Cooking birch pollen–related food: Divergent consequences for IgE- and T cell–mediated reactivity *in vitro* and *in vivo*

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Background: The major birch pollen allergen Bet v 1 cross-reacts with homologous food allergens, resulting in IgE-mediated oral allergy syndromes (OASs). To avoid this food, allergy allergologists and guidebooks advise patients to consume birch pollen–related foods after heating.
Objective: We sought to evaluate whether cooked Bet v 1–related food allergens induce IgE- and T cell–mediated reactions *in vitro* and *in vivo*.
Methods: Recombinant Bet v 1, Mal d 1 (apple), Api g 1 (celery), and Dau c 1 (carrot) were incubated at increasing temperatures. Protein structures were determined by means of circular dichroism. Mediator release was tested in basophil activation assays. PBMCs and Bet v 1–specific T-cell lines with known epitope specificity were stimulated with native and cooked food allergens. Patients with birch pollen allergy who experienced OAS and the exacerbation of atopic dermatitis (AD) on ingestion of fresh apple, celery, or carrot were retested in double-blind, placebo-controlled food challenges with the respective foods in cooked form.
Results: *In vitro*, cooked food allergens lost the capacity to bind IgE and to induce mediator release but had the same potency to activate Bet v 1–specific T cells as native proteins. *In vivo*, ingestion of cooked birch pollen–related foods did not induce OAS but caused atopic eczema to worsen.
Conclusion: T-cell cross-reactivity between Bet v 1 and related food allergens occurs independently of IgE cross-reactivity *in vitro* and *in vivo*. In patients with AD, the resulting immune reaction can even manifest as late eczematous skin reactions. Therefore the view that cooked pollen-related foods can be consumed without allergologic consequences should be reconsidered.
Clinical implications: Symptom-free consumed pollen-related food allergens might cause T cell–mediated late-phase skin reactions in patients with pollen allergy and AD. (J Allergy Clin Immunol 2006;118:242-9.)

Key words: *Birch pollen allergy, food allergy, oral allergy syndrome, atopic dermatitis, pathogenesis-related protein family 10, Bet v 1, cross-reactivity*

Food allergy in adolescent and adult individuals often develops as a consequence of an allergic sensitization to respiratory allergens. The major birch pollen allergen Bet v 1 is a relevant sensitizing protein causing the so-called birch-fruit-vegetable syndrome.[1-4] Bet v 1 belongs to the pathogenesis-related protein family 10 (PR-10).[5] Other members of this protein family are present in different foods, such as fruits of the Rosaceae (eg, Mal d 1 in apple, Pru a 1 in cherry, and Pyr c 1 in pear), vegetables of the Apiaceae (eg, Api g 1 in celery and Dau c 1 in carrot), hazelnut (Cor a 1.04), soybean (Gly m 4), mungbean (Vig r 1), and peanut (Ara h 8).[6-14] These proteins share a high degree of amino acid (aa) sequence similarity with the major birch pollen allergen, and Bet v 1–specific IgE antibodies recognize these dietary proteins and can cause immediate hypersensitivity reactions on ingestion of the foods. In the majority of patients, these reactions are confined to the oropharynx, and this has been termed *oral allergy syndrome* (OAS).[15] Occasionally, systemic IgE-mediated reactions, such as urticaria, asthma, or anaphylactic shock, are observed.[16,17]

In addition to IgE-mediated immediate-type allergic symptoms, ingestion of birch pollen–related food caused the exacerbation of skin lesions in a subpopulation of patients with birch pollen allergy and atopic dermatitis (AD).[18,19] T lymphocytes are important key players in late eczematous reactions, and in the lesional skin of reacting patients with birch pollen allergy, Bet v 1–specific T-cell responses have been detected.[18] Previously, we could demonstrate that Bet v 1–specific T lymphocytes react with related food homologues *in vitro*. This phenomenon is due to cellular cross-reactivity: the major birch pollen allergen contains several distinct T cell–activating

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Abbreviations used
aa: Amino acid
AD: Atopic dermatitis
DBPCFC: Double-blind, placebo-controlled food challenge
OAS: Oral allergy syndrome
PR-10: Pathogenesis-related protein family 10
RBL: Rat basophil leukemia cell
TCL: T-cell line
T_m: Melting temperature

regions, and in the aa sequences of Mal d 1, Cor a 1, and Api g 1, T cell–activating regions matching Bet v 1 epitopes have been identified.[4,20,21] In particular, T cells specific for the highly conserved immunodominant epitope Bet v $1_{142\text{-}156}$ were shown to proliferate and produce cytokines in response to various different PR-10–like food allergens.[22]

After becoming aware of their food allergy, most patients with birch pollen allergy avoid the respective foods. However, to exclude fresh fruits and vegetables from diet during the entire year restricts the quality of life of these individuals. On the other hand, cooked birch pollen–related foods can generally be consumed without difficulty because PR-10–like food proteins are easily denatured by thermal processing.[23-27] Therefore allergologists and guidebooks recommend heating of these aliments to make them safe for consumption. We speculated that thermal processing of PR-10–like allergens causes conformational changes, resulting in diminished IgE-binding capacity, but that the primary protein structure is not affected, resulting in retained linear T-cell epitopes. Thus cooked birch pollen–related food would not induce immediate reactions but could still cause T cell–mediated symptoms. To prove this hypothesis, the effects of thermal processing on the protein structure, IgE binding, and basophil-activating capacity of recombinant Bet v 1, Mal d 1 (apple), Api g 1 (celery), and Dau c 1 (carrot) were analyzed. The ability of heat-treated allergens to activate T cells was analyzed by using PBMCs and Bet v 1–specific T-cell lines (TCLs). We also evaluated which T-cell epitopes of Bet v 1 were relevant for cross-reactivity with each food allergen. Finally, patients with birch pollen allergy with IgE-mediated (OAS) and T cell–mediated (worsening of AD) reactions to fresh apple, celery, and carrot were rechallenged with these cooked foods in double-blind, placebo-controlled food challenges (DBPCFCs) to substantiate our hypothesis *in vivo*.

METHODS

Allergens

Recombinant Bet v 1, Mal d 1, Api g 1, and Dau c 1 were purchased from Biomay (Vienna, Austria). Bacterial endotoxin contents were determined by using the Limulus Amoebocyte Lysate assay (BioWhittaker, Walkersville, Md) and were less than 2.5 EU/μg for each allergen.

Temperature scan and circular dichroism spectra

Circular dichroism measurements were carried out on a Jasco J-715 spectropolarimeter (JASCO Corporation, Tokyo, Japan) with a 0.1-cm path-length cell, with the cooling jacket connected to a water thermostat. Allergens were dissolved in 20 mM Tris buffer at a concentration of 41 μM for Bet v 1, 56 μM for Mal d 1, 51 μM for Api g 1, and 86 μM for Dau c 1. Temperature scans were performed according to a step-scan procedure, during which samples were heated from 20°C to 95°C with a heat rate of 1°C/min and cooled down to 20°C with the same rate. A continuous temperature scan was monitored at a wavelength of 217 nm, with a step resolution of 0.5°C. Spectra were recorded at every 5°C in the range from 20°C to 95°C, with 0.5 nm resolution and a scan speed of 50 nm/min. The average of 3 scans was expressed as the mean residue ellipticity at a given wavelength.

IgE-binding and mediator release assays

Recombinant allergens were heated for 1, 5, 15, 30, 45, and 60 minutes at 20°C, 40°C, 60°C, 80°C, and 100°C, respectively. Thereafter, 1 μg of Bet v 1 and 5 μg of Mal d 1, Api g 1, and Dau c 1 were dotted on nitrocellulose membranes. Dried membranes were incubated with pooled sera from 10 patients with birch pollen allergy overnight at 4°C. After incubation with an iodine 125–labeled anti-human IgE antibody (IBL, Hamburg, Germany), bound IgE was visualized by means of autoradiography. For mediator release assays, the rat basophil leukemia cell (RBL) mediator release assay was used, as described in detail elsewhere.[28] Briefly, the RBL cell subline transfected with the human FcεRI (RBL-30/25) was incubated overnight with sera diluted 1:10 from 4 different patients with birch pollen allergy with OAS to apples, celery, and carrot. After washing, cells were incubated with titrated concentrations (10^1 to 10^{-4} μg/mL) of allergens that had been incubated for 30 minutes at 20°C, 40°C, 60°C, 80°C, and 100°C. The release of β-hexosaminidase was determined according to published protocols.[29]

Proliferative responses of PBMCs and Bet v 1–specific TCLs

Proliferation in PBMCs from 6 patients with birch pollen allergy was analyzed as described previously.[28] Briefly, these patients were characterized by typical case history, CAP/RAST class of greater than 3 to birch (Phadia, Uppsala, Sweden), positive skin prick test reactions (wheal diameter >5 mm) to birch pollen and celery extract (Soluprick; ALK Abello, Hørsholm, Denmark), and positive prick-to-prick test results to fresh apples, celery, and carrots. All individuals experienced OAS to apples and carrots, and 3 of 6 individuals also experienced OAS to celery. The sera contained IgE specific for all allergens under investigation. PBMCs were cultured in triplicate in medium alone or in the presence of titrated concentrations (3-50 μg/mL) of heat-treated (60 minutes at 100°C) or nontreated allergens for 6 days. Proliferation was measured by adding tritiated thymidine during the last 16 hours of culture.

Bet v 1–specific TCLs were established from patients with birch pollen allergy and mapped for Bet v 1 epitopes, as described in detail elsewhere.[22] TCLs were cultured with autologous irradiated PBMCs in duplicate in medium alone and in the presence of heat-treated or nontreated Bet v 1 (5 μg/mL), Mal d 1, Api g 1, and Dau c 1 (all 10 μg/mL). After 48 hours, mean counts per minute of tritiated thymidine uptake were calculated, and a variance of less than 15% was accepted. The response was considered positive when the mean of the allergen-stimulated TCLs was higher than the mean of the medium control plus 5× SD. Stimulation indices were calculated by dividing the mean counts per minute in allergen-stimulated TCLs by the mean counts per minute in the medium control.

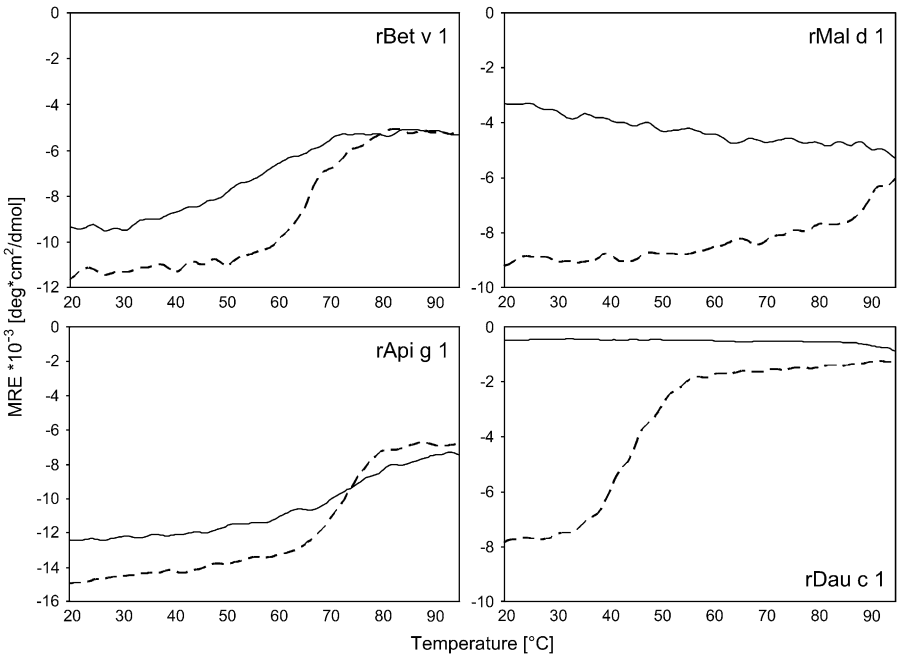

FIG 1. Thermal processing changes the protein structure of Bet v 1–related food allergens. The mean residue ellipticity *(MRE)* was recorded at 217 nm with increasing temperature *(dashed lines)* and during recooling *(continuous lines)*.

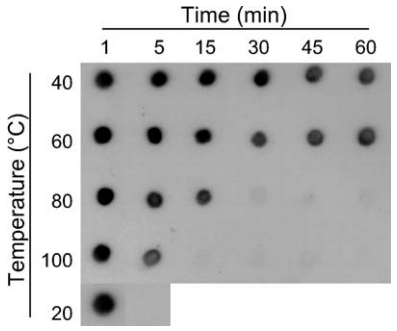

FIG 2. Thermal processing abrogates IgE binding of Mal d 1. Aliquots of Mal d 1 incubated at increasing temperatures for different time periods were dotted on nitrocellulose, and IgE binding was determined by means of immunoblotting. A positive control incubated for 60 minutes at 20°C and the buffer control are shown.

Determination of cytokines

The concentration of IL-5 and IFN-γ in supernatants of Bet v 1–specific TCLs harvested after 48 hours were determined by using ELISA with Endogen Matched Antibody Pairs (Endogen, Woburn, Mass) according to the instructions of the manufacturer (limit of detection: IL-5, 7.4 pg/mL; IFN-γ, 9.5 pg/mL).[22]

Statistics

Cell proliferation and cytokine production were analyzed by using the Wilcoxon signed-rank test.

DBPCFCs

Patients with birch pollen allergy with a typical case history, specific IgE for birch pollen as detected with the CAP RAST FEIA (Phadia), and AD diagnosed according to the criteria of Hanifin and Rajka[30] who suspected birch pollen–related foods as a provocation factor for worsening of AD underwent DBPCFCs with fresh apple, celery, or carrot to identify the single food relevant for the respective individual at the Department of Dermatology and Allergology of the Hannover Medical School. The preparation of verum/placebo and DBPCFCs was performed as described in detail elsewhere.[19] Before the challenges, patients underwent an elimination diet for 4 weeks and 72 hours before challenge antihistamines were withdrawn. Verum and placebo were given on 2 consecutive days. The severity of AD was determined according to the SCORAD score, which is a scoring system combining extent, severity, and subjective symptoms before and the next day after challenge by a physician unaware of the food given.[31] All immediate symptoms, including the OAS, were evaluated. Clinically significant skin reactions were defined as late eczematous reactions when occurring more than 6 hours after ingestion of the last dose. Patients who showed immediate and late allergic symptoms to one of the fresh foods were asked to undergo challenge in DBPCFCs with the respective food after cooking (ie, after boiling until soft).

RESULTS

Thermal processing of food allergens changes their structure

Circular dichroism spectra were recorded during a continuous temperature scan from 20°C to 100°C of Bet v 1, Mal d 1, Api g 1, and Dau c 1 at 217 nm (Fig 1). During heating, all allergens underwent an unfolding process to a random coil structure. Unfolding of Bet v 1 occurred between 50°C and 80°C, with a calculated melting temperature (T_m) of 66°C; unfolding of Api g 1

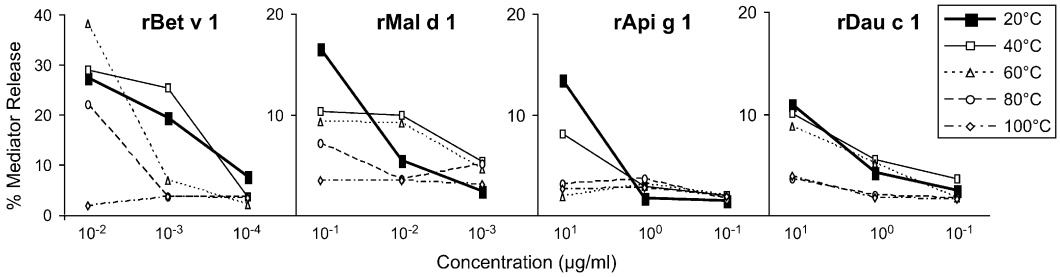

FIG 3. Thermal processing abrogates basophil activation of Bet v 1–related food allergens. RBLs loaded with serum IgE from individuals with birch pollen allergy and OAS to apples, celery, and carrot were activated with allergens incubated for 30 minutes at indicated temperatures. The percentage of mediator release *(x-axis)* induced by titrated concentrations of allergens of one representative individual of 4 is shown.

occurred between 43°C and 80°C, with a calculated T_m of 73°C; and unfolding of Dau c 1 occurred between 28°C and 58°C, with a calculated T_m of 45°C. Mal d 1 underwent a continuous unfolding process, with an apparent transition temperature of approximately 90°C. During recooling, Bet v 1 and Api g 1 partly restored their structure, whereas Mal d 1 and Dau c 1 did not (Fig 1). These data indicate that heating irreversibly destroys the protein structure of the recombinant major apple and carrot allergen, whereas this seems not to be the case for the celery allergen and might explain why cooked celery has been reported to induce type I allergy symptoms.[16]

Thermal processing of food allergens reduces mediator release

In a first set of experiments, recombinant allergens were incubated at increasing temperatures (40°C, 60°C, 80°C, and 100°C) for different time periods (1-60 minutes). Thereafter, their IgE binding was determined in immunodot assays. Fig 2 shows recombinant Mal d 1 as a representative example. In general, the IgE-binding capacity of the recombinant allergens was not affected after incubation at 40°C and 60°C but was markedly reduced after incubation for 15 minutes at 80°C and after 5 minutes at 100°C. Next, allergens were incubated for 30 minutes at 40°C, 60°C, 80°C, and 100°C, respectively, and their ability to trigger degranulation of basophils was evaluated. For this purpose, RBL mediator release assays were performed with titrated concentrations (10^0 to 10^{-4} µg/mL) of heat-treated and nontreated allergens and sera from 4 different individuals with pollen allergy and OAS to the foods under investigation (Fig 3). Incubation at 40°C hardly reduced basophil activation by all allergens. Incubation at 60°C reduced the mediator-releasing capacity of Api g 1 completely and to a lesser extent the capacity of Bet v 1, Mal d 1, and Dau c 1. Treating allergens at 80°C and 100°C abolished their mediator-releasing capacity.

Cooked food allergens cross-react with Bet v 1–specific T cells

Before T cell–stimulation experiments, allergens were cooked for 60 minutes at 100°C to completely abolish IgE binding. PBMCs from 6 different individuals with birch pollen allergy were stimulated with different concentrations of cooked and native allergens. The concentration of native allergens that induced the highest proliferation in each patient was used for comparison with equal concentrations of cooked allergens (Table I). Cooked and native allergens induced a comparable proliferation in PBMCs, demonstrating that T-cell epitopes in these allergens were not destroyed by thermal processing. Also, 7 Bet v 1–specific TCLs reactive with Mal d 1, Api g 1, or Dau c 1 were tested for reactivity with equal concentrations of cooked proteins (Table I). Bet v 1–specific TCLs proliferated to a comparable extent to cooked and native allergens. The levels of IL-5 and IFN-γ were assessed in supernatants of 4 TCLs, revealing that cooked proteins induced a similar T_H2 bias in Bet v 1–specific TCLs as native allergens (Table II).

T-cell epitopes of Bet v 1 relevant for cross-reactivity with food allergens

Bet v 1–specific TCLs with known epitope specificity from patients with birch pollen allergy (described in detail elsewhere[22]) were screened for reactivity with Mal d 1, Api g 1, and Dau c 1 to elucidate which T-cell epitopes of Bet v 1 were relevant for cross-reactivity with each homologue. A stimulation index of greater than 2 in response to the food allergens was considered positive. Epitope recognition patterns of food allergen–reactive and nonreactive TCLs were compared, and the percentage of peptide-reactive cultures was calculated for each group (Fig 4). An epitope was considered as relevant if the percentage in food allergen–reactive TCLs was more than 2 times the percentage in nonreactive TCLs. In 16 (84%) of 19 Mal d 1–positive Bet v 1–specific TCLs, the C-terminal epitope Bet v $1_{142\text{-}153}$ was detected, whereas this peptide was found in only 4 (21%) of 19 Mal d 1–nonreactive TCLs. For none of the other epitopes was an obvious difference between food-reactive and nonreactive TCLs found. In Api g 1–reactive Bet v 1–specific TCLs, a more pronounced reactivity for epitopes located in the regions aa 1-15, aa 4-18, aa 10-24, aa 94-111, and aa 109-123 compared with Api g 1–nonreactive cultures was observed. Dau c 1–reactive Bet v 1–specific TCLs harbored more T cells specific for epitopes located at aa 1-15, aa 79-93, aa 94-111, and aa 109-120 than Dau c 1–nonreactive cultures.

TABLE I. Proliferative responses of PBMCs and Bet v 1–specific TCLs to heat-treated and nontreated allergens

	Medium	Bet v 1	Bet 95°C	Mal d 1	Mal 95°C	Api g 1	Api 95°C	Dau c 1	Dau 95°C
A	6.6*	31.3	36.5	18.2	15.6	14.0	12.0	22.3	14.6
B	4.5	13.9	10.3	10.6	11.2	7.6	7.0	12.1	7.4
C	3.0	27.3	26.8	32.3	18.2	22.9	28.9	32.6	30.6
D	3.1	15.9	15.6	12.1	12.4	17.2	19.4	15.2	14.4
E	3.3	9.8	9.3	14.2	13.4	ND	ND	ND	ND
F	0.9	23.1	4.8	27.8	21.6	4.8	2.5	26.2	11.5
1	4.0	27.0	63.6	91.9	61.2	34.6	39.0	3.6	2.9
2	0.9	94.5	104.0	4.9	5.9	47.8	35.7	8.3	9.2
3	3.6	33.3	41.2	ND	ND	10.9	7.0	ND	ND
4	1.7	47.2	56.0	3.8	7.4	10.8	14.1	2.5	2.1
5	1.6	21.8	36.9	1.8	1.9	9.6	4.8	3.6	3.6
6	1.1	22.7	37.6	ND	ND	4.9	5.0	1.7	1.8
7	3.2	12.1	8.1	3.0	3.3	8.0	5.1	6.6	6.8

Counts per minute in medium controls differed significantly from counts per minute in stimulated cultures ($P_{\text{Bet v 1}} = .001$, $P_{\text{Bet95°}} = .001$; $P_{\text{Mal d 1}} = .005$, $P_{\text{Mal95°}} = .003$; $P_{\text{Api g 1}} = .002$, $P_{\text{Api95°}} = .002$; $P_{\text{Dau c 1}} = .004$ and $P_{\text{Dau95°}} = .008$). No significant difference was found between cultures stimulated with native and cooked allergens (Wilcoxon signed-rank test).
A-F, PBMCs; *1-7*, Bet v 1–specifics TCL from different patients with birch pollen allergy. *ND*, Not determined.
*Mean counts per minute × 1000 of triplicate *(A-F)* and duplicate *(1-7)* stimulations.

TABLE II. Cytokine response of Bet v 1–specific TCLs to heat-treated and nontreated allergens

TCL	Cytokine	Bet v 1	Bet 95°	Mal d 1	Mal 95°	Api g 1	Api 95°	Dau c 1	Dau 95°
1	IL-5	812*	1076	1173	1052	808	891	331	228
	IFN-γ	453	580	258	142	114	85.5	120	56.3
2	IL-5	1358	1730	267	452	1616	1329	891	1767
	IFN-γ	90.2	311	20.1	29.6	20.7	22.3	14.8	6.6
3	IL-5	1980	2587	ND	ND	915	468	ND	ND
	IFN-γ	41.6	121	ND	ND	117	140	ND	ND
4	IL-5	1598	1988	1888	1756	1818	1770	982	843
	IFN-γ	232	98.4	267	299	61.3	51.3	20.2	30.8

No statistical difference was found between cytokine levels stimulated by native and cooked allergens (Wilcoxon signed-rank test).
ND, Not determined.
*Mean picograms per milliliter of duplicates. Cytokine levels in supernatants of unstimulated cultures were subtracted from levels in stimulated cultures.

Cooked birch pollen–related foods do not induce OAS but late-phase reactions in the atopic skin

Individuals with birch pollen allergy with AD experiencing immediate (OAS) and late allergic symptoms (worsening of atopic eczema within 24 hours) in DBPCFCs with fresh birch pollen–related foods (patient 1 with celery and patients 2-4 with carrot) were rechallenged in DBPCFCs with cooked foods. Worsening of AD was measured as the difference in SCORAD points before and after challenge. None of these individuals experienced an OAS on ingestion of the cooked aliment, but interestingly, they responded again with increased SCORAD values (Table III). Patient 5 had experienced a marked worsening of AD after drinking industrially prepared pasteurized apple juice in the absence of OAS. In DBPCFCs with fresh apple (granny Smith), this patient experienced OAS and a SCORAD difference of 20 points before and after the challenge (Table III). None of the 5 patients responded with immediate or late reactions to oral provocations with placebo. All patients were sensitized to Bet v 1 and none of them to Bet v 2, as determined by means of ImmunoCAP (Table III). Furthermore, none of the individuals was allergic to grass and mugwort pollen, respectively.

DISCUSSION

Our work indicates that cooked birch pollen–related foods represent an underestimated provocation factor for the allergic immune response of individuals with birch pollen allergy because thermal processing makes these foods clinically tolerated but does not reduce their capacity to activate allergen-specific T cells.

Three relevant Bet v 1–related food allergens belonging to different botanical families (ie, Mal d 1 from Rosaceae and Api g 1 and Dau c 1 from Apiaceae) were exposed to increasing temperatures and then tested for their ability to induce IgE and T cell–mediated reactions *in vitro*. Thermal processing affected the protein structure (Fig 1), resulting in abrogated IgE-binding and mediator-releasing capacity after incubation at greater than 80°C for a few minutes (Figs 2 and 3). On the other hand, food allergens neither lost their potency to induce

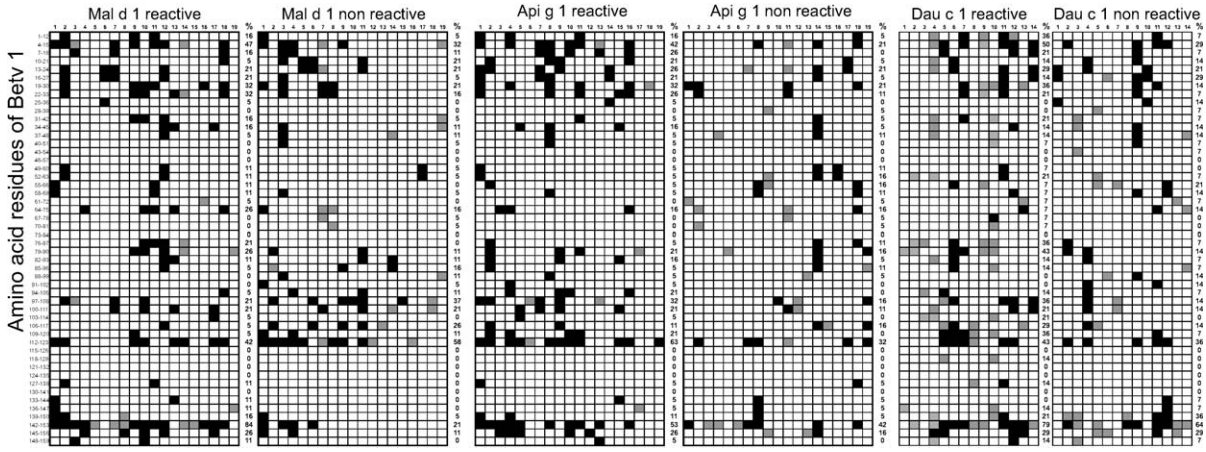

FIG 4. T-cell epitopes of Bet v 1 relevant for cross-reactivity with food allergens. Bet v 1–specific TCLs mapped for epitope recognition[22] were stimulated with Mal d 1, Api g 1. and Dau c 1. A stimulation index of greater than 2 was considered as food allergen reactive. *Gray boxes* indicate peptides inducing a stimulation index of 2.5 to 5, and *filled boxes* indicate peptides inducing a stimulation index of greater than 5. The percentage of peptide-reactive TCLs is shown.

TABLE III. Characteristics of patients with birch pollen allergy and patients with food allergy and AD

			IgE (kU/L)			SCORAD difference (points) before and after DBPCFCs with:	
Patient no.	**Sex**	**Age (y)**	**Total**	**Bet v 1**	**Bet v 2**	**Fresh food**	**Cooked food**
1	F	24	447	28.7	<0.35	16	14
2	F	33	1234	>100	<0.35	12	17
3	F	37	308	>100	<0.35	10	15
4	M	35	137	27.0	<0.35	10	20
5	F	5	55.7	3.5	<0.35	20	ND

M, Male; *F*, female; *ND*, not determined.

proliferation in PBMCs nor to activate Bet v 1–specific T cells after cooking for 1 hour at 100°C (Tables I and II). We also analyzed which epitopes of Bet v 1 were mainly involved in T-cell reactivity with the different food allergens. Interestingly, cross-reactivity between Bet v 1 and Mal d 1 was predominantly restricted to one single epitope, Bet v $1_{142\text{-}153}$, although both allergens share 71% of aa sequence similarity (Fig 4). T-cell cross-reactivity between Bet v 1 and both Api g 1 and Dau c 1 was associated with several different epitopes spreading the entire aa sequence of the pollen allergen (Fig 4). The fact that very similar epitopes were relevant for both Apiaceae allergens might be explained by their aa sequence similarity of 91%. However, in contrast to Mal d 1, Api g 1 and Dau c 1 share only 61% and 57% similarity, respectively, with Bet v 1. Hence the degree of sequence similarity between Bet v 1–related food allergens and the major birch pollen allergen cannot be used as a marker to predict their potential to induce T cell–mediated symptoms.

To substantiate our *in vitro* results *in vivo*, Bet v 1–monosensitized individuals with birch pollen allergy with OAS and AD were tested in DBPCFCs with fresh and cooked apple, celery, and carrot, respectively. Ingestion of cooked foods triggered a comparable worsening of atopic eczema as fresh foods (Table III). These skin reactions occurred in the absence of OAS, which confirms that Bet v 1–related allergens in the natural food matrix had lost their ability to induce IgE-mediated immediate reactions. Food-induced late eczematous reactions in patients with birch pollen allergy have been associated with an increased expression of cutaneous lymphocyte antigen of peripheral blood T cells and the presence of Bet v 1–reactive T cells in the isolated skin lesions.[18] Thus fresh birch pollen–related foods obviously activate Bet v 1–specific T cells to migrate to the skin and to mediate late-phase reactions. The presented data extend these previous findings by showing that (1) cooked birch pollen–related foods induce similar T-cell activation and T cell–mediated symptoms as fresh aliments and (2) T cell–mediated reactions occur also in the absence of IgE-binding and IgE-mediated reactions. The latter hypothesis is further endorsed by our demonstration that after simulated gastrointestinal digestion by means of successive incubation of Bet v 1–related food allergens with pepsin and trypsin, several proteolytic non–IgE-binding fragments have been isolated that induced proliferation and cytokine secretion in Bet v 1–specific T cells.[28] Therefore pollen-related foods should be considered important stimuli for pollen-specific T cells,

even in patients without immediate clinical symptoms of food allergy.

It is well known that inhalation of pollen allergens during the pollen season boosts the allergen-specific immune response (ie, induces local and systemic IgE synthesis and $CD4^+$ memory T cells).[32-34] However, outside the pollen season, specific serum IgE levels typically are maintained at increased levels, although no allergens are encountered. Our data provide strong evidence that symptom-free consumption of birch pollen–related foods leads to activation of pollen-specific T cells *in vivo*. We speculate that eating pollen-related foods outside the pollen season might activate pollen-specific T cells when patients do not inhale pollen allergens and thus support the long-term maintenance of pollen-specific T_H2 cells.[35,36] Perennial activation of pollen-specific T_H2 cells might maintain the synthesis of high amounts of IL-4 off season, which, together with small concentrations of nondegraded, IgE-binding allergens absorbed through the oral mucosa, gastrointestinal mucosa, or both, could then be sufficient to provoke perennial IgE synthesis in B cells.[37,38]

In summary, we have provided *in vitro* and *in vivo* evidence that in contrast to IgE reactivity, the T-cell reactivity of birch pollen–related foods is not abrogated by cooking. The resulting immune reaction might deteriorate acute and chronic manifestations of birch pollen allergy. Consequently, our findings challenge the general view that cooked pollen-related foods can be consumed without allergologic consequences for the patient.

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