ORIGINAL ARTICLE

Alternating *Polistes–Vespula* Venom Immunotherapy: A Therapeutic Strategy to Resolve a Diagnostic Deficiency

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Abstract

Background: In Mediterranean regions, double sensitization to *Polistes* and *Vespula* species is frequent in patients reacting to an unidentified wasp sting. Since both genera are present, it is often difficult to determine which insect is responsible and, consequently, select venom for immunotherapy. When a specific diagnosis cannot be made, a new therapeutic strategy should be undertaken.
Methods: We performed a case-control study in which 37 patients who were allergic to venom from *Vespula* and *Polistes* species received a 3-year schedule of alternating immunotherapy. Twenty monosensitized patients (10 to *Vespula* and 10 to *Polistes*) received conventional venom immunotherapy (VIT) during the same period. All 57 patients received the same number of injections. The effectiveness of VIT was assessed by means of re-sting, which was performed yearly. Serum specific immunoglobulin (Ig) E and IgG4 were also studied.
Results: All the cases tolerated all the stings. One control patient developed a mild systemic reaction after the first-year *Vespula* sting but tolerated subsequent re-stings. Both cases and controls reached significant changes in levels of IgE and IgG4 after VIT ($P<.04$ at minimum). The cases developed a response as expected, although this was less intense than in the control group. In the *Polistes* control subgroup, sIgE to *Polistes* decreased to under baseline levels, after a marked initial increase; this decrease was not observed in the *Vespula* subgroup.
Conclusion: An alternating VIT strategy is appropriate and provides protection to patients sensitized to *Vespula* and *Polistes*.

Key words: Venom Immunotherapy. *Polistes*. *Vespula*. Hymenoptera allergy.

Resumen

Introducción: En regiones mediterráneas es frecuente que los pacientes que sufren reacciones frente a picadura de avispa no identificada estén sensibilizados doblemente a *Vespula* y *Polistes*. La coexistencia de ambos géneros en el medio a menudo hace difícil la identificación del insecto responsable y como consecuencia, la selección de un veneno para inmunoterapia. En los casos en que no es posible el diagnóstico preciso, puede optarse por una estrategia terapéutica sustitutiva.
Metodología: Estudio longitudinal prospectivo de casos y controles. Treinta y siete pacientes alérgicos a venenos de *Vespula sp* y *Polistes sp*, recibieron un programa de inmunoterapia combinada-alterna durante tres años (casos). Veinte pacientes monosensibles (diez a a *Vespula sp* y diez a *Polistes sp*) recibieron inmunoterapia convencional a un veneno durante el mismo período (controles). Los cincuenta y siete recibieron el mismo número de inyecciones. Se comprobó la eficacia de la inmunoterapia mediante un test de repicadura controlada cada año. También se realizó seguimiento annual de los niveles de IgE e IgG4 específicas.
Resultados: Todos los casos toleraron las picaduras realizadas. Un único control desarrolló una reacción sistémica leve tras la picadura de *Vespula* en el primer año, tolerando las sucesivas. La IgE específica y la IgG4 específica mostraron respectivamente descensos y elevaciones significativas tanto en los casos como en los controles ($p<0,04$ como mínimo). La respuesta entre los casos fue de igual significado pero de menor magnitud que entre los controles. En el subgrupo control *Polistes*, la IgE frente a *Polistes* cayó bajo los niveles basales ($p=0,002$) tras una marcada elevación inicial ($p<0,04$). En el subgrupo control *Vespula* no se observó la elevación inicial.
Conclusiones: La estrategia de inmunoterapia combinada-alterna es practicable y garantiza la protección de pacientes sensibilizados a *Vespula* y *Polistes*.

Palabras clave: Inmunoterapia con venenos. *Polistes*. *Vespula*. Alergia a himenópteros.

Introduction

Selection of venom for immunotherapy is usually easy when the patient is allergic to bee sting. However, in the case of allergy to wasp sting, this decision is more difficult, because of the patient's inability to identify the stinging insect and the high degree of cross-reactivity between venoms from vespids.

In the south of Europe, particularly in Spain, *Vespula germanica* and *Polistes dominulus* are evenly distributed [1]. Double sensitization is common in patients who experience anaphylaxis after wasp sting [2,3] and require immunotherapy.

Double sensitization means that it is difficult to identify a specific type of venom. Although component-resolved diagnosis enables specific sensitization profiles to be detected in patients with complex allergies, adequate products for daily practice are not yet commercially available. Furthermore, subsequent episodes of anaphylaxis and high environmental pressure from different species of wasp compound the problem in some regions.

The pattern described above requires an effective reply from clinicians, and 2 complete courses of VIT may be necessary to provide patients with adequate protection. However, the high number of visits to the immunotherapy unit may be inconvenient and render adherence difficult, thus resulting in unsuccessful treatment.

We analyzed a new venom immunotherapy (VIT) strategy based on alternate monthly injections of each extract in patients who are doubly sensitized to wasps (*Vespula* species and *Polistes* species). We assessed the effectiveness of this strategy by means of controlled re-sting challenge. Variations in serum specific immunoglobulin (sIg) E and sIgG4 titers were also monitored. We compared the results with those from patients sensitized to 1 species of wasp only (*Vespula* or *Polistes*) who received conventional immunotherapy with a single extract.

Material and Methods

Design

The study sample was chosen from a cohort of patients sensitized to 2 different species of wasp (cases) who were recruited over a period of 24 months. A group of monosensitized patients (controls) was chosen at random from a database created for follow-up of Hymenoptera venom–allergic patients during the same period.

Patients

The final sample comprised 57 patients living in the south of Spain who had complained of an immediate systemic reaction after wasp sting. All the patients were treated with VIT (Table 1). Of the 57 patients, 37 reported an unidentified wasp sting and were sensitized to *Polistes* and *Vespula* venoms, although it was not possible to establish the responsibility of a particular genus (cases). In the remaining 20 patients (controls), the wasp was identified (10 *Polistes* and 10 *Vespula*). The same diagnostic protocol revealed single or predominant sensitization to the corresponding venom. Sensitization was considered predominant when levels of sIgE were much higher and venom concentrations eliciting cutaneous responses were much lower with the culprit venom than with the other.

Table 1. Patient Characteristics

	Patients	Age	Episodes/Patient	Mueller	Mean (SD) Baseline IgE, kU_A/L
Double					
	Male 24	Min 14	Min 1	II1 4 (38%)	*Polistes* 16.80 (10.6)
	Female 13	Max 73	Max 6	III 20 (54%)	*Vespula* 12.28 (7)
		Mean 45.3	Mean 1.7	IV 3 (8%)	
		SD 12.8	SD 1.6		
Polistes					
	Male 7	Min 12	Min 1	II 4 (40%)	*Polistes* 17.6 (10.25)
	Female 3	Max 61	Max 3	III 6 (60%)	*Vespula* 2.46 (2.02)
		Mean 40.8	Mean 1.3		
		SD 14.7	SD 0.6		
Vespula					
	Male 6	Min 7	Min 1	II 4 (40%)	*Polistes* 6.2 (2.63)
	Female 4	Max 71	Max 2	III 5 (50%)	*Vespula* 26.4 (11.44)
		Mean 49.2	Mean 1.1	IV 1 (10%)	
		SD 21.7	SD 0.3		

Abbreviations: Ig, immunoglobulin.

Diagnosis

The systemic reactions were classified according to Mueller [4].

Every patient underwent a stepwise incremental venom intradermal test, with doses ranging from 0.0001 µg/mL to 1 µg/mL. The recommendations of the European Academy of Allergy and Clinical Immunology (EAACI) were followed [5]. The series were stopped when an increase of 4 mm was recorded in the diameter of the initial wheal up to 20 minutes after injection. *Polistes* venom and *Vespula* venom (ALK-Abelló, Hørsholm, Denmark) were tested.

Serum sIgE and IgG4 (sIgG4) to *Polistes* venom and *Vespula* venom were determined using ImmunoCAP (Phadia, Uppsala, Sweden). Quantitative results were expressed in kU_A/L (IgE) and µg/mL (IgG4).

Immunotherapy

Patients received immunotherapy with Pharmalgen (ALK-Abelló) for 5 years. In the cases, updosing was carried out in 2 separate programs. A maintenance dose of 100 µg was administered monthly in alternating injections, namely, *Polistes* in the even months and *Vespula* in the odd months. In the controls, after a single updosing phase, a maintenance dose of 100 µg was administered following international guidelines (1 dose per month). All the 100-µg doses were administered in 2 simultaneous injections of 50 µg in each upper arm.

Sting Challenge

Patients gave their informed consent (as required by the health authorities) before undergoing a controlled sting test.

The entomology department (animal biology section) of Cordoba University (Cordoba, Spain) provided *Vespula germanica* and *Polistes dominulus* wasps, which were trapped and carried to the hospital in well-ventilated containers. The insects were anesthetized briefly (40-50 seconds) to enable their wings to be cut. They were placed in individual unbreakable containers to avoid accidents. When the wasps awoke and returned to their aggressive state, they were taken to the patients.

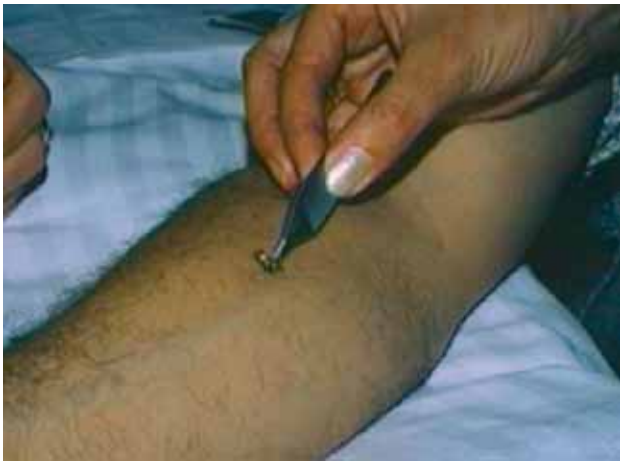

Figure 1. Controlled sting test performance with *Polistes dominulus*.

The insect was placed on the forearm and held with forceps for 30 seconds to ensure that an appropriate amount of venom was injected. The insect was then removed (Figure 1).

The response was classified according to Mueller [4]. "Mueller 0" was defined as onset of systemic symptoms and was milder than "Mueller I".

When the challenge was negative, the patients remained under observation for 2 hours after the sting.

Follow-up

According to the recommendations of the EAACI Immunotherapy Task Force, safety records were kept for every injection [6]. Reactions were recorded using the Mueller classification [4].

Serum sIgE and sIgG4 to *Vespula* and *Polistes* were determined after 1, 2, and 3 years of immunotherapy, and a re-sting was performed with *Polistes* or *Vespula* in the controls and with both *Polistes* and *Vespula* (with an interval of 2 months or more) in the cases.

Statistical Methods

The mean (SD), median (interquartile range), minimum, and maximum were calculated for continuous variables; the percentage by group was calculated for categorical variables.

Categorical variables were compared using the χ^2 test or the nonparametric Fisher exact test when χ^2 assumptions were not reached. Variations in sIgE and sIgG4 during follow-up were evaluated with the percentage of variation over the baseline and evaluated using the *t* test or the nonparametric Mann-Whitney test. A *P* value <.05 was considered statistically significant.

Results

All the controls received 36 doses (72 half doses) of the corresponding venom. The cases received 18 doses (36 half doses) of *Polistes* venom and 18 doses of *Vespula* venom. At the end of the third year, 1026 doses of 100 µg and 2052 half doses of each venom had been administered.

The only systemic reaction recorded was a mild reaction to *Vespula* venom (Mueller 0). The incidence of large local reactions is shown in Table 2.

The changes in levels of immunoglobulin are shown in Figure 2

We performed 109 *Polistes* re-stings and 93 *Vespula* re-stings (Table 3). In year 1, 35/37 cases were stung with

Table 2. Incidence of Large Local Reactions

		Vespula	*Polistes*
Cases	Patients, %	8	8
	Doses, %	0.75	0.45
Controls	Patients, %	20	10
	Doses, %	3.05	0.83

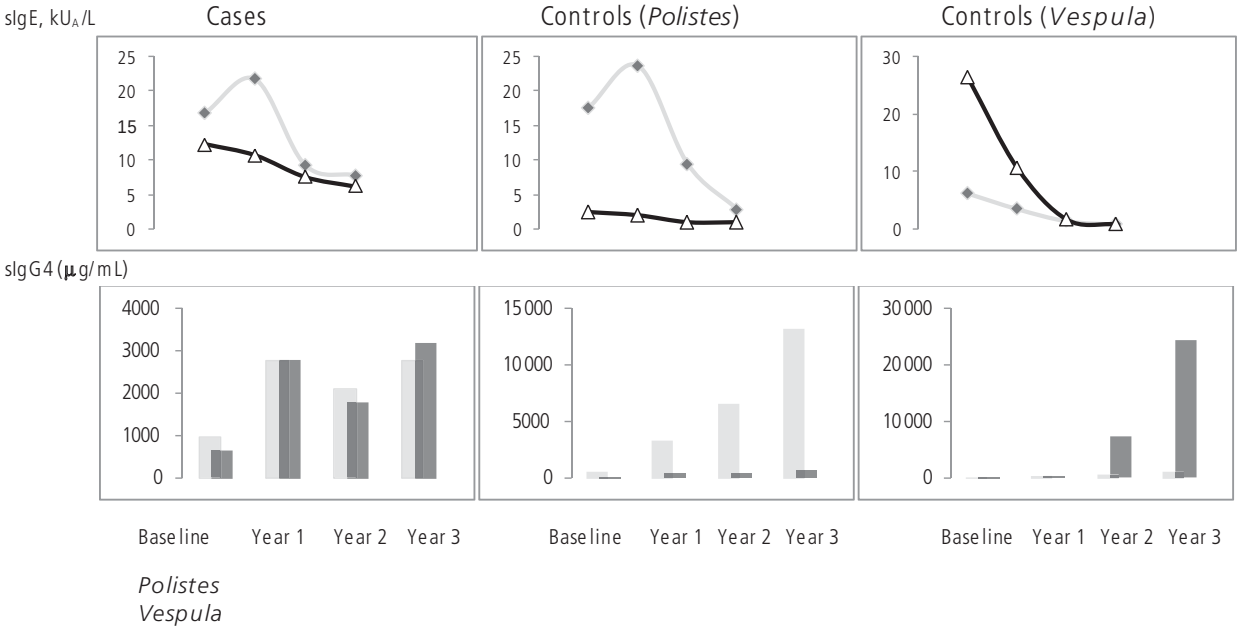

Figure 2. Changes in IgE and IgG4 during immunotherapy in different groups of patients.

Table 3. Re-stings

	Polistes			Vespula		
	N	SR	LLR, No. (%)	N	SR	LLR, No. (%)
Cases						
Year 1	37	0	3 (8%)	35	0	2 (6%)
Year 2	24	0	1 (4%)	19	0	2 (10%)
Year 3	19	0	2 (10%)	16	0	1 (12%)
Controls						
Year 1	10	0	1 (10%)	10	1 M0 (10%)	1 (10%)
Year 2	10	0	0	7	0	0
Year 3	9	0	0	6	0	1 (16%)

Abbreviations: LLR, large local reaction; MO, Mueller grade 0; SR, systemic reaction.

Vespula, and the 2 remaining cases were stung in years 2 and 3 (belonging to the corresponding groups of 19 and 16). The reduction in the number of controlled stings in the second and third years was related to organizational difficulties, although no patients were lost to follow-up.

Discussion

In some Mediterranean regions, the presence of more than 1 genus of allergenic vespids is problematic. When a heavily exposed patient experiences a systemic reaction after a sting by an unidentified wasp, diagnosis is usually made with *Vespula* and *Polistes* extracts. Significant levels of serum IgE to both venoms could be due to cross-reactivity or true double sensitization.

Differences between allergenic compounds from *Vespula* and *Polistes* venoms have been reported [7]. In the absence of a molecular diagnosis, the primary sensitizing venom could be suspected when the amounts of sIgE are very different for both sensitizing venoms; however, when a quantitative interpretation is not possible, diagnostic uncertainty makes it difficult to decide on therapy. Inhibition of IgE is sometimes helpful [8]. The diagnostic controlled sting test has been proposed to distinguish between the sensitizing venom and the culprit venom, but a single negative test has no solid predictive

value for further stings. Moreover, considering the increased risk of further systemic reactions among untreated patients, sting challenge is not recommended for diagnostic purposes [9]. Immunoblotting can identify individual allergenic proteins that serum reacts to [10]. Although this technique is noninvasive, no standardized information is available on its sensitivity and specificity, and its differential diagnostic value remains unclear. Other in vitro tests such as basophil degranulation or interleukin measurement have been performed for research purposes; however, their use in daily clinical practice is not suitable.

Considering this frustrating lack of diagnostic options, the most conservative approach—double sensitization—should be applied to protect patients against further wasp stings.

Although venom doses of 50 µg have been reported to be effective in the treatment of *Apis* and *Vespula* allergy [11], a dose of 100 µg provides better protection [12]. Given these results and the lack of information on *Vespula* and *Polistes* immunotherapy, it seems appropriate to treat our patients with 100 µg of each venom. A conventional maintenance course of immunotherapy, namely, 1 dose every 4-6 weeks, could have been too expensive for many patients and not available at the hospital. Treatment strategies should take account of convenience of administration and expense in order to guarantee good adherence.

The 37 cases received all the planned injections over 3 years, as did the 20 controls. The interval between doses was 8 weeks, and this did not result in a higher number of adverse reactions for *Polistes* or *Vespula*, thus confirming once again the possibility of enlarging the 4-week interval in the absence of systemic reactions and large local reactions [13].

The controlled re-sting test is considered the gold standard for verifying the effectiveness of VIT. In some cases, spontaneous field stings provide information that confirms clinical protection after or during immunotherapy. This advantage is particularly interesting for beekeepers. Nevertheless, a spontaneous sting is always unpredictable: it may be unfinished (low amount of venom injected) and the response is never verified. Furthermore, in the case of our patients, exact identification of the culprit insect was not possible.

The performance of a controlled double sting test allowed us to verify the effectiveness of alternating VIT. Only 1 of the 202 re-sting tests was positive. One 21-year-old woman treated with *Vespula* venom for 1 year reported pharyngeal pruritus and malaise after a sting. Mild redness of neck was observed, but no respiratory symptoms or hypotension were present. The episode was registered as a "Mueller 0" systemic reaction that was easily cured with conventional measures. The patient was discharged after 3 hours. The woman tolerated a new re-sting during the second year.

Interestingly, none of the patients receiving double VIT had a positive re-sting test, suggesting that alternating vespid VIT is as effective as the single injection. Furthermore, the incidence of large local reaction was the same in both groups.

Changes in Ig levels during VIT are not directly associated with individual clinical tolerance to stings, but they have been suggested to be a consequence of interleukin 10–enhanced secretion by regulatory T cells [14].

Since sIgG4 level in untreated patients is considered a marker of exposure [15], the baseline amount in our cases suggests that a high number of previous stings led to the double allergy. Nevertheless, the improved sIgG4 production among the cases was limited in comparison with the controls, suggesting that the 2 types of sensitization are independent. If cross-reactivity were the cause of double sensitization, a boosting effect between the 2 vaccines could be expected [16].

Regarding the dose-effect relationship, our data suggest that some immunological changes during VIT, such as production of sIgG4, depend on the cumulative dose, but that clinical effectiveness, as demonstrated by the re-sting test, could be related to a high dose in every injection.

The recently developed proteomic technologies promise to provide an accurate etiologic diagnosis for anaphylaxis due to hymenoptera stings [17]. However, for patients with clear double sensitization, alternating immunotherapy with *Vespula* and *Polistes* has proven to be an effective and protective therapeutic strategy, irrespective of the insect responsible for the reaction.

References

1. Giuglia D. Les guêpes sociales (Hymenoptera Vespidae) d'Europe occidentale et septentrionale. Coll. Faune de l'Europe et du Bassin Méditerranéen. Tome 6. Centre National de la Recherche Scientifique. Ed. Masson et cie. Paris; 1972.
2. Blanca M, García F, Miranda A, Carmona MJ, García J, Fernández J, Terrados S, Vega JM, Juárez C. Determination of IgE antibodies to Polistes dominulus, Vespula germanica and Vespa crabro in sera of patients allergic to vespids. Allergy. 1991:46 (2):109-14.
3. Navarro LA, Peláez A, de la Torre F, Tenias Burillo JM, Megias J, Martinez I. Epidemiological factors of hymenoptera venom allergy in a Spanish adult population. J Invest Allergol Clin Immunol. 2004;14: 134-41.
4. Mueller HL. Diagnosis and treatment of insect sensitivity. J Asthma Res. 1966;3:331-3.
5. Dreborg S, Frew AJ. EAACI Position paper: Allergen standardisation and skin tests. Allergy. 1993;48 (14 Suppl):48-82.
6. Álvarez-Cuesta E, Bousquet J, Canonica GW, Durham SR, Malling HJ, Valovirta E. EAACI, Immunotherapy Task Force. Standards for practical allergen-specific immunotherapy. Allergy. 2006;Suppl 82:1-20.
7. Caruso B, Bonadonna P, Severino MG, Manfredi M, Dama A, Schiappoli M, Rizzotti P, Senna G, Passalacqua G. Evaluation of the IgE cross-reactions among vespid venoms. A possible approach for the choice of immunotherapy. Allergy. 2007;62(5):561-4.
8. Hamilton RG, Wiesenauer JA, Golden DBK, Valentine MD, Adkinson FA. Selection of Hymenoptera venoms for immunotherapy on the basis of patient's IgE antibody cross-reactivity. J Allergy Clin Immunol. 1993;92:651-9.
9. Bilò MB, Rueff F, Mosbech H, Bonifazi F, Oude-Elberink JNG, Birnbaum J, Bucher C, Forster J, Hemmer W, Incorvaia C, Kontou Fili K, Gawlik R, Muller U, Fernandez J, Jarish R, Jutel M, Wuthrich B. EAACI Position Paper. Diagnosis of hymenoptera venom allergy. EAACI website. 2005. doi:10.1594/eaaci.net/2005/PP/1-210907.

10. Zollner TM, Spengler K, Podda M, Ergezinger K, Kaufmann R, Boehncke WH. The Western blot is a highly sensitive and efficient technique in diagnosing allergy to wasp venom. Clin Exp Allergy. 2001;31:1754-61.
11. Reisman RE, Livingston A. Venom immunotherapy: 10 years of experience with administration of single venoms and 50 micrograms maintenance doses. J Allergy Clin Immunol. 1992.89(6):1189-95.
12. Golden D, Kagey-Sobotka A, Valentine M, Lichtenstein L. Dose dependence of Hymenoptera venom immunotherapy. J Allergy Clin Immunol. 1981;67:370-4.
13. Bonifazi F, Jutel M, Bilò MB, Birnbaum J, Muller U, Bucher C, Forster J, Hemmer W, Incorvaia C, Mosbech H, Oude Elberink JNG, Rueff F, Fernandez J, Senna GE, Jarish R, Wuthrich B. EAACI Position Paper. Prevention and treatment of hymenoptera venom allergy. EAACI website. 2005. doi: 10.1594/eaaci.net/2005/PP/2-210907
14. Akdis CA, Blesken T, Akdis M, Wüthrich B, Blaser K. Role of interleukin 10 in specific immunotherapy. J Clin Invest. 1998;102:98-106.
15. Müller U, Johansson SGO, Streit C. Hymenoptera sting hypersensitivity: IgE, IgG and haemagglutinating antibodies to bee venom in relation to exposure and clinical reactions to bee stings. Clin Allergy. 1978;8:267-72.
16. Juárez C, Blanca M, Miranda A, Sánchez F, Carmona MJ, Ávila MJ, Fernandez S, Fernandez J, Terrados S. Specific antibodies to vespids in the course of immunotherapy with Vespula germanica administered to patients sensitized to Polistes dominulus. Allergy. 1992;47:299-302.
17. De Graaf DC, Aerts M, Danneels E, Devreese B. Bee, wasp and venomics pave the way for a component-resolved diagnosis of sting allergy. J Proteomics. 2009;6;72(2):145-54.

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