-IT) ($P < 0.01$, χ^2 -test). Additionally, the concentration required to provoke a positive response in the IT-group was significantly higher at E-post-IT than at E-pre-IT. ($P < 0.01$, Wilcoxon). There were no such changes in the control group ($P > 0.05$, Wilcoxon).

Cytokine levels in nasal secretions

IL-4 was not detectable in the majority of NS samples. As shown in Fig. 4, NS levels of IL-5 were significantly decreased in S-post-IT (median: 22.7 pg/mL) when compared with S-pre-IT (79.8 pg/mL) in the IT group ($P = 0.007$, Wilcoxon), but not in the control group (73.1 vs 74.2 pg/mL) ($P = 0.789$, Wilcoxon).

Levels of IFN γ in NS significantly increased in S-post-IT (17.2 pg/mL) in comparison with S-pre-IT (7.3 pg/mL) in the IT group ($P = 0.01$, Wilcoxon), while no augmentation was observed in the control group (11.95 vs 5.45 pg/mL) ($P = 0.374$, Wilcoxon).

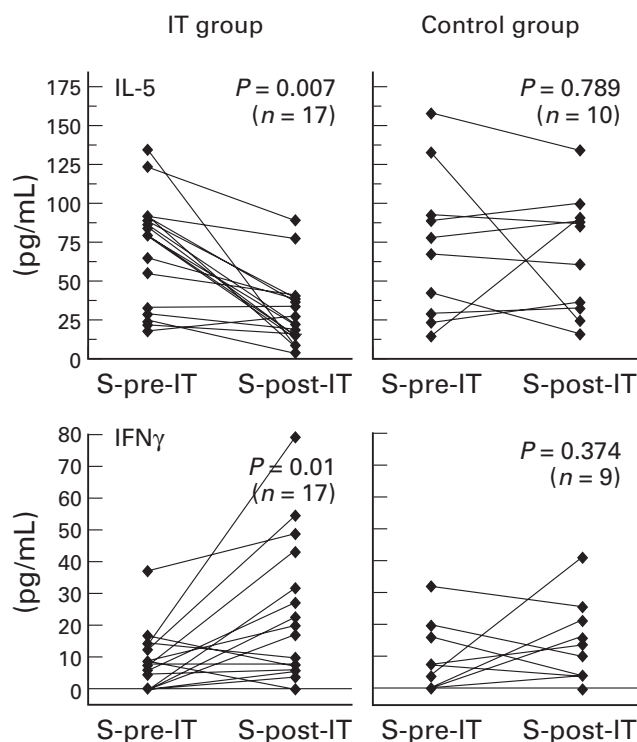


Fig. 4. Decrease in IL-5 levels and increase in IFN γ levels in nasal secretions following IT. Nasal secretions were collected during the pre-IT season (S-pre-IT) and the post-IT season (S-post-IT) as described in the Methods section. Levels of IL-5 (upper panels) and IFN γ (lower panels) were determined by ELISA (P -values after Wilcoxon).

The change in IL-5 between S-pre-IT and S-post-IT was well correlated with the change in the combined symptom/medication scores between both seasons ($r_s = 0.77 \pm 0.18$; $P \leq 0.05$, Spearman-Rank), and a similar, but inverse correlation was seen for IFN γ ($r_s = -0.61 \pm 0.27$; $P \leq 0.05$, Spearman-Rank).

T-cell proliferation and cytokine production

There was no significant effect of IT treatment on the allergen-induced proliferation of TCLs of 10 selected patients of the IT group (Δ c.p.m. pre-IT [E-pre-IT]: 24491 ± 27580 vs Δ c.p.m. post-IT [E-post-IT]: 15984 ± 17523 , $P = 0.203$, Wilcoxon).

Birch pollen extract-induced cytokine production (IL-4, IL-5, IL-10, IFN γ) by TCLs established at E-post-IT vs E-pre-IT in the IT group was comparable (IL-4: 27.6 ± 51.5 pg/mL vs 43.6 ± 75.8 pg/mL, $P = 0.779$, Wilcoxon; IL-5: 975.8 ± 1512.3 pg/mL vs 692.4 ± 976.8 pg/mL, $P = 0.753$, Wilcoxon; IL-10: 79.2 ± 202.2 pg/mL vs 40.2 ± 73.2 pg/mL, $P = 0.715$, Wilcoxon; IFN γ : 251.1 ± 706.5 pg/mL vs 510.1 ± 731.7 pg/mL, $P = 0.463$, Wilcoxon).

Surface marker expression

The frequency of CD4 $^{+}$ and CD8 $^{\text{bright}}$ cells (both $P > 0.05$, Wilcoxon), as well as the ratio of CD4 $^{+}$ /CD8 $^{\text{bright}}$ cells (2.88 ± 1.74 at E-post-IT vs 2.74 ± 1.45 at E-pre-IT, $P = 0.722$, Wilcoxon) was not influenced by IT. The frequency of naive (CD45RA $^{+}$) and memory (CD45RO $^{+}$) T cells within the CD4 $^{+}$ T-cell population did not change between E-pre-IT and E-post-IT, neither did the ratio of CD45RA $^{+}$ /CD45RO $^{+}$ (1.71 ± 0.87 at E-post-IT vs 1.75 ± 0.91 at E-pre-IT, $P = 0.790$, Wilcoxon).

Discussion

Immunotherapy (IT) represents the only specific treatment that can be offered to allergic patients besides allergen avoidance. The underlying mechanism of this treatment is still far from being understood. Previous studies have focused on changes in serum antibodies, including blunting of seasonal rises in specific IgE and increase in specific IgG antibodies, especially of the IgG4 isotype. Recent studies suggest that IT influence the response of T lymphocytes with a shift away from a TH2 response (IL-4, IL-5) towards a TH1 response (IFN γ) [9,10,22]. A shift in the cytokine profile can result in inhibition of IL-4 dependent IgE production, reinforced decreased autocrine effect [8] and of IL-5-dependent eosinophilic inflammation [10].

In the present study, we focused on evaluating the effects of a preseasonal birch pollen allergoid therapy protocol on T cells. The effects on the local immune reaction in the nose are most important from the clinical point of view in patients with allergic rhinitis, because these effects may be expected to have a direct relation to symptoms. Both B and T cells have been found in nasal lavage fluid [23–25], but our initial investigations yielded insufficient numbers of T cells to enable us to perform FACScan analyses or to culture TCLs using nasal lavage or curettage techniques. Thus we decided to analyse local immune reactions by measuring distinct cytokines (IL-4, IL-5 and IFN γ) in NS to evaluate potential effects of IT.

We found a significant decrease of IL-5 in NS after IT when compared with the pre-IT season. This finding is of major relevance, since IL-5 promotes eosinophil differentiation and prolongs survival *in vitro*, whilst also potentiating mediator release and inducing eosinophil activation [26]. IL-5 also enhances IL-4-induced IgE synthesis [27]. A positive correlation has been found between the number of T cells containing mRNA for IL-5 and the number of activated eosinophils in human bronchial mucosa [28]. The *in vivo* application of recombinant human IL-5 induces eosinophilic infiltration and mediator release in the nasal mucosa [29].

IFN γ increased significantly in NS of patients of the IT group when measured during the pollen season following IT

compared with the pre-IT season. The changes in the level of the two cytokines provide evidence for a shift from a more pronounced TH2 response to a predominant TH1 response.

These findings were accompanied by a significant decrease in symptoms and use of anti-allergic drugs under natural pollen exposure and an increase in the threshold dose necessary to induce a positive response in the applied nasal provocation model.

Although IL-4 is an essential proinflammatory cytokine of allergic reactions *in vitro*, NS recovered from most subjects did not contain this cytokine at detectable levels. This finding is similar to results of studies using BAL fluids [30], NS recovered with filter paper strips after allergen challenge [12] and nasal lavage fluid after allergen challenge [17]. However, the absence of detectable IL-4 in NS does not exclude local production of this cytokine. Possible reasons for failing to detect IL-4 in NS may be: (1) insufficient sensitivity of the cytokine assay; (2) binding of IL-4 to its receptor, precluding its recovery in NS; (3) degradation of IL-4 by proteolytic enzymes; and (4) adherence of this protein to container or sampler surfaces.

In addition to measuring cytokines at the site of the allergic inflammatory reaction, TCLs were grown by short-term stimulation of PBMCs with birch pollen extract to obtain a test cell population comprising an allergen-specific T-cell repertoire most similar to the *in vivo* situation. Using this system we found no significant effects of the allergoid IT on TCL proliferation. Additionally, TCLs showed no significant changes in BP extract-induced cytokine production (IL-4, IL-5, IL-10 and IFN γ). The differences between the local and peripheral measurements might be explained by the fact that in the nasal mucosa a stronger pre-activation of the inflammatory cells might have occurred as part of the allergic nasal reaction as compared with peripheral blood T cells.

Similar findings of a possible local immunomodulation of T-cell responses can be observed when the IL-5 production of *Phleum pratense*-specific PBMCs is correlated with cutaneous late-phase responses [31].

It is not possible to identify the sources of the cytokines recovered in the nasal secretions. Although TH2-type T cells are the principal source of IL-4 and IL-5, basophils and mast cells are also potential sources of the cytokines [13,32]. Nasal mucosal biopsies taken from patients with seasonal allergic rhinitis during natural allergen exposure [33] revealed immunoreactive IL-4 in association with mast cells, whilst IL-5 was found in both mast cells and eosinophils. Thus, the changes in local IL-5 production measured in this study may be in part related to influences of IT on effector cells such as mast cells and eosinophils in addition to T cells. This is in congruence with immunological changes that have been found after successful IT providing

evidence that IT influences pathophysiological mechanisms responsible for mediator release and subsequent inflammatory changes in allergic diseases [9,22,34–40]. Mast cells and eosinophils may respond early during IT whilst the influence on T cells might manifest itself somewhat later. The increase in IFN γ in NS under natural pollen exposure is evidence of the modulatory effect of IT on T cells because IFN γ is produced solely by T cells and NK cells [41].

We did not observe an effect of short-term allergoid IT on the ratio of CD4⁺/CD8^{bright} cells within the lymphocyte population in peripheral blood. With the anti-CD8 α antibody employed, CD8^{bright} cells represent T cells, while CD8^{dull} cells represent NK cells (data not shown). Assessment of the influence of IT on this parameter was based on findings reported before, that in patients with bronchial asthma a positive correlation existed between the symptom score and the CD4/CD8 ratio during season, suggesting a reduced frequency of CD8⁺ T-suppressor cells and/or an increased frequency of CD4⁺ T-helper cells [42]. Our results suggest, that within the short period of allergoid IT no change in the frequency of these T-cell populations had occurred. We also assessed the influence of IT on the frequency of CD45RA⁺ and CD45RO⁺ cells. Patients with atopic dermatitis and bronchial asthma were reported to have an augmented ratio of CD4⁺CD45RA⁺/CD4⁺CD45RO⁺ cells [43]. Again, short-term allergoid IT did not modulate the frequency of the two cell populations.

In conclusion, a short-term preseasonal IT with birch pollen allergoid (Allergovit[®]) is able to reduce symptoms, drug use and reactivity in a nasal provocation model in association with decreased local IL-5 production and increased levels of IFN γ in patients with allergic rhinitis.

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References

- 1 Freeman J, Noon L. Further observations on the treatment of hayfever by hypodermic inoculations of pollen vaccine. *Lancet* 1911 814–7.
- 2 Brunet C, Bedard PM, Lavoie A, Jobin M, Herbert J. Allergic rhinitis to ragweed pollen. I. Reassessment of the effects of immunotherapy on cellular and humoral responses. *J Allergy Clin Immunol* 1992; 89:76–86.
- 3 Maasch HJ, Marsh DG. Standardized extracts. Modified allergens. *Allergoids Clin Rev Allergy* 1987; 5:89–106.
- 4 sterballe O. Immunotherapy with grass pollen major allergens. *Allergy* 1982; 37:379–88.

- 5 Varney V, Gaga M, Frew AJ, Aber VR, Kay AB, Durham SR. Usefulness of immunotherapy in patients with severe summer hayfever uncontrolled by antiallergic drugs. *Br Med J* 1991; 302:265–9.
- 6 Walker SM, Varney VA, Gaga M, Jacobson M, Durham SR. Grass pollen immunotherapy: efficacy and safety during a 4-year follow-up study. *Allergy* 1995; 50:405–13.
- 7 Marsh DG, Lichtenstein LM, Campbell DH. Studies on 'allergoids' prepared from naturally occurring allergens. 1. Assay of allergenicity and antigenicity of formalinized rye group 1 component. *Immunology* 1970; 18:705–22.
- 8 Romagnani S. Regulation of the development of type 2 T-helper cells in allergy. *Curr Opin Immunol* 1994; 6:838–43.
- 9 Varney VA, Hamid QA, Gaga M et al. Influence of grass pollen immunotherapy on cellular infiltration and cytokine mRNA expression during allergen-induced late-phase cutaneous responses. *J Clin Invest* 1993; 92:644–51.
- 10 Durham SR, Ying S, Varney VA et al. Grass pollen immunotherapy inhibits allergen-induced infiltration of CD4⁺ T lymphocytes and eosinophils in the nasal mucosa and increases the number of cells expressing messenger RNA for interferon-gamma. *J Allergy Clin Immunol* 1996; 97:1356–65.
- 11 Barnes PJ. Pathophysiology of allergic inflammation. In: Middleton E Jr, Reed CE, Ellis EF, Adkinson NF Jr, Yunginger JW, eds. *Allergy Principles and Practice*, 3rd edn. St Louis: Mosby, 1991:243–66.
- 12 Sim TC, Grant JA, Hilsmeier KA, Fukuda Y, Alam R. Proinflammatory cytokines in nasal secretions of allergic subjects after antigen challenge. *Am J Respir Crit Care Med* 1994; 149:339–44.
- 13 Bachert C, Wagenmann M, Hauser U. Proinflammatory cytokines: measurement in nasal secretion and induction of adhesion receptor expression. *Int Arch Allergy Immunol* 1995; 107:106–8.
- 14 Howarth PH, Bradding P, Feather I, Wilson S, Church MK, Holgate ST. Mucosal cytokine expression in allergic rhinitis. *Int Arch Allergy Immunol* 1995; 107:390–1.
- 15 Durham SR, Kay AB, Hamid QA. Changes in allergic inflammation associated with successful immunotherapy. *Int Arch Allergy Immunol* 1995; 107:282–4.
- 16 Garred IM, De-Graaf-in-'t-Velt T, Nahori MA, Vargaftig BB, Wijk van RG, Zijlstra FJ. Interleukin-5 and eosinophil cationic protein in nasal lavages of rhinitis patients. *Eur J Pharmacol* 1995; 275:295–300.
- 17 Linden M, Greiff L, Andersson M et al. Nasal cytokines in common cold and allergic rhinitis. *Clin Exp Allergy* 1995; 25:166–72.
- 18 Malling HJ, Weeke B. Position Paper Immunotherapy of the EAACI. *Allergy* 1993; 48:9–35 (and appendices).
- 19 Gonsior EC, Bachert C, Berdel D et al. Richtlinien für die Durchführung von nasalen Provokationstests mit Allergenen bei Erkrankungen der oberen Luftwege. Arbeitskreis 'Bronchiale und nasale Provokationstests' der Deutschen Gesellschaft für Allergie- und Immunitätsforschung. *Allergologie* 1990; 13:53–5.
- 20 Klimek L, Riechelmann H. Zellaktivierungsmarker bei Rhinitis allergica. In: Mösges R, Schlöndorff G, eds. *Die Folgen der Allergie – Rhinitis, Asthma, Polyposis Nasi*. Zülrich: Biermann-Thieme-Verlag, 1996:52–9.
- 21 Dormann D, Montermann E, Klimek L et al. Heterogeneity in the polyclonal T cell response to birch pollen allergens. *Int Arch Allergy Immunol* 1997; 114:272–7.
- 22 Iliopoulos O, Proud D, Adkinson NF Jr et al. The effects of immunotherapy on the early, late, and rechallenge nasal reaction to provocation with allergen: changes in inflammatory mediators and cells. *J Allergy Clin Immunol* 1991; 87:855–66.
- 23 Zurcher AW, Derer T, Lang AB, Stadler BM. Culture and IgE synthesis of nasal B cells. *Int Arch Allergy Immunol* 1996; 111:77–82.
- 24 Diaz-Sanchez D, Tsien A, Casillas A, Dotson AR, Saxon A. Enhanced nasal cytokine production in human beings after in vivo challenge with diesel exhaust particles. *J Allergy Clin Immunol* 1996; 98:114–23.
- 25 Pawankar RU, Okuda M, Suzuki K, Okumura K, Ra C. Phenotypic and molecular characteristics of nasal mucosa gamma delta T cells in allergic and infectious rhinitis. *Am J Respir Crit Care Med* 1996; 153:1655–65.
- 26 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; 157:219–25.
- 27 Pene J, Rousset F, Briere F et al. Interleukin-5 enhances interleukin-4-induced IgE production by normal human B cells. The role of soluble CD 23 antigen. *Eur J Immunol* 1988; 18:929–35.
- 28 Bentley AM, Meng Q, Robinson DS, Hamid QA, Kay AB, Durham SR. Increases in activated T lymphocytes, eosinophils, and cytokine mRNA expression for interleukin-5 and granulocyte/macrophage colony-stimulating factor in bronchial biopsies after allergen inhalation challenge in atopic asthmatics. *Am J Respir Cell Mol Biol* 1993; 8:35–42.
- 29 Terada N, Konno A, Tada H, Shirotori K, Ishikawa K, Togawa K. The effect of recombinant human interleukin-5 on eosinophil accumulation and degranulation in human nasal mucosa. *J Allergy Clin Immunol* 1992; 90:160–8.
- 30 Broide DH, Lotz M, Cuomo AJ, Coburn DA, Federman EC, Wasserman SI. Cytokines in symptomatic asthma airways. *J Allergy Clin Immunol* 1992; 89:958–67.
- 31 Till S, Walker S, Dickason R et al. IL-5 production by allergen-stimulated T cells following grass pollen immunotherapy for seasonal allergic rhinitis. *Clin Exp Immunol* 1997; 110:114–21.
- 32 Arok M, Merle-Beral H, Dugas B et al. IL-4 release by human leukemic and activated normal basophils. *J Immunol* 1993; 151:1441–7.
- 33 Desreumaux P, Janin A, Dubucquoi S et al. Synthesis of interleukin-5 by activated eosinophils in patients with eosinophilic heart disease. *Blood* 1993; 82:1553–60.
- 34 Norman PS. Immunotherapy for nasal allergy (symposium). *J Allergy Clin Immunol* 1988; 81:992–6.
- 35 Durham SR. New insights into mechanisms of immunotherapy. *Europ Arch Oto-Rhino-Laryngol* 1995; 252:64–7.
- 36 Rak S, Hakansson L, Venge P. Immunotherapy abrogates the generation of eosinophil and neutrophil chemotactic activity during pollen season. *J Allergy Clin Immunol* 1990; 86:706–13.

- 37 Rak S, Löwenhagen O, Venge P. The effect of immunotherapy on bronchial hyperresponsiveness and eosinophil cationic protein in pollen-allergic patients. *J Allergy Clin Immunol* 1988; 82:470–80.
- 38 Rak S, Björnson A, Hakansson L, Sörenson S, Venge P. The effect of immunotherapy on eosinophil accumulation and production of eosinophil chemotactic activity in the lung of subjects with asthma during natural pollen exposure. *J Allergy Clin Immunol* 1991; 88:878–88.
- 39 Otsuka H, Mezawa A, Onishi M, Okubo K, Seki H, Okuda M. Changes in nasal metachromatic cells during allergen immunotherapy. *Clin Exp Allergy* 1991; 21:115–20.
- 40 Klimek L, Riechelmann H, Prellwitz W. Einfluss einer Hypo-sensibilisierung auf Tryptase und ECP in Nasensekret und Blutserum bei Birkenpollenallergikern. *HNO Informationen* 1995; 20:131.
- 41 Farrar MA, Schreiber RD. The molecular cell biology of interferon-gamma and its receptor. *Annu Rev Immunol* 1993; 11:571–611.
- 42 Monteseirin J, Guardia P, Delgado J, Llamas E, Palma J, Conde A, Conde J. Peripheral blood T lymphocytes in seasonal bronchial asthma. *Allergy* 1995; 50:152–6.
- 43 Nasert S, Burtchen N, Kussebi F et al. Stimulation of IgE and IgA Production by CD45RA T Helper Cells in Atopic Patients. *J Immunol* 1996; 157:441–8.