

Determination of IgE antibodies to *Polistes dominulus*, *Vespula germanica* and *Vespa crabro* in sera of patients allergic to vespids

M. BLANCA, F. GARCIA, A. MIRANDA, M. J. CARMONA, J. GARCIA, J. FERNANDEZ, S. TERRADOS, J. M. VEGA & C. JUAREZ

Research Unit for Allergic Diseases, Carlos Haya Hospital, Malaga, Spain

The study was undertaken to investigate the presence of IgE antibodies to *Polistes dominulus* (PD), *Vespula germanica* (VG) and *Vespa crabro* (VC) in a large group of sera belonging to patients sensitized to Vespids in Spain. RAST values showed that although the majority of patients had IgE antibodies to PD, VG and VC, there was a marked predominance of PD. These results were related to the distribution of the insect in the areas where the sera were obtained. Due to geographical and insect distribution differences, the whole area was divided into three zones: Central, East and South. Comparison of the positive RAST values obtained indicated that, although the positivity to PD predominated over VG and this over VC, there were significant differences in percentage positivities to each vespid in the different regions studied. The results of the RAST absorption studies indicated that in most instances patients were originally sensitized to one vespid and were RAST positive to the other venoms due to cross-reactivity. Only in a minority of cases were coexisting antibodies to two insects present. These results show that PD and VG are the important vespids followed to a lesser extent by VC. This study provides relevant information concerning insect distribution sensitivity in a European country.

Key words: IgE antibodies; *Polistes dominulus*; vespid sensitivity; *Vespa crabro*, *Vespula germanica*.

Accepted for publication 17 June 1990

Amongst the different species of Vespids in Europe are *Polistes dominulus*, *P. gallicus*, *P. nipha*, *P. biglumus*, *Vespula germanica*, *V. vulgaris*, *Dolichovespula saxonica* and *D. silvestris* (1, 3, 15). These insects can be found in various countries and their distribution is variable (1, 5). Previous studies have established that the most common vespids in the south of Spain are PD and VG, with VC being less commonly found (2, 5). VC is limited mainly to areas of the center and north of Spain (5). In addition to these preliminary observations a further study of sera samples obtained from different zones showed that PD and VG are the most relevant (3). To

the best of our knowledge little information is available about the distribution of vespid sensitivities in Europe, particularly in areas where the *Polistes* species are present (1, 4, 6). The purpose of this study was to undertake a survey of a large number of serum samples from out-patient clinics located in geographical areas with differing weather and climate conditions and the existence of different vespids has been reported (5). RAST and RAST absorption studies were carried out in order to establish the original insect inducing sensitivity and also the existence of multiple sensitivities. We showed that PD is the most common followed by VG, with the

RAST value to VC being predominant in only a minority of cases. These results correlated with the reported insect distribution (2, 15).

MATERIAL AND METHODS

Serum collection

Several hospitals located in different parts of Spain were asked to send sera belonging to patients sensitized to vespids and with a clinical history of systemic or large local reactions and the presence of IgE antibodies to at least one of the three vespids: PD, VG, VC. They were grouped in three areas according to geographical location: East (Hospitals of the NIH of Castellon, La Fe of Valencia and Arrixaca of Murcia), Central (Hospital 12 de Octubre, Madrid and Hospital of the NIH Jean) and South (Hospital Carlos Haya of Malaga and Torre Cardenas of Almeria). The Eastern and Southern areas have a mediterranean climate and the Central a continental climate.

Venom collection

PD, VG and VC insects were collected from different parts of Spain where these species are normally found. The venoms were obtained by sac dissection as described (18).

Radioallergosorbent test (RAST)

Dose response curves were carried out with positive control to the three venoms in order to determine the optimal allergen concentration per disc (0.05, 0.1, 0.5, 1, 2 mg/ml). 37 sera from normal and atopic patients with total IgE values ranging from 5 to 1300 IgE units with no history of allergy to hymenoptera and negative skin tests to vespid venoms were used as a negative control. Values over the mean + 2 SD were considered as positive. RAST procedures was carried out as described (2).

RAST absorption studies

To determine whether patient sera positivity to several venoms was due to cross-reactivity or

coexisting non-cross-reacting antibodies, RAST inhibition studies were carried out with all the sera with sufficient label uptake enabling inhibition to be done. A dose response curve was made using discs of PD, VG and VC, and in the fluid phase different concentrations (0.6, 0.1, 0.2, 0.4, 0.6, 2, 4, 6 mg/ml) of PD, VG and VC venoms. The same pools used for titrating the RAST discs were used for performing the RAST absorption studies. Several individual sera positive to each venom and with different RAST values were also used. Once the maximum absorption had been established all the sera were inhibited using one or two venoms in the solid phase depending on RAST value and using the three venoms (PD, VG, VC) in the fluid phase. Results of RAST absorption were expressed according to the following ratio:

$$\frac{\text{RAST non-inhibited sera} - \text{RAST inhibited sera}}{\text{RAST non-inhibited sera}} \times 100$$

The following patterns of response were expected: a) sera with IgE antibodies to one venom and with a variable degree of cross-reactivity with another. In these cases the venom to which the antibodies are directed will absorb most IgE antibodies when used in the fluid phase whatever venom is conjugated to the RAST discs; b) sera with IgE antibodies to specific determinants of each vespid. In this case each venom will absorb in the fluid phase an optimal amount only when the same venom is coupled to the disc; c) sera with IgE antibodies to both common and specific determinants of each species.

Statistical studies

The coefficient of variation (CV) for RAST was determined as described (14). Values over the established CV were considered significant. The percentage of the predominance of the RAST value to each vespid was compared by chi-square analysis. The RAST to the three venoms in all the sera was correlated by linear regression analysis (10, 11).

RESULTS

A total of 187 sera were received from the allergy units of the hospitals involved.

The intrassay CV for the RAST was 12% and the interassay CV was 15%. RAST values > the CV were considered significant. Differences in the RAST value in positive cases gave the following results: East zone (50 cases) 71% were more positive to PD, 11% to VG and 3% to VC. The RAST value was similar (< 15%) to both PD and VG in 14% of the cases (Fig. 1 left). Central zone (45 cases) 62% were more positive to PD, 15% to VG and 11% to VC. RAST value was similar to PD and VG in 10% and to VG and VC in 2% (Fig. 1 center). South zone (92 cases) 58% were more positive to PD, 29% were more positive to VG and 2% to VC. RAST was of similar value to PD and VG in 11% (Fig. 1 right).

RAST was positive to just one woman in four cases in the East zone (all to PD), to 11 cases in the Central zone (6 to PD, 2 to VG and 3 to VC) and 32 cases in the South zone (16 to PD and 15 to VG) (Table 1). Chi-square analysis of the differences in the proportion of percentages of RAST positivity in the three areas mentioned showed that, although positivity to PD predominated over the other vespids in all the zones, in the East zone the proportion of positivity to VG was lower than expected ($P < 0.005$). In the Central zone the proportion of positivity to VC

and the proportion $VG = VC$ was higher than expected ($P < 0.005$).

The optimal concentration selected for RAST absorption was 2 mg/ml, the same for all three venoms. At this concentration all the 72 sera with a RAST value over 8% were inhibited. RAST absorption values showed 40 sera more positive to PD, 18 to VG and 6 to VC. Table 2 shows the mean and SD of the values obtained in the three groups. Eight cases with similar RAST value to PD and VG (< 20%) were inhibited with both discs and venom in the fluid phase in order to establish the primary sensitivity. In five of these the antibodies were more absorbed with PD and in three with VG. In four cases coexisting IgE antibodies to two venoms were found. Results are presented in Fig. 2. Cases 1, 3 and 4 had antibodies to PD and VG and Case 2 had antibodies to PD and VC.

Comparison of the RAST value by linear regression analysis showed that PD versus VG was 0.67, PD versus VC was 0.52 and VG versus VC 0.67. In all cases the association was significant ($P < 0.01$).

DISCUSSION

The present study attempts to provide data about distribution in sensitivity to the three vespids found in the areas studied using sera of patients with systemic or large local reactions to these vespids and with evidence of specific IgE

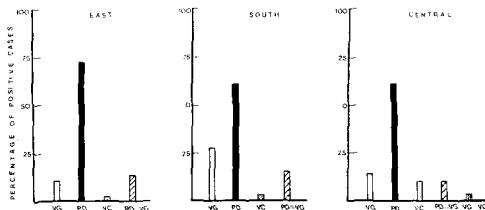


Fig. 1. RAST value to PD, VG and VC in the three regions studied. In the East zone (left graph) 71% of the cases were more positive to PD, 11% to VG and 3% to VC. RAST was of similar value to PD and VG in 14%. In the South zone (central graph) 58% were more positive to PD, 29% to VG and 2% to VC. In 11% PD was similar to VG. In the Central zone (right graph), 62% were positive to PD, 15% to VG and 11% to VC. RAST value to PD and VG was similar in 10% and to VG and VC in 2%.

Table 1

Distribution of RAST results in the group of 46 sera with positivity to one or more of the three venoms

	Number	PD (%)	VG (%)	VC (%)
East	4	4 (8)	0	0
Central	11	6 (13)	2 (4)	3 (6)
South	31	16 (17)	15 (16)	0

The percentage in sensitivity to each vespids was obtained by dividing the total number of cases by number of sera in each zone.

antibodies to at least one of the vespids tested (PD, VG and VC). Based on previous evidence of the predominance in sensitivity to PD in some areas (2, 3) and taking into account entomological data about insect distribution (5) we expected that an important number of cases would be positive to *Polistes* species. The study was carried out in sera available from the units with the purpose of quantifying by RAST and RAST inhibition studies the predominance of sensitivities to the different vespids. Of the 187 sera studied 156 had IgE antibodies to at least two venoms (83%) and only a minority (16%) had IgE antibodies to only one of the venoms tested.

Considering the proportion in percentage in all the sera, PD predominated in all the regions studied, although there were significant percentage differences in each region. The positivity to VG was lower than expected in the East zone

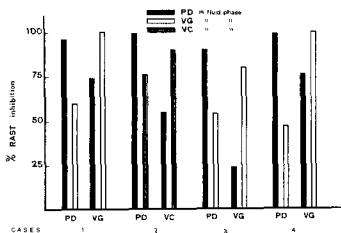


Fig. 2. Cross-absorption studies in the sera that had co-existing antibodies to two venoms expressed as percentage of RAST inhibition. In Cases 1, 3 and 4 IgE cross-reacting and independent antibodies were detected to PD and VG. In each of these cases PD and VG venoms in the fluid phase inhibited more when the same venom was used in the solid phase conjugated to the discs. In Case 2 coexisting antibodies were detected to PD and VC.

($P < 0.005$). VC was higher than expected in the Central zone ($P < 0.005$).

Various degrees of cross-reactivity have been reported by different authors (10, 11, 16). Venoms of *Vespula* and *Vespa* genus are more closely related than with *Polistes* and there is only a limited degree of cross-reactivity between *Polistes* and Vespids venoms (13). With the isolation of proteins from the venoms of the different species this point has been further elucidated and there seem to be allergens shared by all the species and also specific determinants

Table 2

Results of RAST absorption studies determined by optimal inhibition

		Venoms used in the fluid phase			
		PD	VG	VC	
Venoms coupled to discs	PD	91 (7)	32 (26)	19 (16)	40 cases
	VG	84 (8)	76 (17)	31 (23)	
	PD	46 (21)	91 (5)	35 (38)	18 cases
	VG	37 (23)	92 (9)	45 (31)	
VC		39 (23)	72 (13)	94 (3)	6 cases

Results of optimal inhibition in 64 sera which were more positive to one of the venoms tested. The groups more positive to PD and VG were inhibited using both venoms in the solid phase. In the group of VC only this venom was used in the solid phase. The numbers represent the mean value of the inhibitions and the standard deviation in each group.

unique to each specie (12, 13). Rast inhibition studies have been proposed as a method for studying the degree of cross-reactivity (16) and optimal inhibition studies as a means of detecting primary and coexisting sensitivities (9). Our results obtained with a procedure similar to that described by Hamilton et al. (9) indicated that 72 sera were originally sensitized to one vespid specie and cross-reacted with the others. Of these, 62% proved to be primary positive to PD, 29% to VG and 8% to VC, there being only four cases with coexisting antibodies to two venoms, three to PD and VG and one to PD and VC. These data would indicate that, when studying allergic reactions to vespids in the areas mentioned, it is convenient to include both PD and VG, and possibly also VC. If these venoms are not included when serological studies and *in vitro* cross-reactivities are considered, the results may not be adequate.

In most European studies (4, 6, 13) of sensitivity to vespids, the term wasp is mentioned but no specific details are given about VG, PD or any other vespids found in the areas under study. PD is widespread in Europe (1, 3, 17) and should be convenient to use for study purposes. Further, this species has taken on more importance since its introduction to the USA in 1979 (New York and Boston City areas) showing differences with North American species of *Polistes* (12).

Immunotherapy should be given with the correct allergens to which the individuals are sensitized in order to provide the optimal concentration of relevant proteins for desensitization (7, 12). Therapeutic failures have been reported after using inappropriate venom for immunotherapy (8). On the other hand, during the course of immunotherapy IgE antibodies may appear to allergens to which the individuals were not initially sensitized with the corresponding risk of sensitization to new determinants (19).

We are at present extending similar studies to other parts of Europe in order to determine distribution of vespid sensitivity.

ACKNOWLEDGEMENTS

We wish to thank the following doctors who sent us sera for study: Dr. Antonio Pelaez, Hospital la Fe, Valencia. Dr.

Julian Borrás, Hospital Seguridad Social, Castellón. Dr. J. Negro, Hospital Virgen de la Arrixaca, Murcia. Dr. Victoriano Peralta and Dr. Francisco Anguita, Hospital Capitan Cortes Jaen. Dr. Sara Bensabat, Hospital 12 Octubre, Madrid. We are grateful to the following farmers who helped us by collecting the different vespids: Julian Granados, Heladio García, Salvador Navarrete. Finally, we are indebted to the entomologist Robert Jacobson for supervising the insect collection and helping in recognizing the different species.

This work was supported in part by a Grant from the Dirección General Promoción de Investigación (PM88-0089).

REFERENCES

1. Akre RD, Reed HC. Biology and distribution of social hymenoptera. In Tu A (ed.), pp 3-47. Marcel Dekker, New York, 1987.
2. Blanca M, Miranda A, Fernandez J, et al. Allergic reactions to vespids: comparison of sensitivities to two species in a mediterranean area. *Clin Allergy* 18, 21-27, 1988.
3. Blanca M, Miranda A, Garcia J, et al. Distribution of the sensitivity to vespids in south Europe (abstract). *J Allergy Clin Immunol* 81, 170, 1988.
4. Bousquet J, Muller UR, Dreborg S, et al. Immunotherapy with hymenoptera venoms. Position paper of the Working Group on Immunotherapy of the European Academy of Allergy and Clinical Immunology. *Allergy* 42, 401-413, 1987.
5. Ceballos J. Catálogo de los himenopteros de España. Instituto Nacional de Entomología, Madrid, p 231, 1956.
6. Frankland AW. Allergy to insect and arachnids. In Lefsoff MH, Lee TH, Kemney DM (eds.), *Allergy. An international textbook*, pp. 409-401. John Wiley, London, 1987.
7. Golden DBK, Meyers DA, Kagey-Soborka A, et al. Clinical relevance of the venom specific immunoglobulin IgG antibody level during immunotherapy. *J Allergy Clin Immunol* 69, 489-493, 1982.
8. Grant JA, Kokanson JA, Gillaspie JE, Farnan J. Wasp venom immunotherapy. A status report (abstract). *J Allergy Clin Immunol* 57, 32, 1986.
9. Hamilton RG, Wisenauer J, Valentine M, Golden D, Adkinson NF. Hymenoptera venoms RAST inhibition test (RIT) facilitates selection of venoms of immunotherapy. *J Allergy Clin Immunol* 85, 213, 1990. (abstract).
10. Hoffman DR, McDonald CA. Allergens in hymenoptera venom. VII. Immunologic comparison of venoms from six species of vespids (yellow jackets). *Ann Allergy* 48, 78-81, 1982.
11. Hoffman DR. Allergens in hymenoptera venom XV. the immunologic basis of vespid venom cross-reactivity. *J Allergy Clin Immunol* 75, 611-613, 1985.
12. Hoffman DR, Jacobsen R, Blanca M. Allergy to venom of *Polistes dominulus*. A paper wasp introduced from Europe. *J Allergy Clin Immunol* 85, 211, 1990. (abstract).

13. King TP, Joslyn A, Kochounian L. Antigenic cross-reactivity of venom proteins from hornets, wasps and yellow jackets. *J Allergy Clin Immunol* 75, 621-628, 1985.
14. Hunt KJ, Sobotka AK, Valentine M, et al. Sensitization following hymenoptera body extract therapy. *J Allergy Clin Immunol* 61, 48-53, 1978.
15. Jaarisch R, Yman L, Boltz A, et al. IgE antibodies to bee venom, phospholipase A2, melittin and wasp venom. *Clin Allergy* 9, 535-541, 1979.
16. Jeeffcoate SL. Efficiency and effectiveness in the endocrine laboratory. Academic Press, London, 1981.
17. Miranda A, Blanca M, Garcia J, et al. A status report of the insect distribution and sensitivities to vespids in Spain. *Rev Esp Alergol Immunol Clin* 4, 57-66, 1989.
18. Reisman RE, Wypych JL, Mueller CR, Grant JA. Comparison of the allergenicity and antigenicity of *Polistes venomis* and other species venoms. *J Allergy Clin Immunol* 70, 281-287, 1982.
19. Schuller DE, Sutton PL. Venom skin testing and alteration of RAST levels. *Ann Allergy* 47, 84-86, 1981.
20. Sobotka AD, Adkinson NF, Valentine MD, et al. Allergy to insect stings. Diagnosis by radioallergosorbent test. *J Immunol* 122, 2477, 1978.

Address.

Miguel Blanca, M.D.

Research Unit for Allergic Diseases

Carlos Haya Hospital

Malaga

Spain

This document is a scanned copy of a printed document. No warranty is given about the accuracy of the copy. Users should refer to the original published version of the material.