

***Polistes* wasp hypersensitivity: Diagnosis by venom-induced release of histamine in vitro**

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Polistes wasps cause a majority of Hymenoptera-induced anaphylactic reactions in Texas. Using the in vitro release of histamine from basophils of patients allergic to Polistes stings, we have studied the cross-reactivity of venoms from three species of Polistes wasps as well as the cross-reactivity among Polistes, honeybee, and Vespula maculifrons (yellow jacket) venoms. Venom collected by an extrusion technique from Polistes exclamans, Polistes apachus, and Polistes carolina caused release of histamine in seven Polistes-sensitive individuals. The dose-response curves from all three Polistes species were quite similar, suggesting extensive cross-reactivity among these species. None of these patients showed significant release of histamine from leukocytes exposed to yellow jacket or honeybee venom. We conclude that a source of Polistes venom is available for further study and possibly for therapy. It appears that any of three local common species of Polistes wasps could be used. Our studies confirmed earlier reports that Hymenoptera sensitivity is often genus-specific.

POLISTES WASP HYPERSENSITIVITY

Anaphylactic reactions to stinging insects of the order Hymenoptera cause more deaths than all other venomous animals.¹ Because of the serious nature of these reactions, immunotherapy with Hymenoptera whole body extracts has been used in surviving patients for over 45 years.² However, there have been reservations concerning the efficacy of whole body extracts, and these reservations have led to studies of venom for both diagnosis and therapy of this disorder.^{3, 4, 5}

One technique used to study insect hypersensitivity is the in vitro release of histamine from leukocytes. When venom combines with IgE antibodies bound to basophils from Hymenoptera-allergic donors, histamine as well as other mediators of anaphylaxis are released.^{5, 6} It has been found that histamine release correlates with the presence of clinical hypersensitivity and results of venom skin testing.^{7, 8}

Studies performed in the northern, eastern, and midwestern parts of the United States have been concerned principally with the use of venoms in patients allergic to hornets and yellow jackets, both of the genus *Vespula*, and honeybees of the genus *Apis*.^{5, 7, 8} Another group of Hymenoptera, *Polistes* wasps, apparently cause many anaphylactic reactions, especially in southern areas of the country. According to Micks⁹ *Polistes* wasps caused more anaphylactic reactions in Texas than any other Hymenoptera, including honeybees and fire ants. This is probably due to the fact that these wasps prefer to nest on houses and because of their prolific, territorial, and aggressive nature.

To avoid confusion with yellow jackets (genus *Vespula*), it should be noted that *Polistes* nests are formed of a horizontal comb of paper cells and are attached to a support by a pedicel. Some members of this genus, especially *Polistes exclamans* and *Polistes apachus*, have been referred to as "yellow jackets"

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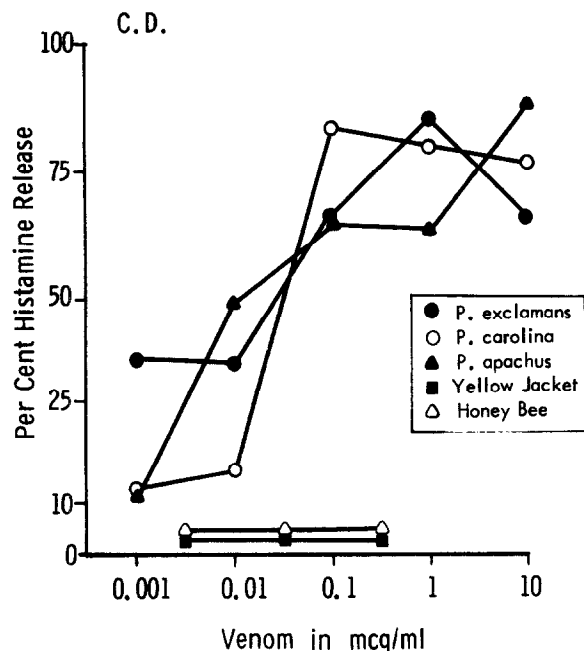


FIG. 1. Release of histamine in vitro from basophils of a *Polistes*-allergic patient.

because of their vertical yellow body markings. However, the term yellow jacket is best reserved for the ground-nesting form of the genus *Vespula*.¹⁰ *Polistes* wasps are also referred to as "paper nest wasps," although many other vespids construct nests of paper-like material. Further confusion occurs because several Hymenoptera genera nest in overlapping geographic regions. Many species of *Polistes* wasps are found within the state of Texas; however few yellow jackets and hornets (genus *Vespula*) have been identified.¹¹ Therefore, within this geographic region, individuals reporting "wasp" or "yellow jacket" hypersensitivity are most likely allergic to *Polistes* species.

In this study the venom from the honeybee, the yellow jacket (*Vespula maculifrons*), and from three separate species of *Polistes* wasps were compared with respect to their ability to cause the release of histamine from basophils of individuals who were allergic to *Polistes* wasps.

MATERIALS AND METHODS

Insect collection and antigen preparation

Three separate species of *Polistes* wasps, *P. exclamans*, *P. apachus*, and *P. carolina*, were collected and identified by J. E. G. Insects were immobilized by chilling. Subsequently, individual wasps were hand-held and induced to extrude venom onto a glass surface. Venoms were dissolved in saline and dialyzed against isotonic HEPES (25

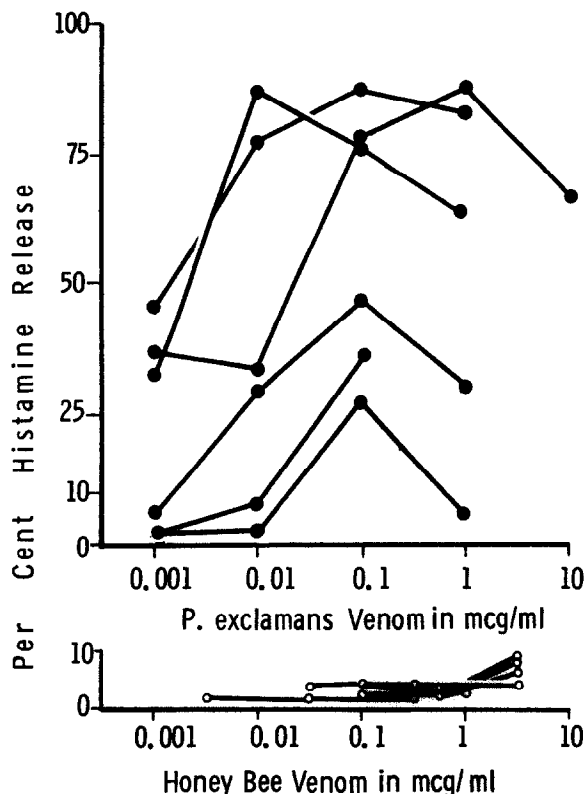


FIG. 2. The histamine release dose-response of six *Polistes*-allergic patients tested with *P. exclamans* venom in the upper panel and bee venom in the lower panel.

mM) buffer pH 7.4 with calcium (2 mM) and magnesium (1 mM) using tubing which retained substances greater than 3,500 daltons in size. Venom was stored at -70°C until used in vitro. Fluorescamine was used to quantitate the number of free amines present in venom samples.¹² An estimate of weight was then determined by comparison with a standard protein, bovine serum albumin (BSA). Yellow jacket venom was kindly provided by Dr. Martin Valentine and honeybee venom by Center Laboratories (Lot No. 310309 FD).

Histamine release studies

Antigen-induced release of histamine from basophils was performed according to the method of Lichtenstein and Osler¹³ as modified by Grant and co-workers.¹⁴ Histamine was assayed in the supernatant of leukocytes using a modification of a radioenzyme technique of Beaven, Jacobsen, and Horakova.

Patients and controls

Patients between the ages of 4 and 45 yr who had experienced symptoms of urticaria, hypotension, bronchospasm, or laryngeal edema in response to wasp or bee stings within the past 1 month to 2 years were studied. Control individuals were normal adults who denied a history of Hymenoptera sensitivity.

TABLE I. Histamine release data

Patient	Maximum histamine release in percent				
	<i>P. exclamans</i>	<i>P. apachus</i>	<i>P. carolina</i>	Yellow jacket	Honeybee
T. T.	87	63	61	<10	<10
D. S.	36	42	36	ND	ND
B. R.	46	41	54	<10	<10
A. G.	27	37	25	<10	<10
G. C.	86	64	70	40	<10
R. C.	37	26	15	ND	<10
C. D.	87	84	94	<10	<10
Three bee-sensitive patients					Range, 38-80
Six normals	<10	<10	<10	<10	<10

ND: not done.

TABLE II. Historical data on patients allergic to *Polistes* wasp sting

Patient	Age-sex	Interval sting-test	Sting-induced symptoms
T. T.	28 M	1 mo	Hives; pruritus; large local
D. S.	4 M	1 mo	Hives; pruritus; large local
B. R.	5 M	1 mo	Distant swelling; large local
A. G.	45 M	6 mo	Hives; distant swelling; laryngeal edema
G. C.	36 M	2 mo	Hives; pruritus
R. C.	6 M	2 mo	Hives; pruritus; distant swelling; rash
C. D.	32 F	2 yr	Distant swelling; hypotension

T. T., A. G., and G. C. are physicians, and C. D. is a physician's wife. All identified the stinging insect as a wasp and described typical *Polistes* nests in the immediate vicinity. D. S. and G. C.'s parents killed and identified the stinging insect as a wasp. B. R.'s mother identified a wasp as it stung the patient.

RESULTS AND CONCLUSIONS

Three individuals who had experienced recent systemic anaphylaxis associated with a honeybee sting were found to have diagnostic histamine release data (Table I) as well as positive skin tests to honeybee venom.⁷ *Polistes* venom was not available at the time these individuals were evaluated.

Seven consecutive patients gave histories of anaphylactic reactions induced by wasp stings within two years (Table II). Leukocytes from these individuals were tested for in vitro histamine release in response to Hymenoptera venoms. *Polistes* venoms caused maximal release of 15% to 94% of intracellular histamine (Table I). Maximal release was noted at venom concentrations of 0.01 mcg to 1 mcg/ml, which is within the range previously reported to

identify anaphylactic sensitivity to other Hymenoptera venoms.^{5, 7, 8} Leukocytes from six normal control adults released less than 10% of intracellular histamine when exposed to *Polistes* venom over the same range of concentrations.

Fig. 1 illustrates a typical dose-response curve showing the release of histamine from basophils of a wasp-sensitive patient. An identical pattern of release was seen with venoms from all three species of *Polistes*. Similar curves were obtained with the six other *Polistes*-sensitive individuals.

Six of the *Polistes*-sensitive patients were studied for response to bee venom (Fig. 2) and 5 to yellow jacket venom (Fig. 3). No significant response was seen with venom concentrations as high as 5 mcg/ml, except in the case of G. C. in whom 40% release occurred at a concentration of yellow jacket venom of 5 mcg/ml. In this individual, comparable levels of histamine were released with a concentration of yellow jacket venom 5,000 times greater than with *Polistes* venom (Fig. 3). Release of histamine occurring at concentrations greater than 1 mg of venom per milliliter may be nonspecific.⁸ Release of twenty percent or less likewise has been considered of borderline significance.⁸

Sequential studies of basophil histamine release following challenge with *P. exclamans* venom were done in 2 patients (Fig. 4). It can be seen that without receiving further stings, Patient G. C. had a comparable pattern of release over a nine-month period of time (Fig. 4, A). The adjoining panel (Fig. 4, B) shows the release of another patient, A. G. Six months after suffering a wasp-induced anaphylactic reaction, the maximal release of histamine was 30%. Later a second *Polistes* sting resulted in urticaria and mild laryngeal edema. Three days after this sting histamine release was not detected. Three weeks after this reaction a remarkable release of over 70% was

Minimum venom concentration for maximum histamine release in mcg/ml

<i>P. exclamans</i>	<i>P. apachus</i>	<i>P. carolina</i>	Yellow jacket	Honeybee
0.1	0.01	0.01	>5	>1
0.01	0.01	0.01	ND	ND
0.1	1	1	>5	>5
0.1	1	0.1	>5	>5
0.01	0.01	0.1	5	>5
0.1	0.1	0.1	ND	>5
1	0.1	1	>0.5	>0.5
	>1	>1	>5	Range, 0.01-1 >5

obtained in what appeared to be an anamnestic response. It was concluded that although histamine release may remain stable, variations in release may also appear coincident to or because of sting-induced anaphylaxis.

In summary, bee venom and *Polistes* wasp venom did not cross-react in six *Polistes*-sensitive individuals. In 4, yellow jacket and *Polistes* venoms also did not cross-react. Venoms from all three *Polistes* species tested cross-reacted in all 7 allergic donors. Therefore, *Polistes* venom hypersensitivity may be genus-specific, but species cross-reactive.

DISCUSSION

Twenty years ago Loveless and Fackler¹⁶ made the earliest reports concerning the use of wasp venom in the therapy of wasp-sensitive patients. Since then few clearly *Polistes*-sensitive subjects have been evaluated. This has hampered evaluation of *Polistes* wasp venom preparations as diagnostic and therapeutic agents. The present study not only identified several *Polistes* wasp-allergic patients, but also demonstrated that *Polistes* venom collected by the extrusion technique caused histamine release from the basophils of individuals recently experiencing anaphylactic reactions to *Polistes* wasps.

The importance of the extrusion technique in *Polistes* venom collection was first noted by O'Connor and Erickson¹⁷ and Dirks.¹⁰ In an immunoelectrophoresis study of dissected poison sac extract, venom antigens were not detected.¹⁰ The author postulated that venom proteins were altered or lost during dissection of the extremely muscular poison sac of *Polistes* wasps. The extrusion technique was therefore chosen for the preparation of venoms used in this study.

It should be noted that Loveless¹⁸ and Kern and co-workers apparently found anaphylactogenic sub-

stances in carefully dissected *Polistes* poison sacs. The reasons for these technical discrepancies remain unclear and are a possible problem for future studies.

The question of cross-reactivity between venoms of different Hymenoptera genera has been investigated previously.^{8, 19, 20, 21} These studies support the concept that many individuals are allergic to members of only one Hymenoptera genus. The majority of individuals allergic to honeybee venom do not react in vitro or by skin tests to yellow jacket or hornet venoms. In a minority, but significant number of cases, cross-reactivity or concurrent allergy to separate genera is seen. Kern and co-workers¹⁹ reported that 10 of 18 patients showed cross-reactivity between yellow jacket and *Polistes* wasp venom. The insect causing the sting-induced anaphylaxis was apparently unknown in these patients. About half of these patients had been treated with whole body mixed Hymenoptera extract prior to histamine release testing. By linear regression analysis, it was found that the concentrations of yellow jacket and *Polistes* wasp venom giving 50% histamine release did not correlate. The authors concluded that allergenic cross-reactivity therefore was not present. To what extent therapy with mixed whole body extract influenced the results is unknown.

It should be noted that patients in the present study were not treated with immunotherapy prior to histamine release testing and all lived in areas where yellow jackets are uncommon. Cross-reactivity was clearly demonstrated between the venom of three *Polistes* species. Convincing cross-reactivity was not seen with either honeybee or yellow jacket venoms.

Previous studies^{10, 17} have demonstrated that *Vespa* and *Polistes* venom have few if any antigenic components in common. All types of immunodiffusion studies of Hymenoptera venoms are complicated by the presence of substances that nonspecifically

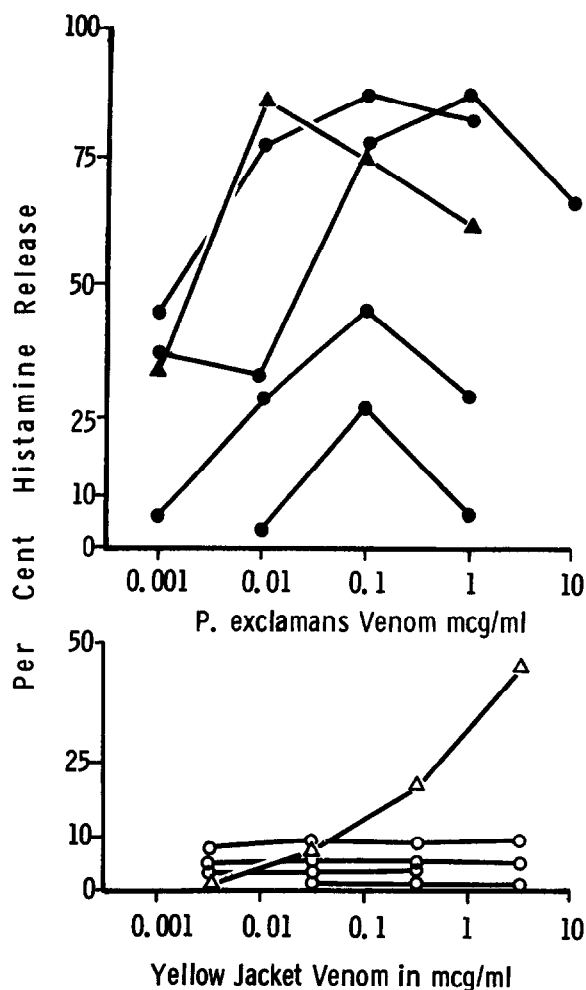


FIG. 3. The histamine release dose-response of 5 *Polistes*-allergic patients tested with *P. exclamans* venom in the upper panel and yellow jacket venom in the lower panel. Data of Patient G. C. are noted in triangles.

precipitate serum components.²² Using immunoelectrophoresis, Dirks¹⁰ separated these artifacts from true immunoprecipitation lines. In his studies different species of *Polistes* venoms did cross-react extensively, but *Vespula* venom did not cross-react with *Polistes* venoms.

Finally, Pinna and co-workers²³ have introduced data supporting the concept that ant sting hypersensitivity may also be genus-specific.

It has been suspected that immediate skin tests to specific venom may become negative shortly after sting-induced anaphylaxis. The sequential histamine release data of Patient A. G. appears to demonstrate basophil nonreactivity 3 days following an insect-induced anaphylactic reaction. These differences in histamine release were not due to variations in the absolute level of intracellular histamine which approximated 100 ng/ml of blood consistently. The

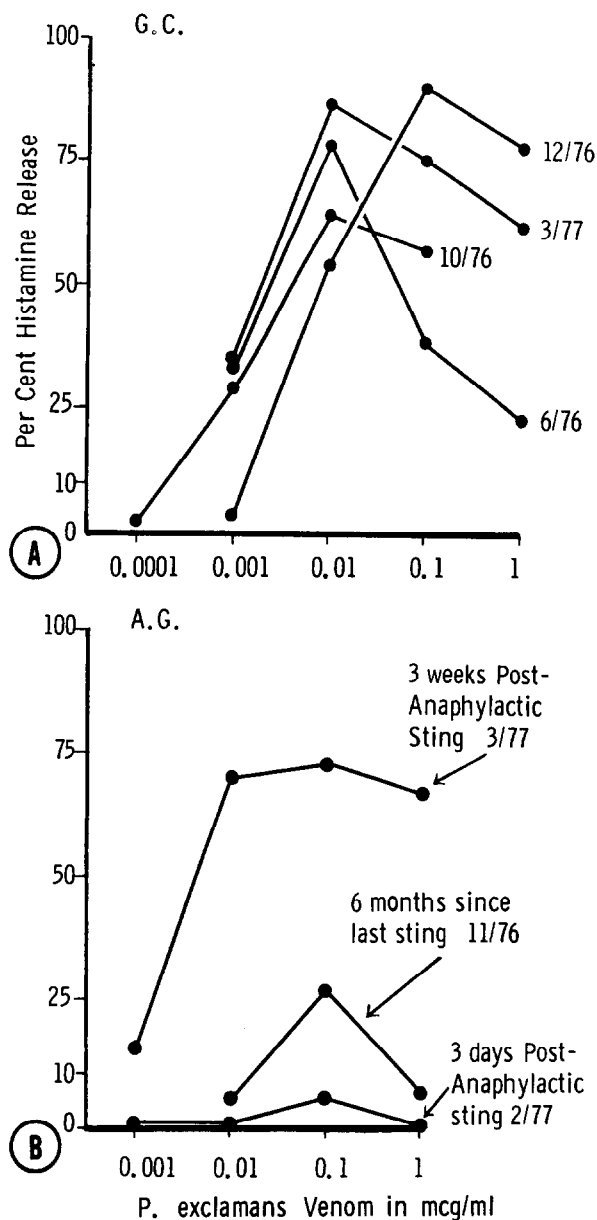


FIG. 4. Serial histamine release dose-response curves from two patients. A, G. C. over 9 mo. B, A. G., 6 mo after first sting, 3 days after second sting, and 3 wk after second sting.

explanation for these findings remains unknown. Perhaps measurements of levels of venom-specific IgE directly before and after anaphylaxis would clarify the problem. The anaphylactic reaction may have depleted or "desensitized" those basophils with venom-specific IgE. The anamnestic response 3 weeks later may parallel a rise in venom-specific IgE reported after sting-induced anaphylaxis.^{20, 24}

In summary, it appears that venom collected by the extrusion technique is valuable for the *in vitro* study of *Polistes* wasp hypersensitivity. Our study indicated

that the *Polistes* venom induces the release of histamine from blood basophils in hypersensitive individuals, and that the venoms of the three species of the genus *Polistes* cross-react significantly. The venoms of genus *Polistes*, however, did not cross-react with venoms of the genus *Vespula* or *Apis*. Considering these results and the large number of *Polistes*-sensitive patients in this geographic area, a study using *Polistes* venom for skin testing and immunotherapy in anaphylactically sensitive subjects appears warranted.

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