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

Tamar Kinaciyan, MD,[a] Beatrice Jahn-Schmid, PhD,[b] Astrid Radakovics,[b] Bettina Zwölfer,[b] Claudia Schreiber, MD,[a] James N. Francis, PhD,[c] Christof Ebner, MD,[d] and Barbara Bohle, PhD[b] *Vienna, Austria, and London, United Kingdom*

Background: Cross-reactivity between the major birch pollen allergen, Bet v 1, and the apple protein, Mal d 1, frequently causes food allergy.
Objective: To investigate the effects of successful sublingual immunotherapy (SLIT) with birch pollen extract on apple allergy and the immune response to Bet v 1 and Mal d 1.
Methods: Before and after 1 year of SLIT, Bet v 1–sensitized patients with oral allergy syndrome to apple underwent nasal challenges with birch pollen and double-blind placebo-controlled food challenges with apple. Bet v 1–specific and Mal d 1–specific serum antibody levels and proliferation in PBMCs and allergen-specific T-cell lines (TCLs) were determined. Bet v 1–specific TCLs were mapped for T-cell epitopes.
Results: In 9 patients with improved nasal provocation scores to birch pollen, apple-induced oral allergy syndrome was not significantly reduced. Bet v 1–specific IgE and IgG_4 levels significantly increased. Bet v 1–specific T-cell responses to all epitopes and those cross-reactive with Mal d 1 significantly decreased. However, neither Mal d 1–specific IgE and IgG_4 levels nor Mal d 1–induced T-cell proliferation changed significantly. In contrast, Mal d 1–specific TCLs showed increased responses to Mal d 1 after 1 year of SLIT.
Conclusion: This longitudinal study indicates that pollen SLIT does not efficiently alter the immune response to pollen-related food allergens, which may explain why pollen-associated food allergy is frequently not ameliorated by pollen immunotherapy even if respiratory symptoms significantly improve.
Clinical implications: SLIT with birch pollen may have no clinical effect on associated apple allergy. (J Allergy Clin Immunol 2007;119:937-43.)

Key words: *Birch pollen allergy, Bet v 1, food allergy, oral allergy syndrome, sublingual immunotherapy, cross-reactivity*

Birch pollen is an important cause for type I allergy in central and northern Europe and northern America.[1] In addition to seasonal respiratory symptoms, patients with birch pollen allergy frequently develop hypersensitivity reactions to apples and other birch pollen-related foods that perennially hamper their quality of life.[2,3] This birch-fruit-vegetable syndrome mainly occurs because IgE antibodies specific for the major birch pollen allergen, Bet v 1, cross-react with homologous proteins in the respective foods.[4,5] Apples contain Mal d 1, a protein sharing 64% amino acid (aa) sequence similarity with Bet v 1.[6] Because of this high homology, both allergens have several B-cell and T-cell epitopes in common.[4,6,7] Bet v 1 was shown to inhibit IgE binding to Mal d 1 potently, but not vice versa.[6,8] When Mal d 1–reactive T-cell lines (TCLs) and clones isolated from peripheral blood of patients with allergy were restimulated with Mal d 1 or Bet v 1, most cultures responded more pronouncedly to the pollen than the apple protein.[7] Thus, the major birch pollen allergen seems to contain all relevant B-cell and T-cell epitopes of the apple protein. These immunologic findings together with the clinical observation that the majority of patients develop hypersensitivity reactions to apple after having developed hay fever to birch pollen led to the conclusion that Bet v 1 initiates sensitization to Mal d 1. Accordingly, one would assume that successful specific immunotherapy (SIT) of birch pollen allergy should also reduce hypersensitivity to apples. Indeed, several studies have reported improvement of associated apple allergy after birch pollen SIT,[9-13] and one study showed that such effect was rather long-lasting.[14] However, some studies have observed no beneficial effect on apple allergy.[15,16] Thus, there is still a need for more efficient treatment strategies for pollen-related food allergy.

The most frequent manifestation of birch pollen-associated food allergy is the oral allergy syndrome (OAS):

From [a]the Department of Dermatology, Division of Immunology, Allergy and Infectious Diseases and [b]the Department of Pathophysiology, Center for Physiology and Pathophysiology, Medical University of Vienna; [c]Upper Respiratory Medicine, National Heart and Lung Institute, Imperial College, London; and [d]Allergy Clinic Reumannplatz, Vienna.

Supported by the Fonds zur Förderung der wissenschaftlichen Forschung (SFB-F1807-B04), Austria.
Disclosure of potential conflict of interest: The authors have declared that they have no conflict of interest.
Received for publication August 17, 2006; revised November 2, 2006; accepted for publication November 3, 2006.
Available online January 3, 2007.

Reprint requests: Barbara Bohle, PhD, Medical University of Vienna, Center for Physiology and Pathophysiology, Department of Pathophysiology, Waehringer Guertel 18-20, AKH-3Q, A-1090 Wien, Austria. E-mail: barbara.bohle@meduniwien.ac.at.

0091-6749/$32.00

doi:10.1016/j.jaci.2006.11.010

Abbreviations used
aa: Amino acid
BU: Biological unit
DBPCFC: Double-blind, placebo-controlled food challenge
dpm: Δ cpm
NPT: Nasal provocation test
OAS: Oral allergy syndrome
SI: Stimulation index
SIT: Specific immunotherapy
SLIT: Sublingual immunotherapy
SPT: Skin prick test
TCL: T-cell line
VAS: Visual analog scale

itching, tickling, blistering, and/or angioedema confined to the oropharynx immediately after contact with fresh fruits.[17] Speculating that administration of birch pollen extract directly at the site of food-allergic symptoms could benefit the therapeutic efficacy on food allergy, we considered sublingual delivery an ideal route. Sublingual immunotherapy (SLIT) is a safe and convenient alternative to subcutaneous SIT that is widely used in many European countries.[18-22] SLIT with birch pollen was demonstrated to achieve a significant benefit of rhinitis, to reduce the eosinophil infiltration in nasal mucosa, and to improve pulmonary function significantly during the birch pollen season.[23,24]

In the current study, we evaluated whether successful birch pollen SLIT cures apple-induced OAS in Bet v 1–sensitized patients. Clinical improvement was assessed in nasal provocation tests (NPTs) with birch pollen and double-blind placebo-controlled food challenges (DBPCFCs) with apple before and after 1 year of SLIT. In patients with improved NPT, Bet v 1–specific and Mal d 1–specific serum IgE and IgG_4 levels and proliferative responses to both allergens were individually monitored. TCLs specific for Bet v 1 or Mal d 1 were generated before and after 1 year of SLIT and tested for cross-reactivity with pollen and food allergens and T-cell epitope recognition patterns.

METHODS

Patients

Twenty patients (5 men and 15 women; mean age, 33.2 years; range, 21-47 years) with a clear history of birch pollen rhinoconjunctivitis and OAS to apples were included. All patients gave written consent before enrollment in the study, which was approved by the local Medical Ethical Committee of Vienna and conducted according to guidelines for Good Clinical Practice. Definitive inclusion criteria were a positive skin prick test (SPT; >5 mm^2) to birch pollen extract (Soluprick; ALK-Abelló, Hørsholm, Denmark), specific serum IgE > 0.7 kU/L to birch (CAP/FEIA; Phadia & Upjohn, Uppsala, Sweden), and a positive DBPCFC with apple. All individuals were exclusively sensitized to Bet v 1 as determined by immunoblotting experiments using birch pollen extract (data not shown). In addition, patients were positive in CAP/FEIA for Bet v 1 but not for Bet v 2 (Phadia; data not shown). During the study, 2 patients moved. One patient was excluded because she discontinued the medication for 8 weeks during vacation. One patient stopped SLIT because she disliked the taste of the preparation. One patient was excluded because of a reaction with the placebo during the DBPCFC after 1 year of SLIT.

SLIT protocol

Pangramin SLIT BU (221) birch pollen extract (provided by ALK- Abelló, Allergie-Service Gmbh, Linz, Austria) was used. Drops were self-administered at home and held under the tongue for 2 minutes before swallowing. According to the manufacturer's protocol, increasing numbers of drops (1, 2, 4, 6, 8, 10, and 10) of increasing strengths of extract were administered daily during 4 weeks. The maintenance dose of 10 drops (equalling 4.5 μg Bet v 1) was applied daily. NPTs with birch pollen and DBPCFCs with apples were performed before SLIT in November 2002 ($t = 0$) and in November 2003 ($t = 52$). At the same time, SPT with titrated concentrations of birch pollen extract, open food challenges with fresh apples, and blood sampling were performed.

NPTs

Before nasal provocation, the nose of each patient was rhinoscopically evaluated. Nonspecific responsiveness was determined by spraying the glycerol diluent on the nasal mucosa of the wider side. If after 10 minutes no significant changes occurred, provocation with birch pollen allergen was started with a concentration of 0.016 biological units (BU)/mL. After 20 minutes, rhinomanometric measurement was performed. This procedure was repeated with increasing concentrations of birch pollen allergen (0.08, 0.4, 2, and 10 BU) until a positive response occurred or the highest concentration was reached. A nasal airflow decrease of >40 % was regarded as positive response.

DBPCFCs

The placebo contained 15 g shredded Golden Delicious apple microwaved for 5 minutes at 95°C, 15 g shredded cabbage turnip, and 3 mL commercially available pasteurized apple syrup. The verum contained 10 g each, microwaved and fresh apple, cabbage turnip, and syrup. Freshly shredded apple was added within 5 minutes before challenge. During the challenge, all patients wore sun glasses. The sequence verum versus placebo or placebo versus verum for each patient was blinded by the dietitian. An interval of 30 minutes was kept between the test meals or longer if symptoms were still present. The skin and oral cavity was inspected for lesions before, during, and after the challenge. Patients scored their OAS using visual analog scales (VASs) ranging from 0 to 10 cm. After DBPCFCs, open challenges were performed with a slice of fresh apple (20 g).

SPTs

Skin prick tests were performed on the flexor aspect of the forearm with different concentrations of birch pollen extract (Pangramin; ALK-Abelló; 500, 250, 100, 50, 20, and 4 BU). All skin reactions were recorded after 20 minutes by copying the wheal reaction onto a transparent adhesive tape, and wheal diameters were measured. Histamine (10 mg/mL) and the glycerol diluent of the allergen extract were used as positive and negative controls (ALK-Abelló).

Allergens

Recombinant Bet v 1 and Mal d 1 were purchased from Biomay (Vienna, Austria). Throughout the entire study, the same batches of allergens were used. Endotoxin levels were <2.5 endotoxin units/μg as determined by limulus amoebocyte lysate assay (BioWhittaker, Walkersville, Md).

Determination of allergen-specific IgE and IgG_4

Microtiter plates (Maxisorp, Nunc, Denmark) were coated with Bet v 1 (1 μg/mL) and Mal d 1 (2 μg/mL) in carbonate buffer (pH 9.6) overnight at room temperature. After saturation with 1% BSA in PBS

for 6 hours, sera were incubated overnight at room temperature. Bound IgE and IgG_4 antibodies were detected using alkaline phosphatase-conjugated antihuman IgE and IgG_4 antibodies, respectively (BD Biosciences Pharmingen, San Diego, Calif). For inhibition experiments, sera from patients were preincubated with titrated concentrations (0.003-1 μg/mL) of each allergen for 6 hours at room temperature before transfer to Bet v 1–coated and Mal d 1–coated microtiter plates, respectively.

T-cell responses

Proliferative responses of PBMCs (2×10^5) were determined as described.[25] Bet v 1 and Mal d 1 were titrated from 3.18 to 25 μg/mL. Data are expressed as Δ counts per minute (cpm) (dpm) = cpm in stimulated cultures minus cpm in those containing medium alone. Allergen-specific TCLs were generated from PBMCs of individuals with birch pollen allergy by initial stimulation with 10 μg/mL Bet v 1 or 10 μg/mL Mal d 1 according to protocols described.[26] In parallel, cultures without allergens served as controls. TCLs were restimulated with Bet v 1 or Mal d 1 (5 μg/mL each), and proliferation was determined after 48 hours. Stimulation indices (SIs) were calculated as ratio between cpm obtained in cultures with T cells plus autologous antigen-presenting cell (APC) plus peptide and cpm obtained in cultures containing T cells and APC alone. T-cell epitope recognition in Bet v 1–specific TCLs was determined as described.[26]

Digestion fragments of Bet v 1

Peptides created by simulated gastrointestinal digestion of Bet v 1 were analyzed as described previously.[25] Briefly, Bet v 1 was digested with pepsin for 30 minutes at 37°C and, after adjusting the pH with 1 mol/L NaOH to pH 8.3, for another 30 minutes at 37°C with trypsin. LC-MS/MS spectra of digested Bet v 1 were recorded on a Micromass Global Ultima Q-Tof instrument (Waters, Milford, Mass).

Statistics

Statistical significance of differences was determined by the Wilcoxon signed rank test. Differences were considered statistically significant for $P < .05$.

RESULTS

Successful birch pollen SLIT does not significantly improve OAS to apple

In 9 of 15 individuals, higher concentrations of birch pollen extract were needed in NPT after 1 year of SLIT ($t = 52$) to reduce the air flow-through equally compared with the respective NPT performed before SLIT ($t = 0$) (Table I). These individuals were defined as responders and selected for further analysis. All these patients also experienced improved seasonal allergic symptoms. Successful SLIT was further reflected by the significant reduction of skin reactivity to titrated concentrations of birch pollen extract (Table I). In parallel to NPT, patients underwent DBPCFC with apple. At both time points, individuals experienced OAS only after provocation with verum and rated their reactions using VAS. In addition, open challenges with apple were performed (Table I). Overall, at $t = 52$, the VAS of neither DBPCFC nor open challenges differed significantly from $t = 0$.

Bet v 1 dominates the IgE response to Mal d 1

To confirm that Bet v 1 was the leading allergen for sensitization to Mal d 1 in the patients under investigation, IgE-inhibition ELISA were performed. Preincubation of sera collected at $t = 0$ with titrated concentrations of Bet v 1 abolished IgE binding to Mal d 1 equally as well as incubation with Mal d 1 in all individuals (Fig 1). In contrast, preincubation with Mal d 1 had no strong effect on IgE-binding to Bet v 1. Equal results were obtained with sera of the same patients collected at $t = 52$ (data not shown). Hence, Bet v 1 contains all epitopes recognized by Mal d 1–reactive IgE in these individuals but not vice versa.

TABLE I. Clinical evaluation of responders *(1-9)* and nonresponders *(A-F)* before and after 1 year of SLIT*

	NPT (BU)		SPT (BU)		DBPCFC (VAS)		OFC (VAS)	
t =	0	52	0	52	0	52	0	52
1	0.4	10	20	20	2.5	2.5	2.5	6
2	2	10	4	20	1.5	0.8	4	3.5
3	2	10	20	20	1	0.8	2	1.8
4	2	10	4	20	2	0	4	0
5	0.4	2	4	20	1	4	5	5
6	0.08	2	4	50	5.8	6	4.2	9
7	0.08	2	4	20	1.5	3	2	1.6
8	0.08	0.4	20	50	1	8	10	10
9	0.08	0.4	20	50	3.5	4	2	4
			$P = .016$		NS		NS	
A	2	2	4	20	1	2	9	4
B	2	2	20	20	3	3	3	3
C	10	10	4	4	4	2.8	3	4
D	10	0.08	4	4	4	3	4	6
E	2	0.4	4	50	7	0	9.2	0
F	2	0.08	20	20	3	4	4	3
	NS		NS		NS		NS	

OFC, Open food challenge; *NS*, not significant.
*Statistical difference between values at $t = 0$ and $t = 52$ was calculated by the Wilcoxon signed rank test.

Successful birch pollen SLIT induces Bet v 1–reactive but not Mal d 1–reactive IgE and IgG_4

Allergen-specific serum IgE and IgG_4 levels were individually measured by ELISA before ($t = 0$) and after 1 year ($t = 52$) of SLIT. Bet v 1–specific IgE levels increased significantly during SLIT ($P = .028$; Fig 2), which was confirmed in CAP/FEIA (data not shown). In addition, Bet v 1–specific serum IgG_4 levels also increased significantly ($P = .038$; Fig 2). Interestingly, Mal d 1–specific serum IgE and IgG_4 levels showed increasing tendency but did not reach statistical significant difference (Fig 2), indicating that the majority of SLIT-induced Bet v 1–specific antibodies were not cross-reactive with Mal d 1.

Successful birch pollen SLIT reduces Bet v 1–specific but not Mal d 1–specific T-cell proliferation

PBMCs obtained before ($t = 0$) and after 1 year ($t = 52$) of SLIT were stimulated with Bet v 1 or Mal d 1. Of each

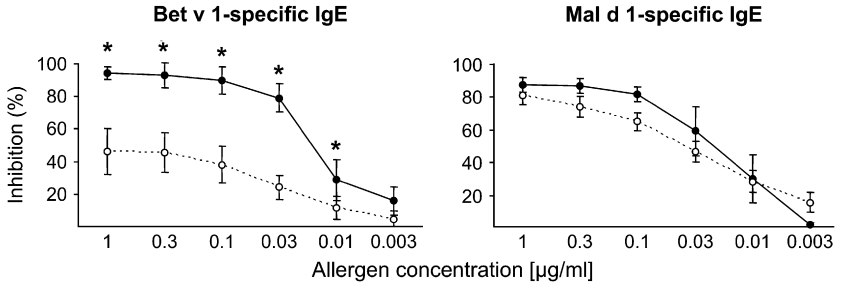

FIG 1. IgE-inhibition ELISA. Sera of 9 patients obtained before SLIT were preincubated with titrated concentrations of Bet v 1 *(solid circles)* or Mal d 1 *(open circles)*. The mean value of inhibition of IgE binding to Bet v 1 *(left)* and Mal d 1 *(right)* is shown. *$P < .05$, Wilcoxon signed rank test.

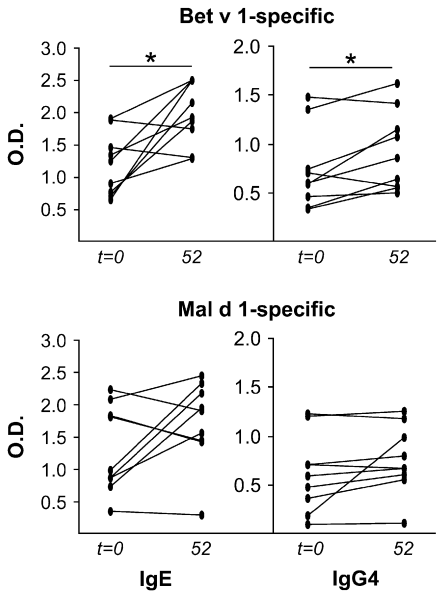

FIG 2. Bet v 1–specific and Mal d 1–specific IgE and IgG_4 levels during SLIT. Sera were tested for allergen-specific antibody levels before ($t = 0$) and after 1 year ($t = 52$) by ELISA. *$P < .05$, Wilcoxon signed rank test.

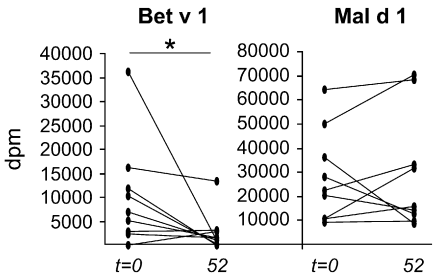

FIG 3. Bet v 1–induced and Mal d 1–induced proliferation during SLIT. PBMCs isolated before ($t = 0$) and after 1 year ($t = 52$) were stimulated with the concentration of allergens individually inducing the highest proliferation at $t = 0$. *$P < .05$, Wilcoxon signed rank test.

allergen, the concentration inducing optimum proliferative responses at $t = 0$ was individually determined and used at $t = 52$. During SLIT, Bet v 1–induced proliferation decreased significantly ($P = .022$), which was not observed for Mal d 1–induced proliferative responses (Fig 3). Cytokine responses are the subject of a separate publication. TCLs were established from each individual by stimulating PBMCs with either Bet v 1 or Mal d 1 and restimulated with both allergens. Bet v 1–specific TCLs were obtained from 7 of 9 individuals and responded significantly less to restimulation with Bet v 1 at $t = 52$ compared with $t = 0$ ($P = .018$; Table II). Four Bet v 1–specific TCLs cross-reacted with Mal d 1 (SI > 2), and 3 of 4 also showed reduced responses to the apple allergen at $t = 52$ (Table II). Mal d 1–specific TCLs were obtained from 5 of 9 individuals at both time points. Four of 5 Mal d 1–specific TCLs responded more pronouncedly to restimulation with Mal d 1 at $t = 52$ compared with $t = 0$ (Table III). In parallel, Bet v 1–induced proliferation was reduced.

Birch pollen SLIT downregulates T cells specific for all Bet v 1 epitopes

We have previously shown that Bet v 1 is immediately degraded by gastrointestinal enzymes.[25] Analyzing and sequencing the fragments created by subsequent digestion with pepsin and trypsin revealed several remaining peptides in the range of 8 to 18 amino acids (Fig 4). To evaluate whether sublingually administered Bet v 1 reduced the T-cell response to all relevant epitopes of the major birch pollen allergen or only to those still present after gastrointestinal passage, Bet v 1–specific TCLs established from the same individual at $t = 0$ and $t = 52$ were mapped with 50 synthetic 12-mer peptides representing the entire aa sequence of Bet v 1.[26] Comparison of proliferative responses to each peptide at $t = 0$ and $t = 52$ in 7 patients revealed that T cells specific for all Bet v 1 epitopes were significantly downregulated during SLIT ($P < .001$; Fig 4). In 6 of 7 TCLs, proliferative responses to the most relevant epitope for cross-reactivity with Mal d 1, Bet v $1_{142\text{-}153}$, were also reduced.[27] Interestingly, the fragments of digested Bet v 1 did not match epitopes recognized by the individuals under investigation (Fig 4).

TABLE II. Restimulation of Bet v 1–established TCLs before and after 1 year of SLIT

	Patient 1		Patient 2		Patient 3		Patient 4		Patient 5		Patient 6		Patient 7	
t =	0	52	0	52	0	52	0	52	0	52	0	52	0	52
Bet v 1	124*	1.7	76.0	21.5	29.1	1.2	6.6	3.5	341	125	6.7	4.4	34.1	1.9
Mal d 1	2.1	1.3	27.9	0.9	2.8	0.9	0.8	0.9	2.3	4.3	1.0	1.1	1.0	1.6

*SIs are shown.

DISCUSSION

The effects of successful SLIT with birch pollen on associated allergy to apple were evaluated. For this purpose, Bet v 1–sensitized individuals with birch pollen allergy with OAS to apple underwent SLIT for 1 year. Because no placebo group was included in our trial, improvement in NPT with birch pollen was applied as sine qua non for clinical success, which reduced the number of individuals defined as responders to 9 (Table I). Allergic reactions to apple were evaluated in DBPCFC. Reduced respiratory symptoms to birch pollen were not associated with significantly reduced apple-induced OAS (Table I). These data may indicate that higher allergen doses are needed to reduce food-induced than pollen-induced reactions.[28] However, in line with previous studies, we conclude that administration of birch pollen directly at the site of food-allergic manifestations does not enhance the therapeutic efficacy of pollen immunotherapy on associated food allergy.[15,16]

Successful birch pollen SLIT was associated with significantly increased Bet v 1–specific IgG_4 levels and significantly reduced Bet v 1–induced proliferation in PBMCs and Bet v 1–specific TCLs (Figs 2 and 3; Table II). These immunologic alterations accord with previous studies monitoring the same parameters for major allergens during grass pollen and house dust mite SLIT in adults.[29,30] Moreover, patients who did not improve in NPT showed neither significantly altered Bet v 1–specific IgG_4 nor proliferative responses (see this article's Fig E1 in the Online Repository at www.jacionline.org). Successful SLIT also clearly reduced cross-reactivity of Bet v 1–specific TCLs with Mal d 1 (Table II). Comparing epitope recognition patterns in Bet v 1–specific TCLs from the same patient before and after 1 year of SLIT revealed that the T-cell response to all individually relevant Bet v 1 epitopes was abolished (Fig 4). Thus, monitoring the effects of SLIT at the level of T-cell epitope recognition demonstrated that sublingually administered allergen induced peripheral tolerance in all respective T cells. Proliferation to T-cell epitopes spreading the entire aa sequence of Bet v 1 was reduced, and not only to those still present after simulated gastrointestinal digestion (Fig 4). These findings provide evidence that sublingually administered allergens enter the oral mucosa as intact proteins, where they are taken up by APC of the local immune system, which then migrate to lymph nodes and induce T-cell tolerance. This hypothesis is also supported by Bagnasco et al,[31] showing *in vivo* that sublingually administered allergens were degraded after reaching the gastrointestinal tract but not before.

TABLE III. Restimulation of Mal d 1–established TCLs before and after 1 year of SLIT

	Patient 1		Patient 2		Patient 3		Patient 4		Patient 5	
t =	0	52	0	52	0	52	0	52	0	52
Mal d 1	1.5*	8.9	1.4	42	4.7	2.2	3.5	7.3	1.8	9.3
Bet v 1	3.3	2.3	3.6	0.8	10.6	0.9	7.5	2.8	0.6	3.2

*SIs are shown.

In all individuals under investigation, birch pollinosis preceded food allergy to apple. IgE inhibition experiments revealed that Bet v 1 contained all IgE epitopes of Mal d 1 recognized in these patients (Fig 1). These clinical and immunologic observations strongly indicate that the patients developed allergy to apple as a consequence of primary sensitization to Bet v 1 and subsequent cross-reactivity with Mal d 1. Thus, one would assume that Bet v 1 determines the allergic response to Mal d 1 in these individuals. However, SLIT-induced alterations of the Bet v 1–specific immune response were paralleled by neither significant changes of Mal d 1–specific antibody nor T-cell responses (Figs 2 and 3). In contrast, after 1 year of SLIT, TCLs generated with Mal d 1 responded more pronounced to restimulation with Mal d 1 compared with those established before therapy and concomitantly lost cross-reactivity with Bet v 1 (Table III). These findings may point to the existence of a Bet v 1–independent T-cell response to Mal d 1. This concept is further supported by the previous isolation of T-cell clones specific for Mal d 1 or other Bet v 1–related food allergens that did not cross-react with Bet v 1.[7,32,33] We thus speculate that the T-cell response to pollen-related food allergens in an individual can consist of 2 arms: a pollen-specific cross-reactive and an exclusively food-specific response. The latter may be difficult to detect because of the dominating pollen-specific response, but in our study, it became evident after SLIT-induced tolerance induction of Bet v 1–specific T cells. The existence of food-reactive T cells that are not modulated by pollen immunotherapy may provide an immunologic explanation for the limited effect of pollen therapy on associated food allergy.

In summary, successful SLIT with birch pollen did not efficiently reduce concomitant allergy to apple because the immune response to Mal d 1 was not significantly altered. Therefore, we propose to combine pollen and related food

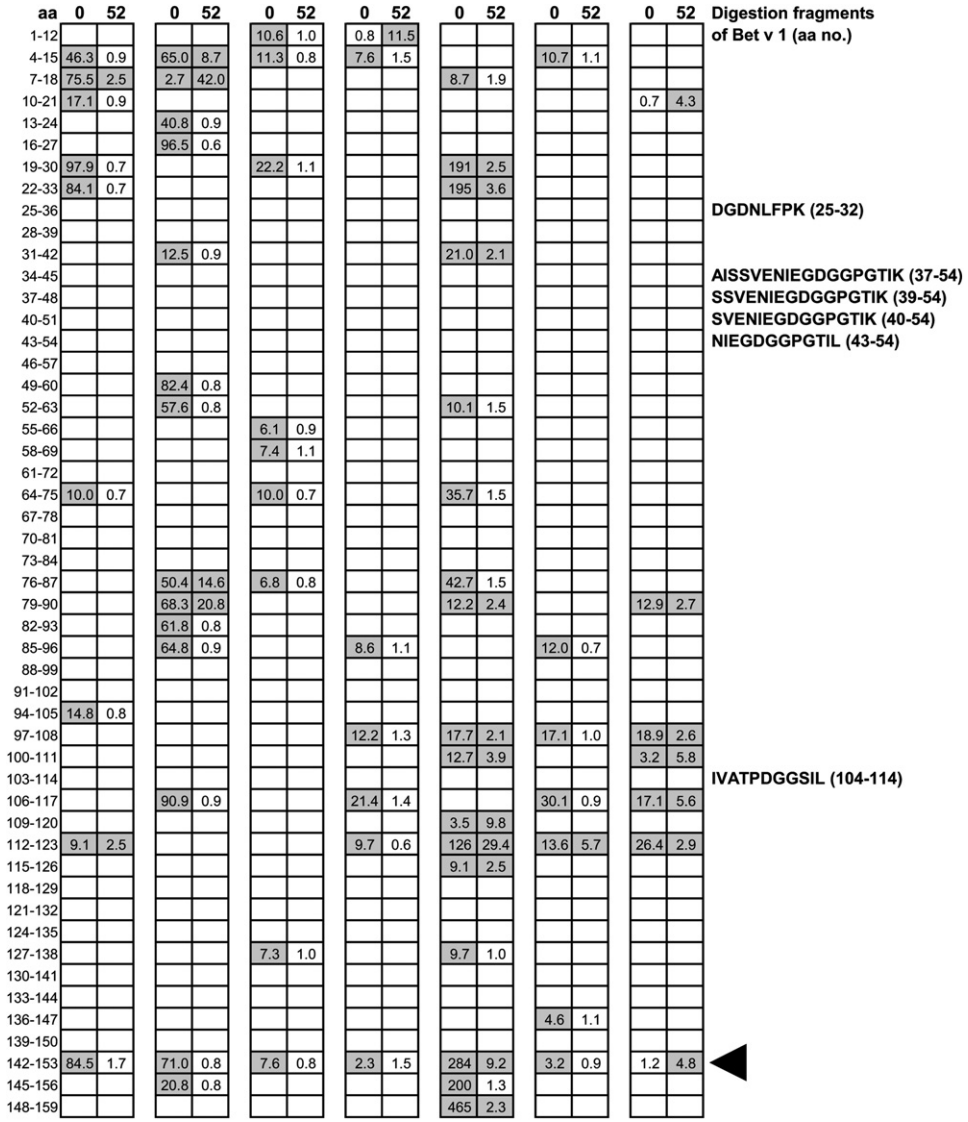

FIG 4. SLIT affects T cells specific for all Bet v 1 epitopes. Bet v 1–specific TCLs from 7 individuals were mapped with 12-mer peptides representing the entire aa sequence of Bet v 1 before ($t = 0$) and after 1 year ($t = 52$). *Numbers* indicate SI to individual peptides, and *gray boxes* mark positive responses (SI > 2). The aa sequences of fragments created by gastrointestinal digestion of Bet v 1 and their aa position are depicted. The *arrow* indicates the most relevant epitope for cross-reactivity with Mal d 1, Bet v $1_{142\text{-}153}$.[27]

allergens in a vaccine for SIT of individuals with birch pollen allergy with associated food allergy.

REFERENCES

1. Wuthrich B, Schindler C, Leuenberger P, Ackermann-Liebrich U. Prevalence of atopy and pollinosis in the adult population of Switzerland (SAPALDIA study). Swiss Study on Air Pollution and Lung Diseases in Adults. Int Arch Allergy Immunol 1995;106:149-56.
2. Eriksson NE. Food sensitivity reported by patients with asthma and hay fever: a relationship between food sensitivity and birch pollen-allergy and between food sensitivity and acetylsalicylic acid intolerance. Allergy 1978;33:189-96.
3. Dreborg S, Foucard T. Allergy to apple, carrot and potato in children with birch pollen allergy. Allergy 1983;38:167-72.
4. Ebner C, Birkner T, Valenta R, Rumpold H, Breitenbach M, Scheiner O, et al. Common epitopes of birch pollen and apples: studies by Western and Northern blot. J Allergy Clin Immunol 1991;88:588-94.
5. Wensing M, Akkerdaas JH, van Leeuwen WA, Stapel SO, Bruijnzeel-Koomen CA, Aalberse RC, et al. IgE to Bet v 1 and profilin: cross-reactivity patterns and clinical relevance. J Allergy Clin Immunol 2002;110:435-42.
6. Vanek-Krebitz M, Hoffmann-Sommergruber K, Laimer da Camara Machado M, Susani M, Ebner C, Kraft D, et al. Cloning and sequencing of Mal d 1, the major allergen from apple (Malus domestica), and its immunological relationship to Bet v 1, the major birch pollen allergen. Biochem Biophys Res Commun 1995;214:538-51.
7. Fritsch R, Bohle B, Vollmann U, Wiedermann U, Jahn-Schmid B, Krebitz M, et al. Bet v 1, the major birch pollen allergen, and Mal d 1, the major apple allergen, cross-react at the level of allergen-specific T helper cells. J Allergy Clin Immunol 1998;102:679-86.

8. Kazemi-Shirazi L, Pauli G, Purohit A, Spitzauer S, Froschl R, Hoffmann-Sommergruber K, et al. Quantitative IgE inhibition experiments with purified recombinant allergens indicate pollen-derived allergens as the sensitizing agents responsible for many forms of plant food allergy. J Allergy Clin Immunol 2000;105:116-25.
9. Asero R. Effects of birch pollen-specific immunotherapy on apple allergy in birch pollen-hypersensitive patients. Clin Exp Allergy 1998;28: 1368-73.
10. Modrzynski M, Zawisza E, Rapiejko P, Przybylski G. [Specific-pollen immunotherapy in the treatment of oral allergy syndrome in patients with tree pollen hypersensitivity]. Przegl Lek 2002;59:1007-10.
11. Herrmann D, Henzgen M, Frank E, Rudeschko O, Jager L. Effect of hyposensitization for tree pollinosis on associated apple allergy. J Investig Allergol Clin Immunol 1995;5:259-67.
12. Bolhaar ST, Tiemessen MM, Zuidmeer L, van Leeuwen A, Hoffmann-Sommergruber K, Bruijnzeel-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;34:761-9.
13. 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;59:1272-6.
14. Asero R. How long does the effect of birch pollen injection SIT on apple allergy last? Allergy 2003;58:435-8.
15. Moller C. Effect of pollen immunotherapy on food hypersensitivity in children with birch pollinosis. Ann Allergy 1989;62:343-5.
16. Hansen KS, Khinchi MS, Skov PS, Bindslev-Jensen C, Poulsen LK, Malling HJ. Food allergy to apple and specific immunotherapy with birch pollen. Mol Nutr Food Res 2004;48:441-8.
17. Ortolani C, Ispano M, Pastorello E, Bigi A, Ansaloni R. The oral allergy syndrome. Ann Allergy 1988;61:47-52.
18. Bousquet J. Sublingual immunotherapy: from proven prevention to putative rapid relief of allergic symptoms. Allergy 2005;60:1-3.
19. Bousquet J. Sublingual immunotherapy: validated! Allergy 2006;61: (suppl 81):5-6.
20. Wilson DR, Lima MT, Durham SR. Sublingual immunotherapy for allergic rhinitis: systematic review and meta-analysis. Allergy 2005;60:4-12.
21. Canonica GW, Passalacqua G. Sublingual immunotherapy in the treatment of adult allergic rhinitis patients. Allergy 2006;61(suppl 81):20-3.
22. Potter PC. Update on sublingual immunotherapy. Ann Allergy Asthma Immunol 2006;96:S22-5.
23. Khinchi MS, Poulsen LK, Carat F, Andre 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.
24. Marogna M, Spadolini I, Massolo A, Canonica GW, Passalacqua G. Clinical, functional, and immunologic effects of sublingual immunotherapy in birch pollinosis: a 3-year randomized controlled study. J Allergy Clin Immunol 2005;115:1184-8.
25. Schimek EM, Zwolfer B, Briza P, Jahn-Schmid B, Vogel L, Vieths S, et al. Gastrointestinal digestion of Bet v 1-homologous food allergens destroys their mediator-releasing, but not T cell-activating, capacity. J Allergy Clin Immunol 2005;116:1327-33.
26. Jahn-Schmid B, Radakovics A, Luttkopf D, Scheurer S, Vieths S, Ebner C, et al. Bet v 1142-156 is the dominant T-cell epitope of the major birch pollen allergen and important for cross-reactivity with Bet v 1-related food allergens. J Allergy Clin Immunol 2005;116:213-9.
27. Bohle B, Zwolfer B, Heratizadeh A, Jahn-Schmid B, Antonia YD, Alter M, et al. Cooking birch pollen-related food: divergent consequences for IgE- and T cell-mediated reactivity in vitro and in vivo. J Allergy Clin Immunol 2006;118:242-9.
28. Asero R. Effects of birch pollen SIT on apple allergy: a matter of dosage? Allergy 2004;59:1269-71.
29. Fanta C, Bohle B, Hirt W, Siemann U, Horak F, Kraft D, et al. Systemic immunological changes induced by administration of grass pollen allergens via the oral mucosa during sublingual immunotherapy. Int Arch Allergy Immunol 1999;120:218-24.
30. Cosmi L, Santarlasci V, Angeli R, Liotta F, Maggi L, Frosali F, et al. Sublingual immunotherapy with Dermatophagoides monomeric allergoid down-regulates allergen-specific immunoglobulin E and increases both interferon-gamma- and interleukin-10-production. Clin Exp Allergy 2006;36:261-72.
31. Bagnasco M, Mariani G, Passalacqua G, Motta C, Bartolomei M, Falagiani P, et al. Absorption and distribution kinetics of the major Parietaria judaica allergen (Par j 1) administered by noninjectable routes in healthy human beings. J Allergy Clin Immunol 1997;100:122-9.
32. Bohle B, Radakovics A, Jahn-Schmid B, Hoffmann-Sommergruber K, Fischer GF, Ebner C. Bet v 1, the major birch pollen allergen, initiates sensitization to Api g 1, the major allergen in celery: evidence at the T cell level. Eur J Immunol 2003;33:3303-10.
33. Bohle B, Radakovics A, Luttkopf D, Jahn-Schmid B, Vieths S, Ebner C. Characterization of the T cell response to the major hazelnut allergen, Cor a 1.04: evidence for a relevant T cell epitope not cross-reactive with homologous pollen allergens. Clin Exp Allergy 2005;35: 1392-9.