

Original article

European *Polistes* venom allergy

The American *Polistes* species venom mixture – that of *P. annularis*, *P. fuscatus*, *P. metricus* and *P. exclamans* – was the only commercially available mixture for diagnosis and therapy until 1996. However, these species of *Polistes* are not present in Europe, where *P. dominulus* and *P. gallicus* and to a lesser extent *P. nimphus* are widespread. The aim of this study was to assess the allergenic differences among the commercial American mix, *P. dominulus* and *P. gallicus* venom in European patients and therefore to verify if this mixture is suitable for diagnosis in these patients. We carried out skin tests, radioallergosorbent tests (RAST) and RAST inhibition in Italian patients with adverse reactions to *Polistes* stings. RAST inhibition results demonstrated that cross-reactivity between the American and European species is only partial and that *P. dominulus* and *P. gallicus* venoms have exclusive allergens. Skin tests and direct RAST confirmed these results and also showed that European *Polistes* venom is more suitable than the American mix in Italian patients. Moreover, we found a high rate of cross-reactivity between *P. dominulus* and *P. gallicus*. To conclude, *P. dominulus* and/or *P. gallicus* venoms are necessary for diagnosis and therefore in the therapy of European patients.

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Polistes is the most common genus among social wasps. *Polistes dominulus* (*Pd*), *Polistes gallicus* (*Pg*) and, to a lesser extent *Polistes nimphus*, are the most widely spread in Europe (1). Only a mixture of the American *Polistes* species (*A Pm*) – *P. annularis*, *P. fuscatus*, *P. metricus* and *P. exclamans* – was commercially available until 1996, when Anallergo (Florence, Italy) started the production of *Pd* venom. The American species are not present in Europe.

The aim of this study was to assess the allergenic differences among the *A Pm*, *Pd* and *Pg* venoms in European patients. We carried out skin tests, radioallergosorbent test (RAST) and RAST inhibition in Italian patients with adverse reactions to *Polistes* stings.

Materials and methods

Patients

We studied 21 patients with a history of adverse reactions to Hymenoptera stings (Table 1). Patients 1–10 had positive tests only to *Polistes* genus. Patients 11–21 also had positive tests to other

genera. However, in the latter group of patients, *Polistes* venom was recognized as the 'primary venom' (2): in three patients (no. 17, 18 and 19), RAST inhibition (not presented) showed that the other positivities were attributable to cross-reactivity with *Polistes* venom. For the remaining eight patients, the data – identification of the culprit insect and/or the results of the skin tests and serum specific IgE – indicated primary sensitization to *Polistes*. Beekeepers monopositive to *Apis mellifera* formed the control group.

Venoms

Apis mellifera, *Vespula* spp. and *A Pm* venoms were purchased from Stallergènes (Milan, Italy) and Alk-Abellò (Milan, Italy). The *Vespa crabro* venom was prepared by Anallergo. *Pd* and *Pg* venoms were obtained from nests collected in Tuscany and stored at –70°C. The insects were individually identified by an entomologist. After defreezing, the venom was collected by dissection and gently squeezing the sac (3). The protein content was assessed using the Lowry method and the yield was 60 µg per sac. The venoms were dialysed for *in vivo* use by removing components with molecular weight under 1000 Da using Centrator protein concentrator tubes from Anallergo. They were then lyophilized and refrigerated at –20°C for further use.

Table 1. clinical data, skin tests and serologic results

Patient no.	Pat.	Age (years)	Sex	Atopy	Reaction (Mueller grade)	Intradermal tests (threshold in µg/ml)*						Serum specific IgE (% retained radioactivity)†						Total IgE (kU/l)
						<i>Apis m.</i>	<i>Vespula</i> spp.	<i>V. crabro</i>	<i>APm</i>	<i>Pd</i>	<i>Pg</i>	<i>Pm</i>	CS	<i>Pd</i>	CS	<i>Pg</i>	CS	
1	RS	73	M	NO	IV	NEG	NEG	NEG	1	0.01	0.01	3.2	1.9	5.1	1.3	4.8	1.6	12
2	CR	37	F	NO	LLR	NEG	NEG	NEG	NEG	0.1	0.1	3.0	1.9	4.0	1.3	4.6	1.6	50
3	BL	62	F	NO	IV	NEG	NEG	NEG	0.1	0.1	0.1	9.3	1.6	17.0	1.1	12.7	1.0	21
4	MS	16	M	NO	LLR	NEG	NEG	NEG	0.1	0.1	0.1	29.4	1.6	40.6	1.1	37.8	1.0	194
5	MA	49	F	NO	II	NEG	NEG	NEG	0.001	0.001	0.001	32.6	1.6	42.8	1.1	34.5	1.0	180
6	MP	69	F	NO	IV	NEG	NEG	NEG	NEG	1	1	3.3	1.7	3.7	2.4	3.5	2.6	75
7	PS	33	M	YES	LLR	NEG	NEG	NEG	0.1	0.1	0.01	8.9	1.7	10.5	2.4	11.2	2.6	31
8	SI	53	M	YES	I	NEG	NEG	NEG	1	0.1	0.1	15.5	1.6	38.9	1.1	37.4	1.0	81
9	SG	20	M	YES	III	NEG	NEG	NEG	0.01	N.D.	N.D.	36.0	1.6	51.8	1.1	48.3	1.0	437
10	CM	38	M	YES	IV	NEG	NEG	NEG	NEG	0.1	N.D.	NEG‡	—	NEG‡	—	N.D.	N.D.	102
11	FL	33	M	NO	III	NEG	NEG	1	1	0.1	0.1	7.7	1.6	13.1	1.1	11.7	1.0	71
12	TS	35	M	NO	III	NEG	NEG	1	1	0.1	0.1	10.4	1.6	16.6	1.1	12.0	1.0	105
13	LF	47	M	NO	IV	NEG	1	NEG	NEG	1	1	3.7	1.9	5.6	1.3	5.9	1.6	90
14	SDR	17	M	NO	II	NEG	0.01	0.01	0.1	0.01	0.01	9.8	1.6	15.8	1.1	12.7	1.0	402
15	ML	25	M	NO	IV	NEG	NEG	1	0.1	1	1	3.5	1.9	2.0	1.3	2.5	1.6	104
16	PF	43	M	NO	IV	NEG	1	0.1	0.1	0.1	0.1	7.0	1.6	8.1	1.1	4.9	1.0	26
17	GG	42	M	NO	IV	1	0.0001	0.01	0.1	0.01	0.01	39.8	2.2	50.3	1.4	48.9	1.2	366
18	BG	35	M	NO	IV	1	0.1	1	0.1	0.01	0.01	15.7	2.2	23.9	1.4	19.3	1.2	315
19	DL	49	M	NO	IV	1	0.01	0.01	0.01	0.001	0.01	24.5	2.2	24.3	1.4	24.8	1.2	134
20	NS	17	M	YES	III	NEG	1	0.1	0.01	0.01	0.001	11.9	1.6	35.7	1.1	18.4	1.0	554
21	CD	50	M	NO	III	NEG	1	0.1	0.1	0.01	0.01	5.7	1.6	9.4	1.1	7.7	1.0	152

*Lowest venom concentration able to induce at least a 5-mm diameter's wheal with erythema.

†We consider positive (bold type) the values superior to double of control serum.

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LLR = large local reaction; APm = American *Polistes* mix; Pd = *Polistes dominulus*; Pg = *Polistes gallicus*; CS = control serum (pool of sera of patients with negative skin tests); N.D. = not done.

Skin tests

Skin tests were performed as previously described (4).

Serum-specific IgE

To compare values among the A *Pm*, *Pd* and *Pg* venoms, RAST was performed using solid-phase nitrocellulose according to Ceska's method (5), and the same concentration for all the three allergens was used (Anallergo).

The test was considered positive when values were more than double those obtained with a pool of sera from patients with negative skin tests to Hymenoptera venom, using the same allergen in the solid phase: 'control serum' (Table 1). The values are expressed as a percentage: bound radioactivity divided by total radioactivity. All the tests were performed twice in the same session and the mean value was reported.

RAST inhibition

We used sera from 10 patients with sufficiently high serum-specific IgE (no. 3, 4, 5, 8, 9, 11, 12, 14, 19 and 21). At low specific IgE levels, RAST inhibition is hardly reliable (6). RAST inhibition was carried out according to Hoffman's method (7). Homologous RAST inhibition was performed for *Pd* and *Pg*: the same venom was used in the solid phase and as an adsorbent. In the same session, specific IgE binding to solid-phase *Pd* and *Pg* was inhibited through serum adsorption with the A *Pm*. Reciprocal RAST inhibition was also carried out between *Pd* and *Pg*.

Statistical analysis

Significance was assessed by the Mann–Whitney *U*-test. Differences below 0.05 were considered significant.

Results

Skin tests

Fifteen of the 20 patients (75%) showed greater skin reactivity to *Pd* and/or *Pg* than to the A *Pm* (Table 1). In four of the 20 patients, reactivity was of the same order. Patient no. 15 had greater skin reactivity to A *Pm*; he had been stung when on holiday in the USA. In patient no. 9, intradermal tests with the European *Polistes* venoms were not performed.

It should be noted that in four patients, skin tests were negative to the A *Pm* and positive to the European *Polistes* (no. 2, 6, 10 and 13). Patient no. 10 had urticaria pigmentosa and a history of very severe anaphylactic shock; the patient was diagnosed in another Allergy Unit with 'non-IgE mediated insect sting anaphylaxis in subjects with urticaria pigmentosa' because a skin test with European *Polistes* sp. had not been performed. Comparison between *Pd* and *Pg* showed the same skin reactivity in 16 of the 19 patients. In the controls, skin tests were negative.

Serum-specific IgE

In all RAST-positive patients (18/21), IgE titres to the European species were higher than those to the A *Pm* ($P < 0.001$). IgE mean values were: *Pd*: 18.4%, *Pg*: 13.5%, A *Pm*: 10.7%. In three patients, RAST was negative to the A *Pm* and positive to the European species (no. 1, 2 and 13). Comparison between *Pd* and *Pg* serum-specific IgE titres showed a high correlation ($r = 0.97$).

RAST inhibition

Nine of 10 patients clearly had specific IgE to allergens of the European species which are exclusive because adsorption of serum with the A *Pm* only partially inhibited the binding of specific IgE to European *Polistes* (Figs 1 and 2). In these nine patients, RAST for *Pd* was inhibited by homologous venom by 80.9% on average, whereas it was inhibited by the A *Pm* by 46.1% on average (Fig. 3). RAST to *Pg* was inhibited by homologous venom by 86.6% on average, whereas it was inhibited by the A *Pm* by 47.5% on average (Fig. 3). In one of the 10 patients, cross-reactivity was complete (Figs 1 and 2: DL): that is, specific IgE tended only towards allergens common to both American and European species. Moreover, serum IgE titres in this patient were virtually the same towards the American and European species (DL; no. 19).

Reciprocal RAST inhibition between *Pd* and *Pg*, carried out in six patients, showed, in all of them, high cross-reactivity between the two species. Specific IgE to *Pd* were adsorbed by *Pg* venom and the values varied from 75% to 98% – average 84% (not presented). Specific IgE to *Pg* were adsorbed by *Pd* venom and the values varied from 62% to 96% – average 81% (not presented). This confirms the results of skin tests and serum-specific IgE.

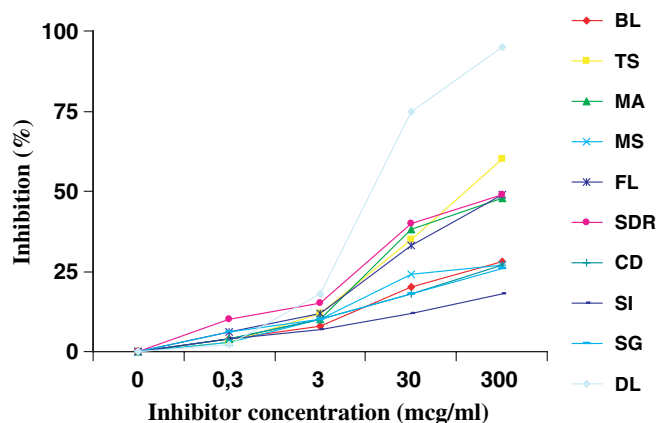


Figure 1. RAST inhibition. IgE binding to *Polistes dominulus* venom inhibited by adsorption with American *Polistes* mix in 10 patients.

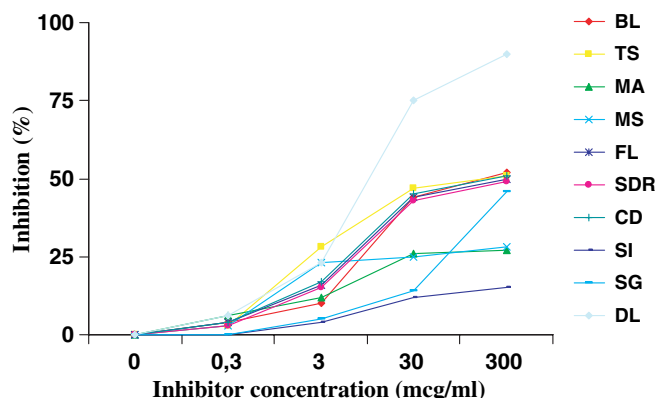


Figure 2. RAST inhibition. IgE binding to *Polistes gallicus* venom inhibited by adsorption with American *Polistes* mix in 10 patients.

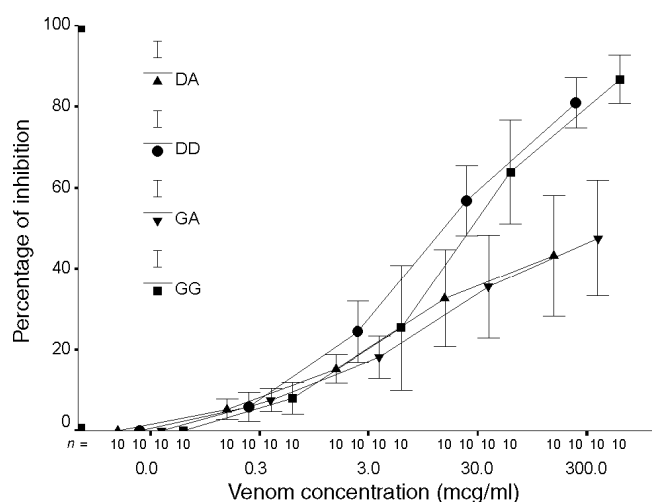


Figure 3. RAST inhibition: mean values of 10 patients. IgE binding to *Polistes dominulus* venom inhibited by American *Polistes* mix (DA) and by *P. dominulus* (DD). IgE binding to *P. gallicus* inhibited by American mix (GA) and by *P. gallicus* (GG).

Discussion

Among the genus *Polistes*, *P. dominulus*, *P. gallicus* and, to a lesser extent, *P. nimphus* are the most widely spread in Europe, while *P. annularis*, *P. fuscatus*, *P. metricus* and *P. exclamans* are present in the USA (1). The latter are not present in Europe.

Recent systematic studies (8, 9) have confirmed that North American and European species of *Polistes* belong to different subgenera and are phylogenetically distant. The most common European species – *P. dominulus*, *P. gallicus*, *P. nimphus* and *P. biglumis* – belong to the subgenus *Polistes sensu stricto* while the most common North American native species – *P. exclamans*, *P. annularis* and *P. metricus* and *P. fuscatus* – are included in the

subgenera *Aphanilopterus* and *Fuscopolistes* (g). We already know that cross-reactivity among European species of *Polistes* is very strong whereas that between European and American species is weaker (4). Our RAST inhibition studies demonstrate that adsorption with the A Pm only slightly inhibits IgE binding to the European venoms. This, therefore, suggests the presence of exclusive allergens in the latter.

No *in vivo* studies in European patients have been carried out to compare the venoms from the American and European *Polistes*. RAST inhibition studies, carried out in only four patients, compared *Pd* with single American species but not with the commercial A Pm (10). Our study is the first to describe *in vivo* tests with *Pd* and *Pg* venom, carried out in European patients.

Our *in vivo* results confirm the data previously obtained *in vitro* on the allergenic differences between American and European *Polistes* (10–13). It is particularly important to note that our four patients, whose skin tests were negative to the A Pm, showed positive skin tests to European *Polistes* venoms; two of them had severe reactions – grade IV of Mueller classification – and skin tests positive to 1 µg/ml. This further stresses that there is no correlation between skin test reactivity and severity of the reaction and also the

need for performing skin tests up to this concentration (4).

The man suffering from urticaria pigmentosa, with a history of a very severe anaphylactic shock after a *Polistes* sting (positively identified by the patient) is an emblematic case. It clearly demonstrates that omitting to perform skin tests for *Pd* venom would have repeated the wrong diagnosis of non IgE-mediated anaphylaxis and deprived the patient of life-saving immunotherapy (14).

So far we have treated 130 patients with the same *Pd* venom used for *in vivo* and *in vitro* tests (Anallergo). As regards safety, we have never observed systemic reactions or other side effects (15). Our data also demonstrate an excellent degree of protection from field stings, numbering 59 of 60 field stings without adverse reactions in 26 patients (M. G. Severino, pers. obs.).

In conclusion, our data raise the problem of the need to introduce the now commercially available European *Pd* venom extract into clinical practice.

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