

Immunotherapy with partially purified and standardized tree pollen extracts. IV. Results from long-term (6-year) follow-up

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The long-term effect of tree pollen extract immunotherapy was investigated 6 years after termination of the treatment. Subjective symptom evaluation of 36 patients 6 years after a 3-year period of immunotherapy showed that rhinitis and asthma symptoms remained at the improved level reached just after termination of the treatment. Some 86% of the rhinitis patients and 68% of the asthma patients maintained improvement. None of the rhinitis patients developed asthma in the study period. Skin prick tests reflected the outcome of the subjective symptom assessment. The skin sensitivity of the patients decreased significantly during immunotherapy, and the skin reactions 6 years after specific immunotherapy were still significantly lower than the pretreatment levels. Total IgE and birch-specific IgE levels were constant throughout the study period, and both the affinity and epitope specificity of the IgE antibodies of the patients were the same before, during, and 6 years after treatment. In conclusion, specific immunotherapy reduces symptoms in patients suffering from rhinitis and asthma, and the effect is maintained 6 years after termination of the treatment. Specific immunotherapy seems to prevent long-term development of asthma in rhinitis patients. IgE measurements do not reflect the overall status of the patients.

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Tree pollens, primarily pollen from birch (*Betula*), are one of the most important allergen sources in the temperate climate zone of the northern hemisphere during spring. The extensive cross-reaction of IgE antibodies of patients against the group 1 allergens (1, 2) of the botanically closely related genera of the *Fagales* order (*Alnus*, *Betula*, *Corylus*, *Carpinus*, etc.) prolongs the exposure time for the patients from the end of January to the beginning of June.

Specific immunotherapy (SIT) has been used throughout this century, but only during the last two decades have standardized allergen extracts been available, and immunotherapy has been documented in controlled studies with regard to the clinical effect in allergic rhinitis and asthma patients. However, only a few studies have been performed to evaluate the long-term efficacy of immunotherapy after termination of the treatment (3, 4).

The present paper reports on the 6-year follow-up from a number of allergic patients. The

patients were treated with SIT for 3 years with characterized and standardized tree pollen extracts (5–7). The clinical benefit of SIT at the end of treatment was a reduction of symptoms to 10% of the initial value. At the termination of SIT, the nasal provocation test showed that 30 times more allergen was needed to elicit the same degree of symptoms as at the initial pretreatment provocation. At the follow-up, the patients were monitored for the following parameters: severity of symptoms, skin prick test (SPT), specific and total IgE, and major allergen-specific inhibitory capacity during SIT and 6 years later.

Material and methods

All patients from two out of three centers were analyzed in the follow-up study. One center dropped out for practical reasons.

Pollen counts

The pollen counts for the three genera *Alnus*, *Betula*, and *Corylus* determined for 1981–9 were kindly provided by the Danish Aerobiologic Group. The corresponding pollen counts from the Swedish center (Malmö) were of the same magnitude as the Danish values.

Patients

The selection of allergic patients and inclusion criteria are described in detail elsewhere (6). Of the 50 rhinitis patients undergoing SIT, 36 were monitored during, at the end of, and 1 and 6 years after termination of immunotherapy. Nineteen patients suffered from mild to moderate asthma in combination with rhinitis.

SIT

SIT was performed as a continuous 3-year treatment with a depot-preparation of standardized and characterized allergen extracts of birch or a mixture of birch, alder, and hazel in accordance with the recommendations of the manufacturer (Alutard SQ, ALK A/S).

Symptom questionnaire

The severity of symptoms was recorded by the individual patients. The question asked in 1983 at termination of SIT was, "How were your symptoms after SIT compared to when you started immunotherapy?", and the question asked in 1989 was, "How are your symptoms today compared to 1983 when you stopped immunotherapy?" The answers were grouped according to the following categories: worse, unchanged, improved (better and much better), and symptom-free.

SPT

SPT was performed as described earlier (7), and the results were evaluated with concentrations of 0.3, 1.0, and 10 HEP of birch Soluprick SQ (ALK A/S). SPT was performed before, during, just after, and 6 years after termination of immunotherapy.

Total IgE and specific IgE

The amount (SU/ml) of birch-specific and total IgE in serum samples obtained before, during, and after immunotherapy was determined by the Magic Lite Allergy System (ALK A/S) (8). The serum samples were stored at -20°C and were all analyzed at the termination of the observation period.

Inhibition experiments

Monoclonal antibodies raised against the major allergens Bet v 1 (birch) and Car b 1 (hornbeam), both species being representative of the Fagales order, were covalently coupled to paramagnetic particles (BioMag, USA), according to the instructions of the manufacturer. Serum samples from five random patients drawn throughout the period (1981–9) were individually diluted (PBS) to contain the same amount of Bet v 1-specific IgE (10 SU/ml) and absorbed on Con A Sepharose (Pharmacia, Sweden): 250 μl Con A Sepharose was mixed with 1000 μl diluted serum for 30 min and centrifuged (2000 rpm, 10 min). The capacity of the IgG-depleted samples to inhibit the binding of Bet v 1 or Car b 1 to a number of immobilized solid-phase monoclonal antibodies was determined as follows. Some 50 μg PMP-mAb was mixed with 50 μl serum sample (10 SU/ml final concentration) and further incubated for 3 h with ^{125}I -labeled Bet v 1 (25 nM) in PBS. The solid phases were separated on a Biomag Separator and counted in a gamma-counter. Zero percent inhibition was determined with buffer in place of serum sample, and all determinations were performed in triplicate.

Statistics

All statistical analyses were performed with Statistica Version 4.5 (StatSoft) with the Shapiro-Wilk W test to assess the distribution characteristics of the data, the Friedman ANOVA, and the Wilcoxon matched pairs test. All two-sided stochastic probabilities less than 0.05 were considered significant.

Results

Fig. 1 illustrates the amounts of alder, birch, and hazel pollens present in the environment during the study period. The allergen "load" differed considerably throughout the study period, reaching the highest level in 1989. During the first year of treatment, the exposure was relatively low. At the end of treatment and at the 6-year follow-up, the exposures were, respectively, 36 and 70 times higher than in the initial year.

There was no difference between the two treatment groups (birch/mixture of birch, alder, and hazel) just after termination of immunotherapy treatment. All but one patient reported relief of rhinitis symptoms just after termination of immunotherapy (Fig. 2A), and 22% of the patients became symptom-free. Six years after termination of SIT, 86% of the patients still showed the same improvement in rhinitis symptoms as the 1983 level, and

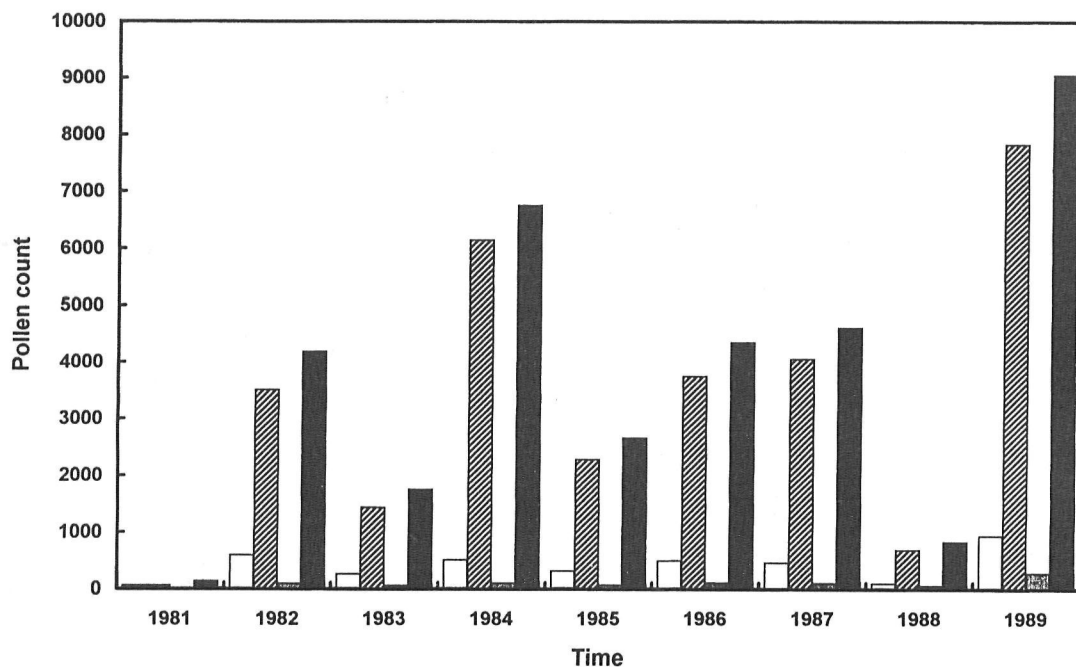


Fig. 1. Tree pollens in environment during study period. Pollen of genera *Alnus* (open bar), *Betula* (light gray), and *Corylus* (hatched), and sum of all genera (solid) are expressed as pollen grains/m³ in various columns.

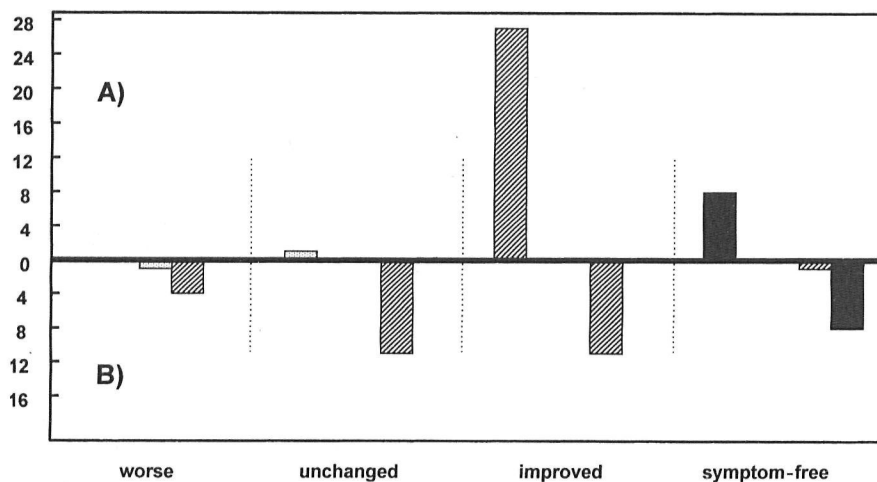


Fig. 2. Distribution of effect on rhinitis symptoms reported by patients ($n=36$) A) just after immunotherapy (1981–3) and B) 6 years later (1983–9). Column fill patterns indicate how patients reporting symptoms in period A reported symptoms in period B; e.g., 27 patients reported improvement of rhinitis symptoms just after SIT (panel A, 1983). Six years later (panel B, 1989), four patients reported worse symptoms, 11 patients unchanged symptoms, 11 patients improved symptoms, and one patient no symptoms.

only 11% of the patients experienced an increase in rhinitis symptoms (Fig. 2B). All patients had fewer symptoms than before SIT. All but two of the asthmatic patients reported improvement of asthma symptoms (Fig. 3A) just after termination of immunotherapy, and 68% of the patients maintained this improvement 6 years after immunotherapy. Some 21% of the asthma patients experienced an increase of symptoms compared with their status just after termination of immunotherapy (Fig. 3B), but symptoms were at a lower

level than before SIT. None of the patients suffering only from rhinitis developed asthma symptoms during the follow-up period.

Skin sensitivity to birch pollen extract (Fig. 4) decreased significantly during SIT, and skin reactivity remained at a significantly lower level compared to the pretreatment level.

Fig. 5 illustrates the levels of total IgE and birch-specific IgE during the test period. There was no significant change in IgE levels throughout the treatment period. Since analyses were performed

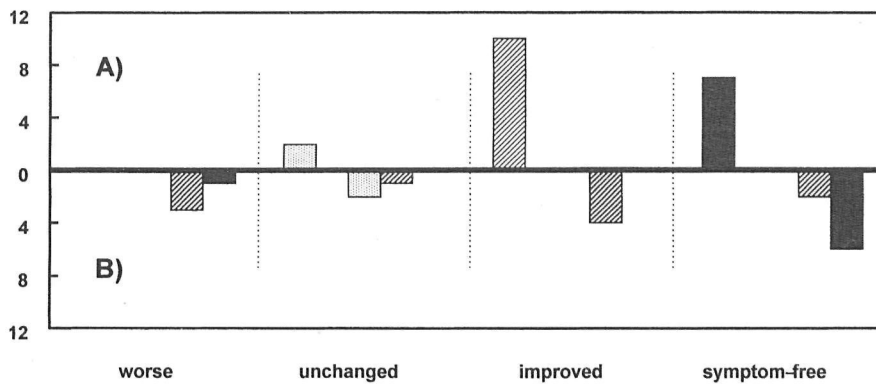


Fig. 3. Distribution of effect on asthma symptoms reported by patients ($n=19$) A) just after immunotherapy (1981-3) and B) 6 years later (1983-9). Column fill patterns indicate how patients reporting symptoms in period A reported symptoms in period B; e.g., 10 patients reported improvement of asthma symptoms just after SIT (panel A, 1983). Six years later (panel B, 1989), three patients reported worse symptoms, one patient unchanged symptoms, four patients improved symptoms, and two patients no symptoms.

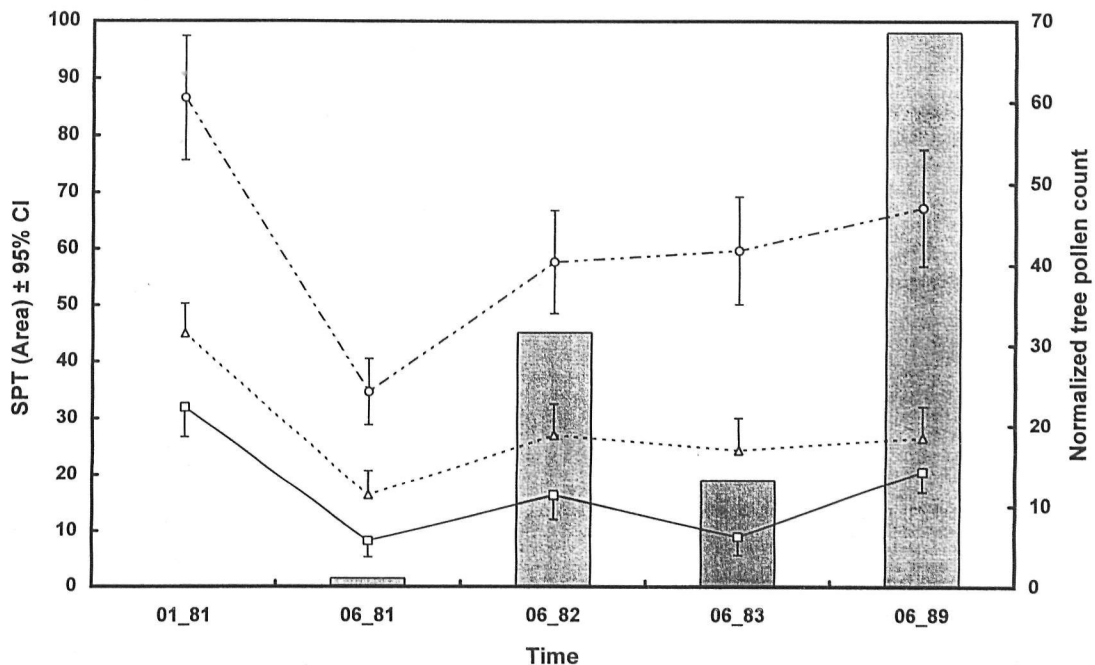


Fig. 4. Mean of SPT reaction recorded before, during, and up to 6 years after immunotherapy with different allergen concentrations; 0.3 HEP (solid line), 1.0 HEP (dotted line), and 10 HEP (dot-dashed line). Error bars represent 95% confidence intervals (CI) of mean. Columns represent sum of relative pollen counts (pollen count 1981=1) of alder, birch, and hazel.

at 12-month intervals, the increase in IgE normally seen during the first 6 months of SIT is not illustrated in this study. High levels of tree pollens during the flowering seasons did not affect the IgE levels of the patients.

Fig. 6 illustrates the inhibition of the binding of ^{125} I-labeled Bet v 1 to a number of solid-phase monoclonal antibodies by patient serum samples depleted of IgG. The inhibitory capacity remained constant throughout the treatment period, indicating that the affinity of the IgE antibodies remained unaffected, and since the monoclonal antibodies were directed against at least three

different epitopes (data not shown), the epitope specificity of the IgE antibodies seemed to be constant.

Discussion

SIT has proved to be effective in the treatment of type 1 allergic diseases caused by a large number of allergen sources; e.g., pollen (5, 9-13), animal hair and dander (14-16), Hymenoptera venom (17, 18), house-dust mite (19-21), and mold (22, 23). All these studies include clinical and serologic parameters before, during, and

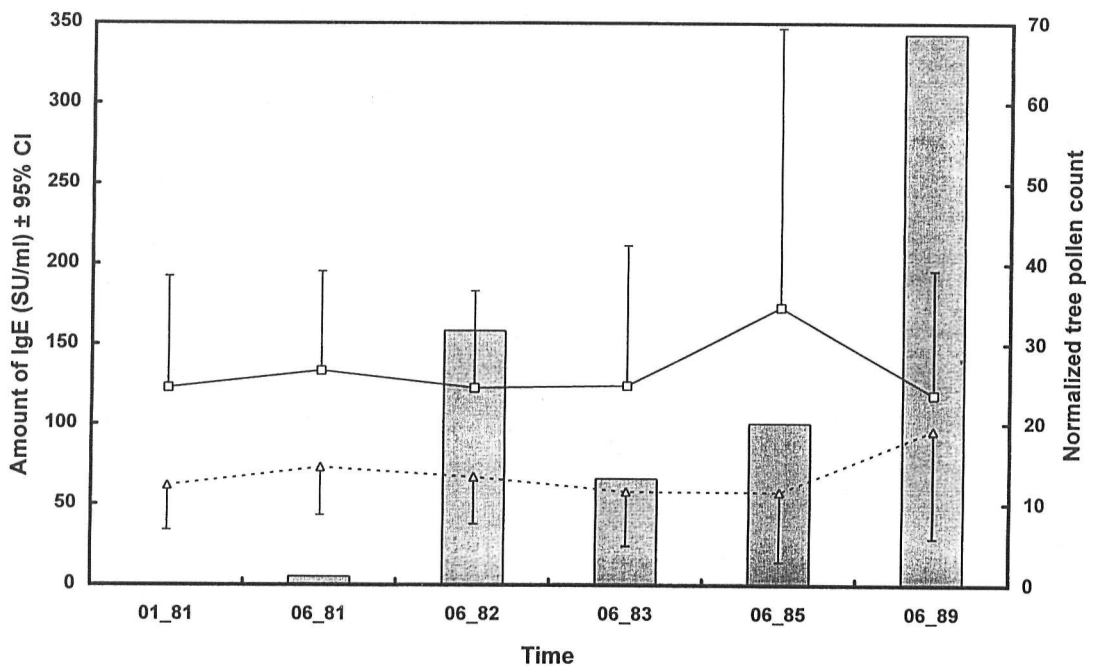


Fig. 5. Mean ($n=16$) of total IgE (solid line) and birch-specific IgE (dotted line) in sera before, during, and 6 years after immunotherapy. Error bars represent 95% confidence intervals of mean. Columns represent relative pollen counts (pollen count 1981=1) of sum of alder, birch, and hazel counts.

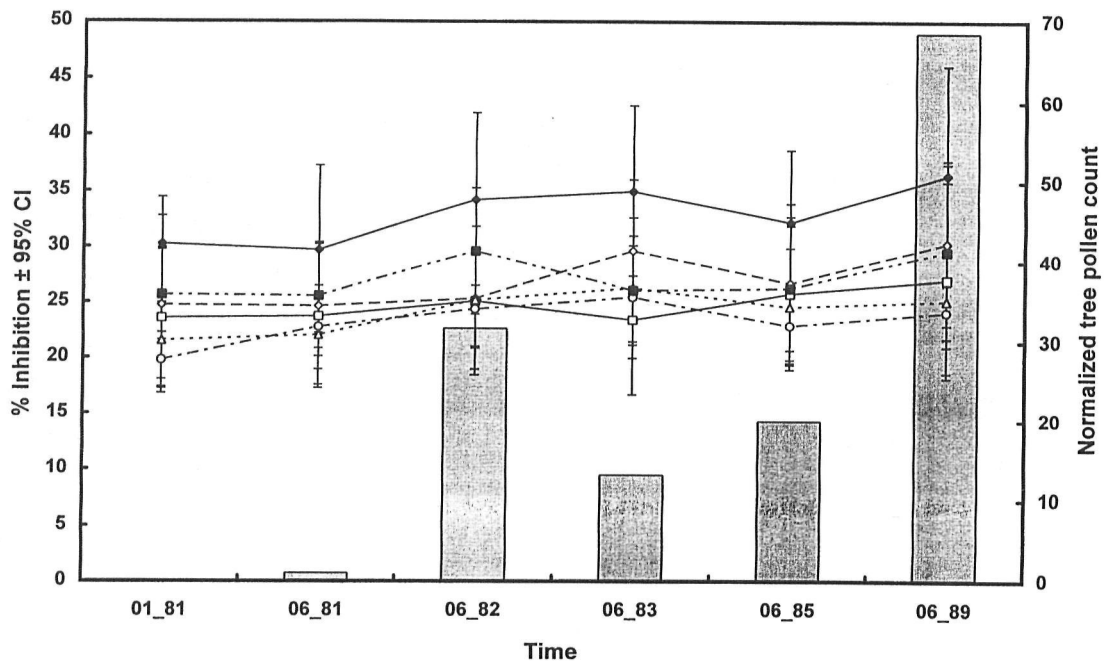


Fig. 6. Inhibition of binding of ^{125}I -labeled Bet v 1 to solid-phase immobilized monoclonal antibodies by IgG-depleted serum samples ($n=5$) containing 10 SU/ml birch-specific IgE. Points represent mean inhibitory capacity of five patients, and error bars represent 95% confidence intervals of mean. Columns represent relative pollen counts (pollen count 1981=1) of sum of alder, birch, and hazel counts.

immediately after termination of immunotherapy. Only a few studies have assessed the long-term effect of immunotherapy. Mosbech & Østerballe (3) concluded that the clinical effect of grass pollen immunotherapy on rhinitis per-

sisted for 6 years after termination of immunotherapy, and in patients with asthma caused by allergy to animal dander, the effect persisted for 5 years after termination of a 3-year course of SIT with cat or dog allergen extracts (4).

Several studies have recently shown not only that SIT reduces the symptoms and the use of symptomatic medication, but also that SIT interferes with more basic immunologic mechanisms and the potential switch between important T-cell-dependent mediators. Suppression of a seasonal exposure increase of ECP in birch-pollen-allergic patients was shown by Rak et al. (9) during the period of immunotherapy, and it was shown by Walker et al. (11) that suppression of the late-phase skin reactivity not only occurred initially during treatment, but also persisted over a 4-year period of immunotherapy in grass-pollen-allergic patients. The clinical efficiency and the late-phase reactivity reduction in these patients followed a reduction in conjunctival sensitivity to allergens (10). Confirmation of the interference of immunotherapy with a basic immunologic mechanism and potential increase in interferon-gamma (24) suggest that SIT may have a long-term effect after termination of the treatment and may inhibit exacerbation of the allergic disease.

In the present study, patients with rhinitis and/or asthma caused by tree pollens received SIT with either birch pollen extract or a mixture of alder, birch, and hazel pollen extract (6). No significant difference was observed between the two treatment groups; consequently, the present study regards the patients as one group. Only data and serum samples from patients who participated in the follow-up study were analyzed.

Just after termination of SIT, 97% of the patients reported improvement or even total relief of their rhinitis symptoms. Six years after termination of SIT, 86% of the patients maintained the reduced level of symptoms. For asthma symptoms, 89% of the patients reported improvement or even total symptom relief. Six years after termination of SIT, 68% maintained the reduced level of asthma. Exposure to tree pollens varies considerably from season to season (25). It is important that the mean yearly pollen exposure in the follow-up season was significantly higher than in the treatment period. The higher exposure to pollen did not affect the qualitative clinical benefits of the treatment.

It has been reported that 25–43% of a population of rhinitis patients develop asthma over a period of 8–10 years (26). In the present study, none of the patients suffering only from rhinitis developed asthma over the 6-year follow-up included in the total 9-year study period. This indicates that SIT not only prevents the onset of asthma in a risk group (rhinitis patients) but also produces a long-term reduction of asthma risk. Since 50–80% of all asthma is caused by allergy, this information has to be further investigated in prospective studies.

The skin sensitivity of the patients were significantly reduced during SIT, and the effect persisted for 6 years after termination of the treatment. The skin sensitivity of the patients to low concentrations of SPT extracts (0.3 HEP) reflected seasonal variations in the pollen counts. There was no observable effect on the total and birch-specific IgE measurements, which both remained at the same level throughout the study period. Furthermore, inhibition experiments performed with IgG-depleted patient serum samples indicated that both the affinity and the epitope specificity of the IgE antibodies of the patients were unaffected by SIT. In accordance with previous studies, the clinical parameters and SPT, but not the antibody measurement, reflected the symptom status and sensitivity of the patients.

Apparently, SIT did not change the IgE responses of the patients with respect to either the amount of total IgE and specific IgE or the affinity and epitope specificity. However, SIT does seem to induce changes in the “shock” organs by lowering the sensitivity of the respiratory tissues of the patients (7, 14, 19) and early as well as late skin reactivity (11).

Numerous SIT studies have shown an increase in non-IgE allergen-specific antibodies, primarily IgG4 (27). The rise in IgG production could not be correlated to the onset of either improvement in clinical parameters or reduction in skin sensitivity for inhalant allergens. If one looks at the IgE responses of the patients to the allergens as the end point of a long process involving refinement and adjustment of both the antibody affinity and specificity, one could speculate that the production of other allergen-specific immunoglobulin isotypes would require an unknown length of time to reach an affinity high enough to compete efficiently with the IgE antibodies for the allergen. This would explain the discrepancy between onset of IgG production and improvement of the patients. Finally, production of allergen-specific IgA could participate in the observed reduction of nasal and lung sensitivity of patients receiving SIT.

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