

Recombinant allergen-based monitoring of antibody responses during injection grass pollen immunotherapy and after 5 years of discontinuation

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Abstract

Background: Subcutaneous injection immunotherapy (SCIT) is considered as antigen-specific and disease-modifying treatment with long-lasting effect.

Methods: We used a panel of recombinant grass pollen allergens for analyzing allergen-specific IgE, IgG₁-IgG₄, IgM, IgA, and light-chain (kappa, lambda) responses in grass pollen-allergic patients who had received one course of injection immunotherapy (SCIT) with an aluminum hydroxide-adsorbed grass pollen extract or only anti-inflammatory treatment. Serum samples were analyzed before and after 5 months of treatment as well as after 5 years.

Results: After 5 months of SCIT but not of anti-inflammatory treatment, IgG₁ > IgG₄ > IgG₂ > IgA antibody responses using both kappa and lambda light chains specific for major grass pollen allergens (Phl p 1, Phl p 5, Phl p 6, Phl p 2) increased significantly, whereas specific IgM or IgG₃ levels were unaltered. Allergen-dependent basophil degranulation was only inhibited with SCIT sera containing therapy-induced allergen-specific IgG antibodies. Likewise, decreases in Phl p 1- and Phl p 5-specific IgE levels and significant ($P < 0.001$) reduction in symptom and medication scores were found only in the SCIT group but not in the group of patients receiving anti-inflammatory treatment. After 5 years, allergen-specific IgG antibody levels in the SCIT group had returned to baseline levels and there was no significant difference regarding symptoms between the SCIT and non-SCIT groups.

Conclusion: The results from our observational study demonstrate that only SCIT but not anti-inflammatory treatment induces allergen-specific IgG and reduces boosts of allergen-specific IgE production but that one SCIT course was not sufficient to achieve long-term immunological and clinical effects.

Subcutaneous injection immunotherapy (SCIT) is considered as antigen-specific, disease-modifying treatment for IgE-mediated allergies (1). It is accompanied by alterations in the humoral and cellular allergen-specific immune response which are thought to reduce allergen-specific hypersensitivity leading to tolerance. Subcutaneous injection immunotherapy has

been reported to have long-lasting clinical effects which can be detected for 5 years and even longer after discontinuation of treatment. In this context, it has been reported that patients having received venom immunotherapy for at least 3 years tolerated stings even after 5 years without treatment (2, 3). Similar findings have been made for grass pollen injection

immunotherapy. It could be demonstrated that SCIT for 3 to 4 years induced prolonged clinical remission for at least 3 years after discontinuation of injections (4). In an independent study, Eng et al. described a significant clinical benefit 6 years after discontinuation of 3 years of preseasonal grass pollen injection immunotherapy (5).

Long-lasting effects have been also described for the disease-modifying potential of SCIT, in particular for the prevention of asthma in patients suffering from allergic rhinoconjunctivitis in the PAT study (6). A preventive effect for the development of asthma was also observed in follow-ups of the PAT study conducted after 5 and 10 years (7, 8). Although several studies have demonstrated long-lasting clinical effects of SCIT, several important questions remain open. For example, it has not been investigated whether long-lasting effects can be achieved when SCIT is performed for shorter periods than 3 years and whether such effects may even be observed after one course of treatment. Furthermore, only few studies demonstrating long-lasting effects have analyzed immunological alterations that might be associated or responsible for the long-lasting clinical effects. The studies by Golden and Lerch have shown for venom SCIT that long-term protection was associated with decreases in allergen-specific IgE levels and skin sensitivity, whereas allergen-specific IgG levels had declined after treatment (2, 3). Similar results were obtained by Durham et al., who found trends toward decreases in immediate-type allergen-specific conjunctival sensitivity, a reduction in late-phase skin responses, and reduced activation of Th2 cells in biopsies taken from allergen-challenged skin (4).

We performed an observational study in a group of patients who had received only one course of preseasonal grass pollen injection immunotherapy and in a group of patients who had received only anti-inflammatory pharmacotherapy. Symptoms and medication were recorded before treatment, shortly after treatment, and 5 years after the single preseasonal SCIT course. Furthermore, we performed a detailed analysis of allergen-specific antibody responses using a panel of recombinant allergens at the same time points.

Materials and methods

Recombinant allergens

Recombinant allergens, i.e., rPhl p 1, rPhl p 2, rPhl p 5, rPhl p 6, rPhl p 7 (timothy grass pollen), and rBet v 1 (birch pollen), were purchased from Biomay (Vienna, Austria), and rPhl p 12 and rPhl p 13 (timothy grass pollen) were purified as described (9, 10).

Patients, blood samples, treatment, and monitoring of clinical effects

Measurements of allergen-specific antibody responses were taken in grass pollen-allergic patients ($n = 19$) who had been recruited in summer 1999.

The diagnosis of grass pollen allergy in all 19 patients was based on case history, specific IgE measurements, and positive skin prick test results. Twelve patients suffered for more than 2 years from allergic rhinoconjunctivitis (one of them from concomitant asthma) and had no contraindications for subcutaneous immunotherapy (SCIT). These patients agreed to receive immunotherapy by subcutaneous injections of a mix of timothy and orchard grass pollen extracts adsorbed to aluminum hydroxide gel (Allpyral; Bayer Corporation, Spokane, WA, USA) in one course of preseasonal treatment (SCIT group) as recommended by the manufacturer (Fig. 1). On average, patients received 13.8 injections (between 11 and 17) and a mean cumulative dose of 5512.9 PNU was administered. Seven patients with allergic rhinoconjunctivitis, two of them with concomitant asthma, received only treatment with anti-inflammatory drugs (non-SCIT group) during the pollinosis season.

Blood samples were taken before treatment in January/February 2000 (time point T0), after one course of SCIT in May/June 2000 (T1), and 5 years later in July/August/September 2005 (T2) (Fig. 1).

The clinical effects of immunotherapy and of anti-inflammatory treatment were evaluated on the basis of symptom scores and the need for rescue medication. Symptoms were determined according to a scale ranging from 0 to 3 points (0 – no, 1 – mild, 2 – moderate, 3 – severe symptoms) for the following symptoms: nasal obstruction, itching, sneezing, rhinorrhea, and eye symptoms (as well as cough and dyspnea). After SCIT, patients kept a diary for 2 weeks during the pollen season (June 2000) where they reported their daily symptoms and medication. For these patients, the mean daily symptom score was calculated as follows: the sum of the daily symptom scores per patient was calculated for the period of the 2-week monitoring and divided by 14.

The scores for patients from the non-SCIT group at T1 were determined during consultations. Symptom scores at T0 as well as at T2 were collected for both groups during consultations.

Patients from both groups were allowed to take the antihistamine loratadine, the nasal glucocorticoid beclomethasone, and the β_2 -adrenergic receptor agonist salbutamol as rescue medications. The number of days with rescue medica-

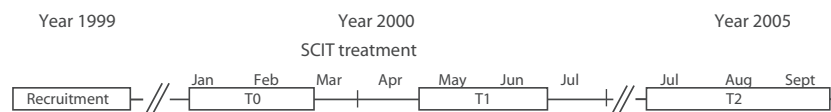


Figure 1 Patients were recruited in summer 1999 to establish a baseline symptom score. At time point T0 (January/February 2000), patients from the subcutaneous injection immunotherapy group

received vaccination treatment. Vaccination was stopped after 5 months in the grass pollen season 2000, and patients were monitored immediately thereafter (T1) and after 5 years (T2).

tion during the 2-week assessment period in summer 2000 (T1) was noted by each patient and used to calculate the total days and mean days medication/patient.

ELISA measurements of IgG₁₋₄, IgA, and IgM

Sera were analyzed for IgG₁₋₄, IgA, and IgM responses against recombinant allergens from timothy grass pollen as described (11). Each allergen (5 µg/ml in 0.1 M bicarbonate buffer, pH 9.6) was coated to ELISA plates (Nunc, Roskilde, Denmark) and was incubated with sera diluted as follows: IgG₁₋₄: 1 : 50, IgA and IgM: 1 : 100, or with buffer alone. IgA and IgM detections were also performed at lower serum dilutions (1 : 10, 1 : 25, 1 : 50). Bound antibodies were detected with mouse monoclonal anti-human IgG₁ (clone JDC-1), IgG₂ (clone G18-21), IgG₄ (clone JDC-14), IgA_{1/2} (clone G18-1), and IgM (clone JDC-15) (PharMingen, San Diego, CA, USA) or mouse monoclonal anti-human IgG₃ (clone HP-6050) (Sigma-Aldrich, St Louis, MO, USA) (12–15). All determinations were performed in duplicates, and results are displayed as mean values with a mean coefficient of variation of 6.5%. Serum from a patient with defined antibody levels was added to each ELISA plate for calibration. Cutoffs were determined using the buffer control, which yielded mean OD values of 0.025 for IgG, 0.023 for IgA, and 0.022 for IgM determinations.

Allergen-specific quantitative IgE measurements

Serum IgE reactivities against recombinant allergens and, for control purposes, against human serum albumin were determined in a quantitative RAST-based, nondenaturing dot blot assay under conditions of allergen excess (16). Allergens were immobilized to nitrocellulose by dot blotting (0.2 µg/dot). Bound IgE antibodies were detected with ¹²⁵I-labeled anti-human IgE (Phadia AB, Uppsala, Sweden) diluted 1 : 15 in blocking buffer and were visualized by autoradiography and quantified (counts per minute: cpm) using a gamma counter (1277 Gammamaster; LKB, Wallac, Gaithersburg, MD, USA) (16).

The percentages of increase or reduction in the median IgE binding were determined according to the following formula: % increase/reduction = 100 × median cpm_{T1}/median cpm_{T0} – 100, where cpm_{T1} represent the cpm after incubation of the dotted allergen with post-treatment sera and cpm_{T0} represent the cpm after incubation with pretreatment sera.

ELISA measurement of allergen-specific light-chain binding

Patients' sera were analyzed for light chain-specific binding to purified recombinant allergens. ELISA plates (Nunc) were coated with allergens (5 µg/ml) and were incubated with 1 : 50 diluted sera (or buffer).

Serum from a patient with defined antibody levels was added to each ELISA plate to allow a comparison of results from different plates. Bound antibodies were detected with mouse monoclonal anti-human kappa or anti-human lambda light-chain antibodies (Sigma) and were traced with a

horseradish peroxidase-labeled sheep anti-mouse antiserum (Amersham Biosciences) diluted 1 : 2000. Results are means of duplicate determinations with a mean coefficient of variation of 1.85%. Cutoff of the ELISA was determined using the buffer control (mean OD value of 0.029).

Basophil degranulation experiments

T0, T1, and T2 sera from two SCIT group patients and from two non-SCIT patients where sufficient serum was available were heat-inactivated for 4 h at 56°C in order to remove IgE reactivity (17). Then, sera were diluted 1 : 10 and pre-incubated overnight with 0.01 µg/ml rPhl p 5 in Tyrode's buffer (18). In parallel, RBL cells expressing human FcεRI (19) (1 × 10⁵/well) were loaded with serum IgE from non-heat-inactivated T0 serum samples at a dilution of 1 : 10 overnight. Cells were washed three times with Tyrode's buffer and exposed to the pre-incubated T0, T1, and T2 serum samples containing allergen immune complexes for 1 h. Supernatants were analyzed for β-hexosaminidase activity as described (18). Results (single determinations owing to serum shortness) are reported as percentages of total β-hexosaminidase released after addition of 10% Triton X-100.

Statistical analysis

Because of the skewed distribution of OD and cpm values, data were log-transformed before analysis, resulting in distributions of residuals not deviating significantly from a normal distribution in the Lilliefors corrected Kolmogorov–Smirnov test. These data were subjected to ANOVA with repeated measurements. Homogeneity of variances was tested by Levene's tests. Comparisons between time points were made by linear contrasts against the baseline. No correction for multiple testing was applied. *P*-values below 0.05 were considered significant.

Results

Subcutaneous injection immunotherapy patients develop IgG₁, IgG₂, and IgG₄ responses primarily against Phl p 1, Phl p 2, Phl p 5, and Phl p 6 but no relevant allergen-specific IgA or IgM antibody levels

As shown in Fig. 2, vaccinated patients significantly increased their IgG₁ responses against each of the analyzed allergens except against Phl p 12 after one course of SCIT. The increases in IgG₁ and IgG₄ against Phl p 1, Phl p 2, Phl p 5, and Phl p 6 were strong (Fig. 2A,C), whereas the increases in allergen-specific IgG₂ were more moderate but nevertheless highly significant (*P* < 0.001) (Fig. 2B). No relevant changes in allergen-specific IgA (serum dilution 1 : 100; Fig. 2D) and IgM (serum dilutions: 1 : 10, 1 : 25, 1 : 50) responses were observed for the vaccinated group. Only a slight increase in allergen-specific IgA was noted at a serum dilution of 1 : 10.

Five years after vaccination, the IgG antibody responses were comparable to those found in the pretreatment serum samples (Fig. 2A–D). Patients from the group receiving only

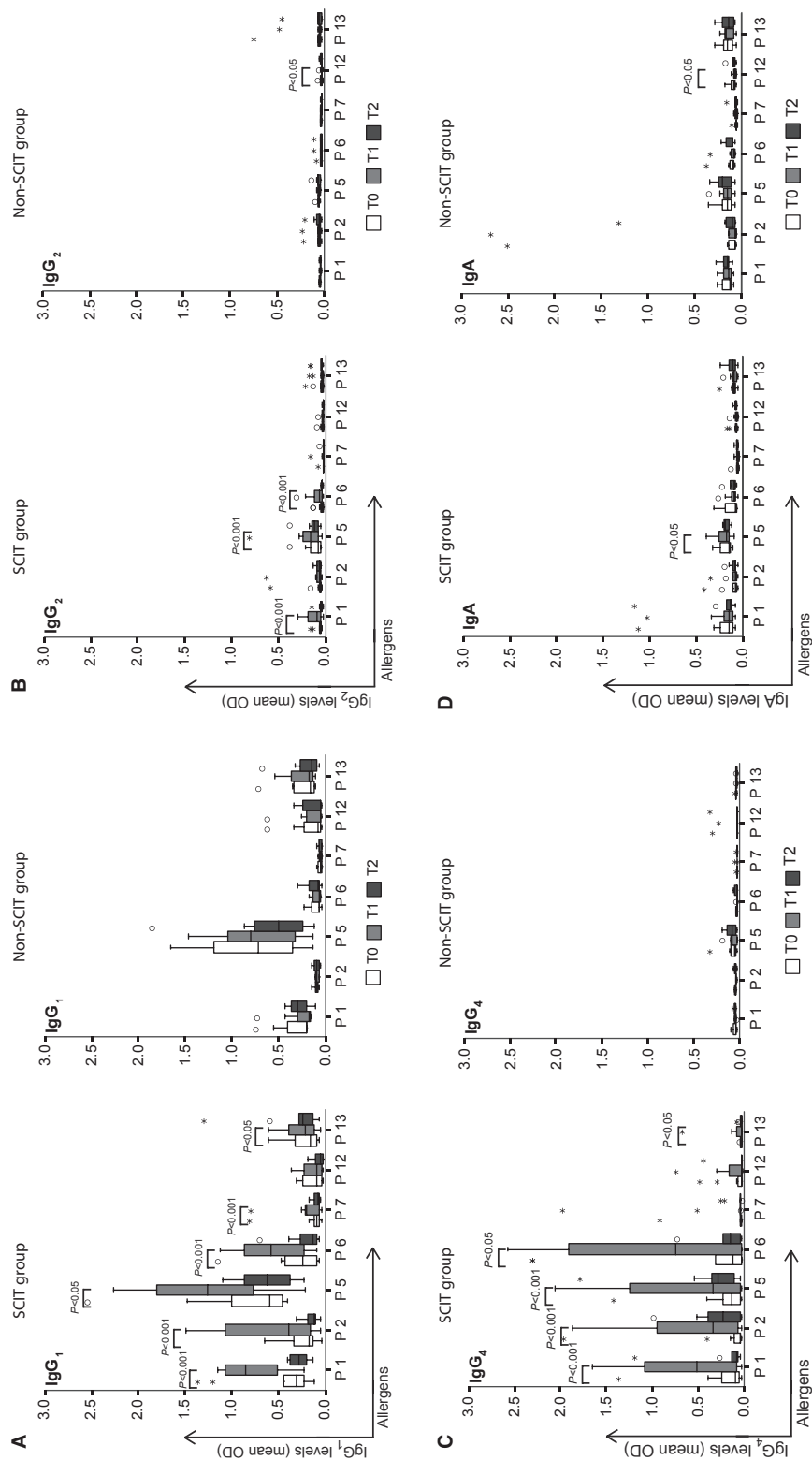


Figure 2 Box plot representation of IgG₁ (A), IgG₂ (B), IgG₄ (C), and IgA (D) reactivities (OD values: y-axes) to recombinant timothy grass pollen allergens (rPhl p 1: P1, rPhl p 2: P2, rPhl p 5: P5, rPhl p 6: P6, rPhl p 7: P7, rPhl p 12: P12, rPhl p 13: P13) are shown for the subcutaneous injection immunotherapy (SCIT) group and non-SCIT group patients. The courses of antibody reactions are shown for three time points: T0: serum obtained at the

start of the trial, T1: serum obtained approximately 5 months after T0, T2: serum 5 years after T0. Boxes include 50% of the values, and nonoutliers are located between the bars. Circles and asterisks indicate outliers and extremes, respectively. Lines within the boxes denote median values. P-values of <0.05 or <0.001 respectively are displayed.

anti-inflammatory treatment showed no relevant increases in allergen-specific IgG, IgA, or IgM responses compared with the vaccinated group (Fig. 2A–D).

Subcutaneous injection immunotherapy blunts the boost of allergen-specific IgE responses

Figure 3 shows the development of allergen-specific IgE levels for the major timothy grass pollen allergens Phl p 1 and Phl p 5 and for the unrelated birch pollen allergen Bet v 1. By comparing the median IgE levels in serum samples at T0 and T1, we found a reduction in the median Phl p 1-specific IgE levels in the range of 16.45% for the vaccinated group, whereas the median Phl p 1-specific IgE levels increased slightly in the group of patients receiving only anti-inflammatory treatment. This effect was much stronger for the highly immunogenic grass pollen allergen Phl p 5 (20). A median decrease in Phl p 5-specific IgE levels in the range of 30% was observed for the vaccinated group, whereas strong boost of Phl p 5-specific IgE of 136% was noted for the patients with only anti-inflammatory treatment. Concerning Bet v 1, the comparison of allergen-specific IgE levels at T0 and T1 showed a similar increase in median IgE levels in the vaccinated group and in patients with anti-inflammatory treatment only (i.e., SCIT group: +122%; non-SCIT group: +139%). Phl p 5-specific IgE levels in T2 were higher in both groups compared with those in T0, whereas Phl p 1-specific IgE levels in T2 were comparable with those in T0 in both groups as observed for the unrelated birch pollen allergen Bet v 1 (Fig. 3).

Therapy-induced antibodies utilize both kappa and lambda light chains

In order to investigate the contribution of kappa and lambda chains in the vaccine-induced IgG responses, we

determined allergen-specific kappa and lambda reactivities in the sera. Figure 4 shows that only vaccinated patients exhibit a significant induction of kappa and lambda responses against the major allergens Phl p 1 and Phl p 5 from T0 to T1. This response decreased after 5 years. For the minor allergens, Phl p 7 and Phl p 12, no significant development of light chain responses could be detected. The time courses/kinetics and levels of allergen-specific IgG₁ and IgG₄ responses shown in Fig. 2A,C are similar to those of the light-chain responses but not to those of the other antibody classes and subclasses, suggesting that the detected light chains belong to the corresponding IgG₁ and IgG₄ heavy chains.

Reduction in allergen-induced basophil degranulation in SCIT-treated patients

We found that serum samples obtained after SCIT which had increased allergen-specific IgG inhibited basophil degranulation strongly (Fig. 5). At T1, serum from patient 4 caused an 89% inhibition of basophil degranulation and serum from patient 5 a 42% inhibition compared with that at T0. Serum from patient 4 showed reduced blocking activity after 5 years, which was associated with lower allergen-specific IgG levels at T2 in this serum (IgG₁: T0: 0.547, T1: 1.673, T2: 0.226; IgG₄: T0: 0.244, T1: 1.646, T2: 0.107; Fig. 5). Serum from patient 5 obtained at T2 showed even stronger inhibition of basophil degranulation than serum obtained at T1. This effect was associated with maintained allergen-specific IgG levels in this serum (IgG₁: T0: 0.463, T1: 1.981, T2: 1.096; IgG₄: T0: 0.116, T1: 0.414, T2: 0.353; Fig. 5).

Sera obtained from patients who had received only anti-inflammatory medication did not show any relevant inhibition of allergen-induced basophil degranulation at T1 or T2 compared with that at T0 (Fig. 5, right).

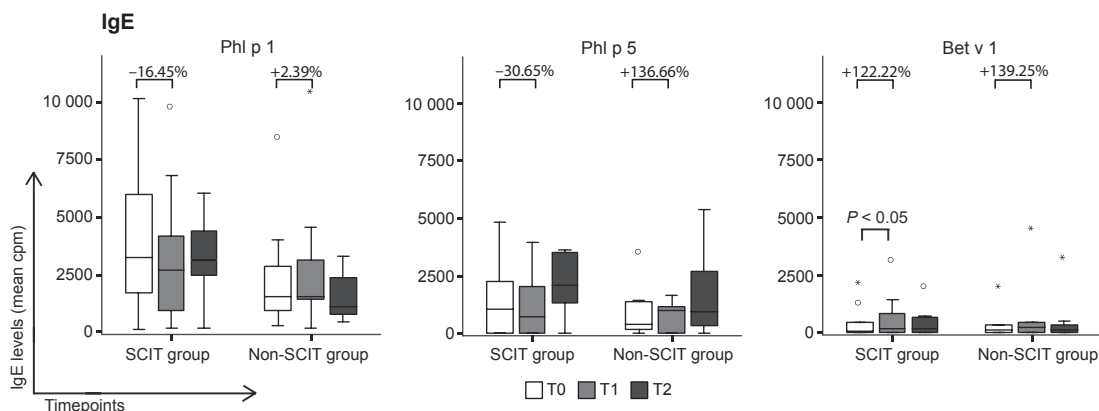


Figure 3 Box plot representation of IgE levels (cpm: y-axes) to rPhl p 1, rPhl p 5, and rBet v 1 is shown for the subcutaneous injection immunotherapy (SCIT) group and non-SCIT group patients before the trial (T0), 5 months after T0 (T1), and 5 years later (T2). Alterations of the median allergen-specific IgE levels between T0 and T1

are expressed as percentage increases (+) or decreases (–). Boxes include 50% of the values, and nonoutliers are located between the bars. Circles and asterisks indicate outliers and extremes, respectively. Lines within the boxes denote median values. *P*-values of <0.05 are displayed.

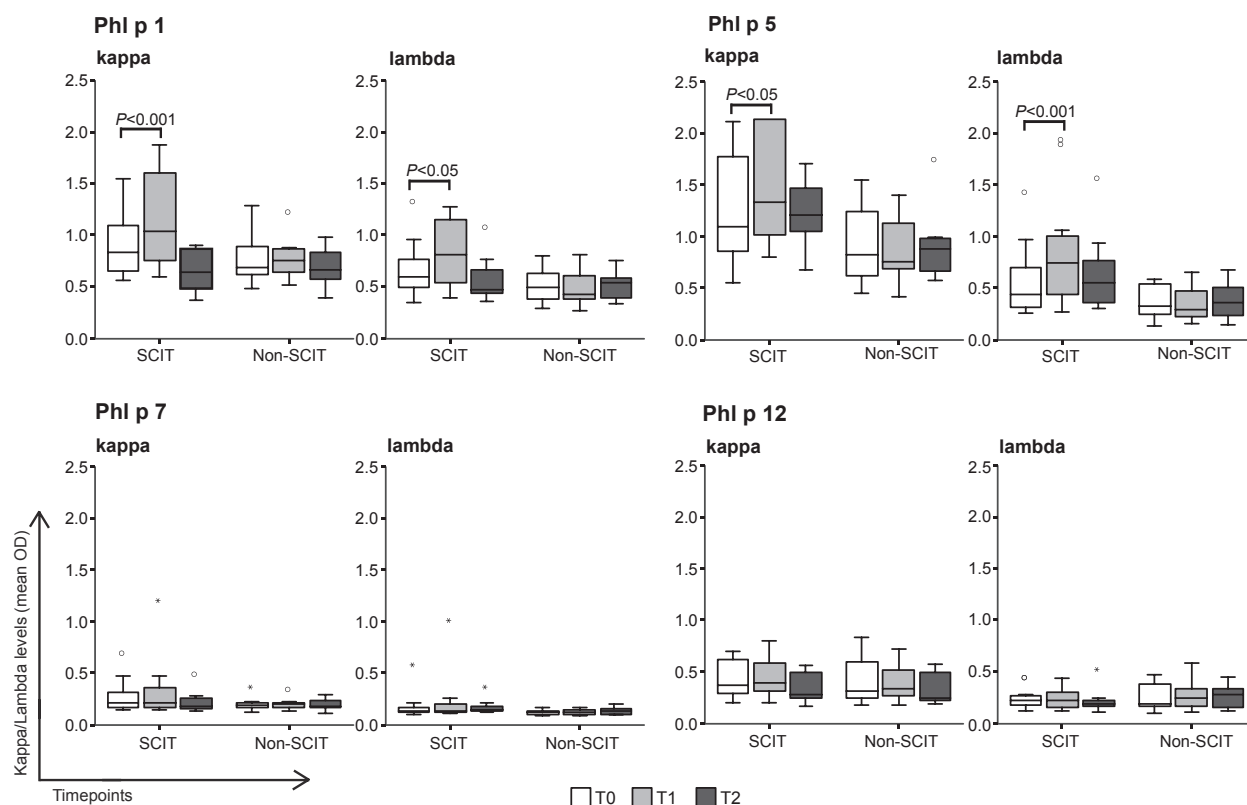


Figure 4 Box plot representation of kappa and lambda light chain levels (OD values: y-axes) to recombinant timothy grass pollen allergens (rPhl p 1, rPhl p 5, rPhl p 7, rPhl p 12) is shown for the subcutaneous injection immunotherapy (SCIT) group and non-SCIT group patients. Reactions are displayed before the trial (T0), 5 months

after T0 (T1), and 5 years later (T2). Boxes include 50% of the values, and nonoutliers are located between the bars. Circles and asterisks indicate outliers and extremes, respectively. Lines within the boxes denote median values. P-values of <0.05 or <0.001 respectively are displayed.

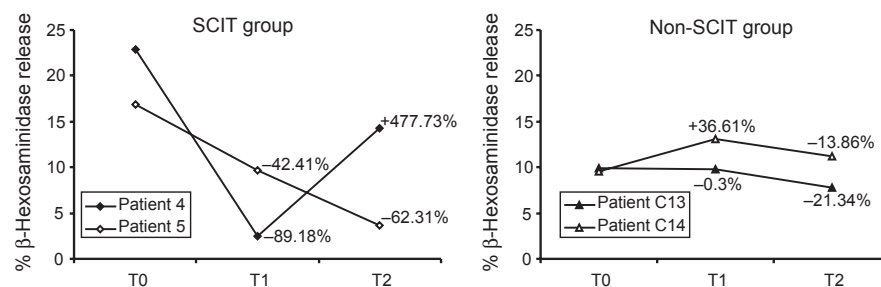


Figure 5 Influence of allergen-specific IgG in patients' sera on rPhl p 5-induced basophil degranulation. RBL cells were loaded with pretreatment serum IgE from two treated (left: 4, 5) and two non-treated (right: C13, C14) patients. Cells that had been loaded with IgE from a patient were then exposed to IgE-depleted sera from

the very same patient obtained before the trial (T0), 5 months after T0 (T1), or 5 years thereafter (T2). β -Hexosaminidase releases are displayed as percentages of total releases. Alterations of the releases are indicated as percentage increase or decrease compared with those of the earlier time point.

Development of symptoms following SCIT treatment and after 5 years

In the SCIT group, median symptom scores declined significantly ($P < 0.001$) from 8.5 points to 2.1 points after 5 months of SCIT (Fig. 6). Five years later, this effect showed

only moderate significance ($P < 0.05$) and the reported median scores went back to 6.5 points. The median score at time point T0 in the non-SCIT group was 9 and thus was very similar to the 8.5 points in the SCIT group before treatment. Patients receiving only anti-inflammatory treatment showed almost no clinical improvement with a symptom score of 8

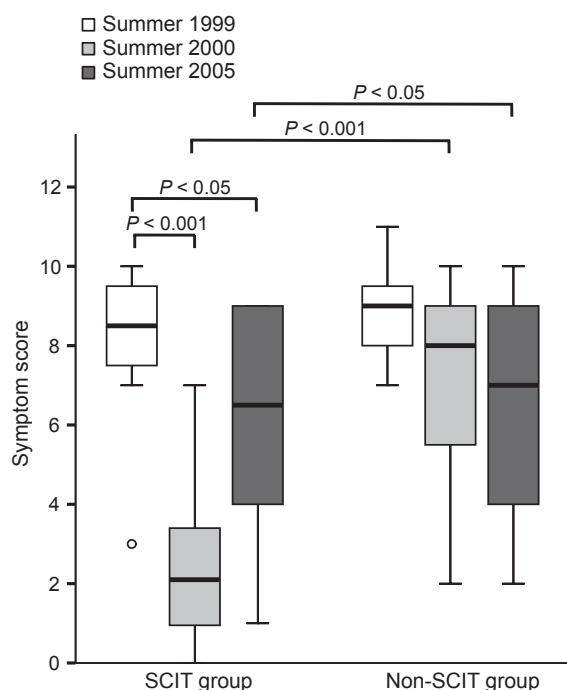


Figure 6 Box plot representation of the development of symptoms in the subcutaneous injection immunotherapy (SCIT) and non-SCIT group. Symptom scores (y-axis) are shown for each group before treatment in summer 1999, at time point T1 in summer 2000, and 5 years later in summer 2005 (T2). Boxes include 50% of the values, and nonoutliers are located between the bars. Circles and asterisks indicate outliers and extremes, respectively. Lines within the boxes denote median values. Significant differences (P -values of <0.05 or <0.001) are displayed.

points in summer 2000 and of 7 points in summer 2005, 5 years after treatment. Five years after discontinuation of the study, patients from the SCIT group showed a lower symptom score than the patients from the non-SCIT group, which was of moderate significance ($P < 0.05$). The number of patients with asthma did not increase in the two groups at T2.

Table 1 shows the number of days during which patients had consumed rescue medication and the mean number of days/patient with rescue medication at time point T1. Patients in the non-SCIT group took more often loratadine

(75 days; mean 10.7 days/patient) than patients from the SCIT group (58 days; mean 4.8 days/patient). This was partly outweighed by a higher consumption of nasal beclomethasone in the SCIT group (38 days; mean 3.2 days/patient) compared with the non-SCIT group (0 days). When the total medication including salbutamol was calculated, we found that the SCIT group had even consumed less often rescue medication (101 days; mean 8.4 days/patient) than the non-SCIT group (77 days; mean 11 days/patient). These observations indicate that clinical improvement was not caused by higher consumption of rescue medication.

Discussion

Here, we performed an observational pilot study in which we used a panel of recombinant timothy grass pollen allergens to study allergen-specific antibody levels in grass pollen-allergic patients who had received one course of injection immunotherapy and in patients who had received anti-inflammatory treatment. We found a strong induction of specific IgG₁ > IgG₄ > IgG₂ but no relevant induction of IgM or IgG₃ levels specific for allergens included in the vaccine (11, 21–26). As described by other authors (27), a slight increase in allergen-specific IgA was noted, but given the fact that it was obvious at a very low serum dilution of 1 : 10, the clinical relevance of this increase is uncertain. IgG antibodies were not induced against all grass pollen allergens, and the levels of allergen-specific IgG varied between the individual allergens. Increases in IgG responses against allergens (e.g., Phl p 7, Phl p 12, Phl p 13) which may be absent from allergen extracts or exhibit poor immunogenicity were low or not detectable (28). The testing for the usage of kappa and lambda light chains provided a hitherto unknown result, i.e., that SCIT induces allergen-specific reactivity of both kappa and lambda light chains. We think that the increase in light-chain reactivity is primarily attributable to the occurrence of lambda and kappa light chains in the allergen-specific IgG fraction because there was a parallel increase in similar magnitudes in IgG and light chain binding. Furthermore, free light chains reportedly have anaphylactic but not protective activity as shown in our basophil experiment (29). This finding indicates that SCIT induces a *de novo* immune response via a balanced activation of kappa and lambda bearing B cells.

In the majority of SCIT-treated patients, allergen-specific IgG responses declined to pretreatment levels 5 years after

Table 1 Days with rescue medication (loratadine, salbutamol, beclomethasone) are reported for subcutaneous injection immunotherapy (SCIT) group ($n = 12$) and non-SCIT group patients ($n = 7$). For each single drug as well as for total medication, the total days of usage are shown and further the mean days per patient are indicated

		Days with loratadine		Days with salbutamol		Days with beclomethasone		Total	
		Total days	Mean days/patient	Total days	Mean days/patient	Total days	Mean days/patient	Total days	Mean days/patient
SCIT group	$n = 12$	58	4.8	5	0.42	38	3.2	101	8.4
Non-SCIT group	$n = 7$	75	10.7	2	0.3	0	0	77	11

treatment. In a study performed with hypoallergenic derivatives of the major birch pollen allergen, Bet v 1, it was also found that therapy-induced IgG decreased already 1 year after treatment and that at this time point patients had regained sensitivity when subjected to nasal allergen provocation (24, 30). Using a recently established basophil degranulation assay that allows determining the effects of blocking antibodies on basophil sensitivity for serum samples obtained before and after treatment, we show that the reduction in basophil degranulation depends on the presence of allergen-specific IgG antibodies, because no relevant alterations of allergen-specific antibody levels were noted for other immunoglobulin classes. Pauli et al. recently showed in a double-blind placebo-controlled SCIT trial performed with purified recombinant Bet v 1, natural Bet v 1, and birch pollen extract that clinical improvement and reduction in skin/mast cell sensitivity are associated with the induction of allergen-specific IgG (26).

Three studies demonstrated that patients who have developed allergen-specific IgG during SCIT exhibit reduced boosts of allergen-specific IgE (23, 24, 31) or a decrease in allergen-specific IgE when treatment was performed for several years (2, 3). One possible explanation for the decrease in allergen-specific IgE is that patients develop allergen-specific IgG which prevent boosts of allergen-induced IgE production (32–34).

If elevated allergen-specific IgG can be maintained for a long period by SCIT, allergen-specific IgE levels may decline as under lack of allergen exposure. This may lead to reduced

loading of mast cells with IgE antibodies and reduced clinical sensitivity. The latter mechanisms may play a role for the long-lasting effects of SCIT, which have been reported to be associated with reductions of allergen-specific IgE levels and skin sensitivity (2, 3). Also, the decrease in Th2 cell activation observed for grass pollen immunotherapy (4) may be associated with the effects of blocking antibodies that block IgE-facilitated allergen presentation to T cells (35–39).

Although it is known that SCIT and SLIT can induce rises in allergen-specific IgE, we found a trend toward a reduction in allergen-specific IgE levels after one course of SCIT but not in the patients receiving anti-inflammatory treatment. This effect may be explained by the suppression of boosts of IgE production owing to grass pollen exposure by SCIT-induced IgG as reported earlier (23, 24, 31). However, the reduction in IgE levels was only detected shortly after treatment but not after 5 years, which may explain why only a moderate long-term clinical effect was achieved in this study.

In summary, our results may indicate that more than one course of preseasonal SCIT is necessary to maintain high levels of allergen-specific IgG for a period long enough to achieve a sustainable reduction in allergen-specific IgE levels leading to long-lasting and relevant clinical effects.

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