

## The new Api m 11.0301 isoallergen from *Apis mellifera* is a food allergen from honey

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Cases of allergic reactions have been reported following honey ingestion, including symptoms as bronchial asthma, generalized urticaria, angioedema or anaphylaxis. However, this is a food allergy with unknown scope due 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 come from bee venom (Api m 1-10 and Api m 12), while two allergenic isoforms or isoallergens of Api m 11 are derived from the bee secretions from the royal jelly-producing glands (Api m 11.0101 and Api m 11.0201), corresponding with 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 showed eight allergens were detected of which Api m 10 (icarapin) and Api m 11.0201 (MRJP9) were in all the honeys. The others 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 the honey allergy.

Sera from seven patients with symptoms after honey ingestion and positive tests, and sera from two non-allergic subjects as negative controls, were studied. Informed consent was obtained from all the study participants. Patients were studied by skin prick tests (SPTs), prick-to-prick tests (PPTs) performed with artisanal and industrial honeys and royal jelly (RJ), and specific IgE determinations by ImmunoCAP.

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

The allergen was separated by reverse phase high performance liquid chromatography (RP-HPLC) from the honey extract (6 mg). Elution was performed using a 60 min 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 1). The images of the sera from patients 2, 3, 5, 6 and 7 resulted weak, despite the effort when scanning their nitrocellulose strips, but in agreement with their low or negative CAP values to honey (Table 1).

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

For characterization of the honey allergen, a shotgun sequencing by reverse-phase liquid

chromatography tandem mass spectrometry (RP-LC-MS/MS) was performed followed by a proteomic analysis. The 50-kDa protein band was subjected to trypsin digestion and the tryptic peptides were analyzed. The resulting spectra were used to launch a search, using the search engine PEAKS, against the *A. mellifera* reference proteome downloaded from the UniprotKB repository (<https://www.uniprot.org/uniprot/?query=proteome:UP000005203>). A multiple protein sequences alignment was performed by 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 Table 2 (Online-supplemental file). We considered a percentage of sequence coverage greater than 25% as a criterion for a further protein sequences alignment analysis. Six proteins fulfilled that criterion, 4 belonged to the MRJP family. The peptides obtained covered the 78% of the MRJP1 sequence, followed by 62% of MRJP2, MRJP3 (38%) and MRJP5 (29%). Alpha-amylase and alpha-glucosidase were the remaining proteins. The alignment performed with the four MRJPs to compare with the sequences of the peptides obtained from the 50-kDa allergen showed regions of high similarity with some zones of amino acid identity (Online-supplemental file, Figure 2).

Thus, the common allergen detected by the seven patients resulted to belong to the MRJP family from *A. mellifera*. The allergen has been designated as Api m 11.0301 isoallergen and incorporated into database as a food allergen.

Reviewing the literature, nine proteins have been characterized from honeybee larval jelly with MW ranging 49-87 kDa and identified as members of the MRJP family [12,13]. Regarding the presence of MRJPs in honey, they have been classified according to their relative amount [10]. The four more abundant (MRJP1, MRJP2, MRJP3 and MRJP5) coincide with those detected in the 50-kDa allergen. The most abundant, MRJP1 or Apalbumin 1 is an authentic protein of honey whose quantification has been considered as a tool for evaluation of honeys quality [14] and was described as an IgE-binding protein in honey by the serum of a honey

allergic patient [15].

Finally, since the allergen detected by our patients resulted to belong to the MRJP family, cross-reactivity between honey and RJ proteins was studied by IgE-immunoblotting inhibition assays performed with both honey and RJ extracts, and a pool of patients sera previously mixed with each of the extracts or with phosphate buffered saline as negative control. The result was that IgE reactivity of the RJ extract was inhibited by the honey extract used as an inhibitor and *vice versa*, the IgE reactivity of the honey extract was inhibited by the RJ extract (Online-supplemental file, Figure 3). These results suggest cross-reactivity between the honey and RJ proteins.

In conclusion, the results of our study have led to the identification of a new isoallergen, the first *A. mellifera* food allergen, which should help to diagnosis of food-allergic patients.

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#### **Conflicts of Interest**

The authors declare that they have no conflicts of interest to disclose.

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## References

1. 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.
2. 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.
3. 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.
4. 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.
5. Ibero M, Castillo MJ, Pineda F, Palacios R, Martínez J. Whole bee for diagnosis of honey allergy. *Allergy*. 2002;57:557-8.
6. 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.
7. Aguiar R, Duarte FC, Mendes A, Bartolomé B, Barbosa MP. Anaphylaxis caused by honey: a case report. *Asia Pac Allergy*. 2017;7:48-50.
8. 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.
9. Burzyńska M, Piasecka-Kwiatkowska D. A Review of Honeybee Venom Allergens and Allergenicity. *Int J Mol Sci*. 2021;22:8371.
10. Erban 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.

11. 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.
12. Schmitzová J, Klaudivy 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.
13. 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.
14. 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.
15. 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.

**Figure 1.** Panel A, Protein separation by SDS-PAGE and Coomassie staining. Panel B, IgE-immunoblotting performed with sera of seven honey allergic patients. Lanes: M) molecular weight marker; ex) honey extract; fr) HPLC fraction corresponding to the allergen around 50 kDa.

