Successful Adaptation of Bee Venom Immunotherapy in a Patient Monosensitized to Api m 10

Ruiz-León B[1,2,5,*], Navas A[1,2,5,*], Serrano P[1,2,5], Espinazo M[2,5], Labrador-Horrillo M[3,5], Monsalve RI[4], Jurado A[1,2,5], Moreno-Aguilar C[1,2,5]

[1]*Department of Immunology and Allergy, Reina Sofia University Hospital, Cordoba, Spain*

[2]*Maimonides Biomedical Research Institute of Cordoba (IMIBIC)/ Reina Sofia University Hospital/University of Cordoba, Cordoba, Spain*

[3]*Allergy Section, Internal Medicine Department, Vall d'Hebron University Hospital, Barcelona, Spain*

[4]*Department of Research and Development, ALK-Abelló, Madrid, Spain*

[5]*National Network ARADyAL, Carlos III Health Institute, Madrid, Spain*

[*]*Both authors contributed equally*

J Investig Allergol Clin Immunol 2020; Vol. 30(4): 296-298
doi: 10.18176/jiaci.0498

Key words: Bee venom allergy. Api m 10. Effectiveness of venom immunotherapy. Molecular diagnosis.

Palabras clave: Alergia al veneno de abeja. Api m 10. Efectividad de la inmunoterapia con veneno. Diagnóstico molecular.

Bee venom immunotherapy (BVIT), although highly effective, does not protect 10%-15% of patients allergic to bee stings [1]. Even though the production of allergenic extracts is standardized, the real content of major components is not completely known, given the total content of allergenic proteins and the enzymatic activity of phospholipase A2 (Api m 1) and hyaluronidase (Api m 2).

To date, 12 allergens have been described as components of *Apis mellifera* venom (AMV). Api m 1, Api m 2, Api m 3, Api m 5, and Api m 10 are considered major allergens, and their specific IgE (sIgE) can be determined using commercially available techniques [1]. Api m 1 was the first described and is the most important; indeed, the presence of IgE to rApi m 1 is regarded as an unequivocal sign of sensitization to AMV. Nevertheless, undetectable sIgE to rApi m 1 does not exclude sensitization to AMV [2]. Therefore, sensitization to AMV can be extremely complex, and some of the many profiles defined have been associated with therapeutic failure [3]. In order to optimize the diagnosis of AMV allergy, it seems appropriate to consider determination of sIgE to the whole AMV extract, together with the available molecular compounds.

A 46-year-old part-time beekeeper who had reported large local reactions after bee stings and tolerance to wasp stings developed palmoplantar pruritus and generalized erythema with a sensation of oppression in the throat a few minutes after a honeybee sting on his right ear. He went immediately to the nearest hospital, where he experienced dizziness, tachycardia, and hypotension. He was successfully treated with intramuscular adrenaline, intravenous methylprednisolone, and dexchlorpheniramine. His REMA score was 2 [4]. The intradermal skin test performed with *A mellifera*, *Polistes dominula*, and *Vespula* species (ALK-Abelló SA) was negative consecutively at 1 µg/mL both 1 month after the sting reaction and 3 weeks later. sIgE and sIgG4 levels to whole AMV and its allergenic components (rApi m 1, rApi m 2, rApi m 3, rApi m 4 [manufacturer's prototype], rApi m 5, and rApi m 10; ImmunoCAP, Thermo Fisher Scientific) were quantified (Table). The basal tryptase value (ImmunoCAP) was 5.98 µg/L.

IgE-immunoblot was performed using a lyophilized preparation obtained from raw bee venom (In-House Reference [IHR], ALK-Abelló, Madrid, Spain) and the patient's serum (Supplementary Figure 1). The results showed specific recognition of 2 bands (50-55 kDa), which matched the main molecular variants of Api m 10 [5].

The basophil activation test (BAT) was performed by incubating 0.1 and 1 µg/mL of AMV (Pharmalgen, ALK-Abelló) with whole blood and staining with the CD63-FITC/ CD123-PE/anti-HLA-DR-PerCP cocktail (BD FastImmune, Becton, Dickinson) before starting BVIT and 1 year later (Table).

Table. sIgE and sIgG4 Levels and Percentage of CD63+ Basophils

	T0	T1	T2
sIgE, kU_A/L			
Apis mellifera	38.6	11.1	5.12
rApi m 1	0.08	0	0
rApi m 2	0.01	0	0
rApi m 3	3.55	1.32	1.32
Api m 4[a]	0	0	0
rApi m 5	1.10	0.91	0.5
rApi m 10	65	14.8	12.3
sIG4, mg/L			
Apis mellifera	163	7322	11735
rApi m 1	<1.00	3357	8231
rApi m 2	<1.00	1858	2129
rApi m 3	<1.00	145	276
Api m 4[a]	<1.00	341	1430
rApi m 5	38.4	98.8	286
rApi m 10	<1.00	<1.00	33.1
Basophils CD63+, %			
Negative control[b]	0.7	1.1	ND
Positive control[b]	49.3	36.0	ND
0.1 µg/mL AMV	9.3	7.1	ND
1 µg/mL AMV	75.8	22.1	ND

Abbreviations: AMV, *Apis mellifera* venom; T0, baseline; T1, 1 year after starting VIT; T2, 2 years after starting venom immunotherapy.
[a]Api m 4 (melittin sequence: H-GIGAVLKVLTTGLPALISWIKRKRQQ-OH from Schafer-N ApS) was coupled into CAPs, which were activated by Thermo Fisher Scientific Inc., to be able to quantify sIgE and sIgG4 levels.
[b]Phosphate-buffered saline and fMet-Leu-Phe were used as negative and positive controls, respectively

Sensitization was diagnosed based on the AMV sIgE level and a positive BAT result at 1 µg/mL of AMV (this high concentration was possibly adequate to provide enough Api m 10 to stimulate the basophils). Molecular sIgE and immunoblot results, together with clinical data, led to a final diagnosis of Müller grade IV anaphylaxis to honeybee venom, with major sensitization to Api m 10 (Table).

Before selecting the best therapeutic approach, 4 commercial extracts were analyzed to detect which most successfully inhibited sIgE of Api m 10 [6]. The best result (31% inhibition) was obtained when 20 µg of Pharmalgen AMV extract was reconstituted immediately and incubated with 100 µL of the patient's serum (ImmunoCAP inhibition). Treatment with Pharmalgen AMV from the same test batch was then started without premedication and in a cluster schedule to reach the therapeutic dose in 4 weeks. An arbitrary dose of 300 µg was planned in order to protect this patient with double the risk of therapeutic failure (predominant sensitization to a very rare protein and beekeeping). No adverse events were recorded. Since then, the patient has been taking 300 µg monthly as a maintenance dose; tolerance has been good for the last 2 years. All vials were reconstituted immediately before use to avoid degradation of Api m 10, although Blank et al [7] demonstrated the stabilizing effect for Api m 10 of human serum albumin, which is used as a diluent in commercial therapeutic extracts.

A controlled sting challenge was performed 1 and 2 years after starting BVIT, according to Moreno et al [8], with negative results in both cases. Moreover, the patient experienced a field sting 15 months after starting BVIT, with no reaction. The result of the intradermal test with AMV remained negative. The progress of sIgE and sIgG4 levels, as well as BAT results, is shown in the Table.

Api m 10, a 23-kDa glycosylated protein, is considered a genuine and relevant major allergen, despite the fact that it only represents <1% of the venom dry weight. Some patients are exclusively or predominantly sensitized to Api m 10, which has been associated with failure of BVIT [5]. Nevertheless, studies performed to date do not include a molecular analysis of sensitization to honeybee venom components before starting BVIT [9,10] or propose a solution for treatment.

We report the case of a patient who was predominantly (almost exclusively) sensitized to Api m 10 and treated using an effective specific BVIT strategy. He tolerated 2 controlled in-hospital stings and a field sting without anaphylactic reactions. We observed the intended decrease in sIgE and increase in sIgG4 throughout BVIT, both to the whole venom extract and to its specific allergenic components, even though production of rApi m 10 sIgG4 was lower than that of rApi m 1 and rApi m 2 sIgG4. A progressive decrease in the percentage of $CD63^+$ basophils was also detected. The persistence of positive sIgE values and degranulated basophils with a negative response to a controlled-sting challenge suggests that the latter remains the gold standard for assessment of the effectiveness of BVIT.

The strategy used to achieve protection was the selection of a nonpurified AMV extract, which had previously showed the strongest IgE inhibition to Api m 10, and an arbitrary chosen triple maintenance dose to reach a potentially protective dose. BVIT in patients predominantly sensitized to Api m 10 is challenging owing to the low presence of this protein in the whole extract. We present a therapeutic approach based on 3 points: (*1*) molecular diagnosis using both whole venom extract and all commercially available molecular allergens; (*2*) tailored selection of the best available extract in terms of Api m 10 content; and (*3*) a high dose of BVIT.

Additional cases are necessary to validate these results, together with examination of other possibilities to improve the effectiveness of BVIT.

Funding

This study was cofunded by the Spanish Health Ministry (PI1502170 to CM, Carlos III Health Institute), the Spanish Society of Allergology and Clinical Immunology, and the Andalusian Society of Allergology and Clinical Immunology.

Conflicts of Interest

Dr. Labrador-Horrillo reports personal fees from Alk-Abelló S.A. outside the submitted work.

Dr. Monsalve currently works at Alk-Abello S.A.

The remaining authors declare that they have no conflicts of interest.

References

1. Alfaya-Arias T, Soriano-Gomis V, Soto-Mera T, Vega-Castro A, Vega-Gutierrez JM, Alonso-Llamazares A, et al. Key Issues in Hymenoptera Venom Allergy: An Update. J Investig Allergol Clin Immunol. 2017;27:19-31.
2. Kohler J, Blank S, Muller S, Bantleon F, Frick M, Huss-Marp J, et al. Component resolution reveals additional major allergens in patients with honeybee venom allergy. J Allergy Clin Immunol. 2014;133:1383-9.
3. Frick M, Fischer J, Helbling A, Rueff F, Wieczorek D, Ollert M, et al. Predominant Api m 10 sensitization as risk factor for treatment failure in honey bee venom immunotherapy. J Allergy Clin Immunol. 2016;138:1663-71.
4. Alvarez-Twose I, Gonzalez de Olano D, Sanchez-Munoz L, Matito A, Esteban-Lopez MI, Vega A, et al. Clinical, biological, and molecular characteristics of clonal mast cell disorders presenting with systemic mast cell activation symptoms. J Allergy Clin Immunol. 2010;125:1269-78.
5. Blank S, Seismann H, Michel Y, McIntyre M, Cifuentes L, Braren I, et al. Api m 10, a genuine A. mellifera venom allergen, is clinically relevant but underrepresented in therapeutic extracts. Allergy. 2011;66:1322-9.
6. Straumann F, Bucher C, Wuthrich B. Double sensitization to honeybee and wasp venom: immunotherapy with one or with both venoms? Value of FEIA inhibition for the identification of the cross-reacting ige antibodies in double-sensitized patients to honeybee and wasp venom. Int Arch Allergy Immunol. 2000;123:268-74.
7. Blank S, Etzold S, Darsow U, Schiener M, Eberlein B, Russkamp D, et al. Component-resolved evaluation of the content of major allergens in therapeutic extracts for specific immunotherapy of honeybee venom allergy. Hum Vaccin Immunother. 2017;13:2482-9.