

Original article

Efficacy of sublingual swallow immunotherapy in children with severe grass pollen allergic symptoms: a double-blind placebo-controlled study

Background: Local application of allergen extracts in specific immunotherapy is accompanied by increased compliance and significantly reduced side effects. However, efficacy of local immunotherapy in children has yet not been sufficiently demonstrated. This study was performed to determine clinical efficacy of high dose sublingual swallow immunotherapy (SLIT) by a double-blind placebo-controlled study in children with grass pollen allergy using high dose allergen extracts.

Methods: A total of 161 children with seasonal rhinoconjunctivitis of which, 68 had also asthma symptoms were enrolled in a multicenter double-blind placebo-controlled study for 1 year and treated on a daily basis with sublingually applied allergen drops. After 1 year all children were given treatment for another 2 years in an open-controlled setting. Symptom scores and medication were assessed during the pollen seasons with structured interviews. Applied allergen dosage, compliance, and side effects were documented by daily diary cards. Primary endpoint was a clinical index (CI) combining symptom scores with medication index. Titrated skin prick tests (SPT) and specific antibody measurements were performed each year.

Results: Combining symptom with medication scores to CI was highly reliable (reliability coefficient = 0.89, standard error = 9.6%). Allergen-specific IgE- and IgG-subclass antibodies increased significantly in patients treated with SLIT indicating an activation of the immune response induced by the locally applied grass pollen extract. SPT reactivity did not change during therapy. After 1 year of SLIT in the original design we observed no significant difference in the CI between treatment and placebo analyzing all patients included in the study per intention to treat and per protocol. However, subgroup analysis in a repeated measures model revealed that patients with SLIT and severe symptoms before the beginning of treatment (CI > mean/ > 1.51) showed a significant improvement of clinical symptoms after 3 years.

Conclusion: In this study SLIT was accompanied by a significant placebo effect. Efficacy of treatment could only be seen in children with severe clinical symptoms and this became clinically marked after 3 years of therapy.

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Local allergen-specific immunotherapy for the treatment of allergic diseases applied via nasal or sublingual mucosa has been accepted in a WHO-position paper as a possible alternative to subcutaneous treatment as the rate of dangerous side effects is substantially minimized (1). Although early controlled studies analyzing oral applica-

tion of allergens did not demonstrate a clear clinical effect, sublingual immunotherapy (SLIT) proved to be of some reproducible value in adults (2). This observation is consistent with experiments showing that pollen allergens are completely degraded when passing through the gastrointestinal tract whereas complete degradation does not occur on the respiratory mucosa (3). The best uptake of intact allergens was seen via the nasal and sublingual epithelium (4).

Local immunotherapy can suppress allergic mucosal inflammation in terms of early- and late-phase reaction (5)

Abbreviations: AUC, area under the curve; BAU, biological activity units; CI, clinical index; IgE, immunoglobulin E; MI, medication index; SI, symptom index; SLIT, sublingual immunotherapy; SPT, skin prick test.

leading to a reduction of eosinophils in the epithelium. Furthermore the elevation of specific IgG antibodies induced by immunotherapy with allergens usually seen in subcutaneous treatment is, when determined, also often observed in SLIT (6, 7). However, the quantity of serious side effects seen with systemic immunotherapy is significantly reduced when using SLIT. Therefore children, whose compliance for subcutaneous injections is generally low, would clearly profit from SLIT. Most recently a Cochrane analysis has described a benefit for SLIT compared with placebo with the reservation that those studies involving only children did not show a significant reduction in symptoms and medication (8). Thus, reliable data on the clinical efficacy of sublingual swallow immunotherapy in a greater number of children are missing.

Here we present the results of a double-blind, placebo-controlled randomized multicenter clinical study with children on sublingual swallow immunotherapy with grass pollen extracts. By modification of an acknowledged symptom and medication score we were able to enhance score indices to a clinically relevant level. Specific immune response was analyzed before and after immunotherapy by measuring immunoglobulin (Ig)E- and IgG subclass antibodies. Change of sensitivity towards allergens during therapy was determined by titrated skin prick test (SPT).

Methods

Study design

A double-blind, placebo-controlled randomized study design was used for the first year, followed by an open controlled phase in the second and third year of treatment for all patients. The study included 33 centers mainly in the north and west of Germany. Patients were enrolled from January to June 1998 and the study was continued over 3 years until December 2001. After selection, patients, and parents of patients gave written informed consent. The study was approved by the ethical committees of involved regions. Patients were selected according to a history of summer hay fever. Inclusion criteria were doctors' diagnosis of rhinoconjunctivitis, allergic asthma or both and positive-SPT towards grass pollen allergen extract. Symptoms had to be restricted to the grass pollen season from May to September. The diagnosis of asthma was given by the investigators according to patients history of repeated wheezing, clinical assessment, and reduction of FEV1 in lung function tests of >20% from age-related values during clinical signs of bronchial obstruction. Patients with parallel SPT reactivities to three or more other allergen groups (i.e. animal dander, mites, and moulds), with chronic asthma, with previous immunotherapy or other systemic diseases such as diabetes, leukemia, immunodeficiency etc. were excluded from the study.

Patients and study design

Patients were divided in two groups by blockwise randomization to ensure homogenous distribution of treatment within the centers: treatment group ($n = 82$) and placebo ($n = 79$). Treatment started in December 1998 for all patients after a run-in period, in which

clinical symptoms were assessed (March–November 1998). Before and 1 year after the onset of treatment, a titrated SPT was performed and blood samples were drawn. Treatment was performed according to the recommendations of the WHO-position paper on local immunotherapy (2). Patients were either given grass pollen extracts (Sublivac B.E.S.T.TM, HAL-Allergy, Haarlem, the Netherlands) or placebo containing the solvent used for the treatment extract. Drops were placed daily under the tongue for at least 1 minute and afterwards swallowed (sublingual-swallow-design). The build-up phase involved the administration of increasing doses of extract [from 100 allergic units (AU) to 2500 AU/day] for 3 weeks. In the maintenance phase patients received 2500 AU every day. After 3 years patients had ingested a mean of 5250 allergen drops (2.625.000 AU). This was equivalent to a cumulative dosage of 9.6 mg major allergen Phl p 5 (timothy grass pollen, data given by HAL-Allergy). This dosage is about 10 times higher than the usual cumulative dosage reached during 3 years subcutaneous treatment. Documentation of patients keeping to the study protocol was performed by regular diary control, control of returned allergen drop bottles and professional monitoring of investigators. Furthermore increase of IgG4 or IgG1 indicated application of allergen extracts. Patients were allowed to take drugs for relief of symptoms if needed. Data on pollen-counts were obtained from the 'Stiftung Deutscher Pollen-Informationsdienst' (Bad Lippspringe, Germany).

Clinical index (CI) score

During the course of the study clinical symptoms and use of medication of all subjects were assessed by questionnaires in a structured interview by an independent person on the telephone at six different time points before, during and after the season (interview in the time of 1st–7th of every month: February, June, July, August, September, and October, always asking about the peak symptoms of the previous month). Scores were used according to the FDA recommendations on monitoring clinical symptoms in allergic diseases (<http://www.fda.gov/cder/guidance/index.htm>). Score 1: patients were asked to estimate symptoms in four grades (no = 0, mild = 1, medium = 2, strong = 3) for the last 4 weeks affecting eyes (itching, redness, discharge, and swelling), nose (itching, sneezing, congestion, and discharge) and lung (breathlessness, cough, wheeze, and tightness). Score 2: at the same time patients had to put down the degree of their complaints on an open scale ranging from 'no symptoms' to 'severe symptoms' (visual-analogue-score from 1 to 8) for each of the three organs. For every organ the grades from score 1 and the scales from score 2 were transformed into comparable numbers ranging from 0 (no symptoms) to 3 (severe symptoms). Then the symptom index (SI) for every organ was defined as the mean of the respective score 1 and 2. Score 3: use of medication and its frequency of applications during the time in question were documented. To not simply count the number of medications but to document the suppressive effects, which the different drugs would have on the severity of symptoms, we introduced a medication index (MI). The assumed mean-suppressive effect of drugs used in anti-allergic and anti-asthmatic treatment was calculated according to the clinical significance, which the national and international societies had given major drug groups in their guidelines. Table 1 shows the percentage of suppressive effects, which were used in our study for every group of drugs, for asthma also depending on the mode of application. The maximal long-term therapy was taken as a 100% standard of the special suppressive effect either for eye and nose or for lung symptoms. This standard is highlighted in the table.

To determine the MI, first the difference of 3 (highest possible SI) minus the individual SI is calculated. The MI is given as the

Table 1. Symptom-suppressive effect of drugs used in the context allergic rhinitis, conjunctivitis and bronchial asthma in percentage of maximal symptoms. Drugs are distinguished by local and systemic application and by 'on demand' and 'regular' prescription

Drugs	Local (%)		Systemic (%)	
	On demand	Regular	On demand	Regular
Chromones	5	10	—	—
Anti-histamines	10	12	10	15
α -Mimetics	15	20	—	—
β 2-agonists (only for lung)	15	15	20	15
Long-acting β 2-agonists	3	15	—	—
Anti-cholinergics	10	10	—	—
Theophylline	—	—	15	15
Montelukast	—	—	5	10
Corticosteroids (only for eye and nose symptoms)	25	45	—	—
Corticosteroids (only for lung symptoms)	10	20	10	25
Others	3	3	3	3
Maximal therapy				
For rhinitis/conjunctivitis	100			
For asthma	100			

Values in bold indicate drugs recommended for rhinitis/conjunctivitis symptoms and values in italics indicate drugs for asthma symptoms.

percentage (drug effect) of the above-mentioned difference (MI = % drug effect of 3 minus SI).

Finally the CI score (CI = SI + MI) was calculated as the individual SI from that organ (eye, nose, or lung) which showed the highest SI plus the MI. By adding the MI to the SI we described the 'true' symptoms a patient would expect to have without taking anti-allergic drugs. For further analysis CIs were either used as single values or for the calculation of the area under the curve (AUC) between June and August (mean of both values).

Skin prick test

Titred SPT were performed according to the standards of clinical diagnosis of allergies (9). Test solutions were provided by HAL-Allergy. SPT were performed with three different concentrations of grass pollen extract containing 100, 1.000, and 10.000 biological activity units (BAU) per milliliter; histamine dihydrochloride solutions at 1 and 0.1 and a 0.9% sodium chloride solution served as

controls. They were tested as duplicates on the volar surface of the forearms. After 15 min two diameters of the wheal reaction were measured. The area was calculated in mm^2 [(medium diameter/2) $^2 \times \pi$]. Calculation of sensitivity towards allergens was performed as described earlier (7).

Antibody tests

Blood samples were collected before the beginning of the treatment and 1, 2, and 3 years later. Sera were kept frozen at -20°C until the end of the study and then tested altogether in one experiment every year for each antibody class. Total IgE were measured by IgE-antibody ELISA (Euroimmun, Luebeck, Germany). The measurements of timothy grass pollen-specific IgE were performed with Specific IgE ELISA purchased from Allergopharma (Reinbek, Germany). IgG4 and IgG1 antibodies were determined by ELISA technique: Timothy grass pollen was extracted as described (10). 10 $\mu\text{g}/\text{ml}$ allergen extract (=1 $\mu\text{g}/\text{well}$) were coated onto microtiter plates (MaxiSorb, Nunc, Roskilde, Denmark) over night. Plates were blocked with 5% defatted milk in NaCl/Tris, pH 7.4 for 2 h, followed by incubation with patients' sera (diluted 1 : 3 and 1 : 6 in 2% defatted milk in TBS, pH 7.4) for 2 h at 37°C . After washing with 0.05% Tween in NaCl/Tris pH 7.4, the plates were incubated with the respective antibodies for 2 h at 37°C . The antibodies were alkaline phosphatase-coupled (AP). The dilutions were 1 : 4000 AP-anti-human IgG4, and 1 : 2000 for anti-human IgG1 (Pharmingen, Becton-Dickinson, Hamburg, Germany). After washing, bound conjugate was detected using 4-nitrophenyl phosphate substrate (Merck, Darmstadt, Germany). Reaction was stopped with 1 M NaOH and read at 405 nm on an ELISA reader (MR 7000, Dynatech, Billingham, England).

Statistical analysis

Statistical analysis was carried out using programs SPSS (SPSS GmbH Software, Munich, Germany) and SAS (Proc Mixed, SAS Version 8.0, SAS Institute, Cary, NC, USA). Unpaired data in Table 2 as well as Figs 1 and 2 were analyzed by Mann-Whitney *U*-test, the contingency data in Table 2 by chi-square test. For all tests significance level of 5% was chosen. Responsiveness as a rough measure for content validity of the score system was determined. Using visual analog score of the nose ranging from 1 to 8 as classification (see above), CIs of patients with severe (VAS-nose > 5) and without symptoms (VAS-nose = 1) were compared and differences were analyzed by Mann-Whitney *U*-test. Retest reliability was tested by calculating the coefficient of variation (CV) of the CIs

Table 2. Demographic data of all patients before the beginning of treatment

Variable	Placebo		Treatment		P
	i.t.t. (n = 78)	p.p. (n = 64)	i.t.t. (n = 83)	p.p. (n = 68)	
Age (years)	8.97 (± 2.71)	9.0 (± 2.8)	9.5 (± 3.1)	9.6 (± 3.2)	ns
Weight (kg)	30.6 (± 11.0)	30.5 (± 10.9)	35.4 (± 14.1)	36.6 (± 14.7)	ns
Length (cm)	133.6 (± 17.2)	134.2 (± 16.4)	138.9 (± 18.9)	139.6 (± 18.8)	ns
Total IgE (u/ml)	528.0 (± 686.0)	536.3 (± 721.2)	465.3 (± 668.9)	499.7 (± 727.3)	ns
slgE (u/ml)	35.7 (± 65.8)	36.6 (± 69.9)	33.8 (± 76.4)	38.4 (± 83.9)	ns
slgG4 (ru/ml)	19.8 (± 19.8)	19.0 (± 18.1)	24.8 (± 42.9)	26.4 (± 45.5)	ns
Diagnosis rhinitis	76	63	79	63	ns
Conjunctivitis	73	61	76	60	ns
Asthma	35	28	33	28	ns

i.t.t., intention to treat; p.p., per protocol; ns, not significant.

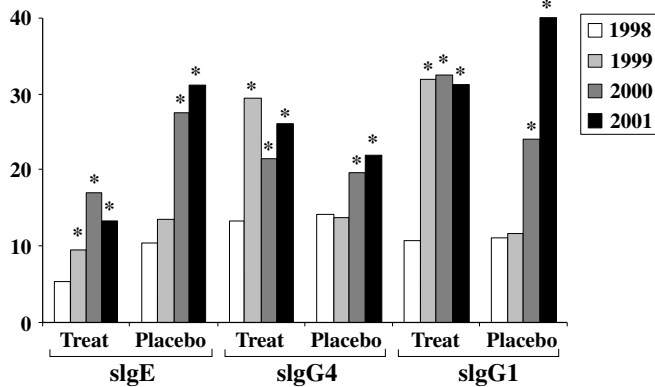


Figure 1. Mean of antibody titer before and during the course of treatment in both study groups (*indicates a statistically significant increase of antibody titer compared with 1998, before the beginning of treatment).

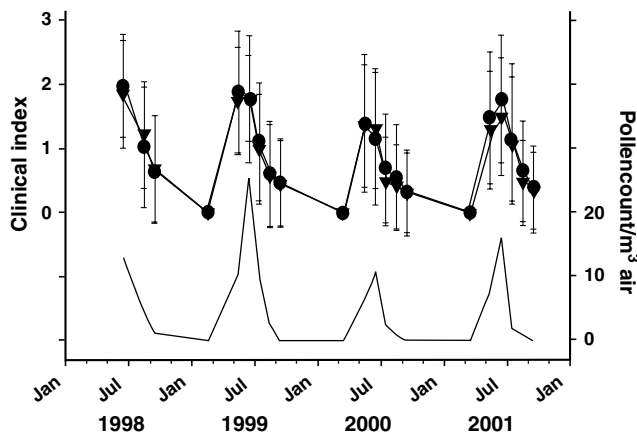


Figure 2. Change of allergic symptoms (AUC of CI) from 1998 to 2001. AUC of 2001 minus 1998 for all patients (per protocol), children with mild (AUC 1998 < 1.51) and severe (AUC 1998 ≥ 1.51) symptoms. Values for symptom reduction are: all-placebo CI = -0.27; (SI = -0.26); all-treatment CI = -0.55; (SI = -0.45); mild-placebo CI = -0.24 (SI = -0.21); mild-treatment CI = -0.25 (SI = -0.09); severe-placebo CI = -0.33 (SI = -0.30); severe treatment CI = -1.02 (SI = -0.98). *P*-values were calculated by Mann-Whitney *U*-test.

determined in patients with VAS-nose > 5 at two time points (June 1998 and August 1998). The reliability of the CI was calculated as described: the coefficient of reliability (r_H) is the squared standard deviation of the true values (s_{VAS}^2) divided by the squared standard deviation of total values (s_{CI}^2). The standard error (S_e) equals the standard deviation (s_{CI}) multiplied with square root of 1 minus coefficient of reliability (r_H).

For additional subgroup analysis efficacy of SLIT was assessed by a repeated measures model (ANOVA). This procedure can be used to estimate the effect of several independent variables such as treatment, severity etc. both directly on the CI as the dependant variable as well as on its change over time. The relative influence of each independent on the dependant variable is described by the variance of the CIs among patient groups with different levels of this independent variable compared with the total variance of CI. As

treatment was suspected to be more effective in severe cases three independent variables were included in this repeated measures model: the treatment group of patients (0, placebo; 1, verum), the baseline severity of allergic symptoms in 1998 (0, CI below mean CI; 1, CI above mean CI, that is > 1.51) and finally the presence of asthma as a possible confounder (0, no asthma; 1, asthma). In addition the possible interaction between the independent variables 'severity' and 'treatment' was analysed.

The calculations were performed for two different sets of dependent variables: the complete set of CI measurements at all four time points (model 1) and the score at baseline and after 3 years of treatment (model 2).

Results

Patients

A total of 132 of 161 enrolled patients completed the study per protocol (18% dropped out). Fifteen patients withdrew during the first year (seven from placebo and eight from treatment group) whereas 14 patients had to be excluded for poor compliance (seven placebo and seven treatment group). No systemic anaphylactic reactions were observed, one patient showed an urticaria reaction in his face, 17 patients had a mild oral allergy syndrome. The remaining patients completed the study after 1 year (placebo $n = 64$ and treatment group $n = 68$). Before the beginning of treatment both groups were matched for age, weight, length, diagnosis, total IgE, specific IgE, and specific IgG4-independent for drop out (Table 2). In the second treatment year a further 29 patients dropped out (18%; 17 former placebo and 12 former treatment). For those patients with CIs between 0.75 and 1.875 the drop-out rate in the placebo group after 1 year of SLIT was significantly higher than in the treatment group ($P = 0.032$). In the third year no further drop out was observed.

Evaluation of the CI score

As a modification of the usually applied symptom scores was introduced with the CI, it was evaluated for responsiveness and retest reliability. Responsiveness as a rough measure for content validity showed significant difference between groups of patients with severe and without symptoms, as determined by Visual Analogue Scale (see Methods): severe symptoms group in June 1998 had a mean CI of 2.29 (± 0.36 SD) vs no symptoms group February 1999 with a mean CI of 0.15 (± 0.39 SD; $P < 0.0001$). The coefficient of variation (CV) as a measure for retest reliability was 15.7% for patients with severe symptoms in June 1998 and 20% in August 1998. This resulted in a coefficient of reliability for the CI of 0.89, indicating a standard error of 0.29 CI, which was equivalent to 9.6%. This demonstrates that results of the CI score vary in an acceptable range for a subjective symptom measurement system and that changes of CI notably > 0.29 or 9.6% indicate actual differences.

Antibody induction and SPT

First it was important to determine whether the local application of allergen extracts led to a measurable immune response on the level of specific antibody production. As Fig. 1 shows, children in the respective treatment groups had a significant increase in specific IgG4 and IgG1 titers 1 year after the onset of treatment. This could also be seen in patients of the placebo group who received treatment in the open controlled phase 1 year later. In the second and third year of treatment both IgG subclasses remained elevated. Interestingly, also specific IgE increased during treatment, indicating that local application of allergens led to a general activation of specific adaptive immune response. No significant changes in SPT reactivity after treatment were seen (data not shown).

Effect of SLIT

Results of CIs (primary endpoint) are shown in Fig. 3. In both groups CIs increased parallel to pollen counts, indicating that patient's symptoms were related to grass pollen concentration in the air. By analyzing the course of CIs during the three seasons no difference between placebo and treatment group was seen. This demonstrated that according to the original design of the study SLIT did not show clinical efficacy.

Subgroup analysis

Further analysis of the data revealed that severity of allergic symptoms could be a confounder for effectivity of SLIT. The percentage of patients with CIs above 2.5 after 1 year of SLIT turned out to be significantly lower in the

treatment compared with the placebo group (data not shown). This effect was not recognized when calculating the mean CIs.

Therefore baseline CI distribution before treatment was used to define the following subgroups: a severe symptoms group with CIs above and a mild symptoms group with CIs below mean CI of 1.51. Figure 3 shows the change of symptoms measured by CI before and after 3 years of treatment in all patients and those with severe as compared with mild symptoms. These results indicate that after 3 years SLIT has an observable effect of 30% improvement in patients with severe symptoms whereas the comparable placebo group improves by only 10%. After 1 year of treatment no significant improvement compared with placebo was observed (data not shown). To strengthen this finding a standard repeated measures model (Table 3) was calculated on an intention to treat basis, analyzing the influence of different independent variables such as treatment, the diagnosis of asthma, severity of symptoms and the interaction of both treatment and severity on the course of symptoms. The variables asthma and severity have a significant influence on the CI in general, an expected effect because symptoms of patients with asthma are stronger than in patients only suffering from rhinoconjunctivitis. As severity depends itself on the CI, it has to be significant in the repeated measures model. The influence of the treatment variable on general CI in model 2 indicates that the variances of CIs for placebo and verum were different although patient groups were randomized. When analyzing the change of CI instead of general CI in the repeated measures model this effect does not occur. Furthermore, while no significant influence of the therapy can be shown in the model including all four time points (model 1), the effect of treatment on the severe symptoms group can be

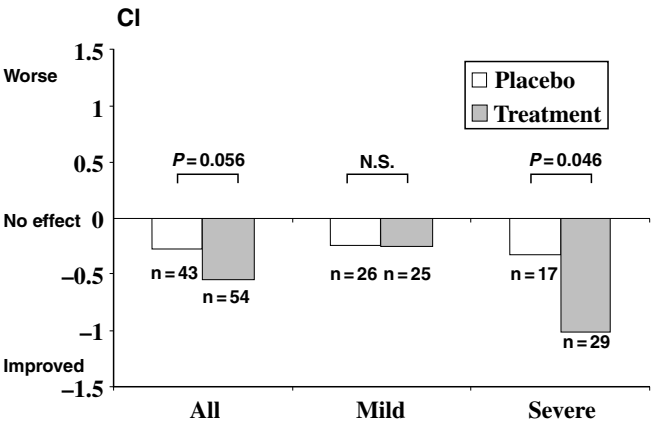


Figure 3. Course of clinical index (mean $\pm$ SD) during 4 years in treatment group (closed triangles) and placebo group (closed circles). Pollen counts (pro m³ air) in Germany are given as a mean of all measurement centres ($n = 14$).

Table 3. Repeated measures model for the reduction of allergic symptoms (CI). Calculation of anova (see methods). Variables included are treatment, diagnosis of asthma, severity of symptoms before beginning of treatment (severe = CI > 1.51) and the combination of treatment and severity. *F*-statistic indicates the ratios of variances between the respective variable and the effect on the CIs. All calculations as intention to treat ($n = 161$)

Variables	All four time-points ($n = 161$) model 1		Only 1998 and 2001 ($n = 161$) model 2	
	<i>F</i> -statistic	<i>P</i> -value	<i>F</i> -statistic	<i>P</i> -value
Effect on CI				
Treatment	2.5	0.115	6.0	0.016*
Asthma diagnosis	8.2	0.005*	5.5	0.021*
Severity	80.2	< 0.001*	83.1	<0.001*
Treatment and severity	1.8	0.181	5.1	0.026*
Effect on change of CI				
Treatment	1.0	0.397	2.0	0.163
Asthma diagnosis	1.3	0.275	2.0	0.156
Severity	12.5	< 0.001*	52.5	<0.001*
Treatment and severity	1.9	0.136	4.5	0.037*

* indicates statistical significance of $P < 0.05$.

seen in the two-time point variant (model 2, effect of treatment and severity on CI-change). However, no severity-independent effect of treatment can be seen in either case. The same calculation using patients per protocol leads to nearly the same results but is statistically not significant and therefore only a trend (data not shown). Asthma diagnosis as a further independent variable for severity shows no influence. Further confounders such as mite sensitization were not different between the two groups (19% in treatment vs 26% in placebo). In summary, subgroup analysis revealed that a significant effect of SLIT in this study could only be seen in patients with severe symptoms after 3 years whereas in children with mild symptoms a marked placebo effect is predominant.

Discussion

Sublingual swallow immunotherapy is readily given to allergic patients in European countries including Germany, France, Italy, Spain, and England, but only in a small number of studies it has been proven to be effective in children (2, 6, 11, 12). The study presented here demonstrates that SLIT with grass pollen extracts given in a dosage 10 times the amount for subcutaneous application can only improve the course of allergic diseases in a subgroup of severely allergic patients.

In contrast to many other studies which analyzed clinical symptom scores and the use of medication (5) separately we combined the effect of symptomatic therapy with the given complaints. We thus measured the expected symptoms one would see in case of no treatment at all. Our procedure enabled us to analyze the influence of SLIT on the natural course of allergic symptoms and consequently CIs turned out to show generally higher scores than the SI alone. Furthermore it could be demonstrated that the CI, which was used as the primary outcome had good responsiveness and an acceptable retest reliability and standard error.

Using this endpoint no clinical efficacy of SLIT during 3 years according to the original design was observed, at first sight suggesting that SLIT is no effective treatment. When reanalyzing the data in more detail a significant therapeutic effect of SLIT could be discovered after 3 years when patients had severe symptoms (CI above the mean) before the beginning of treatment. This difference was still present but lost significance when only symptoms without medication scores were considered. Three important aspects may have led to this observation: first combining symptoms and medication indices may increase sensitivity of symptom measurements, explaining why in other studies on local immunotherapy the use of medication has often been more indicative for actual improvement than the symptom scores themselves (6). Secondly, severity of symptoms is a confounder when using subjective clinical markers as an end point in

clinical trials because, as seen in the repeated measures model, patients with severe symptoms show generally greater improvement than those with mild symptoms. In the future clinical studies should take this observation into consideration. However, as the subgroup analysis involves special cases of a nonsignificant general effect, the statistical significance should be handled with care. Finally, the standard error of the CI score may hide a possible positive effect of SLIT on a mild symptoms level. In this context it is important to note that a significant number of children in the placebo group improved without treatment. This reflects the well-known placebo effect which is seen in many other immunotherapy studies (13) and for which the reason is still not understood.

In the present study SLIT showed clinical efficacy in patients with severe symptoms only after 3 years and this effect amounted to about 30% improvement. With these results this study is in the range of what was observed by other authors after 1 year performed mainly with adults (2, 6, 11, 12). It also demonstrates that the effect of SLIT in this study is rather weak, although long-term efficacy might have been more obvious if the placebo phase in this study had been longer. Still, this observation is consistent with data of studies which have shown substantial improvement induced by local immunotherapy only after a longer period of time (14, 15).

It is important to analyze the specific immune response during immunotherapy because only a significant elevation of IgG antibodies would prove a specific effect of the given allergen extract. A highly significant increase of specific anti-grass pollen IgG4 and IgG1 antibodies in the treatment group can be demonstrated. But it has to be noticed that the IgG-subclass elevation in local immunotherapy by average is only a tenth of what is seen in subcutaneous application (7). There is evidence that the change of allergic inflammation correlates to the differential elevation of IgG-subclasses under specific immunotherapy (16, 17). It is yet unknown whether the changes of IgG-subclass antibodies are only indicators for a certain immune response induced by high dose specific allergens or whether they are directly involved in the suppression of the allergic response.

In the present study those children who clinically improved by SLIT did not show a significant reduction of sensitivity in SPT. Once more this demonstrates that the allergic reaction in the skin is not directly related to the immune response seen by the increase of the specific antibodies.

In conclusion, this study shows clinical efficacy of local immunotherapy in a group of children with severe clinical symptoms only after 3 years. No dangerous systemic side effects must be expected with SLIT using grass pollen allergens. Local application of allergens is comparable with systemic treatment in immunologic terms. Only long-term treatment and comparative studies will show whether local immunotherapy can compete with the established subcutaneous treatment.

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