Allergen-specific immunotherapy with recombinant grass pollen allergens

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Background: Allergen-specific immunotherapy uses aqueous extracts of natural source materials as a basis for preparations to downregulate the allergic response. Recombinant DNA technology has enabled the cloning of many allergens, thus facilitating investigations aimed at improving efficacy and safety of immunotherapy.

Objective: To determine the effectiveness of a mixture of 5 recombinant grass pollen allergens in reducing symptoms and need for symptomatic medication in patients allergic to grass pollen.

Methods: A randomized, double-blind, placebo-controlled study of subcutaneous injection immunotherapy was performed in subjects with allergic rhinoconjunctivitis, with or without asthma. Primary endpoint was a symptom medication score compiled from separate symptom and medication scores. Secondary endpoints included a rhinitis quality of life questionnaire, conjunctival provocation, and specific antibody responses.

Results: The symptom medication score showed significant improvements in subjects receiving recombinant allergens as opposed to placebo, with reductions in both symptoms and medication usage. The rhinitis quality of life questionnaire revealed clinically relevant significant improvements in overall assessment and in 5 of 7 separate domains, and conjunctival provocation showed a clear trend in favor of active treatment. All treated subjects developed strong allergen-specific IgG_1 and IgG_4 antibody responses. Some patients were not sensitized to Phl p 5 but nevertheless developed strong IgG antibody responses to that allergen.

Conclusion: A recombinant allergen vaccine can be a effective and safe treatment to ameliorate symptoms of allergic rhinitis. The clinical benefit is associated with modification of the specific immune response with promotion of IgG_4 and reduction of IgE antibodies consistent with the induction of IL-10–producing regulatory T cells. (J Allergy Clin Immunol 2005;116:608-13.)

Key words: *Allergic rhinitis, allergy, allergen, immunotherapy, recombinant allergens, grass pollen*

The incidence of allergic diseases is increasing, and associated socioeconomic burdens place them among the most important chronic diseases. Allergen-specific immunotherapy is effective in ameliorating the symptoms of allergic disease. Controlled clinical studies with grass pollen in rhinoconjunctivitis have shown that both symptoms and the need for medication can be effectively reduced.[1-5] Benefits can be maintained for at least 6 years after discontinuation of treatment,[6,7] which can also have preventative effects on development of new sensitizations and asthma.

Therapeutic vaccines are produced from extracts of natural source materials such as grass pollen. Eleven different grass pollen allergens have been identified.[8,9] Their relative concentrations in an extract differ, reflecting the composition of the raw material, and their relative importance varies from patient to patient. Extracts also contain numerous nonallergenic proteins that are not thought to be relevant to the treatment. Recombinant DNA technology enables allergens to be produced to high pharmaceutical standards, resulting in preparations with improved quality in terms of purity, consistency, composition, and dosage. Theoretically, there is also the possibility to formulate vaccines to include only the most relevant allergens in defined concentrations and to match them to allergen sensitizations of individual patients.[10]

Phleum pratense (timothy grass) is representative of grasses found in to temperate regions. It belongs to the subfamily *Pooideae*, the members of which show very substantial allergenic cross-reactivity. The allergens most frequently inducing sensitization and high specific IgE concentrations are groups 1 and 5, exemplified by Phl p 1 and Phl p 5 of *Phleum pratense*, with 90% and 65% to 85% sensitization rates, respectively. Some subjects show reactivity to only 1 of 2 isoforms of Phl p 5 (Phl p 5a and Phl p 5b). The Phl p 2 and Phl p 6 allergens are reactive in 40% to 60% and 60% to 70% of subjects allergic to grass pollen, respectively.[8,9]

Here we have used a mixture of 5 *Phleum pratense* allergens in approximately equimolar concentrations in a

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Abbreviations used
BU: Biological unit
CPT: Conjunctival provocation test
GINA: Global Initiative for Asthma
RQLQ: Rhinitis quality of life questionnaire

pilot placebo-controlled immunotherapy study with patients with grass pollen–induced allergic rhinitis, with or without asthma, with the objective of determining efficacy and safety.

METHODS

Study design

A randomized, double-blind, placebo-controlled study was performed in Wroclaw Medical University, Poland, with approval of the local ethics committee and the Medical Council for Schleswig-Holstein, Bad Segeberg, Germany. Subjects provided informed written consent, and the study was conducted in accordance with Guidelines for Good Clinical Practice.[11] The study physician allocated treatment sets previously randomized and labeled by the manufacturer using computer generated random number tables. The code was concealed until all data-related queries had been resolved at the end of the study. Treatment commenced in January 2002 and continued until August/September 2003. The dosage was increased progressively with 10 subcutaneous injections at 7-day intervals. Once the maximum or maintenance dose had been achieved, the interval was increased stepwise to 14, 28, and finally 42 days. The maintenance dose was reduced by 50% during the pollen seasons.

Subjects

Subjects were recruited who had a history of grass pollen–associated moderate to severe seasonal allergic rhinitis, with or without asthma (Global Initiative for Asthma [GINA] step 1 and 2),[12] that required medication during the previous pollen season. Sensitization was confirmed by skin prick test (weal $\geq$ 3 mm) and specific IgE determination (RAST-CAP $\geq$ 2) and clinical relevance of grass pollen by a conjunctival provocation test (CPT). Subjects with clinically relevant sensitizations to pollen of midspring flowering trees (birch, oak, beech, and plane tree) and perennial allergens including mites, cat, dog and molds were excluded. Further exclusion criteria were unstable bronchial asthma or GINA steps 3 and 4, generalized eczema, severe atopic dermatitis, other severe acute or chronic diseases, or allergen-specific immunotherapy with grass or a cross-reacting allergen within the last 3 years.

Immunotherapy preparations

The 5 grass pollen allergens were cloned from a cDNA expression library derived from pollen of *Phleum pratense* (timothy grass), and the respective cDNAs were subcloned into appropriate expression vectors and expressed in *Escherichia coli*. Recombinant allergens were purified by using various chromatographic techniques including hydrophobic interaction, ion exchange, and size exclusion, and analyzed to confirm identity and purity.[13] Total endotoxin content of the maximum dose of the study preparation corresponded to 0.43 endotoxin units, 20 to 180 times less than mean corrected values of natural timothy extracts.[14] Proteins were adsorbed to aluminium hydroxide to achieve a depot effect and enhance processing by antigen-presenting cells. Adsorbates of the 5 allergens were combined in approximately equimolar amounts and supplied in 3 dilutions. The highest concentration (strength 3) contained 50 μg/mL total protein. The initial dose contained 0.02 μg total protein. The dose was increased to 0.16 μg in the second injection and then doubled at subsequent injections to a maximum of 40 μg total protein (0.8 mL) 10 μg Phl p 1 (=0.38 nmoles), 5 μg Phl p 2 (=0.48 nmoles), 10 μg Phl p 5a (=0.35 nmoles), 10 μg Phl p 5b (=0.38 nmoles), and 5 μg Phl p 6 (=0.42 nmoles), together with 1 mg/mL Al^{3+} in physiological saline. A matching placebo contained aluminium hydroxide and histamine dihydrochloride (0.125 mg/mL in strength 3), which was included to assist in blinding the study.

Procedures

Symptom Medication Score in the second year of the study was the primary outcome measure to assess efficacy. Subjects kept diaries for 3 months over each grass pollen season to record nature and severity of symptoms and type and dose of any medication. Symptoms considered were eyes (itching, tear flow, redness); nose (sneezing, running, blocked); and chest (cough, wheezing, asthma/dyspnea). Intensity was documented as 0 = no symptoms, 1 = mild, 2 = moderate, and 3 = severe. All subjects had access to the same rescue medication. Only short-acting and not prophylactic basic medication was scored on a daily basis: topical antihistamines (levocabastine), disodium cromoglycate, and topical α-mimetic (0.5% oxymetasoline), 1 (0.5 per nostril); oral antihistamines (loratadine), 6; nasal steroids (budesonide), 3; β-mimetic (salbutamol), 1; inhaled corticosteroid (budesonide), 6; and oral corticosteroid (prednisolone), 4 per 5 mg. Subjects were instructed to use short-acting topical medication as a first-line treatment, oral antihistamine only when more severe symptoms occurred, and nasal steroids only under exceptional circumstances. Asthma symptoms were to be treated with a short-acting bronchodilator, and inhaled steroid treatment was to be administered constantly when appropriate and scored only when dosage was changed. Final evaluation was based on a 42-day period encompassing the main pollen exposure, starting 15 days before and ending 26 days after the maximum pollen count. A validated rhinitis quality of life questionnaire (RQLQ)[15] was a secondary endpoint and was completed by subjects during the baseline visit, before each pollen season, and every 2 weeks during the seasons. The questionnaire after the maximum pollen count was used for analysis.

CPTs were performed before therapy (inclusion criterion) and after the second grass pollen season (2003) by using a standardized lyophilized 6-grass allergen extract containing *Phleum pratense*, *Poa pratensis*, *Lolium perenne*, *Holcus lanatus*, *Dactylis glomerata*, and *Festuca pratensis* (Allergopharma Joachim Ganzer KG, Reinbek, Germany). Subjects were free of any infectious disease or allergic symptoms and had discontinued antiallergic agents, oral or inhaled, 1 week before and eye drops 24 hours before. Initial test concentration was 5 biological unit (BU)/mL (1 drop), and this was increased in half-log steps until a positive reaction was obtained or a maximum concentration of 5000 BU/mL (1 drop), corresponding to approximately 0.18 μg group 5 allergen/drop, was reached.

Blood samples were collected during the screening visits, before and during each grass pollen season, and at the end of each study year. Serum was stored at −20°C before antibody assay. *Phleum pratense* specific IgE antibodies were measured by using the Allervance system (Allergopharma Joachim Ganzer KG). Specific IgG_1 and IgG_4 antibodies were detected by using microtiter plates coated with either *Phleum pratense* pollen extract 10 μg/mL (Allergopharma Joachim Ganzer KG), natural Phl p 1 (1 μg/mL), natural Phl p 5a/b (1 μg/mL) or monoclonal anti-IgG (5 μg/mL), and anti-IgG_4 (0.5 μg/mL), respectively (BD Biosciences, Heidelberg, Germany) in 0.05 mol/L carbonate buffer, pH 9. Antibody-coated wells were incubated with purified IgG_1 or IgG_4 (Sigma-Aldrich, Taufkirchen, Germany) as references, with concentrations of 4 to

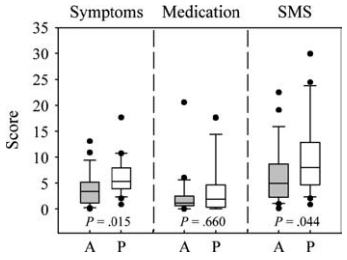

FIG 1. Symptom scores, medication scores, and symptom-medication scores (SMS) (per protocol set). Evaluation based on a 42-day period encompassing the main pollen exposure, starting 15 days before and ending 26 days after the day with the maximum pollen count. Median values with 25th, 75th *(boxes)*, 10th, and 90th *(error bars)* percentiles, and outliers *(points)*. *A,* Active treatment *(gray bars)*; *P,* placebo treatment *(white bars)*. Mann-Whitney *U* test.

2000 μg/L, and allergen-coated wells with serum samples diluted at least 1:2. Biotinylated anti-IgG_1 and anti-IgG_4 (BD Biosciences; 1 μg/mL) and alkaline phosphatase–labeled streptavidin (Sigma-Aldrich; 1 μg/mL) with para-nitrophenylphosphate as substrate were used for detection. Plates were read at 405 nm after 15 minutes of substrate incubation.

Grass pollen (*Poaceae*) counts were conducted on a daily basis between May 1 and July 30 in Wroclaw in each year of the study. Counts were expressed as *Poaceae* pollen grains per cubic meter.

Statistical analysis

It was planned to include 80 subjects, anticipating a 15% dropout and a target 30% difference in combined symptom medication score. Data from a study with a natural allergen preparation indicated a power of approximately 60% for a 2-sided *U* test, which was considered adequate for a pilot proof-of-concept study. Data management was undertaken by using SPSS Data Entry 3.0 and statistical data analysis with SPSS 10.0 for Windows (SPSS Inc, Chicago, Ill). Global assessments of clinical efficacy in terms of symptom, medication, and combined scores were tested by applying the hypothesis of differences between the mean area under the curve in both groups with a confirmatory 2-sided Wilcoxon-Mann-Whitney *U* test, assuming a significance level of 5%. Analysis of the full data set required that missing diary data from 2003 for 5 active treatment and 3 placebo subjects was compensated for by including the data from 2002. These 8 subjects were excluded from the per protocol data set. The 2-sided Wilcoxon-Mann-Whitney *U* test was applied in analysis of all other parameters. Figures were prepared by using SigmaPlot 8.0 (SYSTAT Software GmbH, Erkrath, Germany).

RESULTS

A total of 64 subjects was enrolled, and 62 were assigned randomized treatment sets and included in the safety evaluation. Five subjects were subsequently excluded from the main data set because more than 25% of diary entries were missing, making assessment of the primary endpoint impossible, thus leaving 29 active treatment and 28 placebo subjects in the full analysis set. Groups were well matched for age (25 years; 21-30, interquartile range; vs 24.5 years; 22-26.5) and sex (8 F/21 M vs 12 F/16 M). The median duration of allergy symptoms was 7 years (5-15) and 11 years (5.25-16.75), respectively, and median diameter of skin prick test responses was 10 mm (7.75-12.63) and 12 mm (9.25-13.75). Five subjects in each group had GINA 1 asthma, and 1 in the active group was diagnosed GINA 2. Eight subjects were excluded from the per protocol population because diary data was not available for the second pollen season.

The grass pollen counts exceeded 20 grains/m^3 (high) on 46 days in 2002, and 50 grains/m^3 (very high) on 21 of those days. In 2003, the count exceeded 20 on 28 days and 50 on 16 of those days.

The maximum dose achieved did not differ between the groups, with 27 of 31 (87.1%) of subjects on active treatment and 28 of 31 (90.3%) on placebo receiving 0.8 mL of strength 3. The median cumulative dose was 490 μg total protein, or 122.5 μg each of Phl p 1, Phl p 5a, and Phl p 5b, and 61.25 μg of Phl p 2 and Phl p 6. A total of 1479 injections were administered, 731 active treatment and 748 placebo. The median number of injections per subject in each group was 25. A total of 153 adverse events were recorded, 94 with active treatment and 59 with placebo, of which 78 (10.7% of 731 injections) and 44 (5.9% of 748 injections), respectively, were treatment-related. Local reactions involving erythema and swelling with or without pruritus in the vicinity of injection sites accounted for 71 of 78 and 42 of 44 reactions, respectively. The 7 systemic reactions (0.96% of injections) observed with the active preparation included 1 general urticaria, treated intravenously with 200 mg hydrocortisone and 2 mg clemastine, 1 general urticaria together with dyspnea, 2 cases of local urticaria of upper extremities treated with cetirizine and loratadine, 1 rhinoconjunctivitis, and 1 asthma exacerbation 2 days after an injection treated intravenously with 200 mg hydrocortisone and 250 mg aminophylline. The reactions occurred in different individuals, 5 of 7 in the updosing period and 2 with the maximum dose. All abated without consequence, and all subjects continued therapy without further problems.

Evaluation of symptoms alone for the per protocol set showed a 36.5% lower median average symptom score for active treatment compared with placebo (3.38 vs 5.32; $P = .015$). There was also a reduction in the need for medication, reflected in a 36.5% lower medication score. The combined symptom medication score showed a significant difference between groups after the 18-month treatment period, with median average daily scores of 4.92 versus 8.05 for active and placebo treatment, respectively, a difference of 38.9% ($P = .044$; Fig 1). Evaluation of the full data set revealed symptom medication scores of 4.6 versus 7.5, a difference of 38.5%, which just failed to reach statistical significance ($P = .051$).

The RQLQ was evaluated for 7 domains and also as a whole (28 questions). Assessment completed in the second pollen season (2003) showed significant and clinically relevant differences in favor of active treatment for the whole questionnaire and 5 of 7 domains (Fig 2). These differences were improvements on those already seen in the first pollen season. All subjects fulfilled the inclusion criterion of a positive CPT, and the 2 study groups were

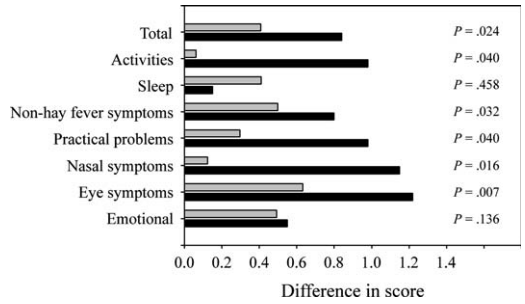

FIG 2. Rhinitis quality of life questionnaire: differences between the means for the 2 treatment groups in the first and second year (full analysis set). The questionnaires completed following the maximum pollen counts in 2002 *(gray bars)* and 2003 *(black bars)* were used for analysis. Mann-Whitney *U* test and *P* values for 2003 season.

TABLE I. Conjunctival provocation responses before and at the end of the study

		Response at final assessment			
	Response at baseline assessment	500 BU/mL	1600 BU/mL	5000 BU/mL	Negative
Active	500 BU/mL	1	0	2	1
	1600 BU/mL	0	2	9	5
	5000 BU/mL	0	0	4	4
Placebo	500 BU/mL	2	2	0	1
	1600 BU/mL	1	6	5	3
	5000BU/mL	0	1	2	3

Gray cells indicate patients whose threshold responsiveness did not change.

well matched in terms of threshold responses. At the end of the study, there was a clear trend to a higher threshold allergen dose, although this was not statistically significant ($P = .081$). In the active treatment group, 21 of 28 patients tolerated higher allergen concentrations, as opposed to 14 of 26 in the placebo group, 13 by 1 allergen concentration step, 7 by 2 steps, and 1 by 3 steps (Table I).

Active treatment induced increases in both IgG_1 and IgG_4 *Phleum pratense* specific antibody concentrations (Fig 3). IgG_1 concentrations increased approximately 60-fold, peaking during the first 12 months of the study. IgG_4 concentrations showed a continuing upward trend, achieving an approximately 4000-fold increase by the end of treatment. Comparisons between the groups showed statistically significant differences at all time points after the commencement of immunotherapy ($P < .001$).

Specific IgE levels were not significantly different between groups at the beginning of the study (sample 1), but thereafter, those of the active treatment group were significantly less than placebo. Concentrations showed a downward trend, with values significantly less than baseline (Fig 3).

All subjects in the active treatment group showed specific IgG isotype responses to natural Phl p 1 and Phl p 5a/b. IgG1 responses peaked before the first pollen season and declined very slightly thereafter, whereas IgG_4

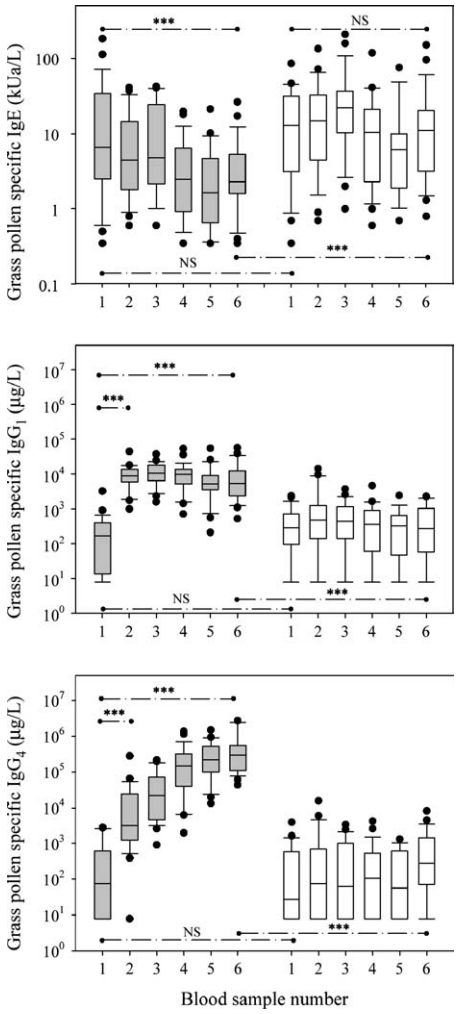

FIG 3. Grass pollen specific IgE, IgG_1, and IgG_4 antibody concentrations (full analysis set). Median values with 25th/75th and 10th/90th percentiles represented by *boxes* and *error bars*, respectively, and outliers by *points*. Active *(gray bars)* and placebo *(white bars)* groups. Time points: *1,* before immunotherapy, 1/2002-2/2002; *2,* after initial dosage increase and before pollen season, 4/2002-5/2002; *3,* after the pollen season, 7/2002-9/2002; *4,* after 12 months, 1/2003-3/2003; *5,* before the pollen season, 4/2003-5/2003; *6,* at the end of the study, 8/2003-9/2003. ***$P < .001$; *NS,* nonsignificant.

increased progressively to a maximum before the second pollen season. Responses to Phl p 5a/b were stronger than those to Phl p 1, with medians 290 and 23 µg/mL, respectively, for IgG_4, and 13 and 2.1 µg/mL for IgG_1. Four subjects in each study group had no Phl p 5a/b specific IgE before the study but had IgE antibodies to Phl p 1 and other grass pollen allergens. None of these subjects developed Phl p 5a/b IgE antibodies during the study, although 4 subjects receiving active treatment developed strong IgG_4 and IgG_1 Phl p 5a/b responses. IgG_4 was not detectable before therapy but reached a median concentration of 282 µg/mL serum (interquartile range, 274-621) during treatment. Specific IgG1 concentration peaked in the second serum sample at 26.7 µg/mL (8.18-58.99).

DISCUSSION

This first clinical study of immunotherapy using a cocktail of 5 recombinant grass pollen allergens for the treatment of hay fever has demonstrated the clinical efficacy and good tolerance of the preparation, together with the induction of strong allergen-specific IgG antibody responses.

Allergic patients have both the clinical manifestations of their disease and the burden of having to take antiallergic medication.[16] The combined symptom medication score took account of both considerations and showed a statistically significant clinical improvement with active treatment by comparison with placebo in the second grass pollen season, with a relative difference of 38.9%. This result is of the same order as the mean 45% additional improvement in disease severity above the response to placebo seen in 43 rhinitis studies including 1120 actively treated patients.[17] Furthermore, the improvement compares favorably with results for oral antihistamines and topical corticosteroids, particularly when taking into account the well recognized large placebo effect associated with immunotherapy.[18,19]

The RQLQ provides a new tool for assessing the clinical efficacy of immunotherapy,[15] although it has the disadvantage that it cannot be corrected to take account of the contribution of symptomatic medication. Statistically significant differences between active and placebo groups support the symptom and medication score findings. Improvements in excess of 0.5 for individual subjects are considered clinically relevant,[20,21] and the mean difference of 0.84 in the total score between the study groups is in line with such improvement, as are differences of 1.15 and 1.22 for nasal and eye symptoms.

Conjunctival provocation has been shown to be an effective diagnostic method, even in those patients who have symptoms of rhinitis without conjunctivitis,[22] and changes in this objective measure have been seen in several immunotherapy studies as indicative of changes in sensitivity.[5,7,23,24] The trend for increased tolerance observed in this study serves to substantiate the clinical findings, although the fact that changes were not significant suggests that CPT may not be the best marker. Whereas CPT is dependent on induction of IgE-dependent immediate responses, the clinical improvement in allergic rhinitis is attributable to suppression of inflammatory mechanisms. IgE synthesis is promoted through activation of T_H2 cells, and inadequate T-regulatory cell (Tr1) activity is probably a crucial factor in the development of the allergic phenotype.[25,26] Specific immunotherapy results in a deviation in the T lymphocyte response and a modified T_H2 response. An increase in T-regulatory cells (Tr1) contributes to this process, and their production of IL-10 and TGF-β directly favors a suppression of IgE production and a simultaneous increase in IgG_4 and IgA antibodies, respectively.[27-30] The moderate downward trend in specific IgE seen over the course of the study is probably not an important mechanism in immunotherapy, because the clinical improvement is seen much sooner. The increase in IgG_4 antibodies is indicative of a normal immune response,[29,31] and the increase in allergen-specific IgG_1 clearly reflects the immunogenic activity of the therapeutic preparation. The fact that the nonglycosylated recombinant Phl p 1 molecule elicits IgG antibodies that are reactive with natural Phl p 1 indicates that its immune competence is essentially intact. Although various studies propose roles for blocking antibodies, data are derived largely from *in vitro* studies, and a physiological role has yet to be convincingly demonstrated.[32]

Some subjects in the active treatment group had no Phl p 5a/b specific IgE at the outset of the study and failed to develop IgE antibodies despite repeated injections of the allergen, but nevertheless, they developed IgG_1 and particularly IgG_4 responses. It has been suggested that subjects without specific IgE against a particular allergen fail to mount a significant IgG_4 response,[33] but our results do not support this view and are consistent with induction of a tolerant immune response. Furthermore, suppression of the IgE response speaks against the development of new sensitizations reported by others.[34]

A favorable safety profile was demonstrated. The majority of the reactions involved transient erythema and swelling in the vicinity of the injection sites consistent with allergic responses or possibly mild trauma caused by the aluminium hydroxide suspension. Reactions to placebo were probably attributable in large part to histamine included to mimic allergen-induced reactions. Systemic reactions were isolated in nature, and the fact that all subjects continued therapy with either the same or higher doses without further problems indicates that the preparation is generally well tolerated. Grass pollen immunotherapy in a study with 47 subjects was shown to induce systemic reactions including asthma and urticaria in 45% of cases and in association with 3.3% of injections.[35] The dosage schedule was more aggressive than that used in the current study, which was in turn less conservative than conventional schedules used with most aqueous and depot allergen preparations. In a study of 628 patients receiving conventional immunotherapy, 7% had a systemic reaction within 6 hours of an injection, with generalized pruritus and urticaria most common.[36] A prospective study with 488 subjects showed systemic reactions in only 3.7% of subjects in association with 0.3% of injections,[37] but the diversity of allergen preparations from different sources and lack of a common standardization make direct comparison difficult.

Sensitizations to the group 1, 5a, and 5b allergens are the most prevalent and potentially most clinically relevant,[8,9] and here we proved that a cocktail containing these and 2 additional allergens is sufficient to achieve clinical benefit in subjects allergic to grass pollen. The precise dosage formulation possible with recombinant allergens has to be seen as a distinct advantage over natural extracts. There is a possibility that some patients might gain additional benefit from the inclusion of further allergens, but whether it is worthwhile will have to be determined. The most prevalent sensitization profiles should be

identified and allergen combinations established to treat the majority of patients allergic to grass pollen. For selected individuals, the concept of patient-tailored diagnosis and allergen-specific immunotherapy may be appropriate.[38]

A larger study has now been initiated in an attempt to substantiate the current data on both safety and clinical efficacy and to obtain more detailed immunological data to confirm the adequacy of the allergen mixture and provide further insight into the mechanisms of the immunotherapy.

Clinical coinvestigators were Dr M. Wrzyszcz, Dr D. Kuliczkowska, Dr E. Liebhart, Dr A. Dor, Dr J. Miecielica, Dr A. Gawlik, Dr K. Solarewicz, and Dr K. Gietkiewicz. Dr B. Weber, PhD, Ms C. Fritz, and Ms S. Buchhop performed the specific antibody determinations. Double-data entry was performed by Ms E. Zoller and Ms A. Keles.

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