

The new Api m 11.0301 Isoallergen From *Apis mellifera* Is a Food Allergen From Honey

Rodríguez-Pérez R¹, Carretero P², Brigido C², Nin-Valencia A³, Carpio-Hernández D³, Tomás M^{1,3}, Quirce S^{1,3}, Caballero ML^{1,3}

¹Allergy Research Group, La Paz Hospital Institute for Health Research (IdiPAZ), Madrid, Spain

²Department of Allergy, Hospital Universitario, Burgos, Spain

³Department of Allergy, La Paz University Hospital, Madrid, Spain

J Investig Allergol Clin Immunol 2022; Vol. 32(6): 492-493
doi: 10.18176/jiaci.0799

Key words: *Apis mellifera*. Honey allergy. IgE. Mass spectrometry. Major royal jelly proteins.

Palabras clave: *Apis mellifera*. Alergia a la miel. IgE. Espectrometría de masas. Principales proteínas de la jalea real.

Allergic reactions to honey are characterized by symptoms such as bronchial asthma, generalized urticaria, angioedema, and anaphylaxis. However, the scope of this food allergy is unknown owing to the lack of current prevalence data [1-8].

Twelve allergens from *Apis mellifera* have been identified and registered in the allergen database of the World Health Organization and International Union of Immunological Societies (WHO/IUIS) (<http://www.allergen.org>). Eleven are from bee venom (Api m 1-10 and Api m 12), while 2 allergenic isoforms or isoallergens of Api m 11 are from secretions of the royal jelly-producing glands (Api m 11.0101 and Api m 11.0201), corresponding to the major royal jelly proteins (MRJPs) MRJP8 and MRJP9 (UniProt accession B3GM11 and Q4ZJX1, respectively) [9]. Regarding presence in honey, a proteomic study of 13 honeys revealed 8 allergens, of which Api m 10 (icarapin) and Api m 11.0201 (MRJP9) were in all the honeys tested. The other allergens detected were Api m 2 (hyaluronidase), Api m 7 (CUB serine protease), Api m 3 (acid phosphatase), Api m 1 (phospholipase A2), Api m 4 (melittin), and Api m 12 (vitellogenin) [10]. The aim of this study was to identify and characterize the allergens involved in honey allergy.

We studied sera from 7 patients with symptoms after honey ingestion and positive test results and sera from 2 nonallergic persons as negative controls. Informed consent was obtained from all the study participants. Patients were assessed using commercial skin prick tests, prick-to-prick tests with artisanal and industrial honeys and royal jelly, and specific IgE determinations by ImmunoCAP.

Extracts from artisanal honey and royal jelly, both obtained from local beekeepers, were prepared as described [2,11]. Proteins were separated using 12% SDS-PAGE under standard conditions to perform IgE-immunoblotting. An allergen with an apparent molecular weight of around 50 kDa from honey extract was detected by 7 patients. The clinical data of the positive patients are summarized in Supplementary Table 1.

The allergen was separated by reverse-phase high-performance liquid chromatography of the honey extract (6 mg). Elution was performed using a 60-minute increasing linear gradient 0%-100% of 70% acetonitrile containing 0.09% trifluoroacetic acid (TFA) and milliQ water containing 5% acetonitrile and 0.1% TFA at a flow rate of 2 mL/min. Peaks containing the allergen were identified by SDS-PAGE and IgE-immunoblotting (Figure). The images of the sera from patients 2, 3, 5, 6, and 7 were weak, despite the effort required to scan their nitrocellulose strips. However, findings were consistent with their low or negative CAP values for honey (Supplementary Table 1).

Sugar residues were oxidated to determine whether cross-reactive carbohydrate determinants (CCDs) were implicated in IgE-mediated reactivity of the allergen detected. Periodate treatment did not cause a loss of IgE-binding, indicating that the sugar residues were not involved in allergenicity (data not shown). A serum sample from a patient with positive IgE to the ImmunoCAP allergen MUXF3 CCD bromelain was used as a positive control CCD (data not shown).

Honey allergen was characterized using shotgun sequencing by reverse-phase liquid chromatography tandem mass spectrometry followed by a proteomic analysis. The 50-kDa protein band underwent trypsin digestion, and the tryptic peptides were analyzed. The resulting spectra were used to launch a search with the search engine PEAKS against the *A mellifera* reference proteome downloaded from the UniprotKB repository (<https://www.uniprot.org/uniprot/?query=proteome:UP000005203>). Multiple protein sequence alignment was performed using CLUSTAL O (1.2.4) to compare *A mellifera* proteins with the sequences of the peptides obtained from the allergen. The results are shown in Supplementary Table 2. We considered a percentage of sequence coverage greater than 25% as a criterion for further protein sequence alignment analysis. Six proteins fulfilled that criterion; of these, 4 belonged to the MRJP family. The peptides obtained covered 78% of the MRJP1 sequence, followed by 62% of MRJP2, 38% of MRJP3, and 29% of MRJP5. The remaining proteins were α -amylase and α -glucosidase. The alignment performed with the 4 MRJPs to compare with the sequences of the peptides obtained from the 50-kDa allergen showed regions of high similarity with some areas of amino acid identity (Supplementary Figure 2).

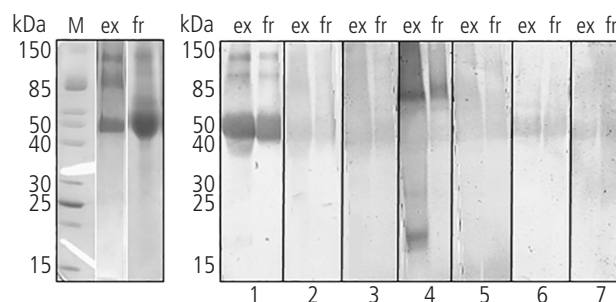


Figure. A, Protein separation by SDS-PAGE and Coomassie staining. B, IgE-immunoblotting performed with sera from 7 honey-allergic patients. Lanes: M, molecular weight marker; ex, honey extract; fr, high-performance liquid chromatography fraction corresponding to the allergen around 50 kDa.

Thus, the common allergen detected by the 7 patients belonged to the MRJP family from *A mellifera*. The allergen has been registered as a new Api m 11.0301 isoallergen and incorporated into the database as a food allergen.

A review of the literature revealed that 9 proteins have been characterized from honeybee larval jelly (with molecular weights ranging from 49 kDa to 87 kDa) and identified as members of the MRJP family [12,13]. MRJPs in honey have been classified according to their relative amount [10]. The 4 most abundant (MRJP1, MRJP2, MRJP3 and MRJP5) are consistent with those detected in the 50-kDa allergen. The most abundant, MRJP1 or apalbumin 1, is an authentic honey protein whose quantification has been considered a tool for evaluation of honey quality [14]. MRJP1 was described as an IgE-binding protein in honey in the serum of a honey-allergic patient [15].

Finally, since the allergen detected in the study patients belonged to the MRJP family, cross-reactivity between honey and royal jelly proteins was assessed using IgE-immunoblotting inhibition assays performed with both honey and royal jelly extracts and a pool of patient sera previously mixed with each of the extracts or with phosphate-buffered saline as a negative control. The result was that IgE-mediated reactivity of the royal jelly extract was inhibited by the honey extract used as an inhibitor and vice versa, ie, the IgE-mediated reactivity of the honey extract was inhibited by the royal jelly extract (Supplementary Figure 3). These results suggest cross-reactivity between the honey and royal jelly proteins.

In conclusion, our study led to the identification of a new isoallergen, the first *A mellifera* food allergen, which should help physicians in the diagnosis of food allergy.

Acknowledgments

We thank Marta Ramiro for her technical laboratory support and Dr Alberto Paradela of the Proteomics department of National Centre for Biotechnology (CNB-CSIC) for his assistance with the proteomic analysis.

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

- Helbling A, Peter C, Berchtold E, Bogdanov S, Müller, U. Allergy to honey: relation to pollen and honey bee allergy. *Allergy*. 1992;47:41-9.
- Bauer L, Kohlich A, Hirschwehr R, Siemann U, Ebner H, Scheiner O, et al. Food allergy to honey: pollen or bee products? Characterization of allergenic proteins in honey by means of immunoblotting. *J Allergy Clin Immunol*. 1996;97:65-73.
- De la Torre F, García JC, Martínez A, Martínez J, Palacios R. IgE binding proteins in honey: discussion on their origin. *J Investig Allergol Clin Immunol*. 1997;7:83-9.

- Lombardi C, Senna GE, Gatti B, Feligioni M, Riva G, Bonadonna P, et al. Allergic reactions to honey and royal jelly and their relationship with sensitization to compositae. *Allergol Immunopathol (Madr)*. 1998;26:288-90.
- Ibero M, Castillo MJ, Pineda F, Palacios R, Martínez J. Whole bee for diagnosis of honey allergy. *Allergy*. 2002;57:557-8.
- Vezir E, Kaya A, Toyran M, Azkur D, Dibek Mısırlıoğlu E, Kocabaş CN. Anaphylaxis/angioedema caused by honey ingestion. *Allergy Asthma Proc*. 2014;35:71-4.
- Aguiar R, Duarte FC, Mendes A, Bartolomé B, Barbosa MP. Anaphylaxis caused by honey: a case report. *Asia Pac Allergy*. 2017;7:48-50.
- Di Costanzo M, De Paulis N, Peveri S, Montagni M, Berni Canani R, Biasucci G. Anaphylaxis caused by artisanal honey in a child: a case report. *J Med Case Rep*. 2021;15:235.
- Burzyńska M, Piasecka-Kwiatkowska D. A Review of Honeybee Venom Allergens and Allergenicity. *Int J Mol Sci*. 2021;22:8371.
- Erbán T, Shcherbachenko E, Talacko P, Harant K. The Unique Protein Composition of Honey Revealed by Comprehensive Proteomic Analysis: Allergens, Venom-like Proteins, Antibacterial Properties, Royal Jelly Proteins, Serine Proteases, and Their Inhibitors. *J Nat Prod*. 2019;82:1217-26.
- Thien FCK, Leung R, Baldo BA, Weiner JA, Plomley R, Czarny D. Asthma and anaphylaxis induced by royal jelly. *Clin Exp Allergy*. 1996;26:216-22.
- Schmitzová J, Klaudiny J, Albert S, Schröder W, Schreckengost W, Hanes J, et al. A family of major royal jelly proteins of the honeybee *Apis mellifera* L. *Cell Mol Life Sci*. 1998;54:1020-30.
- Rosmilah M, Shahnaz M, Patel G, Lock J, Rahman D, Masita A, et al. Characterization of major allergens of royal jelly *Apis mellifera*. *Trop Biomed*. 2008;25:243-51.
- Bíliková K, Simúth J. New criterion for evaluation of honey: quantification of royal jelly protein apalbumin 1 in honey by ELISA. *J Agric Food Chem*. 2010;58:8776-81.
- Hayashi T, Takamatsu N, Nakashima T, Arita T. Immunological characterization of honey proteins and identification of MRJP 1 as an IgE-binding protein. *Biosci Biotechnol Biochem*. 2011;75:556-60.

■ Manuscript received December 20, 2021; accepted for publication February 25, 2022.

María Luisa Caballero

Department of Allergy La Paz University Hospital, IdiPAZ
Paseo de la Castellana 261
28046 Madrid, Spain
E-mail: mlcsoto@hotmail.com