

Gastrointestinal digestion of Bet v 1-homologous food allergens destroys their mediator-releasing, but not T cell-activating, capacity

Eva Maria Schimek, MD,^a Bettina Zwölfer,^a Peter Briza, PhD,^b Beatrice Jahn-Schmid, PhD,^a Lothar Vogel, PhD,^c Stefan Vieths, PhD,^c Christof Ebner, MD,^d and Barbara Bohle, PhD^a *Vienna and Salzburg, Austria, and Langen, Germany*

Background: Food allergy to apples, hazelnuts, and celery is frequent in individuals with birch pollen allergy because IgE antibodies specific for the major birch pollen allergen, Bet v 1, cross-react with structurally related allergens in these foods. In addition, T lymphocytes specific for Bet v 1 also cross-react with these dietary proteins.

Objective: We sought to evaluate the effects of simulated gastrointestinal degradation of Bet v 1-related food allergens on their mediator-releasing and T cell-activating capacity. **Methods:** Recombinant Mal d 1, Cor a 1.04, and Api g 1 were incubated separately with pepsin and trypsin. Binding of IgE was tested in immunoblots. After successive incubation with both enzymes, allergens were tested in mast cell mediator release assays and used to stimulate PBMCs and Bet v 1-specific T-cell lines and clones. Proteolytic fragments of allergens were analyzed and sequenced by means of mass spectrometry.

Results: Pepsin completely destroyed IgE binding of all allergens within 1 second, and trypsin completely destroyed IgE binding of all allergens within 15 minutes, except for the major hazelnut allergen, which remained intact for 2 hours of trypsinolysis. Allergens after gastrointestinal digestion did not induce basophil activation but induced proliferation in PBMCs from allergic and nonallergic individuals. Digested Mal d 1 and Cor a 1.04 still activated Bet v 1-specific T cells, whereas digested Api g 1 did not. Different proteolytic fragments of Mal d 1 and Cor a 1.04 matching relevant Bet v 1 T-cell epitopes were found.

Conclusion: Gastrointestinal degradation of Bet v 1-related food allergens destroys their histamine-releasing, but not T cell-activating, property. Our data emphasize that birch pollen-related foods are relevant activators of pollen-specific T cells. (*J Allergy Clin Immunol* 2005;116:1327-33.)

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

Pollen-related food allergy represents the most frequent food allergy in adult individuals in Europe. More than 70% of patients with birch pollen allergy experience allergic symptoms after the ingestion of birch pollen-related foods (eg, apples, hazelnuts, and celery).^{1,2} This association is due to cross-reactive IgE antibodies because both birch pollen and birch pollen-related foods contain homologous structures sharing IgE-binding sites.^{3,4} IgE antibodies specific for the major birch pollen allergen, Bet v 1, were shown to be of highest relevance for the induction of the birch-fruit syndrome.⁵ Bet v 1 belongs to the pathogenesis-related protein family 10 (PR-10), and homologous molecules were isolated in fruits of the Rosaceae family (eg, Mal d 1 in apple), in vegetables of the Apiaceae family (eg, Api g 1 in celery), in hazelnut (Cor a 1.04), in soybean (Gly m 4), and recently also in peanut (Ara h 8).⁶⁻¹¹ The symptoms characteristically associated with sensitization to this protein family appear immediately after contact with the fresh food, are confined to the oropharynx, and are characterized by tingling and angioedema of the lips, tongue, palate, and throat. This manifestation was termed oral allergy syndrome (OAS).¹² In addition, systemic and more severe reactions than OAS can occur, particularly after ingestion of celery and soy-containing dietary products.^{13,14}

In addition to IgE cross-reactivity, PR-10-like proteins also cross-react at the T-cell level. The major allergens of apple, celery, and hazelnut contain T-cell epitopes located in regions corresponding to relevant T cell-activating regions of Bet v 1.¹⁵⁻¹⁹ Consequently, Bet v 1-specific T cells are activated by these food allergens to proliferate and produce cytokines. After ingestion of birch pollen-related food, a marked deterioration of atopic eczema was observed in individuals with birch pollen allergy and atopic dermatitis.²⁰ Bet v 1-specific T cells were detected in the respective skin lesions. Hence pollen-related food allergens might activate Bet v 1-specific T cells *in vivo*, which then migrate to the skin and exert effector functions.²¹

From ^athe Department of Pathophysiology, Center for Physiology and Pathophysiology, Medical University of Vienna; ^bthe Department of Molecular Biology, University of Salzburg; ^cPaul-Ehrlich-Institut, Langen; and ^dAllergy Clinical Reumannplatz, Vienna.

Supported by the Fonds zur Förderung der wissenschaftlichen Forschung, Austria (SFB-F1807-B04; FSP-S8808-MED), and Biomay, Austria.

Received for publication June 15, 2005; revised September 2, 2005; accepted for publication September 12, 2005.

Available online November 2, 2005.

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/\$30.00

© 2005 American Academy of Allergy, Asthma and Immunology

doi:10.1016/j.jaci.2005.09.007

Abbreviations used

OAS: Oral allergy syndrome
 PR-10: Pathogenesis-related protein family 10
 RBL cell: Rat basophil leukemia cell
 TCC: T-cell clone
 TCL: T-cell line

It is generally assumed that PR-10-like proteins undergo rapid gastric degradation, leading to the loss of their IgE-binding capacity.^{22,23} In contrast to the IgE-binding sites of these proteins, which are conformational epitopes depending on the tertiary protein structure,²⁴⁻²⁷ T-cell epitopes are short linear peptides.²⁸ These short amino acid sequences might survive gastric, as well as pancreatic, digestion. Therefore we investigated the effects of enzymatic digestion on the T cell-stimulatory capacity of Bet v 1-related food allergens. Furthermore, we analyzed whether the fragments created by means of gastrointestinal digestion were capable of activating pollen-specific T lymphocytes. For this purpose, the recombinant major allergens of apple (rMal d 1), celery (rApi g 1), and hazelnut (rCor a 1.04) were incubated with pepsin followed by trypsin to simulate gastrointestinal degradation.²⁹ Thereafter, the T-cell response to digested allergens was tested in PBMCs from individuals with and without birch pollen allergy. T-cell lines (TCLs) and T-cell clones (TCCs) established with the major birch pollen allergen were stimulated with the food allergens and their digested products to investigate the cross-reactivity with Bet v 1-specific T cells.

METHODS

Allergic patients

Allergic individuals ($n = 18$) had a history of hay fever during early spring, a specific IgE ImmunoCAP class of greater than 3 to birch (mean, 24.9 kU/mL; Pharmacia Diagnostics, Uppsala, Sweden), and positive skin prick test reactions (wheal diameter >5 mm) to birch pollen (Soluprick; ALK Abello, Hørsholm, Denmark). All patients displayed positive wheal-and-flare reactions when tested with fresh apples, celery, and hazelnuts in a prick-to-prick test.¹ OAS was evaluated in standardized interviews. Sera of all allergic patients contained IgE specific for the allergens under investigation, as determined by means of immunoblotting (data not shown). Nonallergic individuals ($n = 6$) were nonatopic and not sensitized to any allergen under investigation. The study was approved by the local medical ethics committee (Vienna, Austria).

Protease digestion of allergens

Recombinant Bet v 1, rMal d 1, and rApi g 1 were purchased from Biomay (Vienna, Austria), and rCor a 1.04 was produced as previously described.⁹ Bacterial endotoxin contents were detected with a Limulus Amoebocyte Lysate assay (BioWhittaker, Walkersville, Md) and were less than 2.5 EU/ μ g for each allergen. Proteases were obtained from Sigma Aldrich (St Louis, Mo). Allergens were digested according to the protocol of Sen et al.³⁰ Briefly, each allergen (50 μ M) was incubated at 37°C with 10 μ M

pepsin in 0.1 M HCl (pH 1.0) and 0.1 μ M trypsin in 76 mM NaHCO₃ (pH 8.3). Aliquots were taken at the indicated time points and subjected to SDS-PAGE. For all tissue culture experiments, each allergen was first digested with pepsin for 30 minutes at 37°C and after adjusting the pH with 1 M NaOH to 8.3 for another 30 minutes at 37°C with trypsin during continuous shaking. Thereafter, the samples were immediately cooled and maintained at 4°C.

SDS-PAGE and immunoblotting

Allergens and digested samples (2 μ g per lane) were separated by 20% SDS-PAGE and either stained with Coomassie Brilliant blue or blotted onto a nitrocellulose membrane. For immunodot experiments, 2 μ g of digested and nondigested allergens were dotted on the membrane. Membranes were incubated with a serum pool of 10 patients overnight at 4°C. The pool contained greater than 100 kU/mL rBet v 1-specific, 46.1 kU/mL rMal d 1-specific, 15.5 kU/mL rApi g 1-specific, and 84.6 kU/mL rCor a 1-specific IgE. All individuals experienced OAS to apples and hazelnuts, and 3 of 10 experienced OAS to celery. After incubation with an iodine 125-labeled anti-human IgE antibody (IBL, Hamburg, Germany), bound IgE was visualized by means of autoradiography.

Rat basophil leukemia cell mediator release assay

The permanently growing rat basophil leukemia (RBL) cell subline transfected with the human Fc ϵ RI (RBL-30/25) was maintained in Eagle's Minimum Essential Medium with 10% FCS. Mediator release assays were performed as previously described.³¹ Briefly, RBL cells were harvested in the stationary phase and plated in flat-bottomed 96-well plates (1×10^5 per well; Nunclone, Nunc, Denmark) in the presence of patient sera diluted 1:10 overnight at 37°C in a humidified CO₂ (5%) atmosphere. Thereafter, the cell layer was washed twice with Tyrode buffer (130 mM NaCl, 5 mM KCl, 1.4 mM CaCl₂, 1 mM MgCl₂, 5.6 mM glucose, 10 mM HEPES, and 0.1% BSA, pH 7.4), and 100 μ L per well of a serial dilution of digested and nondigested allergens in Tyrode buffer containing 50% D₂O was added for 60 minutes at 37°C to trigger mediator release. Total release was determined in cells treated with buffer containing 1% Triton X-100, and spontaneous release was determined in cells treated with Tyrode buffer alone. All tests were performed in triplicate. Mediator release was quantified by measurement of the β -hexosaminidase activity. Cell-free supernatants (30 μ L) were transferred to a microtiter plate, and the enzymatic activity of β -hexosaminidase was detected by means of hydrolysis of *p*-nitrophenyl-N-acetyl- β -D-glucosaminide for 50 minutes. Absorbance was measured at 405 nm. Allergen-induced releases were expressed as a percentage of the total release after subtraction of the spontaneous release.

Proliferation assays

PBMCs were isolated from 6 allergic patients with OAS to apples and hazelnuts (3 of 6 had OAS to celery) and 6 nonallergic individuals. PBMCs (2×10^5) were cultured in triplicate in 96-well plates (Nunclone) in 200 μ L of Ultra Culture Medium (BioWhittaker) supplemented with 2 mM glutamine and 2×10^{-5} M β -mercaptoethanol in the presence of titrated concentrations (3-50 μ g/mL) of digested and nondigested allergens for 6 days. As negative controls, PBMCs were cultured in medium alone and in medium containing proteases, respectively. Proliferation was measured by adding tritiated thymidine (0.5 μ Ci per well) during the last 16 hours of culture. Delta counts per minute are counts per minute in cultures stimulated with allergens minus counts per minute in negative controls. Statistical significance of differences was determined by using the Mann-Whitney *U* test. Differences were considered statistically significant at a *P* value of less than .05.

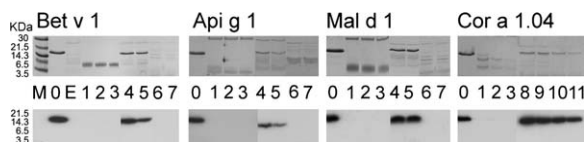


FIG 1. Pepsin and trypsin digestion profiles of PR-10-like allergens. *M*, Marker; *lane 0*, untreated allergen; *lane E*, both enzymes alone; *lanes 1-3*, allergen plus pepsin for 1, 5, and 30 seconds; *lanes 4-7*, allergen plus trypsin for 1, 5, 15, and 30 minutes; *lanes 8-11*, allergen plus trypsin for 30, 45, 60, and 120 minutes. The *upper panels* show the Coomassie staining of a 20% gel. The *lower panels* depict the IgE binding of a serum pool of 10 individuals with birch pollen allergy, as determined by means of immunoblotting.

Bet v 1-specific TCLs and TCCs

PBMCs ($1.5 \times 10^6/\text{ml}$) from individuals with birch pollen allergy were stimulated with $10 \mu\text{g/mL}$ rBet v 1 in 24-well culture plates (3524 Costar, Cambridge, Mass) under conditions as described above. After 5 days, 10 U/mL human rIL-2 (Boehringer, Mannheim, Germany) was added. At day 7, TCLs were fed with irradiated PBMCs and rIL-2, followed by another feeding after 1 week. After 10 days, TCLs (5×10^4) were stimulated with digested and non-digested allergens ($5 \mu\text{g/mL}$) in the presence of 1×10^5 autologous irradiated (60 Gy) PBMCs. After 48 hours, proliferation was measured as described above.

Bet v 1-specific TCCs with known epitope specificity were stimulated with the respective peptide of Bet v 1 ($5 \mu\text{g/mL}$; Mimotopes/Biotrend, Köln, Germany) or food allergens after gastrointestinal digestion for 48 hours.¹⁸ The stimulation index was calculated as the ratio between counts per minute obtained in cultures with TCCs plus autologous irradiated PBMCs plus peptide and counts per minute obtained in cultures containing TCCs and PBMCs.

Identification of proteolytic fragments by means of liquid chromatography and mass spectrometry

Liquid chromatographic and mass spectrometric spectra of digested allergens were recorded on a Micromass Global Ultima Q-ToF instrument (Waters, Milford, Mass) connected online to a Waters CapLC system. Peptides were first trapped on a precolumn (PepMap100, C18, $5 \mu\text{m}$, $100 \text{ \AA}$, $300 \mu\text{m} \times 5 \text{ mm}$; LC Packings, Amsterdam, The Netherlands) and washed with 0.1% vol/vol formic acid at a flow rate of $20 \mu\text{L/min}$. After 5 minutes, the sample was backflushed to the analytic column (PepMap100, C18, $3 \mu\text{m}$, $100 \text{ \AA}$, $75 \mu\text{m} \times 15 \text{ cm}$; LC Packings) and separated by an acetonitrile gradient at a flow rate of 300 nL/min (solvent A: 0.1% vol/vol formic acid/5% vol/vol acetonitrile; solvent B: 0.1% vol/vol formic acid/95% vol/vol acetonitrile). The gradient was 5% to 40% B in 40 minutes and 40% to 95% B in 5 minutes. The Micromass nanoflow spray head (Waters) was used for electrospray ionization, with the capillary voltage set to 3.4 kV. Data were acquired with MassLynx 4.1 software (LC Packings) in the survey mode. Doubly charged peptides were automatically detected by the software and subjected to fragmentation by increasing the collision energy to values optimized for the specific mass of the ion. The sequences of fragmented peptides were determined and identified by means of comparison with a custom-made database consisting exclusively of the 4 allergens in question by using ProteinLynx Global Server 2.0 (Waters). Singly charged peptides were identified by mass by using the BioLynx Protein/Peptide Editor software (Waters).

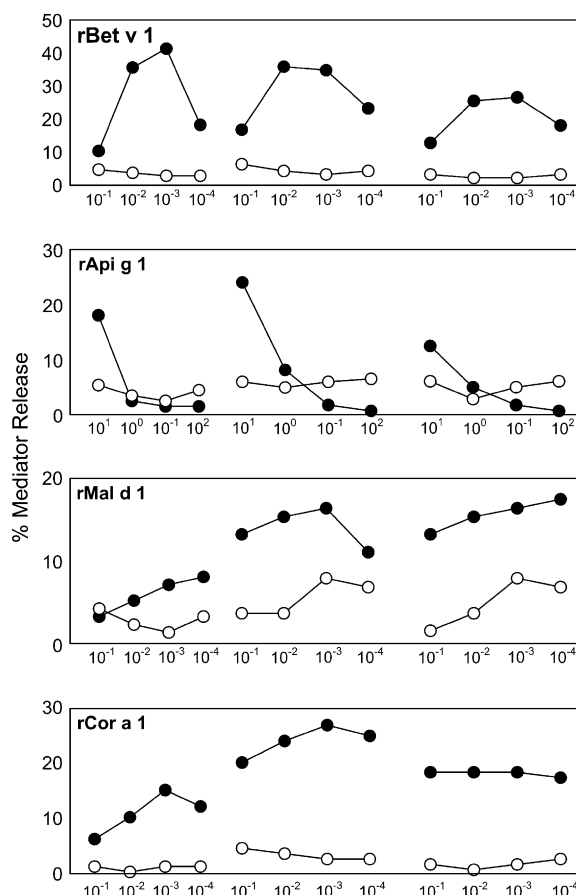


FIG 2. Mediator release induced by digested allergens. RBL cells were loaded with serum IgE from 3 different individuals with OAS to apples, hazelnuts, and celery. The percentage of mediator release (x-axis) induced by equal concentrations of intact allergens (filled circles) and allergens after gastrointestinal digestion (open circles) is shown.

RESULTS

Gastric and pancreatic digestion profiles of PR-10-like allergens

Pepsin (gastric) and trypsin (pancreatic) digestion profiles were evaluated for rBet v 1, rCor a 1.04, rMal d 1, and rApi g 1 (Fig 1). All allergens were destroyed after incubation for 1 second with pepsin. With the exception of Cor a 1.04, the allergens were also degraded after 15 minutes of incubation with trypsin (Fig 1, upper panel). None of the fragments created by either enzyme bound allergen-specific IgE, as determined by means of immunoblotting (Fig 1, lower panel). Of note, a certain amount of non-degraded Cor a 1.04 still bound IgE antibodies after 2 hours of trypsinolysis. These results were reproduced in 3 experiments.

Gastrointestinal digestion of food allergens eliminates mediator release

Each allergen was first treated with pepsin for 30 minutes followed by trypsin for another 30 minutes at

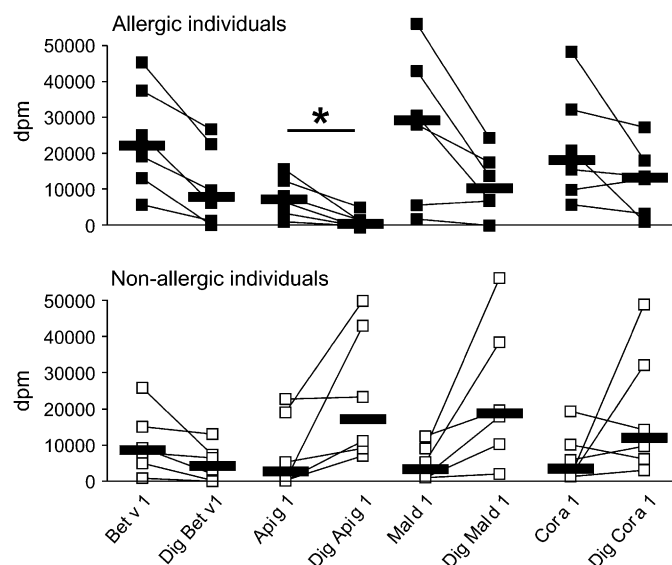


FIG 3. Proliferative responses to digested allergens. PBMCs of allergic (upper panel) and nonallergic (lower panel) individuals were stimulated with entire allergens and allergens after gastrointestinal digestion. Dpm, Delta cpm. Background levels ranged from 893 to 6566 cpm in allergic individuals and from 1215 to 8086 cpm in healthy individuals. * $P < .05$, Mann-Whitney U test.

TABLE I. Proliferative response of Bet v 1-specific TCLs with nondigested and digested allergens

TCLs	Med	Bet v 1	Digested Bet v 1	Cor a 1	Digested Cor a 1	Mal d 1	Digested Mal d 1	Api g 1	Digested Api g 1
1	0.94*	94.47	16.64	15.65	0.66	4.94	0.62	47.80	1.22
2	1.07	16.60	3.20	36.12	1.84	13.76	5.00	17.83	1.11
3	4.04	27.03	34.82	32.46	1.03	91.89	2.09	34.64	6.60
4	4.18	17.42	5.52	8.50	2.65	10.25	3.95	10.24	1.00
5	0.14	11.00	0.17	0.19	0.12	0.13	0.19	1.93	0.11
6	7.59	121.40	36.25	81.59	28.75	15.95	2.15	80.20	8.62
7	1.90	77.88	0.88	33.29	18.76	3.21	15.44	ND	ND
8	0.66	41.56	0.30	1.89	0.31	ND	ND	26.57	0.31
9	0.48	3.04	4.45	0.57	0.56	0.98	1.49	0.89	0.56
10	1.68	227.24	1.75	1.94	1.39	3.77	11.09	10.75	1.09
11	3.57	33.25	20.76	7.31	11.61	ND	ND	10.94	3.51
12	1.06	22.68	1.64	3.73	1.38	ND	ND	4.94	0.77
13	1.68	21.19	1.68	2.42	1.91	1.83	0.38	9.55	0.67
14	1.55	44.68	5.46	3.56	1.54	ND	ND	ND	ND
15	6.47	109.98	7.05	37.32	2.46	ND	ND	34.5	4.49
16	0.46	28.33	ND	0.78	0.47	2.84	0.28	0.65	ND
17	3.22	27.31	ND	ND	ND	12.70	1.82	ND	ND
18	2.40	17.6	9.22	ND	ND	11.68	1.32	ND	ND

ND, Not determined.

*cpm $\times 10^{-3}$; positive reactions (mean cpm after allergen stimulation $>$ mean cpm of unstimulated cultures $+ 10 \times$ SDs) are indicated in gray boxes.

37°C to simulate the enzymatic degradation in the gastrointestinal tract. Thereafter, degraded allergens were tested for IgE binding in immunodot assays, which confirmed that none of the proteolytic fragments bound IgE (data not shown). In addition, allergens after gastrointestinal digestion were tested for their ability to trigger mast cell degranulation. For this purpose, transgenic RBL cells loaded with human IgE were incubated with titrated concentrations (10^0 to 10^{-4} μ g/ml) of digested and

nondigested allergens. The concentrations inducing maximum mediator release for each allergen are shown in Fig 2. None of the proteolytic fragments cross-linked surface-bound allergen-specific IgE resulting in mediator release.

Food allergens after gastrointestinal digestion induce proliferation in PBMCs

PBMCs from allergic and nonallergic individuals were stimulated with different concentrations of untreated

allergens and allergens after gastrointestinal digestion. Stimulation of cells with the gastrointestinal enzymes alone induced no effects and revealed the same results as observed in medium controls. The concentration of each allergen that induced the highest proliferation in the respective individual was compared with an equal concentration of digested sample (Fig 3). As expected, all allergens induced higher proliferation in cells from allergic than from nonallergic individuals. These differences were significant for rBet v 1 ($P = .041$), rMal d 1 ($P = .041$), and rCor a 1.04 ($P = .026$). PBMCs from allergic individuals responded in a more pronounced manner to intact than to digested allergens (Fig 3, upper panel). The proliferative responses to rApi g 1 and digested rApi g 1 differed significantly ($P = .041$). In contrast, proliferation of PBMCs from nonallergic individuals was more pronounced in response to allergens after gastrointestinal digestion. In general, digested food allergens induced a slightly higher proliferation in PBMCs from nonallergic than allergic subjects.

Food allergens after gastrointestinal digestion activate Bet v 1-specific TCLs

Bet v 1-specific TCLs generated from allergic patients were stimulated with intact allergens and allergens after gastrointestinal digestion (Table I). All TCLs proliferated in response to Bet v 1, confirming their specificity. Digested Bet v 1 induced proliferation in 8 (50%) of 16 TCLs. Cor a 1.04 induced proliferation in 10 (63%) of 16 TCLs, of which 3 (19%) responded to digested Cor a 1.04. Mal d 1 induced proliferation in 9 (69%) of 13 Bet v 1-specific TCLs, of which 4 (31%) responded to digested Mal d 1. Most of the cultures responded in a less pronounced manner to the digested than to the entire allergens. The major celery allergen induced proliferation in 12 (86%) of 14 Bet v 1-specific TCLs, of which none responded to digested Api g 1.

Different proteolytic fragments of Mal d 1 and Cor a 1.04 cross-react with Bet v 1-specific T cells

Fragments created by gastrointestinal degradation of all allergens were analyzed and sequenced by means of mass spectrometry with a liquid chromatography and mass spectrometry instrument. Several peptides consisting of 5 to 18 amino acid residues of each allergen were identified. Seven residues were defined as cutoffs because this length is usually necessary for the activation of CD4⁺ T cells.³² Three distinct peptides were found after digestion of Bet v 1, 5 peptides after digestion of Cor a 1.04 (C1-C5), and 4 peptides after digestion of Mal d 1 (M1-M4) and Api g 1 (A1-A4, Fig 4). Comparing the fragments of Mal d 1 and Cor a 1.04 with known T-cell epitopes of Bet v 1¹⁹ revealed that 4 of 5 Cor a 1.04 fragments and 3 of 4 Mal d 1 fragments matched Bet v 1 epitopes (Fig 4). In Table II the amino acid sequence similarity between the food fragments and Bet v 1 epitopes is shown. Fragments C1, C4, M1, M2, and M4 were found to be



FIG 4. Proteolytic fragments detected after gastrointestinal digestion of allergens. Peptides of greater than 7 amino acid residues are highlighted in gray in the sequence of each allergen. Amino acids identical with Bet v 1 are indicated in bold, and homologous residues are indicated in italics. The frames highlight known T-cell epitopes of Bet v 1 matching proteolytic fragments.

highly similar to Bet v 1 epitopes, which represent frequently recognized epitopes.¹⁹ TCCs specific for these relevant Bet v 1 epitopes were stimulated with digested Mal d 1 and Cor a 1.04 (Table III). Both digested food allergens stimulated Bet v 1-specific TCCs, which confirms that their fragments contain epitopes cross-reactive with pollen-specific T cells.

DISCUSSION

Birch pollen-related food allergy predominantly manifests as OAS, an IgE-mediated form of contact allergy. This clinical appearance concurs with the high lability of Bet v 1-related food proteins to gastric digestion, which immediately destroys their conformational epitopes.^{22,23} We sought to separately analyze the effects of pepsin and trypsin on Mal d 1, Api g 1, and Cor a 1.04. Pepsinolysis degraded all allergens within 1 second (Fig 1). Trypsinolysis was as effective after 15 minutes, except for the Bet v 1 homologue in hazelnuts, and notable amounts of Cor a 1.04 remained undegraded for up to 2 hours (Fig 1). Bet v 1-related food allergy is the consequence of a primary inhalant sensitization to the birch pollen allergen. However, it has been shown that antilucer treatment with antacid drugs can promote sensitization to hazelnuts through the oral route *in vivo*.³³ For this phenomenon, the particular resistance of the major hazelnut allergen to pancreatic digestion might also be relevant: after drug-induced malfunction of pepsin, intact Cor a 1.04 might reach the gut-associated lymphoid tissue and cause food allergy independently of a previous sensitization to birch pollen.

Before use in T-cell activation experiments, all allergens were incubated for 30 minutes with pepsin, followed by incubation with trypsin for 30 minutes. Certainly these *in vitro* digestion conditions cannot predict the effects occurring *in vivo* because pure recombinant allergens were used, and any influences of the respective food

TABLE II. Similarity of proteolytic fragments of the major apple and hazelnut allergens with T-cell epitopes of Bet v 1

Bet v 1 epitope (aa)	Prevalence*	Fragment	Sequence (aa)†	Similarity‡
NYETETTSVIPAARL	27%	M 1	<u>T</u> /ENEFTSEIPSR	57%
FKAFILDGDNLFPAKV	20%	C 1	SFVLDADNLIPK	75%
		M 2	VLDADNLIPK	94%
AISSVENIEGNG	9%	C 2	<u>TSA</u> ENLEGNGGPGTIK	80%
		M 3	QAEI <u>LE</u> GGGPGTIK	80%
FKYNYSVIEGGPIGDTLE	16%	C 4	S/IEGGPL	100%
		M 4	<u>YTL</u> IEGDALDT/EK	67%
EVKAEQVKASKE	3.6%	C 5	S/NEEE/KA	63%

aa, Amino acid.

*Prevalence of the epitope-reactive T cells in Bet v 1-specific TCLs from 57 different patients with birch pollen allergy.¹⁹

†Amino acids matching Bet v 1 epitopes are underlined.

‡Identical amino acids are shown in bold, and similar amino acids are shown in italics.

TABLE III. Proliferation of Bet v 1-specific TCCs in response to the Bet v 1 peptide and to major allergens after gastrointestinal digestion in apple and hazelnut

TCCs	Bet v 1 epitope (aa)	SI*	Digested allergen	SI	Fragment (aa)
1	<u>NYETETTSVIPA</u>	112	Mal d 1	8.1	<u>T</u> /ENEFTSEIPP
2	FKAFIL <u>DGDNLFPAKV</u>	12.2	Cor a 1	1.4	SFVLDADNLIPK
			Mal d 1	13.4	VLDADNLIPK
3	FKYNYSVIEGG <u>PI</u>	12.9	Cor a 1	3.3	S/IEGGPL

aa, Amino acid; SI, stimulation index.

*Background levels varied from 130 to 3570 cpm. The amino acids matching the Bet v 1 epitope are underlined, identical amino acids are shown in bold, and similar amino acids are shown in italics.

matrices were excluded. However, this experimental approach is useful for identifying the fragments involved in T-cell activation, which would not be possible if whole foods were used. PBMCs from individuals with birch pollen allergy clearly reacted with the intact allergens and, to a lesser extent, with the digested proteins (Fig 2). Referring to our previous studies on the T-cell response to Bet v 1-homologous food allergens, proliferation induced by these proteins can mostly be attributed to activation of cross-reactive but originally Bet v 1-specific T cells.^{15,16} Obviously this cellular cross-reactivity is partly retained after gastrointestinal digestion. In contrast, nonallergic individuals barely reacted with intact allergens but responded to their digestion products. Hence T cells specific for Bet v 1-related food allergens in nonallergic individuals seem to recognize peptides created by means of gastrointestinal digestion, which differ from those created by means of antigen processing of intact allergens. This observation might indicate that these dietary allergens mainly contact the human immune system as degraded and not as complete proteins. The slightly higher response to digested food allergens in nonallergic subjects compared with that seen in allergic individuals might reflect the presumably higher consumption of these food allergens by healthy persons. Allergic patients supposedly avoid these foods because of their hypersensitivity reactions.

To demonstrate that digested Mal d 1, Api g 1, and Cor a 1.04 activate pollen-specific T cells, we used Bet v 1-specific TCLs and TCCs. The major celery allergen induced proliferation in 86% of the tested Bet v 1-specific TCLs but completely lost its cross-reactive potential after incubation with pepsin and trypsin (Table I). This finding

might be explained by the enzymatic destruction of the dominant T-cell epitope Api g 1₁₀₉₋₁₂₆, which was found to be particularly relevant for cellular cross-reactivity with Bet v 1 (Fig 4).¹⁶ Mal d 1 and Cor a 1.04 after gastrointestinal digestion induced proliferation in 31% and 19% of the Bet v 1-specific TCLs, respectively (Table II), and responding TCLs synthesized the same cytokine patterns compared with entire food allergens and Bet v 1 (data not shown). This retained cellular cross-reactivity could be attributed to different fragments derived from the food allergens that match relevant T-cell epitopes of Bet v 1 (Tables II and III). Our results provide a biochemical and immunologic mechanism underlying the clinical observation that ingestion of birch pollen-related foods can trigger the worsening of atopic eczema in patients with cutaneous T-cell responses to Bet v 1.^{20,34} After passing through the stomach and duodenum, fragments of pollen-related dietary allergens activate Bet v 1-specific T cells to upregulate homing receptors, move to the skin, and induce effector reactions.²⁰ We even speculate that the late reactions in the skin could be elicited independently of an IgE-mediated mechanism, similar to late-phase reactions in the lung that occurred in patients with cat-induced asthma after subcutaneous administration of peptides from the major cat allergen Fel d 1.³⁵ In accordance with the Fel d 1 peptides, none of the fragments of Bet v 1-related food allergens after gastrointestinal digestion were able to bind IgE (Fig 1) or to induce basophil degranulation (Fig 2).

In summary, exposure of PR-10-like proteins in apple, celery, and hazelnut to gastrointestinal proteases does not completely abolish their ability to cross-react with Bet v 1-specific T cells. Our observations emphasize the role of

birch pollen-related food as relevant stimuli for pollen-specific T cells.

We thank Dr Jonas Lidholm, Pharmacia Diagnostics, Uppsala, Sweden, for providing ImmunoCAPs of rMal d 1, rCor a 1.04, and rApi g 1.

REFERENCES

- Dreborg S, Foucard T. Allergy to apple, carrot and potato in children with birch pollen allergy. *Allergy* 1983;38:167-72.
- Wuthrich B, Stager J, Johansson SG. Celery allergy associated with birch and mugwort pollinosis. *Allergy* 1990;45:566-71.
- Ebner C, Hirschehr R, Bauer L, Breiteneder H, Valenta R, Ebner H, et al. Identification of allergens in fruits and vegetables: IgE cross-reactivities with the important birch pollen allergens Bet v 1 and Bet v 2 (birch profilin). *J Allergy Clin Immunol* 1995;95:962-9.
- Bircher AJ, Van Melle G, Haller E, Curty B, Frei PC. IgE to food allergens are highly prevalent in patients allergic to pollens, with and without symptoms of food allergy. *Clin Exp Allergy* 1994;24:367-74.
- 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.
- Breiteneder H, Radauer C. A classification of plant food allergens. *J Allergy Clin Immunol* 2004;113:821-30.
- 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.
- Breiteneder H, Hoffmann-Sommergruber K, O'Riordan G, Susani M, Ahorn H, Ebner C, et al. Molecular characterization of Api g 1, the major allergen of celery (*Apium graveolens*), and its immunological and structural relationships to a group of 17-kDa tree pollen allergens. *Eur J Biochem* 1995;233:484-9.
- Luttkopf D, Muller U, Skov PS, Ballmer-Weber BK, Wuthrich B, Skamstrup Hansen K, et al. Comparison of four variants of a major allergen in hazelnut (*Corylus avellana*) Cor a 1.04 with the major hazel pollen allergen Cor a 1.01. *Mol Immunol* 2002;38:515-25.
- Mittag D, Akkerdaas J, Ballmer-Weber BK, Vogel L, Wensing M, Becker WM, et al. Ara h 8, a Bet v 1-homologous allergen from peanut, is a major allergen in patients with combined birch pollen and peanut allergy. *J Allergy Clin Immunol* 2004;114:1410-7.
- Mittag D, Vieths S, Vogel L, Becker WM, Rihs HP, Helbling A, et al. Soybean allergy in patients allergic to birch pollen: clinical investigation and molecular characterization of allergens. *J Allergy Clin Immunol* 2004;113:148-54.
- Amlot PL, Kemeny DM, Zachary C, Parkes P, Lessof MH. Oral allergy syndrome (OAS): symptoms of IgE-mediated hypersensitivity to foods. *Clin Allergy* 1987;17:33-42.
- Ballmer-Weber BK, Vieths S, Luttkopf D, Heuschmann P, Wuthrich B. Celery allergy confirmed by double-blind, placebo-controlled food challenge: a clinical study in 32 subjects with a history of adverse reactions to celery root. *J Allergy Clin Immunol* 2000;106:373-8.
- Kleine-Tebbe J, Vogel L, Crowell DN, Haustein UF, Vieths S. Severe oral allergy syndrome and anaphylactic reactions caused by a Bet v 1-related PR-10 protein in soybean, SAM22. *J Allergy Clin Immunol* 2002;110:797-804.
- 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.
- 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.
- Bohle B, Radakovics A, Luttkopf D, Jahn-Schmid B, Vieths S, Ebner C. Characterisation of the T cell response to the major allergen in hazelnuts, Cor a 1.04: evidence for a T cell epitope not cross-reactive with homologous pollen allergens. *Clin Exp Allergy* 2005;35:1392-9.
- Ebner C, Schenk S, Szepfalusi Z, Hoffmann K, Ferreira F, Willheim M, et al. Multiple T cell specificities for Bet v 1, the major birch pollen allergen, within single individuals. Studies using specific T cell clones and overlapping peptides. *Eur J Immunol* 1993;23:1523-7.
- 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.
- Reekers R, Busche M, Wittmann M, Kapp A, Werfel T. Birch pollen-related foods trigger atopic dermatitis in patients with specific cutaneous T-cell responses to birch pollen antigens. *J Allergy Clin Immunol* 1999;104:466-72.
- Werfel T, Reekers R, Busche M, Schmidt P, Constien A, Wittmann M, et al. Evidence for a birch pollen-specific cutaneous T-cell response in food-responsive atopic dermatitis. *Int Arch Allergy Immunol* 1999;118:230-1.
- Jensen-Jarolim E, Wiedermann U, Ganglbauer E, Zurcher A, Stadler BM, Boltz-Nitulescu G, et al. Allergen mimotopes in food enhance type I allergic reactions in mice. *FASEB J* 1999;13:1586-92.
- Vieths S, Reindl J, Müller U, Hoffmann A, Haustein D. Digestibility of peanut and hazelnut allergens investigated by a simple in vitro procedure. *Eur Food Res Technol* 1999;209:379-88.
- Spangfort MD, Mirza O, Ipsen H, Van Neerven RJ, Gajhede M, Larsen JN. Dominating IgE-binding epitope of Bet v 1, the major allergen of birch pollen, characterized by X-ray crystallography and site-directed mutagenesis. *J Immunol* 2003;171:3084-90.
- Neudecker P, Lehmann K, Nerkamp J, Haase T, Wangorsch A, Fotisch K, et al. Mutational epitope analysis of Pru av 1 and Api g 1, the major allergens of cherry (*Prunus avium*) and celery (*Apium graveolens*): correlating IgE reactivity with three-dimensional structure. *Biochem J* 2003;376:97-107.
- Wiche R, Gubesch M, König H, Fotisch K, Hoffmann A, Wangorsch A, et al. Molecular basis of pollen-related food allergy: identification of a second cross-reactive IgE epitope on Pru av 1, the major cherry (*Prunus avium*) allergen. *Biochem J* 2005;385:319-27.
- Mittag D, Batori V, Neudecker P, Wiche R, Esben P, Ballmer-Weber B, et al. A novel approach for investigation of specific and cross-reactive IgE epitopes on Bet v 1 and homologous food allergen in individuals patients. *Mol Immunol* 2006;43:268-78.
- Ebner C, Schenk S, Najafian N, Siemann U, Steiner R, Fischer GW, et al. Nonallergic individuals recognize the same T cell epitopes of Bet v 1, the major birch pollen allergen, as atopic patients. *J Immunol* 1995;154:1932-40.
- Kopper RA, Odum NJ, Sen M, Helm RM, Steve Stanley J, Wesley Burks A. Peanut protein allergens: gastric digestion is carried out exclusively by pepsin. *J Allergy Clin Immunol* 2004;114:614-8.
- Sen M, Kopper R, Pons L, Abraham EC, Burks AW, Bannon GA. Protein structure plays a critical role in peanut allergen stability and may determine immunodominant IgE-binding epitopes. *J Immunol* 2002;169:882-7.
- Vogel L, Luttkopf D, Hatahet L, Haustein D, Vieths S. Development of a functional in vitro assay as a novel tool for the standardization of allergen extracts in the human system. *Allergy* 2005;60:1021-8.
- Jahn-Schmid B, Fischer GF, Bohle B, Fae I, Gadermaier G, Dedec A, et al. Antigen presentation of the immunodominant T-cell epitope of the major mugwort pollen allergen, Art v 1, is associated with the expression of HLA-DRB1*01. *J Allergy Clin Immunol* 2005;115:399-404.
- Scholl I, Untersmayr E, Bakos N, Roth-Walter F, Gleiss A, Boltz-Nitulescu G, et al. Antiulcer drugs promote oral sensitization and hypersensitivity to hazelnut allergens in BALB/c mice and humans. *Am J Clin Nutr* 2005;81:154-60.
- Breuer K, Wulf A, Constien A, Tetau D, Kapp A, Werfel T. Birch pollen-related food as a provocation factor of allergic symptoms in children with atopic eczema/dermatitis syndrome. *Allergy* 2004;59:988-94.
- Haselden BM, Kay AB, Larche M. Immunoglobulin E-independent major histocompatibility complex-restricted T cell peptide epitope-induced late asthmatic reactions. *J Exp Med* 1999;189:1885-94.