

Antigenic cross-reactivity of venom proteins from hornets, wasps, and yellow jackets

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The venoms of hornets, wasps, and yellow jackets have similar protein compositions. The three major venom proteins are antigen 5, hyaluronidase, and phospholipase A₁. These proteins, which are allergens in man, have been isolated and compared for their biochemical and antigenic properties. The homologous proteins from these vespids have very similar biochemical properties that include their molecular size and charge, their amino acid composition, and, where it is applicable, their enzymatic activities. They were studied for their antigenic cross-reactivity with specific mouse antibodies. The phospholipases demonstrated no detectable antigenic cross-reactivity, whereas the antigen 5s and hyaluronidases demonstrated varying extents of cross-reactivity. (J ALLERGY CLIN IMMUNOL 75:621-8, 1985.)

Most vespid venom-allergic people demonstrate positive reactions to more than one vespid venom by skin test, RAST, or RAST inhibition.¹⁻⁷ The multiple reactions can be due to multiple sensitizations by different vespids or to antigenic cross-reactivity of the vespid venoms. It is not possible to ascertain by tests with venoms which of the two conditions applies because venoms are mixtures of protein allergens, and people can demonstrate varying immune responses to the different antigens of each venom.⁸⁻¹¹

We have previously identified three proteins of vespid venoms to be allergens in sensitive patients. They are antigen 5, hyaluronidase, and phospholipase A₁. The vespids studied included hornets, yellow jackets, and wasps.⁸⁻¹⁰ In this article we designate as hornets, those insects belonging to the genus *Dolichovespula*, wasps, those insects belonging to the genus *Polistes*, and yellow jackets, those of the genus *Vespula*. There is disagreement among entomologists on the use of these common names for insects of different genera.¹²

We will report in this article our comparative observations on the biochemical and the antigenic properties of the purified venom proteins from hornets, wasps, and yellow jackets. The homologous venom proteins from these vespids are found to have very similar biochemical properties. Nonetheless, they can

be readily differentiated with specific mouse antibodies. The findings suggest that the purified venom proteins may be better reagents than whole venoms for unambiguous diagnosis of vespid allergy.

MATERIAL AND METHODS

Venom sac extracts of white-face hornets (*D. maculata*), yellow hornets (*D. arenaria*), yellow jackets (*V. vulgaris*), and wasps (*P. annularis*) were products of Vespa Laboratories, Spring Mills, Pa. The lot numbers of extracts used were 12218207 BF, 01058002 YH, 12038048 YJ, and 011980106 PW, respectively. Venom proteins were isolated after our published procedures.^{9, 10} Unless it is noted otherwise, all methods used are cited in ref. 10. Hyaluronidase activity was assayed by a turbidimetric method as well as by following the release of reducing groups of *N*-acetylglucosamine.¹³

Mouse antibodies specific for the purified venom proteins were obtained from the ascitic fluids of BALB/c mice. Groups of four female mice were immunized at week 0, 3, 4, 5, 8, 10, and 12 with intraperitoneal injections of the antigen (2 to 5 µg) in 0.2 ml of a 9:1 mixture of complete Freund's adjuvant and buffer.¹⁴ The ascitic fluids were collected beginning on week 5, and they were examined by immunodiffusion in 1% agarose gel (agarose immunodiffusion tablets, Bio-Rad Laboratories, Richmond, Calif.). Equal volumes (10 µl) of the antigen solution (about 0.05 mg per milliliter) and the ascitic fluid were applied to gel, and a second addition of all reagents was made after about 20 hr. After another 20 hr the gels were stained with dye after drying and washing. For the studies reported in this paper, pools of ascitic fluids collected during weeks 13 to 14 were used for antigen 5-specific antibodies, and pools from weeks 7 to 10 were used for hyaluronidase and phospholipase-specific antibodies.

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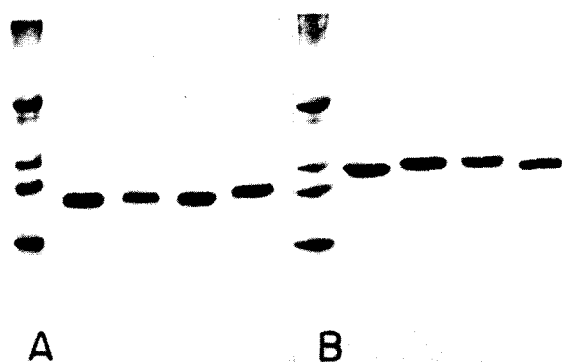


FIG. 1. Sodium dodecyl sulfate gel electrophoresis of vespid venom proteins. **A**, Left to right, a standard protein mixture and antigen 5 samples from yellow jacket, yellow hornet, white-face hornet, and wasp, respectively. **B**, Phospholipase samples from these same vespids. About 5 μ g of each protein was reduced with mercaptoethanol and then applied to gel. The molecular weights of the standard proteins are from top to bottom 66, 35, 29, and 14 kilodaltons, respectively.

Inhibition of binding of mouse antibodies to solid-phase antigen by a competing antigen was measured by enzyme immunoassay.^{10,15} Wells of vinyl flat-bottom plates (Falcon 39R, Falcon Labware Division, Becton-Dickinson, Rutherford, N.J.) were coated with 50 μ l aliquots of 2 μ g per milliliter antigen solution in 0.05 mol/L Tris-HCl buffer of pH 7.95 for use as solid-phase antigens. After saturation of wells with 0.01% albumin and washing, 30 μ l aliquots of inhibition mixture were added. The mixture contained a fixed concentration of specific mouse antibodies and a variable concentration of competing antigen in pH 7.95 buffer. The antibody concentration chosen corresponded to that required for about 30% saturation of binding of solid-phase antigen, which from other studies¹⁰ generally was about 100 ng per milliliter of antibody. This desired concentration of specific antibodies was obtained by 3000 to 10,000-fold dilution of the ascitic fluids. The competing antigen solution ranged from 2 to 200,000 ng per milliliter. The amounts of antibodies bound to the wells were detected by further treatments with rabbit anti-mouse immunoglobulins, peroxidase-labeled sheep anti-rabbit immunoglobulins, and finally peroxidase substrate. The extents of inhibition of antibody binding are expressed as percentage of maximal absorbance change in the absence of competing antigen, which was in the range of 0.4 to 0.8 after 30-minute development.

In this article the weights of all purified proteins were determined by amino acid analysis. The weights of venom sac extracts in Table I were supplied by the manufacturers.

RESULTS

Isolation of vespid venom proteins

Venom proteins of hornets, yellow jackets, and wasps were isolated from their venom sac extracts as

described in our recent publications.^{9,10} Our earlier procedure⁸ was useful for isolation of the antigen 5 component. However, that procedure was not useful for the hyaluronidase and phospholipase components, probably because of proteolysis under the conditions used. In the new procedure^{9,10} protease inhibitor was added to suppress proteolysis, and affinity chromatography was used for the selective isolation of these two enzyme components.

The isolation procedures used are, briefly, as follows: Phospholipase A₁ was isolated from the venom sac extract by affinity chromatography on Sepharose gel containing a substrate analog of phospholipase. The venom sac extract, depleted of phospholipase, was next separated by affinity chromatography on heparin Sepharose that is a specific adsorbent for hyaluronidase to yield antigen 5 and hyaluronidase. Each of the isolated venom proteins was further purified by ion exchange chromatography on carboxymethyl cellulose, and when it was necessary, by gel filtration on Sephadex G-100. The isolation procedures were reproducible because the same venom proteins isolated from separate experiments were obtained in identical yields and degrees of purity that was judged by the biochemical tests related below.

The amounts of these three venom proteins that were isolated per 100 mg of venom sac extracts are listed in Table I. The vespids studied included one species each of yellow jacket (*V. vulgaris*) and wasp (*P. annularis*) and two species of hornets (*D. maculata* and *D. arenaria*). The common names of these two hornets are white-face and yellow hornet, respectively. These two hornets were studied because their venom proteins are antigenically dissimilar.^{8,16} One species each of yellow jacket and wasp was studied because most species of yellow jackets^{9,17,18} and wasps^{5,10,19} that have been examined demonstrate strong antigenic identity. The yellow jacket *V. squamosa* is an exception. Its venom^{17,18} is reported to be antigenically dissimilar to those of other common yellow jackets, e.g., *V. maculifrons* and *V. vulgaris*. *V. squamosa* is present in the southeastern part of the United States, whereas *V. maculifrons* and *V. vulgaris* are widely distributed in this country.¹²

Biochemical comparison of vespid venom proteins

We had three sets of homologous proteins from four different vespids, which is indicated in Table I. Within each set the homologous proteins demonstrate very similar but not identical biochemical properties. Their similarity in size is indicated by their mobility on sodium dodecyl sulfate gel electrophoresis. This is illustrated in Fig. 1 for the homologous antigen 5 and

TABLE I. Amounts of proteins isolated per 100 mg of venom sac extract

Vespid	Antigen 5	Hyaluronidase	Phospholipase A ₁
White-face hornet	4.5 mg (2.3)	0.3 mg (2.5)	0.3 mg (1.6)
Yellow hornet	1.5 mg (1.9)	0.2 mg (2.4)	0.7 mg (0.89)
Yellow jacket	1.7 mg (1.9)	0.3 mg (1.9)	2.0 mg (0.89)
Wasp	0.5 mg (1.9)	0.3 mg (2.4)	0.6 mg (1.7)

Values in parentheses represent absorbances at 280 nm of indicated proteins at 1 mg per milliliter (light path 1 cm); yields of proteins are in the range of 30% to 50%.

TABLE II. Amino acid compositions of vespid venom phospholipases

Amino Acid	White-face hornet	Yellow hornet	Yellow jacket	Wasp
Lys	30	28	24	30
His	9	6	9	10
Arg	17	13	12	10
Asp	32	38	27	35
Thr	21	19	24	17
Ser	23	29	26	21
Glu	29	26	32	21
Pro	13	12	13	10
Sly	27	26	23	25
Ala	20	17	21	26
Cys	10	10	12	13
Val	21	17	18	23
Met	4	2	4	2
iLeu	17	23	22	17
Leu	19	29	22	24
Tyr	11	10	13	15
Phr	15	15	14	15

Values are recorded to the nearest integer and were calculated by assuming the Asp and the Gly contents of white-face hornet enzyme to be 32 and 27 residues per mole, respectively, and those of the yellow hornet enzyme to be 38 and 26 residues per mole, respectively. The analytical error is $\pm 6\%$. The values recorded for the yellow jacket and the wasp enzymes are taken from references 9 and 10.

phospholipase samples. The homologous hyaluronidase samples also illustrated very similar mobilities; these results are not illustrated. The molecular weights of antigen 5, hyaluronidase, and phospholipase are estimated to be about 22, 44, and 37 kilodaltons, respectively, from their sodium dodecyl sulfate gel electrophoresis.

In addition to the gel electrophoretic data, the purity of the venom proteins was ascertained by immunodiffusion against rabbit anti-venom antibodies, and a single line was obtained in each case. These results are not presented here because they have been reported previously.⁸⁻¹⁰ The purity of the venom proteins used in this study is estimated to be better than 95%.

The homologous proteins of each set have very similar charge properties that are indicated by their similar ion exchange chromatographic elution position. For example, the phospholipases of white-face hornet, yellow jacket, and wasp venoms are eluted at the same position when they are separated on a car-

boxymethyl cellulose column at pH 4.75, whereas that of yellow hornet is eluted ahead of the others.

The homologous vespid venom proteins have similar amino acid compositions. This is illustrated in Table II for the homologous phospholipases. The results for antigen 5 samples also demonstrate a strong degree of similarity; they are not illustrated here as they were previously reported.⁸⁻¹⁰

The specific activities of the hyaluronidases from hornets, yellow jackets, and wasps are illustrated in Table III. The homologous hyaluronidases were found to have the same specific activities within experimental error of $\pm 20\%$, and these activities were determined by following the depolymerization of hyaluronic acid. Nearly identical specific activities of the homologous hyaluronidases were also found by following the release of reducing groups of *N*-acetylglucosamine from hyaluronic acid. Furthermore, the later findings indicate that the vespid hyaluronidases have the same specificity and that they are of a type known

TABLE III. Specific activities of hyaluronidases and phospholipases of vespid venoms

Vespids	Hyaluronidase (units/mg)	Phospholipase (units/mg)	
		- Triton	+ Triton
White-face hornet	1.7×10^4	4.8×10^2	1.0×10^3
Yellow hornet	1.8×10^4	7.6×10^2	0.9×10^3
Yellow jacket	1.6×10^4	7.0×10^2	5.7×10^3
Wasp	1.9×10^4	8.1×10^2	5.7×10^3

Hyaluronidase activity was measured by a turbidimetric method to follow the depolymerization of hyaluronic acid. Phospholipase activity was measured by a titrimetric method to follow the release of acids from egg yolk phospholipid in the presence or absence of 0.5% triton.

as endo-*N*-acetylhexosaminidase.²⁰ We found that the bee venom hyaluronidase has the same specificity as that of vespid hyaluronidase.

The specific activities of the phospholipases from hornets, yellow jackets, and wasps are also illustrated in Table III. The homologous enzymes were found to have similar specific activities when the assays were carried out with 10% egg yolk as substrate. Striking changes in the specific activities of the homologous phospholipases were observed when the assays were carried out with 10% egg yolk containing 0.5% triton. The specific activities of the wasp, yellow jacket, white-face hornet, and yellow hornet enzymes increased by about 800%, 500%, 200%, and 120%, respectively.

We have demonstrated that the purified phospholipases from yellow jackets and wasps have dual specificities of the A₁ and B types¹⁰; that is, hydrolysis takes place at the 1-acyl residue of phosphatidyl choline and at the 1- or 2-acyl residue of lysophosphatidyl choline. The specificity of the purified phospholipases from white-face and yellow hornets was determined in this work by following the release of fatty acids from the 1- or 2-position of synthetic phospholipid substrates, which was done previously.¹⁰ The results demonstrated that the hornet phospholipases have the same specificity as that of yellow jacket or wasp enzyme. It is of interest to note that the phospholipase from the honey bee venom has the specificity of A₂ type (21); that is, hydrolysis occurs only at the 2-acyl residue of the phospholipid.

Immunochemical comparison of vespid venom proteins

Antibodies specific for each of three purified venom proteins from the four vespids were obtained from hyperimmunized mice. Mouse antibodies were used because they can be prepared in adequate amounts with minimal expenditure of the immunogens. Ten of the 12 mouse antibodies, which were prepared for this

study, were specific for the immunogen when it was tested by immunodiffusion (results not demonstrated). The two exceptions are that mouse antibodies raised against yellow hornet hyaluronidase or phospholipase each contained yellow hornet antigen 5-specific antibodies in addition to the antibodies specific for the immunogen. These two exceptions were due to the presence of contaminating antigen 5 in the immunogen samples used.

The immunodiffusion patterns of the antigen 5 samples against white-face hornet, yellow hornet, yellow jacket, and wasp antigen 5-specific antibodies are illustrated in Fig. 2, A to D, respectively. Each antigen 5 sample formed a strong precipitin line with its own specific antibodies. The white-face hornet and yellow hornet antigen 5 samples also demonstrated faint precipitin lines of partial identity with antibodies specific for each other, but they did not form precipitin lines with antibodies specific for yellow jacket or wasp antigen 5. The yellow jacket antigen 5, as well as the wasp sample, demonstrated a precipitin line with its own specific antibodies and not with any of the antibodies specific for the homologous antigen 5 samples.

The immunodiffusion patterns for hyaluronidase samples against their specific antibodies are illustrated in Fig. 3, A to D. The results are qualitatively similar to those of antigen 5 samples with the one difference that the white-face hornet and yellow hornet hyaluronidase samples demonstrated stronger lines of partial identity than their respective antigen 5 samples.

The patterns for phospholipase samples are illustrated in Fig. 4, A to D. Each phospholipase sample formed a strong precipitin line only with its own specific antibodies and did not form a precipitin line with antibodies specific for the homologous phospholipase.

The immunodiffusion results of Figs. 2 to 4 help to support the thesis that the purified homologous venom proteins are free of cross contamination and that the mouse antibodies are specific for the proteins

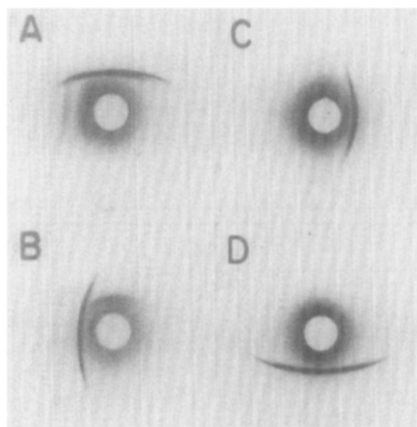


FIG. 2. Immunodiffusion of vespid antigen 5 samples. **A-D**, Center wells are filled with mouse antibodies specific for antigen 5 samples from white-face hornet, yellow hornet, wasp, and yellow jacket, respectively. The four peripheral wells starting from the *top* and continuing in counterclockwise direction are filled with antigen 5 samples from white-face hornet, yellow hornet, yellow jacket, and wasp, respectively.

of interest. If this were not the case, multiple precipitin lines would have been observed in these patterns.

Inhibition of binding of specific antibodies to solid-phase reference venom proteins by the reference or homologous proteins was next studied. The results for antigen 5 samples are illustrated in Fig. 5, *A* to *D* that were obtained with mouse antibodies specific for white-face hornet, yellow hornet, yellow jacket, and wasp antigen 5 samples, respectively. Complete inhibition, which was expected, was observed in each case in which the antibodies and the reference antigen 5 had the same specificity. Significant inhibition by the homologous antigen 5 samples was observed with antibodies specific for white-face hornet antigen 5 (Fig. 5, *A*), to a lesser extent with antibodies specific for yellow hornet and yellow jacket antigen 5 samples (Fig. 5, *B* and *C*), and very little if any with antibodies specific for wasp antigen 5 (Fig. 5, *D*).

The results for hyaluronidase samples are illustrated in Fig. 6, *A* to *D*. They are qualitatively similar to those of antigen 5 samples. Significant inhibition by the homologous proteins was only observed with antibodies specific for white-face or yellow hornet hyaluronidase (Fig. 6, *A* and *B*), but no inhibition was observed with antibodies specific for yellow jacket or wasp hyaluronidases (Fig. 6, *C* and *D*).

Similar studies were also made with phospholipase samples; within experimental error ($\pm 10\%$) there was no inhibition by homologous proteins, and these results are not given.

The highest concentration of venom proteins tested

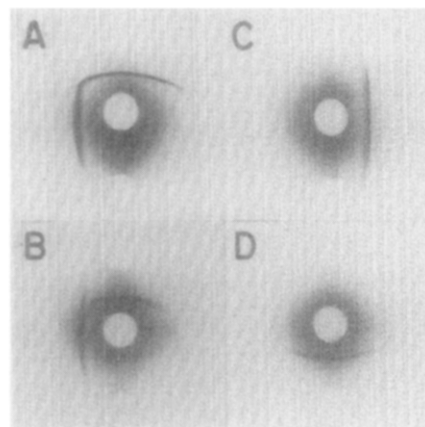


FIG. 3. Immunodiffusion of vespid hyaluronidase samples. The antibody and the antigen wells are filled in the same order as illustrated in Fig. 2 for antigen 5.

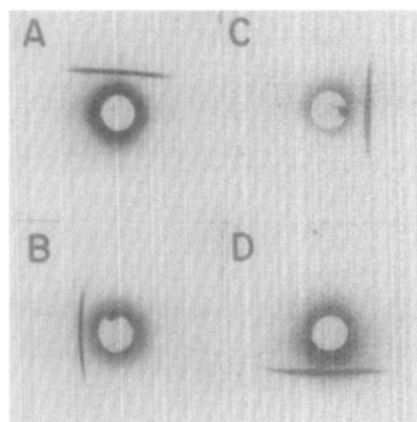


FIG. 4. Immunodiffusion of vespid phospholipase samples. The antibody and the antigen wells are filled in the same order as illustrated in Fig. 2 for antigen 5.

for inhibition in Figs. 5 and 6 was 0.1 mg per milliliter. Higher concentrations were not used because of the limited availability of protein samples. Nonetheless, the data in these two figures suggest that maximal extents of inhibition were nearly attained for all proteins tested. In comparing the inhibition data of homologous proteins, the more similar they are in their antigenic determinants, the more alike will be their extents of maximal inhibition and the concentrations required for half maximal inhibition. The concentrations of homologous proteins required for half maximal inhibition in Figs. 5 and 6 ranged from tenfold to 1000-fold greater than that of the reference protein. This variability may reflect different affinities of the homologous proteins for specific antibodies, or it may reflect low levels of cross contamination. The second possibility is considered unlikely because the proteins

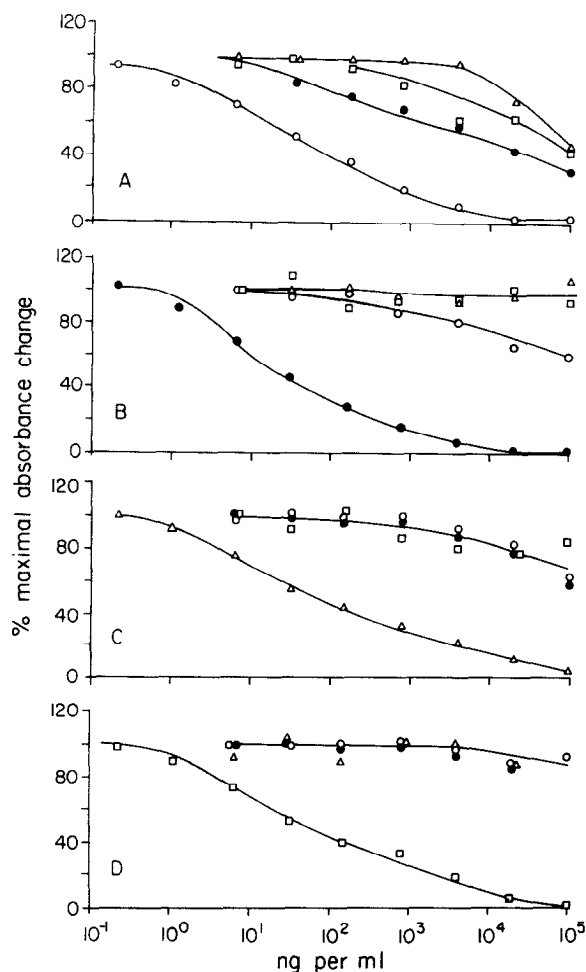


FIG. 5. Inhibition of binding of mouse antibodies specific for reference antigen 5 to solid-phase reference antigen 5 by reference or homologous antigen 5 samples. **A-D**, Reference antigen 5 samples are from white-face hornet (○), yellow hornet (●), yellow jacket (△), and wasp (□) venoms, respectively.

studied were isolated from the same lots of vespid venoms. For example, if the inhibition data in Fig. 6, *B* with yellow jacket hyaluronidase were due to cross contamination with yellow hornet enzyme, one would expect that similar inhibition would be observed with the yellow jacket antigen 5 or phospholipase, but this was not the case with antigen 5 in Fig. 5, *B* or for phospholipase (results not given).

The inhibition data of the homologous venom proteins on the basis of their relative extents of maximal inhibition are summarized in Table IV. The homologous antigen 5 samples of these four vespids share partial antigenic cross-reactivity, their homologous hyaluronidase samples also demonstrate partial cross-reactivity, but the homologous phospholipase samples do not. The cross-reaction of yellow jacket or wasp

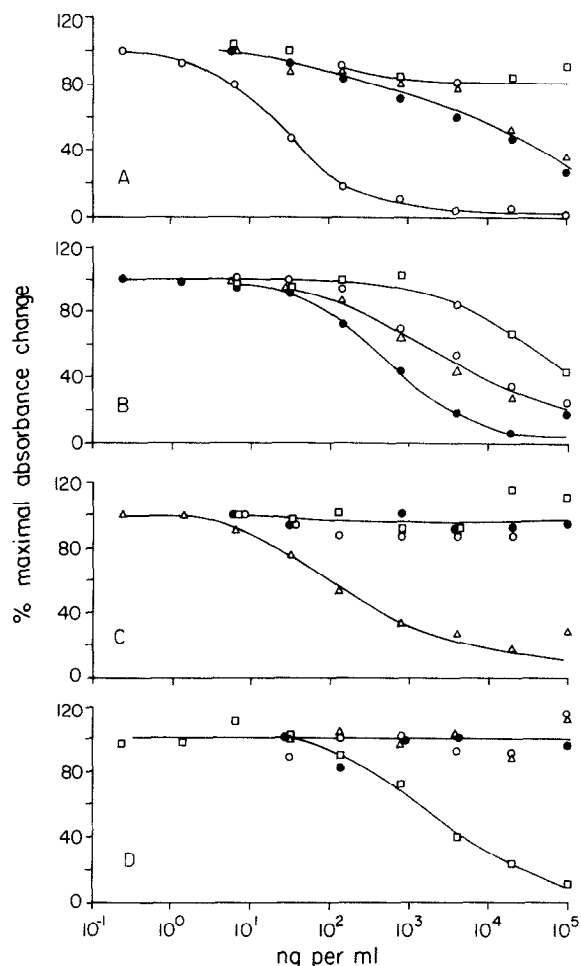


FIG. 6. Inhibition of binding of mouse antibodies specific for reference hyaluronidase to solid-phase reference hyaluronidase by reference or homologous hyaluronidase samples. **A-D**, Reference hyaluronidase samples are from white-face hornet (○), yellow hornet (●), yellow jacket (△), and wasp (□) venoms, respectively.

antigen 5 samples or of their hyaluronidase samples with antibodies specific for hornet proteins was detected by inhibition experiments but not by immunodiffusion experiments, even though the same antibody samples were used in both types of experiments. The difference probably reflects the sensitivity of the two methods.

We had previously studied the antigenic cross-reactivity of partially purified venom proteins from white-face and yellow hornets and yellow jackets by immunodiffusion with venom-specific rabbit antibodies.⁸ The findings are in general agreement with those in Table IV obtained with mouse antibodies. There is one exception that partially purified yellow hornet phospholipase reacted weakly with rabbit antibodies specific for white-face hornet venom, al-

TABLE IV. Summary of inhibition analysis of antigenic cross-reaction of vespid venom proteins

Antigen 5 from venom of	Mouse antibodies specific for antigen 5 of			
	White-face hornet	Yellow hornet	Yellow jacket	Wasp
White-face hornet	+++	+	+	—
Yellow hornet	++	+++	+	—
Yellow jacket	+	—	+++	—
Wasp	+	—	+	+++

Hyaluronidase from venom of	Mouse antibodies specific for hyaluronidase of			
	White-face hornet	Yellow hornet	Yellow jacket	Wasp
White-face hornet	+++	++	—	—
Yellow hornet	++	+++	—	—
Yellow jacket	++	++	+++	—
Wasp	+	++	—	+++

Phospholipase from venom of	Mouse antibodies specific for phospholipase of			
	White-face hornet	Yellow hornet	Yellow jacket	Wasp
White-face hornet	+++	—	—	—
Yellow hornet	—	+++	—	—
Yellow jacket	—	—	+++	—
Wasp	—	—	—	+++

The degree of cross-reactivity was estimated from the inhibition data, and the symbols —, +, ++, and +++ represent respectively 0% to 10%, 11% to 50%, 51% to 90%, and 91% to 100% maximal inhibition.

though white-face hornet phospholipase did not react with yellow hornet venom-specific rabbit antibodies. We had repeated these experiments with the better purified phospholipase samples and the previously used rabbit antibodies, and identical results were obtained.

Nair et al.²² reported that rabbit antibodies specific for white-face hornet venom bound the phospholipases of white-face hornet, yellow hornet, and yellow jacket with decreasing affinity that was measured by neutralization of phospholipase activity. Their data would suggest that there is weak cross-reactivity of the three vespid phospholipases, but our data with rabbit antibodies suggest that there is only weak cross-reactivity of the hornet phospholipases.

DISCUSSION

We have compared the antigenic properties of three sets of homologous proteins from white-face and yellow hornets, yellow jackets, and wasps with their specific mouse antibodies. Our findings indicate that the homologous antigen 5s from these vespids, as well as their hyaluronidases, have varying extents of antigenic cross-reactivity, but the phospholipases do not have detectable cross-reactivity. The four vespid phospholipases were found to have striking changes in their

enzymatic activities when they were assayed in the presence or absence of the detergent triton (Table III). These results also suggest that the phospholipases differ in their topographical features.

The cross-reaction of the hornet, yellow jacket, and wasp antigen 5 samples that is summarized in Table IV was detected with mouse antibodies specific for white-face hornet antigen 5 and for yellow jacket antigen 5 and was not detected with antibodies specific for yellow hornet or wasp antigen 5. With the hyaluronidase samples cross-reaction was detected with antibodies specific for white-face or yellow hornet hyaluronidases but not with antibodies specific for yellow jacket or wasp hyaluronidases. These results suggest that the cross-reactions of the antigen 5 samples, as well as those of the hyaluronidases, are not reciprocal. That is, they depend on the antibodies studied. Any explanation for these results will have to wait until more is known about the chemical structures of these homologous proteins.

By skin tests⁶ and by RAST² with venoms as reagents, most vespid-sensitive patients are reported to demonstrate both hornet and yellow jacket sensitivity, and to a lesser extent, these patients also demonstrated wasp sensitivity. This pattern of multiple sensitivity is believed to be due in part to antigenic cross-reac-

tivity of vespid venoms, since it is unlikely that so many patients were stung by more than one vespid. If our findings in the mouse system are applicable to man, the cross-reactivity of vespid venoms in man will be due to their antigen 5 and hyaluronidase components. It follows that a white-face hornet-sensitive patient will be more likely to demonstrate multiple sensitivity than will a yellow hornet- or yellow jacket-sensitive patient because his antibodies specific for both antigen 5 and hyaluronidase can cross-react with the homologous proteins of other vespids. A yellow hornet-sensitive patient will demonstrate multiple sensitivity because of cross-reactivity of his hyaluronidase-specific antibodies, whereas a yellow jacket-sensitive patient will do so because of cross-reactivity of his antigen 5-specific antibodies. A wasp-sensitive patient will not demonstrate multiple sensitivity because both his antigen 5- and hyaluronidase-specific antibodies do not cross-react with the homologous proteins of other vespids.

All vespid-sensitive patients who have been tested thus far were found to have varying levels of IgG and IgE antibodies specific for the three venom protein components studied in this article.⁸⁻¹¹ Since the homologous antigen 5 and phospholipase samples from different vespids have less cross-reactivity than the venoms do, our results suggest that the purified proteins can be used for unambiguous diagnosis of a patient's true sensitivity. This can be of importance for the proper choice of vespid venom for immunotherapy in order that maximal antibody response of the desired specificity be attained.

There are articles suggesting that the specificity of antibody response to a protein may vary with the species of the immunized hosts.²³ That is, different antigenic determinants of a protein may be immunogenic in man or in mouse. Therefore, it remains to be demonstrated whether or not the present findings in the mouse system are directly applicable to man. Such studies are being presently pursued.

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