

Comparison of the allergenicity and antigenicity of *Polistes* venom and other vespid venoms

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The crossantigenicity of Polistes venom with other vespid venoms was examined with rabbit and human antisera. Venom preparations from various Polistes species were obtained by electrical stimulation of individual insects and venom sac dissection. Rabbit antibodies were raised to the venom (P. apachus) and venom sac extract (P. exclamans). Human antisera were obtained from patients allergic to Polistes and other vespid venoms. The venom appeared to be more potent than the venom sac preparations in reactions with rabbit IgG and human IgE antibodies. Among the Polistes species, P. exclamans, P. instabilis, and P. apachus venoms showed several lines of precipitation with rabbit antisera, and P. annularis and P. fuscatus venoms only one line, suggesting quantitative or qualitative antigenic differences. In RAST analysis, most sera reacted equally to all Polistes species but occasional exceptions were noted, again suggesting differences in venom allergens. P. exclamans-coupled discs gave the most consistent results. In gel diffusion experiments, there was no crossreactivity between Polistes and yellow jacket venoms and only limited crossreactivity between Polistes and hornet venoms. Patients sensitive to Polistes venom showed varying degrees of reactivity to yellow jacket and hornet venoms in RAST analysis. Patients sensitive to other vespid venoms also showed varying degrees of sensitivity to Polistes venom. Polistes venom appears to contain a genus-unique antigen (allergen). In addition, there appear to be some crossreacting antigens in Polistes and other vespid venoms but to a much lesser degree than found previously in the analysis of the relationship of yellow jacket and hornet venoms. (J ALLERGY CLIN IMMUNOL 70:281, 1982.)

Yellow jackets, hornets, and wasps are the common stinging insects in the vespid family that are responsible for acute allergic reactions. Studies of the unique or crossallergenicity of these species are of great practical concern because of the current recommendations regarding testing and treatment of patients with stinging insect sensitivity. Our previous studies have suggested strong crossreactivity between the various yellow jacket species, with one exception,¹ and between the two major North American hornet species, *Vespa maculata* and *V. arenaria*.² Sub-

sequently, major crossantigenicity between hornet and yellow jacket species was also found.³

The purpose of this study was to examine the crossantigenicity of the *Polistes* species (wasp) venom with the other vespid venoms. As a preliminary aspect of this study, it was necessary to determine whether there were any differences in the antigenicity among different species of *Polistes*. The results of these studies suggest that there is limited crossreactivity between *Polistes* and other venoms and that within the *Polistes* genus there is strong crossreactivity among the various species.

MATERIALS AND METHODS

Antigens

Venoms obtained by individual insect milking,⁴ kindly supplied by Dr. James Gillaspay (Texas A and I University,

From the Allergy Research Laboratory, Buffalo General Hospital, State University of New York at Buffalo, and University of Texas Medical Branch, Adult Allergy Section, Galveston, Tex. Supported in part by NIH grant NIH-NIAID, AI 01303, and by NIH grant AI-12621 and Academic Career Development Award AI-00110 to Dr. Grant.

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Abbreviation used

RAST: Radioallergosorbent test

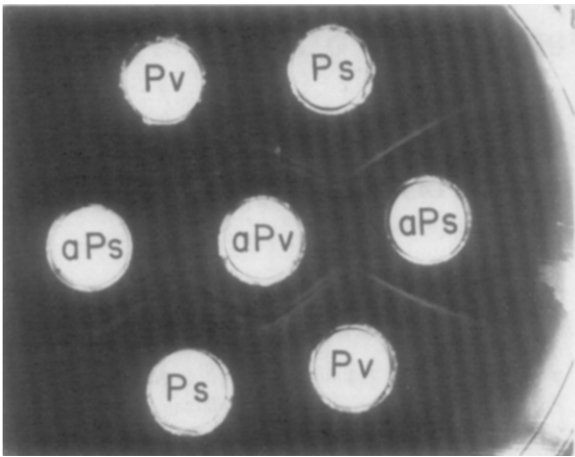


FIG. 1. Double-diffusion gel precipitation showing the reaction of *P. apachus* venom (Pv) and *P. exclamans* venom sac extract (Ps) and their respective rabbit antisera (aPv and aPs). The antigen concentration was 400 µg/ml.

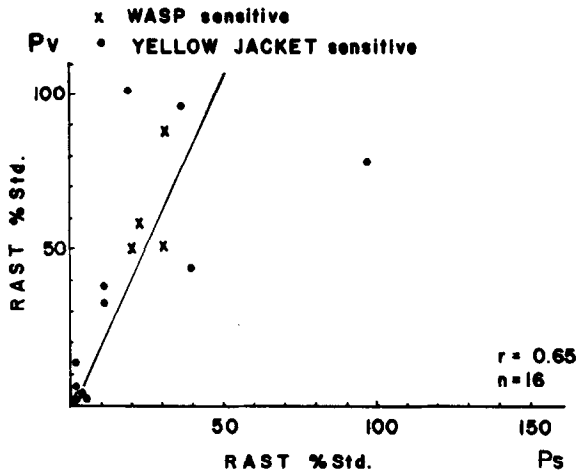


FIG. 2. Comparison of RAST results using *P. apachus* venom (Pv) and *P. exclamans* venom sac extract (Ps) as coupling antigens. Sera were obtained from *Polistes*- and yellow jacket-sensitive patients. Results are expressed as percent of a positive standard.

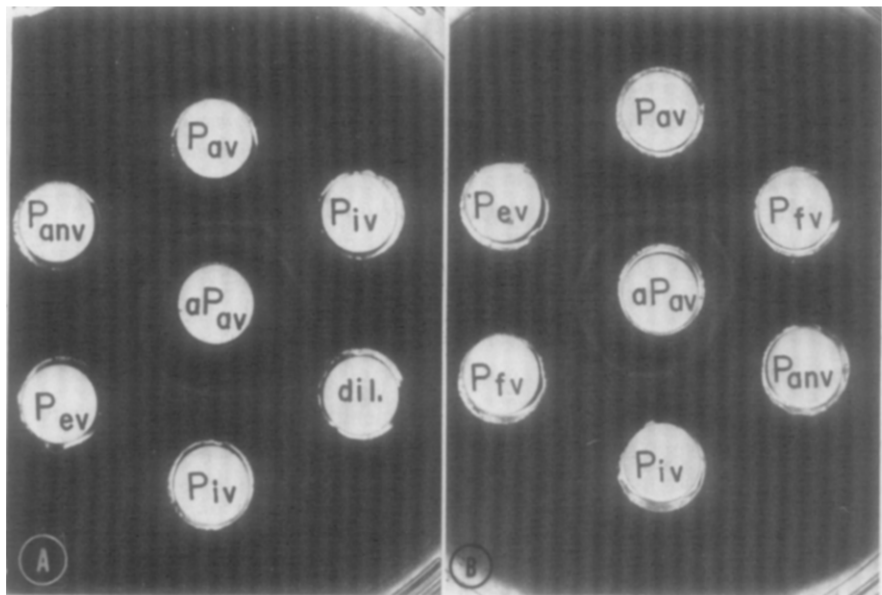


FIG. 3. Double-diffusion gel precipitation showing the reaction of *P. apachus* venom rabbit antisera (aPav) with venom obtained from *P. apachus* (Pav), *P. exclamans* (Pev), *P. instabilis* (Piv), *P. annularis* (Panv), and *P. fuscatus* (Pfv). All venom concentrations were 400 µg/ml.

Kingsville, Tex.) and Dr. Andrew Grant, were obtained from the following *Polistes* species: *P. fuscatus*, *P. annularis*, *P. instabilis*, *P. exclamans*, and *P. apachus*. The venoms were supplied lyophilized. Prior to use, they were reconstituted in buffered saline, the protein concentration was determined by the method of Lowry et al.^{4a} using a bovine serum albumin standard, and the solution was adjusted to a concentration of 1 mg protein/ml.

Venom obtained by venom sac extraction (*P. exclamans*) was supplied by Dr. Daniel Mullally, Head, Collaborative Product Section, IAIDP-NIAID, NIH, Bethesda, Md. In-

dividual venom sac preparations obtained from the following species were supplied by Dr. Allan Benton (Vespa Laboratories, Spring Mills, Pa.): *P. fuscatus*, *P. annularis*, *P. exclamans*, *P. metricus*, and *P. perplexus*. These venoms were supplied in a lyophilized state and reconstituted just prior to use.

Rabbit IgG antibodies

Immunization of rabbits. New Zealand white rabbits weighing 8 to 10 pounds were immunized by intradermal injections at multiple sites at 2 to 3 week intervals with

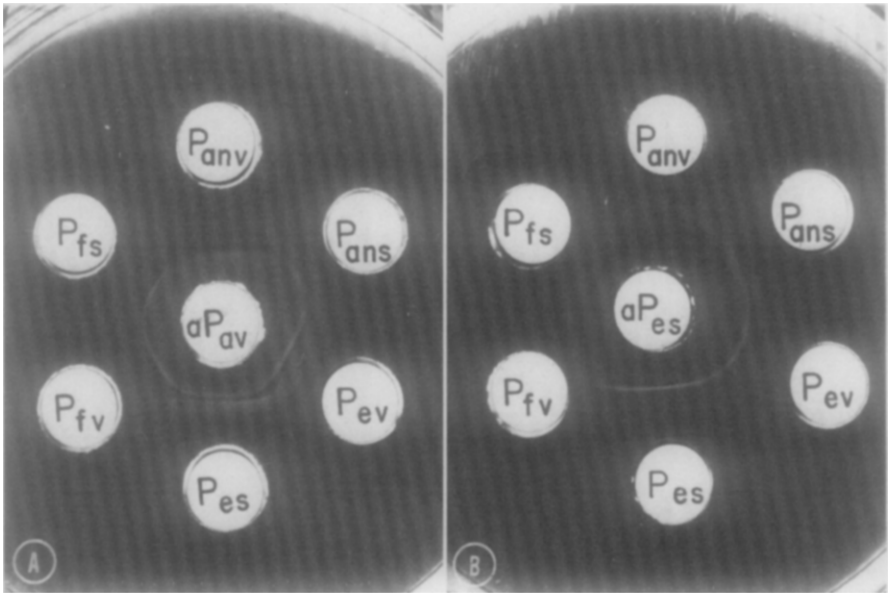


FIG. 4. Double-diffusion gel precipitation showing the reaction of rabbit antisera to *P. apachus* venom (aPav) and *P. exclamans* venom sac extract (aPes) with venom and venom sac preparations from *P. annularis* (Panv and Pans), *P. exclamans* (Pev and Pes), and *P. fuscatus* (Pfv and Pfs).

TABLE I. Comparison of RAST results* of discs coupled with different *Polistes* species venoms

Patient	<i>P. instabilis</i>	<i>P. exclamans</i>	<i>P. annularis</i>	<i>P. apachus</i>	<i>P. fuscatus</i>
<i>Polistes</i> sensitive					
J. H.	93	80	41†	81	73
S. T.	89	76	42	53	42
R. M.	71	52	61	48	29
J. M.	63	51	42	20	39
R. J.	53	69	57	18	56
S. W.	36	36	22	26	24
E. P.‡	27	31	40	47	37
S. C.‡	18	44	44	45	22
T. G.‡	8	8	4	6	11
L. W.	2	1	0	0	2
Yellow jacket sensitive					
J. B.	177	116	24	23	114
S. S.	90	109	65	101	93
W. S.	88	65	29	79	69

*RAST results are expressed in percent of a positive standard.
†Figures in italics indicate lowest RAST result for each profile.
‡*Polistes*-sensitive patients from New York State. All other *Polistes*-sensitive patients are from Texas.

P. apachus venom and *P. exclamans* venom sac extract. Two rabbits were immunized with each antigen. For each injection, 0.3 to 1 mg of *P. exclamans* venom sac extract or 0.2 to 0.4 mg of *P. apachus* venom in 0.5 ml saline were mixed with equal volumes of complete Freund's adjuvant. Higher immunization doses were given to provide adequate antibody stimulation. Bleedings were obtained 14 days after the seventh injection of the *P. exclamans* venom sac extract and after the third injection of the *P. apachus* venom.

Double-diffusion gel precipitation. Double-diffusion gel

precipitation in 1% agarose was done as described previously.¹ Preliminary experiments indicated that 400 µg/ml of the venom preparations was the optimal concentration.

Immunoelectrophoresis. Plates 8 by 10 cm were coated with 12 ml of 1% agarose in Tris barbital buffer, pH 8.3. The optimal amount of *Polistes* venom was found to be 5 mg/ml. No additional lines were found at lower concentrations. The electrophoresis was run at 10 V/cm for 50 min. Troughs were filled with 150 µl of the respective antisera. Immunodiffusion was carried out for 24 hr at room tempera-

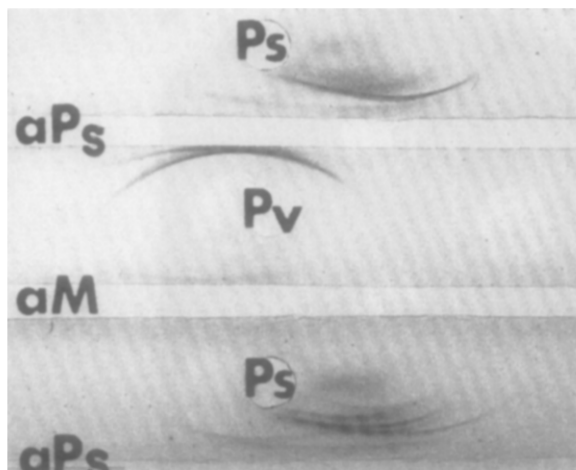


FIG. 5. Immunoelectrophoresis of *P. apachus* venom (Pv) and *P. exclamans* venom sac extract (Ps) with rabbit antisera to *P. exclamans* venom sac extract (aPs), and *V. maculata* (bald-faced hornet) venom (aM). The anode is to the right, the cathode to the left.

ture in a moist chamber. The plates were then washed, dried, and stained with 0.2% Coomassie Brilliant Blue. When the troughs were filled with normal rabbit serum rather than antiserum, no precipitation lines resulted.

Human IgE antibodies

Sera from seven patients from Texas and three patients from New York State with systemic allergic reactions after wasp stings were obtained before starting immunotherapy. In addition, sera were also obtained from patients with systemic allergic reactions after yellow jacket and hornet stings. Specific IgE antibodies to these venoms were measured by the RAST as described previously.³⁻⁵ For the *Polistes* venom discs, 2 mg of venom protein was used to couple 200 cyanogen bromide-activated discs. Results were expressed in percent of the net binding of a serum pool from ragweed-sensitive patients to ragweed discs. A net binding to the venom discs of >5% of the positive standard was considered significant. A binding of the ragweed standard varied between 11.5% and 12.8% of the total activity added. The background activity bound when normal human serum was assayed was 0.7% for the ragweed discs and approximately 1% of the total activity added for the venom discs.

RESULTS

Comparison of venom and venom sac preparations

Rabbit IgG antibodies. Fig. 1 shows the reaction of antisera prepared to *Polistes* venom (*P. apachus*) and *Polistes* venom sac extract (*P. exclamans*) with *P. exclamans* venom sac extract and *P. apachus* venom. There are several lines of precipitation between the antisera and the two venom preparations. In these reactions, the antisera appeared to be of approx-

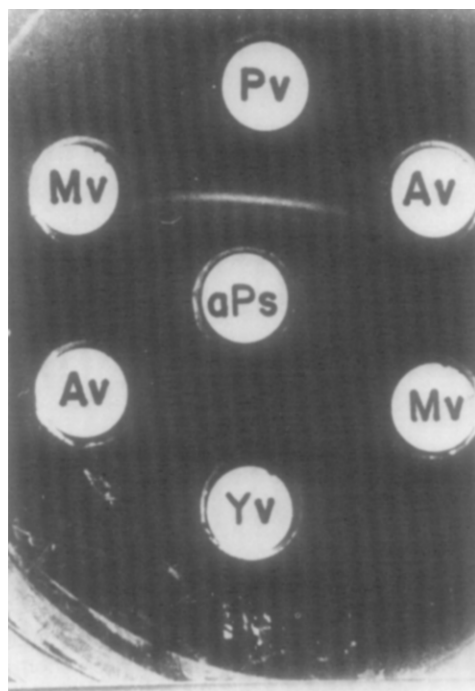


FIG. 6. Double-diffusion gel precipitation showing the reaction between *P. exclamans* rabbit antisera (aPs) and *P. apachus* (Pv), yellow jacket (Yv), bald-faced hornet (Mv), and yellow hornet (Av) venoms. Venom concentrations were 400 μ g/ml.

imately equal potency. Some differences were noted between the venom preparations of individual species, which will be discussed in more detail below.

Human IgE antibodies. Fig. 2 shows the RAST results with sera obtained from four patients clinically sensitive to wasps and 12 patients clinically sensitive to yellow jackets. There was a significant but not close correlation ($r = 0.65$) between the RAST results using *P. apachus* venom and *P. exclamans* venom sac extract as the coupling antigens. The RAST results were somewhat higher with the *P. apachus* venom.

Comparison of venom from different *Polistes* species

Before initiating studies of crossreactivity, it was necessary to examine the relative antigenicity of the various *Polistes* species.

Rabbit IgG antibodies. Fig. 3, A and B, shows the gel-diffusion experiments of the reaction between antisera to *P. apachus* and the various *Polistes* venom preparations. There is one strong line of identity with all of the venom species. Another common line is found with *P. exclamans*, *P. instabilis*, and *P. apachus* venoms. There is a definite spur between *P. instabilis* and *P. apachus* venoms, suggesting perhaps

another unique antigen. Only the common line of precipitation was clearly found with *P. annularis* and *P. fuscatus* venoms.

Fig. 4, A and B, shows the reaction of *P. apachus* venom and *P. exclamans* venom sac extract rabbit antisera with available paired venom and venom sac extracts. Stronger lines are noted with venom antiserum. *P. annularis* venom sac extract shows the presence of the second precipitin line common between *P. exclamans* and *P. instabilis* venoms but not detected in *P. annularis* venom. In other experiments, *Polistes* venom antisera reacted with one line of precipitation with *P. metricus* venom sac extract and reacted with two bands of precipitation with *P. perplexus* venom sac extract.

Immunoelectrophoresis of the two *Polistes* venom preparations (*P. apachus* venom, *P. exclamans* venom sac extract) with *Polistes* venom sac antisera is shown in Fig. 5. *Polistes* venom reacts with two strong arcs on the cathode side. The venom sac extract shows only two weak arcs in a similar position. On the anode side, there are multiple lines in the reaction between the venom sac extract and its antisera. These are absent from the pattern with venom. The anodic arcs should correspond to nonvenom components of the venom sac extract.

Human IgE antibodies. Sera from 10 *Polistes*-sensitive patients and three patients sensitive to other vespids were examined in RAST analysis, using discs coupled with venom from five different *Polistes* species. The results are shown in Table I. In general, the sera reacted about equally with all venoms, although scattered exceptions are noted.

The most consistent reactions were obtained with *P. exclamans*-coupled discs. Reactions to a lesser degree were found with two sera (J. H., S. T.) with *P. annularis*, three sera (S. T., R. M., S. C.) with *P. fuscatus*, two sera (J. M., R. J.) with *P. apachus*, and one serum (S. C.) with *P. instabilis*. No serum failed to react with one *Polistes* species venom and did react with others. The three sera from yellow jacket-sensitive patients all reacted with each *Polistes* species venom. However, all three reacted to a lesser degree with *P. annularis* and one to a lesser degree with *P. apachus*.

Crossreactivity of *Polistes* venom

Rabbit antibodies. Fig. 6 shows the reaction between antisera to *Polistes* venom sac extract (*P. exclamans*) and venoms from *Polistes* (*P. apachus*), yellow jackets, yellow hornets, and bald-faced hornets. The only lines of precipitation evident are those between the *Polistes* venom and its antiserum.

Fig. 7 shows the reaction between *Polistes* venom

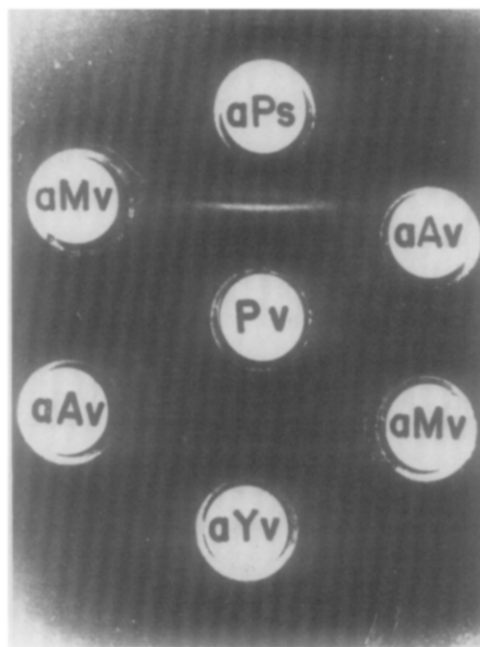


FIG. 7. Double-diffusion gel precipitation showing the reaction between *P. apachus* venom (*Pv*) and rabbit antisera to yellow jacket venom (*aYv*), bald-faced hornet venom (*aMv*), yellow hornet (*aAv*), and *Polistes* venom sac extract (*aPs*). The *Polistes* venom concentration was 400 $\mu\text{g/ml}$.

and rabbit antisera prepared against yellow jacket, yellow hornet, and bald-faced hornet venoms and *Polistes* venom sac extract. *Polistes* venom reacts with the antisera to *Polistes* venom sac extract with two lines. There is also one line of identity with antisera prepared to yellow hornet venom. No reaction is noted with antisera to yellow jacket venom or to bald-faced hornet venom.

In the electrophoretic pattern shown in Fig. 5, there is a very weak cathodal line of precipitation shown between *Polistes* venom and antisera to hornet venom (*aM*).

Human IgE antibodies. Nine sera from wasp-sensitive patients and 14 sera from patients with yellow jacket sensitivity were compared in RAST analysis using discs coupled with each of the vespid venoms. Fig. 8 shows the reaction of these sera with *Polistes* and yellow jacket venoms. With one exception, all sera from *Polistes*-sensitive patients reacted with *Polistes* venom. Five of these sera failed to react to yellow jacket venom and four reacted with various titers. The sera from yellow jacket-sensitive patients reacted over a wide range with *Polistes* venom. Similar results also were found when bald-faced hornet (Fig. 9) and yellow hornet venoms were used as the coupling antigens.

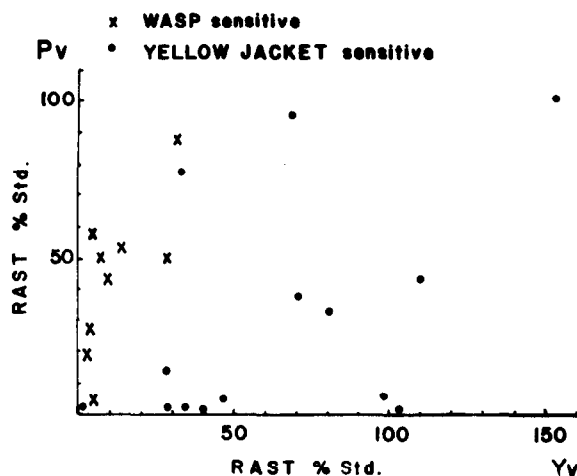


FIG. 8. Comparison of RAST results with *P. apachus* venom (Pv) and yellow jacket venom (Yv) as coupling antigens. Sera were obtained from *Polistes*- and yellow jacket-sensitive patients. Results are expressed as percent of a positive standard.

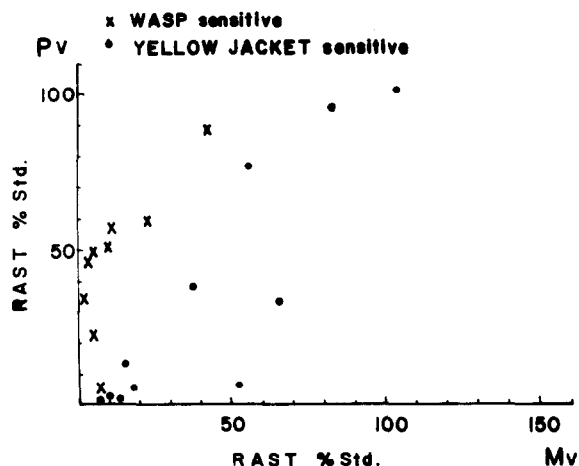


FIG. 9. Comparison of RAST results using *P. apachus* venom (Pv) and bald-faced hornet venom (Mv) as coupling antigens. Sera were obtained from *Polistes*- and yellow jacket-sensitive patients. Results are expressed as a percent of a positive standard.

DISCUSSION

Polistes venom obtained by electrical stimulation appeared more potent than the *Polistes* venom sac extract. In immunoelectrophoresis, stronger lines were found with the reaction of rabbit antisera with *Polistes* venom. In RAST analysis, IgE antibodies from sensitive patients reacted in higher titers with the venom. The *Polistes* venom sac extract does contain body protein not present in the venom obtained by electrical stimulation, similar to the results found in the analysis of other vespid venoms collected by electrical stimulation and venom sac extraction.⁶

There appear to be quantitative antigenic differences between corresponding venoms and venom sac extracts, and between venoms from different *Polistes* species. In gel-diffusion experiments, several antigens were detected in venoms from *P. exclamans*, *P. instabilis*, and *P. apachus*. *P. annularis* venom sac extract, but not *P. annularis* venom, appears to show the same reactivity. *P. instabilis* venom may have an increased amount of a third antigen. *P. fuscatus* venom and venom sac extracts showed only one precipitin line in these experiments.

In RAST analysis, most sera from *Polistes*-sensitive patients reacted with venom from all of the specific *Polistes* species. There were occasional exceptions in the degree of reactivity. For example, of the 10 sera from the *Polistes*-sensitive patients, two reacted to *P. annularis*, two to *P. apachus*, one to *P. instabilis*, and three to *P. fuscatus* to a much lesser degree than with the other *Polistes* species venoms. The *P. exclamans* venom gave the most consistent RAST result. This is the major *Polistes* species in the South Texas

area, from which several sera were obtained. The three sera from yellow jacket-sensitive patients showed similar results reacting with all *Polistes* species venoms. *P. annularis* reacted to a lesser degree with all three sera and *P. apachus* reacted to a lesser degree with one serum. These results are generally consistent with the data reported by Findlay et al.,⁴ who found that venoms from three *Polistes* species (*P. apachus*, *P. exclamans*, and *P. carolina*) had similar activity in their ability to release histamine from basophils of sensitive individuals. Our data do suggest the likelihood of quantitative differences in allergenic components in the different *Polistes* species venoms. This generally extensive crossreactivity among the various *Polistes* species suggests that commercially available *Polistes* venom, composed of venom from mixed species, is reliable for diagnosis and treatment of *Polistes* venom hypersensitivity.

In gel-diffusion experiments examining the crossreactivity of different vespid venoms, antisera raised to *Polistes* venom sac extract reacted only with the *Polistes* venom and failed to react with yellow jacket, yellow hornet, and bald-faced hornet. *Polistes* venom did show some reaction with antisera prepared to yellow hornet venom. In immunoelectrophoresis, there was also one line of precipitation between *Polistes* venom and antisera to bald-faced hornet.

In RAST analysis, sera of wasp-sensitive patients showed various degrees of reactivity with yellow jacket and hornet venoms. Similarly, the sera of patients sensitive to other vespids reacted with *Polistes* venom. In the study of Findlay et al.,⁴ yellow jacket venom did not release histamine from basophils of

Polistes-sensitive patients. Somewhat different findings were observed by Kern et al.,⁷ who found that basophils of over half of 18 insect-sensitive patients did release histamine when challenged with both *Polistes* and yellow jacket or hornet venoms. However, linear regression analysis showed no correlation in the amount of venom required for 50% histamine release.

These results suggest that there is limited cross-reactivity between *Polistes* venom and other vespid venoms. There does appear to be an antigen in vespid venoms that crossreacts among all vespids. In addition, it would appear that *Polistes* venom does have a unique antigen of its own. This suggests that patients sensitive to *Polistes* venom will require *Polistes* venom immunotherapy. On the other hand, some patients sensitive to yellow jacket or hornet venoms may also react to *Polistes* venoms. In these situations there appears to be a crossreacting antigen and it is doubtful that specific *Polistes* immunotherapy would be necessary. Unfortunately, unless the insect causing the sting reaction can be reliably identified, venom therapy with both *Polistes* and other vespid venoms may be the only practical solution. RAST inhibition studies that might resolve the question of unique or cross-sensitivity are too expensive and time-consuming to do on an individual patient basis. These remain a research tool.

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