

Selection of Hymenoptera venoms for immunotherapy on the basis of patient's IgE antibody cross-reactivity

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Background: Positive skin test results to multiple venoms in patients with Hymenoptera venom allergy may result from IgE antibody cross-reactivity among venom proteins. To avoid treatment with unnecessary and costly venoms, we have developed a venom RAST inhibition test that identifies individuals in whom a positive venom IgE RAST result is due to cross-reactive venom-specific IgE antibody.

Methods: Serum samples ($n = 412$) were collected over 5 years from patients with clinically characterized Hymenoptera venom allergy who had positive skin test results to more than one venom. Venom allergosorbent was added to serum containing IgE antivenin and buffer or 100 μ g of homologous or heterologous venom. Bound IgE was detected with radiolabeled anti-human IgE. Intraassay variation less than 10% coefficient of variation and homologous venom inhibition greater than 80% were required for acceptance of data. A "cross-reactivity index" (CRI) was computed as a ratio of percent inhibition produced by heterologous versus homologous venom.

Results: Of the 412 sera-venom combinations analyzed, 41 (10%) were excluded because of incomplete homologous venom inhibition. Of the 371 remaining sera, 82% ($n = 305$) were studied for IgE anti-Polistes wasp venom (PWV) cross-reactivity with yellow jacket venom (YJV) and the other 66 for other venom specificity cross-inhibitions. Of the serum samples tested, 36.4% (111 of 305) contained IgE anti-PWV venom of which the binding to solid-phase PWV was inhibited with soluble YJV to a level that produced CRIs greater than 95%. We believe that this constitutes complete inhibition and demonstrates exclusively YJV cross-reactive antibody in these samples. The remaining 63.6% had CRIs from 0% to 95%, indicating IgE specific for a spectrum of unique and cross-reactive PWV allergens. Only 4.3% (13 of 305) had CRIs less than 5%, which is consistent with IgE restricted to PWV unique allergens. The degree of the IgE anti-PWV inhibition to solid-phase PWV by YJV was independent of the IgE anti-PWV level.

Conclusions: This study shows that one third of patients with Hymenoptera venom allergy evaluated with positive YJV- and PWV-reactive IgE in the skin and/or serum were identified as candidates for exclusion of PWV from their immunotherapy regimen because their IgE anti-PWV was more than 95% cross-inhibitable with YJV. Cost analysis of the venom RAST inhibition test and a conventional 5-year Hymenoptera venom immunotherapy program indicates that this serologic evaluation is cost-effective. (*J ALLERGY CLIN IMMUNOL* 1993;92:651-9.)

Key words: Hymenoptera venom, venom immunotherapy, human IgE antibody, cross-reactivity, RAST inhibition

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Venoms from a medically important Hymenoptera species (Table I) can induce life-threatening type I hypersensitivity reactions in predisposed individuals who have been previously stung.¹ Once confirmed with a careful clinical history and positive in vivo provocation (skin test) and/or in vitro IgE antibody test results,^{2, 3} the patient may be advised to start venom immunotherapy. Selection of the appropriate venoms for therapy is based on those specificities identified during the diagnostic screening process.^{4, 5} An

Abbreviations used

- CRI: Cross-reactivity index
PWV: *Polistes* wasp venom
VRIT: Venom RAST inhibition test
YJV: Yellow jacket venom

individual may, however, exhibit sensitivity to multiple insects as a result of either concomitant allergies to *unique* allergens in each of the venoms or *cross-reactivity* among structurally similar venom proteins. If a venom is included in a therapy regimen to which an individual has skin reactivity strictly as a result of cross-reactivity to structurally similar allergens in different venoms, then cost of therapy is needlessly increased. Moreover, the administration of venom allergens to which the patient was not originally allergic may in theory induce *de novo* sensitization to these components. Selection of the proper venoms for immunotherapy may be particularly difficult in patients with weak skin reactivity to *Polistes* wasp venom (PWV), which may be due to specific IgE to cross-reactive allergens in vespid venoms, most commonly yellow jacket venom (YJV).⁴⁻⁸

During the past 5 years, we have evaluated a soluble venom competitive inhibition format of the Hymenoptera venom RAST for its utility in identifying individuals with a primary YJV sensitivity whose IgE binds strictly to cross-reactive allergens in PWV. We report here on the optimization of the venom RAST inhibition test for selection of venoms for immunotherapy and on our experience with this procedure over 5 years of use. Although the primary indication for this test is for use in individuals who have strong skin reactivity to YJV and weak reactivity to PWV, selected cross-reactivity patterns with different patterns of Hymenoptera venom reactivity were also examined. This study shows that 36% of the 305 patients with Hymenoptera venom allergy evaluated with positive YJV- and PWV-reactive IgE in the skin and/or serum had IgE anti-PWV that was more than 95% cross-inhibitable with YJV, identifying these patients as candidates for exclusion of PWV from their immunotherapy regimens.

METHODS**Hymenoptera venoms**

The purified venoms obtained by dissection and/or electrical stimulation from yellow jacket (*Vespula* spp.), white-faced hornet (*Dolichovespula maculata*), yellow

hornet (*D. arenaria*), *Polistes* wasp (*Polistes* spp.), and honeybee (*Apis mellifera*), as previously described,⁹ were purchased in lyophilized form from Vespa Laboratories (Spring Mills, Pa.). Each venom was reconstituted with phosphate-buffered saline (to 1 mg/ml of total protein and either coupled to CNBr-activated Sepharose 4B-CL (Pharmacia, Uppsala, Sweden) at 0.5 mg/ml packed Sepharose as previously described¹⁰ or used as soluble antigen for inhibition. All venoms, once reconstituted, were stored frozen at -20° C in 200 µl aliquots until used in the inhibition study.

Patient specimens

A total of 500 consecutive serum samples were accepted from patients across the United States who had a suspected Hymenoptera venom sensitivity as determined by clinical history. In most cases skin test data were available to confirm sensitivity to two or more Hymenoptera venoms. On receipt, all samples were analyzed with a previously described venom RAST^{10, 11} to confirm the presence of IgE antibody to the specified venoms. Of the 500 serum samples evaluated, 88 had detectable IgE antibody (>1 ng/ml) to only one venom. The remaining 412 had detectable venom-specific IgE antibodies to two or more venoms: of these, 41 (10%) were excluded due to incomplete homologous venom inhibition, and 305 had a level of IgE anti-YJV in nanograms per milliliter that was higher than their IgE anti-PWV, which made them candidates for YJV inhibition of IgE anti-PWV binding to PWV-solid phase allergosorbent. The remaining 66 subjects were evaluated for other venom cross-reactivities on the basis of clinical history and specific requests by the referring allergist.

Venom RAST inhibition test

The venom RAST inhibition test (VRIT) that was used to evaluate sera for the presence of IgE antibody binding to cross-reactive components among the vespid and *Polistes* wasp venoms is a modification of a previously reported assay.^{11, 12} In its most general format, 0.1 ml of buffer (no inhibition condition), soluble homologous venom (identical to that on the solid phase), or soluble heterologous venom (1 mg/ml) is added to 0.1 ml of human serum (undiluted to 1:20) in a 12 × 75 mm plastic tube. The diluent used throughout the assay, unless otherwise stated, is RAST buffer, phosphate-buffered saline with 0.05% Tween 20, 0.1% bovine serum albumin, and 0.01% sodium azide. All chemicals, unless otherwise specified, were purchased from Sigma Chemical Company (St. Louis, Mo.). After a 2- to 4-hour preincubation at 23° C, an optimal concentration of solid-phase venom allergosorbent (0.5 ml, 1% to 2% vol/vol) was added, and the mixture was rotated at a 45-degree angle for 16 to 20 hours at 23° C. Unbound proteins were removed with three 2.5 ml washes, each followed by centrifugation at 1000 g and removal of supernatant by suction with a collared vacuum system. Bound human IgE was detected with iodine-125-la-

TABLE 1. Medically important Hymenoptera

Family	Genus and species	Common name	Treatment venom
<i>Apidae</i>	<i>Apis mellifera</i>	Honeybee	HBV
<i>Apidae</i>	<i>Bombus</i> (39 spp in NA)	Bumblebee	NA
<i>Apidae</i>	<i>Halictus</i> spp.	Sweatbee	NA
<i>Vespidae</i>	<i>Vespula</i> (13 spp in NA)	Yellow jacket	YJV*
<i>Vespidae</i>	<i>Dolichovespula maculata</i>	White-faced hornet	WFHV*
<i>Vespidae</i>	<i>Dolichovespula arenaria</i>	Yellow hornet	YHV*
<i>Vespidae</i>	<i>Polistes</i> (100-200 spp in NA)	Paper wasp	PWV
<i>Formicidae</i>	<i>Solenopsis invicta</i>	Fire ant	NA

HBV, Honeybee venom; NA, not available; WFHV, white-faced hornet venom; YHV, yellow hornet venom.

*Treatment may be performed with mixed vespid venom.

beled rabbit anti-human IgE Fc (150,000 cpm/tube, specific activity = 10,000 cpm/ng, labeled by the chloramine-T method¹³) with rotation for 16 to 18 hours at 23° C. Unbound anti-IgE was removed with four RAST buffer washes, and bound radioactivity was counted to statistical precision in a Capintec CAP-RIA, 16-well gamma counter (Capintec Inc., Ramsey, N.J.).

All measurements were performed in duplicate, and intraassay percent coefficients of variation greater than 15% were repeated to ensure good precision. A calibration curve was constructed in each assay with the YJV-specific IgE system by analyzing in triplicate 11 serial dilutions of a standard serum pool ($n > 40$ patients) containing 50 ng/ml of IgE anti-YJV as determined by depletion analysis. The level of radioactivity bound in each test specimen tube was interpolated into IgE anti-YJV equivalents from the standard curve, and these antibody levels were used to compute the percentage inhibition produced by preincubation of the serum with the homologous and heterologous venoms. The analytical sensitivity of the VRIT was defined in each assay as that level of IgE anti-YJV antibody in the standard serum that produced a response signal (counts per minute bound), which was statistically higher (according to Student's t test) than the response produced by undiluted serum analyzed in the same assay from a nonatopic adult (total serum IgE = 15 ng/ml). Parallelism, or the ability of test sera to dilute in parallel to the reference serum, has been previously demonstrated by interdilutional coefficients of variation of less than 15% in the working range of the RAST assay. Serum from a nonatopic individual was run in each assay to determine the analytical sensitivity of the assay. Three IgE antivenin-positive control serum samples with varying levels of IgE anti-YJV were analyzed in each VRIT analysis with and without homologous venom inhibition to ensure overall assay performance (precision with interassay variation less than 20% and proper functioning of the venom preincubation step). The other heterologous venom-specific IgE-containing positive control sera that were analyzed in the assay differed depending on the specificities of the venom insolubilized on the allergosorbent that was required in those VRIT analyses.

Data analysis

The extent of homologous and heterologous venom inhibition was individually computed with the following formula: $[(\text{ng/ml IgE anti-venom (buffer)} - \text{ng/ml IgE antivenin (homologous or heterologous venom)}) / \text{ng/ml IgE antivenin (buffer)}]$. Serum samples that produced a homologous venom inhibition less than 80% were repeated. If the homologous inhibition was greater than 80%, a cross-reactivity index (CRI) was computed as the ratio of heterologous inhibition to homologous inhibition. CRIs greater than 95% were defined as complete inhibition, and they identified sera in which the IgE antivenin was essentially all cross-reactive. The majority of serum samples produced CRIs that ranged from 5% to 95%, indicating the presence of IgE antibody to both cross-reactive and heterologous venom unique allergens.

RESULTS

Optimization of the venom inhibition test

Optimal concentrations of soluble venom and solid-phase venom were identified to ensure that all IgE antivenin is bound in the absence of soluble venom and that there is sufficient soluble venom to inhibit the binding of cross-reactive IgE antibody. These studies were performed in two stages. Experiments were performed first to identify the optimal venom allergosorbent concentration and second to identify the optimal soluble venom for maximal inhibition. Fig. 1 displays the results of first box-titration studies in which 0.1 ml of a serum pool containing five of the highest levels of IgE anti-PWV (70 ng/ml) was preincubated with 0.1 ml of buffer or high, variable amounts of added soluble (heterologous) YJV (100, 200, 400 μg). After 1 hour, 0.5 ml of varying concentrations of PWV allergosorbent (1%, 2%, or 4% vol/vol) were added to their respective tubes, and the assay was completed as discussed in the Methods section. Optimal binding conditions were observed with a 2% vol/vol PWV-

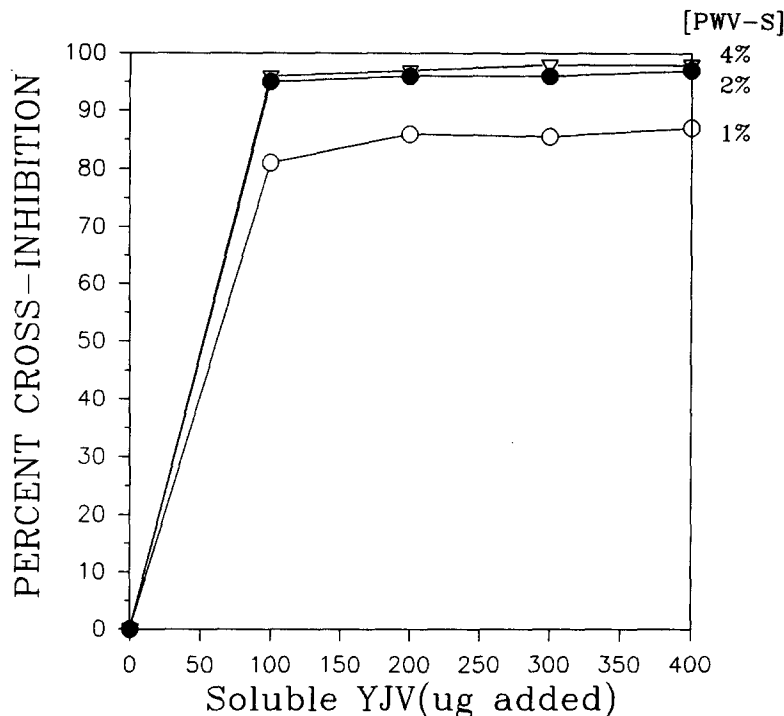


FIG. 1. Results of the first set of optimization experiments in which the percentage of inhibition of IgE anti-PWV binding to PWV allergosorbent was evaluated in a box-titration manner with three PWV-allergosorbent concentrations (1%, 2%, and 4% vol/vol) and three concentrations of heterologous soluble YJV (1, 2, and 4 mg/ml, 0.1 ml added). YJV venom inhibited the binding of IgE anti-PWV binding to PWV allergosorbent by 95% with the addition of 100 μ g of soluble YJV (0.1 ml of 1 mg/ml) and 0.5 ml of 2% vol/vol PWV allergosorbent. These results were followed by a more extensive examination of the degree of cross-inhibition produced by preincubation of serum with added levels of soluble YJV lower than 100 μ g (Fig. 2).

allergosorbent concentration, and this was used for all subsequent analyses.

The second set of optimization experiments involved the preincubation of 0.1 ml of three serum samples with 0.1 ml of buffer or lower amounts of soluble YJV (1, 10, 25, 50, 76, 100, 150, and 200 μ g added) than were used in the first study. Of the three samples, two (D7859 and D8155) contained IgE anti-YJV that was known to be more than 95% cross-reactive with PWV from previous analyses, and the third (E5231) contained IgE anti-PWV that was 80% cross-reactive with YJV and 20% reactive to PWV unique allergens. Fig. 2 shows the percentage of cross-inhibition observed in this study. Twenty-five micrograms of soluble heterologous YJV protein was able to maximally inhibit cross-reactive IgE antibody in all three serum samples. A conservative, fourfold higher concentration (100 μ g) of YJV was then selected for all subsequent competitive inhibition studies to ensure that excess soluble heterologous venom was available for

maximal inhibition. One hundred micrograms of homologous and heterologous venoms and 2% vol/vol venom allergosorbent were the reagent concentrations used in all IgE anti-PWV/soluble YJV cross-inhibition VRITs. Five different heterologous soluble venom/venom allergosorbent combinations (Table II) were similarly optimized, and 100 μ g of added soluble venom was observed to maximize heterologous venom inhibition in these VRITs.

Frequency of complete cross-inhibition in study population

The number of serum samples that were identified as containing exclusively cross-reactive antibody as evidenced by a CRI greater than 95% are presented in Table II. First, homologous venom preincubation with the serum (e.g., IgE anti-YJV vs soluble YJV or IgE anti-PWV vs soluble PWV) produced more than 95% inhibition of binding, which provided intraassay quality control of the reagents and the assay's optimization. One third

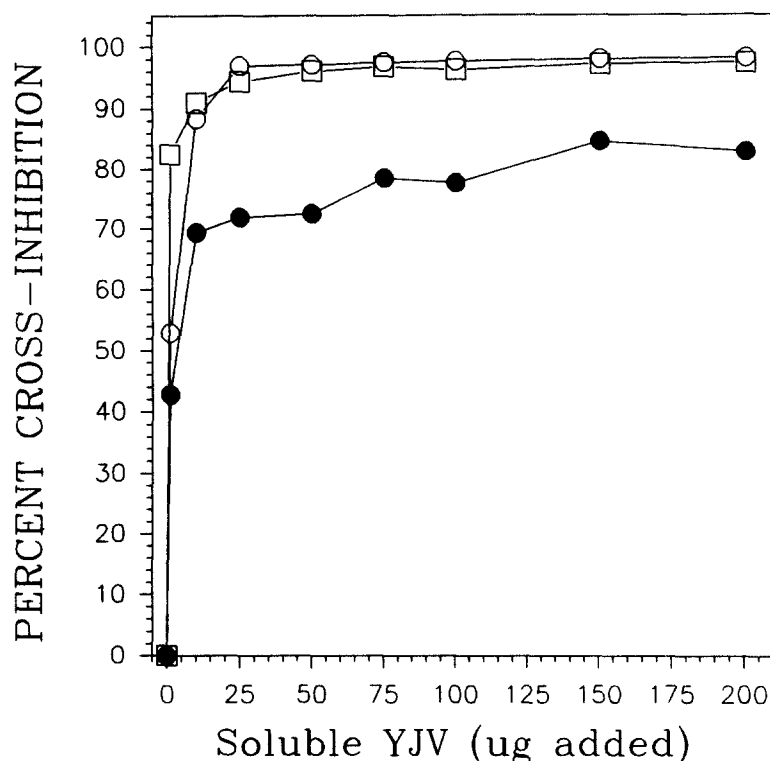


FIG. 2. Results of the second set of optimization experiments in which IgE anti-PWV binding from three serum samples onto PWV allergosorbent was competitively inhibited by the preincubation with increasing amounts of soluble YJV (1, 10, 25, 50, 75, 100, 150, and 200 μ g added). IgE anti-PWV in both pooled and individual serum samples were optimally inhibited with 25 μ g of added protein. A 100 μ g level of soluble inhibiting heterologous venom was selected together with a 2% vol/vol venom allergosorbent as optimal for all clinical studies of the VRIT. Similar optimization studies with the other venom specificities examined in this study (see Table II) identified 100 μ g of added heterologous venom protein as sufficient to obtain more than 95% heterologous inhibition with the sera studied.

TABLE II. Frequency of complete venom IgE cross-reactivity (YJV and PWV specificities)

Solid-phase venom	Soluble YJV inhibition	Soluble PWV inhibition
	CRI > 95% (%I N/total N)	CRI > 95% (%I N/total N)
YJV	100% (19/19)*	58% (11/19)
WFHV	50% (6/12)	50% (2/4)
YHV	38% (3/8)	ND
PWV	36% (111/305)	100% (305/305)*
HBV	0% (0/19)	ND

This table shows the percentage of total patients that had CRIs >95%, indicating complete cross-inhibition with the heterologous venom.

WFHV, White-faced hornet venom; YHV, yellow hornet venom; ND, not done; HBV, honeybee venom.

*Homologous venom inhibition was 100% by definition in both the YJV and PWV analyses.

to one half of the small numbers of patients tested with white-faced hornet venom or yellow hornet venom-specific IgE had antibody that was exclusively cross-reactive with YJV. The binding of IgE anti-honeybee venom to honeybee venom solid

phase was not inhibited by YJV in any of the 19 study subjects. The largest group studied included 305 serum samples that contained higher levels (in nanograms per milliliter) of IgE anti-YJV than of IgE anti-PWV. Of these samples, 36% had IgE

TABLE III. Extent of anti-PWV cross-reactivity with YJV as a function of the IgE anti-PWV concentration

Quartile	IgE anti-PWV concentration* (ng/ml)	Patients with CRIs > 95% with YJV		Patients with CRIs > 90% with YJV	
		No.	%	No.	%
(<1 ng/ml)	0.5 ± 0.01	14/30	46.7	14/30	46.7
I 0-25%	2.1 ± 0.1	23/69	33.3	28/69	40.6
II 26-50%	5.3 ± 0.02	22/69	31.9	31/69	44.9
III 51-75%	14.6 ± 0.06	20/68	29.4	27/68	39.7
IV 76-100%	111.3 ± 1.78	32/69	46.4	38/69	55.1
Total group	Range 0.3-529	111/305	36.4	138/305	45.2

Chi-square test = NS.

*Mean ± SEM.

anti-PWV of which binding to PWV-solid phase was more than 95% cross-inhibitable by soluble YJV, which identified these patients as candidates for exclusion of PWV from their venom immunotherapy regimen. Reversing the venom specificities, 58% of 19 patients with IgE anti-PWV greater than IgE anti-YJV had IgE anti-YJV of which binding to YJV solid phase was cross-inhibitable by more than 95% after preincubation with soluble PWV (Table II).

Relationship between antibody levels and cross-reactivity

To determine whether the absolute level of IgE anti-PWV influenced the extent to which cross-reactivity could be measured in the VRIT, the CRI results were subdivided into five groups depending on the IgE anti-PWV concentration in serum (Table III). The first group that contains IgE anti-PWV levels between 0.3 ng/ml (the assay's minimal detectable dose) and 1 ng/ml had the same percentage of serum samples (47%) that were more than 95% cross-inhibitable by soluble YJV as the highest IgE anti-PWV group (111.3 ± 1.78 ng/ml, mean ± SEM; 46%). These results indicate that the ability of the assay to identify sera with IgE anti-PWV that is more than 95% cross-reactive with YJV is independent of the absolute level of IgE anti-PWV measured in the serum.

Distribution of CRIs and criterion for specifying cross-reactivity

Fig. 3 displays the spectrum of CRIs obtained in the 305 patients with YJV/PWV allergy presented in Table III. There is a continuum of CRIs from 0% to 110% across the IgE anti-PWV range

(0.3 to 529 ng/ml), which indicates a heterogeneous distribution of IgE anti-PWV antibodies that span from reacting solely with PWV unique allergens (CRI < 5%) to those reacting exclusively with YJV cross-reactive allergens (CRI > 95%). Only 4.3% of patients' sera (13 of 305 samples) produced (CRIs of less than 5%, which is consistent with their IgE anti-PWV being restricted to PWV unique allergens. A conservative CRI of 95% has been used throughout this study to ensure that only those individuals with a predominance of IgE anti-PWV that binds to cross-reactive YJV are identified for elimination of PWV from their treatment regimen. Up to 12% more individuals would fall into this category if a CRI threshold limit of 90% instead of 95% were used as the criterion for defining cross-inhibition and for identifying patients who are candidates for elimination of PWV from their immunotherapy regimen (Table III, right column).

DISCUSSION

Since the 1978 controlled trial of immunotherapy with purified Hymenoptera venoms,¹⁴ venom immunotherapy has been widely recognized for its ability to reduce the risk of adverse reactions that result from a re-sting in venom-sensitive individuals. Despite the widely accepted practice of including all venoms that elicit positive skin test results in the treatment regimen,^{4, 15} Reisman and Livingston⁵ have recently proposed the use of single venoms, even in patients with positive skin test results to multiple venoms. All agree, however, that the selection of the proper venoms for therapy in an allergic patient with multiple venom sensitivities is complicated by the high degree of structural similarity among components in vespid

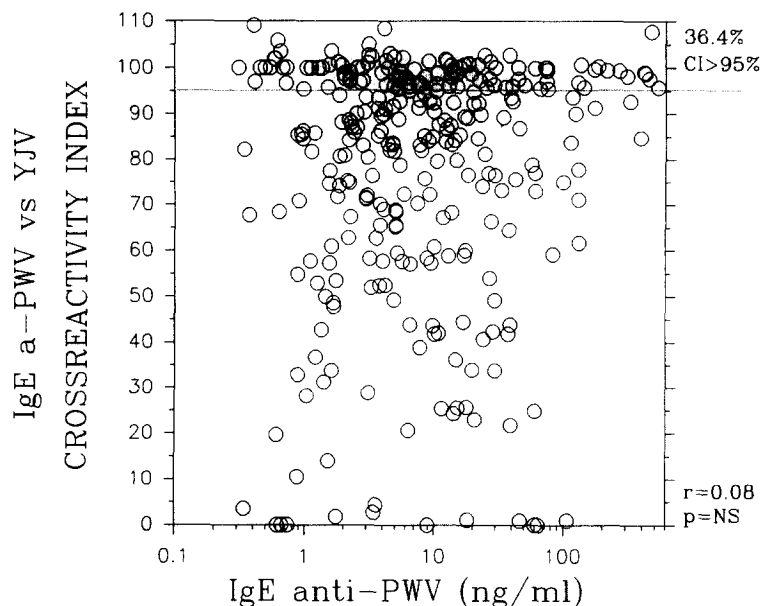


FIG. 3. Distribution of CRIs obtained with 305 serum samples analyzed in the VRIT for IgE anti-PWV binding to PWV allergosorbent and cross-inhibited by preincubation with soluble YJV. Table III presents these results subdivided into five groups on the basis of their levels of IgE anti-PWV in the serum. This figure shows a continuum of CRIs from 0% to 100% with 36.4% of the total population exceeding 95% inhibition, the mark of essentially complete cross-reactivity. Linear regression analysis shows no clear association of the CRI with the level of IgE anti-PWV in the serum ($r = 0.08$, $p = \text{NS}$).

and *Polistes* wasp venoms. The major vespid venom allergens include the nonenzymatic protein antigen 5, and phospholipase A, hyaluronidase, and acid phosphatase, all of which express enzymatic activity. YJV antigen 5 or *Ves g V* is reported to be the predominant allergen, and it has been shown to be structurally similar and antigenically cross-reactive with *Polistes* wasp antigen 5 (*Pol a V*).^{6, 7, 16-18} Moreover, structural similarity between vespid and *Polistes* wasp phospholipase A1/B (*Ves g I*, *Pol a I*) and hyaluronidase (*Ves g II*, *Pol a II*) can lead to IgE antibody cross-reactivity.^{6, 7}

In this report we demonstrate the ability of a serologic method, the VRIT, to aid in the selection of venoms for immunotherapy by identifying patients in whom IgE antibody is largely specific for cross-reactive as opposed to venom unique allergens. Although the most extensive cross-reactivity is known to exist among the vespid venoms, there is little practical use for determining whether a patient's IgE anti-yellow hornet venom or IgE anti-white-faced hornet venom results from IgE antibody binding to exclusively cross-reactive proteins in YJV. In most cases mixed vespid venoms would be used to economically

TABLE IV. Cost-effectiveness of the venom inhibition test

Venom for IT	Cost	Difference
YJV only	\$2000/5 yr	
YJV + PWV	\$3500/5 yr	\$1500
MVV only	\$3400/5 yr	
MVV + PWV	\$4500/5 yr	\$1100

Estimate of venom inhibition expense based on 5-year immunotherapy program with single or multiple venoms.

IT, Immunotherapy; MVV, multiple vespid venoms.

treat such patients. This is not the case, however, for patients who have sensitivity to PWV and one of the vespid venoms. The most common situation is the individual who has strongly positive skin test reactivity or a high serum IgE antibody level to YJV and a weaker skin reactivity or lower level of IgE antibody in serum to PWV. We have shown during the past 5 years that 36% of 305 individuals who have concomitant skin reactivity to YJV and PWV had CRIs greater than 95%, indicating that their IgE anti-PWV was essentially exclusively cross-reactive to allergens present in YJV. This percentage is increased to 45% when a

slightly more liberal criterion of cross-reactivity is used (CRI $\geq 90\%$, Table III). We believe that patients with CRIs greater than 95% are candidates for elimination of PWV from their venom treatment regimens. The remaining 64% of patients have produced IgE antibody reactive with a spectrum of PWV cross-reactive and unique allergens. On the basis of these serologic analyses, only 10% of the study patients had IgE antibody to PWV unique allergens as evidenced by less than 5% inhibition of IgE anti-PWV binding to PWV allergosorbent with YJV. The percentage of individuals with low IgE anti-PWV that had CRIs greater than 95% was similar to that for venom-allergic patients with high levels of IgE anti-PWV (33%-I quartile vs 46% IV quartile, Fig. 2, Table III). This lack of association between the level of IgE anti-PWV and the degree of cross-reactivity (CRI) indicates that the heterogeneity of the immune response is independent of the quantity of IgE antibody produced in serum.

This study provides evidence that the inhibition format of the venom RAST technically performs well and can provide precise data with which to make a decision to include or exclude PWV from a treatment regimen of an individual with multiple-venom (vespid and PWV) sensitivities. Two other issues, however, need to be addressed if this procedure is to be considered clinically useful. The first issue relates to the cost-effectiveness of the RAST inhibition measurement in relationship to the cost of including PWV in an immunotherapy program with mixed vespoid venom or YJV. Cost-effectiveness of the VRIT must be viewed within the context of the cost of an average conventional venom immunotherapy regimen, which ranges from \$2000 to \$4500 for a 5-year program depending on the number of venoms included. Eliminating PWV from a YJV or mixed vespoid venom treatment regimen would save approximately \$1100 to \$1500 (Table IV), which would make the VRIT (about \$200 in our laboratory for a PWV and YJV RAST and a separate venom inhibition assay) well justified economically, even if only one patient in three will qualify to have PWV excluded.

The second issue that affects the utility of the VRIT relates to the clinical outcome of YJV-PWV-sensitive patients who have been treated by excluding PWV because their VRITs' CRI was greater than 95%, indicating IgE anti-PWV that is cross-reactive with YJV. The clinical outcome of such patients has been examined by sting challenge in a group of patients that had concomitant

YJV and PWV skin reactivity and were treated only with YJV on the basis of their CRI after a VRIT. Although the results of this study are the subject of a separate article, the general observations of this study show that over 95% of patients with multiple vespoid (yellow jacket, white-faced hornet, yellow hornet) and PWV sensitivities were clinically protected by immunotherapy with YJV alone.¹⁹⁻²⁰ Moreover, treatment with mixed vespoid venoms conferred a higher level of protection in this group of patients, presumably as a result of the higher dose of YJV allergens that resulted from the administration of YJV cross-reactive allergens alone.

Although the VRIT is proposed as an aid in the selection of venoms for the newly diagnosed individual who is initiating venom immunotherapy, there are other rare circumstances in which the test may provide useful information. One such case involves the venom-allergic individual who has been started on a regimen of YJV or mixed vespoid venom together with PWV and for whom the allergist is considering changes in the venoms used for immunotherapy as a result of new diagnostic (skin test or serologic) information. Such an individual most commonly has high levels of IgE anti-YJV and very low levels of PWV-specific IgE antibody in his or her blood. A VRIT may be able to identify those individuals in whom the IgE anti-PWV is more than 95% cross-reactive with YJV. There is evidence that venom-sensitive children, who began receiving YJV or mixed vespoid venom and PWV as a part of an epidemiologic study of insect allergy²¹ and had PWV withdrawn after an observed loss of skin reactivity to PWV, did not have an increased number of adverse reactions (personal communication, Dr. Kenneth Schuberth, March 1993).

In conclusion, the Hymenoptera VRIT is a technically robust and cost-effective procedure, which defines the extent of an IgE antibody response that is due to cross-reactive antibody. This knowledge aids the allergist in selecting the optimal panel of venoms for immunotherapy for those individuals who have multiple skin test reactivity to vespoid and *Polistes* wasp venoms.

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