# Food allergy to apple and specific immunotherapy with birch pollen

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Conflicting results concerning the effect of specific pollen immunotherapy (SIT) on allergy to plant foods have been reported. The aim of this study was to investigate the effect of SIT using a birch pollen extract on food allergy with focus on allergy to apple. Seventy-four birch pollen-allergic patients were included in a double-blind, double-dummy, and placebo-controlled comparison of sublingual-swallow (SLIT) and subcutaneous (SCIT) administration of a birch pollen extract. Sixty-nine percent of these patients reported allergy to apple. The clinical reactivity to apple was evaluated by open oral challenges with fresh apple and a questionnaire. The immunoglobulin E (IgE)-reactivity was assessed by skin prick test (SPT), specific IgE, and leukocyte histamine release (HR). Forty patients were included in the final evaluation of the effect of SIT. The challenges were positive in 9 (SCIT), 6 (SLIT), and 8 (placebo) patients after treatment compared to 10, 4, and 10 patients, respectively, before SIT. The symptom scores to apple during challenges decreased in all groups, but only significantly in the placebo group ($p = 0.03$). As evaluated by the questionnaire, the severity of food allergy in general did not change and there were no differences between the groups. In spite of a significant effect on seasonal hay fever symptoms and use of medication and decrease in IgE-reactivity, SIT was not accompanied by a significant decrease in the severity of allergy to apple compared to placebo. Therefore, oral allergy syndrome (OAS) to apple should not be considered as a main criterion for selecting patients for birch pollen immunotherapy at present.

**Keywords:** Apple allergy / Birch pollen / Food allergy / Immunotherapy

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## 1 Introduction

Clinical manifestations of allergenic cross-reactions between pollen and plant foods are frequent in birch pollen allergic individuals [1–6]. Apples, together with hazelnuts, are among the most prevailing offending foods in a majority of studies [1, 6, 7] and investigations have revealed a high degree of structural homology between the major birch pollen allergen Bet v 1 and allergens from different plant foods, *e.g.*, Mal d 1 from apple, Cor a 1.04 from hazelnut and Pru av 1 from Prunoideae fruit [8–13]. According to the literature, the level of specific immunoglobulin E (IgE) to pollen seems to influence the probability of clinical manifest allergy to plant foods [6, 8, 14] and, furthermore, the severity of pollen related food allergy has been shown to correlate with the clinical severity of the primary inhalation allergy [15].

The potential effect of specific immunotherapy (SIT) with tree pollen extracts on associated allergy to plant foods has been addressed by various authors [16–21] and most studies have included apple in the investigations [16, 17, 19–25]. Due to differences in design, selection of patients, method of administration, and extract quality, the studies are difficult to compare and the efficacy of SIT regarding pollen-related food allergy is therefore still under debate. Only a few controlled studies have been conducted [17, 19–21, 25] and even fewer have used a placebo-controlled design [17, 20, 21]. In some studies all included patients had clinical allergy to apple [19, 21, 23, 25], in others, the inclusion criteria was birch pollen allergy with or without concomitant food allergic manifestations [17, 20, 22]. Three publications are casuistic reports on only a few

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**Abbreviations:** **DBPCFC**, double-blind, placebo-controlled food challenge; **GD**, Golden Delicious apple; **HR**, leukocyte histamine release; **IgE**, immunoglobulin E; **OAS**, oral allergy syndrome; **SCIT**, subcutaneous immunotherapy; **SIT**, specific immunotherapy; **SLIT**, sublingual immunotherapy; **SPT**, skin prick test

 

patients [16, 18, 26]. Regarding SIT with traditional birch pollen extracts and the influence on allergy to apple, most open clinical trials have found an effect [19, 20, 22, 23, 25], whereas two blinded, placebo-controlled trials failed to demonstrate a reduction in symptoms elicited by ingestion of apple [17, 21]. One recent study, applying recombinant allergens for skin tests, demonstrated a significant effect on food allergy to apple evaluated by double-blinded, placebo-controlled food challenges (DBPCFC). The immunotherapy was performed in a randomized design but without placebo control [25].

The aims of the present study were to investigate the effect of specific birch pollen immunotherapy (SIT) on food allergy to apple and to correlate the severity of food allergy with the seasonal hay fever symptoms and the IgE-reactivity to apple and birch pollen.

## 2 Materials and methods

### 2.1 Design

The immunotherapy trial was conducted as a double-blind, double-dummy, and placebo-controlled comparison of sublingual-swallow (SLIT) and subcutaneous administration (SCIT) of a birch pollen extract [27]. The patients were allocated to either SCIT, SLIT or placebo by means of a randomization algorithm to ensure equal distribution regarding severity of rhinoconjunctivitis symptoms during a baseline season, age, and gender. Before and after the immunotherapy treatment, the patients were subjected to open food challenges with Golden Delicious apple (GD). They were also asked to fill in a questionnaire regarding plant food allergy. During the study period the patients recorded seasonal symptoms and medication. The IgE-reactivity to apple was assessed by skin prick test (SPT), measurement of specific IgE, and leukocyte histamine release (HR). The study was approved by the local ethics committee ((KF)02-156/96 & 01-075/97) and all subjects gave written informed consent before entering the study.

### 2.2 Study population

Seventy-four patients with a history of rhinoconjunctivitis during the birch pollen season, combined with positive specific IgE (CAP-class $\geq 2$), positive SPT, and a positive conjunctival provocation test to birch pollen using standardized extracts (ALK-Abelló, Hørsholm, Denmark), were included in this part of the study. The mean age was 30 years (range 21 – 58) and the male/female ratio was 42/32. All patients were recruited, treated, and examined at the Allergy Clinic. At inclusion, 70 patients were subjected to oral challenge with apple; one patient did not want to go through a food challenge and three patients did not comply with the program. Forty-two patients were re-evaluated two years later and 40 patients were subjected to oral challenge with apple both before and after treatment.

### 2.3 Immunotherapy

The active immunotherapy treatment consisted of standardized birch pollen extracts administered sublingually as a glycerol-saline solution (Staloral, Stallergènes, Antony, France) or subcutaneously adsorbed on calcium phosphate (Phostal, Antony, France). The treatment was conducted perennially according to the treatment schedules suggested by the manufacturer. The treatment period was approximately two years from autumn 1997 to 1999, preceded by a baseline registration of symptoms and medication in the birch pollen season 1997. The maintenance dose was adjusted in accordance with the tolerance of the patient. The cumulative dose per patient of the major allergen Bet v 1 was approximately 11 mg for the sublingual administration and 0.05 mg for the subcutaneous administration. The results of the immunotherapy trial showed a statistically significant effect of both types of active treatment (SCIT and SLIT) on seasonal rhinoconjunctivitis and use of medication compared to placebo [27].

### 2.4 Medication

Anti-allergic medication was discontinued prior to food challenges and SPT, according to the guidelines on skin testing from the European Academy of Allergy and Clinical Immunology (EAACI) [28]. At inclusion none of the patients were treated continuously with inhaled corticosteroid, but 11 patients reported asthma symptoms during the baseline pollen season. At the final challenge test, one patient in the placebo group received inhaled corticosteroid against asthma.

### 2.5 Oral challenges

The challenges were performed as open oral challenge tests with fresh GD apples (France), purchased at the local market. A slice of apple of approximately 10 g was administered to the patients. In case of no or very mild symptoms, the patient was allowed to eat a whole apple, bite for bite. The patients graded the severity of the symptoms on a scale from zero to three, three being the most pronounced symptoms.

 

### 2.6 Questionnaire

Before and after the treatment, the patients filled in a questionnaire regarding allergy to fruits, nuts, and vegetables with emphasis on allergic manifestations upon ingestion of apple. A list of specific plant foods was presented and the patients were asked to describe and grade the severity of the symptoms to each food on a scale from zero to three. After the treatment but prior to breaking of the code of the immunotherapy trial, patients were asked to evaluate any possible effect of SIT on their food allergic symptoms. The options were: "If you still have symptoms when you eat fruit, nuts, and vegetables, how are your symptoms now compared to before treatment: much worse, worse, unchanged, improved, highly improved." Further, the patients were asked to evaluate whether the perennial food allergy was less important, more important or equally important to their well-being than the hay fever symptoms during the birch pollen season. In the pretreatment questionnaire the age at debut of rhinoconjunctivitis and food allergy were registered.

### 2.7 Skin prick test

The SPT was performed according to the EAACI guidelines [28]. All patients were tested with a commercial birch pollen extract (Soluprick, ALK-Abelló, Hørsholm, Denmark) and fresh GD apples. The SPT with fresh apples was performed by the prick-prick technique [2, 3]. The tests were applied in duplicate or quadruplicate and skin wheal areas were determined by computer scanning [29].

### 2.8 Leukocyte histamine release

The histamine release experiment was performed by the glass-fiber method [30]. Fresh GD and the birch pollen extract (Soluprick) were applied for HR. Ten gram of fresh apple was crunched in a Stomacher 80 (Seward Lab System, United Kingdom) at high speed for 120 s with 10 mL PIPES-AMC, pH 7.4 (10 mM piperazine-*N,N*-(bis-ethanesulfonic acid), 140 mM sodium acetate, 5 mM potassium acetate, 0.6 mM $CaCl_2$, 1.1 mM $MgCl_2$, 1 mg/mL glucose, 0.3 mg/mL human serum albumin, 15 IU/mL heparin (Leo, Ballerup, Denmark)). After centrifugation ($2000 \times g$; 10 min) the supernatant was used as stock solution. The fresh preparation was applied within 15 min. For technical reasons, the birch pollen extract was diluted 10 times with PIPES buffer. Aliquots of 25 µL washed blood (containing approximately 500–2000 basophil leukocytes) was incubated with allergen extract or fresh preparation. The extracts were added to the plates in nine 3.5-fold dilutions in PIPES buffer. A histamine release test was considered positive when the maximal release ≥ 14 ng/mL. The HR was calculated as the mean value of the maximal release (ng/mL), and as the number of 3.5-fold dilution of the apple extract at which the release was 50% of the maximal release ($^1/_2$ max HR), *e.g.*, the higher number, the higher the allergenic potency of the extract [31].

### 2.9 Specific IgE

Specific IgE against apple and birch pollen was measured using the CAP-system (Pharmacia-Upjohn, Uppsala, Sweden) according to the instructions from the manufacturer.

### 2.10 Statistics

The results were compared by nonparametric methods (Mann-Whitney rank sum test, Wilcoxon signed rank test, Kruskal-Wallis one-way analysis of variance on ranks, Spearman rank order).

## 3 Results

### 3.1 Food allergy

Of the 74 patients included in the study, 64 (86%) had a history of allergic symptoms to plant foods; hazelnut was as the most frequent offending food reported by 55 (74%) of the patients and 51 (69%) reported allergy to apples. None of the patients had symptoms to red apples only. The mean number of positive foods was 8 (range 1–21). The mean duration of rhinoconjunctivitis to birch pollen was 14 years (range 4–31) and the duration of food allergy to apple was 11 years (1–28) (birch pollen > apple, $p < 0.001$). Of the 71 subjected to food challenges before treatment, 41 patients had a positive open challenge test. The pretreatment clinical, serological and SPT data were compared (data not shown). The symptom score and medication scores of the baseline pollen season did not correlate with the severity of food allergy to apple, evaluated by either the scores of the oral challenges or from the questionnaires. The apple challenge scores showed a correlation ($p$ corrected for the number of correlated parameters $<0.05$) with the results of SPT with fresh apple ($r = 0.54$) and a weaker correlation with specific IgE to apple ($r = 0.47$) and birch ($r = 0.44$). The symptom scores from the questionnaires correlated with IgE to apple ($r = 0.46$) and SPT with fresh apple ($r = 0.46$) and a weaker relationship was found with IgE to birch ($r = 0.38$) and HR to apple ($r = 0.38$). The patients with asthmatic symptoms during the pollen season did not differ significantly from the total group in any of the tested parameters, but among patients with a positive oral challenge with apple 9/41 patients had seasonal asthma compared to 2/33 in the challenge-negative group. Forty-two of the originally

 

**Table 1.** Symptoms to apple

| | SCIT ($n$ = 16) | | SLIT ($n$ = 12) | | Placebo ($n$ = 14) | |
|---|---|---|---|---|---|---|
| | Pre[a)] | Post | Pre | Post | Pre | Post |
| *Questionnaire* | | | | | | |
| Positive | 13 | 11 | 6 | 8 | 10 | 12 |
| Negative | 3 | 5 | 6 | 4 | 4 | 2 |
| Score/positive | 1.9 | 2.2 | 1.7 | 1.8 | 2.2 | 2.1 |
| *Change in questionnaire* | | | | | | |
| Positive → negative | 2 | | 0 | | 1.0 | |
| Negative → positive | 0 | | 2 | | 3.0 | |
| *Food challenges* | ($n$ = 15) | | ($n$ = 11) | | ($n$ = 14) | |
| Positive | 10 | 9 | 4 | 6 | 10 | 8 |
| Negative | 5 | 6 | 7 | 5 | 4 | 6 |
| Score/positive | 1.8 | 1.2 | 1.5 | 1.2 | 2.0 | 1.5[b)] |
| *Change in challenge result* | | | | | | |
| Positive → negative | 2 | | 1 | | 2 | |
| Negative → positive | 1 | | 3 | | 0 | |

a) Values before the treatment indicated in the column 'pre', values after treatment 'post'.
b) ↓ Challenge scores of the placebo group ($p$ = 0.03)

included 74 patients were re-evaluated after 2 years of SIT. The mean age of the 42 patients was 30 years (range 22 – 50) and the male/female ratio was 23/19.

Challenges were repeated in 15, 11, and 14 patients in the active subcutaneous, the active sublingual-swallow, and the placebo group, respectively. Table 1 summarizes the results of the open challenges and the questionnaire. The anamnestic symptom score per positive patient and the score of the challenges before immunotherapy did not differ significantly between the groups. The food challenges were positive in 10 (SCIT), 4 (SLIT), and 10 (placebo) patients before immunotherapy, and in 9, 6, and 8 after treatment. The symptoms during the challenges were mild and in most patients confined to OAS [32]. The scores decreased during treatment in all three groups, but this only reached statistical significance in the placebo group ($p$ = 0.03). Evaluated by the responses to the questionnaire, the apple symptom score did not change and there were no differences between the groups. In addition, Table 1 shows the number of patients in each group changing from positive reaction to apple to negative and vice versa, according to the questionnaire and the oral challenges.

According to the questionnaire, three patients changed from apple intolerant to tolerant during the trial (two in the SCIT group and one in the placebo group), whereas five patients developed food allergy to apple during the trial (two in the SLIT group and three in the placebo group). Eight out of the 42 patients asked reported that the symptoms elicited by foods were more important to their well-being than the seasonal hay fever symptoms and four patients reported that the symptoms were equally important.

Figure 1 shows the changes in severity of food allergy in general evaluated by the answers to the questionnaire, *i. e.*, the overall perception of severity of food allergic symptoms. In all, 34 patients completed this part of the questionnaire (14 SCIT, 8 SLIT, 12 placebo). There was a tendency towards improvement in all three groups, but for most patients the symptoms were unchanged and there were no differences between the groups.

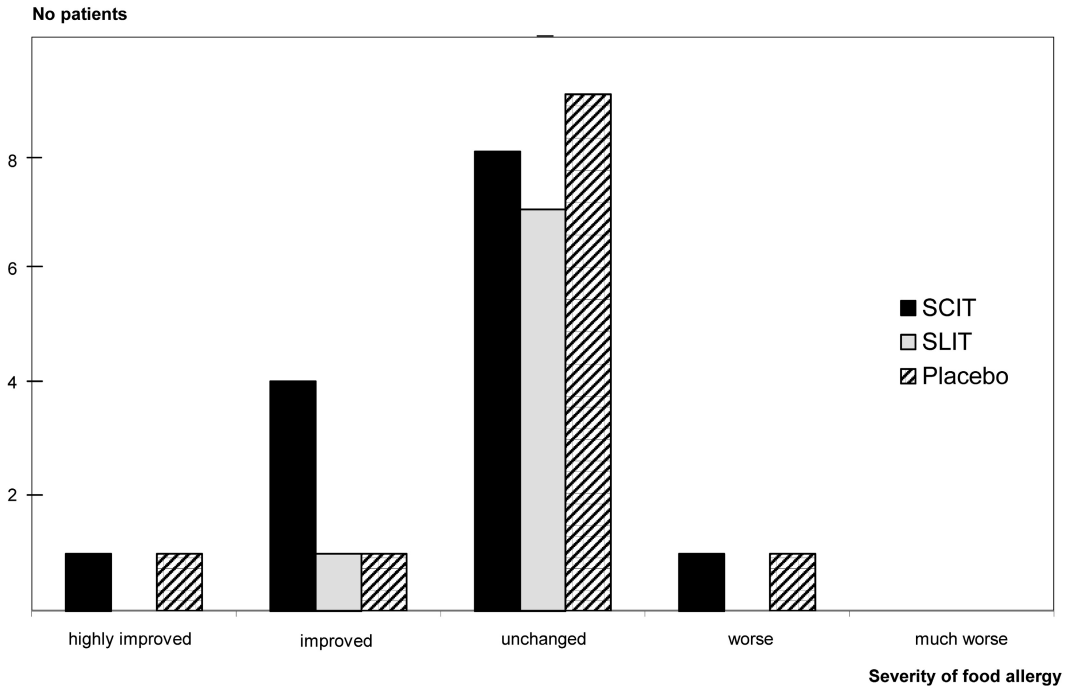


**Figure 1.** Change in severity of food allergy in general in the three groups ($n$ = 34).

 

**Table 2.** SPT and specific IgE

| | | SCIT ($n = 16$) | | SLIT ($n = 12$) | | Placebo ($n = 14$) | |
|---|---|---|---|---|---|---|---|
| | | Pre[a)] | Post | Pre | Post | Pre | Post |
| *SPT* | | | | | | | |
| Apple ($mm^2$) | Mean | 21.4 | 14.5 | 10.0 | 16.5 | 21.1 | 30.5 |
| | Range | 1.0 – 56.8 | 2.2 – 28.6 | 0.5 – 22.1 | 0 – 27.5 | 3.1 – 52.0 | 5.2 – 86.0 |
| Birch | | 42.7 | 11.7[a)] | 41.8 | 32.8 | 47.4 | 44.0 |
| | | 22.5 – 99.6 | 0.7 – 22.2 | 21.3 – 126.8 | 8.2 – 79.5 | 12.2 – 174.6 | 11.8 – 148.1 |
| Histamine 10 mg/mL | | 21.9 | 24.6 | 24.7 | 30.6 | 20.0 | 23.8 |
| | | 10.8 – 38.2 | 11.6 – 42.1 | 4.5 – 50.6 | 10.5 – 52.8 | 7.5 – 28.6 | 13.8 – 33.1 |
| *Specific IgE* | | | | | | | |
| Apple (kU/L) | Mean | 5.9 | 1.8[b)] | 0.8 | 1.4 | 2.6 | 2.1 |
| | | 0 – 33.3 | 0.5 – 0.8 | 0 – 4.8 | 0 – 3.4 | 0 – 13.4 | 0 – 7.4 |
| Birch | | 34.1 | 21.8[c)] | 10.5 | 22.7[d)] | 32.8 | 31.3 |
| | | 1.0 – 102.0 | 1.4 – 66.4 | 1.3 – 21.4 | 1.3 – 75.8 | 1.1 – 83.5 | 1.1 – 86.9 |

a) ↓ SPT birch ($p = 0.00003$); b) ↓ IgE apple ($p = 0.009$); c) ↓ IgE birch ($p = 0.04$); d) ↑ IgE birch ($p = 0.001$)

### 3.2 Skin prick test and specific IgE

Table 2 shows the changes in SPT and specific IgE. There were no differences in skin wheal areas to either birch pollen or apple between the groups before treatment. Regarding specific IgE before treatment, the SLIT group had lower level of specific IgE to apple than the placebo group ($p = 0.02$) and lower level of specific IgE to birch than both the placebo group ($p = 0.004$) and the SCIT group ($p = 0.02$). Skin wheal area to birch ($p = 0.00003$) and specific IgE to apple and birch decreased significantly during treatment in the SCIT group ($p = 0.009/0.04$). In the SLIT group, IgE to birch increased significantly ($p = 0.001$), whereas the placebo group showed no significant changes in either SPT or specific IgE.

### 3.3 Histamine release

Figure 2 illustrates the changes in maximal HR to apple (2A) and birch (2B) during the immunotherapy treatment. During the high-level pollen season 1998, the SCIT group showed significantly lower release to birch (max HR, $p = 0.02/^1/_2$ max HR, $p = 0.03$) and apple (max HR, $p = 0.045$) than the placebo group. There were no significant differences between the SLIT group and the placebo group. There were one, zero, and three nonresponders in the SCIT, SLIT and placebo group, respectively, during the seasonal HR testing of 1998.

## 4 Discussion

In contrast to the findings of other investigations [15], the severity of the birch pollen induced rhinoconjunctivitis did not correlate with the severity of the symptoms elicited by ingestion of apple during food challenges or to the reported severity from the questionnaire. Further, seasonal asthmatic symptoms did not imply more severe food allergy. On the other hand, seasonal asthma was most frequent among patients with challenge verified food allergy to apple. Twelve patients assessed the perennial allergic symptoms to food above or equal to the seasonal pollen induced hay fever symptoms. These results could be ascribed to perennial nature of the pollen related food allergies, and perhaps the incoherence in severity of rhinoconjunctivitis and food allergy some way is connected to the observed lack of effect on food allergy of the pollen immunotherapy.

Among birch pollen-allergic individuals, a prevalence of food allergy between 20% and 90% has been reported [1, 2, 17, 33, 34]. The range can be explained by selection biases, geographical differences in pollen occurrence, eating habits *etc*. If there is a connection between the level of sensitization to birch pollen and food allergic symptoms, the high rate of pollen-associated food allergy to plant foods in this study can partly be explained by the inclusion criteria's since only patients with specific IgE to birch pollen CAP class 2 ($\geq 0.7$ kU/L) or higher were included [27].

In food allergy to plant foods caused by resemblance between birch pollen and food allergens, it has generally been considered that immunotherapy should aim at the primary inhalation allergen rather than the food allergen. In this study, two types of active treatment were compared: subcutaneous and sublingual-swallow immunotherapy. Regarding seasonal rhinitis, the sublingual-swallow application is less investigated than the subcutaneous application, but it seems to be effective [35] . One short-term SLIT trial with a birch pollen extract has been published [36], showing an effect on nasal and eye symptoms. As recently published, the immunotherapy trial confirmed an effect of SCIT and SLIT on seasonal symptoms and use of medication [27]. Evaluated by the change in symptom score during

 

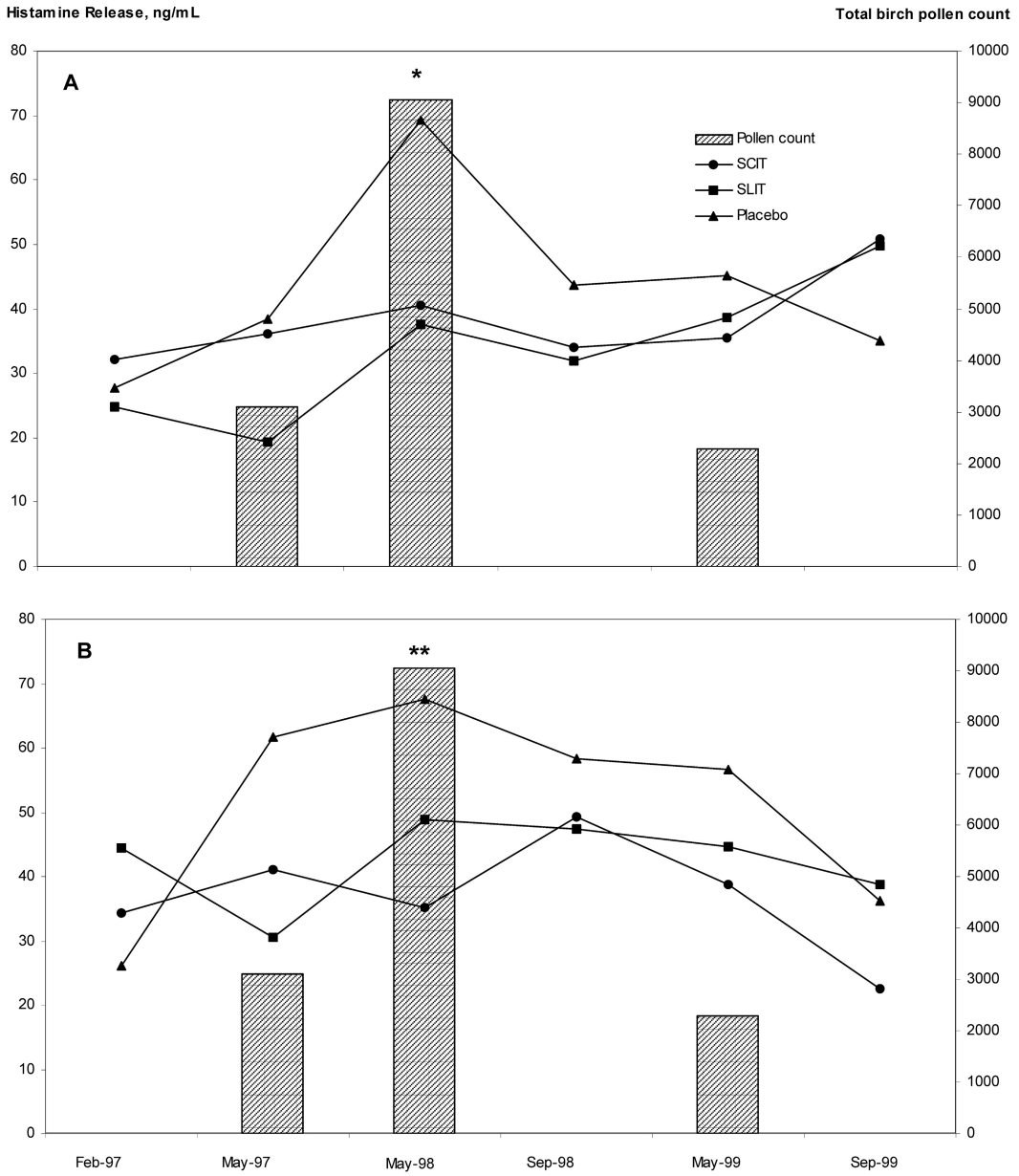


**Figure 2.** Leukocyte histamine release in ng/mL (curves) to (A) apple and (B) birch. SCIT ($n$ = 16), SLIT ($n$ = 12), placebo ($n$ = 14). * Max HR apple: placebo group > SCIT group ($p$ = 0.045). **Max HR and $^1/_2$ max HR birch: placebo group > SCIT group ($p$ = 0.02/0.03). The total birch pollen counts (bars) are indicated under the in-season values.

open challenge with apple, the results showed no effect of either subcutaneous or sublingual birch pollen immunotherapy on food allergy to apple. Only two actively treated patients and one patient in the placebo group previously allergic to apple developed tolerance during the trial.

During the last years, recombinant allergens produced by DNA technology have been investigated in a number of studies. It has been shown that the use of such recombinant allergens can improve the overall diagnostic and therapeutic possibilities regarding pollinosis [37, 38]. Recombinant Bet v 1 and Mal d 1 has been applied for skin testing in one study on SIT with a birch pollen extract and food allergy to apple and a significant reduction in severity of allergy to apple was documented by DBPCFC with apple [25]. A serious draw-back in the study was the lack of placebo control of the immunotherapy treatment. It remains unclear whether SIT with recombinant Bet v 1 will have a more pronounced effect on pollen related food allergy than the traditionally used whole allergen extracts. High doses of modi-

 

fied and thereby less immunogenic recombinant Bet v 1 would perhaps have a more convincing impact on the pollen related food allergy but it might also be necessary to include the relevant food allergens in modified form. Recent results could support the latter since food specific epitopes not shared by pollen allergens has been shown in studies on carrot [39] and hazelnut allergy (Vieths and co-workers, personal communication). The answers from the questionnaires and changes in challenge symptom scores showed a tendency towards an overall decrease in clinical reactivity to plant foods in all three groups. This emphasises the need for placebo control when subjective assessments are included in clinical trials.

The groups displayed differences regarding baseline-specific IgE to apple and birch pollen, and the SLIT group comprised fewer patients with clinical allergy to apple and they also had a lower challenge score per patient than the other groups. The inclusion criteria and randomization for this study did not address pollen related food reactions and, therefore, the patients with allergy to apple might have been unevenly distributed in regard to severity of apple-induced symptoms. Furthermore, the withdrawal of patients during the trial may have influenced the distribution. It is generally accepted that the diagnosis of food allergy must relay on DBPCFCs [40], but regarding apple there are several methodological difficulties and at the time of this study no valid DBPCFC with apple was available. As a consequence, open challenges were performed.

In conclusion, the study confirmed that allergy to plant foods is very common among birch pollen allergic individuals in Scandinavia. The severity of seasonal symptoms and use of medication during the birch pollen season did not correlate with the severity of the food allergy to apple. The answers from the questionnaires showed a tendency towards an overall decrease in clinical reactivity to plant foods in all groups, including the placebo group. In spite of an effect on seasonal rhinoconjunctivitis and a decrease in IgE-reactivity to apple, SIT with a birch pollen extract was not accompanied by a decrease in oral sensitivity to apple. The results of this study can not rule out beneficial effect on food allergy in some patients, but at present OAS to apple should not be considered as a main criterion for selection of patients for birch pollen immunotherapy.

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