

Birch-Apple Syndrome Treated with Birch Pollen Immunotherapy

Marina Mauro^a Marina Russello^a Cristoforo Incorvaia^b Gianbattista Gazzola^a
Franco Frati^{c, d} Philippe Moingeon^f Gianni Passalacqua^e

^aAllergy Unit, Sant'Anna Hospital, Como, ^bAllergy/Pulmonary Rehabilitation Unit, ICP Hospital, and
^cMedical and Scientific Department, Stallergenes, Milan, ^dPediatrics Unit, University Department of Medical and
Surgical Specialties and Public Health, Perugia, and ^eAllergy and Respiratory Diseases, University Department of
Internal Medicine, Genoa, Italy; ^fScientific Department, Stallergenes, Antony, France

Key Words

Birch pollen • Apple • Birch-apple syndrome • Subcutaneous immunotherapy • Sublingual immunotherapy • Apple challenge • Mal d 1 • Specific IgE

Abstract

Background: The most common pollen-fruit cross-reaction is the birch-apple syndrome. Allergen immunotherapy (IT) is clearly effective for birch allergy, but its efficacy on apple allergy is controversial. We performed a randomized study on patients with birch-apple syndrome to evaluate the outcome of subcutaneous immunotherapy (SCIT) and sublingual immunotherapy (SLIT). **Methods:** Forty patients underwent IT with a birch extract (Staloral; Stallergenes, Antony, France), 20 by SCIT and 20 by SLIT. After 1 year of treatment, 15 patients (8 for SCIT and 7 for SLIT) accepted to undergo an oral apple challenge. Measurements of specific IgE to Bet v 1 and Mal d 1 and related allergens Api g 1 and Dau c 1 were obtained in 10 patients, at baseline and after IT. **Results:** Two of 8 SCIT-treated patients (25%) and 1 of 7 SLIT-treated patients (14.2%) developed complete tolerance to apple. In the remaining patients, an increase in the provocative dose was found in 3 of the SCIT-treated (37.5%) and 2 of the SLIT-treated patients (28.6%). Changes in the levels of specific IgE to

Mal d 1 were unrelated to clinical results. **Conclusions:** These findings suggest that different doses of birch extract may be needed in different patients to improve the associated apple allergy and that a finer diagnostic work-up in selecting patients with birch-apple syndrome who are candidates to respond to birch pollen IT also concerning apple allergy is required.

Copyright © 2011 S. Karger AG, Basel

Introduction

Clinical syndromes characterized by pollinosis and allergic reactions to vegetable foods are caused by cross-reacting molecules present in different allergen sources [1]. In recent years, new laboratory techniques have better clarified the molecular mechanisms and characteristics of cross-reactions [2–4]. The most extensively studied model is the so-called birch-apple syndrome, with respiratory symptoms caused by birch pollen and concomitant oral allergy syndrome (OAS) to apples [1]. The birch-apple syndrome is explained by the high structural homology of the major birch allergen Bet v 1 and the major apple allergen Mal d 1 [5].

The only treatment able to act on sensitization and thus modify the natural history of respiratory allergy is specific immunotherapy (IT) [6]. Among the numerous studies dealing with IT, there are controlled trials demonstrating its effectiveness in birch pollinosis either by subcutaneous immunotherapy (SCIT) [7, 8] or by sublingual immunotherapy (SLIT) [9, 10], and 1 study compared the 2 routes of administration reporting a higher, although not significant, efficacy for SCIT, but a better safety for SLIT [11].

Taking into account the homology of the major allergens from birch and apple, it was reasonable to hypothesize that IT with birch extracts was also able to improve apple allergy. In fact, early open studies with SCIT for birch rhinoconjunctivitis reported a favorable outcome for OAS symptoms [12–15]. However, subsequent studies performed with either SCIT or SLIT provided controversial or inconclusive results [16–22], and so the possibility to cure apple allergy by birch IT is nowadays questioned.

In order to provide further data on this aspect, we assessed the effect of SCIT or SLIT in apple-induced OAS in a randomized study by means of a standardized oral challenge.

Patients and Methods

Patients

Subjects were recruited from the adult outpatients who were referred to the Allergy Unit of the Sant'Anna Hospital in Como, Italy, for seasonal rhinitis. The inclusion criteria were as follows: (1) age between 18 and 60 years; (2) symptoms of rhinitis during the seasonal period of tree pollens (from February to April) in the last 2 years, with or without apple-induced OAS; (3) positive IgE assay to the major allergen of birch, namely Bet v 1. For patients with OAS, the sensitization to apple had to be confirmed by a prick-to-prick test with fresh apples. Prick tests with the standard panel of food commercial extracts and prick-to-prick test with fresh fruits and vegetables were also done to assess the cross-reactions with Bet v 1-related food allergens or the sensitization to other food allergens. The exclusion criteria were as follows: (1) sensitization to other allergens interfering with clinical symptoms (such as cypress pollen, house dust mites, and animal epithelia); (2) previous IT in any form; (3) contraindications to allergen IT according to the guidelines [6].

IT Treatment

Patients were randomized to receive SCIT or SLIT according to a computer-generated randomization list, resulting in 24 SCIT and 23 SLIT treatments. Tree pollen (birch, alder and hazelnut) extracts from Stallergenes (Antony, France), standardized in index of reactivity (IR), were used. The schedules of treatment and administered doses have already been reported [23]. Briefly, with SCIT the maintenance dose of 10 IR was reached by a build-up in

12 weeks, and with SLIT the maintenance dose of 100 IR was reached by a build-up in 11 days.

Symptom-Medication Score

All patients registered their symptoms and drug consumption in diary cards during the month of March in the year before the start of IT and in the same month after IT.

Challenge with Apple

The oral challenge with fresh apples was performed openly, using the standardized technique described by Pastorello et al. [24]. In brief, increasing amounts from 4 to 64 g of fresh apples were administered at 15-min intervals. For safety reasons, the first dose was preceded by putting a thin apple slice in contact with the lips for 2 min. The challenge was stopped when symptoms appeared. The grade of reaction was classified as follows: grade 1, only oral mucosal symptoms (itching and burning); grade 2, oral mucosal and gastrointestinal symptoms (nausea, vomiting, abdominal pain and diarrhea); grade 3, oral mucosal and systemic symptoms (urticaria, rhinoconjunctivitis and asthma); grade 4, oral mucosal and anaphylactic symptoms (laryngeal edema, shock). The challenge was performed before randomization and after 1 year of IT.

Measurement of IgE to Bet v 1 and Related Allergens

Blood samples were collected at baseline and after 1 year of IT. Sera were frozen and stored at -80°C until assayed. A microarray, recombinant-allergens test (Immuno Solid Allergen Chip, ISAC; Genosense Diagnostics GmbH, Vienna, Austria) [25], including 90 allergen molecules, was used. For the purpose of this study, specific IgE were determined for Bet v 1, Cor a 1, Aln g 1, Mal d 1, Api g 1 and Dau c 1. The tests were performed directly in the Genosense Diagnostic Laboratories in Vienna on the sera from 10 patients (4 SCIT-treated and 6 SLIT-treated patients).

Results

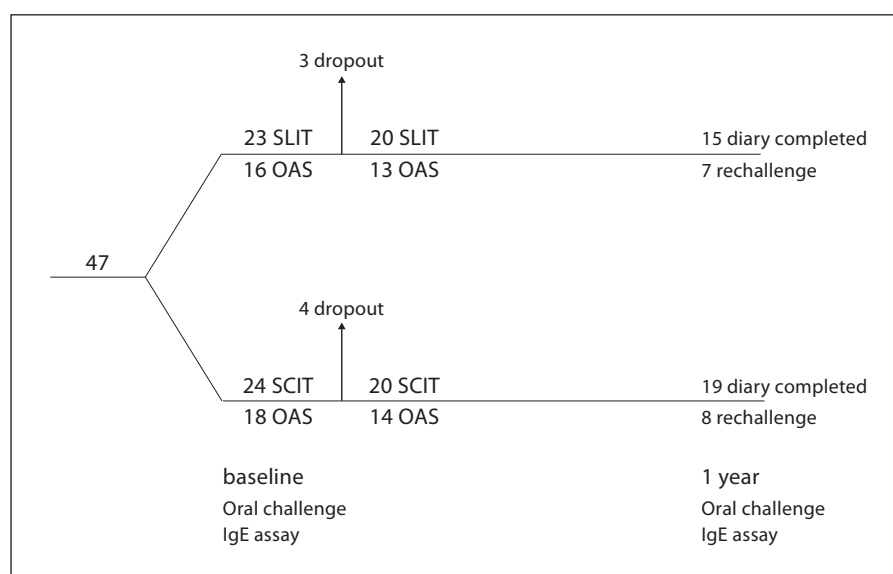
Forty-seven patients (25 males; mean age 39.8 years; age range 18–60 years) fulfilled the inclusion criteria to be randomized, and 34 of these patients resulted to have an apple-induced OAS. Seven patients refused to start IT (1 because of pregnancy, 1 intercurrent disease, 1 moving and 4 personal unspecified reasons). The patients' disposition is summarized in figure 1.

The characteristics of the 40 patients starting IT (20 for SLIT and 20 for SCIT) are shown in table 1. Thirty-four subjects (19 for SCIT and 15 for SLIT) completed the registration of symptoms and drug consumption before IT during the month of March. As already reported [23], the mean symptom-medication score was 4.77 ± 1.41 in SCIT-treated and 3.63 ± 1.08 in SLIT-treated patients, this difference not being significant. Systemic reactions occurred in 16% of the SCIT-treated but in none of the SLIT-treated patients, and no serious adverse event oc-

Table 1. Baseline characteristics of patients

	SLIT	SCIT
Number	20	20
Gender	11 males, 9 females	10 males, 10 females
Mean age, years	38.7	36.9
OAS	16	18
Mean duration of pollinosis, years	6.2 (1–18)	7.4 (1–19)
Mean duration of OAS, years	3.8 (1–13)	4.4 (1–9)
SPT positivity to carrot/celery	9	8

Figures in parentheses are ranges.

**Fig. 1.** Flow chart describing the number of patients at different time points and withdrawals.

curred. Concerning the immunological evaluation, only Bet v 1-specific IgG4 in SCIT-treated patients showed a significant increase ($p = 0.001$ versus baseline) [23]. After 1 year of treatment, 15 patients (8 for SCIT and 7 for SLIT) accepted to undergo oral apple challenges. Figure 2 reports the results of the challenge with fresh apples. Two of 8 SCIT-treated patients (25%) and 1 of 7 SLIT-treated patient (14.2%) developed a complete tolerance to apples (64 g of apples). In the remaining patients, an increase in the provocative dose was found in 3 of the SCIT-treated (37.5%) and 2 of the SLIT-treated (28.6%) patients.

Concerning specific IgE to Bet v 1 and related allergens, complete data, reported in table 2, were obtained in 10 subjects. Patients developing higher tolerance to the apple challenge had mean IgE levels of 5.01 kU/l to Bet v 1 and of 2.64 kU/l to Mal d 1 before IT, compared to 7.25

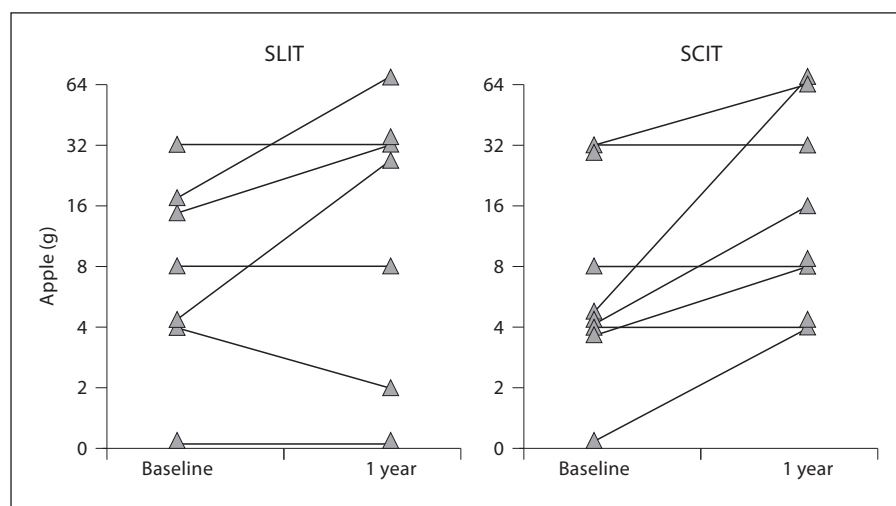
kU/l to Bet v 1 and 3.46 kU/l to Mal d 1 after IT. Patients with unchanged or lower tolerance to the apple challenge had mean IgE levels of 12.1 kU/l to Bet v 1 and of 4.1 kU/l to Mal d 1 before IT compared to 14.1 kU/l to Bet v 1 and 2.3 kU/l to Mal d 1 after IT. Insignificant levels of specific IgE to Api g 1 and Dau c 1 were detected both before and after IT. No patient was sensitized to lipid transfer proteins, such as Pru p 3, Cor a 8, Art v 3 and Par j 2.

Discussion

Food allergy is generally managed by avoiding causative foods, but inadvertent consumption of very small amounts of these foods may occur and cause severe reactions [26]. This warrants effective preventive treatments.

Table 2. Specific IgE measured in kU/L to Bet v 1 and to related allergens before and after IT

Patients	Bet v 1		Cor a 1		Aln g 1		Mal d 1		Api g 1		Dau c 1	
	before	after	before	after	before	after	before	after	before	after	before	after
SCIT												
1	2.41	9.88	2.44	2.23	0.85	1.88	1.62	2.32	0.00	0.00	0.00	0.00
2	3.35	6.34	6.51	5.00	4.61	4.59	2.01	1.74	0.00	0.00	0.00	0.00
3	22.68	23.78	19.45	6.01	12.63	8.36	7.74	7.30	0.00	0.00	0.00	0.00
6	9.84	7.04	8.64	6.28	4.05	7.47	5.28	6.00	2.97	2.30	0.00	0.00
SLIT												
1	2.46	3.27	2.24	3.00	1.82	3.60	0.00	0.00	0.00	0.00	0.00	0.00
2	28.15	24.3	16.72	10.20	14.27	14.30	10.18	2.80	1.03	0.50	0.00	0.00
4	4.11	5.86	2.74	2.84	1.81	3.63	2.36	2.58	0.00	0.00	0.00	0.00
5	6.23	10.22	9.61	8.15	9.18	9.22	3.96	6.44	0.76	0.52	0.73	0.00
6	1.46	1.47	2.46	1.01	1.58	1.24	0.71	0.00	0.00	0.00	0.00	0.00
7	4.90	7.63	1.27	0.96	5.25	6.35	0.00	0.00	0.81	0.00	0.00	0.00

**Fig. 2.** Changes in the tolerated apple amounts by challenge with fresh apples before and after SLIT and SCIT.

It was demonstrated that the immunologic treatment by anti-IgE antibody was able to increase the tolerance in peanut-allergic subjects from half a peanut to about 9 peanuts [27]. Concerning allergen IT, there is a solid evidence of effectiveness in respiratory allergy and in *Hymenoptera* venom allergy [6], whereas the data available for food allergy are contradictory. SCIT was effective but unsafe in peanut-allergic patients [28], while the sublingual route demonstrated both efficacy and safety in hazelnut-allergic patients, who tolerated significantly higher amounts of the culprit food following SLIT [29]; recently, a study using an extract quantified in major allergen in peach-allergic patients has shown similar results [30].

Cross-reactivity between pollens and foods is a particular field of IT application; initial reports on the effects of pollen IT on allergy to cross-reactive foods were optimistic [12–15], but the overall results of the studies so far available have not confirmed the possibility to cure food allergy by this approach. The clinical cross-reactivity most investigated under this aspect is the birch-apple syndrome. The global analysis of the studies on the effects of IT with birch extracts on apple allergy gives a general picture that in most reports apple allergy improves (that is, a total or partial disappearance of OAS occurs) in about 50% of the patients undergoing SCIT and somewhat less with SLIT. However, when only placebo-controlled or randomized studies are analyzed [17, 18,

Table 3. Results in the reported studies of the challenge in relation to the apple amount administered

Authors	Kind of IT	Effects on OAS	Apple amount administered for challenge
Asero [14]	SCIT	Disappearance or improvement in 87% of patients	10 g
Bucher et al. [16]	SCIT	No disappearance, increase of tolerance in 84% of patients	128 g
Bolhaar et al. [17]	SCIT	Disappearance in 23%, improvement in 70% of patients	120 g
Skamstrup Hansen et al. [18]	SCIT SLIT	Disappearance in 10% of patients No effect	A whole apple
Kinaciyan et al. [19]	SLIT	No effect	64 g

20], the effect of IT on apple allergy is apparently insufficient, especially concerning SLIT.

To further confound the issue, different methods to assess the efficacy on apple allergy were used, on the basis of the patient's perception by visual analogue scale, questionnaires and symptom scores. Moreover, the amount of apple used for the challenge varies, as listed in table 3, from only 10 g, with excellent clinical results [14], to a whole apple (about 200 g), the latter being tolerated only by 10% of the patients [18]. We chose to use the standardized challenge technique described in an article on identification of apple allergens [24], in which a final amount of 64 g was reached, but only one other study used the same amount and did not find any effect on apple allergy [19].

Of course, the basis to expect an improvement in apple allergy is the clinical efficacy of IT on birch pollen allergy. The first part of this study was addressed on this goal, comparing SLIT and SCIT with extracts from the same producer, and similar good efficacy was found with regard to respiratory symptoms caused by birch pollen [23]. This was one of the few studies directly comparing SCIT and SLIT, analyzed by Frew [31], which globally show a similar effectiveness on respiratory allergy, provided that the allergen at much higher doses is administered with SLIT rather than with SCIT.

Concerning OAS, which included 15 (8 SCIT-treated and 7 SLIT-treated patients) of the original 34 patients, we found a complete tolerance to apple in 25% of the SCIT-treated and in 14% of the SLIT-treated patients and an incomplete but increased tolerance in 25% of the SCIT-treated and in 28% of the SLIT-treated patients. Thus, 63% of the SCIT-treated and 42% of the SLIT-treated patients had an improvement of apple allergy. A trend in the favor of SCIT, in line with the global results reported in

the literature as mentioned above, is apparent, statistical significance being prevented by the low number of patients. Immunological data should help in explaining why some patients respond and some others do not respond to birch IT by improving their tolerance to apples.

Among the patients we studied, the nonresponders had a higher mean baseline level of specific IgE to Bet v 1 (12.1 kU/l) and to Mal d 1 (4.1 kU/l) than the responders (5.1 and 2.6 kU/l, respectively). However, some patients showed an increased tolerance to apple notwithstanding a rise of Mal d 1-specific IgE after IT, while others had a decreased tolerance despite a reduction in the Mal d 1-specific IgE. Concerning specific IgE to other Bet v 1-related allergens, that is, Dau c 1 from carrot and Api g 1 from celery, they were absent or very low in almost all patients, and this does not allow any hypothesis on the degree of cross-reactivity of the antibodies to Bet v 1 in our patients [32].

The main limitation of the present study is that despite an initial sufficiently high population of patients, complete clinical and immunological data for only 10 patients were available, and a reliable statistical analysis was not feasible.

In any case, one must consider other important aspects that influence the results. First, there is considerable variation in the expression of Mal d 1. Yet, in 1999, Son et al. [33], using cDNA of Mal d 1 from various apple strains amplified by a PCR strategy, found 12 Mal d 1 clones that were sequenced from 7 apple varieties and concluded that the divergent allergenicity of apple strains mainly depends on different expression levels of the major allergen. Second, allergens other than Mal d 1 may be relevant in inducing allergic symptoms to apple but may not be concerned with the effects of birch pollen IT; this is true for Mal d 2, a thaumatin-like protein, and Mal d 3, a lipid

transfer protein [24, 34], the latter showing around 100-fold differences in its levels between different apple cultivars [35]. In the present study, a role for such allergens was excluded on the basis of the negative results of the microarray test. Finally, the dose of birch pollen extract may be critical in its efficacy on apple allergy. As noted by Asero [36], the amount of cross-reacting allergen administered with SCIT or SLIT is very variable in different studies, and it is possible that in some patients the doses needed to improve the associated food allergy are higher than those sufficient to improve pollen-related symptoms.

These issues suggest the need of a much finer diagnostic work-up in selecting patients with birch-apple syndrome who are candidates to respond to birch pollen IT also concerning apple allergy. In particular, future studies should evaluate in single patients the apple strains responsible for clinical symptoms, and allergens expressed in such strains, in order to accurately investigate the factors underlying the positive or negative response to IT.

References

- ▶ 1 Pastorello EA, Ispano M, Pravettoni V, Farioli L, Incorvaia C, Ansaloni R, Ortolani C: Cross-reacting allergens in clinical syndromes. In Wuthrich B, Ortolani C (eds): *Highlights in Food Allergy*. Monogr Allergy, Basel, Karger, 1996;32:57–62.
- ▶ 2 Breiteneder H, Radauer C: A classification of plant food allergens. *J Allergy Clin Immunol* 2004;113:821–830.
- ▶ 3 Radauer C, Breiteneder H: Pollen allergens are restricted to few protein families and show distinct patterns of species distribution. *J Allergy Clin Immunol* 2006;117:141–147.
- ▶ 4 Aalberse RC: Assessment of allergen cross-reactivity. *Clin Mol Allergy* 2007;5:2.
- ▶ 5 Ebner C, Birkner T, Valenta R, Rumpold H, Breitenbach M, Scheiner O, Kraft D: Common epitopes of birch pollen and apples. Studies by Western and Northern blot. *J Allergy Clin Immunol* 1991;88:588–594.
- 6 Bousquet J, Lockey RF, Malling HJ (eds): *WHO Position Paper. Allergen immunotherapy: therapeutic vaccines for allergic diseases*. *Allergy* 1998;53(suppl 54):1–42.
- ▶ 7 Arvidsson MB, Lowhagen O, Rak S: Effect of 2-year placebo-controlled immunotherapy on airway symptoms and medications in patients with birch pollen allergy. *J Allergy Clin Immunol* 2002;109:777–783.
- ▶ 8 Bodtger U, Poulsen LK, Jacobi HH, Malling HJ: The safety and efficacy of subcutaneous birch pollen immunotherapy – a one-year, randomised, double-blind, placebo-controlled study. *Allergy* 2002;57:297–305.
- ▶ 9 Horak F, Stubner P, Berger UE, Marks B, Toth J, Jager S: Immunotherapy with sublingual birch pollen extract. A short-term double-blind placebo study. *J Invest Allergol Clin Immunol* 1998;8:165–171.
- ▶ 10 Voltolini S, Modena P, Minale P, Bignardi D, Troise C, Puccinelli P, et al: Sublingual immunotherapy in tree pollen allergy. Double-blind, placebo-controlled study with a biologically standardized extract of three pollens (alder, birch and hazel) administered by a rush schedule. *Allergol Immunopathol* 2001;29:103–110.
- ▶ 11 Khinchi MS, Poulsen LK, Carat F, André C, Hansen AB, Malling HJ: Clinical efficacy of sublingual and subcutaneous birch pollen allergen-specific immunotherapy: a randomized, placebo-controlled, double blind, double dummy study. *Allergy* 2004;59:45–53.
- ▶ 12 Moller C: Effect of pollen immunotherapy on food hypersensitivity in children with birch pollinosis. *Ann Allergy* 1989;62:343–345.
- ▶ 13 Herrmann D, Henzgen M, Franck E, Rudeschko O, Jager L: Effect of hyposensitization for tree pollinosis on associated apple allergy. *J Invest Allergol Clin Immunol* 1995;5:259–267.
- ▶ 14 Asero R: Effects of birch pollen-specific immunotherapy on apple allergy in birch pollen-hypersensitive patients. *Clin Exp Allergy* 1998;28:1368–1373.
- 15 Modrzynski M, Zawiska E, Rapiejko P, Przybylski G: Specific-pollen immunotherapy in the treatment of oral pollen syndrome in patients with tree pollen hypersensitivity. *Przegl Lek* 2002;59:1007–1010.
- ▶ 16 Bucher X, Pichler WJ, Dahinden CA, Helbling A: Effect of tree pollen specific, subcutaneous immunotherapy on the oral allergy syndrome to apple and hazelnut. *Allergy* 2004;59:1272–1276.
- ▶ 17 Bolhaar ST, Tiemessen MM, Zuidmeer L, van Leeuwen A, Hoffmann-Sommergruber K, Bruijnzell-Koomen CA, et al: Efficacy of birch-pollen immunotherapy on cross-reactive food allergy confirmed by skin tests and double-blind food challenges. *Clin Exp Allergy* 2004;34:761–769.
- ▶ 18 Skamstrup Hansen K, Sondergaard Khinchi M, Stahl Skov P, Bindslev-Jensen C, Poulsen LK, Malling HJ: Food allergy to apple and specific immunotherapy with birch pollen. *Mol Nutr Food Res* 2004;48:441–448.
- ▶ 19 Kinaciyan T, Jahn-Schmid B, Radakovics A, Zwolfer B, Schreiber C, Francis JN, et al: Successful sublingual immunotherapy with birch pollen has limited effects on concomitant food allergy to apple and the immune response to the Bet v 1 homolog Mal d 1. *J Allergy Clin Immunol* 2007;119:937–943.
- ▶ 20 Niederberger V, Reisinger J, Valent P, Krauth MT, Pauli G, van Hage M, et al: Vaccination with genetically modified birch pollen allergens: immune and clinical effects on the oral allergy syndrome. *J Allergy Clin Immunol* 2007;119:1013–1016.
- ▶ 21 Czarnecka-Operacz M, Jenerowicz D, Silny W: Oral allergy syndrome in patients with airborne pollen allergy treated with specific immunotherapy. *Acta Dermovenerol Croat* 2008;16:19–24.
- 22 Bergmann KC, Wolf H, Schnitker J: Effect of pollen-specific sublingual immunotherapy on oral allergy syndrome. An observational study. *WAO J* 2008;1:79–84.
- ▶ 23 Mauro M, Russello M, Incorvaia C, Gazzola GB, Di Cara G, Frati F: Comparison of efficacy, safety and immunologic effects of subcutaneous and sublingual immunotherapy in birch pollinosis: a randomized study. *Eur Ann Allergy Clin Immunol* 2007;39:119–122.
- ▶ 24 Pastorello EA, Pravettoni V, Farioli L, Ispano M, Fortunato D, Monza M, et al: Clinical role of a lipid transfer protein that acts as a new apple-specific allergen. *J Allergy Clin Immunol* 1999;104:1099–106.
- ▶ 25 Deinhofer K, Sevcik H, Balic N, Harwanegg C, Hiller R, Rumpold H, et al: Microarrayed allergens for IgE profiling. *Methods* 2004;32:249–254.
- ▶ 26 Sampson HA: Food anaphylaxis. *Br Med Bull* 2000;56:925–935.

- ▶ 27 Leung DY, Sampson HA, Yunginger JW, Burks AW Jr, Schneider LC, Wortel CH, et al: Effect of anti-IgE therapy in patients with peanut allergy. *N Engl J Med* 2003;348:986–993.
- ▶ 28 Nelson HS, Lahr J, Rule R, Bock A, Leung D: Treatment of anaphylactic sensitivity to peanuts by immunotherapy with injections of aqueous peanut extract. *J Allergy Clin Immunol* 1997;99:744–751.
- ▶ 29 Enrique E, Pineda F, Malek T, Bartra J, Basagna M, Tella R, et al: Sublingual immunotherapy for hazelnut food allergy: a randomized double-blind, placebo-controlled study with a standardized hazelnut extract. *J Allergy Clin Immunol* 2005;116:1073–1079.
- ▶ 30 Fernandez-Rivas M, Garrido-Fernandez S, Nadal JA, Diaz de Durana MD, Garcia BE, Gonzalez-Mancebo E, et al: Randomized double-blind, placebo-controlled trial of sublingual immunotherapy with a Pru p 3 quantified peach extract. *Allergy* 2009;64:876–883.
- ▶ 31 Frew A: Sublingual immunotherapy. *N Engl J Med* 2008;358:2259–2264.
- ▶ 32 Aalberse RC: Assessment of allergen cross-reactivity. *Clin Mol Allergy* 2007;5:2.
- ▶ 33 Son DY, Scheurer S, Hoffman A, Hausten D, Vieths S: Pollen-related food allergy: cloning and immunological analysis of isoforms and mutants of Mal d 1, the major apple allergen, and Bet v 1, the major birch pollen allergen. *Eur J Nutr* 1999;38:201–215.
- ▶ 34 Oberhuber C, Ma Y, Marsh J, Rigby N, Smole U, Radauer C, et al: Purification and characterisation of relevant natural and recombinant apple allergens. *Mol Nutr Food Res* 2008;6:1–12.
- ▶ 35 Sancho AI, van Ree R, van Leeuwen A, Meulenbroek BJ, van de Weg EW, Gilissen LJ, et al: Measurement of lipid transfer protein in 88 apple cultivars. *Int Arch Allergy Immunol* 2008;146:19–26.
- ▶ 36 Asero R: Effects of birch pollen SIT on apple allergy: a matter of dosage? *Allergy* 2004;59:1269–1271.