

Profilin is a relevant melon allergen susceptible to pepsin digestion in patients with oral allergy syndrome

Rosa Rodriguez-Perez, PhD,^a Jesus F. Crespo, MD,^b Julia Rodríguez, MD,^b and Gabriel Salcedo, PhD^a Madrid, Spain

Background: Melon allergy has been documented by means of double-blind, placebo-controlled food challenges. The most common clinical feature associated with melon allergy is oral allergy syndrome (OAS). However, no relevant allergens of melon have been identified to date.

Objective: We sought to identify melon allergens and analyze their digestibility in human saliva and simulated gastric fluid (SGF) to provide a rationale for the OAS.

Methods: Melon, zucchini, cucumber, and watermelon allergens were identified by means of IgE immunoblotting of sera from 21 patients with OAS after melon ingestion confirmed by means of double-blind, placebo-controlled food challenge. Further characterization was performed with rabbit antisera against sunflower pollen profilin and anticomplex glycans.

Lability of allergens was assayed by incubation of melon extract in human saliva and SGF.

Results: Several IgE-binding components between 15 and 60 kd and a main reactive band of 13 kd were detected in melon extract with the pooled sera from patients with melon allergy. As in melon, 13-kd components of zucchini, cucumber, and watermelon extracts were strongly recognized by the IgE antibodies of the patients with melon allergy and were identified as profilins. Putative cross-reacting carbohydrate determinants were also detected. Sera from 71% of patients recognized the melon profilin, and therefore profilin is considered a major allergen. Melon allergens were unaffected by crude human saliva. In contrast, most melon proteins, predominantly the 13-kd component (profilin), were quickly digested in the SGF.

Conclusion: In patients with OAS, a 13-kd protein identified as a profilin is a major melon allergen highly susceptible to pepsin digestion. (*J Allergy Clin Immunol* 2003;111:634-9.)

Key words: Melon allergens, profilin, oral allergy syndrome, digestibility

Oral allergy syndrome (OAS) is considered a form of contact allergy that is confined almost exclusively to the oropharynx and rarely affects other target organs.¹ It is mainly caused by sensitization to fresh fruits and vegeta-

Abbreviations used

DBPCFC: Double-blind, placebo-controlled food challenge

LTP: Lipid-transfer protein

OAS: Oral allergy syndrome

PVDF: Polyvinylidene difluoride

SGF: Simulated gastric fluid

bles in subjects with hypersensitivity to pollens, especially to birch, mugwort, and ragweed pollens (pollen-food allergy syndrome).² It has been estimated that more than half of the patients with birch pollinosis might be sensitized to fresh fruits (mainly apples) and less frequently to Apiaceae (celery and carrot).³⁻⁵ In most cases birch pollen-food syndromes inducing oral symptoms are due to the cross-reactivity of IgE antibodies to conserved plant allergens, particularly the major birch pollen allergen Bet v 1 and the actin-binding protein profilin (Bet v 2).⁶ Moreover, an association between ragweed pollen sensitization and oropharyngeal symptoms after contact with fresh melon and banana was recognized by Anderson et al⁷ in 1970. Furthermore, immunochemical studies have proved that approximately half of the sera from individuals with ragweed allergy contained IgE specific for any single gourd family member, including watermelon, melon, or cucumber.⁸ In addition, cross-reactivity between watermelon and ragweed pollen has been shown in ELISA-based IgE-inhibition experiments. More recently, Cuesta Herranz et al⁹ have confirmed that melon allergy occurred mainly in patients with pollinosis, even in ragweed-free areas. In addition, CAP-RAST and immunoblotting inhibition experiments have identified IgE-binding components shared by melon and *Plantago lanceolata* and orchard grass pollen allergens.¹⁰ However, potentially cross-reactive relevant melon allergens have not been characterized. Our previous studies addressed the clinical features of IgE-mediated melon allergy confirmed by means of double-blind, placebo-controlled food challenges (DBPCFCs).¹¹ The most prominent findings included an association with pollinosis and other fruit allergies and short-lived symptoms confined exclusively to the oropharynx as the most common clinical manifestations. An intriguing characteristic in these patients is the lack of systemic manifestations after oral symptoms occurring after ingestion of the food. The lability and susceptibility of some plant-derived food allergens to digestion has been suggested as

From ^aUnidad de Bioquímica, Departamento de Biotecnología, E.T.S. Ingenieros Agrónomos, Madrid; and ^bServicio de Alergia, Hospital Universitario Doce de Octubre, Madrid.

Supported by Dirección General de Enseñanza Superior e Investigación Científica, MEC (grant PB98-0735).

Received for publication March 29, 2002; revised July 29, 2002, and September 3, 2002; accepted for publication September 18, 2002.

Reprint requests: Gabriel Salcedo, PhD, Unidad de Bioquímica, Departamento de Biotecnología, E.T.S. Ingenieros Agrónomos, Ciudad Universitaria, 28040 Madrid, Spain.

© 2003 Mosby, Inc. All rights reserved.

0091-6749/2003 \$30.00 + 0

doi:10.1067/mai.2003.74

a cause of isolated oral symptoms, but little experimental evidence has been provided. In this study we have characterized a major cross-reactive melon allergen and analyzed its digestibility in human saliva and simulated gastric fluid (SGF) to provide a rationale for OAS.

METHODS

Patient sera

Sera from 21 adult patients given a diagnosis of OAS induced by melon in the Food Allergy Unit of the Hospital Universitario Doce de Octubre (Madrid, Spain) were selected for this study. The diagnosis was made on the basis of history and confirmed by positive results of skin tests, melon-specific IgE antibody assessments, and oral challenges. Subjects underwent skin prick testing with fresh melon according to the procedure described by Dreborg and Foucard,¹² and their serum was assayed for IgE antibodies specific for melon in the ImmunoCAP System (Pharmacia). Levels of melon-specific IgE antibodies ranged from 0.7 to 46 kUA/L. Actual clinical reactivity was established on the basis of DBPCFC with melon, as described previously.¹¹ All patients had concomitant seasonal rhinitis, asthma, or both in addition to positive results on skin prick tests with commercial extracts to several grass, weed, and tree pollens.

Fruit extracts and purified allergens

Melon (*Cucumis melo*), zucchini (*Cucurbita pepo*), cucumber (*Cucumis sativus*), and watermelon (*Citrullus lanatus*) were purchased from the local market. Samples were defatted with acetone (2 × 1:10 [wt/vol] for 1 hour at 4°C), followed by ethanol/ether (1:3 [vol/vol]; 2 × 1:10 [wt/vol] for 1 hour at 4°C). The dried material was then extracted with PBS buffer (0.1 mol/L sodium phosphate [pH 7.0] and 0.15 mol/L NaCl; 1 × 1:5 [wt/vol] for 1 hour at 4°C). After centrifugation (10,000 rpm for 30 minutes at 4°C), the supernatant was dialyzed against H₂O (cutoff point of 3.5 kd) and freeze-dried.

Determination of the protein concentration was performed according to the method of Bradford.¹³

Recombinant sunflower pollen profilin (rHel a 2) was obtained from Bial-Aristegui Laboratories,¹⁴ and recombinant birch pollen profilin (rBet v 2) was obtained from Biomay. The peach lipid-transfer protein (LTP) Pru p 3 was isolated as previously described.¹⁵

Immunodetection and immunoblot inhibition assays

Fruit extracts were fractionated by means of SDS-PAGE in accordance with the method of Laemmli¹⁶ on Bio-Rad Miniprotein II System gels (15% polyacrylamide) and then electrotransferred onto polyvinylidene difluoride (PVDF) membranes, as described elsewhere.¹⁷ After washing and blocking, membranes were incubated with a serum pool or individual sera from patients with melon allergy or with control sera (1:3 dilutions) and then with alkaline phosphatase-conjugated monoclonal anti-human IgE (clon GE-1, Sigma; 1:500 dilution) and developed by adding a 5-bromo-4-chloro-3-indolyl phosphate/nitro blue tetrazolium solution. Alternatively, replica-blocked membranes were incubated with rabbit antisera against sunflower pollen profilin (1:5000 dilution, Bial-Aristegui Laboratories) to detect antigenic profilin in fruit extracts¹⁴ or to complex asparagine-linked glycans of plant glycoproteins (1:80,000 dilution; kindly provided by Dr M. J. Chrispeels, University of California, San Diego, Calif).^{17,18} Membranes were then treated with alkaline phosphatase-conjugated anti-rabbit IgG (1:500 dilution, Sigma) and finally with a 5-bromo-4-chloro-3-indolyl phosphate/nitro blue tetrazolium solution.

Immunoblot inhibition assays with rBet v 2 and BSA (negative control) as inhibitors were performed as described above. The

serum pool or each individual serum was preincubated for 3 hours at 25°C with the corresponding inhibitor (5 µg of protein per lane).

Analysis of stability of melon and purified allergens to digestion in vitro

Human saliva was directly collected from the mouth with a Pasteur pipette, and its α -amylase activity was automatically determined (EPS method, Boehringer Mannheim), obtaining a level of 422 IU/mL. The melon extract (680 µg of protein) was dissolved in 200 µL of crude human saliva and then incubated at 37°C with continuous rocking. Aliquots of this solution (corresponding to 15 µg of protein) were periodically withdrawn at 0.5, 5, and 15 minutes. The digestibility of the melon extract and purified proteins in the SGF was analyzed essentially as described by Astwood et al.¹⁹ The melon extract (105 µg of protein), purified rHel a 2, Pru p 3, and BSA (10 µg of protein) were dissolved in 50 µL of prewarmed 0.7% (vol/vol) HCl with 30 mmol/L NaCl and in the same solution containing 0.32% (wt/vol) pepsin A (Sigma). Digestion was performed with continuous shaking for 30 minutes at 37°C. Aliquots of these solutions (corresponding to 15 µg of melon extract and 2 µg of purified proteins) were withdrawn at 0.5 and 30 minutes. Aliquots from both human saliva and SGF treatments were promptly frozen in liquid nitrogen, freeze-dried, redissolved in sample buffer, and separated by means of SDS-PAGE. After electrotransfer to PVDF membranes, immunodetection was carried out with a serum pool from patients with melon allergy, rabbit antiserum against sunflower pollen profilin (rHel a 2), and rabbit antiserum against peach LTP (Pru p 3), as described above.

RESULTS

Identification of melon allergens

SDS-PAGE and immunoblot analysis of melon and other members of the gourd family are shown in Fig 1. Protein staining (Fig 1, A) revealed a complex pattern of components from approximately 6 to 80 kd in all allergenic extracts. Immunodetection of melon-blotted extract with the serum pool from patients with OAS recognized several IgE-binding components between 15 and 60 kd and a major reactive band of 13 kd (Fig 1, B, lane Me). This serum pool also reacted with a number of components in the other blotted-fruit extracts tested. As in melon extract, 13-kd components of zucchini, cucumber, and watermelon extracts were strongly recognized by the IgE antibodies of the patients with melon allergy. These bands were identified as profilins by using rabbit anti-sunflower pollen profilin (Fig 1, C). The presence of cross-reacting carbohydrate determinants in the fruit extracts was assayed by immunoblotting with a specific serum to complex asparagine-linked glycans (Fig 1, D). Multiple bands from 12 to 70 kd were found in most blotted extracts, with several of them probably corresponding to those recognized by the serum pool from allergic patients, as suggested by the comparison of the patterns presented in Fig 1, B and D.

To determine the relative importance of profilin as a melon allergen, we examined melon immunoblots with individual sera from the 21 patients. The blots are shown in Fig 2. At least 15 (71%) sera reacted with the 13-kd band (profilin), and therefore it was identified as a major melon allergen. This band was the strongest IgE-binding component in 6 of these sera. No band was detected when a negative control serum pool was assayed (not shown).

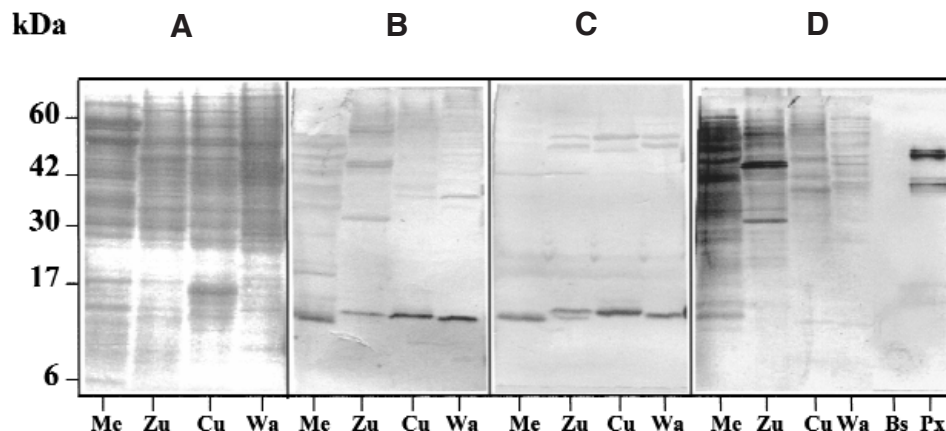


FIG 1. Protein stain (**A**) and IgE (**B**), profilin-like (**C**), and complex asparagine-linked glycan (**D**) immunoblots of PBS extracts from melon (*Me*), zucchini (*Zu*), cucumber (*Cu*), and watermelon (*Wa*) fruits. BSA (*Bs*) and horseradish peroxidase (*Px*) were used as negative and positive controls, respectively (**D**).

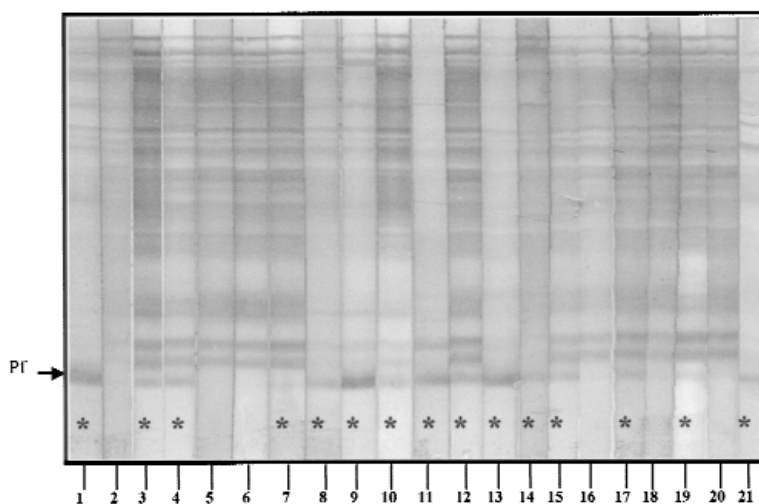


FIG 2. IgE immunoblot analysis of the melon extract with individual sera (*lanes 1-21*) from patients with melon allergy. The position of melon profilin (*Pr*) is indicated, and those sera reacting with the profilin band are marked (*).

To further confirm the identification of the 13-kd melon component as profilin, immunoblot inhibition assays were performed, with recombinant birch profilin rBet v 2 as the inhibitor (Fig 3). The serum pool from patients with melon allergy clearly recognized rBet v 2, which completely inhibited the specific IgE binding to the 13-kd melon band. Similar results were obtained with 2 individual sera (Fig 2, nos. 12 and 13). Interestingly, rBet v 2 did not decrease the IgE binding to other low-molecular-weight (15-17 kd) bands of the melon extract (Fig 3).

Susceptibility of melon profilin to human saliva and SGF

The effect of treatment with human saliva and SGF on melon profilin is depicted in Fig 4. Immunoblotting with the pooled sera of patients with melon allergy showed that melon allergens were unaffected by crude human

saliva. Likewise, concomitant experiments with antisera against sunflower pollen profilin demonstrated that this antigen was unaltered when treated with human saliva (Fig 4, A).

Most melon proteins, predominantly the 13-kd component (profilin) and the recombinant sunflower pollen profilin, were digested in the SGF (Fig 4, B and C). In correspondence with these findings, a lack of reactivity of melon and sunflower pollen profilins with the pooled sera of patients with melon allergy and rabbit anti-sunflower pollen profilin antiserum, respectively, was demonstrated by means of immunoblotting. This effect was accomplished only when the digestibility of melon extract and sunflower pollen profilin was assessed in the SGF containing pepsin. On the contrary, the purified peach LTP (Pru p 3), used as a control, was very stable in the SGF (Fig 4, D).

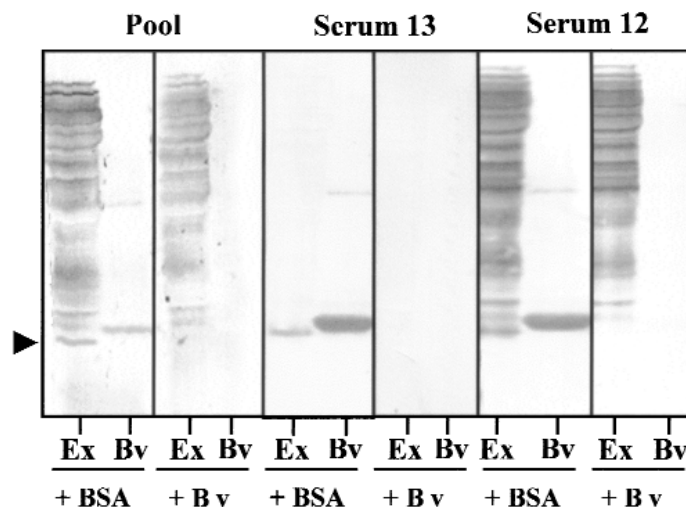


FIG 3. Immunoblot inhibition assays of the melon PBS extract (Ex) and rBet v 2 (Bv) with BSA (+BSA; negative control) and rBet v 2 (+Bv) as inhibitors. A pool of sera and 2 selected individual sera (Fig 2, nos. 12 and 13) from patients with melon allergy were tested.

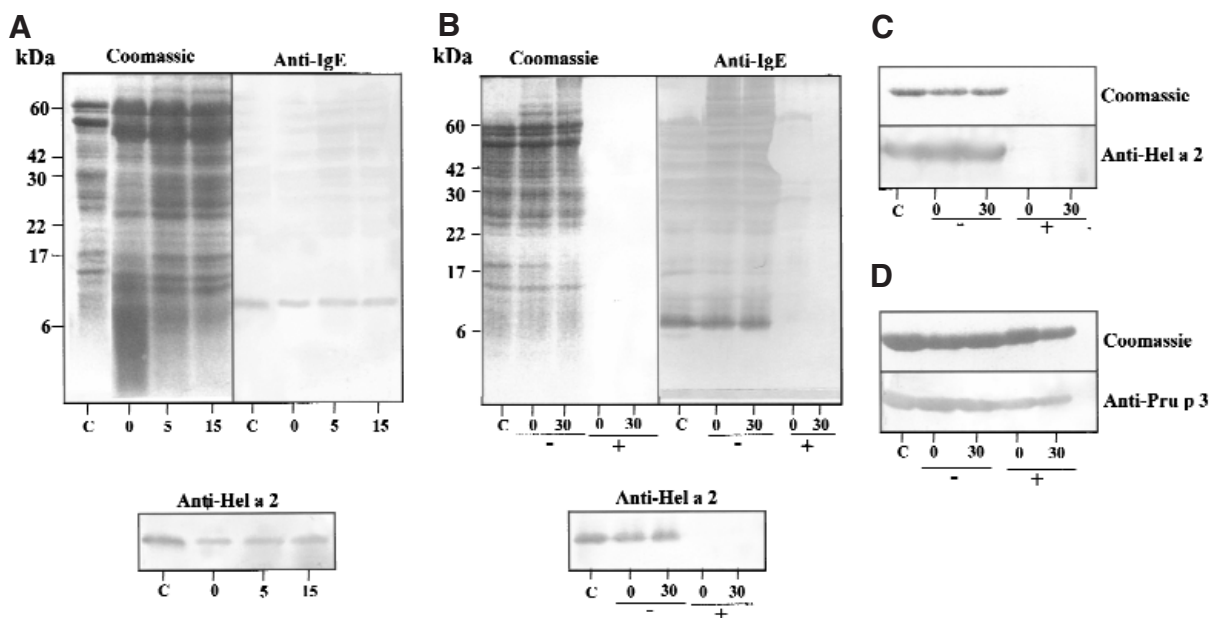


FIG 4. A, Effect of human saliva on melon profilin reactivity. Untreated (c) or treated samples of melon extract for 0.5 (0), 5, or 15 minutes were separated by means of SDS-PAGE, and replica gels were stained with Coomassie Blue or electrotransferred to PVDF membranes and immunoblotted with a serum pool from patients with melon allergy (Anti-IgE) or with polyclonal antibodies to rHel a 2 (Anti-Hel a 2). B-D, Effect of SGF on the reactivity of melon profilin (Fig 4, B), purified rHel a 2 (Fig 4, C), and purified Pru p 3 (Fig 4, D). Untreated (c) or treated samples (melon extract in Fig 4, B) with SGF, containing (+) or not containing (-) pepsin, for 0.5 (0) or 30 minutes were separated by means of SDS-PAGE. Replica gels were stained with Coomassie Blue or electrotransferred to PVDF membranes and immunoblotted with a serum pool from patients with melon allergy (Anti-IgE) or with polyclonal antibodies to rHel a 2 (Anti-Hel a 2) or Pru p 3 (Anti-Pru p 3).

DISCUSSION

Melon hypersensitivity has been reported to occur mainly in patients with allergy to pollens.⁷⁻¹¹ Consistent with other pollen-fruit allergy syndromes, the most common clinical feature associated with melon allergy is OAS. However, the lack of identification of its allergens

still prevents explanation of the immunologic relationship with pollens and other fruits. In this way the aim of this study was to identify relevant allergens of melon to which allergic reactions were objectively confirmed by means of recently performed DBPCFCs.¹¹ From this clinical assessment, 21 patients with OAS and a positive SPT response and CAP-FEIA result for melon were

included in the present study. Sera from 71% of the patients recognized a 13-kd component that was tentatively identified as melon profilin by comparing its reactivity to the pooled sera from the patients with melon allergy and the rabbit antiserum against sunflower profilin. Unfortunately, we did not succeed in isolating this 13-kd melon allergen by means of subjecting the melon extract (or gel-filtration fractions derived from it) to either chromatographic (affinity chromatography on polyproline columns or reverse-phase HPLC) or semi-preparative electrophoretic fractionation. Moreover, no clear membrane-transferred band was obtained to N-terminal amino acid sequencing. The apparent low level of the allergen in the crude melon extract, as well as its tendency to associate with other components, seem to be the main constraints to its isolation. In this context it should be mentioned that virtually no natural profilins from fruits or vegetables have been purified, and only isolated recombinant forms have been reported.²⁰ Taking into account the limitations discussed above, an alternative approach was performed to support the identification of the 13-kd melon allergen as profilin. This identification was confirmed by means of immunoblot inhibition assays with rBet v 2 (birch profilin), which completely inhibited the IgE binding to the 13-kd melon component. In concordance with the sensitization frequency of patients with melon allergy against profilin, it can be considered as a major allergen. In addition, IgE from the pooled sera of patients with melon allergy and the rabbit antiprolin serum showed strong reactivity to proteins with a molecular size of approximately 12 to 14 kd in other Cucurbitaceae fruits, all of which suggests that profilin could be not only an important allergenic compound in melon but also in other members of the gourd family. These results are consistent with the findings from Reindl et al,²¹ who identified zucchini profilin as a relevant allergen in 2 of 4 patients with zucchini allergy confirmed by means of DBPCFC. Profilins were described as panallergens in botanical-related and botanical-unrelated plants.²² Cross-reaction of IgE against melon profilin with its homologues from associated pollens could be an explanation for the pollen-melon allergy syndrome, the immunologic basis of which has been scarcely investigated.¹⁰ In addition, a number of components with molecular sizes ranging from 60 to 15 kd were identified as allergenic in melon, zucchini, cucumber, and watermelon by using the serum pool from patients with melon allergy. Most of them seem to be glycoproteins harboring complex asparagine-linked glycans comprising xyloxy residues, fucosyl residues, or both, which constitute cross-reactive IgE-binding determinants.^{23,24} These carbohydrate determinants have also been identified as allergens in zucchini fruit.²¹

In our experiments melon profilin was stable in human saliva but was digested by the SGF within a few seconds. This difference can be explained by the virtual lack of digestive proteases in human saliva and the strong effect of pepsin on most plant food proteins. Immunoblotting with the pooled sera of patients with OAS caused by

melon and with rabbit antiserum against sunflower pollen profilin lacked any reactivity with the melon profilin when melon extract was treated with SGF. These results are consistent with previous findings from Yagami et al,²⁵ who have investigated the digestibility of allergens extracted from natural rubber latex and vegetable foods. Regarding melon, the authors demonstrated that the allergenic proteins recognized by the IgE antibodies of a serum pool from 8 patients with a history of OAS caused by several fruits were rapidly digested by SGF. It is possible to speculate that this allergen could be responsible for local oral symptoms and could induce positive skin prick test responses without systemic responses in other target organs because although this protein would remain intact in the oral cavity, it could be quickly degraded in the gastrointestinal tract after ingestion. However, it should be considered that about 10% of patients with melon allergy have severe anaphylactic reactions.¹¹ These clinical findings suggest that some patients allergic to melon could be sensitized concomitantly to allergens highly resistant to pepsin digestion, such as LTPs.²⁶ In this line the serum pool used in this study strongly recognized Pru p 3, and consequently, a search to identify allergenic melon LTPs is currently being undertaken.

We thank Dr M. J. Chrispeels for kindly providing the specific serum to complex glycans and Mr J. Garcia-Guijarro and Mrs D. Lamonedá for technical assistance.

REFERENCES

- Ortolani C, Ispano M, Pastorello E, Bigi A, Ansaloni R. The oral allergy syndrome. *Ann Allergy* 1988;61:47-52.
- Sampson HA. Food allergy. Part 1: immunopathogenesis and clinical disorders. *J Allergy Clin Immunol* 1999;103:717-28.
- Kremser M, Lindemayr W. Frequency of the so-called "apple allergy" ("apple contact urticaria syndrome") in patients with birch pollinosis. *Z Hautkr* 1983;58:543-52.
- Rossi RE, Monasterolo G, Operti D, Corsi M. Evaluation of recombinant allergens Bet v 1 and Bet v 2 (profilin) by Pharmacia CAP System in patients with pollen-related allergy to birch and apple. *Allergy* 1996;51:940-5.
- Wutrich B, Dietschi R. The celery-carrot-mugwort-condiment syndrome: skin test and RAST results. *Schweiz Med Wochenschr* 1985;115:258-64.
- Ebner C, Hirschwehr 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.
- Anderson LB, Dreyfuss EM, Logan J, Johnstone DE, Glaser J. Melon and banana sensitivity coincident with ragweed pollinosis. *J Allergy Clin Immunol* 1970;45:310-9.
- Enberg RN, Leickly FE, McCulloch J, Bailey J, Ownby DR. Watermelon and ragweed share allergens. *J Allergy Clin Immunol* 1987;79:867-75.
- Cuesta Herranz J, Lazaro M, Figueredo E, Igea JM, Umpierrez A, De las Heras M. Allergy to plant-derived fresh foods in a birch- and ragweed-free area. *Clin Exp Allergy* 2000;30:1411-6.
- Garcia Ortiz JC, Cosmes Martin P, Lopez Asunolo A. Melon sensitivity shares allergens with Plantago and grass pollens. *Allergy* 1995;50:269-73.
- Rodriguez J, Crespo JF, Burks W, Rivas-Plata C, Fernandez-Anaya S, Vives R, et al. Randomized, double-blind, crossover challenge study in 53 subjects reporting adverse reactions to melon (*Cucumis melo*). *J Allergy Clin Immunol* 2000;106:968-72.
- Dreborg S, Foucard T. Allergy to apple, carrot and potato in children with birch pollen allergy. *Allergy* 1983;38:167-72.
- Bradford MM. A rapid and sensitive method for the quantitation of

- microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* 1976;72:248-54.
14. Asturias JA, Arilla MC, Gomez-Bayon N, Aguirre M, Martinez A, Palacios R, et al. Cloning and immunological characterization of the allergen Hel a 2 (profilin) from sunflower pollen. *Mol Immunol* 1998;35:469-78.
 15. Diaz-Perales A, Garcia-Casado G, Sanchez-Monge R, Garcia-Selles FJ, Barber D, Salcedo G. cDNA cloning and heterologous expression of the major allergens from peach and apple belonging to the lipid-transfer protein family. *Clin Exp Allergy* 2002;32:87-92.
 16. Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 1970;227:680-5.
 17. Diaz-Perales A, Collada C, Blanco C, Sanchez-Monge R, Carrillo T, Aragoncillo C, et al. Cross-reactions in the latex-fruit syndrome: a relevant role of chitinases but not of complex asparagine-linked glycans. *J Allergy Clin Immunol* 1999;104:681-7.
 18. Lauriere M, Lauriere C, Chrispeels MJ, Johnson KD, Sturm A. Characterization of a xylose-specific antiserum that reacts with the complex asparagine-linked glycans of extracellular and vacuolar glycoproteins. *Plant Physiol* 1989;90:1182-8.
 19. Astwood JD, Leach JN, Fuchs RL. Stability of food allergens to digestion in vitro. *Nat Biotechnol* 1996;14:1269-73.
 20. Scheurer S, Wangorch A, Nerkamp J, Skov PS, Ballmer-Weber B, Wüthrich B, et al. Cross-reactivity within the profilin panallergen family investigated by comparison of recombinant profilins from pear (Pyr c 4), cherry (Pru ar 4) and celery (Api g 4) with birch pollen profilin Bet v 2. *J Chromatogr B* 2001;756:315-25.
 21. Reindl J, Anliker MD, Karamloo F, Vieths S, Wüthrich B. Allergy caused by ingestion of zucchini (*Cucurbita pepo*): characterization of allergens and cross-reactivity to pollen and other foods. *J Allergy Clin Immunol* 2000;106:379-85.
 22. Valenta R, Duchene M, Ebner C, Valent P, Sillaber C, Deviller P, et al. Profilins constitute a novel family of functional plant pan-allergens. *J Exp Med* 1992;175:377-85.
 23. Garcia-Casado G, Sanchez-Monge R, Chrispeels MJ, Armentia A, Salcedo G, Gomez L. Role of complex asparagine-linked glycans in the allergenicity of plant glycoproteins. *Glycobiology* 1996;6:471-7.
 24. Van Ree R, Cabanes-Macheteau M, Akkerdaas J, Milazzo JP, Loutelier-Bourhis C, Rayon C, et al. $\beta(1,2)$ -xylase and $\alpha(1,3)$ -fucose residues have a strong contribution in IgE binding of plant glycoallergens. *J Biol Chem* 2000;275:11451-8.
 25. Yagami T, Haishima Y, Nakamura A, Osuna H, Ikezawa Z. Digestibility of allergens extracted from natural rubber latex and vegetable foods. *J Allergy Clin Immunol* 2000;106:752-62.
 26. Asero R, Mistrello G, Roncarolo D, de Vries SC, Gautier MF, Ciurana CL, et al. Lipid transfer protein: a pan-allergen in plant-derived foods that is highly resistant to pepsin digestion. *Int Arch Allergy Immunol* 2000;122:20-32.