

ORIGINAL RESEARCH

The Effects of Short-Term Immunotherapy Using Molecular Standardized Grass and Rye Allergens Compared With Symptomatic Drug Treatment on Rhinoconjunctivitis Symptoms, Skin Sensitivity, and Specific Nasal Reactivity

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BACKGROUND: The efficacy and safety of short-term immunotherapy with molecular standardized allergens (STI) has been demonstrated by double-blind placebo-controlled clinical trials. The aim of this study was to compare STI with symptomatic drug treatment.

METHODS: Forty-eight patients with rhinoconjunctivitis to grass and/or rye pollen were treated either with STI (ALK⁷, n = 24) plus anti-allergic drugs or anti-allergic drugs, alone (n = 24) in a prospective, randomized study. Symptoms and use of drugs were reported in patient diaries and titrated nasal provocation and skin prick tests were performed at baseline, before, and after season.

RESULTS: Median overall symptom ($P = 0.022$, U test) and medication scores ($P = 0.003$) were significantly lower in the STI group, as was the result for a simultaneous analysis of conjunctival, nasal, and bronchial symptom scores and medication ($P = 0.005$). Sensitivity in the nasal provocation test decreased in the STI group but not in the drug-treated group. These differences became significant directly after STI ($P = 0.027$) as well as after the grass pollen season ($P < 0.001$). Skin sensitivity did not change in the STI group but increased in the drug-treated group after season, with a significant difference between the two groups for the erythema ($P < 0.001$).

CONCLUSIONS: STI reduces grass pollen–induced rhinoconjunctivitis symptoms and drug use, and specific nasal reactivity and skin sensitivity, more efficiently than a standard symptomatic treatment.

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Short-term immunotherapy (STI) is a new immunotherapy protocol that consists of 7 preseasonal injections of molecular standardized allergens over a period of 6 weeks. STI has been shown to be efficacious and safe in two double-blind placebo-controlled multicenter studies with grass pollen and tree pollen allergic patients.^{1,2} In these studies, patients treated with STI had significantly lower nasal and overall symptoms compared with placebo and patients with considerable pollen exposure used significantly less amounts of topical nasal drugs. Skin sensitivity was reduced after STI for patients who received the maximum dose. Specific IgE and IgG4 were significantly increased after STI compared with placebo.¹ Additionally, we were able to demonstrate an effect of STI on eosinophil cationic protein and tryptase in nasal secretions before and after titrated nasal provocation test (t-NPT).³

The intention of a prophylactic treatment with a course of 7 preseasonal injections is to prevent the patient from developing a high level of symptoms in the following pollen season and to reduce the need for symptomatic drugs to control the residual symptoms. Additionally, the sensitivity of the target organ and the inflammatory status of the nasal mucosa should be affected in rhinoconjunctivitis patients.

The nonprophylactic alternative is to control the symp-

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toms during exposure to allergen by symptomatic drug treatment alone. It was the aim of our study to compare these two treatment strategies regarding allergen-specific reactivity of the nasal mucosa measured by titrated nasal provocation test (t-NPT) and of the skin by titrated skin prick test (t-SPT).

MATERIAL AND METHODS

Patients

Forty-eight patients in the age range of 15 to 50 years, allergic to grass and/or rye pollen with typical rhinoconjunctivitis symptoms during the grass pollen season, were enrolled into the study. The symptoms were confirmed by a wheal size ≥ 5 mm in skin prick test (SPT). Patients with a need for treatment of allergic asthma, perennial rhinitis, or acute or chronic infected nasal mucosa; patients on systemic glucocorticosteroids; and patients treated by specific immunotherapy in the past 3 years were excluded. Other exclusion criteria were the known contraindications of immunotherapy according to the European Academy of Allergology and Clinical Immunology (EAACI) Immunotherapy Position Paper.⁴

The study was performed according to EC-GCP guidelines⁵ and the Declaration of Helsinki.⁶ The study was approved by the ethical committee of Landesärztekammer Rheinland-Pfalz, Mainz, Germany.

Allergen Extracts

The grass and rye allergen extracts used for short-term immunotherapy were partially purified and standardized extracts⁷ composed of equal parts of the 6 grasses and rye: *Dactylis glomerata*, *Lolium perenne*, *Avena elatior*, *Phleum pratense*, *Poa pratensis*, *Festuca pratensis*, and *Secale cereale*.

Extracts for short-term immunotherapy, SPT, t-NPT, and t-SPT were manufactured by ALK A/S, Horsholm, Denmark.

The allergen extracts are standardized relative to an internal standard by qualitative and quantitative immunoelectrophoresis for characterization and quantitation of the major allergens, luminescence immunoassay (LIA) inhibition for total allergenic activity, and the biologic activity of the extracts used was determined by t-SPT, as previously described.^{8,9} The potency was expressed in arbitrary SE units (specific units); 1000 SE contains ≈ 1.5 μ g (between 1.0 and 2.0 μ g for individual grasses) grass major allergen.

For STI, a depot extract adsorbed to aluminium hydroxide (0.5% phenol) was used (ALK⁷, Gräsermischung und Roggen, ALK-SCHERAX, Hamburg, Germany), and an identically manufactured, partly purified extract of equal components of the 6 grasses without rye was used for t-SPT (ALK-prick SQ/soluprick SQ, 10 HEP [histamine equivalent in skin prick test], 10,000 BU [biological units]/mL, dissolved in 50% glycerol) and for t-NPT (Aquagen SQ/

ALK-lyophilisiert SQ, 10-100,000 SQ units/mL). STI was applied out of 2 vials with the concentrations 30 and 1000 SE/mL. All extracts were stored at 4°C until use; the stability assigned by the manufacturer was much longer than the total duration of the study.

Short-Term Immunotherapy

Patients treated by STI received 7 injections in weekly intervals before the expected beginning of the grass pollen season with ascending concentrations (3, 9, 30, 100, 300, 600, 1000 SE). Dose modifications were allowed for medical indications according to the routine procedure of specific immunotherapy.⁴ 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 grass pollen exposure.

Symptomatic Treatment

For patients in both groups, disodium cromoglycate (DSCG) eyedrops and nasal sprays, local or systemic antihistamines, nasal decongestants, and local glucocorticosteroids were prescribed according to the stepwise approach recommended by the International Consensus Report on the Diagnosis and Management of Rhinitis;¹⁰ simultaneous use of different drug types was allowed. Drugs were given on an on-demand basis, so that patients were able to adjust the drug intake according to their individual needs after thorough explanation.

Study Design

Patients were randomly assigned either to STI or to symptomatic treatment with a block size of 4 (2 STI-treated and 2 drug-treated patients). Four visits (T0, T1, T2, and T3) were scheduled: before (T0) and after (T1) STI, and during (T2) and after season (T3). Patients either were preseasonally treated with STI and treated by drugs as needed during grass pollen season (STI group) or were exclusively treated by drugs as needed during grass pollen season (drug-treated group). During the initial investigation (T0), the duration and severity of symptoms of the eyes, nose, and bronchi in the pretreatment year (scored 0-3, based on memory data), the general health status, and a sensitization to other aeroallergens by SPT were recorded by the investigator. Baseline values for nasal and skin reactivity were determined by t-SPT and t-NPT and specific antibodies were measured. The determinations were repeated during the following control investigations directly after STI (T1) and after season (T3), respectively. No provocation tests were performed during the season (T2). Symptoms and use of symptomatic drugs were recorded by the patient in diaries during the grass pollen season.

Data Analysis

As clinical parameters, severity scores 0-3 of each single symptom of the eyes (itching, running, reddening), nose

(congestion, itching, rhinorrhea, sneezing), and bronchi (0 = no, 1 = mild, 2 = moderate, 3 = severe symptoms) were evaluated. The daily symptom scores were cumulated over 1 week. The weekly score range, therefore, was 0-21 score points for each individual symptom. For further analysis, a weekly nasal score (range, 0-84), a conjunctival score (range, 0-63), and a bronchial score (range, 0-21) were generated.

Symptomatic drugs were scored separately for the different drug categories: antihistamines, corticosteroids, and DSCG/sympathomimetics. DSCG/sympathomimetics include α - and β -sympathomimetics, herbal drugs, saline solutions, etc. The use of drugs was recorded daily by the patients in the diaries. Antihistamines and corticosteroids were scored as 0 = no use, 1 = topical application, 2 = systemic application. DSCG/sympathomimetics were scored as 0 = no drugs; 1 = DSCG, herbal drugs, saline solutions, etc.; 2 = α -, β -sympathomimetics. The scores for the different types of drugs were cumulated weekly.

Titrated Skin Prick Test

The skin reactivity of the patients was analyzed by t-SPT. The test was performed twice on both forearms with concentrations of 1, 3, and 10 HEP (histamine equivalent in SPT). A 0.9% NaCl solution was used as negative and 1% histamine dihydrochloride solution as positive control. The areas of wheal and erythema caused by allergen and positive control were determined planimetrically and expressed as skin index (wheal area related to area of the positive control).

Titrated Nasal Provocation Test

Nasal reactivity of the patients was determined by t-NPT using active anterior rhinomanometry as an objective parameter. Nasal volume flow (cm^3/s) was measured with a transnasal pressure difference of 150 Pa (V_{150}) (Rhinomanometer 200, Atmos Inc., Lenzkirch, Germany). After an adaptation period to the test environment of at least 30 minutes, the basal value was determined 15 minutes after application of the suspension buffer without allergens (ALK-diluent) to the middle turbinate by a nasal spray applicator. The reactivity to allergen was then measured 15 minutes after each application in concentration steps of 10, 100, 1000, 10,000, and 100,000 SQ units/mL. The test was assessed as positive either by a decrease in nasal volume flow greater than 40%, or by reaching a symptom score greater than 4 for rhinorrhea (no secretion = 0, little secretion = 1, much secretion = 2), irritation (0-2 sneezings = 0, 3-5 sneezings = 1, >5 sneezings = 2), itching (no itching = 0, mild itching = 1, severe itching = 2), and distant symptoms (no = 0, tear flow and/or palatal itching and/or itching of the ears = 1, conjunctivitis and/or chemosis and/or urticaria and/or coughing and/or shortness of breath = 2).

Statistics

Dependent on the characteristics of the respective variable, data were statistically analyzed for minimum, maximum, median, arithmetic, and geometric mean; standard deviation and standard error of mean; and frequency distributions. In addition to tests for homogeneity of baseline data, the following analytical procedures were used: analysis of covariance (ANCOVA) for the repeated-measurement model and baseline data as a covariate; *U* test in the nonparametric version of O'Brien's procedure for comparing samples with multiple endpoints, including the closed test procedure according to Lehman.^{11,12}

RESULTS

Patients

A total of 48 patients ($n = 24$ in the STI group and $n = 24$ in the drug-treated group) could be evaluated (females $n = 31$, males $n = 17$, mean age 30.6 years, range 15-50 years). The median conjunctival score (range 0-9) in the prestudy year was 7.0 (range 3-8) in the STI group and 5.0 (range 1-8) in the drug-treated group; the median nasal score (range 0-12) was 8.5 (range 5-10) in the STI group and 8.0 (range 5-10) in the drug-treated group. In the STI group 10 of 24 patients had mild ($n = 5$) or moderate ($n = 5$) bronchial symptoms, and 8 of 24 patients in the drug-treated group (mild, $n = 2$; moderate, $n = 6$). Diaries were obtained for 35 patients (STI, $n = 18$; drugs, $n = 17$). Both patient groups did not differ with regard to sex, age, and duration of the allergic disease (all $P > 0.05$). The severity of symptoms in the prestudy year was comparable (all $P > 0.05$), except that conjunctival symptoms were more severe in the STI group ($P < 0.05$).

Clinical Symptoms and Drug Use

Symptoms of the STI-treated group were significantly lower than in the drug-treated group during the period with maximum grass pollen exposure (weeks 21-33), as shown in Figure 1 (total score: $P = 0.022$, *U* test). The median nasal score in the STI group was significantly reduced compared with the drug-treated group, by 59.7% (STI 106.5 versus 264.0 drugs, $P = 0.020$). The median score levels for conjunctival and bronchial symptoms were low; the differences between the two groups were not significant (conjunctival score STI 21.0, drugs 40.0, $P = 0.099$; bronchial score STI 0.0, drugs 1.0, $P = 0.290$). Significantly less symptomatic drugs were used as assessed by the simultaneous analysis of the single scores for antihistamines, corticosteroids, and DSCG/sympathomimetics for weeks 21-33 ($P = 0.003$). Antihistamines were the most frequently used drugs in both groups. The median scores for antihistamines were significantly lower in the STI group, with 13.0, than in the drug-treated group, with 92.0 ($P = 0.005$). The H mean scores for glucocorticosteroids ($P = 0.637$) and for DSCG/

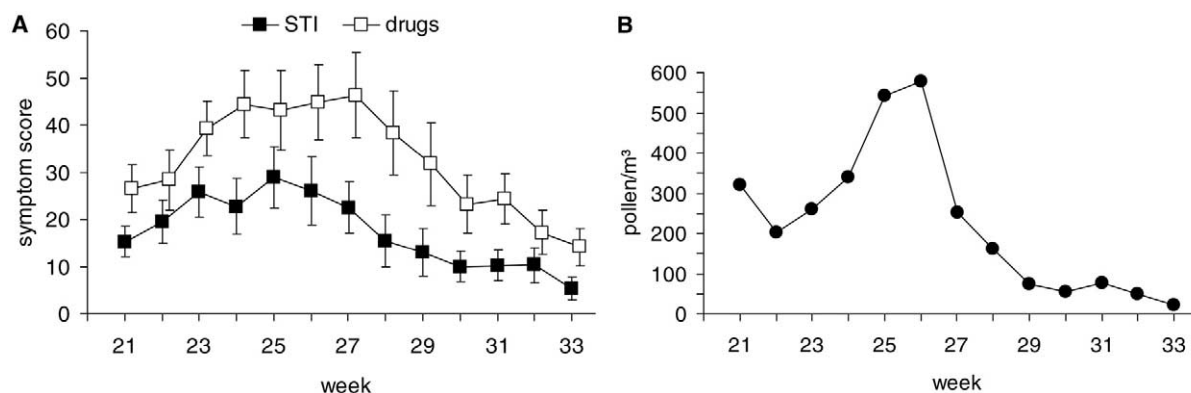


Figure 1 Median overall symptom scores (A) and pollen counts (B) for weeks 21-33 with maximum grass pollen exposure. Symptoms were recorded by the patients daily in diaries. The differences between the symptom scores of the two groups were significant ($P = 0.022$, one-tailed U test).

sympathomimetics ($P = 0.153$) were not significantly different (Fig 2).

The multiple endpoint analysis according to the method of O'Brien¹⁰ resulted in significant differences for the simultaneous analysis of the endpoints' conjunctival, nasal, and bronchial symptoms and medication ($P = 0.005$) and for the 3 symptom scores without medication ($P = 0.041$). The single endpoint analysis resulted in significantly lower symptom scores for nasal symptoms with STI ($P = 0.020$).

Titrated Nasal Provocation Test

From the individual values of each patient we calculated the allergen threshold dose for a reduction of the nasal volume flow by 40% for the two groups.

The threshold dose did not change at any control investigations in the drug-treated group. The threshold dose for the STI group significantly increased at T1 (directly after STI, $P = 0.027$) and at T3 (after season, $P < 0.001$) compared with the drug-treated group (Fig 3). This increase

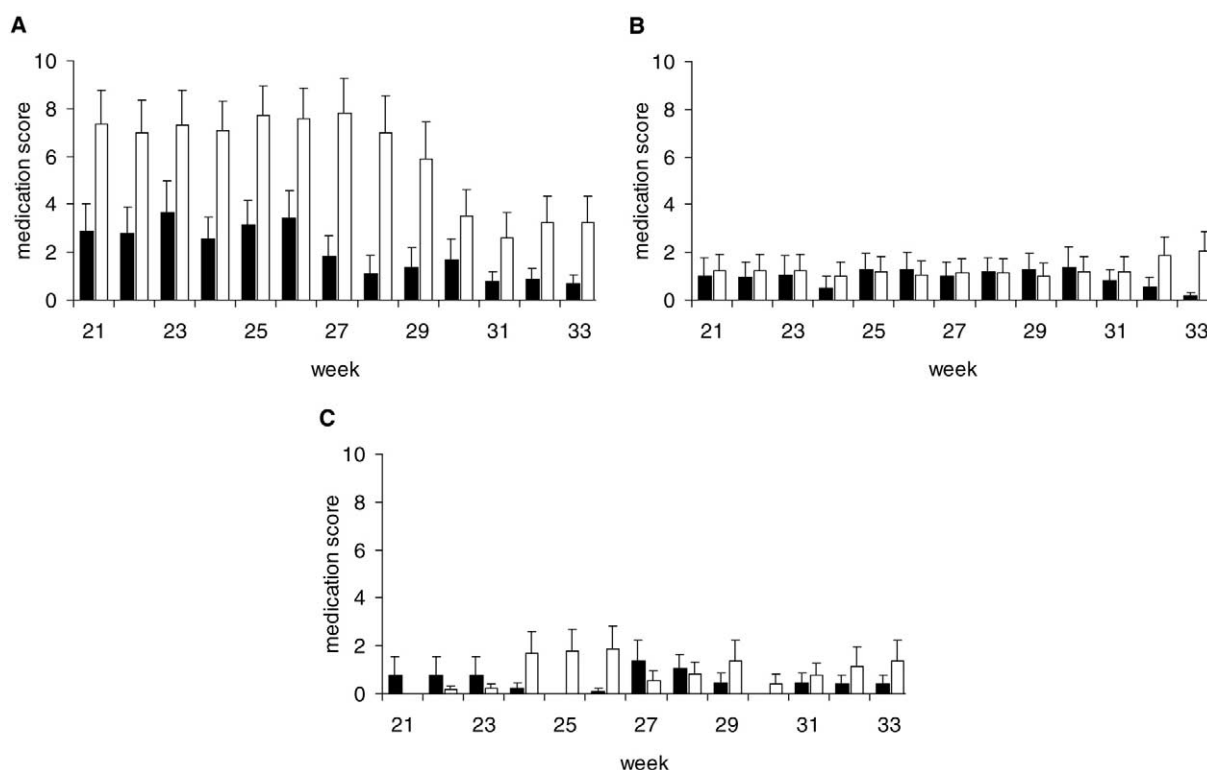


Figure 2 Mean medication scores in weeks 21-33 for antihistamines (A), glucocorticosteroids (B), and DSCG/sympathomimetics (C). The differences between the STI group (black bars) and the drug-treated group (white bars) were significant for antihistamines ($P = 0.005$) and not significant for glucocorticosteroids ($P = 0.637$) and for DSCG/sympathomimetics ($P = 0.153$) as analyzed by U -test.

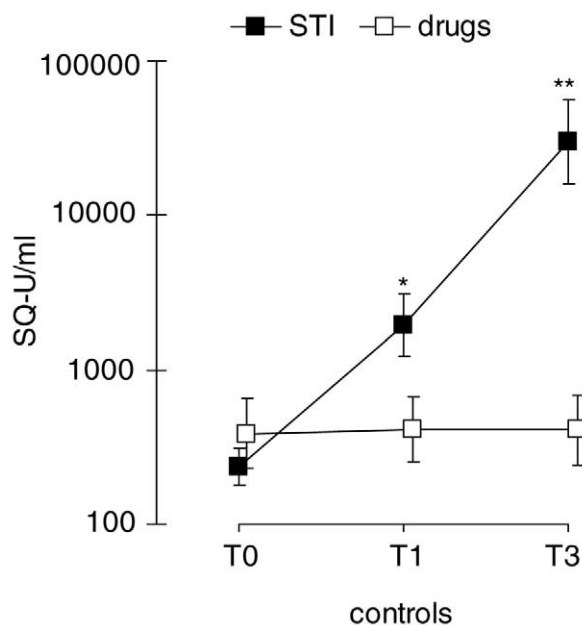


Figure 3 Nasal reactivity. The threshold dose in nasal provocation test for a decrease in peak flow of 40% is displayed for the STI and the drug-treated group at baseline (T0), directly after STI (T1), and after season (T3). The difference between the two groups was significant at T1 (* $P = 0.027$) and at T3 (** $P < 0.001$) as analyzed by t test.

corresponds to an average increase of the test concentrations that had to be applied for a positive test result from 1000 SQ U/mL at baseline (T0) to 10,000 SQ U/mL at T1 (directly after STI) and to 100,000 SQ U/mL at T3 (after season).

Titrated Skin Prick Test

Wheal and erythema after skin prick test were evaluated as skin index (Fig 4).

The skin index of the erythema was slightly lower in both groups at T1 compared with the baseline value (T0). In

the drug-treated group the skin index was significantly higher ($P < 0.001$) at T3, while in the STI group it had not considerably changed compared with the values for T0 and T1 (Fig 4A).

The skin index of the wheal was not different between the two groups at T1 (directly after STI), but was slightly higher in the drug-treated group at T3 (after season). This difference was not significant (Fig 4B).

Safety of STI

Major local reactions (>5 cm) were observed in 9.1% of 175 total injections; weak systemic reactions were documented in 2.9%. No severe systemic reactions occurred.

DISCUSSION

It was the aim of this study to compare two patient groups, one group treated preseasonally by a short-term immunotherapy with molecular standardized grass and rye allergens and the other group treated by standard symptomatic drug treatment in grass pollen season.

The recordings for the use of symptomatic drugs from the patients' diaries showed significant differences between the two groups, as observed in other trials with specific immunotherapy with grass pollen allergens.¹³⁻¹⁵ Patients in the drug-treated group took their medication continuously throughout the grass pollen season while patients in the STI group applied considerable amounts of symptomatic drugs only in the period with maximum pollen exposure. Differences in drug use between the two groups were expected because the STI group received a preseasonal prophylactic treatment so that patients in this group expected to have fewer symptoms compared to their experiences in previous grass pollen seasons while patients in the drug-treated group were aware to control their symptoms by symptomatic drugs. The application of higher amounts of symptomatic

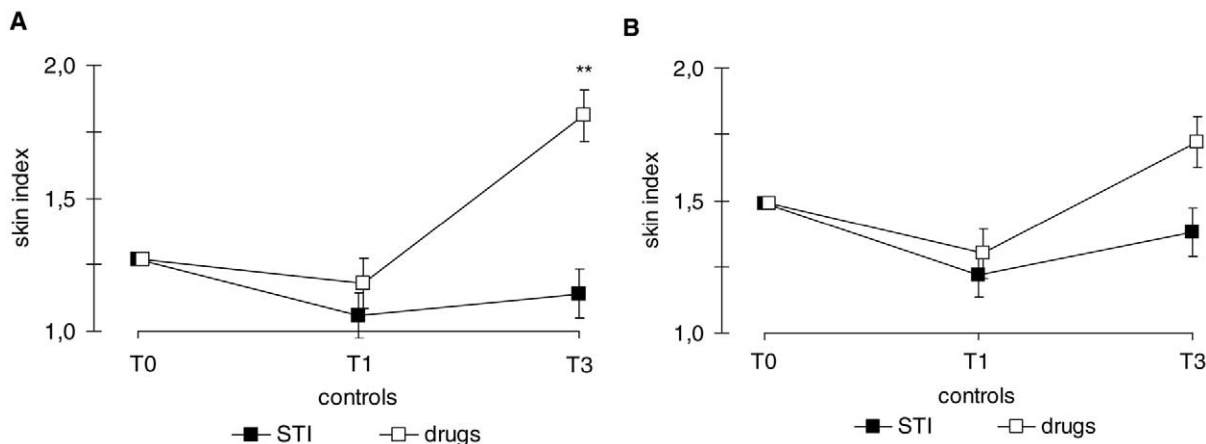


Figure 4 Skin index for the erythema (A) and the wheal (B). Skin reactivity was measured by skin prick tests as area of the allergen-induced reaction related to the reaction of the histamine control (skin index) at baseline (T0), before season (T1), and after season (T3). The difference between the two groups was significant for the erythema at T3 (** $P < 0.001$) as analyzed by t test.

drugs in the drug-treated group was not able to reduce the symptoms to a level as low as the one documented by the patients in the STI group, which had concomitantly used considerably less amounts of drugs. This was shown by significant differences in favor of the STI group for the simultaneous statistical analysis of conjunctival, nasal, and bronchial symptoms and medication according to the method of O'Brien. Therefore, patients were more efficiently treated by STI than by an exclusive symptomatic drug treatment according to subjective clinical parameters.

These findings were supported by the data for objective clinical parameters. Patients in the STI group showed a significant reduction of reactivity to grass pollen allergens in nasal provocation testing compared with the drug-treated group. This is consistent with other results reported for specific immunotherapy.¹⁶⁻¹⁸ In the t-NPT technique used in this study, the test result demonstrates a direct measure of the individually needed allergen concentration that provokes a positive reaction. Thus, a direct relation of the therapeutic efficacy with nasal sensitivity could be demonstrated. Skin reactivity was significantly lower in the STI group compared with the drug-treated group only for the erythema after the grass pollen season. We could not find a significant reduction of the wheal response as was found with long-term immunotherapy protocols.⁴ A conclusion might be that a longer treatment might be needed to achieve such changes. In addition to these effects on organ reactivity to allergen, we already showed that STI is able to influence immunological parameters.³

These results show that STI is capable to influence not only symptoms of pollen rhinitis but also the underlying allergic disease.⁴

Finally, long-term immunotherapy treatment has been shown to be effective up to 6 years after discontinuing treatment.⁴ At present we do not know if the effects observed for STI extend only to the season following treatment or are more persistent. Observation of the patients is continued to address this question.

In conclusion, patients are more efficiently treated by a preseasonal short-term immunotherapy with molecular standardized allergens and additional on-demand drug therapy during the season than by symptomatic drug treatment alone.

REFERENCES

1. Zenner HP, Baumgarten C, Rasp G, et al. Short-term immunotherapy: a prospective, randomized, double-blind, placebo-controlled multicenter study of molecular standardized grass and rye allergens in patients with grass pollen-induced allergic rhinitis. *J Allergy Clin Immunol* 1997;100:23-9.
2. Balda BR, Baumgarten C, Klimek L, et al. Tree pollen allergy can be efficiently treated by short-term immunotherapy (STI) with 7 pre-seasonal injections of molecular standardized unmodified allergens. *Allergy* 1997;53:740-8.
3. Klimek L, Wolf H, Mewes T, et al. The effect of short-term immunotherapy with molecular standardized grass and rye allergens on eosinophil cationic protein and tryptase in nasal secretions. *J Allergy Clin Immunol* 1999;103:47-53.
4. Malling HJ, Weeke B. Position Paper Immunotherapy of the EAACI. *Allergy* 1993;48:9-35 (and Appendices).
5. European Economic Community (EEC). Note for Guidance, Good Clinical Practice for Trials on Medicinal Products in the European Community, CPMP Working Party on Efficacy of Medicinal Products. Final Approval 11.07.1990, Date for Coming into Operation 01.07.1991. *Pharm Toxicol* 1990;67:361-72.
6. World Medical Association. Declaration of Helsinki. 1989. Hong Kong.
7. Gjessing B, Jaeger L, Marsh DG, et al. The international collaborative study establishing the first international standard for timothy (*Phleum pratense*) grass pollen allergenic extract. *J Allergy Clin Immunol* 1985;75:258-67.
8. Sundin B, Lilja G, Graff-Lonnevig V, et al. Immunotherapy with partially purified and standardized animal dander extracts. I. Clinical results from a double-blind study on patients with animal dander asthma. *J Allergy Clin Immunol* 1986;77:478-87.
9. Aas K, Backman A, Belin L, et al. Standardization of allergen extracts with appropriate methods. The combined use of skin prick testing and radio-allergosorbent tests. *Allergy* 1978;33:130-7.
10. Lund VJ, Aaronson D, Bousquet J, et al. International consensus report on the diagnosis and management of rhinitis. *Allergy* 1994;49:1-34.
11. O'Brien PC. Procedures for comparing samples with multiple endpoints. *Biometrics* 1984;40:1079-87.
12. Lehman W, Wassmer G, Reitmeir P. Procedures for two-sample comparisons with multiple endpoints controlling the experimentwise error rate. *Biometrics* 1991;47:511-21.
13. Østerballe O. Immunotherapy with grass pollen major allergens. *Allergy* 1982;37:379-88.
14. Varney VA, Gaga M, Frew AJ, et al. Usefulness of immunotherapy in patients with severe summer hay fever uncontrolled by antiallergic drugs. *Br Med J* 1991;302:265-9.
15. Dolz I, Martinez-Cócerca C, Bartolomé JM, et al. A double-blind, placebo-controlled study of immunotherapy with grass-pollen extract Alutard SQ during a 3-year period with initial rush immunotherapy. *Allergy* 1996;51:489-500.
16. Wihl JA, Ipsen H, Nüchel Petersen B, et al. Immunotherapy with partially purified and standardized tree pollen extracts. *Allergy* 1988;43:363-9.
17. Iliopoulos O, Proud D, Adkinson NF Jr. Effects of immunotherapy on the early, late, and rechallenge reaction to provocation with allergen: changes in inflammatory mediators and cells. *J Allergy Clin Immunol* 1991;87:855.
18. Van Metre TE, Adkinson NF. Immunotherapy for aeroallergen disease. In: Middleton E, Reed CE, Ellis EF, et al, editors. *Allergy principles and practice*. Baltimore: Mosby St. Louis; 1993. p. 1489-509.