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Allergens in Hymenoptera venom XXV: The amino acid sequences of antigen 5 molecules and the structural basis of antigenic cross-reactivity

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The complete amino acid sequences have been determined by solid-phase protein sequencing for eight different vespid venom antigen 5 molecules. These include five species of yellow jackets, *Vespula squamosa*, *V. flavopilosa*, *V. germanica*, *V. pensylvanica* and *V. vidua*, representing all three species groups; two variants from the European hornet, *Vespa crabro*; and a species of paper wasp, *Polistes fuscatus*, from a second subgenus. The new sequences were compared with the seven previously published sequences from yellow jackets, hornets, and wasps, and to that of *Solenopsis invicta* 3 allergen from imported fire ant venom. These comparisons provided structural evidence to support the observed high degree of cross-reactivity among the antigens of the common group of yellow jackets and among those of the two common North American subgenera of paper wasps studied. The antigen 5 of *V. squamosa* and of *V. vidua* were significantly different from those of the *vulgaris* group. Common features that could generate immunologic cross-reactivity were seen among the antigen 5 molecules of hornets of both genera and among those of yellow jackets, hornets, and paper wasps. The imported fire ant allergen has only minimal conserved areas in common with the vespid allergens, which explains the lack of observed IgE cross-reactivity. These results provide the structural basis for the cross-reactivity patterns observed in clinical practice and suggest that the commercial extracts of yellow jacket and paper wasp could be prepared with fewer carefully selected species. (J ALLERGY CLIN IMMUNOL 1993;92:707-16.)

Key words: Allergen, antigen 5, cross-reactivity, fire ant, hornet, sequence, venom, vespid, wasp, yellow jacket

The two major allergens of venoms from vespid wasps are antigen 5 and phospholipase A₁.¹⁻⁵ Hyaluronidase and some minor proteins are also venom allergens.¹⁻⁵ The imported fire ant allergens *Solenopsis invicta* (Sol i) 3 has also been found to be a member of the antigen 5 family by comparison of protein sequences.⁶ In the early studies to establish venoms for use as skin testing and in vitro diagnostic reagents, it was noted that many persons are multiply reactive to vespid venoms.⁷⁻⁹ Without formal proof this was assumed to be due to cross-reactivity. Extensive RAST inhibition studies demonstrated the existence in many persons of true immunologic cross-reactivity of

Abbreviations used

cDNA: Complementary DNA
Dol a: *Dolichovespula arenaria* allergen
Dol m: *Dolichovespula maculata* allergen
PIR: Protein Identification Resource
Pol a: *Polistes annularis* allergen
Pol e: *Polistes exclamans* allergen
Pol f: *Polistes fuscatus* allergen
Sol i: *Solenopsis invicta* allergen
TFA: Trifluoroacetic acid
Ves f: *Vespula flavopilosa* allergen
Ves g: *Vespula germanica* allergen
Ves m: *Vespula maculifrons* allergen
Ves p: *Vespula pensylvanica* allergen
Ves s: *Vespula squamosa* allergen
Ves v: *Vespula vulgaris* allergen
Ves vi: *Vespula vidua* allergen
Vesp c: *Vespa crabro* allergen

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IgE antibodies among the groups of yellow jackets,^{10, 11} among species of paper wasps,¹² between *Dolichovespula* hornets, among yellow jackets and hornets, among yellow jackets and paper wasps,¹³ and among *Vespa* hornets, *Dolichovespula* hornets,

TABLE I. Peptides sequenced to establish structures of antigen 5 molecules

Fraction	Residues
A. <i>Ves s 5</i>	
N-Terminal	1-62
Staph V8 protease	
#21	201-203
#47	152-168
#52	62-83
#54	1-41
#56	138-148
#58	136-148
#61	45-61 +
#66	126-138
#73	169-200
Insoluble	84-118 +
Endo Asp N	
#66 and 67	178-205
#82	51-99 +
#93	140-170 +
Insoluble	104-129 +
Trypsin	
#53	155-165
#59	140-154
#69	176-201 +
#84	64-73
#87	74-96
#91	74-96
Insoluble	108-132 +
B. <i>Ves vi 5</i>	
N-Terminal	1-63
Endo Asp N	
#58	52-82
#59a	105-125
#59b	101-116 +
#63a	141-179
#63b	180-206
#64	126-140
#75	83-100
C. <i>Ves g 5</i>	
N-terminal	1-60
Endo Asp N	
#15	99-102
#16	135-138
#48	103-123
#55	178-204
#59	50-80
#65	124-134
#78	139-177
#81	81-98

TABLE I. cont.

Fraction	Residues
D. <i>Ves f 5</i>	
N-terminal	1-60
Endo Asp N	
#15	99-102
#16	135-138
#21	200-204
#23	199-204
#29	43-49
#49	103-123
#51	1-42
#52	179-199
#53	168-178
#61	139-167
#62	50-80
#69	124-134
#75	81-98
#79	139-204
Staph V8 protease	
#23	200-204
#52	179-198
#53	168-178
#60	139-167
E. <i>Ves p 5</i>	
N-Terminal	1-60
Endo Asp N	
#16	121-123
#19	135-138
#20	99-102
#31	43-50
#44	103-124
#49	1-42
#57	51-80
#62	124-134
#78	81-98
#85	139-198 +
Trypsin of Endo-Asp-N #85	
#37	175-181
#52	153-174
#56	182-204
F. <i>Vesp c 5</i>	
N-Terminal	1-63
Staph V8 Protease	
#24	44-50
#36	51-60
#48	166-185 +
#50a	166-175
#50b	1-25 +
#54	61-82
#56a	137-165

+, Peptide continues beyond last residue sequenced; *Endo Asp N*, endoproteinase *Asp N*; *staph V8 protease*, staphylococcal V8 protease.

*Additional peptides were sequenced that duplicated those listed.

TABLE I. cont.

Fraction	Residue
#56b	51-82
#58	176-202
#60	83-115
#71	116-139 +
#77	116-136
G. <i>Pol f</i> 5	
N-Terminal	1-38
Endo Asp N	
#45	1-29 +
#49	102-121 +
#55	75-82
#56	30-64 +
#84a	83-101
#84b	140-169 +
#88	126-139
#93	102-131 +
Staph V8 Protease of	
Endo-Asp-N #84	
#65	169-190 +
Staph V8 Protease of	
Endo-Asp-N #45	
#21	63-74
#33	30-41
Insoluble staph V8 protease	
#66	169-205

and yellow jackets.¹⁴ However, IgE antibodies against *Sol i* 3 did not cross-react with vespid venoms.¹⁵ Many cases of multiple reactivity were not due to cross-reactivity, because dose-dependent inhibition could not be demonstrated,¹³ and many sera were reactive with only venom from a single species or species group.^{10, 14}

Most modern taxonomists place the social wasps in the superfamily Vespidae. Many solitary wasps are in the superfamilies Pompilidae and Sphecidae, whereas bees are in the superfamily Apoidea. Yellow jackets of the genus *Vespula*, hornets of the genera *Dolichovespula* and *Vespa*, and paper wasps of the genus *Polistes* are all members of the family Vespidae. Although traditionally ants have been placed in the superfamily Formicoidea, much contemporary evidence indicates that ants belong in the superfamily Vespoidea.

The complete amino acid sequences have been reported for antigen 5 molecules from six species of vespids. *Dol m* 5.01 and 5.02 from the white-faced hornet, *Dolichovespula maculata*⁶; *Dol a* 5 from the yellow hornet, *Dolichovespula arenaria*; *Ves v* 5 from the yellow jacket, *Vespula vulgaris*; and *Pol a* 5 from the paper wasp, *Polistes annu-*

*laris*¹⁷ have been sequenced from complementary DNA clones. *Ves m* 5 from the yellow jacket, *Vespula maculifrons*, and *Pol e* 5 from the paper wasp, *Polistes exclamans*, have been sequenced by solid-phase amino acid sequencing of overlapping peptide fragments.¹⁷

In this article we present the complete amino acid sequences of antigen 5 from seven additional species of vespids, including an additional genus and representatives of three other species groups. The proteins sequenced include *Ves f* 5, *Ves g* 5, and *Ves p* 5 from the vulgaris group of yellow jackets, *Vespula flavopilosa*, *V. germanica*, and *V. pennsylvanica*; *Ves s* 5 from *V. squamosa*, and *Ves vi* 5 from the rufa group yellow jacket, *V. vidua*. *Ves p* 5.01 and 5.02 were isolated from the venom of the European hornet, *Vespa crabro*, and *Pol f* 5 from the venom of the paper wasp, *Polistes fusca-tus*, of the subgenus *Fuscopolistes*. The 16 antigen 5 sequences were compared, to determine the structural relationships that generate the immunologic cross-reactivity.

METHODS

Venom

Commercial grade imported fire ant venom was a gift from Vespa Laboratories, a division of ALK (Spring Mills, Pa.). Fire ant venom allergens were purified by Sephadex G-75 superfine gel filtration and high-performance cation exchange chromatography as previously described.¹⁸ Antigen 5 was prepared from hand-milked venom of *D. maculata*, *V. maculifrons*, *V. squamosa*, *Vespa crabro*, and *P. exclamans*, as previously described,¹⁹ by gel filtration and cation exchange chromatography. The allergens from the other species were prepared from commercial grade venom sac extract obtained from Vespa Laboratories. Purity of allergens was assessed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, and N-terminal sequencing runs of 50 to 60 cycles.

Reduction and alkylation

Purified proteins were reduced in either 6 mol/L guanidine HCl, 0.5 mol/L Tris-HCl (pH 7.4), 0.01 mol/L ethylenediamine tetraacetic acid, or 8 mol/L urea in 0.1 mol/L ammonium bicarbonate (pH 7.8), with 5 mg of dithiothreitol under nitrogen for 2 hours at 56° C. The reduced proteins were alkylated either with 13.4 mg of iodoacetic acid (Pierce Chemical Co., Rockford, Ill.), freshly dissolved in 0.1 ml of 0.5N sodium hydroxide, in the dark under nitrogen at room temperature for 1 hour, or with 2 µl of 4-vinylpyridine (Sigma Chemical Co., St. Louis, Mo.) for 2 hours under nitrogen at room temperature.²⁰ Denaturing agents and excess reactants were removed either by dialysis or with the use of a Rapidchanger desalting cartridge (Promega, Madison, Wis.).

TABLE II. Computer-generated comparison of amino acid sequences of antigen 5 molecules expressed as percent of identical residues

	% Identical residues										
Sequence	Ves v 5	Ves f 5	Ves m 5	Ves g 5	Ves p 5	Ves s 5	Ves vi 5	Dol a 5	Dol m 5.0	Dol m 5.02	Vesp c 5.01
Ves v 5		98	95.1	93.6	93.6	71.1	74	67.8	64.9	64	67.3
Ves f 5	98		95.1	93.1	93.1	70.6	74	67.3	64.9	63.5	66.8
Ves m 5	95.1	95.1		95.1	93.1	72.1	73	67.3	64.9	64.5	66.8
Ves g 5	93.6	93.1	95.1		94.1	70.6	74	66.8	64.4	62.6	66.8
Ves p 5	93.6	93.1	93.1	94.1		72.5	74.5	69.3	64.9	64.5	68.8
Ves s 5	71.1	70.6	72.1	70.6	72.5		80.5	68.6	67.2	65.3	68.1
Ves vi 5	74	74	73	74	74.5	80.5		68.6	70.6	66.8	71.6
Dol a 5	67.8	67.3	67.3	66.8	69.3	68.6	68.6		76	85.1	71.6
Dol m 5.01	64.9	64.9	64.9	64.4	64.9	67.2	70.6	76		77.3	72.1
Dol m 5.02	64	63.5	64.5	62.6	64.5	65.3	66.8	85.1	77.3		67.8
Vesp c 5.01	67.3	66.8	66.8	66.8	68.8	68.1	71.6	71.6	72.1	67.8	
Vesp c 5.02	65.9	65.4	65.4	66.3	67.3	66.7	70.6	70.1	71.1	66.3	97
Pol e 5	58.5	58.5	58	58.9	59.9	60.6	61.1	66	60.7	63.7	61.2
Pol a 5	57	57	58	57.5	58.9	61.1	61.1	65	60.7	63.7	61.2
Pol f 5	58.9	58.9	58.9	58	59.4	61.1	61.5	62.6	59.7	64.2	60.7
Sol i 3	43.3	43.3	44.3	44.3	43.8	44.8	45.8	47.4	46.2	49.8	49.1

Protein fragmentation

Cyanogen bromide cleavage was performed in 70% formic acid with at least a 100-fold excess of cyanogen bromide, in the dark under nitrogen at room temperature for 24 hours.²⁰ Ten volumes of water were added, and the mixture was dried in a centrifugal vacuum evaporator. Sequencing grade staphylococcal V8 protease, trypsin, chymotrypsin, and endoproteinase-Asp-N were all purchased from Sigma Chemical Co. Digestions were performed in 0.1 mol/L ammonium bicarbonate (pH 7.8), or Tris-HCl (pH 7.4), with up to 2 mol/L urea added if necessary for solubility, with 2 to 4 µg of enzyme per milligram of protein at 37° C.^{20, 21} Enzyme hydrolyses were stopped by adding trifluoroacetic acid (TFA) (Pierce Chemical Co.).

Separation of peptides

Peptide digests were separated by high-performance chromatography with an 0.46 × 25 cm Vydac 218TP54 reverse-phase column (The Separations Group, Hesperia, Calif.) with a gradient starting at 0.1% TFA in water to 70% of 0.07% TFA in acetonitrile. The flow rate was 0.6 ml/min and 0.5-minute fractions were collected in microcentrifuge tubes. The separation was monitored at 220 nm.

Protein sequencing

Sequencing was performed with a Milligen-Bioscience Model 6625 Prosequencer (Millipore Corp., Marlborough, Mass.) after the manufacturer's procedures with cycles J3. This is a solid-phase, fully automated sequencer/phenylthiohydantoin analyzer. Proteins were coupled to either 1,4-phenylene diisothiocyanate-derivatized disks or arylamine disks, and the covalently bound protein was reduced and alkylated

in the presence of 6 mol/L guanidine HCl as described previously. The disks were then washed five times each in water and methanol, and air dried. Peptides separated from digests were coupled to arylamine disks for sequencing. In rare cases peptides were coupled to 1,4-phenylene diisothiocyanate-derivatized disks to verify aspartic or glutamic acid residues.

Alignment and database comparisons

The Hitachi Software Engineering America, Ltd. (Brisbane, Calif.) Hibio Prosis programs and compact disk edition of the Protein Identification Resource (PIR) (National Biomedical Research Foundation) and SWISS-PROT (European Molecular Biology Laboratory) data bases were used on an MS-DOS computer system. The program can automatically align pairs of sequences, and a manual alignment routine is provided for multiple sequences. The PIR was release 29, September 1991, and SWISS-PROT release 20, November 1991. PIR release 35, January 1993, was also compared by the Atlas program, which can search only for strings of 30 amino acids at a time.

RESULTS

The complete amino acid sequence of *Ves s 5* was determined by solid-phase sequencing of the N-terminal 60 residues and sequencing each of the isolated peptides from endoproteinase-Asp-N, staphylococcal V8 protease, and trypsin digestions (Table I). The complete set of overlapping peptides were sequenced to establish unambiguously the sequence without reference to related molecules. The sequences of *Ves g 5* and *Vespula vidua* (*Ves vi*) 5 were determined from N-terminal runs

% Identical residues			
<i>Vesp c 5.02</i>	<i>Pol e 5</i>	<i>Pol a 5</i>	<i>Pol f 5</i>
65.9	58.5	57	58.9
65.4	58.5	57	58.9
65.4	58	58	58.9
66.3	58.9	57.5	58
67.3	59.9	58.9	59.4
66.7	60.6	61.1	61.1
70.6	61.1	61.1	61.5
70.1	66	65	62.6
71.1	60.7	60.7	61.5
66.3	63.7	63.7	64.2
97	61.2	61.2	60.7
	60.2	60.2	59.7
60.2		98.5	92.7
60.2	98.5		93.2
59.7	92.7	93.2	
49.5	44.9	45.8	45.8

and by sequencing each of the peptides from endoproteinase-Asp-N digestions. The sequence of *Ves p 5* was determined from an N-terminal run, sequencing all the peptides from an endoproteinase-Asp-N digestion and from the tryptic digestion of the C-terminal Asp fragment. The sequence of *Ves f 5* was determined from an N-terminal run, which sequenced the complete set of endoproteinase-Asp-N peptides, and the staphylococcal V8 digest of the C-terminal Asp peptide. The two variants of *Vesp c 5* did not separate by ion exchange chromatography and were sequenced together by an N-terminal run and by sequencing each of the peptides from a staphylococcal V8 protease digestion. The sequence of *Pol f 5* was determined from an N-terminal run of 37 residues, which sequenced each of the endoproteinase-Asp N-generated peptides, with further digests of the N- and C-terminal fragments by staphylococcal V8 protease. Because of the similarity of antigen 5 molecules, it was not necessary to generate sets of overlapping peptides for each protein, because 5 cDNA sequences and four protein sequences with complete overlaps have been determined. The set of peptides used to determine each sequence is shown in Table I. The new sequences have been deposited in the PIR database under acquisition numbers A44522 through H44522.

The 16 amino acid sequences of antigen 5 molecules were compared pairwise with the Prosis program. The percent identity between each pair is given in Table II. The sequences were also

aligned by computer, and these are illustrated in the figures. The sequences of the yellow jacket antigen 5 are shown in Fig. 1. The vulgaris group proteins, *Ves v 5*, *Ves f 5*, *Ves m 5*, *Ves g 5*, and *Ves p 5*, were all closely related with from 93.1% to 98% identical residues. Almost all the substitutions were conservative, involving only a single base change. There was only one nonconservative substitution from *Ves v* to *f* and two from *v* to *g*, *v* to *p*, *f* to *m*, *f* to *g*, *f* to *p*, *m* to *g*, and *g* to *p*. There were three nonconservative substitutions from *Ves v* to *m* and four from *m* to *p*.

The sequence of *Ves s 5* contained an additional amino acid residue, a threonine residue at position 21. Identity was only 70.6% to 72.5% with the vulgaris group of antigen 5 (Table II). From *Ves s 5* to *Ves p 5* there were 57 amino acid substitutions, 13 of which were nonconservative. *Ves s* is usually placed in a different subgenus from the vulgaris group of yellow jackets.²² This is also consistent with studies of immunologic cross-reactivity that show that *Ves s* venom can be recognized as different from the vulgaris group venoms by both human IgE^{10, 11} and animal IgG⁸ antibodies. The sequence of *Ves vi 5* contained the additional threonine seen in *Ves s 5* and an additional lysine at the N-terminus. The sequence was 73% to 74.5% identical to those from the vulgaris group and 80.5% identical to *Ves s 5*. Only five of the 40 amino acid differences between *Ves vi 5* and *Ves s 5* were not conservative. *Ves vi* is normally placed in the rufo species group,²² which have smaller nests and are usually predators. The rufo group insects rarely sting human beings. These sequence data suggest that *Ves s* is more closely related to the rufo group than to the vulgaris group but is clearly distinct.

The sequences of antigen 5 molecules from three species of *Polistes* (paper wasp) venoms are shown in Fig. 2. *Pol a* and *Pol e* come from insects in the subgenus *Aphanilopterus*, and *Pol f* from the subgenus *Fuscopolistes*. All three molecules are 205 residues long, with only three amino acid differences between *Pol a 5* and *Pol e 5*, 14 between *Pol a 5* and *Pol f 5*, and 15 between *Pol e 5* and *Pol f 5*. All these differences represent conservative substitutions. These findings are consistent with the high degree of immunologic similarity seen among paper wasp venoms from these subgenera.¹² Some immunologic differences have been noted between the venoms of the New World genera of *Polistes*, discussed previously, and venom from the Old World species *P. dominulus*,²³ for which no sequence data are available at present.

The amino acid sequences of the antigen 5

Ves v 5	1	-NNYCKIKCL KGGVHTACKY	G-SLKPNCNG KVVVS YGLTK	QEKQDILKEH	50
Ves f 5	1	*****	*****	*****	50
Ves m 5	1	*****	*-*****	*K*****	50
Ves g 5	1	*****	E-*****A*	*K**A*****	50
Ves p 5	1	*****	*-*****	*I*****	50
Ves s 5	1	-VD*****	*T*T*****	M**K**V*Q A**E**I*	50
Ves vi 5	1	KV*****	*T*T*****	M**K**A**E A**E**V*	50
Ves v 5	51	NDFRQKIARG LETRGNPGPQ	PPAKNMKNLV WNDELAYVAQ	VWANQCQYGH	100
Ves f 5	51	*****	*****	*****	100
Ves m 5	51	*****	*****	*S*****I**	100
Ves g 5	51	*****	*****	*S*****I**	100
Ves p 5	51	*****	*****	*****	100
Ves s 5	51	***N*V**	*****N**	*N**N**I** I**S**G**	100
Ves vi 5	51	*****V*K*	*****N**	*****N** I**S**N**	100
Ves v 5	101	DTCRDVAKYQ VGQNVALTGS	TAAKYDDPVK LVKMWEDEVK	DYNPKKFFSG	150
Ves f 5	101	****I****	*****	*****	150
Ves m 5	101	*****	***V*N***	*****E	150
Ves g 5	101	*****P	*****N**	*****E	150
Ves p 5	101	*****P	***D***N**	*****E	150
Ves s 5	101	***K*TT*N	****I*VSS*	***V*ENVGN ***A**N**	150
Ves vi 5	101	***K*TE*P	****I*KRST	***LF*S*G* *****	150
Ves v 5	151	NDFLKTGHYT QMVWANTKEV	GCGSIKIYQE KWHKHYLVCN	YGPNGFMNE	200
Ves f 5	151	*N*****	*****F**	*****Q**	200
Ves m 5	151	*N***I****	*****N*****	*****Q**	200
Ves g 5	151	*N***I****	*****D*****	*****G**	200
Ves p 5	151	*N*N*I****	*****N*****	*****G**	200
Ves s 5	151	*E*K*I****	*****K***I	*****VDN N*YT*****	200
Ves vi 5	151	*NLK*****	*****K***I	***V*VKD E*YT*****	200
Ves v 5	201	ELYQTK			
Ves f 5	201	*****			
Ves m 5	201	*****			
Ves g 5	201	*****			
Ves p 5	201	*****			
Ves s 5	201	*V*ER*			
Ves vi 5	201	K*EK*			

FIG. 1. Comparison of amino acid sequences of antigen 5 molecules from seven species of yellow jackets. Dashes indicates gaps inserted to maximize homology, and asterisks indicate residues identical to those of top sequence. Sequence of Ves v 5 was determined from translation of the cDNA sequence¹⁷ and others by amino acid sequencing of overlapping peptides.

molecules from three species of hornets are compared in Fig. 3. The sequences of *Dol m* 5.01 and 5.02¹⁶ and *Dol a* 5¹⁷ have been presented and compared previously. *Vesp c* 5 was found as two variants, differing by six amino acid residues, of which four are conservative substitutions. Two of three residues were also deleted between positions 143 and 152 relative to the *Dolichovespula* hornet antigen 5. *Vesp c* 5 is 66.3% to 72.1% identical to *Dolichovespula* and 65.4% to 71.6% identical to yellow jacket venom antigen 5 molecules. *Vesp c* 5 is also 59.7% to 61.2% identical to paper wasp antigen 5's. This is consistent with the immunologic observations that *Vespa crabro* venom is as closely related to yellow jacket venom as it is to *Dolichovespula* venoms.¹⁴

The sequences of antigen 5 molecules from a representative species of each genus including *Sol*

i 3 from imported fire ants and *Ves s* 5 are compared in Fig. 4. A number of gaps have been inserted to maximize homology. It is possible to align all the cysteine residues. The pattern shows several regions are relatively conserved among the vespid wasp antigen 5. These include fairly long stretches of sequence such as residues 49 to 99. By contrast, only short regions are in common between *Sol i* 3 and the vespid venom proteins. *Sol i* 3 also has two regions of inserted amino acids from residues 11 to 13, and 151 to 154. Extensive immunologic studies of human IgE antibodies have not revealed any detectable cross-reactivity between *Sol i* 3 and vespid antigen 5.¹⁵ In addition to the changes discussed within the individual groups, a glycine insertion is present after residue 11 in *Vespula* antigen 5's. Paper wasp antigen 5 molecules have an insertion of serine and lysine at

Pol e 5	1	VDYCKIKCPS	GIHTVCQYGE	STKPSKNCAG	KVIKSVGPTE	EEKKLIVSEH	50
Pol a 5	1	*****S*	*****D	*****N**			50
Pol f 5	1	*****S*	*****D	*****N**			50
Pol e 5	51	NRFRQKVAQG	LETRGNPGPQ	PAASDMNDLV	WNDELAHIAQ	VWASQCQFLV	100
Pol a 5	51	*****S*	*****D	*****N**	*****I**		100
Pol f 5	51	*****S*	*****D	*****N**	*****I**		100
Pol e 5	101	HDKCRNTAKY	PVGQNIAYAG	GSKLPDVVSL	IKLWENEVKD	FNNTGKITQK	150
Pol a 5	101	*****S*	*****D	*****N**	*****K*****		150
Pol f 5	101	*****S*	*****D	*****N**	*****K*****		150
Pol e 5	151	NFAKIGHYTQ	MVWGKTKKEIG	CGSLKYIENK	MQNHLYLCNY	GPAGNYLGQL	200
Pol a 5	151	*****S*	*****D	*****N**	*****H*****		200
Pol f 5	151	*****S*	*****D	*****N**	*****H*****		200
Pol e 5	201	PYTKK					
Pol a 5	201	*****					
Pol f 5	201	*****					

FIG. 2. Comparison of amino acid sequences of antigen 5 molecules from three species of paper wasps. Symbols are as in Fig. 1. Sequence of *Pol a 5* was determined by translation of cDNA sequence.¹⁷ Sequences of *Pol e 5*¹⁷ and *Pol f 5* were determined by amino acid sequencing.

		10	20	30	40	50	
Dol a 5	1	NNYCKI-CPK	GHTTLCKYGT	SMKPNCGGKI	VKSYGVTNDE	KNEIVKRHNE	50
Dol m 5.01	24	*****K*R*	*****F**	*****RNV	***A**L***	***L***D	73
Dol m 5.02	11	*****K*SR	*****F**	*****S*L	***VH**S***	*****N***Q	60
Vesp c 5.01	1	*****K*RS	*****F**	*****KNV	***AS*L*KQ*	NL**L*Q***	50
Vesp c 5.02	1	*****K*RS	*****F**	*****KNV	***AS*L*KQ*	NL**L*Q***	50
		60	70	80	90	100	
Dol a 5	51	FRQKVAQGLE	TRGNPGPQPP	AKNMNLLVWN	DELAKIAQTW	ANQCNGFHDQ	100
Dol m 5.01	74	*****K***	*****K*****	*****V***	*****D*N**D		123
Dol m 5.02	61	*****K***	*****K*****	*****V***	*****S*****		110
Vesp c 5.01	51	*****R***	*****K*****	*****S**T***	*****Q***V*	*****Y***N	100
Vesp c 5.02	51	*****R***	*****K*****	*****S**T***	*****Q***V*	*****Y***N	100
		110	120	130	140	150	
Dol a 5	101	CRNTAKYPVG	QNVIASTTG	NSYQTMSTYLI	KMWEDEVKDY	NPHKDLMHNN	150
Dol m 5.01	124	*****Q**	***I**S***A	TQFDRP*K**	*Q*****TEF	*YKVG*QNS*	173
Dol m 5.02	111	*****Q**	*****K**	E***N***F	**K*GT*GD*		160
Vesp c 5.01	101	***S***S**	***I*EG***A	DNFGSV*NMV	*****QY--GSPK--		150
Vesp c 5.02	101	***S***S**	***I*EG***A	DNFVNV*NMV	*****QY--GSPK--		150
		160	170	180	190	200	
Dol a 5	151	-FSKVGHYTQ	MVWGKTKKEIG	CGSVKYIENK	WHTHYLCNY	GPAGNYMNQP	200
Dol m 5.01	174	-R*****	*****I*****	*****Y*****	*****G**DF**		223
Dol m 5.02	161	N*****	*****I*****	*****Y*****	*****D**		210
Vesp c 5.01	151	KLN*****	***A*****	***I*****C	**R*****	*****VG*E*	200
Vesp c 5.02	151	KLN*****	***A*****	***ED***DG	**R*****	*****VG*E*	200
Dol a 5	201	YIERK					
Dol m 5.01	224	I****					
Dol m 5.02	211	I*					
Vesp c 5.01	201	I****					
Vesp c 5.02	201	I****					

FIG. 3. Comparison of amino acid sequences of antigen 5 molecules from three species of hornets. Symbols are as in Fig. 1. Sequences of *Dol a 5*¹⁷ and *Dol m 5.01* and *5.02*¹⁶ were determined by translation of the cDNA sequences. Sequences of *Vesp c 5.01* and *5.02* were determined by direct amino acid sequencing.

	10	20	30	40	50
Ves v 5	NNYCKIKCLK	GG--VHTACK	YG-SLKP--N	CGNKVVVSYG	LTQKEKQDIL
Ves s 5	VD*****	**--*****	**T*T*---	***M**K**	V*QA***E**
Dol m 5.01	*****R*	*---I**L**	F*T*M*---	**RN**KA**	***ND**NE**
Vesp c 5.01	*****RS	*---I**L**	**T*T*---	**KN**KAS*	*****NLE**
Pol e 5	VD*****PS	*---I**V*Q	**E*T**SK*	*AG**IK*V*	P*EE**KL*V
Sol i 3	T***NLQSC*	RNNAI**M*Q	*TSPTPGP-M	*LEYSN*GFT	DAEKDAIVNK
	60	70	80	90	100
Ves v 5	KEHNDFRQKI	ARGLETRGNP	GPQPPAKNMK	NLVWNEDELAY	VAQVWANQCQ
Ves s 5	*I*****NV	*****N	*****N	*****N	I**I**S**K
Dol m 5.01	*R*****NV	*K*****K*	*****N	V*****K	I**T*****D
Vesp c 5.01	*Q**E***V	*****S*N	T*****Q	I*****N	*****S**S
Pol e 5	S***R***V	*Q*****A	SD*N	D*****H	I*****S**S
Sol i 3	HNELRQ*VAS	GKEMRGTTNG*	Q*PAV--K*P	**T*DP***T	I**R*****T
	110	120	130	140	150
Ves v 5	-YGHDTCRDV	AKYQVGQNV	LTGSTAAKYD	DPVKLVKMWE	DEVKDYNPCK
Ves s 5	-----K*T	T**N*****I	VSS*****V	EVGN*****A	N*****F**TI
Dol m 5.01	-FN**D**NT	*****S**I	ISST**TQF*	R*S**I*Q**	***TEF*Y*V
Vesp c 5.01	-----N**NS	***S*****I	EGST**DNFG	SVSNM*****	*****QYGS
Pol e 5	FLV**K**NT	***P*****I	YA*GSKLP--*	VVS--*I*L*	N*****F*YNT
Sol i 3	-FE**A**N*	ERFA*****I	A*S*SGKNKS	T*NEMILL*Y	N*****FDNRW
	160	170	180	190	200
Ves v 5	----KFSGND	FLKTGHYTQM	VWANTKEVGC	GSIKYI-QEK	WHKHVLVCNY
Ves s 5	----SWEQ*E	*K*I*****	***K***I**	*****V-DNN	*YT*****
Dol m 5.01	----GLQNSN	*R*V*****	**GK***I**	*****-EDN	*YT*****
Vesp c 5.01	-----PK*K	LN*V*****	***K***I**	*****-ENG	**R*****
Pol e 5	----GITKQN	*A*I*****	**GK***I**	**L***-EN*	MQN***I***
Sol i 3	ISSFPSDD*I	LM*VE*****I	***K*SKI**	AR*MFKEPDN	*T*****
	210				
Ves v 5	GPSGNFMNEE	LYQTK			
Ves s 5	**A***G*Q*	V*ER*			
Dol m 5.01	**G**DF*QP	I*ER*			
Vesp c 5.01	**A**VG**P	I*ER*			
Pol e 5	**A**YLGQL	P*TK*			
Sol i 3	**A**VLGAP	I*EI*K			

FIG. 4. Comparison of amino acid sequences of antigen 5 molecules from two species groups of yellow jackets, both genera of hornets, paper wasps, and *Sol i 3* from imported fire ants. Symbols are as in Fig. 1. Sequence of *Sol i 3* was determined by amino acid sequencing of overlapping peptides.⁶

28 and 29, phenylalanine at 101, and deletions at positions 129 and 134 relative to the other vespids. The calculations of identity in Table II show that the yellow jacket antigen 5 molecules are more related to hornet antigen 5 than to paper wasp antigen 5. The paper wasp antigen 5 is slightly closer to the hornet antigen 5 than to yellow jacket, but only marginally so. The range of vespid antigen 5 identity with *Sol i 3* is from 43.3% to 49.8%.

DISCUSSION

The antigen 5 family of wasp venom proteins all possessed a number of distinguishable features. The molecules were all from 202 to 212 amino acids long, with eight cysteine

residues. The predicted isoelectric points were 8.6 to 9.5, except for *Sol i 3*, which is 8.04. As shown in Fig. 4, it is possible to align the cysteines with only a relatively small number of insertions and deletions. All the vespid molecules showed at least 57% identity, and the imported fire ant molecule showed from 43% to 49.5% identity with the vespid molecules. Many regions of the molecules were conserved among the vespids and partially conserved in fire ant allergen. This high degree of conservation of many of the areas around the cysteines, the similar locations of cysteines, and the existence of relatively long conserved regions suggest that the tertiary conformations may be similar for the vespid venom molecules.

The structural features correlated with the observed cross-reactivity patterns of human IgE antibodies. The allergens from the vulgaris group of yellow jackets were structurally very similar and essentially totally cross-reactive serologically. *Ves s 5* was significantly different structurally and can be distinguished by some sera from allergic patients.^{10, 11} No serologic data were available for *Ves vi 5*, which was also structurally different from the vulgaris group. The antigen 5 molecules from species representing both major North American subgenera of paper wasps were very closely related, with no nonconservative amino acid substitutions. Serologic testing also showed very little difference in allergen recognition by human IgE antibodies among the species of these two subgenera.¹² The structures of the two variants of antigen 5 from the European hornet showed significant differences from both yellow jacket and *Dolichovespula* hornet venoms, which was consistent with the serologic observations of partial cross-reactivity with both groups.¹⁴ Enough common features were shared among all the vespid venoms to explain the partial cross-reactivities observed in many allergic patients among the four genera.^{13, 14} Enough differences were present among the genera, yellow jacket species groups, and hornet species to explain the not infrequent occurrence of IgE antibodies that are species, group, or genus specific.¹³

Sol i 3 showed a much lower degree of sequence identity with the vespid antigen 5 molecules and is significantly less basic in charge. Extensive serologic studies with human IgE antibodies have not revealed any significant immunologic cross-reactivity with vespid antigen 5.¹⁵ This is similar to the situation observed with the ragweed pollen allergens *Amb a 5* and *Amb t 5*, which have 45% sequence homology and are essentially non-cross-reactive immunologically.²⁴

As amino acid sequence data become available for more groups of related allergens and additional studies of tertiary structure are performed, it may become possible to quantify the sites and probabilities of cross-reactions at both the B and T cell levels. This information will allow the rational design of synthetic peptides to modulate specific allergen responses²⁵ to cross-reactive allergen groups. Other allergens such as grass pollens and some tree pollens also are composed of mixtures of several classes of structurally related protein molecules^{26, 27} such as the vespid venoms, so studies of this nature may aid in the development of class-specific peptide mixtures such as vespid venom and grass pollen.

The eight additional vespid antigen 5 sequences described in this article bring the number of complete vespid venom antigen 5 sequences to 15, in addition to *Sol i 3*, which is also a member of this protein family. This is the largest series of related allergen sequences known.

The function of antigen 5 is unknown, although it has been postulated to be an invertebrate neurotoxin.^{28, 29} Although all these insects are known to sting vertebrates either in defense of their colonies or themselves, this may be a secondary role. Other wasps use their stings to paralyze prey for food. *V. squamosa* is a social parasite of *V. maculifrons*, and the queens of each species use their stings for offense and defense, respectively.²² Some of the protein components in vespid venoms may have evolved for use against other insects or invertebrates rather than vertebrates. This may help to explain why variation in the amino acid sequences within a restricted framework of antigen 5 structure is so significant. The venom proteins may have become optimized for use against specific invertebrates. No proteins similar to antigen 5 have been described from venoms of the predatory sphecoid and pompiloid wasps. Because an antigen 5 molecule is found in fire ants (formicoid wasps), it would not be unexpected in other wasps.

These studies provide a structural basis for the cross-reactivity of antigen 5 molecules from vespid venoms that can be seen clinically by both skin testing and in vitro testing,^{1, 4, 7, 9, 15} and in laboratory studies with animal antisera.^{1, 4, 5, 8} Recently reported studies of the sequences of vespid venom phospholipases^{30, 31} indicate that these enzymes are very similar in closely related species of yellow jackets and significantly different between yellow jackets and white-faced hornets. Presently, commercial venom extracts sold in North America contain a mixture of venoms from several species of yellow jackets and paper wasps. These studies and the cross-reactivity studies previously reported^{10, 12} suggest that these venom protein extracts could be more simply formulated. Simpler formulations would be easier to standardize and control. All the vulgaris group of yellow jackets have allergens that are highly cross-reactive and very similar structurally, and only a single representative is probably needed. *V. squamosa* venom allergens are significantly different from those of the vulgaris group, so they need to be present in the mix. Stings by insects of the rufa group such as *V. vidua*, are rare, and this venom is probably not needed. All the important paper wasps found in the United States and Canada belong to the two subgenera studied. Their allergens are also very

similar structurally and highly cross-reactive. A single-species venom would probably be sufficient for diagnosis and therapy. The three hornet venom antigen 5 molecules all show significant differences in structure and are not fully cross-reactive.^{1, 4, 5, 8, 9, 13, 14} It is probably necessary to continue to have all three available, although yellow hornet and European hornet should have only limited use in areas where these insects are not found¹⁴ and where patient reactivity results from cross-reactivity with other vespid venoms.

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