

Effects of birch pollen-specific immunotherapy on apple allergy in birch pollen-hypersensitive patients

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Summary

Background Most patients with birch pollen allergy report oral allergy symptoms after eating fresh apples and other vegetable foods. Major birch pollen and apple allergens, Bet v 1 and Mal d 1, are highly homologous; as a consequence, pollen-specific immunotherapy (SIT) might be expected to improve apple hypersensitivity.

Objective To evaluate the clinical and immunological effects of birch pollen SIT on oral allergy syndrome (OAS) induced by apples.

Methods A prospective study carried out in 49 birch pollen-sensitive patients with apple-induced OAS who received injection immunotherapy for 12, 24, or 36 months. Twenty-six patients not submitted to SIT and followed up for 12–48 months were used as controls. Both SPT and open oral challenges with fresh golden delicious apple were performed, as well as specific IgE measurements, before and after SIT.

Results Forty-one patients (84%) vs no control (0%) reported a significant reduction (50–95%) or a total disappearance (100%) of OAS symptoms after SIT ($P < 0.001$). Similar responses were observed in patients treated for 12, 24, or 36 months. SIT also induced a marked reduction in skin reactivity against fresh apple in 43 patients (88%). The effect of SIT was inversely related with baseline skin reactivity: 50% and 8% patients with a weakly or strongly positive baseline apple skin prick tests (SPT), respectively, did not report changes in OAS severity after SIT ($P < 0.01$). In contrast, baseline birch pollen-specific or apple-specific IgE antibodies levels did not influence SIT effectiveness on OAS. SIT induced a marked decrease in birch pollen-specific IgE levels ($P < 0.001$), whereas apple-specific IgE showed an unexpected variability (reduction in 21%, no change in 43%, increase in 38%). No control subject reported a reduction in OAS severity or showed a decrease in skin reactivity at follow-up ($P < 0.001$).

Conclusions SIT with birch pollen extracts effectively reduces clinical apple sensitivity and skin reactivity in most cases after only 1 year of treatment; these effects are not paralleled by a similar reduction in apple-specific IgE. These findings suggest a decrease in activability of effector cells as the mechanism underlying clinical benefit.

Keywords: apple allergy, birch pollen allergy, cross-reactivity, specific immunotherapy

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Introduction

Up to 80% of patients with birch pollen allergy (BPA) develop the so called oral allergy syndrome (OAS), an IgE-mediated food allergy characterized by immediate itching in

the mouth and throat and eventually angioedema after eating various fresh fruits, nuts, and/or vegetables, due to cross-reactivity between pollen and food allergens [1–4]. Apple is one of the most frequently involved foods, and this is thought to be due to the high homology between its major allergen, Mal d 1, and major birch pollen allergen, Bet v 1 [5–7], and specific IgE against apple allergens is detectable in sera from virtually all birch pollen-sensitive patients [5].

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Since several lines of evidence show that, in patients with birch pollen allergy and OAS induced by apples, most allergenic epitopes of the apple are present in birch pollen [4,6], one could reasonably expect that birch pollen specific immunotherapy (SIT) may influence apple hypersensitivity (and possibly OAS caused by other vegetable foods) both clinically and immunologically. This controlled study was carried out to investigate these potentially beneficial effects of SIT.

Patients and methods

Patients

Forty-nine adult birch pollen-allergic patients (M/F 18/31, mean age 34.4 years) with a clear history of OAS after the ingestion of apples were included in this prospective study.

A baseline evaluation was carried out in October (just before the start of SIT) in order to avoid possible bias caused by changes in symptoms, SPT results, and specific IgE levels during the pollen season: (a) SPT which were performed with fresh apple by the prick-prick technique [8] using a 1 mm tip disposable prick lancetter (Bayer). Weals < ++ by comparison with histamine 10 mg/mL were considered negative [9]. (b) The severity of OAS was graded by an open challenge: each patient was asked to chew about 10 g of fresh apple for 1 min and then to spit it out; subjects were taken under control during the following 15 min. Baseline OAS severity was considered 100%.

In view of the marked differences in allergenicity between apple varieties [10], all SPTs and oral challenges were performed using the Golden Delicious strain.

Baseline IgE specific for birch pollen were measured in 36 subjects by an immunoenzymatic assay (Allercoat, Kallestad); levels were expressed in Allercoat arbitrary units per millimetre (AU/mL). Baseline IgE specific for apple were measured in 24 subjects by CAP-system (Pharmacia); levels were expressed in kilounits per litre.

Patients were all re-evaluated in October 1997, after 12 ($n = 15$), 24 ($n = 21$), and 36 ($n = 13$) months of SIT, when SPTs and open oral challenges with fresh apple were repeated. On that occasion, patients were asked to grade OAS severity by comparison with the baseline (100%) as worse or unchanged, or better; in this case patients were asked to quantify symptom reduction on a visual analogue scale; only reductions equal or better than 50% were considered significant. Apple-specific IgE were re-measured in 14 patients.

Controls

Two separate groups of controls were used. The first group

consisted of 26 birch pollen-allergic patients with typical OAS after the ingestion of apples not submitted to SIT who were followed-up for 12 ($n = 7$), 24 ($n = 5$), 36 ($n = 11$), or 48 ($n = 3$) months. The second group comprised 16 patients submitted to SIT who were followed-up for 12 ($n = 8$), 24 ($n = 4$), 36 ($n = 1$), or 48 ($n = 3$) months before SIT was started. All these patients underwent SPTs with fresh apple at the time when OAS was diagnosed and when subsequent follow-up visits were performed.

Specific immunotherapy (SIT)

All patients underwent injection SIT with commercial depot aluminium hydroxide-adsorbed birch pollen extracts by two manufacturers (Allergopharma retard [$n = 33$], and Bayer Alhydrox [$n = 16$]). SIT was administered following a perennial schedule: maintenance doses were reduced by half during the pollen season. Weekly doses were given during the 'build-up' (induction) phase in order to reach the planned maintenance dose which was 1.0 mL of vial III (corresponding to 5000 TU for Allergopharma and to 10 000 AlhU for Bayer); however, in practice, this dose could not be achieved in all patients and subjects were therefore maintained on the highest tolerated dose that did not elicit side-effects. Others have proposed that this corresponds with the optimal dose for SIT [11]. Maintenance doses were given at 3 week intervals.

Statistical methods

Proportions were compared using a two-tailed chi-square test with Yates' correction. Because of their skewed distribution, specific IgE levels were analysed by non-parametric means. Probability (P) values less than 0.05 were considered statistically significant.

Results

Baseline findings

In 36/49 cases, apple-induced OAS was characterized by oral itching alone, whereas in 13 patients itching was associated with immediate angioedema of the lips and/or of the tongue or with contact urticaria after apple peeling. All 49 patients were positive on SPT with fresh apple; skin reactivity was ++, +++, or ++++ in 10, 22, and 17 subjects, respectively. Median birch pollen IgE level was 10 AU/mL (mean 10.6; range 3–18 AU/mL). Median apple IgE level was 1.85 kU/L (mean 6.95, range <0.35–46.4 KU/L).

All 26 controls were positive on SPT with fresh apple; skin reactivity was ++, +++, or ++++ in four, eight and 14 subjects, respectively.

SIT effects

Overall 41/49 (84%) patients reported a significant reduction or a total disappearance of OAS symptoms on open challenges performed after SIT; in 22 (45%) cases re-challenge with golden delicious apple did not elicit OAS symptoms at all. Moreover, none of 13 patients in which apple caused angioedema or contact urticaria reported such symptoms after SIT: apple challenges elicited only a slight oral itching in 10 of these patients and no symptoms at all in three subjects. All 22 patients who did not report any reactivity were asked to introduce apples in their normal diets. To date (April 1998) none of them reported adverse reactions but two who reported a slight relapse of OAS coincident with seasonal respiratory symptoms (i.e. apple intolerance changed from perennial into seasonal in these patients); several other patients report eating apples almost every day without any problem.

The analysis of challenge test results (Table 1) did not show statistically significant differences between patients

Table 1. Results of challenge tests with fresh golden delicious apple after SIT

	No.	Unchanged <i>n</i> (%)	Better <i>n</i> (%)	Cured <i>n</i> (%)
Whole population	49	8 (16)	19 (39)	22 (45)
<i>SIT duration</i>				
12 months	15	2 (13)	7 (47)	6 (40)
24 months	21	3 (14)	6 (29)	12 (57)
36 months	13	3 (23)	6 (46)	4 (31)
<i>Baseline SPT</i>				
++	10	5 (50)	4 (40)	1 (10)
+++	22	2 (9)	7 (32)	13 (59)
++++	17	1 (6)	8 (47)	8 (47)
<i>Baseline birch IgE (median 10 AU/mL)</i>				
< 10 AU/mL	18	5 (28)	6 (33)	7 (39)
> 10 AU/mL	18	2 (11)	9 (50)	7 (39)
<i>Baseline apple IgE (median 1.85 kU/L)</i>				
< 1.85 kU/L	12	3 (25)	3 (25)	6 (50)
> 1.85 kU/L	12	2 (17)	6 (50)	4 (33)

Unchanged, No reduction of OAS severity; Better, 50–95% reduction of OAS severity; Cured, Total disappearance of apple-induced OAS symptoms.

submitted to SIT for 12, 24, or 36 months, or patients treated with extracts from different manufacturers.

SIT induced a marked reduction in skin reactivity to fresh apple in 43/49 (88%) patients: at the follow-up evaluation SPTs were negative (i.e. <++) in 34 (69%) patients, ++ in 13, and +++ in two cases; no patient showed a++++ apple SPT after SIT. In 3/6 (50%) cases, unchanged SPT was associated with unchanged OAS severity.

The effect of SIT on OAS was inversely related with baseline skin reactivity against fresh apple: the proportion of patients who did not observe changes in OAS severity after SIT was significantly higher among subjects whose baseline SPT ranked ++ than among those with a higher baseline skin reactivity (5/10 [50%] vs. 3/39 [8%]; $P < 0.01$). In contrast, baseline birch pollen-specific IgE levels or apple-specific IgE levels did not influence SIT effectiveness (Table 1).

Apple- and birch-specific IgE levels before and after SIT, challenge test results, and SPT results in 14 patients are shown in Table 2. Most patients showed a significant reduction in birch pollen-specific IgE levels after SIT ($P < 0.01$; Wilcoxon rank sum test). In contrast, after SIT, IgE levels were significantly reduced in three patients (21%), unchanged in six (43%), and significantly increased in five subjects (38%). Statistical analysis for paired samples did not show significant differences between apple-specific IgE levels before or after SIT ($P = 0.54$; Wilcoxon rank sum test).

Three of 14 patients did not observe changes in OAS severity; apple specific IgE levels were decreased, increased, or unchanged, respectively, after SIT in these subjects. Twelve (86%) patients showed a reduction in skin reactivity against fresh apple which was independent from apple-specific IgE levels.

Controls

No control patients reported disappearance or reduction of OAS at follow-up visits: on the contrary, in many cases they reported a worsening of OAS symptoms after unintentional ingestion of raw apples or whenever they attempted to eat apples (data not shown). The difference with patients submitted to SIT was highly significant (84% vs 0%; $P < 0.001$). Moreover, no control subjects showed any appreciable reduction of fresh apple SPT at the follow-up visits; again, the difference between patients and controls was highly significant (88% vs 0%, respectively; $P < 0.001$).

SPT results during the pre-SIT and post-SIT follow-up in 16 patients are shown in Table 3. Only one patient (S.B., Table 3) showed a slight spontaneous reduction in apple skin reactivity at the end of the follow-up period before the start of SIT; in contrast, all patients but one (Po.E) showed a marked drop in apple skin reactivity after only 1 year of SIT.

Table 2 Clinical and immunological changes after SIT

Patient	Before SIT/After SIT			SIT	Changes in OAS severity
	Apple SPT	Apple IgE	Birch IgE	Duration (months)	
C.S.	+++/-	1.4/<0.35	7.2/ND	36	Unchanged
R.S.	+++ +/-	1.5/3.4	16.9/1.2	36	- 100%
B.I.	+++ +/-	0.5/8.0	>17.5/ND	36	- 50%
A.P.	++/-	1.8/2.3	16.0/12.0	24	- 100%
S.G.	++++	0.6/2.7	12.2/4.4	24	- 100%
P.C.	++++/+	4.2/27.7	>17.5/9.2	24	- 100%
P.L.	+++ +/-	1.9/<0.35	16.0/<0.35	24	- 100%
S.D.	++++/+++	4.3/3.3	>17.5/11.3	24	- 50%
B.R.	++++	18.5/10.7	10.2/4.8	24	- 100%
D.S.	++++	2.2/1.9	6.0/4.3	12	- 80%
M.C.	+++++	4.8/3.8	15.9/10.2	12	Unchanged
S.B.	+++/-	0.8/0.5	2.9/1.3	12	- 100%
Po.E.	++++	20.7/20.8	9.4/12.5	12	- 50%
Pa.E.	++++	46.4/67.6	12.3/11.0	12	Unchanged

SPT are graded by comparison with the weal induced by histamine 10 mg/mL [9]. Apple-specific IgE are expressed in kU/L. Birch pollen-specific IgE are expressed in AU/mL. ND, not done; OAS severity: - 100%. Total disappearance of apple-induced OAS symptoms; Unchanged, no difference from baseline severity. $P < 0.001$ for birch-specific IgE levels before and after SIT (Wilcoxon rank sum test).

Table 3 Reactivity on SPT with fresh apple in patients followed-up before SIT

Patient	SPT at 1st visit	Pre-SIT follow-up (months)	SPT at SIT start	SIT duration (months)	SPT after SIT	Changes in OAS severity (%)
C.S.	+++	12	+++	36	-	Unchanged
M.A.	++++	24	++++	36	+	- 50
R.S.	++++	24	++++	36	-	- 100
M.I.	+++	12	++++	36	++	- 60
B.I.	+++	12	++++	36	-	- 50
S.G.	+++	48	+++	24	+	- 100
P.E.	++++	12	++++	24	-	- 90
P.Cr.	++++	36	++++	24	+	- 100
R.S.	++++	48	++++	24	-	- 100
Po.E.	+	12	++	12	++	- 50
S.B.	++++	24	+++	12	-	- 100
M.C.	+++	48	+++	12	++	Unchanged
P.Ch.	+++	12	+++	12	+	- 100
C.M.	++++	12	++++	12	-	- 80
D.T.S.	+++	12	+++	12	+	- 80
C.L.	++++	24	++++	12	-	- 60

Discussion

This study aimed to investigate the clinical and immunological effects of birch pollen SIT on apple allergy. There are few studies dealing with this problem in the literature. A recent single case report showed the disappearance of OAS after 1 year of pollen immunotherapy [12]; in that case there was a marked reduction in both skin tests and food-specific IgE levels, and immunoblot analysis revealed a decline of IgE binding to most components of food extracts. In contrast, no effect by subcutaneous or oral immunotherapy on food allergy was observed in a previous study performed on 72 children with birch pollinosis [13]. The present work was carried out on a large population to allow a comprehensive evaluation of the possible effects of SIT on OAS induced by apple. To avoid bias caused by differences in the concentration of cross-reactive allergens between various fruits or vegetables, all the patients included in this study were evaluated for their reactivity against fresh golden delicious apple only, irrespective of other coexisting vegetable food allergies.

The main findings of this study were as follows. 1) A reduction in symptoms of apple hypersensitivity in most patients who received injection SIT with birch pollen extracts. The clinical and immunological effects of SIT appear to have reached a plateau after as little as one year of treatment. 2) A marked reduction in skin reactivity against fresh apple in most patients, although this effect was not always associated with a reduced severity of OAS. 3) The decreases in OAS severity and skin reactivity were not associated with a significant fall of apple-specific IgE levels; on the contrary, an increase in IgE levels was observed in a significant proportion of SIT patients. 4) Birch pollen SIT was more effective in attenuating apple-induced OAS in those with a more marked baseline skin reactivity or with more severe OAS symptoms. No control patient reported a spontaneous reduction or disappearance of OAS. The comparison between SIT patients and controls is even more impressive if one considers that patients had more severe respiratory allergy, and hence more severe OAS [14], than controls. Some of these findings are not unexpected: in most previous studies of SIT, a significant reduction in cutaneous reactivity in response to the relevant allergen and an extreme variability in IgE antibody response to immunotherapy were observed [15,16]. While it is generally accepted that changes in specific IgE are not related to clinical outcome in patients submitted to SIT [15], in this study there was an impressive discrepancy between the response of birch pollen-specific IgE and apple-specific IgE to SIT; this fact is interesting but difficult to explain. One possible explanation might be that apple-specific IgE measured was not relevant to the causation of OAS. While effectiveness of specific immunotherapy is widely accepted,

its mechanisms remain incompletely defined. The findings in this study seem to suggest a decrease in activability of effector cells (as shown by the marked reduction of weals induced by SPT with fresh apple) in the presence of unchanged levels of specific IgE as one of the mechanisms underlying clinical benefit. In effect, reduction of inflammatory cell recruitment, activation or mediator secretion are considered some of the main changes induced by SIT; a previous study performed on ragweed sensitive patients showed that SIT inhibited immediate release of mast cell mediators [17]. This study shows that OAS induced by birch-related foods can be effectively treated by pollen SIT; as a consequence, a similar approach might be reasonably attempted also in patients with other, more dangerous food allergies caused by cross-reactivity between pollens and vegetables. Subjects with mugwort pollinosis and celery allergy [18,19] or with ragweed pollinosis and watermelon allergy [20] might represent good candidates.

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