

Allergic reactions to vespids: comparison of sensitivities to two species in a Mediterranean area

M. BLANCA, A. MIRANDA, J. FERNANDEZ†, S. TERRADOS, J. M. VELA*, J. M. VEGA, J. J. GONZALEZ and C. JUAREZ

Allergy Section, Research Unit for Allergic Diseases, Carlos Haya Department and *Department of Entomology, Faculty of Sciences, Malaga and †Allergy Section, Torre Cardenas Hospital, Almeria, Spain

(Received in original form 2 October 1986 and in revised form 15 April 1987; accepted for publication 26 June 1987)

Summary

We have studied a group of twenty-seven patients who suffer allergic reactions to vespids stings. Specific IgE antibodies to venom extracts from *Polistes gallicus* and *Vespula germanica* were measured by RAST, and the crossreactivity between the two venoms was compared using the RAST inhibition technique. We concluded that, in southern Spain, sensitization to *P. gallicus* was more prevalent than that to *V. germanica*, with 44% of the subjects in this study reacting to *P. gallicus* compared with 33% to *V. germanica*. However, there was a considerable degree of crossreactivity between the two species. It is evident that *Polistes* is an important species in this area; however, both in Spain and other Mediterranean countries, *V. germanica* venom is used almost exclusively for diagnosis and immunotherapy.

Introduction

In the Vespidae family there are several subfamilies, which can be distinguished both entomologically and in terms of antigenic and allergenic differences, that are of clinical relevance to subjects who suffer allergic reactions to vespids [1]. Based upon the prevalence of allergic sensitivity to vespids in the United States of America, there is a clear geographical distribution of species with hornets and yellow jackets predominating in the northern, eastern and midwestern areas, and *Polistes* in the south [2-4].

Most studies suggest that yellow jackets (*Vespula* sp.) are the most important stinging insect, but detailed information about distribution in other parts of the world does not exist. *Vespula germanica* and *Polistes gallicus* are the most common species

found in Spain, although less common species of the genus *Vespa* and subgenus *Dolichovespula* also exist [5]. *Polistes* is found near sites of human habitation around roofs, walls and gardens, making aerial nests. In contrast, *Vespula* live in country areas on both cultivated and wild land, where they nest in the ground. In this study we report cases of sensitivity to both venoms in a population of patients who suffer anaphylactic or large local reactions.

Subjects and methods

Subjects

Twenty-seven individuals sensitized to vespids were studied. All had a clinical history of allergic reactions to vespid stings, and specific IgE an antibody to *P. gallicus* or *V. germanica* venom. Four subjects had suffered only large local reactions, whereas seventeen had anaphylactic symptoms consisting of generalized urticaria, angioedema and pruritus, and a further six developed anaphylactic shock. The age range was 8–69 years, with a mean of 36 years, and there were nineteen males and eight females. Three lived in urban areas of more than one thousand inhabitants whilst the majority resided in small rural communities.

Venom collection

Venom sacs of *V. germanica* and *P. gallicus* were removed from the abdomen, and other anatomical structures were excluded. Venom was extracted using a syringe and needle, and filtered through a Millipore filter. The non-venom proteins were precipitated in acid buffer and eliminated by centrifugation [6]. Protein concentration was estimated by the Lowry method [7].

Specific IgE determination

The venom was diluted in 0.1 M sodium bicarbonate and coupled to cellulose discs previously activated with cyanogen bromide, according to the method of Ceska & Lundkvist [8]. The optimal concentration of allergen per disc was determined using ten-fold dilutions of venom protein starting at an initial concentration of 5 mg/ml. A serum pool derived from ten patients sensitized to yellow jacket was used as a positive control. RAST was carried out following the procedure outlined in the Phadebas RAST kit (Pharmacia, Uppsala, Sweden). The background activity was estimated using the assay buffer alone (BSA 1% plus Tween 0.1% in PBS), and the negative control was comprised of five RAST negative sera from non-sensitized individuals with different IgE levels ranging from 43 to 2000 IU/ml. The number of counts bound was corrected by subtraction of the background count and expressed as a percentage of the total number of counts added.

RAST inhibition

Inhibition studies were performed using either individual sera from patients sensitized to *P. gallicus* or *V. germanica*, or pools of such sera, using the procedure of Yman *et al.* [9]. Ten-fold dilutions of venom starting at a maximum concentration of 200 µg/ml were used. In each case discs sensitized with either *P. gallicus* or *V. germanica* venom extracts were employed. The inhibition was carried out in the fluid phase prior to the addition of the venom disc. The results were expressed as the percentage inhibition. In two experiments, commercial yellow jacket venom (Sigma V 4129) was used to make a comparison with our *vespula* venom.

In IgE binding to either *P. gallicus* or *V. germanica* a five-fold higher concentration of venom was used in the inhibition phase. Results were expressed as a percentage of the maximum inhibition.

Results

The results of RAST appeared to indicate that all twenty-seven subjects had IgE antibodies against both species (Table 1). If a difference of >20% was used to determine the predominant RAST binding, sera from twelve patients reacted more strongly to *P. gallicus* (44%), nine to *V. germanica* (33%), and in six cases (23%) there was no difference. Eight sera had >50% difference in binding, and of these five were more reactive to *Polistes* and three to *Vespula*. These results provided evidence of sensitivity to both venoms and *P. gallicus* venom elicited higher IgE binding than *V. germanica*.

The RAST inhibition of the pool of serum with a predominance of specific IgE to *P. gallicus* is presented in Fig. 1. The inhibition studies showed that 200 µg of *P. gallicus* venom inhibited autologous RAST binding by 76%, whereas the same amount of *V. germanica* venom inhibited binding to the *Polistes* discs by <38%. Conversely, in a *V.*

Table 1. Clinical data and RAST values of the group of twenty-seven patients studied

Patient	Age	Sex	Clinical manifestation	RAST PG	RAST VG
R.M.V.	30	M	Anaphylactic shock	14.22	5.6
E.A.C.	35	F	Large local reaction	9.4	2.2
J.L.H.	46	M	Anaphylaxis	8.6	3.5
J.C.O.	33	M	Anaphylactic shock	32.7	16.9
S.T.G.	37	M	Anaphylactic shock	15.9	3.6
J.M.V.	50	M	Anaphylactic shock	12.5	3.4
I.P.M.	8	F	Anaphylaxis	11.5	3.59
J.C.P.	39	F	Anaphylaxis	18.5	10.2
J.C.V.	43	M	Large local reaction	11.28	5.9
A.P.G.	50	M	Anaphylaxis	20.3	7.4
M.S.L.	17	M	Large local reaction	8.8	3.6
J.M.C.	40	M	Anaphylaxis	7.42	2.44
J.C.F.	42	M	Large local reaction	5.8	11.0
J.B.R.	38	M	Anaphylaxis	14.6	26.3
A.G.T.	52	F	Anaphylaxis	8.2	19.1
J.M.V.	49	F	Anaphylaxis	2.59	8.11
F.B.Y.	26	M	Anaphylaxis	5	7.54
F.G.L.	40	M	Anaphylaxis	3.8	5.8
A.A.L.	49	M	Anaphylaxis	2	5.4
A.R.C.	40	M	Anaphylaxis	2.7	9.0
M.R.S.	28	F	Anaphylaxis	4.48	14.72
M.D.R.	25	F	Anaphylaxis	3.6	4.2
N.V.G.	14	M	Anaphylactic shock	7.6	6.8
J.G.R.	20	M	Anaphylactic shock	7.17	6.85
M.C.C.	20	F	Anaphylaxis	4.4	3.8
J.V.L.	38	M	Anaphylaxis	4.2	3.6
A.G.M.	69	M	Anaphylaxis	4.51	5.23

Specific IgE to *Polistes gallicus* (PG) and *Vespula germanica* (VG) is expressed as the percentage of the maximum counts added.

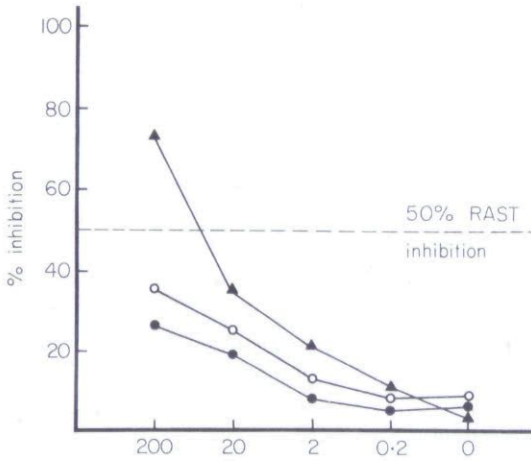


Fig. 1. RAST inhibition using a pool of serum from twelve patients with higher specific IgE to *Polistes gallicus* than to *Vespa germanica*. Discs were sensitized with *P. gallicus* venom. Ten-fold dilutions of (▲) *P. gallicus*, (●) *V. germanica* and (○) commercial *V. germanica* were added to the fluid phase starting at a concentration of 200 µg/ml.

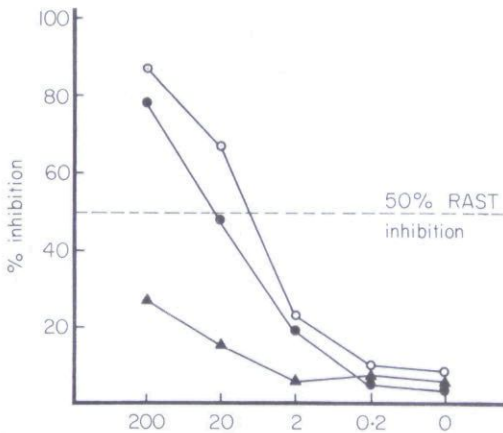


Fig. 2. RAST inhibition using a pool of serum from eight patients with higher specific IgE to *Vespa germanica* than to *Polistes gallicus*. Discs were sensitized with *V. germanica* venom. Ten-fold dilutions of (●) *V. germanica*, (○) commercial *V. germanica* and (▲) *P. gallicus* venoms were added to the fluid phase at concentrations of 200 µg/ml or less.

germanica RAST system, *P. gallicus* venom inhibited binding by <29%, whereas *V. germanica* venom achieved 79% inhibition, and 84% in the case of the commercial preparation (Fig. 2). The inhibition of an individual serum from a patient allergic to *Polistes* is shown in Fig. 3. *Polistes gallicus* venom gave 78% inhibition in contrast with *V. germanica*, which failed to inhibit. The RAST inhibitions of the twelve sera showing greater reactivity towards *P. gallicus* are given in Fig. 4. Inhibition varied from 3 to 73%, mean value 38%. Accepting values lower than 15% as failure to inhibit, three sera had specific IgE to both venoms with no cross reactivity (numbers 9,11 and 12 in Fig. 4). The RAST inhibition of eight sera with higher levels of specific IgE to *V. germanica* than to *P. gallicus* are shown in Fig. 5. The mean inhibition was 47% with only one sera inhibiting less than 15% (number 5).

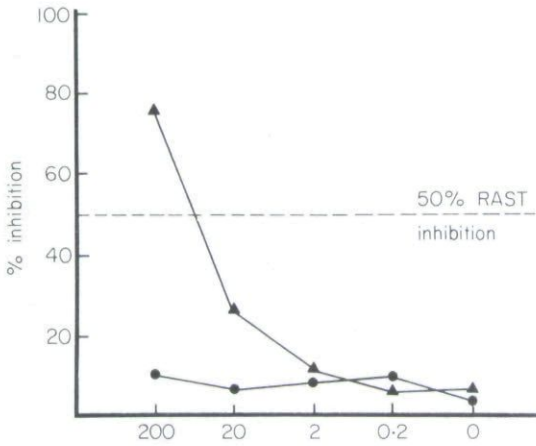


Fig. 3. RAST inhibition using serum from a patient with specific IgE to *Polistes gallicus* and *Vespa germanica*. *Polistes gallicus* venom was coupled to the discs and the inhibition was made with either (▲) *P. gallicus* or (●) *V. germanica* in the fluid phase.

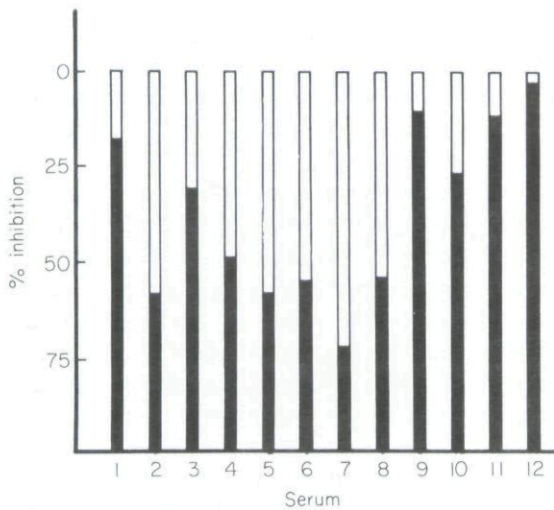


Fig. 4. RAST inhibition of twelve sera with a higher RAST titre to *Polistes gallicus*, by an excess of *Vespa germanica* venom (1 mg/ml) added to the fluid phase. Black columns indicate the maximum percentage inhibition in each case. The maximum inhibition was 73% (serum 7) and the minimum 3% (serum 12). Values lower than 15% were considered to be indicative of no inhibition and, therefore, binding of sera 9, 11 and 12 was not inhibited. The mean value of inhibition of the twelve sera was 38%.

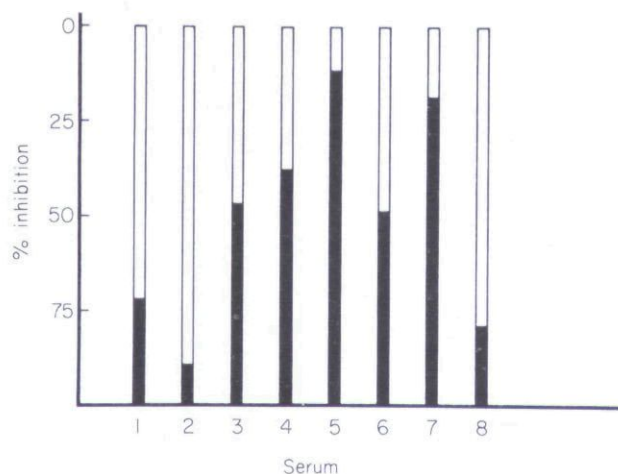


Fig. 5. RAST inhibition using eight sera with a higher RAST titre to *Vespula germanica*. Inhibition was achieved with *Polistes gallicus* venom in the fluid phase at a concentration of 1 mg/ml. The maximum inhibition was reached with serum 2 (86%) and the minimum with serum 5 (13%). The mean value of inhibition of the eight sera was 47%.

Discussion

Hymenoptera taxonomy is complex with many recognized species. Those most relevant clinically belong to the genera *Polistes* (american wasp), *Dolichovespula* (hornets) and *Vespula* (yellow jackets) [10]. Although some authors report that there is crossreactivity between *Polistes* and *Vespula* [2], others have shown that wasp-sensitive patients have different degrees of reactivity with yellow jackets and hornets [11]. In this study we have shown that in patients sensitized to either *V. germanica* or *P. gallicus*, there is crossreactivity between the venoms, and in only four cases did we fail to find conclusive evidence of crossreactivity, three being sensitized mainly to *P. gallicus* and one sensitized to *V. germanica*. The remaining patients showed varying degrees of crossreactivity (range 3–87%) between the venoms.

The identification of the venom to which the patients are allergic is crucial in diagnostic studies and when considering immunotherapy. In some parts of the world, in particular the southern United States and southern Asia, *Polistes* have been reported to cause more anaphylactic reactions than any other hymenoptera [2]. In the population investigated in the present study the differences were not as striking, although sensitization to *Polistes* seemed to be the more common [5]. Indeed, it should be emphasized that the most common species found in the whole Mediterranean region is *P. gallicus*. However, the venoms used in Spain and in other parts of Europe for diagnosis and immunotherapy are mainly *V. germanica*, or a mixture of different yellow jacket species. The results presented here show that both venoms should be used for skin testing and RAST in many areas of Spain, and possibly other Mediterranean countries. The variability of crossreactivity between venoms makes it necessary to use the correct ones for treatment as injection of irrelevant proteins can cause sensitization [12]. A complete epidemiological study of the distribution of the sensitivity to both venoms is in progress.

References

- 1 Kern F, Sobotka AK, Valentine MD, Lichtenstein LM. Allergy to insect stings. Allergic crossreactivity to vespid venom. *J Allergy Clin Immunol* 1976; 57:554-9.
- 2 Findlay RS, Gilaspy JE, Lord R, Weiner LS, Grant JA. Polistes wasps hypersensitivity. Diagnosis by venom induced release of histamine *in vitro*. *J Allergy Clin Immunol* 1977; 60:230-5.
- 3 Hoffman DR, Miller JS, Sutton JL. Hymenoptera venom allergy: a geographic study. *Ann Allergy* 1980; 45:276-9.
- 4 Reisman RE, Wypych JI, Mueller UR, Grant JA. Comparison of the allergenicity and antigenicity of Polistes venom and other vespid venoms. *J Allergy Clin Immunol* 1982; 70:281-7.
- 5 Blanca M, Miranda A, Fernandez J *et al*. Studies of the antigens and allergens of two vespids in the mediterranean area. XII National Congress of Immunology 1986, H 11.
- 6 Sobotka AD, Adkinson NF, Valentine MD, Lichtenstein LM. Allergy to insect stings. Diagnosis by radioallergosorbent test. *J Immunol* 1978; 122:2477-84.
- 7 Lowry CH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the folinphenol reagent. *J Biol Chem* 1951; 193:265-75.
- 8 Ceska M, Lundkvist U. A new and simple radioimmunoassay method for the determination of IgE. *Immunochemistry* 1972; 9:1021-30.
- 9 Yman L, Ponterius G, Brandt R. RAST based allergen assays methods in development of biological standardisation. Vol 29: Standardisation and Control of Allergens Administered to Man. Basel: S Karger, 1975.
- 10 Mueller U, Elliott W, Reisman R *et al*. Comparison of biochemical and immunologic properties of venom from four hornets species. *J Allergy Clin Immunol* 1981; 67:290-8.
- 11 Hoffman DR. Allergens in hymenoptera venom VI. Cross reactivity of human IgE antibodies to the three vespid venoms and between vespid and paper wasp venom. *Ann Allergy* 1981; 47:303-9.
- 12 Grant JA, Hokanson JA, Gillaspy JE, Farnan J. Wasp venom immunotherapy. A status report. *J Allergy Clin Immunol* 1981; 67:32.

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.