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

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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]

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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/μg 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 μM) was incubated at 37°C with 10 μM pepsin in 0.1 M HCl (pH 1.0) and 0.1 μM trypsin in 76 mM $NaHCO_3$ (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 μg 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 μg 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 FcεRI (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 CO_2 (5%) atmosphere. Thereafter, the cell layer was washed twice with Tyrode buffer (130 mM NaCl, 5 mM KCl, 1.4 mM $CaCl_2$, 1 mM $MgCl_2$, 5.6 mM glucose, 10 mM HEPES, and 0.1% BSA, pH 7.4), and 100 μL per well of a serial dilution of digested and nondigested allergens in Tyrode buffer containing 50% D_2O 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 μL) 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 μL 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 μg/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 μCi 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.