

DEVELOPMENT OF NEW IGE SPECIFICITIES TO HYMENOPTERA ALLERGENS DURING VENOM SPECIFIC IMMUNOTHERAPY*

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SUMMARY:

Background: Despite the benefits of specific immunotherapy (SIT) being clinically well documented for allergic diseases, new IgE specificities to SIT extract allergens could be induced during the treatment. The authors evaluated these changes in patients allergic to *Hymenoptera*.

Methods: Six patients allergic to *Hymenoptera* venom were included in the study. Specific IgE (sIgE) levels determination and IgE immunoblots to *Apis mellifera*, *Vespula* spp. and *Polistes* spp. venom were performed before and after one year of SIT.

Results: All patients had sIgE levels reduction, after the first year of treatment, except one in whom there was an increase in sIgE levels to *Apis mellifera* venom, and two patients that maintained a similar value for *Vespula* spp. venom sIgE before and after one year of treatment.

The immunoblot analysis revealed that most of the bands detected before beginning SIT, decreased in intensity or disappeared after one year of treatment. 3/6 patients developed new IgE specificities to venom extracts: one patient to the venom allergens in the treatment, other patient to allergens in other venom and another patient to both. After one year of treatment one of these patients tolerated a field sting by the corresponding insect. The newly recognised proteins were all *minor* allergens.

Conclusion: These results confirm that sIgE levels tend to reduce during SIT, and the bands identifying some allergens in the blot tend to decrease or disappear. Nonetheless venom SIT can be responsible for the induction of new sensitisations to other venom allergens, apparently without clinical relevance.

Key-words: New IgE specificities - Specific immunotherapy - *Vespula*, *Apis mellifera*, *Polistes*.

INTRODUCTION

Specific immunotherapy (SIT) is a highly effective treatment for allergic diseases in selected patients with sensitivity to one or a limited number of allergens (1, 2). Its efficacy is clinically well-documented in rhinoconjunctivitis and asthma due to inhalant allergens (2) as well as in allergy to *Hymenoptera* venom (3). Immunotherapy provides long-term benefit for at least several years after discontinuation (4,5) and it has been shown to modify the natural history of allergic disease (6) as well as to prevent the onset of new sensitizations (7). The aim of SIT is to induce immunological tolerance against the administered allergen (2). The mechanisms underlying this effect are not fully understood and are still under investigation. During the treatment, there is an increase in allergen specific IgG production, particularly the IgG4 isotype (8-10). After a transitory increase

of allergen specific IgE, often reported during the early phase of SIT, it decreases throughout time (11-13). The immunological mechanism behind the initial increase of specific IgE is not known, but elevated IL-4 production by T cells during the early phase of SIT has been reported (14,15). Immunotherapy changes the allergen-specific T cells cytokine profile from Th2 to Th1 (16-19) and reduces the T cell proliferative response to allergens (16, 18, 20-22). More recently, the induction by SIT of IL-10 producing T cells, which might be regulatory T cells, was evidenced (23-25). Despite all these benefits, some studies have shown that SIT did not exert any preventive effect against de novo sensitization to other allergens (26, 27) and new IgE specificities to SIT extract allergens could be induced during immunotherapy (11,22,28,29). In some patients, these IgE levels are low with clinical relevance unknown (29), but in other patients the levels can be high and elicit other allergies (28). The appearing of new IgE specificities in patients on Vespid venom SIT, by means of RAST absorption assays was previously described (30). The aim of this study was to assess by Western blot technique, the changes in venom specific IgE reactivities

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* MEETING of GALL Alcacer-Portugal 23-25 April 2005.

to *Apis mellifera*, *Vespula* spp. and *Polistes* spp. venoms of patients allergic to Hymenoptera after one year on venom immunotherapy.

METHODS

■ **Study design:** The sera from patients allergic to *Hymenoptera* venom were analysed by means of both specific IgE level determination and western blot analysis before and after one year of treatment with *Hymenoptera* venom extract.

■ **Patients:** Six patients (3 female, 3 male; age 19-51 years old, mean: 43 ± 12 years), allergic to *Hymenoptera* venom were included in the study. Three of them, allergic to honeybee (P1, P2 and P3), underwent *Apis mellifera* venom SIT (Albay/Stallergens); the other three patients, allergic to yellow jacket (P4, P5 and P6), underwent *Vespula* spp. venom SIT (Albay/Stallergens), according to a rush protocol. They all reached monthly 100µg maintenance dose and alleged not having suffered any field bite during treatment (Table 1).

■ **Venom skin tests:** Skin prick tests (SPT; concentration: 10µg/ml) and intradermal skin tests (IDT; concentrations: 0.1ng/ml – 1µg/ml) were performed using commercially available extracts (ALK Abelló) in all patients, before starting SIT. IDT being performed whenever the SPT showed negative (wheal diameter < 3mm above the negative control) and were considered positive for a wheal diameter ≥ 5 mm above the negative control.

■ **Specific IgE level determination:** Specific IgE (sIgE) levels determination to *Apis mellifera*, *Vespula* spp. and *Polistes* spp. venom were performed before and

after one year of treatment, utilising the UniCAP® immunoassay system (Pharmacia Diagnostics) according to the manufacturer's instructions. The cut off value of the assay was 0.35 KU/L.

■ **Western blot analysis:** IgE immunoblots to *Apis mellifera*, *Vespula* spp. and *Polistes* spp. venom were performed before and after one year of treatment, with the AlaBLOT® specific IgE test system according to the manufacturer's instructions (DPC® Diagnostic Products Corporation). The immunoblot bands were determined by a combination of visual and computer based evaluations. Immunoblots were scanned and evaluated qualitatively and semi-quantitatively by means of densitometry to detect the bands. By measuring the distance of each band from the dye front, the molecular weights (MW) of the extract components were calculated according to a formula using molecular mass markers ranging from 14 to 200 kDa.

RESULTS

■ **IgE levels:** In general all patients had sIgE levels reduction after the first year of treatment, except P6 in whom there was an increase in sIgE levels to *Apis mellifera* venom, and P3 and P4 that maintained a similar value for *Vespula* spp. venom sIgE before and after one year of treatment (table 2).

IMMUNOBLOT ANALYSIS

Before the treatment all the patients sera recognised a 16 kDa protein in the *Apis mellifera* blot, a protein with a MW similar to that of phospholipase A2. Five patients sera detected a 23 kDa protein in the Vespid

Patient nr	Sex	Age (yr)	Hymenoptera allergy	Müller class (31)	Total IgE (UI/mL)	Atopic status ¹	Skin tests ²	Venom extract for SIT
1	M	19	<i>A. mellifera</i>	IV	396	-	IDT+ for A. mel	<i>A. mellifera</i>
2	M	45	<i>A. mellifera</i>	IV	56	-	IDT+ for A. mel	<i>A. mellifera</i>
3	F	49	<i>A. mellifera</i>	IV	97	-	IDT+ for A. mel	<i>A. mellifera</i>
4	F	49	<i>Vespula</i> spp.	IV	32	-	ST+ for Vesp	<i>Vespula</i> spp.
5	M	47	<i>Vespula</i> spp.	III	564	RA	IDT+ for Vesp	<i>Vespula</i> spp.
6	M	51	<i>Vespula</i> spp.	III	386	-	IDT+ for Vesp	<i>Vespula</i> spp.

Table 1: Demographic and immunological data of the patients included in the study.

1: Atopic status – Individual history of allergic rhinitis (R), allergic conjunctivitis (C), atopic dermatitis (AD) or allergic bronchial asthma (A). – no individual history of atopic diseases.

2: SPT – skin prick test; IDT – intra-dermal test; ST – SPT and IDT ; + - positive; A. mel – *Apis mellifera*; Vesp – *Vespula* spp.

All the patients had positive IDT for the correspondent venom at the concentration 0.1 ng/ml.

blots, a protein with MW similar to that of antigen 5. Proteins with MW similar to that of the other *major* venom allergens were less frequently recognised (32). All the patients had sIgE to other allergens with MW in the range of *minor* allergens, except P4 that had only specific IgE antibodies to a protein with 16 kDa for *Apis mellifera* venom and two patients allergic to honeybee that had only sIgE to proteins with 23 kDa and 35 kDa for *Polistes* venom. Table 3 summarizes the blot analysis.

The IgE specificities to various allergens, changed during SIT. Most of the bands detected before beginning SIT, decreased in intensity or disappeared after one year of treatment. The proteins with MW similar to *major* allergens were included in this group, except for P6 in whom sIgE to a protein with 16 kDa rose in the *Vespid* blots and sIgE to proteins with 23 kDa and 44 kDa rose in the *Polistes* blot. Three out of six patients developed new IgE specificities to venom extracts. One of them, to the allergens in the venom for which he was treated; other patient, to the aller-

gens present in other venom and another patient for both venoms (figure 1). P1 revealed after one year of treatment with honeybee venom, a reduction of all the three bands detected in the *Apis mellifera* immunoblot. The analysis of *Vespula* immunoblot showed that the band with 47 kDa disappeared and three new bands with 25, 31 and 44 kDa respectively appeared (Figure 2a). Bands with MW similar to these new ones were not found in the other blots. P5 treated with *Vespula* venom, revealed in *Apis mellifera* immunoblot analysis, the disappearance of two bands with 12 and 44 kDa, the reduction of one band with 16 kDa, and the appearance of a new IgE specificity to an allergen with 47 kDa (Figure 2b). The analysis of *Vespula* immunoblot showed that after one year of SIT, six bands with 24, 31, 38, 42, 43 and 92 kDa respectively disappeared, while two bands with 45 and 47 kDa appeared de novo. The last one had a similar MW to the one detected in *Apis mellifera* immunoblot (Figure 2b). P6 revealed a more complex specific antibody pattern. After one year of treatment with *Vespula* venom extract, there was a reduction of

Patient nr	<i>Apis mellifera</i> Class (KU/L)		<i>Vespula</i> spp. Class (KU/L)		<i>Polistes</i> spp. Class (KU/L)	
	T0	T1	T0	T1	T0	T1
1	5 (62.4)	4 (19.0)	0 (<0.35)	0 (<0.35)	2 (0.96)	0 (<0.35)
2	2 (2.1)	2 (1.5)	0 (<0.35)	0 (<0.35)	0 (<0.35)	0 (<0.35)
3	3 (6.9)	2 (3.3)	2 (1.9)	2 (1.8)	2 (1.36)	1 (0.6)
4	0 (<0.35)	0 (<0.35)	1 (0.6)	1 (0.5)	0 (<0.35)	0 (<0.35)
5	3 (7.8)	3 (3.6)	3 (10.1)	3 (4.6)	3 (6.1)	2 (2.9)
6	3 (10.9)	3 (15.1)	4 (24.5)	3 (16.7)	3 (7.01)	3 (5.5)

Table 2: Specific IgE levels to *Apis mellifera*, *Vespula* spp. and *Polistes* spp. venoms before (T0) and one year after beginning SIT (T1).

Patient	Venom extract for SIT	A. mellifera					Vespula spp.					Polistes spp.				
		T0	T1	–	–	N	T0	T1	–	–	N	T0	T1	–	–	N
			D					D					D			
P1	A. mel	3 PA2,M	3 PA2,M				1 M	1 M			3 H,M					
P2	A. mel	2 PA2,M	1 PA2	1 M			2 A,M	2 A,M				1 A	1 A			
P3	A. mel	2 PA2,M	1 PA2	1 M			6 A,H,M	5 A,H,M	1 M			2 A,PA1	2 A,PA1			
P4	Vesp.	1 PA2	1 PA2				3 A,PA1,M	3 A,PA1,M				3 A,PA1,M	3 A,PA1,M			
P5	Vesp.	3 PA2,H,M	2 H,M	1 PA2		1 M	6 A,H,M	6 A,H,M			2 M	3 A,M			3 A,M	
P6	Vesp.	5 PA2, M	1 M	1 PA2	3 M		8 A,PA1,H,M	3 M	2 A,M	3 PA1,M	4 M	8 A,PA1,H,M	3 M		5 A,PA1,H,M	

Table 3: Immunoblot analysis before (T0) and after one year of treatment (T1). Allergens with MW similar to the known venom allergens were regarded as the same proteins. The results are expressed in number of bands and below, specific bands for phospholipase A2 (PA2), hyaluronidase (H), phospholipase A1 (PA1), antigen 5 (A) and *minor* antigens (M).

D – Bands that have disappeared; – - increased the intensity; - - decreased the intensity; N – new bands.

A. mel – *Apis mellifera*; Vesp. – *Vespula* spp.;

some bands and total disappearance of others in immunoblot to *Vespula*, *Polistes* and *Apis mellifera*. Some bands in immunoblot to *Vespula* and *Apis mellifera* showed increased intensity and four new bands with 30, 77, 82 and 101 kDa appeared, in immunoblot to *Vespula* (Figure 2c).

The MW of the newly recognised allergens differed from the MW of the *major* known venom allergens, except for P1 serum that identified a protein of 44 kDa in immunoblot to *Vespula*, possibly the hyaluronidase.

After one year of treatment, P5, that showed new IgE sensitivities for *Vespula* venom, tolerated a yellow jacket sting with only a local reaction. P6, allergic to *Vespula* venom, tolerated a honey bee sting despite an increase of intensity of some bands in *Apis mellifera* immunoblot.

DISCUSSION

In this study we found in almost all patients a reduction in the venom sIgE levels after the first year of treatment. Only one patient (P6) allergic to *Vespula* venom, revealed an increase in sIgE levels to *Apis mellifera* venom, and two patients (P3 and P4) maintained a similar value for *Vespula* venom sIgE before and after one year of treatment. These findings are in accordance with those found in literature. After a temporary rise in allergen sIgE level, often reported during the early phase of SIT, it decreases throughout time (11–13).

Before treatment all patients sera recognised a protein with MW similar to phospholipase A2 (16 kDa) in

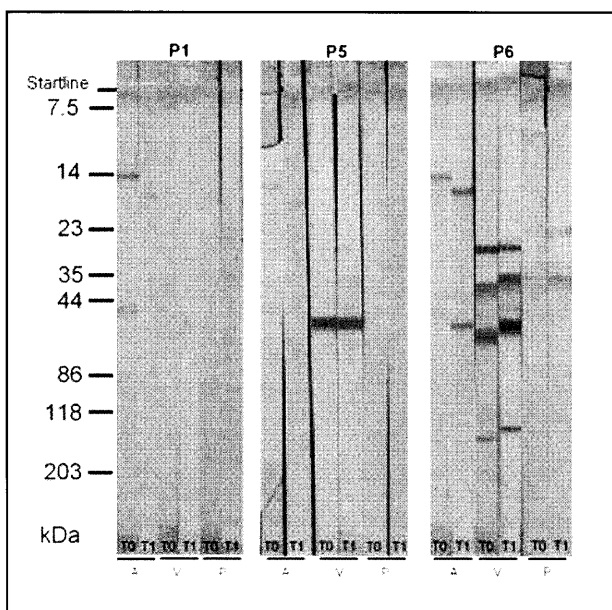


Figure 1.

the *Apis mellifera* blot. Five patients detected a protein with MW similar to antigen 5 (23 kDa) in the Vespid blots. Proteins with MW similar to that of the other *major* allergens in honeybee venom (hyaluronidase - 44 kDa) and in Vespid venoms (phospholipase A1 - 34-35 kDa and hyaluronidase - 44 kDa) (32) were less frequently recognised. These data agree with the findings described in other papers.

Whether the administration of allergen extracts in the course of immunotherapy can induce new IgE specificities and clinically relevant sensitizations to new allergens have been studied in the past years. Immunoblotting is a powerful technique for evaluating visually and semi-quantitatively, specific reactivities to individual allergenic components. This issue makes this assay a useful technique for evaluating the antibody profile of specific allergens.

Juarez C et al. showed in a previous study, the induction of specific antibodies to Vespids in the course of SIT with *Vespula germanica* administered to patients sensitized to *Polistes dominulus* (30). In this study determinations of venom sIgE were performed by means of RAST absorption method and RAST inhibition assays. The authors found that after SIT, 41% of the patients had IgE antibodies directed to specific epitopes of *Vespula germanica* venom that were not

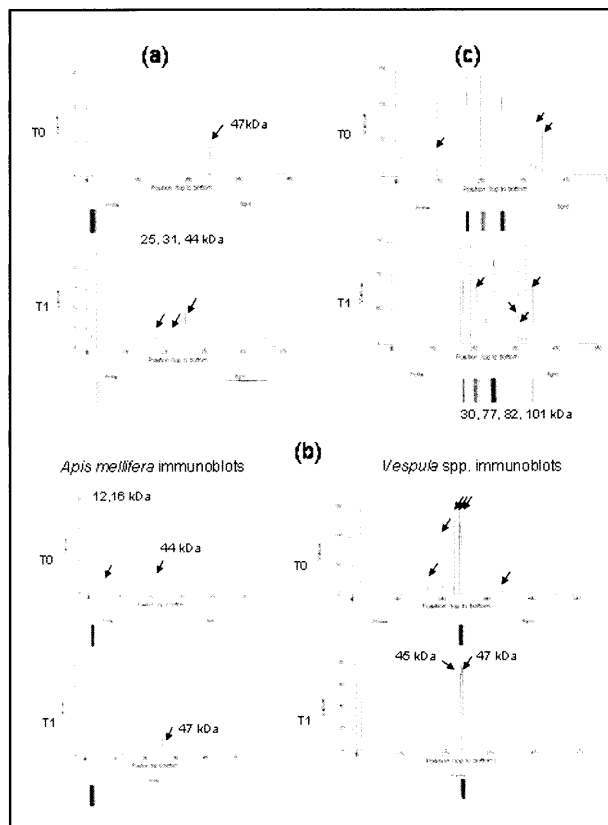


Figure 2.

initially detected in the basal sera. In one case the antibodies increased only to *Vespula germanica* venom without a corresponding rise to *Polistes dominulus*. In our study, despite the disappearance or decrease of most bands after SIT, half the patients developed new IgE specificities to venom extracts. One honeybee allergic patient serum (P1), detected after the treatment in *Vespula* blot, a protein with MW similar to hyaluronidase and two other bands correspondent to *minor* allergens, not found in the other venom blots. These findings could raise the question if this band of 44 kDa does actually correspond to hyaluronidase. If this patient was not really stung during the treatment by any Hymenoptera one wonders why he developed new IgE specificities to allergens present in a different venom from that used for treatment. Allergen lacking in the *Apis mellifera* and *Polistes* blots could be the answer.

The other two patients allergic to *Vespula* venom revealed after the treatment, new bands correspondent to *minor* allergens in the *Vespula* blot. A protein of 47 kDa detected by one of the patients (P5), was also detected in the *Apis mellifera* blot by the same serum. This is probably a cross-reactive allergen present in both venoms.

The development of new IgE specificities to SIT extract allergens has been proved to occur during immunotherapy with airborne allergen extracts (11,22,28,29). In some patients, the IgE levels are low with clinical relevance unknown (29), but in other patients the levels can be high and elicit other allergies. The possible induction of food allergy during SIT has been documented by Van Ree et al. (28). In this study, some patients submitted to house dust mite SIT, developed new IgE antibodies to snail and shrimp, probably cross-reactive antibodies to tropomyosin, and to aggravated clinical symptoms when eating shrimp. In our study, after one year of treatment P5 that showed new IgE sensitivities for *Vespula* venom, tolerated a yellow jacket sting with only a local reaction. P6 allergic to *Vespula* venom tolerated a honey bee sting despite an increase of intensity of some bands in *Apis mellifera* immunoblot.

The immunological mechanism behind the induction of new IgE specificities is not known. Movérare R et al. suggested that as the establishment of clones of Th2 cells with the capacity to produce high levels of IL-4 is believed to be an early crucial step in the development of allergen specific IgE sensitization, one cannot exclude that new clones of allergen-specific Th2 cells might have been established earlier, preceding the switch of allergen-specific B cells (29).

The results of our study confirm that sIgE levels tend to reduce during SIT, and the bands identifying some allergens in the blot tend to decrease or disappear. Nonetheless venom SIT can be responsible for the induction of new sensitisations to other venom allergens, apparently without clinical relevance.

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