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

Hans Peter Zenner, MD,[a] Claus Baumgarten, MD,[b] Gerd Rasp, MD,[c] Thomas Fuchs, MD,[d] Gert Kunkel, MD,[b] Bettina Hauswald, MD,[e] Johannes Ring, MD, PhD,[f] Isaak Effendy, MD,[g] Wolfram Behrendt, MD,[h] Peter J. Frosch, MD,[i] Bernhard Przybilla, MD,[j] Franz X. Brunner, MD,[k] Hans F. Merk, MD,[l] Alexander Kapp, MD,[m] Jörg Schnitker, PhD,[n] and Hendrik Wolf, PhD[o] *Tübingen, Berlin, München, Göttingen, Dresden, Marburg, Leipzig, Dortmund, Würzburg, Aachen, Hannover, Bielefeld, and Hamburg, Germany*

Background: Short-term immunotherapy (STI) can be beneficial for patients who are noncompliant with long-term specific immunotherapy.
Objective: The efficacy and tolerance of STI with seven preseasonal injections of molecular standardized allergens from grass and rye pollen has been investigated in a double-blind, placebo-controlled multicenter study with 87 patients at 12 German University hospitals.
Methods: Symptoms of the eyes, nose, and bronchi and use of symptomatic drugs were documented daily in diaries by patients with allergic rhinitis to grass and/or rye pollen and without bronchial asthma. Patients were monitored by skin prick test titration and measurement of levels of specific IgE and IgG4.
Results: The median nasal score for the 10 weeks with the strongest symptoms during the grass pollen season was significantly lower ($p = 0.014$) with 35.0 for STI ($n = 41$) versus 69.0 for placebo ($n = 40$); the overall symptom score was 54.0 for STI versus 97.5 for placebo ($p = 0.020$). Only STI-treated patients exposed to less than 40 pollen grains per cubic meter per week showed a significantly lower nasal symptom score of 39.0 versus 75.0 for placebo ($p = 0.006$); these patients also had fewer nasal symptoms and less use of topical nasal drugs ($p < 0.001$). The threshold dose in skin prick tests was significantly higher, being 9.06 histamine equivalent for skin prick test (HEP) for STI-treated patients who received the maximum dose ($n = 22$) versus 4.33 HEP for placebo ($p = 0.005$). Specific IgE levels were significantly higher, being 55.9 SU/ml for STI versus 39.2 SU/ml for placebo after seven injections ($p = 0.006$) and level of specific IgG4 was 5.36% for STI versus 1.28% for placebo ($p < 0.001$). No severe systemic reactions were observed.
Conclusion: STI with seven preseasonal injections with molecular standardized allergens is effective and well tolerated.
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Key words: Short-term immunotherapy, specific immunotherapy, allergic rhinitis, grass pollen, rye pollen, skin prick test, specific IgE, specific IgG4

The efficacy and safety of specific immunotherapy with molecular standardized allergen preparations has been shown in a large number of controlled clinical studies.[1-9] Specific immunotherapy is not accepted by all patients because of the long period of injections. Therefore, for patients who do not comply with specific immunotherapy, short-term immunotherapy (STI) that is completed before the pollen season but that also allows a dose level sufficient to induce a considerable clinical effect is attractive.

A dose response study of specific immunotherapy with house dust mite allergens has shown that a significant improvement in symptoms after the first year of treatment could be achieved by a dose lower than the routinely applied maximum dose.[10] Another study with grass pollen allergens showed that a therapeutic effect was obtained in the pollen season following the initiation of therapy.[11]

These results suggest that a preseasonally initiated STI with molecular standardized allergens may lead to a significant improvement of symptoms in the subsequent

From the [a]Universitäts-HNO-Klinik Tübingen; [b]Abteilung für klinische Immunologie und Asthmapoliklinik, Universitätsklinikum Rudolf Virchow, Berlin; [c]HNO-Klinik und Poliklinik der Maximilians Universität, Klinikum Großhadern, München; [d]Universitäts-Hautklinik, Göttingen; [e]HNO-Klinik der Medizinischen Akademie Carl Gustav Carus, Dresden; [f]Dermatologische Klinik und Poliklinik der Technischen Universität München; [g]Medizinisches Zentrum für Hautkrankheiten, Klinikum der Philipps Universität Marburg; [h]Universitäts-HNO-Klinik, Leipzig; [i]Hautklinik der Städtischen Kliniken, Dortmund and Universität Witten/Herdecke; [j]Dermatologische Klinik und Poliklinik der Universität München; [k]Universitäts HNO-Klinik und Poliklinik, Würzburg; [l]Hautklinik der Rheinisch-Westfälischen Technischen Hochschule Aachen; [m]Dermatologische Klinik und Poliklinik der Medizinischen Hochschule Hannover; [n]Institut für Angewandte Statistik, Bielefeld; and [o]Klinische Forschung, Scherax Arzneimittel GmbH, Hamburg.
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Reprint requests: Hendrik Wolf, PhD, Scherax Arzneimittel GmbH, Sülldorfer Landstr. 128, D-22589 Hamburg, Germany.

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Abbreviations used

ANCOVA:	Analysis of covariance
BU:	Biologic units
DSCG:	Disodium cromoglycate
HEP:	Histamine equivalent in skin prick test
LIA:	Luminescence immunoassay
MD:	Maximum dose applied by short-term immunotherapy
SE:	Specific units of short-term immunotherapy
SPT:	Skin prick test
STI:	Short-term immunotherapy
T0:	Initial investigation
T1:	Control investigation after therapy
T2:	Control investigation in season
T3:	Control investigation after season
TD:	Threshold dose in skin prick test titration

pollen season. In order to investigate the safety and efficacy of STI, we performed a prospective, double-blind, multicenter study with patients allergic to grass pollen.

METHODS

Patients

Eighty-seven patients in the age range of 16 to 53 years who were allergic to grass and/or rye pollen as confirmed by a wheal size of at least 5 mm in the skin prick test (SPT) were enrolled into the study. Patients had typical symptoms of rhinoconjunctivitis in the grass pollen season from May to August (Table I). Patients needing treatment for allergic asthma, perennial rhinitis, or acute infected nasal mucosa, patients receiving 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.[12]

The study was performed according to European Community good clinical practice guidelines[13] and the Declaration of Helsinki.[14] The study was approved by the ethics committee of the medical faculty of the University of Tübingen, Germany.

Allergen extracts

The grass and rye allergen extracts used for STI were partially purified and standardized extracts[15] composed of equal parts of the six grasses *Dactylis glomerata, Lolium perenne, Avena elatior, Phleum pratense, Poa pratensis,* and *Festuca pratensis* and rye, *Secale cereale.*

Extracts for STI and SPT, placebo preparations, and allergens for specific IgE and IgG4 analysis were manufactured by ALK A/S (Horsholm, Denmark). The allergen extracts were standardized relative to an internal standard by qualitative and quantitative immunoelectrophoresis for characterization and quantitation of the major allergens and by luminescence immunoassay (LIA) inhibition for determination of total allergenic activity; the biologic activity of the extracts was determined by SPT as previously described.[16, 17] The potency was expressed in arbitrary specific units of short-term immunotherapy (SE); 1000 SE contains approximately 1.5 μg of grass group 5 major allergen (between 1.0 and 2.0 μg for individual grasses).

For STI, a depot extract adsorbed to aluminium hydroxide (0.5% phenol) was used (ALK7 Gräsermischung und Roggen, Scherax, Hamburg, Germany) and for SPT, an identically manufactured, partly purified extract of equal components of the six grasses without rye (ALK prick SQ/soluprick SQ; 10 histamine equivalents in skin prick test [HEP], 10,000 biologic units [BU]/ml, dissolved in 50% glycerol) was used. STI was applied out of 3 vials with concentrations 10, 100, and 1000 SE/ml. Placebo consisted of the suspension buffer with ascending concentrations of histamine dihydrochloride (0.01, 0.1, and 1.0 μg/ml) to simulate potential local reactions. STI and placebo were of identical appearance. All extracts were stored at 4° C (39° F) until use; the stability assigned by the manufacturer was much longer than the total duration of the study.

TABLE I. Clinical data on patients

	Treatment	
	STI	**Placebo**
Total no. of patients	45	41
Sex		
Male	30	29
Female	15	12
Mean (range) age (years)	27.6 (18–53)	29.4 (16–49)
Median (range) duration of allergy (years)	13 (2–29)	12 (1–29)
Eye symptoms (no. of patients)		
none	0	2
mild	10	11
moderate	26	23
severe	9	5
Nasal symptoms (no. of patients)		
none	0	0
mild	3	3
moderate	28	29
severe	14	9
Bronchial symptoms (no. of patients)		
none	29	25
mild	12	12
moderate	4	2
severe	0	2

Short-term immunotherapy

Patients received seven injections at weekly intervals before the expected beginning of the grass pollen season with ascending concentrations (3, 10, 30, 100, 300, 600, and 1000 SE) of STI or placebo equivalent. Dose modifications were allowed for medical indications according to the routine procedure of specific immunotherapy.[12] 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 exposure to grass pollen. Disodium cromoglycate (DSCG) eyedrops and nasal sprays, local or systemic antihistamines, sympathomimetics, and local glucocorticosteroids were allowed as symptomatic treatment.

Study design

During the initial investigation (T0), the duration and severity of symptoms of the eyes, nose, and bronchi in the pretreatment year (scored on a scale of 0 to 3 and based on the patients' memory), the general health status, and sensitization to other aeroallergens as determined by SPT were recorded by the

investigator. Baseline values for skin sensitivity (by a titrated SPT) and for specific antibodies were determined. Symptoms were scored and specific antibodies were determined during three subsequent control investigations at the following times: (1) after therapy (T1), (2) in season (T2), and (3) after season (T3). Skin sensitivity was determined at T1 and T3. Symptoms and use of symptomatic drugs were recorded by the patient in a diary during the grass pollen season.

Random assignment

Patients were randomly assigned either to STI or to placebo treatment with a block size of six (three STI and three placebo preparations). According to the capacity for patient recruitment, a single block or multiple blocks were allocated to each individual trial center. Patient groups (see Table I) were comparable with respect to sex, age, duration of the allergic disease, and severity of symptoms in the previous year.

Data analysis

The main parameter was the severity score, on a scale of 0 to 3, of symptoms of the eyes, nose, and bronchi (0 = no symptoms, 1 = mild symptoms, 2 = moderate symptoms, and 3 = severe symptoms) assessed from the patients' diaries. The daily symptom scores for 1 week were added; the cumulative weekly score range, therefore, was 0 to 21 points for each individual symptom. For further analysis, 10 weeks with the strongest symptoms from the period of grass pollen exposure were selected for each individual patient and for each individual symptom. The weekly scores were added and provided three single endpoints with a theoretical range of 0 to 210 representing the seasonal symptoms of the eyes, nose, and bronchi. The ranked data for each single endpoint were added and analyzed as a primary study endpoint by the Mann-Whitney U test according to O'Brien's multivariate procedure.[18, 19]

The use of nasal drugs and antihistamines, respectively, was scored daily on a scale of 0 to 3 (0 = no drugs, 1 = DSCG, 2 = topical corticosteroids or antihistamines, 3 = nasal decongestants) and corrected by a dose factor. The daily symptom scores for 1 week were added over 10 weeks corresponding to the 10 weeks with the strongest individual overall symptom score. Both medication scores were analyzed as secondary endpoints by the nonparametric O'Brien multiple test procedure for five single endpoints (three symptom scores, two medication scores) in order to control the experimentwise error rate ($\alpha = 0.05$).

SPT

The skin sensitivity of the patients was analyzed by SPT responses. The test was performed twice on both forearms with concentrations of 1, 3, and 10 HEP. A 0.9% sodium chloride solution was used as the negative control and 1% histamine dihydrochloride solution as the positive control. The area of wheal and erythema caused by allergens and positive control were determined planimetrically and expressed as the skin index (wheal area related to area of the positive control). The mean dose of allergen (in HEP) necessary to generate a skin index of 1 was derived from the three allergen concentrations tested and was evaluated as the threshold dose (TD).

Measurement of IgE and IgG4 levels

Serum samples were analyzed for specific IgE and IgG4 by a luminescence immunoassay (LIA) with a solid phase of paramagnetic particles (Magic Lite SQ, ALK) according to the instructions of the manufacturer. Levels of specific IgE are expressed as standardized units per milliliter and those of specific IgG4 as percentage of total activity added in the immunoassay.

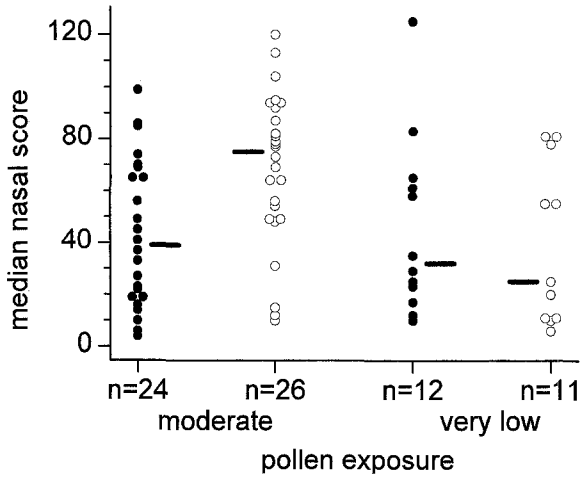

FIG. 1. Median nasal symptom scores (cumulative score over the 10 weeks with strongest symptoms) for each individual patient (STI, *filled circles;* placebo, *open circles*) and for the subgroups of patients *(bars)* with moderate and very low pollen exposure, respectively. The difference in medians for STI and placebo with moderate exposure was significant ($p = 0.006$) with the U test.

Statistics

A decrease in the overall symptom score of at least 30% compared with placebo and a pooled standard deviation of 50% was assumed to estimate the sample size. With a power ($1\text{-}\beta = 0.80$) and an error rate $\alpha = 0.05$ in the one-tailed t test, at least 72 patients (36 per group) were needed to finish the trial according to protocol. The use of a potentially less powerful nonparametric procedure and possible deviations from uniformly equidirected treatment differences led to a recruitment plan of 12 trial centers with 6, 12, and 18 patients.

The baseline characteristics of the STI and placebo groups were compared by the t test and the Mann-Whitney U test for continuous variables, and by the χ^2-test for categorical variables.

Data were statistically analyzed by minimum, maximum, and median values, 95% confidence interval of the median, arithmetic and geometric means, standard deviation and standard error of the 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 Lehmacher.[19]

RESULTS

Patients

Eighty-six patients could be evaluated. One patient withdrew his consent before the first injection, so that 45 patients received STI and 41 patients received placebo. Evaluable diaries were obtained for 81 patients (STI, $n = 41$; placebo, $n = 40$). Clinical data of the patients at inclusion are shown in Table I.

TABLE II. Symptom scores for patients undergoing STI or placebo treatment

Symptom score	STI		Placebo		p value (U-test)*
	Median score (95% confidence interval)	Mean score (± SEM)	Median score (95% confidence interval)	Mean score (± SEM)	
Eyes	17 (12-27)	26.6 (± 4.3)	24 (12-31)	28.3 (± 3.9)	0.256
Nose	35 (22-61)	44.5 (± 5.0)	69 (54-78)	63.3 (± 6.1)	0.014
Bronchi	0 (0-5)	12.5 (± 4.9)	2 (0-9)	26.0 (± 7.6)	0.192
Overall	54 (39-96)	82.2 (± 10.1)	97.5 (81-117)	116.0 (± 13.2)	0.020

*One-tailed test.

Dosage

Most of the patients received seven injections in accordance with the prescribed dosage scheme; three patients (7%) in each group received eight injections, and one patient (2.2%) received nine injections of STI because of dose modifications. The maximum dose of 1000 SE or its placebo equivalent was not received by 23 patients (51%) in the active group and 16 patients (39%) in the placebo group because of dose modifications followed by the early beginning of the grass pollen season. Dose modifications were performed in five cases with STI (11%) and in four cases with placebo (10%) because of systemic reactions, and in three STI cases (6.6%) because of local reactions. Other reasons for dose modifications were dropout (STI, $n = 1$), infection or disease (STI, $n = 5$; placebo, $n = 1$), deviation from injection interval (STI, $n = 2$; placebo, $n = 2$), symptoms resulting from grass pollen exposure (STI, $n = 4$; placebo, $n = 6$), and dose modification without medical need (STI, $n = 3$; placebo, $n = 3$).

Clinical efficacy

Median nasal symptom scores and overall symptom scores were significantly lower in the STI group ($n = 41$) compared to the placebo group ($n = 40$) ($p = 0.014$ for nasal symptoms and $p = 0.020$ for overall symptoms). Median conjunctival and bronchial symptoms were not significantly different if tested as single endpoints (Table II). The median overall symptom score was reduced by 45% with STI. The largest reduction (52%) of mean symptom scores was observed for bronchial symptoms.

The difference between STI and placebo groups for the simultaneous analysis of median nasal, conjunctival, and bronchial symptoms was significant ($p = 0.041$) by testing multiple endpoints with O'Brien's procedure. Therefore significant equidirected treatment differences were obtained with regard to the three individual symptom scores (of the eyes, nose, and bronchi) in the confirmatory statistical analysis. Significance was obtained with simultaneous analyses of nasal and conjunctival symptoms ($p = 0.042$) and of nasal and bronchial symptoms ($p = 0.031$). The difference for the simultaneous analysis of conjunctival and bronchial symptoms was not significant ($p = 0.128$).

Pollen exposure

The amount of grass pollen detected between May and August 1993 in Germany was extremely low due to rainy weather. Because of regional variations, trial centers could be classified into seven centers with moderate pollen exposure (average weekly pollen count more than 40 pollen grains per cubic meter) and three centers with very low pollen exposure ($n = 73$ patients). As expected, there was no significant difference for STI ($n = 12$) and placebo patients ($n = 11$) with very low pollen exposure. The nasal symptom score for the subgroup of patients with moderate pollen exposure (STI, $n = 24$; placebo, $n = 26$) was lower with STI than with placebo (Fig. 1) and was more significantly reduced ($p = 0.006$, two-tailed analysis) than for the entire group of patients ($p = 0.014$). The score level of STI-treated patients with moderate pollen exposure was only slightly different from the basal score level for very low exposure. Median overall symptoms throughout the grass pollen season for patients with moderate pollen exposure are shown in Fig. 2. The differences between the STI and placebo groups were significant if analyzed week by week with one-tailed U tests for weeks 19 to 29 ($p < 0.05$).

Use of symptomatic drugs

The use of symptomatic drugs depended significantly on the severity of symptoms ($p < 0.001$). Symptomatic drugs were used in the STI group for 26.6% of the 70 days (10 weeks) with strongest symptoms during the grass pollen season and for 33.0% of the days in the placebo group ($p = 0.296$). The different types of drugs were used in the following frequencies: antihistamines (STI, 19.0%; placebo, 16.6%; $p = 0.760$), topical nasal drugs (STI, 11.6%; placebo, 18.9%; $p = 0.066$); ocular therapeutics (STI, 10.6%; placebo, 11.1%); and bronchial therapeutics (STI, 4.1%; placebo, 6.4%). Analyzing patients with moderate pollen exposure by O'Brien's procedure, significant differences ($p = 0.025$) for the simultaneous analysis of conjunctival, nasal, and bronchial symptoms together with topical nasal drugs and antihistamines were obtained, demonstrating equidirected multiple effects of STI with respect to these five single endpoints. From the multivariate point of view, differences in antiallergic drug consumption between the two groups were only observed if analyzed simulta-

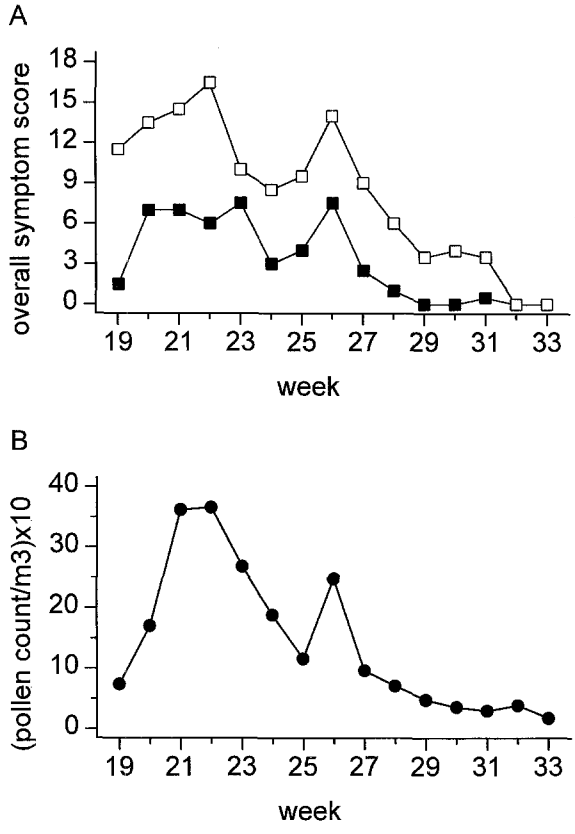

FIG. 2. **(A)** Median overall symptom scores (conjunctival, nasal, and bronchial symptoms) for patients with moderate pollen exposure (more than 40 pollen particles per cubic meter of air) (STI, $n = 24$; *filled squares;* placebo, $n = 26$; *open squares*). Symptoms were continuously displayed over the grass pollen season (weeks 19 to 33). The differences between the two groups were significant in weeks 19 to 29 ($p < 0.05$), as analyzed week by week with one-tailed U tests. **(B)** Median grass pollen count (pollen particles per cubic meter) in weeks 19 to 33 for patients from the seven centers with moderate pollen exposure.

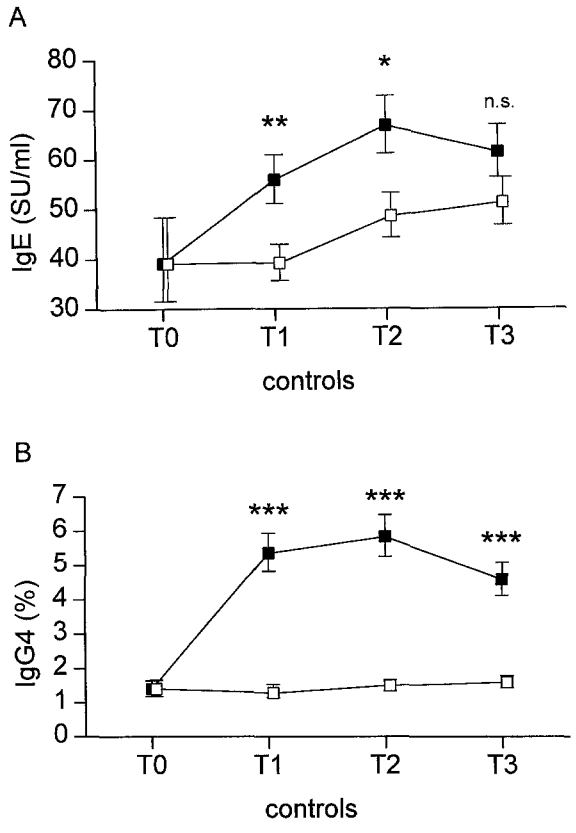

FIG. 3. Geometric means ± SEM of levels of specific IgE **(A)** and specific IgG4 **(B)** in patient sera (STI, $n = 37$, *filled squares;* placebo, $n = 33$, *open squares*) before therapy *(T0)*, after therapy *(T1)*, in season *(T2)*, and after season *(T3)*. Values for STI and placebo were adjusted to T0 levels. Statistics were performed by ANCOVA for the repeated measurement model $*p < 0.05$; $**p < 0.01$; $***p < 0.001$; *n.s.* not significant.

neously, with the inclusion of nasal symptoms and topical nasal drugs. Accordingly, the simultaneous analysis of nasal symptoms and topical nasal drug consumption indicated highly significant reductions with STI ($p < 0.001$).

SPT responses

The TD was higher in the actively treated group than in the placebo group at T1. This was not significant ($p = 0.091$) for the total number of patients but was significant ($p = 0.005$) for patients who reached the maximum dose of 1000 SE during the injection phase ($n = 22$; TD: STI, 9.06 HEP; placebo, 4.33 HEP).

IgE and IgG4 levels

Levels of specific IgE adjusted to T0 significantly increased at T1 ($p = 0.006$) for STI-treated patients but remained unchanged for placebo (Fig. 3, *A*). Specific IgE levels showed a parallel increase for both groups at T2. Levels continued to increase in the placebo group, but decreased slightly in the STI group at T3. Specific IgG4 levels with STI were increased at T1 by more than 400% ($p < 0.001$) and continued at this level up to T3 (Fig. 3, *B*). Patients treated with the maximum dose of 1000 SE had higher levels of IgG4 at T1, T2, and T3 compared to patients who received lower doses. Geometric means of IgG4 levels at T1, T2, and T3, respectively, were the following (expressed as percent relative units): 3.67, 3.69, and 2.87 with 300 SE; 5.19, 5.85, and 4.54 with 600 SE; and 6.66, 7.35, and 5.85 with 1000 SE, ($p < 0.001$ by ANCOVA).

Safety

For estimation of safety, potentially allergic reactions were recorded carefully after every injection. Local reactions at the injection site with swelling and erythema greater than 5 cm in diameter were observed in 30 of 309 (9.7%) STI injections and in 6 of 284 (2.1%) placebo injections. Systemic reactions such as occasionally moderate exacerbations of rhinoconjunctivitis, urticaria, and edema of the eyelid were documented for 9 patients and 12 injections with STI and for 5 patients and 7 injections with placebo. A shortness of breath 1 hour after STI injection documented as bronchospasm was reversible

within 2 hours. Other reactions in the STI group were one transient episode of tachycardia and one episode of paleness and anxiety; both reactions were completely reversible and were classified as minor by the investigators. Sickness was reported for one patient in the placebo group. No severe systemic reactions were observed in either group.

DISCUSSION

This study demonstrates that STI with seven injections of a molecular standardized grass and rye preparation is effective and safe. Nasal symptoms in the STI group were significantly reduced during the maximum pollen exposure compared to the placebo group, despite a low pollen count throughout the study.

Grass pollen exposure had to exceed a threshold of 40 pollen grains per cubic meter per week to demonstrate a therapeutic effect of STI in terms of symptom and medication scores. Patients exposed to lower levels of pollen had a comparable basal level of nasal symptoms. Nasal symptoms increased with higher pollen exposure in the placebo but not in the STI group. Eye symptoms were significantly influenced only if they were accompanied by nasal symptoms. Bronchial symptom scores could be described more adequately with means rather than medians. In the small group of patients with bronchial symptoms, bronchial symptom scores were reduced by 52%. This observation suggests that STI with molecular standardized allergens may also have an influence on allergic asthma.

Patients did increase their use of symptomatic drugs depending on the severity of their symptoms. In the subgroup of patients with moderate exposure to grass pollen, the multiple analysis indicated a significant reduction of symptoms together with the use of symptomatic drugs. The effect of STI was highly significant ($p < 0.001$) with respect to nasal symptoms in combination with nasal drug consumption. This is consistent with the reduction of the symptoms by effective specific immunotherapy.[12]

The prescribed maximum dose of 1000 SE units could not be applied to all patients because of dose modifications and the immediate beginning of the grass pollen season. Therefore, therapy should be initiated at an appropriate time in advance of the prospective beginning of the season for routine use.

The achieved maximum dose had an influence on the skin sensitivity and on the generation of specific IgE and IgG4 antibodies. These results suggest that a continuation of treatment up to the maximum dose of 1000 SE may be favorable after dose modifications to obtain optimum efficacy.

The active treatment by STI triggered a significant increase of specific IgE and IgG4 antibodies in the injection period, but no change was observed with placebo, as found in other studies with specific immunotherapy.[20-22] IgE and IgG4 levels cannot be correlated with the therapeutic efficacy or be used to predict the clinical outcome.[23, 24] The large increase of the IgG4-antibody level observed in this study with STI, however, demonstrates a strong immunomodulatory effect of the treatment, and thus the application of a relatively high cumulative dose.[12]

The schedule for STI used in this study was safe. Apart from local reactions, mild systemic allergic symptoms of the eyes and nose, or urticaria, no severe anaphylactic reactions were observed.

In conclusion, STI was efficacious and safe for the pollen season following treatment. Possible long-term effects have to be investigated by further studies.

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