Successful adaptation of bee venom immunotherapy for a monosensitized patient to Api m 10

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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, considering the total content of allergenic proteins and the enzymatic activity of A2-phospholipase (Api_m_1) and hyaluronidase (Api_m_2), the real content of these major components is not completely known.

To date, twelve 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, being the determination of specific IgE (sIgE) commercially available [1]. Api_m_1 is the first described and the most important; and indeed, the presence of IgE to rApi_m_1 is regarded as an unequivocal sign of sensitization to AMV; nevertheless, the undetectable sIgE to rApi_m_1 does not exclude sensitization to AMV [2]. Therefore, sensitization to AMV can be extremely complex and many profiles have been defined, some of them specifically associated with therapeutic failure [3]. In order to optimize the diagnosis of AMV allergy it seems appropriate to consider the determination of sIgE to the whole AMV extract together with the available molecular compounds.

A 46-year-old man, part-time beekeeper, reporting large local reactions after bee stings and tolerance to wasp stings, developed palmoplantar-pruritus and generalized erythema with a throat oppression feeling a few minutes after a honeybee sting on his right ear.

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The patient immediately attended the nearest hospital, where he suffered dizziness, tachycardia and hypotension. He was successfully treated with intramuscular adrenaline, intravenous methylprednisolone and dexchlorpheniramine. REMA score was 2 [4]. The intradermal skin test (IST) performed with *Apis mellifera, Polistes dominula and Vespula species* (ALK-Abelló S.A) was negative consecutively at 1µg/ml, both one 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 (manufactor prototipe), rApi_m_5 and rApi_m_10; ImmunoCAP, Thermo Fisher) were quantified (Table 1). Basal tryptase value (ImmunoCAP) was 5.98µg/L.

Complementing this, IgE-Immunoblot was performed using a lyophilized preparation obtained from raw bee venom (named IHR-In house Reference ALK-Abelló Madrid, Spain) and the patient serum (Supplementary Figure 1). The results showed specific recognition of two bands (50-55kDa), which matched the main molecular variants of Api_m_10 [5].

In addition, BAT was performed incubating 0.1 and 1µg/mL of AMV (Pharmalgen® ALK-Abelló S.A) with patient total blood and staining with the CD63-FITC/CD123-PE/anti-HLA-DR-PerCP cocktail (BD FastImmune^{TM}, Becton, Dickinson and Company) before starting BVIT, and one year later (Table 1).

Diagnosis of sensitization was performed according to the sIgE level to AMV and to the BAT positive at 1µg/mL of AMV (this high concentration was possibly adequate to provide enough content of Api_m_10 to stimulate basophils). Molecular sIgE and immunoblot results, together with clinical data led to the final patient diagnosis of Müller Grade IV-Anaphylaxis to honeybee-venom, with major sensitization to Api_m_10 (Table I).

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Before selecting the best therapeutic approach, four commercial extracts were analyzed to detect which reached the highest sIgE Api_m_10 inhibition [6]. The best result (31% of inhibition) was obtained when 20µg of Pharmalgen® AMV extract immediately reconstituted, which was incubated with 100µl of the patient serum (InmunoCAP inhibition). Treatment with Pharmalgen® AMV from the same tested batch was then started, without pre-medication and in a *cluster schedule* to reach the therapeutic dose in 4 weeks. An arbitrary dose of 300µg was planned in order to protect this patient with double the risk of therapeutic failure: predominant sensitization to a very scarce protein and the beekeeping exposure. No adverse events were recorded. Since then, 300µg as maintenance dose were implemented monthly for 2 years, and well tolerated. All vials were reconstituted immediately before their use, to avoid any possible Api_m_10 degradation, albeit Blank et al. demonstrated the stabilizing effect for Api_m_10 of human serum albumin, used as dilutent in commercial therapeutic extracts [7].

A controlled-sting-challenge was performed one and two years after starting BVIT, according to Moreno et al. [8], with negative results in both cases. Moreover, the patient suffered a field sting 15 months after starting BVIT, without any reaction. The IST with AMV persisted negative. Evolution of sIgE and sIgG4 levels, as well as BAT results, are shown in Table1.

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 BVIT failure [5]. Nevertheless, neither the studies to date include a molecular analysis of the sensitization pattern to the honeybee venom components before starting BVIT [9, 10], nor propose a solution to treat these patients.

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We describe the prospective case report of a patient predominantly (near exclusively) sensitized to Api_m_10, demonstrating the effectiveness of BVIT using a particular strategy, by tolerating two intra-hospital-controlled stings and a field sting without anaphylactic reactions. The intended sIgE decrease and sIgG4 increase throughout BVIT both to the whole venom extract and to its specific allergenic components, were observed, even though the production of rApi_m_10 sIgG4 was less 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 controlled-sting challenge, suggest that the latter remains as the gold-standard to assess the BVIT effectiveness.

The strategy used to achieve protection was the selection of a non-purified AMV extract, which previously showed the strongest IgE inhibition to Api_m_10 and an arbitrary triple maintenance dose to reach a dose potentially protective. BVIT in patients predominantly sensitized to Api_m_10 is challenging due to the scarce presence of this protein in the whole extract. We present a therapeutic approach based on three points: 1) molecular diagnosis using both whole venom extract and all the commercially available molecular allergens, 2) selecting for each patient the best available extract in terms of Api_m_10 content and 3) giving a high BVIT dose.

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

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Conflicts of interest

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

Dr. Monsalve reports and I currently work at the company alk abello S.A.

The remaining authors have no conflicts of interest to declare.

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Table1. sIgE and sIgG4 levels and percentage of $CD63^+$ basophils

	T0	*T1*	*T2*
sIgE (kU/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

T0, baseline; T1, one year after starting VIT; T2, two years after starting VIT; AMV, *Apis mellifera* venom.

[a] Api m 4 (melittin sequence: H-GIGAVLKVLTTGLPALISWIKRKRQQ- OH from Schafer-N ApS, Denmark) was coupled into CAPs, which were activated by Thermofisher Scientific Inc., to be able to quantify sIgE and sIgG4 levels.

[b] Phosphate buffer saline and fMet-Leu-Phe were used as negative and positive controls, respectively.

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