

Allergy caused by ingestion of zucchini (*Cucurbita pepo*): Characterization of allergens and cross-reactivity to pollen and other foods

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Background: Allergy to zucchini (*Cucurbita pepo*), a member of the Cucurbitaceae family, has not previously been reported. We examined 4 patients complaining of allergic symptoms, such as oral allergy syndrome, nausea, diarrhea, or pruritus, after the intake of zucchini.

Objective: After the confirmation of food allergy, we wanted to characterize zucchini allergens and examine possible cross-reactions to pollen and food.

Methods: The patients underwent skin prick and prick-to-prick-testing with different allergens, including zucchini, latex, and birch, ragweed, and grass pollen. Moreover a double-blind, placebo-controlled, food challenge was performed to confirm food allergy. Total and specific serum IgE levels were determined by using CAP-FEIA and the enzyme allergosorbent test method (EAST), respectively. Proteins from zucchini reacting with patient IgE were detected by means of immunoblotting. To characterize cross-reacting IgE antibodies, immunoblot- and EAST-inhibition assays were carried out.

Results: All patients in this study had positive reactions to zucchini both in prick-to-prick tests and double-blind, placebo-controlled, food challenges. Specific serum IgE levels to zucchini were found in all cases. In blot- and EAST-inhibition assays IgE from two patients revealed binding to zucchini profilin at about 15 kd. Furthermore, in two cases, including one of the profilin-positive patients, IgE directed against cross-reacting carbohydrate determinants was detected. For one patient, no cross-reacting IgE could be found, but IgE from this patient reacted strongly with a zucchini protein at 17 kd.

Conclusions: We report the first 4 cases of food allergy to zucchini. Zucchini allergens can cause systemic reactions and are at least partially heat stable. We suggest that allergy to zucchini can occur as a result of primary sensitization to zucchini, as well as to cross-reactions to the panallergen profilin and cross-reacting carbohydrate determinants. (J Allergy Clin Immunol 2000;106:379-85.)

Key words: Zucchini; allergy; cross-reactivity; double-blind, placebo-controlled, food challenge; cross-reacting carbohydrate determinant; profilin

Symptoms of food allergy in most cases are of an immediate IgE-mediated type and present themselves as an oral allergy syndrome (local reaction)¹ or gastrointestinal symptoms, rhinoconjunctivitis, asthma, angioedema, or anaphylactic shock (systemic reactions).² We distinguish between cross-reactive allergies caused by primary sensitization to pollen (pollen-associated food allergy), latex, and house dust mites; cross-reactions between related foods (taxonomic reaction); and primary sensitization to a defined food. Often so-called panallergens, such as profilin³⁻⁶ or cross-reactive carbohydrate determinants (CCDs),⁷ are involved in cross-reactivity. Until now, allergic reactions to ingestion of seeds and vegetables of the Cucurbitaceae family have rarely been reported.⁸⁻¹¹ Here we report the first 4 cases of allergy to ingestion of zucchini (*Cucurbita pepo*), including double-blind, placebo-controlled, food challenges (DBPCFCs); detection of specific IgE to zucchini; and characterization of allergens and their cross-reactivity to pollen and food allergens.

METHODS

Skin prick tests, prick-to-prick tests, and DBPCFCs

The patients underwent skin prick tests (SPTs) with routine allergens (*Dermatophagoides pteronyssinus*; *Dermatophagoides farinae*; goose feathers; dog, cat, and horse dander; *Alternaria*, *Aspergillus*, *Cladosporium*, and *Penicillium* species; *Candida albicans*; grass, mugwort, birch, alder, ash, hazel, and rye pollen; latex; and cow's milk [ALK, Hørsholm, Denmark]). Testing and interpretation were performed according to the position paper of the European Academy of Allergy and Clinical Immunology.¹² SPTs were also performed for ragweed, as well as for pollen-related foods (Stallergènes, Cédex, France). Zucchini, celery, carrot, potato, and, when available, cucumber and pumpkin were tested by using prick-to-prick tests (PPTs).

DBPCFCs were conducted with raw zucchini with cooked broccoli used as a placebo and to disguise the taste of zucchini.¹³ The placebo drink contained 50 g of cooked broccoli in a total of 143.5 g of drink (broccoli broth, cream, yogurt, water, and salt). The zucchini drink contained 20 g of raw zucchini (approximately 0.7 g per teaspoon) and 30 g of cooked broccoli in a total of 143.5 g of drink (same ingredients as above). No intake of antihistamines, tranquilizers, or antidepressant agents were allowed 5 days before testing. Four control persons were tested in the procedure and could not distinguish between zucchini and placebo.

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Abbreviations used

CCD:	Cross-reacting carbohydrate determinant
DBPCFC:	Double-blind, placebo-controlled, food challenge
EAST:	Enzyme allergosorbent test
PPT:	Prick-to-prick test
SPT:	Skin prick test

For DBPCFCs, we used a spit-and-swallow protocol if necessary, which was similar to a method described elsewhere.¹³ In the spit protocol the patients were advised to keep the drink in the oral cavity for 1 minute and spit it into a basin thereafter. Reactions were monitored for 15 minutes or longer until they subsided. After inspecting the mouth for erythema or swelling, the next dose was given. Between switching the double-blind-labeled drinks, the patient was instructed to rinse the oral cavity with water.

First, the patients were challenged with one teaspoon of placebo and continued with the first drink (one teaspoon of zucchini or placebo drink) if no reaction occurred. The dose was doubled up to 8 teaspoons at intervals of 15 minutes. Then the drinks were switched, and the second drink was given in the same setting. If reactions were mild and not visible to the physician, we tried to reproduce them twice by switching drinks again. If no reaction occurred at all or no difference could be made between the drinks, the spit protocol was continued up to 8 teaspoons. Patients without reactions in the spit protocol underwent the swallow protocol, only allowing one drink to be taken a day and starting with one tablespoon and doubling the dose every 15 minutes up to a total of 143.5 g of the drink. The second drink was consumed in the swallow protocol on another day. If reactions were observed clinically (eg, erythema, urticaria, asthma, and edema) or were very bothering for the patient (nausea), we allowed the protocol to be shortened.

After the DBPCFC, a PPT with both drinks was performed to exclude a sensitization to components of the placebo drink.

Extracts and allergens

Zucchini, melon, and skim milk extract were prepared from fresh material by using a low-temperature extraction procedure, as described elsewhere.^{14,15} Latex and birch pollen extracts were purchased from Allergopharma (Reinbek, Germany), ragweed extract was purchased from MAST Diagnostica (Reinfeld, Germany), and grass pollen extract was a gift from Borstel Research Center (Dr A. Petersen, Borstel, Germany). Recombinant (r)Bet v 1 and rBet v 2 were obtained from Biomay (Linz, Austria).

Immunochemicals

Rabbit antisera against celery profilin and ragweed pollen profilin (kindly supplied by Dr P. Deviller, Lyon, France) were used to detect antigenic profilin in zucchini extract. Serum from nonimmunized rabbits (Riedel de Haën, Seelze, Germany) was applied as a negative control. Goat polyclonal, affinity-purified, biotinylated, anti-rabbit IgG (g α -biotin) was purchased from DAKO (Copenhagen, Denmark), and streptavidin-alkaline phosphatase conjugate was purchased from Medac (Hamburg, Germany). IgE antibodies were detected with mouse anti-human IgE conjugated with alkaline phosphatase from PharMingen (San Diego, Calif).

Total and specific IgE and EAST inhibition

Total serum IgE, Sx1-multi-RAST (*D pteronyssinus*, cat and dog dander, *Cladosporium* species, and grass, mugwort, birch, and rye pollen), and specific serum IgE to rBet v 1 and rBet v 2 was measured with the CAP-FEIA system (Pharmacia & Upjohn, Uppsala, Swe-

den). Specific serum IgE against zucchini was determined by a commercially available enzyme allergosorbent test (EAST; Allergopharma, Reinbek, Germany). EAST classes correspond to RAST classes (Pharmacia, Freiburg, Germany). For EAST and EAST-inhibition experiments, allergen extracts were coupled to cyanogen bromide-activated paper disks (3 μ g/disk), as previously described.¹⁵ The EAST was performed according to the manufacturer's instructions. For EAST inhibitions, the sera of patients 148, 225, and 291 were diluted 1:3 with incubation buffer (0.05 mol/L TRIS-hydroxymethyl-aminomethane, 0.15 mol/L NaCl, 1.0% Tween-20, and 0.3% BSA [pH 7.4]), and for each disk, 50 μ L of serum dilution was incubated with 50 μ L of inhibitor solution (zucchini, birch pollen, grass pollen, mugwort, latex, melon, and skim milk extract: 500, 250, or 50 μ g/mL; rBet v 1, rBet v 2, bromelain glycopeptide, fibrin glycopeptide [see below]: 100, 10, or 1 μ g/mL diluted in incubation buffer).

Immunoblotting and immunoblot-inhibition experiments

Electrophoresis, immunoblotting, and detection of allergens were carried out as described elsewhere.¹⁶⁻¹⁸ Rabbit sera were diluted 1:2500.

For blot inhibitions, sera were diluted 1:6.7 in incubation buffer and preincubated with 100 μ g of protein from plant extracts or skim milk or 10 μ g of rBet v 2 or glycopeptides (see below). Incubations of the blot strips and detection were performed as above.

N-glycans

To examine whether CCDs play a role in zucchini allergy, we used an N-linked glycopeptide with the glycan structure Man α 1-6(Xyl β 1-2)Man β 1-4GlcNAc β 1-4(Fuc α 1-3)GlcNAc and just 2 to 4 amino acid residues for our inhibition experiments. We used this particular glycan because the main IgE-binding epitopes in cross-reactivity to glycoproteins are α 1,3-linked fucose and β 1,2-linked xylose.¹⁹ Such glycoproteins may play a role in food allergy.²⁰ The preparation of the glycopeptide was performed as previously described.^{20,21} To check the purity of the sample, it was analyzed by means of mass spectrometry.

As a negative control, a glycopeptide with the common pentasaccharide core Man α 1-6(Man α 1-3)Man β 1-4GlcNAc β 1-4GlcNAc was used. It was prepared from bovine fibrin as previously described.¹⁹ Both glycopeptide from bromelain and glycopeptide from fibrin were produced and supplied by Professor Friedrich Altmann (Vienna, Austria).

To verify the lack of protein contamination of the glycopeptides, SDS-PAGE under reducing conditions with final silver staining was carried out with BSA as a control. No bands caused by proteins could be detected with 10 μ g of the glycopeptides, whereas with BSA, the expected main band at about 67 kd and many bands caused by contaminations of the commercial product were detected (not shown).

RESULTS

Patients

The personal history of all patients and performed skin tests are summarized in Tables I and II. The results of IgE determinations are given in Table III.

Case A (patient 148). The 20-year-old female patient started 8 years ago to notice an itching of the mouth and pharynx minutes after eating raw and cooked zucchini. After a recent ingestion, she also had stomachache and reacted with urticaria and angioedema. PPT results for zucchini were strongly positive (+++). Also, specific IgE to zucchini was positive (class 3), whereas specific IgE to birch, rBet v 1, and rBet v 2 was negative.

TABLE I. Personal history: Atopy, food allergy, and skin tests

Patient No.	Age (y)	Sex	Rhinoconjunctivitis	Asthma	Food allergy	Positive skin test results
148	20	F	May-June in recent years	Intrinsic	OAS: zu, to, wm, pu for 8-10 y	SPT: gr (+++), rp (+++), rw (+); PPT: zu (+++), ca (+), po (+); zucchini drink (+), placebo drink (negative)
225	47	M	March-September for >30 y	Mixed	zu, cru, ca, ce, cu, av, nuts in recent years	SPT: gr (++), rp (++), as (+); Dpt (+), tu (+), sh (+), lo (+); PPT: zu (+), ce (+++), ca (+++), po (+++), to (+), ma (+), bp (+), zucchini drink (+), placebo drink (negative); IC: bi (+), al (+), mu (+), hp (+)
291	26	M	April-July for >15 years	Animal hair	OAS: ap, pe, nuts; urticaria and asthma: zu, wm, cu, to in recent years	SPT: bi (+++); gr (++); rp (+); al (+++), as (+++), hp (+++), Dpt (+++), Dfa (+++), la (+), ha (+), to (+); PPT: zu (+++), cu (+++), wm (+++), ap (+++), ce (+++), ca (+++), po (+++), zucchini drink (+), placebo drink (negative); IC: mu (+)
652	17	M	March-August for 3 y	None	zu, bp, ap, cu, nuts for 1-2 y	SPT: bi (++), gr (+++), rp (+++), al (++), as (++), hp (++), Dpt (+), Dfa (+), fi (+); PPT: zu (++), cu (++), bp (++), ap (+), ce (+), ca (+), po (++), zucchini drink (+), placebo drink (negative)

OAS, Oral allergy syndrome; IC, intracutaneous test; al, alder pollen; ap, apple; as, ash pollen; av, avocado; bi, birch pollen; bp, bell peppers; ca, carrot; ce, celery; cu, cucumber; Dfa, *Dermatophagoides farinae*; Dpt, *Dermatophagoides pteronyssinus*; fi, *Ficus benjamina*; gr, grass pollen; ha, hazelnut; hp, hazelnut pollen; lo, lobster; ma, mango; mu, mugwort pollen; po, raw potato; pu, pumpkin; rp, rye pollen; rw, ragweed pollen; sh, shrimp; to, tomato; tu, tuna; wm, watermelon; zu, zucchini.

The DBPCFC with zucchini provoked an oral allergy syndrome 5 minutes after contact with 0.7 g of zucchini (spit protocol). The reactions were reproduced twice (0.7 and 2.8 g of zucchini). No reaction occurred to the placebo.

Case B (patient 225). After eating raw zucchini, a 47-year-old male patient felt sick and dizzy and had to vomit. PPT results with zucchini were positive (+). Determination of specific IgE to zucchini resulted in an EAST class of 2. In contrast to birch, no specific IgE to rBet v 1 and rBet v 2 could be detected.

DBPCFC results were twice positive for zucchini. The patient reacted with nausea 10 minutes after a dose of 0.7 g of zucchini in the spit protocol. No nausea was felt after the placebo protocol.

Case C (patient 291). A 26-year-old male patient felt pruritus in both his hands and inguina after eating raw and cooked zucchini. In addition, he had urticaria and bronchial asthma. PPT results with zucchini, cucumber, and watermelon were strongly positive. Specific IgE against zucchini (class 2), birch, rBet v 1, and rBet v 2 could be detected.

In the DBPCFC the patient reacted with nausea, feeling sick to his stomach 5 minutes after 6 g of zucchini in the swallow protocol (about 35 minutes after starting with 1.5 g and after a total amount of about 10.5 g of zucchini in the swallow protocol). The swallow protocol was repeated on another day, leading to pruritus 14 minutes after 3 g of zucchini (30 minutes after starting with 1.5 g), a swollen lip (60 minutes), and persistent diarrhea over 2 days within hours after the beginning of the swallow protocol. No symptoms occurred in the spit-and-swallow (twice) protocol for the placebo.

TABLE II. Personal history: Symptoms to ingestion of zucchini and to DBPCFCs

Patient No.	Zucchini	Symptoms to ingestion	Symptoms to DBPCFCs
148	Raw and cooked	OAS, urticaria, angioedema	OAS
225	Raw	Nausea, vomitus	Nausea
291	Raw and cooked	Palmoplantar pruritus, urticaria	Persistent diarrhea, nausea, pruritus
652	Raw and cooked	OAS, stridor	Swelling of the throat, nausea

OAS, Oral allergy syndrome.

Case D (patient 652). A 17-year-old male patient reacted with stridor within 5 to 10 minutes after eating either raw or cooked zucchini. PPT results with zucchini were strongly positive (++). Specific IgE to zucchini was weak, resulting in an EAST class of 1. Also positive were determinations for birch, rBet v 1, and rBet v 2.

The result of a DBPCFC was positive for zucchini. The patient reacted with nausea, itching, and swelling inside the throat after 0.7 g of zucchini in the spit protocol and again after 2.8 g after switching drinks in between. However, the placebo also led to mild itching of the oral cavity. In a control test PPT results were positive (wheal of 3-5 mm) for the zucchini drink and negative for the placebo. Specific IgE determined by using CAP-FEIA was negative for milk proteins (<0.35 kU/L) and weak for broccoli (0.41 kU/L).

TABLE III. Results of total and specific IgE determinations

Patient No.	Total IgE (kU/L)	Sx1* (kU/L)	EAST, [†] zucchini (kU/L)	CAP (kU/L) [†]						
				Melon	Ragweed	Celery	Latex	Birch	rBet v 1	rBet v 2
148	162	41.4 (4)	3.65 (3)	<0.35	<0.35	<0.35	ND	<0.35	<0.35	<0.35
225	125	35.3 (4)	1.16 (2)	2.25 (2)	2.51 (2)	2.25 (2)	2.02 (2)	2.46 (2)	<0.35	<0.35
291	207	80.5 (5)	2.08 (2)	1.03 (2)	ND	ND	0.85 (2)	32.2 (4)	31.9 (4)	8.1 (3)
652	156	53.4 (5)	0.38 (1)	<0.35	1.49 (2)	0.76 (2)	0.91 (2)	1.86 (2)	0.36 (1)	0.85 (2)

ND, Not determined.

*Sx1 is an IgE screening test for a mix of common inhalant allergens.

[†]EAST and CAP classes, respectively, are shown in parentheses.

EAST inhibition

For characterization of the zucchini cross-reactivity pattern and confirmation of the specificity of the assay, dose-dependent EAST inhibitions were carried out with various plant extracts, the recombinant allergens Bet v 1 and Bet v 2, and the bromelain and fibrin glycopeptides as inhibitors. Skim milk was used as a negative control. EAST inhibitions were carried out with the 3 sera with EAST classes of 2 or greater.

The results of the inhibition tests are presented in Fig 1, A and B. Patient sera 225 and 291 show a very similar inhibition pattern to the examined plant extracts (Fig 1, A). IgE antibodies from these patients were strongly inhibited by all extracts except skim milk. The opposite situation was found for patient serum 148. Only zucchini extract strongly inhibited the IgE binding to the allergen disks, which also demonstrates the specificity of the assay (Fig 1, A). With watermelon extract, an inhibition of approximately 45% was obtained.

Although no inhibition occurred with rBet v 1 as the inhibitor, IgE from serum 291 was inhibited by rBet v 2. Sera 148 and 225 showed no reaction to this allergen (Fig 1, B). Besides this, serum 291 was inhibited by the bromelain glycopeptide. The sum of the maximal inhibition of rBet v 2 and the glycopeptide adds up to about 100% in this serum. In serum 225 a high amount of IgE antibodies directed to CCDs was detected by using the inhibition assay. The bromelain glycopeptide produced a maximal inhibition of about 60%, whereas the fibrin glycopeptide, as in all sera, produced only weak inhibitions.

Immunoblot and immunoblot inhibition

To confirm the specificity of the detection and to obtain further information of proteins involved in cross-reactivity, immunoblot-inhibition tests were carried out. The results from all 4 sera included in this test are presented in Fig 2. Serum 148 strongly reacted with 3 proteins at about 16 to 17 kd (lane 1). Besides this, weaker bands ranging from 14.4 to 67 kd were detected. Similar to the EAST inhibition, an almost complete inhibition was obtained only with zucchini extract for this serum. Also, the blot inhibition with sera 225 and 291 corresponded to the results from the EAST inhibition. Multiple bands above 29 kd were recognized from IgE of these sera. Most of these bands were inhibited by all plant

extracts (lanes 2, 5, and 7) and the bromelain glycopeptide (lane 3) but not with the control inhibitors skim milk extract (lane 8) and fibrin glycopeptide (lane 4). This points to a high cross-reactivity caused by IgE against carbohydrate determinants. Serum 652 showed a totally different binding pattern. Only two bands at approximately 15 kd were specifically detected. A complete inhibition of these bands was obtained with rBet v 2 (lane 6) and all plant extracts (lanes 2, 5, and 7). The same two bands were also recognized with serum 291, but the staining intensity was weak when compared with high molecular weight bands, resulting in these bands being invisible in Fig 2. Hence the blot inhibition method was optimized to solve this problem. The low molecular end of blotted nitrocellulose strips were cut off and used for blot inhibition so that the final staining could be carried out for a longer period. As seen in Fig 3, under these conditions the bands at 15 kd could be visualized with serum 291 (lane 1). They were successfully inhibited by zucchini and birch extract, as well as by rBet v 2 (lanes 2-4). In another experiment two bands at the same mass range were detected with rabbit sera against celery profilin or ragweed pollen profilin, respectively (not shown). The sensitization of patients 291 and 652 to profilin was confirmed by a positive CAP result to Bet v 2 (Table III).

DISCUSSION

This is the first report on confirmed food allergy to zucchini (*Cucurbita pepo*). Thus far, few investigations about reactions to other members of the Cucurbitaceae family (cucumber and watermelon) and cross-reactivity between them have been made. Ingestive food allergy to pumpkinseed has been reported in individuals who were sensitized through an inhalative pathway by using pumpkinseed flour as bait in fishing.⁹ Allergen characterization showed a cross-reactive band between pumpkinseed and birch pollen at 14 kd. Cross-reactivity has also been documented between watermelon and ragweed¹⁰ and between melon and grass pollen.¹¹

In our study sensitization to zucchini could be demonstrated by means of positive PPT results in all 4 patients. A DBPCFC is required for the clinical diagnosis of food intolerance¹³; a positive DBPCFC was demonstrable in 3 patients with positive PPT results and specific IgE to zucchini. The symptoms seen in DBPCFCs were similar to

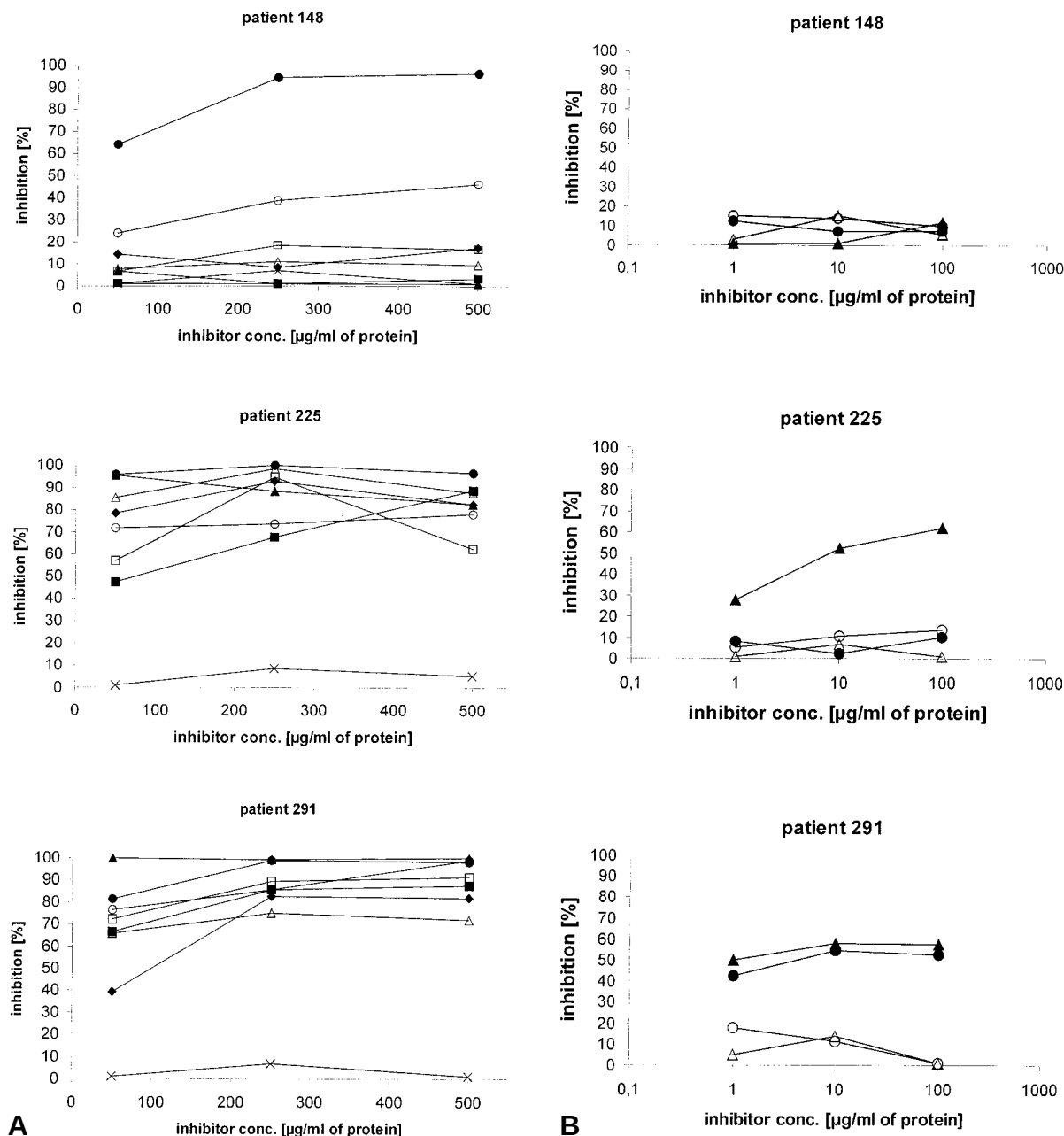


FIG 1. A, Dose-related EAST inhibitions with sera from patients 148, 225, and 291: inhibition with plant extracts. Inhibitors are as follows: filled circles, zucchini; open circles, watermelon; filled squares, birch; open squares, ragweed; filled triangles, grass; open triangles, mugwort; filled diamonds, latex; and x, skim milk. **B,** Dose-related EAST inhibitions with sera from patients 148, 225, and 291: inhibition with recombinant allergens and glycopeptides. Inhibitors are as follows: filled circles, rBet v 2; open circles, rBet v 1; filled triangles, bromelain glycopeptide; and open triangles, fibrin glycopeptide.

those previously reported by the patients. One patient with a positive PPT result and elevated specific IgE had symptoms to the zucchini drink but also to the placebo. However, only mild symptoms were elicited by the placebo. Despite a negative PPT response to the placebo drink, symptoms could have occurred as a result of weak sensitization to cross-reacting broccoli profilin in this case.

The personal history indicates that the allergens in zucchini are heat stable and can lead to systemic reactions (eg, nausea), as demonstrated in DBPCFCs. Symptoms were noticed at doses as low as 0.7 g of zucchini in the spit protocol. However, DBPCFCs were only conducted with raw zucchini. For one patient serum, no cross-reaction to the examined plant extracts, glycopeptides, or

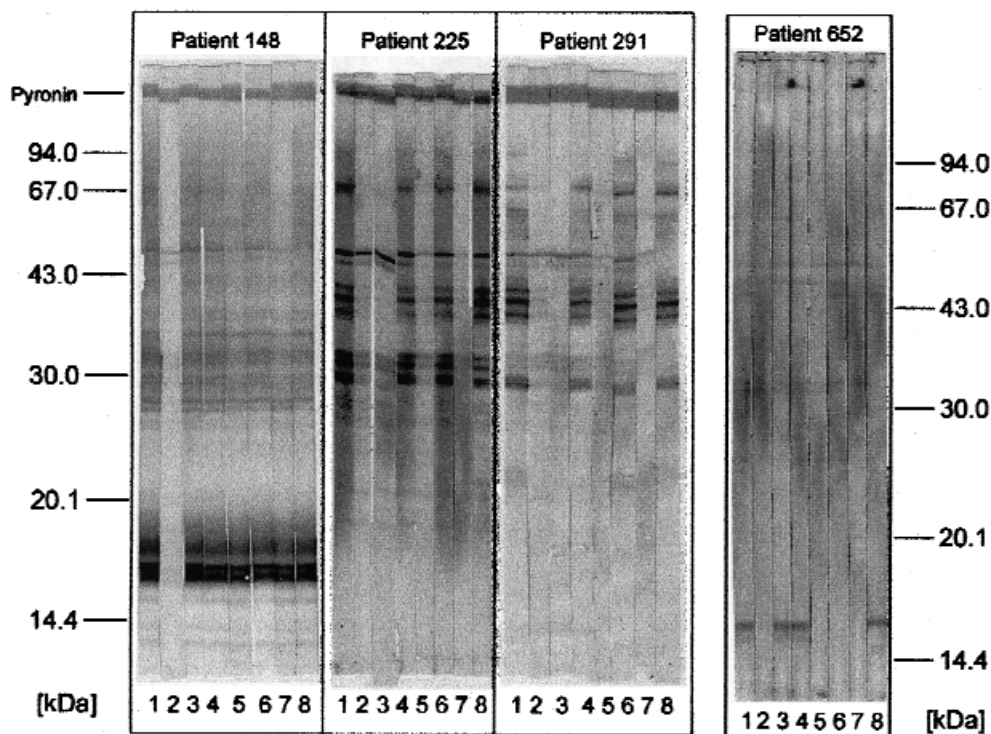


FIG 2. Blot-inhibition experiments with sera from patients 148, 225, 291, and 652. Inhibitors are as follows: *lane 1*, no inhibitor; *lane 2*, 100 μ g of protein from zucchini extract; *lane 3*, 10 μ g of bromelain glycopeptide; *lane 4*, 10 μ g of fibrin glycopeptide; *lane 5*, 100 μ g of protein from birch extract; *lane 6*, 10 μ g of rBet v 2; *lane 7*, 100 μ g of protein from melon extract; and *lane 8*, 100 μ g of protein from skim milk extract.

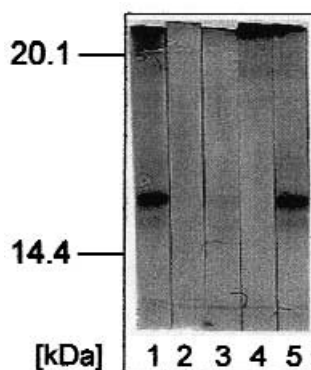


FIG 3. Optimized blot inhibition with serum 291. Inhibitors are as follows: *lane 1*, no inhibitor; *lane 2*, 100 μ g of protein from zucchini extract; *lane 3*, 100 μ g of protein from birch extract; *lane 4*, 10 μ g of rBet v 2; and *lane 5*, 100 μ g of protein from skim milk extract.

recombinant allergens was detected in our EAST-inhibition and blot-inhibition studies, respectively. Only extract from melon, another member of the gourd family, produced a partial inhibition of the zucchini EAST. IgE from this serum showed strong reactivity with proteins with a mass range of approximately 16 to 17 kd. These results suggest the existence of zucchini-specific allergens and the possibility of homologues existing in melon.

Another allergen with a molecular mass of about 15 kd was detected with sera from two patients. It was identified as zucchini profilin because in blot-inhibition experiments the reaction to the double band was inhibited by rBet v 2, and moreover, rabbit sera against celery profilin and ragweed profilin reacted with these bands. The positive CAP result to rBet v 2 verified the sensitization of both patients to birch profilin. These data confirm the importance of profilin as a panallergen in plants.³⁻⁶

In two patient sera a high amount of IgE directed against CCDs was found. With the bromelain glycopeptide (containing α 1,3-linked fucose and β 1,2-linked xylose) as inhibitor, an inhibition up to 60% was reached in both sera in EAST experiments. The possibility of the reaction to protein contaminations of the glycopeptide was excluded by means of mass spectrometry and gel electrophoresis analyses of the carbohydrate moiety isolated from bromelain. In blot inhibition various protein bands from 29 to 96 kd were inhibited by the bromelain glycopeptide, whereas the control glycopeptide from fibrin showed no inhibitory effect in both experiments. In addition, the cross-reactivity to several plant extracts resulting from high degrees of EAST and blot inhibition confirm the reaction to CCDs from IgE of these sera. The reactive glycan structure is not only present in different extracts but also on various proteins of the same plant, which corresponds with the findings of a recently published study about carbohydrate epitopes in celery.²⁰

Because all patients reported here showed clear positive symptoms of food allergy, there is the possibility of CCDs being involved in triggering allergy against zucchini.

A taxonomic cross-reactivity is likely between different vegetables of the Cucurbitaceae family (watermelon, cucumber, pumpkin, and zucchini). Two of the patients tested with PPTs in this respect showed sensitization to cucumber, and one showed sensitization to watermelon. In summary, no major allergen was identified in the protein extract of zucchini. Pollen-related structures, such as profilin and CCDs, as well as proteins that show no relationship to known and important allergens are candidates for explaining the zucchini allergy of the patients. It has to be discussed, at least in one case, whether we are dealing with a primary sensitization to zucchini because one patient did not show any cross-reaction to known panallergens or pollen and had clinical symptoms caused by ingestion of zucchini long before having hay fever. By contrast, the 3 other patients reacting to pollen-related allergens in zucchini had a history of pollen allergy before having sensitivity to zucchini (Table I).

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