

ORIGINAL ARTICLE

AIRWAY DISEASES

A randomized placebo-controlled trial of rush preseasonal depigmented polymerized grass pollen immunotherapy*

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Abstract:

Background: Specific subcutaneous immunotherapy (SCIT) for seasonal rhinoconjunctivitis with unmodified allergen extracts is effective, but limited by risk of side-effects and involves treatment over 3 years. We examined a depigmented polymerized grass pollen extract for immunogenicity and for clinical efficacy in a rush preseasonal regimen.

Methods: Depigmented polymerized grass pollen extract was tested for proliferation and cytokine production by peripheral blood mononuclear cells. A prospective, double-blind, placebo-controlled trial of 195 grass pollen allergic patients treated with preseasonal rush immunotherapy using depigmented polymerized allergenic extract of mixed grass pollen was performed over 2 years. Primary outcome was combined symptom and medication score (SMS) during the peak of the second grass pollen season. Secondary outcomes included combined score over the whole season, during the first grass pollen season, individual symptom and medication scores, quality of life, well days/hell days and responder analysis. Adverse events were classified using the EAACI scale. Grass pollen-specific IgE and IgG4 were measured before and during treatment.

Results: Depigmented polymerized extract stimulated dose-dependent T-cell proliferation and cytokine production. Patients treated with preseasonal SCIT showed improved combined scores during peak season at year 2 (median 3.93, interquartile range 0.77–6.27 vs median 5.86 for placebo, 3.11–8.36, $P < 0.01$). Most secondary outcomes were significantly better for active treatment. Side-effects were minimal, with no grade 3 or 4 reactions.

Conclusions: Depigmented polymerized grass pollen extract is immunogenic and clinically effective in rush preseasonal SCIT. This form of immunotherapy may be an attractive option for some patients.

Allergic rhinoconjunctivitis affects 25% of western populations (1). Subcutaneous immunotherapy (SCIT) with inhalant allergens induces clinical and immunologic tolerance, improves quality of life, and modifies the natural course of disease (2–4). However, injecting allergen extracts has the disadvantage of potentially causing anaphylactic reactions in sensitized patients (5, 6). A rate of systemic reactions between 0.8% and 46.7% of patients (mean 12.92%) was reported in a review of 24 SCIT studies with conventional build-up schedules (7). In a multicenter study of SCIT for grass pollen-induced seasonal rhinoconjunctivitis, the rate of

grade 3 systemic reactions was 4.4% (8). One approach to minimize these risks is polymerizing allergen extracts with glutaraldehyde to produce allergoids (9) with reduced IgE binding (mediating immediate systemic reactions) yet sustained T-cell reactivity to induce tolerance (10). Mild acid hydrolysis before polymerization removes pigments and results in an increased solubility and in a deactivation of the enzymatic activity (11). Clinical trials of SCIT with depigmented polymerized vaccines have documented the feasibility of rapid dose escalation (12) as well as safety and clinical efficacy in patients with pollen- and house dust mite-induced

symptoms (13–16). Despite this clinical data, a laboratory study suggested that depigmented, polymerized extracts retain only minimal T-cell reactivity (17).

Specific subcutaneous immunotherapy treatment conventionally involves up-dosing, then monthly injections over 3 years. Each injection carries a risk of anaphylaxis and must be given in a specialized center with observation for at least 30 mins (4). One option for reducing cost and time required for SCIT is preseasonal immunotherapy where injections are given before each season. Preseasonal treatment for grass pollen-induced rhinoconjunctivitis has demonstrated efficacy for conventional and allergoid allergen extracts, although in one study of unmodified extracts in five of 45 subjects dose modification was necessary because of systemic reactions (18–20).

The objectives of this study were to examine T-cell activity of depigmented polymerized grass pollen extracts *in vitro* and to evaluate clinical efficacy and safety of a 2-year ultra-short preseasonal rush SCIT with a depigmented polymerized allergenic extract of grass pollen.

Methods

In vitro evaluation of depigmented polymerized grass pollen extract

Six volunteers (aged 18–55) with rhinoconjunctivitis and positive skin-prick test (SPT) and/or specific IgE to *Phleum pratense* were recruited. This study was approved by Guy's Hospital Ethics Committee and written informed consent was obtained. Peripheral blood mononuclear cells (PBMC) were separated from 120 ml of blood (with 10% sodium citrate) by Lymphoprep density centrifugation (Axis Shield, Oslo, Norway). Cells were resuspended in RPMI 1640 media with L-glutamine, gentamicin (all from Invitrogen, Paisley, UK), and 5% AB human serum (PAA, Pasching, Austria), then cultured in triplicate in 96-well plates at 2×10^6 cells/ml for 7 days with a range of concentrations of native grass pollen extract (*P. pratense*), depigmented polymerized extract (Laboratorios LETI, SL, Tres Cantos, Spain) or medium alone in a 5% CO₂ incubator at 37°C. Proliferation was assessed by incorporation of titrated thymidine (1 µCi) and IL-13 production by cytometric bead array (BD Biosciences, Oxford, UK).

Patients and clinical study design

This was a randomized, double-blind placebo-controlled study, including patients (age ≥12) with moderate to severe intermittent allergic rhinoconjunctivitis with or without allergic asthma because of sensitization against grass pollen (ARIA classification, 21).

One hundred and ninety-five patients were enrolled at 30 centers in Germany (Fig. 1). Randomization was 2 : 1 active treatment to placebo. Positive SPTs to grass pollen (*P. pratense* pollen; Laboratorios LETI, SL) and serum-specific IgE against grass pollen mix ≥0.7 kU/l were required.

Patients were excluded if they had symptoms because of sensitization to other allergens or asthma graded at 2 or more on GINA guidelines. Written informed consent of

patients was obtained. The study was approved by the relevant Ethics Committees and conducted in accordance with the latest version of the Declaration of Helsinki.

Immunotherapy

Specific subcutaneous immunotherapy was performed using Depiquick® (Laboratorios LETI SL, Tres Cantos, Spain), a standardized depigmented and glutaraldehyde-polymerized grass pollen mix adsorbed onto aluminum hydroxide. This contained *Dactylis glomerata*, *Festuca glauca*, *Lolium perenne*, *P. pratense*, and *Poa pratense* pollens in equal parts. Depiquick® was provided at 1000 DPP/ml. The biological activity unit of 1 DPP is the result of depigmenting and polymerizing 1 histamine equivalent prick test unit (HEP) of allergen extracts. SCIT was performed before grass pollen season. Patients initially received doses of 0.2 and 0.3 ml with an interval of 30 mins at initial up-dosing visit, followed by 5 weekly injections of 0.5 ml (Fig. 1). The maintenance dose corresponded to 31.5 µg Phl p 5 before polymerization (ELISA; Indoor Biotechnologies, Charlottesville, VA, USA). All patients were observed for 30 mins after each injection (4).

Pollen counts

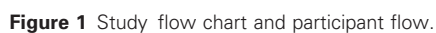
The local grass pollen counts were determined using a volumetric pollen trap (Burkard Scientific Ltd, Uxbridge, UK).

The start of the pollen season was defined as when the grass pollen count was ≥5 pollen grains/m³ per day for more than 3 consecutive days. Efficacy was evaluated until the pollen count fell to ≤5 pollen grains/m³ per day for 3 consecutive days, indicating the end of the relevant pollen exposure. The peak pollen season was defined 1 week before and 2 weeks following the day of maximum grass pollen exposure in the respective season.

Assessment of efficacy

The patients were instructed to enter their individual rhinitis, conjunctivitis, and asthma symptoms on a daily basis in diaries: sneezing, nasal itch, rhinorrhea, nasal congestion, ocular itch, increased lacrimation, and eye redness. Individual symptoms were scored on a scale from 0 to 3, with no symptoms = 0, mild = 1, moderate = 2, and severe = 3 (22–24).

Sufficient rescue medication was provided to all patients and could be taken according to the recommendation of the manufacturer. Other medication was not permitted. Each dose of rescue medication was scored as follows: 0 = no medication; 1 = systemic antihistamines (Levocetirizine one 5 mg tablet), or salbutamol (one puff) for asthma, 2 = nasal or inhaled corticosteroids (mometasone nasal spray two puffs in both nostrils twice daily, budesonide inhaler 200 µg); 3 = systemic corticosteroids for uncontrolled rhinitis or asthma (Prednisone tablet 50 mg) (23). The daily rescue medication score was set as sum of points for each medication used by each patient. The combined symptom and medication score (SMS) was defined as the time weighted area under curve (AUC) of the sum of the daily rescue medication score



and the daily symptom score for all days of the predefined pollen season (15).

Well days were days with a symptom score ≤ 2 and no rescue medication (25). Hell days were days with a symptom score ≥ 10 and additional use of rescue medication.

Quality of life was analyzed at peak grass pollen season in both years using the RQLQ (26).

Safety

Systemic reactions were graded according to the EAACI classification (4).

Statistics

The primary outcome was the time-weighted AUC of the combined symptom and rescue medication scores (SMS) during the peak pollen season in the second treatment year for the intention to treat (ITT) population. The ITT population includes all patients who received study medication at least once and for whom SMS values of the 2nd season were available.

The Wilcoxon rank-sum test was used for the treatment comparison, and the Hodges-Lehmann estimation was used to calculate median differences with confidence intervals. Antibody concentrations were compared by parametric statistics. Response rates were compared using Fisher's exact test. The one-sided significance level was set to 2.5%.

Results

In vitro T-cell activity

Depigmentation and polymerization of the *P. pratense* extract tested resulted in >95% reduction in IgE binding (data not shown). Stimulation of PBMC with both native and depigmented-polymerized extracts resulted in dose-related proliferation and IL-13 production (Fig. 2A, B). Maximal proliferation and cytokine production by PBMC stimulated by depigmented polymerized extract was a median of 48.7% that of native extract for proliferation and 100% for IL-13.

Clinical study

One hundred and ninety-five patients aged 11–69 years (mean: 33 years) were randomized, and 179 patients (ITT) received either SCIT with depigmented polymerized extract (126) or placebo (53). In total, 27 patients (25 of them were adults) discontinued the study prematurely (Fig. 1). Baseline characteristics were comparable (mean age for active treatment 32.9 years, SD 13.8; for placebo 33.8 years, SD 13.3; and timothy grass pollen-specific IgE 43.3 SD 33.6 kU/l and 37.3 SD 32.3, respectively).

Efficacy

In the second grass pollen season, the median combined SMS at the peak of the second season was 3.93 (inter quartile range 0.77–6.27) compared with 5.86 for those receiving

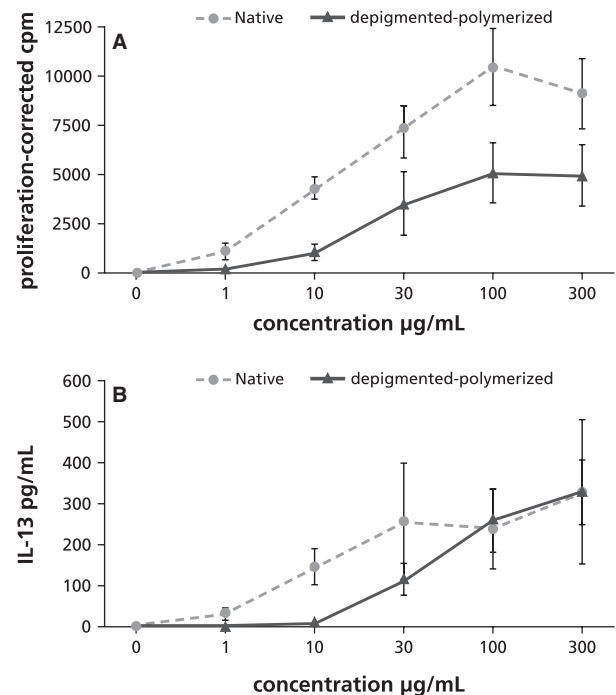


Figure 2 (A) Proliferation as assessed by incorporation of titrated thymidine (counts per minute, CPM) and (B) Interleukin-13 production in pg/ml as measured by cytometric bead array by peripheral blood mononuclear cells stimulated with depigmented polymerized (dark gray line) or native unmodified (light gray line) grass pollen extracts. Data are plotted as mean $\pm$ SEM from six atopic volunteers sensitized to grass pollen.

placebo (3.11–8.36, $P < 0.01$) for the ITT population, 33% less for active vs placebo. For the per protocol population, the SMS for peak season for actively treated patients was 4.25 (1.86–6.64), and for placebo treated patients, it was 6.61 (3.16–8.66), $P < 0.01$, (35.7% less). Significant reductions were also seen for the whole pollen season [median SMS 3.33 for active (IQR 1.44–6.15) vs 5.50 (3.0–7.97) for placebo, $P < 0.01$, 39.5% less for active treatment for the ITT group], (Fig. 3). Data for peak season for the first and second seasons are shown in Table 1: RQLQ improved, and asthma symptom scores were significantly less in year 2.

For adolescent patients (aged under 18), reductions in combined SMS in the second peak season (SMS for active 4.41, IQR 1.60–5.60, $n = 20$ vs 5.36, IQR 0.82–7.39, $n = 9$) and across the whole season (SMS for actively treated patients 4.12, IQR 2.27–6.2 vs 6.0, IQR 2.92–7.34 for placebo, ITT population) were similar to those for adult patients, but these differences were not statistically significant, reflecting small numbers in this subgroup.

Responder analysis

Using a 30% 'reduction' as a response (15), there were 58 responders of 103 in the per protocol group for active treatment (56.3%) and 15 of 45 treated with placebo (33%, $P < 0.01$ by Fisher's exact test).

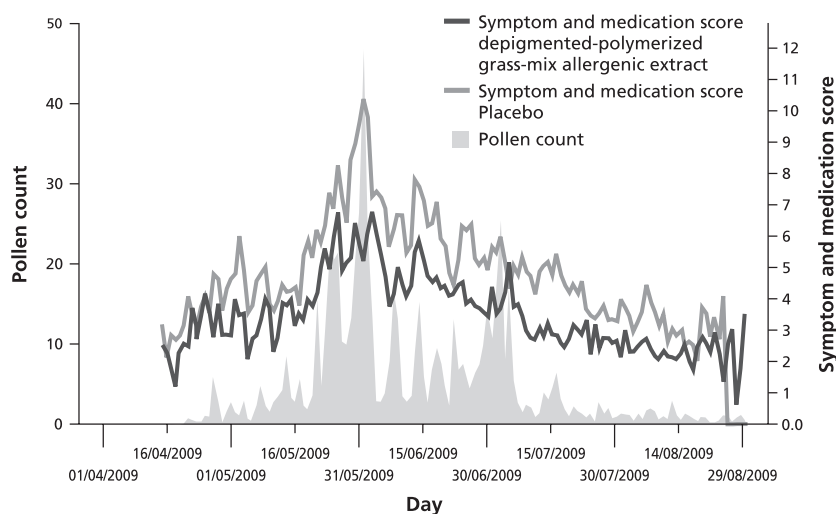


Figure 3 Combined symptom and medication scores (SMS) for actively treated (dark gray line) and placebo-treated patients (light gray line) over the second pollen season. Pollen counts are shown

in solid gray. Data are for the intention to treat dataset and shown mean, time-weighted values.

Table 1 Efficacy data from peak season for year 1 and 2

	Active	Placebo	<i>P</i> -value
Year 1			
SMS	5.68 (3.77–7.25)	6.73 (4.7–10.06)	<0.01
Symptom score	4.65 (3.01–6.08)	5.26 (3.86–6.69)	0.05
Medication score	0.83 (0.28–1.65)	1.49 (0.51–2.52)	<0.01
Asthma score	0.22 (0.0–1.46)	0.64 (0.0–1.76)	0.07
RQLQ			
Activities	3.0 (2.0–4.0)	3.67 (3.0–4.33)	0.02
Symptoms*	1.64 (0.79–3.0)	2.07 (1.57–3.14)	0.05
Emotion	1.0 (0.25–2.25)	1.5 (0–3.0)	0.12
Overall	1.93 (1.29–2.96)	2.46 (1.68–3.32)	0.03
Year 2			
SMS	3.93 (0.77–6.27)	5.86 (3.11–8.36)	<0.01
Symptom score	4.16 (2.30–5.68)	5.03 (3.41–6.57)	0.02
Medication score	0.55 (0.09–1.18)	1.19 (0.41–2.60)	<0.01
Asthma score	0.10 (0.0–0.95)	0.83 (0.0–2.22)	0.03
RQLQ			
Activities	3.0 (2.0–4.0)	3.33 (2.67–4.33)	0.18
Symptoms*	1.43 (0.71–2.57)	2.07 (1.14–3.0)	0.05
Emotion	1.25 (0.25–2.0)	2.0 (1.0–2.5)	0.04
Overall	2.02 (1.32–2.82)	2.30 (1.55–3.16)	0.14

SMS, symptom and medication score.

Data are medians and interquartile ranges from the ITT population. Statistical comparison was by Wilcoxon rank-sum test. RQLQ is rhinitis quality-of-life questionnaire: domains shown are those with significant differences or a trend for difference between active and placebo, symptoms (*) are non-nose symptoms (RQLQ-data given for adults only).

Well days/Hell days

In both pollen seasons, actively treated patients experienced a higher percentage of well days during peak season than the placebo-group (first season, median 9.1, IQR 0–27.3 for active treatment and 0, IQR 0–4.6 for placebo, $P < 0.01$ and second season: 9.1, IQR 0–27.2 for active, and 0, IQR 0–11.4 for placebo, $P < 0.01$, ITT dataset). The percentage of hell days in peak season was significantly reduced in the first year for active treatment (0, IQR 0–11.4) compared with placebo (4.6, IQR 0–18.2, $P = 0.02$), but there were less hell days in peak season for the second year and no significant difference between groups (data not shown).

Safety

Local reactions at the injection site were common, occurring in 95 patients (70.4%) for active and 24 (40%), for placebo, but did not require treatment or change in dosing schedule. Systemic reactions occurred in 16 patients treated with active treatment (27 occasions), and three placebo (seven occasions). Of these, all were grade 1 and 2, and there were no grade 3 or 4 systemic reactions.

Immunological effects

From the beginning of the first to the end of the second pollen season, mean grass-specific IgE decreased in the active group (from 43.3 to 42.9 kU/l), but increased in the placebo group (from 37.3 to 45.6 kU/l).

After the first course of SCIT and before the first grass pollen season, grass-specific IgG4 increased from a mean of 186.5 ng/ml (SD 372.5) to 996.1 (SD 1024) for active treatment and from 195.4 (SD 412.7) to 205.6 (SD 503.4) for placebo. After the second year of treatment,

concentrations were 638.6 (SD 772.6) and 275.7 (SD 543.3), respectively.

Discussion

Here, we initially showed that the depigmented polymerized extract, which has greatly reduced IgE binding, preserved immunogenicity as assessed by T-cell cytokine production. Then, in a 2-year, randomized, double-blind, placebo-controlled study, preseasonal rush SCIT with seven injections of depigmented polymerized mixed grass pollen extract was effective and well tolerated, improving SMSs for both rhinoconjunctivitis and asthma.

Previous studies of PBMCs, T cell lines, and clones have shown some reduction in T-cell stimulation by allergoids, possibly because of loss of T-cell epitopes or altered antigen presentation (27–29). One previous study examined various allergoids and concluded that depigmented polymerized extract had greatly reduced T-cell activity (17). Our current data suggest that depigmented polymerized extracts retain ability to induce IL-13, although with reduced proliferation. It is not known which *in vitro* assay reflects ability to induce tolerance, but extensive clinical data, including the current study, show that depigmented polymerized extracts are clinically effective and presumably induce tolerance. The immunological effects of rush preseasonal SCIT with depigmented and polymerized grass pollen extracts, including increased IgG4, support immunogenicity and are in line with previous reports (2, 8, 14–16).

In an attempt to make immunotherapy more acceptable, short preseasonal treatment regimens have been described. Preseasonal treatment with unmodified extracts was clinically effective, but systemic side-effects resulted in dose modification in 11% of subjects (18). Use of allergoids in preseasonal regimens has previously shown efficacy for grass pollen allergy (19, 20). Here, we show efficacy in reducing SMSs over two consecutive years of preseasonal immunotherapy up to 33% less SMS for primary outcome, using a regimen with no grade 3 or 4 reactions and no requirement for dose reduction. The rush up-dosing regimen (0.2 ml followed by 0.3 ml on day 1 then 5 weekly injections of 0.5 ml) allowed rapid treatment with minimal systemic reactions. A recently published study also supported rush up-dosing with depigmented polymerized extracts and underlines our results (12).

There has been no direct comparison of preseasonal treatment with either year-long subcutaneous or sublingual immunotherapy, but our findings, together with other studies, suggest that all three regimens are options for treatment of severe seasonal rhinoconjunctivitis. A question for future studies is whether preseasonal therapy has long-term effects after stopping treatment, as for other modalities (30, 31).

If the whole grass pollen season is analyzed for symptom scores and concomitant medication, days with minimal pollen exposure causing only marginal allergic symptoms are also included. Therefore, the use of SMS may not reproduce the real impact of the disease as patients tend to indicate much higher scores on days of high pollen exposure (15). We there-

fore analyzed SMSs in peak grass pollen season as our primary outcome. Using data across the whole pollen season also showed significant benefit, and there was no suggestion that disease control was lost toward the end of the season (Fig. 3).

In a recently published guidance, the European Union regulators (EMA) recommend that the efficacy analysis based on SMS should be endorsed by a responder analysis (http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500003605.pdf) (22). We recently described a modified responder analysis which does not require a baseline observation period (15). Using the placebo value as the reference point in our current trial, a 30% 'reduction' occurred in 56% of actively treated patients, and 33% receiving placebo. These results are similar to our data of year-long birch pollen immunotherapy with depigmented polymerized extracts (15). Responder rates for other subcutaneous or sublingual treatments have not been published. Further studies will be required to determine whether these are true nonresponders to active treatment or include apparent nonresponse because of a change in underlying disease severity.

In addition, the EMA guidance suggests 'the number of days with symptom control' is 'an alternative approach for combining symptom score and intake of rescue medication' (22). Recent trials have used this outcome with sublingual immunotherapy in both adult and pediatric patients (25, 32, 33). In our study, the rate of 'well-days' improved in both peak grass pollen seasons. Moreover, quality of life was significantly better even in the first year.

In conclusion, we show that preseasonal SCIT with standardized depigmented polymerized mixed grass pollen extract with rush up-dosing was clinically and immunologically effective, and well tolerated.

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Conflict of interest

The authors declare no conflicts of interest.

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