

Bell Pepper Allergy: Different Sensitization Profiles

Callero A¹, Cabrera-Hernandez V¹, Perez-Rodríguez E¹, Jimeno-Nogales L², Martínez-Tadeo JA¹, Plata-Rodríguez E¹, García-Robaina J¹

¹Nuestra Señora de la Candelaria University Hospital, Service of Allergy, Santa Cruz de Tenerife, Spain

²ALK, Biochemical Research Department, Madrid, Spain

J Invest Allergol Clin Immunol 2018; Vol. 28(5): 340-342
doi: 10.18176/jiaci.0278

Key words: Bell pepper allergy. Immunoblotting. Anaphylaxis. Eosinophilic esophagitis. Cross-reactivity.

Palabras clave: Alergia al pimiento. Immunoblotting. Anafilaxia. Esofagitis eosinofílica. Reactividad cruzada.

Bell pepper (*Capsicum annuum*) belongs to the *Solanaceae* family. Horticultural strains differ in color, size, and taste (sweet or hot). Bell pepper is also consumed as pimenton or paprika, spices that are obtained by drying and powdering.

The 2 major bell pepper allergens that have been sequenced to date are osmotin, or thaumatin-like protein (Cap a 1, 23 kDa), and profilin (Cap a 2, 14 kDa) [1,2]. These proteins act as plant panallergens involved in cross-reactivity between pollen and various vegetable foods. For example, Cap a 2 cross-reacts with latex profilin (Hev b 8) [3].

Solanaceae can be involved in celery-birch-mugwort-spice syndrome through the action of Bet v 1 homologous proteins such as Cap a 4 (PR10 group) [1]. Other allergens involved in bell pepper allergy are glucanase (35 kDa) [4] and peroxidase (30 kDa) [5]. Glucanase cross-reacts with latex 1,3 beta glucanase (Hev b 2) [2]. Latex hevein (Hev b 6) also cross-reacts with bell pepper in sera from sensitized patients [6]. Vicilin (50 kDa) has been described as a possible cause of sensitization to spices from the genus *Capsicum* [7].

We describe a series of cases of bell pepper allergy. The patients were studied using in vivo and in vitro techniques in order to determine clinically relevant allergens.

The study population comprised patients diagnosed with allergy to bell pepper or pimenton during the study period (2012-2016). Diagnosis of bell pepper allergy required a compatible clinical history and a positive skin test and/or specific IgE result.

We identified 7 patients who were sensitized to bell pepper and/or pimenton. Three patients were diagnosed with eosinophilic esophagitis (EE) by endoscopic features and positive biopsy associated with bell pepper or pimenton. Three patients presented with anaphylaxis and 1 with oral allergy syndrome.

All patients underwent prick testing with commercial extracts of airborne allergens, pimenton, latex, nuts, vegetables (legumes, flours, Brassicaceae, and Solanaceae), and fruits (Rosaceae, banana, grape, kiwi, and Cucurbitaceae) (ALK Abelló). Patients with EE also underwent skin testing for milk, egg, fish, and meats. Prick-by-prick testing was performed

with fresh red and green bell pepper (mix of peel and pulp) and pimenton diluted in 0.9% sodium chloride solution (1/10). Prick and prick-by-prick test results were considered positive if the larger diameter of the wheal was ≥ 3 mm, as recommended by the European Academy of Allergology and Clinical Immunology guidelines [8]. A total of 14 healthy patients were used as negative controls.

Total and specific IgE were determined using the ImmunoCAP system (Thermo Fisher Scientific). Specific IgE >0.15 kIU/L was considered positive.

Immunoblotting was performed as previously described [4]. Briefly, bell peppers were cut into pieces and extracted for 90 minutes in 5% phosphate buffer with magnetic agitation. Sweet pimenton from a local supplier was extracted in 10% phosphate buffer. After that, both extracts were separated, filtrated, and stored at -20° C until use.

Extracts were analyzed under nonreducing conditions using tricine SDS-PAGE. Proteins were transferred to nitrocellulose membranes and incubated with patients' sera for 18 hours. After that, they were incubated with antihuman IgE monoclonal antibodies for 2 hours, washed, and incubated for an hour with antimouse peroxidase-labeled rabbit IgG antibodies (Calbiochem). IgE-binding proteins were detected by chemiluminescence (Amersham Bioscience) following the manufacturer's instructions.

EE patients were instructed to avoid bell pepper and pimenton for 3 months. After that period, both foods were reintroduced, and symptoms were recorded daily for a month.

The pimenton commercial extract yielded a positive result in 6 cases. Three patients presented positive skin test results for fresh bell pepper. Skin test results were positive for pimenton but not for fresh bell pepper in EE patients. All patients except one were cosensitized to other vegetable foods. Sensitization to pollens was diagnosed in all patients except in those with EE. All healthy patients presented a negative skin test result. Data are shown in the table.

Reintroduction of pimenton and bell pepper in patients with EE elicited symptoms of dysphagia in 1 patient, although all of the patients were on treatment with oral fluticasone and proton pump inhibitors at the time of the study.

All patients recognized at least 1 band for green pepper and/or bell pepper. Pimenton was recognized in serum from only 1 patient, despite the fact that skin test results were positive in most cases. This could be due to technical problems with allergen extraction in pimenton powder.

We identified various bands, some of which are consistent with known allergens such as profilin (15 kDa) in 2 patients, osmotin (25 kDa) in 4 patients, and peroxidase (30 kDa) in 5 patients. Five patients recognized 50 kDa bands, which may correspond to vicilin or other allergens.

Serum from patient #5 recognized a 10-kDa band, for both green and red bell pepper. The band may correspond to lipid transfer protein (LTP). Few studies suggest sensitivity to LTP [9], although in silico models indicate that it could elicit an immune response [10]. The clinical features of this patient—anaphylaxis and relevant sensitization to mugwort and peach—suggest a role for LTP.

A 35 to 37-kDa band was recognized by 6 patients. The allergen could be a glucanase. Bell pepper glucanase has not

Table. Clinical Characteristics and study results

Pt	Age	Sex	Clinical symptoms	Prick by prick			Commercial SPT positive results		Total IgE, IU/mL	Positive IgE results, kIU/L	Immunoblotting		
				Red pepper	Green pepper	Pimenton	Positive inhalant allergens	Positive foods			Red pepper	Green pepper	Pimenton
1	32	M	EE	-	-	+	None	Sunflower seed, fish, bean, asparagus, endive, pimenton	31.18	None	30-75 kDa (weak)	-	-
2	23	M	EE	-	-	+	None	Pimenton, mustard, curry	126.10	Pimenton (0.17)	15 kDa 25 kDa 30 kDa 37 kDa 50 kDa	15 kDa 25 kDa 30 kDa 37 kDa 50 kDa	25-37 kDa > 100 kDa
3	26	F	EE	-	-	+	Mites, cat and dog dander	Mustard and pimenton	423.60	Pimenton (0.48)	50 kDa 75 kDa	37 kDa 100 kDa 100 kDa	-
4	49	M	Anaph	NT	NT	+	Mites and grass	Pimenton	237.7	Pimenton (0.25)	25 kDa 30 kDa 37 kDa 50 kDa	25 kDa 30 kDa 37 kDa 50 kDa	-
5	42	F	Anaph	+	+	+	Mugwort and ragweed	Nuts, sunflower seed, tomato, peach, banana, onion, and cauliflower	317.22	Mugwort, ragweed, peanut, walnut, chestnut, tomato, peach, sunflower seed, pimenton (1.77), carrot, onion, cauliflower	10 kDa 25 kDa 37 kDa 50 kDa 75 kDa	10 kDa 25 kDa 30 kDa 37 kDa 50 kDa 75 kDa	-
6	34	F	Anaph	+	-	NT	Mugwort, ragweed and <i>Olea</i>	Tomato, nuts, sunflower seed, Rosaceae, kiwi, and latex	106	Pimenton (2.7), peach, almond, peanut, latex, walnut, chestnut	35 kDa	-	-
7	14	F	OAS	+	+	+	Grass and mugwort	Pimenton, tomato, watermelon	147.8	NT	15 kDa 25 kDa 30 kDa 37 kDa 50 kDa	15 kDa 25 kDa 30 kDa 37 kDa 50 kDa	-

Abbreviation: Anaph, anaphylaxis; EE, eosinophilic esophagitis; NT, not tested; OAS, oral allergy syndrome; Pt, patient.

yet been sequenced, although it has been implicated in bell pepper allergy for both oral allergy syndrome and systemic reactions [4] and seems to be a relevant allergen in our study population. We detected bell pepper glucanase in patients presenting with EE and with immediate IgE-mediated reactions.

In EE patients, immunoblotting results are less consistent. Patient #1 showed only weak recognition of 30 to 75kDa bands, which could correspond to peroxidase and/or glucanase. Some of the higher-molecular-weight bands could correspond to vicilin. Similarly, patient #3 recognized a 37-kDa band for green bell pepper that may have been a glucanase. Serum from patient #2 reacted to a variety of bands that may correspond to profilin, osmotin, peroxidase, glucanase, and, possibly vicilin. Interestingly, this patient presented clinical symptoms and positive skin test results with pimenton and other spices and was the only one in whom pimenton IgE-binding bands were detected.

Glucanase and peroxidase seem to be the most prevalent allergens in our population, together with allergens such as osmotin and profilin. Sensitization to the LTP of bell peppers seems less frequent.

Funding

The authors declare that no funding was received for the present study.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

References

1. Ebner C, Jensen-Jarolim E, Leitner A, Breiteneder H. Characterization of allergen in plant derived spices: Apiaceae spices, pepper (Piperaceae), and Paprika (bell peppers, Solanaceae). *Allergy*. 1998;53 (Suppl 46):52-4.
2. Willeroider M, Fuchs H, Wallmer-Weber BK, Focke M, Susani M, Thalhamer J, et al. Cloning and molecular and immunological characterisation of two new food allergens: Cap a 2 and Lyc e 1, profilins of bell pepper (*Capsicum annum*) and tomato (*Lycopersicon esculentum*). *Int Arch Allergy Immunol*. 2003;131:245-55.
3. Wagner S, Radauer C, Hafner C, Fuchs H, Jensen-Jarolim E, Wüthrich B, et al. Characterization of cross reactive bell pepper allergens involved in latex-fruit syndrome. *Clin Exp Allergy*. 2004;34:1739-46.
4. Callero A, Pérez E, Ledesma A, Martínez-Tadeo JA, Hernández G, Rodríguez-Plata E, et al. A case report of bell pepper anaphylaxis: could be 1,3 beta glucanase the culprit allergen. *Ann Allergy Asthma Immunol* 2012;109:474-5.
5. Allergome database. Available at http://www.allergome.org/script/dettaglio.php?id_molecula=2423 Accessed November 23, 2017.
6. Gallo R, Roncarolo G, Mistrello G. Cross reactivity between latex and sweet pepper due to prohevein. *Allergy*. 1998;53:1007-8.
7. Airaksinen L, Riekkö R, Vuokko A, Puustinen A. Paprika rhinoconjunctivitis case reveals new occupational Capsicum allergens. *Am J Ind Med*. 2015;58:791-4.
8. Position paper: Allergen standardization and skin tests. The European Academy of Allergology and Clinical Immunology. *Allergy*. 1993;48(14 Suppl):48-82.
9. Asero R, Mistrello G, Roncarolo D, Amato S, Caldironi G, Barocci F, et al. Immunological cross-reactivity between lipid transfer proteins from botanically unrelated plant-derived foods: a clinical study. *Allergy*. 2002;57:900-6.
10. Garino C, Coisson JD, Arlorio M. In silico allergenicity prediction of several lipid transfer proteins. *Comput Biol Chem*. 2016;60:32-42.

Manuscript received March 5, 2018; accepted for publication May 25, 2018.

Ariel Callero Viera

H. Universitario Nuestra Señora de Candelaria
Carretera del Rosario, 145
38010 Santa Cruz de Tenerife, Spain
E-mail: arielcallero@hotmail.com