
Heterogeneity of banana allergy: characterization of allergens in banana-allergic patients

Martin Grob, MD*; Jürgen Reindl, PhD†; Stephan Vieths, PhD†; Brunello Wüthrich, MD*; and Barbara K. Ballmer-Weber, MD*

Background: Banana is a frequent cause of food allergy, particularly in latex-sensitized patients.

Objective: The aim of the study was to get insights in immunoglobulin (Ig)E antibody responses of patients with a history of allergic reaction to banana but not to latex.

Methods: In four patients who complained about symptoms after banana consumption, skin prick tests (SPTs) with aeroallergens, latex, banana, avocado, and kiwi were performed. Total and specific serum IgE to birch pollen, rBet v 1 and rBet v 2, latex, banana, avocado, and kiwi were determined by the CAP method (Pharmacia Diagnostics, Uppsala, Sweden). Allergens were identified by immunoblotting with banana extract and recombinant banana profilin. Two patients underwent double-blind, placebo-controlled food challenges (DBPCFC) with banana.

Results: All patients showed a positive SPT to banana, and three were IgE-CAP positive ($\geq$ class 2). Two patients were also sensitized (SPT and CAP) to latex, avocado, kiwi, and birch pollen. In the immunoblot these two patients' sera reacted to 32- to 34-kDa proteins, which had already been described as major banana allergens. In both patients banana allergy was confirmed by DBPCFC. The third patient also had a sensitization to avocado, but not to latex or pollen. Immunoblot analysis detected a single band at 70 kDa. The fourth patient was sensitized to birch pollen, rBet v 1 and rBet v 2, but not to latex. Immunoblot analysis in this patient's serum was positive with recombinant banana profilin.

Conclusions: The relevance of banana as a source of food allergy was confirmed in two patients by DBPCFC. In 1 of 2 patients, in whom banana allergy was not a consequence of latex sensitization, a 70-kDa protein was identified as a banana allergen, and in the other patient profilin was detected as a putative cross-reactive allergen.

Ann Allergy Asthma Immunol 2002;89:513-516.

INTRODUCTION

Food allergy-induced reactions can sometimes be life-threatening, and patients with food allergy need to receive an accurate diagnosis to provide sound preventive measures.¹ The double-blind, placebo-controlled food challenge (DBPCFC) is presently the only valid test for diagnosing food allergy with a high degree of certainty.^{2,3}

Allergy to banana is well known, sometimes with severe anaphylactic reactions.^{4,5} Banana is one of the fruits which is highly associated with sensitization to latex and other foods such as avocado, chestnut, and kiwi, called latex-fruit syndrome.⁶⁻⁸ Two proteins at 32 and 34 kDa, possibly representing class I chitinases, have been described as major allergens in banana by Sanchez-Monge et al.⁹ and Mikkola et al.¹⁰ Further, Delbourg et al.¹¹ have been describing two allergens at 33 and 37 kDa as banana allergens in latex-allergic patients. Whether these allergens represent identical compounds as those described by Sanchez and Mikkola remains unclear.

By immunoblotting with banana extract, many other proteins with molecular weights of 40, 52, 58, 88 and 94 kDa could be visualized.¹² There are also reports of banana-allergic patients with no latex allergy or sensitization.⁵ To date, there has been no detailed report of challenge testing for banana allergy under double-blind, placebo-controlled conditions.

The purpose of this study was to get insights in immunoglobulin (Ig)E antibody responses of patients with a history of an allergic reaction to banana fruit but not to latex, and to confirm banana as a food allergen by DBPCFC.

MATERIALS AND METHODS

Patients

Four outpatients of our allergy unit with a history of banana allergy but not of latex allergy were included into the study. All patients provided written informed consent.

Patient 1. A 22-year-old male patient felt itching of the mouth and throat during eating a banana. Thirty minutes later he developed urticaria, angioedema, and a drop in blood pressure. No symptoms occurred after eating avocado or kiwi, but he developed an oral allergy syndrome (OAS) after consuming nuts, mainly peanuts. Further, he suffered from a rhinoconjunctivitis pollinosa from May to June.

Patient 2. The 25-year-old male patient noticed oral itching a few minutes after eating a piece of banana. He also com-

* Allergy Unit, Department of Dermatology, University Hospital, Zurich, Switzerland.

† Department of Allergology, Paul-Ehrlich-Institut, Langen, Germany.

Received for publication November 29, 2001.

Accepted for publication in revised form May 6, 2002.

plained about an OAS to kiwi and dates and had a seasonal rhinoconjunctivitis from May to July.

Patient 3. This 8-year-old girl developed urticaria and conjunctivitis as well as an OAS after eating a banana. She reported no allergic rhinoconjunctivitis and had no history of other food allergies, particularly not to avocado or kiwi.

Patient 4. A 15-year-old female who complained of a rhinoconjunctivitis pollinosa from February to April reacted with itching of the mouth, the throat, and the ears after intake of banana. In addition, after consumption of carrot, kiwi, and tomato she reacted with an OAS too. She had never consumed avocado.

Allergy tests

The patients underwent skin prick tests (SPTs) with extracts from different pollens (grass, mugwort, birch, alder, ash, hazel, and rye pollen) and latex (ALK, Hørsholm, Denmark). SPTs were also performed for banana, avocado, and kiwi (Stallergenes SA, Antony Cedex, France). Testing and interpretation were performed according to the position paper of the European Academy of Allergology and Clinical Immunology.¹³

Total serum IgE and specific serum IgE to birch pollen, rBet v 1 (major allergen of birch), rBet v 2 (profilin), banana, latex, kiwi, and avocado were measured with the CAP FEIA system (Pharmacia Diagnostics, Uppsala, Sweden). Specific serum IgE against recombinant banana profilin was determined by a commercially available enzyme allergosorbent test (EAST; Allergopharma, Reinbek, Germany). EAST classes correspond to radioallergosorbent classes (Pharmacia, Freiburg, Germany).

Extracts and allergens

Banana extract was prepared by a low-temperature method as described recently with minor modifications.¹⁴ Briefly, a food grade enzyme mixture containing a pectinase-hemicellulase-complex (dilution 1:10,000) was added to the extraction buffer, phosphate-buffered saline, to improve the yield of protein, and to decrease the high viscosity of banana protein extract. Cloning, expression, and purification of recombinant banana profilin are described elsewhere.¹⁵

Immunoblotting

Electrophoresis, immunoblotting, and detection of allergens were performed as described elsewhere.^{14,16,17} The amount of banana extract protein applied to the sodium dodecyl sulfate-polyacrylamide gel was 15 µg/cm, in the case of banana profilin 0.5 µg/cm. Patient sera were diluted 1:6.7 in 600 µL of incubation buffer.

DBPCFC

Table 1 shows the ingredients of the active and the placebo meal. The taste of banana was imitated by using a commercial banana flavor (No. 74702-35; Givaudan Dübendorf AG, Dübendorf, Switzerland) and the consistence by blackberry and Nesvital (carob flour; Nestle SA, Vevey, Switzerland). All ingredients were mixed in a blender.

Table 1. Recipe for DBPCFC with Banana

Active meal	Placebo meal
6 g oatmeal	10 g oatmeal
90 g water	100 g water
7 g cocoa powder	10 g cocoa powder
60 g banana mashed	a pinch Nesvital (carob flour)
5 drops banana flavor	16 drops banana flavor
30 g blackberry	40 g blackberry
30 g peppermint syrup	35 g peppermint syrup
10 g lemon juice	5 g lemon juice
2 g gelatin	2 g gelatin

The patients were allowed to have a light breakfast on the day of testing. Treatment with antihistamines was suspended at least 3 days before the challenge. Patients were first challenged with a placebo pudding (to eliminate possible placebo reactors). Thereafter, DBPCFC was performed in a two-step procedure as described previously.¹⁸ First, the patients had to keep pudding 1 in their mouth and spit it out after 1 minute. The amount was doubled every 15 minutes (5, 10, 20, 40 g). After 1 hour the same procedure was repeated with pudding 2. If the patients consistently reported symptoms of an OAS to the active but not to the placebo pudding three times, they were considered to be responders. If the patients did not complain about symptoms during this "spit"-phase, they continued with step two of the DBPCFC, ie, the patients now had to swallow the puddings in increasing amounts (5, 10, 20, 40 g) at intervals of 15 minutes. Between the challenge with puddings 1 and 2 there was a break of at least 24 hours. Again, if the patients consistently reported symptoms to the active pudding three times but never to the placebo pudding, or if they had objective signs of an allergic reaction they were considered to be responders. Ten grams of pudding contained 2.6 g of banana.

RESULTS

All four patients showed a positive SPT to banana. One patient (4) was banana CAP negative, the other three were positive ($\geq$ class 2; Tables 2 and 3). Two of the four patients (1, 2) had a sensitization to latex, avocado, kiwi, and birch pollen. In both patients specific IgE to birch pollen and rBet v 1 but not to rBet v 2 were detected. In banana extract immunoblotting, sera of these two patients reacted strongly with many proteins, including a 32- to 34-kDa band (Fig 1). In both patients banana allergy was confirmed by DBPCFC.

Table 2. Results of SPTs with Banana, Latex, Avocado, and Kiwi

Pat No.	SPT			
	Ba	La	Av	Ki
1	+	+	+	++
2	+++	+	+	+
3	+	—	—	—
4	+	—	—	—

Ba: banana; La: latex; Av: avocado; Ki: kiwi.

Table 3. Results of Total IgE and Specific IgE Determinations to Birch, Birch Allergens, Fruits, and Latex

Pat No	IgE Tot	CAP-class (kU/L)							EAST CAP-class (kU/L)
		Bi	rBet v1	rBet v2	Ba	La	Av	Ki	Ba
1	544	3 (8.85)	3 (5.42)	0 (<0.35)	3 (5.24)	3 (6.60)	4 (18.30)	3 (4.97)	3 (4.76)
2	253	2 (2.30)	1 (0.37)	0 (<0.35)	3 (5.21)	3 (9.17)	3 (5.66)	2 (3.34)	2 (1.15)
3	383	0 (<0.35)	0 (<0.35)	0 (<0.35)	2 (2.08)	0 (<0.35)	2 (0.96)	0 (<0.35)	0 (<0.35)
4	110	4 (23.70)	4 (29.20)	2 (1.14)	0 (<0.35)	0 (<0.35)	0 (<0.35)	0 (<0.35)	0 (<0.35)

Ba: banana; La: latex; Av: avocado; Ki: kiwi, Bi: birch.

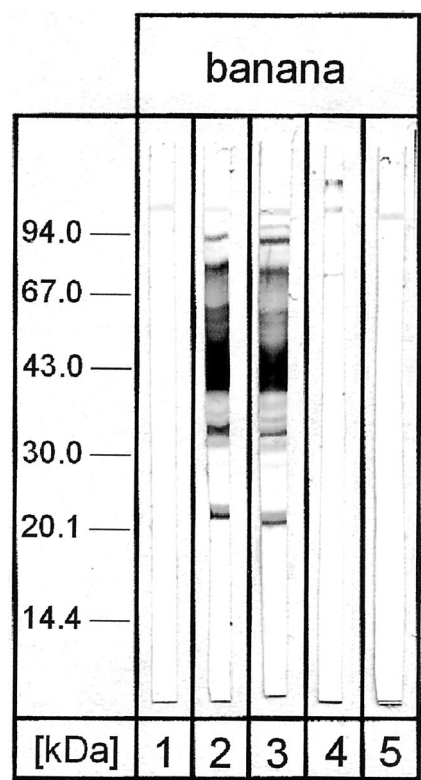


Figure 1. Immunoblot with native banana extract tested with nonallergic human control serum 0 (lane 1) and patient's sera 1 to 4 (lanes 2 to 5).

Patient 1 noticed an OAS 10 minutes after contact with 2.6 g banana. After 10.4 g of banana he developed urticaria and tachycardia. The second patient reacted 5 minutes after a dose of 1.3 g of banana with an OAS. The third patient (3) was sensitized to avocado (CAP class 2) but not to latex, kiwi, or birch pollen. The CAP was negative with rBet v 1 and rBet v 2. By immunoblotting, only a single band at 70 kDa was detectable (Fig 1). The fourth patient was sensitized to birch pollen, rBet v 1, and rBet v 2, but not to latex, avocado, and kiwi (Table 3). In contrast to the other patients (1 to 3), banana-profilin EAST was positive (class 2) and immunoblot analysis with banana extract was negative, but strongly positive with recombinant banana profilin (Fig 2). The last two patients refused a DBPCFC.

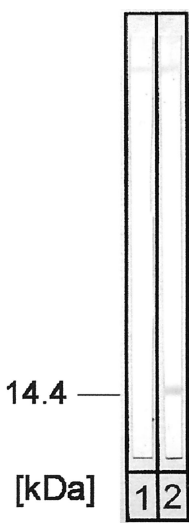


Figure 2. Immunoblot with recombinant banana profilin of nonallergic human control serum 0 (lane 1) and patient's serum 4 (lane 2).

DISCUSSION

Despite the fact that DBPCFC is the only scientifically accepted test for the confirmation of food allergy, no DBPCFC-based study for banana has been carried out until now. Here, we report the first two cases of allergy to ingestion of banana, confirmed by DBPCFC. Both patients (1, 2) were sensitized to latex, but did not experience symptoms after latex contact. In the immunoblot analysis with banana extract, both sera reacted with many proteins. Dominant bands appeared also at 32 to 34 kDa, possibly representing class I chitinase with a hevein-like domain, which has been described as a major allergen in banana.^{9,10} Further, class I chitinases have been purified from chestnut and avocado,¹⁹ and their cross-reactivity with hevein has been demonstrated.²⁰ Whether these allergens described in chestnut and avocado are related to the allergens identified in the present study can not be deduced from our results. Because patients 1 and 2 were negative to Bet v 2, the positive CAP results to latex, avocado, and kiwi are not a consequence of their pollinosis,²¹ but may represent a typical latex-fruit syndrome.

Patient 3 with a positive banana-SPT and -CAP (class 2) and a positive avocado-CAP (class 2) showed in the immu-

noblot analysis a single band at 70 kDa. As she was not sensitized to latex or profilin, we suppose that the allergy to banana possibly occurred as a result of primary sensitization to this fruit.

Patient 4 had a positive SPT to banana, but a negative banana-CAP (class 0). However, her serum displayed specific IgE to Bet v 2 and recombinant banana profilin, and reacted strongly with the banana profilin in the immunoblot assay. Considering the fact that this patient had birch pollinosis, it is likely that banana allergy resulted from the cross-reaction of IgE primarily directed against birch pollen profilin in this case.²²

CONCLUSION

We were able to confirm banana allergy in two latex-sensitized patients by using a DBPCFC protocol for the first time. Further, our serologic analyses revealed a heterogeneous IgE response to banana allergens in four patients. In addition to the well known cross-reacting allergens in latex, we detected a 70-kDa protein as new banana allergen and identified profilin as another potentially cross-reactive allergen in banana. Because of the relatively high incidence of banana allergy and the possibility of anaphylaxis, we recommend further work with the focus on the characterization of the allergens recognized by IgE from patients with a positive DBPCFC to banana.

REFERENCES

1. Wuthrich B. Lethal or life-threatening allergic reactions to food. *J Invest Allergol Clin Immunol* 2000;10:59–65.
2. Bruijnzeel-Koomen C, Ortolani C, Aas K, et al. Adverse reactions to food. European Academy of Allergology and Clinical Immunology Subcommittee. *Allergy* 1995;50:623–635.
3. Bock S, Sampson H, Atkins F, et al. Double-blind, placebo-controlled food challenge (DBPCFC) as an office procedure: a manual. *J Allergy Clin Immunol* 1988;82:986–997.
4. Linaweaver WE, Saks GL, Heiner DC. Anaphylactic shock following banana ingestion. *Am J Dis Child* 1976;130:207–209.
5. Savonius B, Kanerva L. Anaphylaxis caused by banana. *Allergy* 1993;48:215–216.
6. Garcia-Ortiz JC, Moyano JC, Alvarez M, Bellido J. Latex allergy in fruit-allergic patients. *Allergy* 1998;53:532–536.
7. Kim KT, Hussain H. Prevalence of food allergy in 137 latex-allergic patients. *Allergy Asthma Proc* 1999;20:95–97.
8. Blanco C, Carrillo T, Castillo R, et al. Latex allergy: clinical features and cross-reactivity with fruits. *Ann Allergy* 1994;73:309–314.
9. Sanchez-Monge R, Blanco C, Diaz-Perales A, et al. Isolation and characterization of major banana allergens: identification as fruit class I chitinases. *Clin Exp Allergy* 1999;29:673–680.
10. Mikkola JH, Alenius H, Kalkkinen N, et al. Hevein-like protein domains as a possible cause for allergen cross-reactivity between latex and banana. *J Allergy Clin Immunol* 1998;102:1005–1012.
11. Delbourg MF, Guilloux L, Moneret-Vautrin DA, Ville G. Hypersensitivity to banana in latex allergic patients. Identification of two major banana allergens of 33 and 37 kDa. *Ann Allergy Asthma Immunol* 1996;76:321–326.
12. Moller M, Kayma M, Vieluf D, et al. Determination and characterization of cross-reacting allergens in latex, avocado, banana, and kiwi fruit. *Allergy* 1998;53:289–296.
13. The European Academy of Allergology and Clinical Immunology. Position paper: Allergen standardization and skin tests. *Allergy* 1993;48(Suppl):48–82.
14. Anliker MD, Reindl J, Vieths S, Wuthrich B. Allergy caused by ingestion of persimmon (*Diospyros kaki*): detection of specific IgE and cross-reactivity to profilin and carbohydrate determinants. *J Allergy Clin Immunol* 2001;107:718–723.
15. Reindl J, Rihs HP, Scheurer S, et al. IgE reactivity to profilin in pollen-sensitized subjects with adverse reaction to banana and pineapple allergy. *Int Arch Allergy Immunol* 2002;128:105–114.
16. Kyhse-Andersen J. Electrophoretic blotting of multiple gels: a simple apparatus without buffer tank for rapid transfer of proteins from polyacrylamide to nitrocellulose. *J Biochem Biophys Methods* 1984;10:203–209.
17. Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 1970;227:680–685.
18. Ballmer-Weber BK, Vieths S, Luttkopf D, et al. 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;106:373–378.
19. Diaz-Perales A, Collada C, Blanco C, et al. Class I chitinases with hevein like domain, but not class II enzymes, are relevant chestnut and avocado allergens. *J Allergy Clin Immunol* 1998;102:127–133.
20. Chen Z, Posch A, Cremer R, et al. Identification of hevein (Hev b 6.02) in Hevea latex as a major cross-reacting allergen with avocado fruit in patients with latex allergy. *J Allergy Clin Immunol* 1998;102:476–481.
21. Diez-Gomez ML, Quirce S, Cuevas M, et al. Fruit-pollen-latex cross-reactivity: implication of profilin (Bet v 2). *Allergy* 1999;54:951–961.
22. Caballero T, Esteban M. Association between pollen hypersensitivity and edible vegetable allergy: a review. *J Invest Allergol Clin Immunol* 1998;8:6–16.

Requests for reprints should be addressed to:

Brunello Wüthrich, MD

Department of Dermatology/Allergy Unit

University Hospital

Gloriastrasse 31

CH-8091 Zurich, Switzerland

E-mail: wuethric@derm.unizh.ch